ASIP Meritorious Trainee Award Lectures

Abstract 001

Inflamed Intestinal Endothelial Cells Establish Self-Regulatory Niches via IL16 Dependent Recruitment of CD4 Expressing Vessel-associated Macrophages

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Introduction: Macrophages play important roles in initiation and resolution of inflammation. Notably, a subset of tissue macrophages strategically localizes to the blood vessels, exerting essential regulatory functions at the blood-tissue interface, including regulation of vascular permeability and neutrophil (PMN) transendothelial migration (TEM). Although the number of vessel-associated macrophages (VAMs) is increased in inflamed tissue, the underlying mechanisms of VAMs recruitment are unknown. Our current study uncovers a novel mechanism whereby inflamed intestinal vascular endothelial cells (ECs) release IL-16 to selectively recruit $CD4^+$ tissue macrophages to become VAMs. **Methods and Results:** Whole-mount confocal microscopy studies in CX3CR1-EGFP macrophages reporter mice confirmed significant increases in VAMs numbers in several murine models of intestinal inflammation. Surprisingly, the majority of VAMs were found to express a classical T cell marker, CD4 and to be of an inflammatory phenotype, enriched for ICAM-1 and TNFα expression. VAMs-derived TNFα was previously found to prime ECs to from PMN TEM hot spots. Whole genome mRNA sequencing, and flow cytometry/western blot protein expression analyses revealed heightened expression of the CD4 ligand, IL-16 by inflamed intestinal lamina propria ECs. In vitro, using transwell assays, recombinant IL-16 and supernatants derived from murine (bEnds.3) or human (HUVEC) endothelial cells induced robust migration of murine, LPS/IFNγ-stimulated bone marrow-derived or human THP-1 cell line-derived macrophages. Whereas antibody-mediated IL-16 or CD4 neutralization in cultures and in vivo reduced VAMs recruitment. **Conclusion:** Collectively, our findings establish CD4 as a novel marker of VAMs and identify the regulatory role of ECs IL-16 and macrophages $CD4^+$ axis in VAMs recruitment in inflamed mucosal tissues.

Abstract 002

***Acinetobacter calcoaceticus* Stimulates Inflammation in the Gastrointestinal Tract**

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Introduction: Inflammatory bowel disease (IBD), which encompasses the subsets Crohn's Disease and Ulcerative Colitis, is a life-long condition characterized by chronic inflammation of the gastrointestinal tract. It has been speculated that gut microbes in IBD patients drive or exacerbate inflammation, but few studies have identified bacterial candidates. Analysis of 3,853 publicly available RNA-Seq samples from 26 independent studies revealed that *Acinetobacter calcoaceticus* was one of the top 10 highest elevated bacteria in Crohn's Disease patients. *Acinetobacter* are well-characterized by their antibiotic resistance, but little is known about its relationship to the intestine. We hypothesize that *A. calcoaceticus* colonizes the gut and promotes intestinal inflammation. **Methods and Results:** We grew commercially available and clinical isolates of *A. calcoaceticus in vitro* in minimal media with stressors found in the gut. All strains grew in a range of pHs (4-7), osmolarity (0.1-1 M NaCl), ethanol (1- 5%) and hydrogen peroxide (0.05-0.1%); indicating that *A. calcoaceticus* is well-adapted to withstand the harsher conditions of the gastrointestinal tract. To further investigate the ability of *A. calcoaceticus* to colonize the gut, we utilized *Caenorhabditis elegans* as a model. All *A. calcoaceticus* strains colonized the nematode's gut. We likewise found that all *A. calcoaceticus* strains colonized human stool-based bioreactors; suggesting that *A. calcoaceticus* can colonize the gastrointestinal tract. To identify whether *A. calcoaceticus* stimulated inflammation, we incubated intestinal organoids and macrophages with live *A. calcoaceticus* or LPS derived from our *A.*

calcoaceticus strains and found that both conditions stimulated pro-inflammatory cytokines. In order to confirm our *in vitro* findings, we oral gavaged adult BalbC mice with *A. calcoaceticus* and examined fecal levels using selective agar and qPCR. *Acinetobacter* was observed at low levels in the vehicle control mice and at high levels in our mice gavaged with *A. calcoaceticus* (average 9.9x 10^7 CFU); suggesting that *A. calcoaceticus* colonized the murine gut. To examine the ability of *A. calcoaceticus* to enhance inflammation, we oral gavaged mice with either a vehicle control (PBS) or *A. calcoaceticus* and the following day we administered 2,4,6-trinitrobenzene sulfonic acid (TNBS) to induce colitis. Mice treated with *A. calcoaceticus* and TNBS lost more weight and had worse histological scores than mice treated with vehicle control and TNBS; indicating *A. calcoaceticus* worsens intestinal inflammation. **Conclusions:** These data show that *A. calcoaceticus* can promote intestinal inflammation and may be a contributing factor to the inflammation experienced by IBD patients. We speculate that traditional IBD treatments, such as anti-TNF therapy, could be complemented with *A. calcoaceticus* specific antibiotics to improve patient well-being.

Abstract 003

Noradrenergic Stimulation Reconfigures Brain States

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Introduction: The noradrenergic system (NS) modulates brain-wide neurophysiology, “brain states”, through its projections from the locus coeruleus (LC) throughout the brain. While loss of LC neurons is a characteristic of neurodegenerative diseases such as Alzheimer’s disease (AD), heightened LC signaling may play a role in anxiety disorders and addiction. We are defining brain-states as fractional activation volumes and coordinated cross-correlated neural activity. To learn how NS modulates brain-wide activity we adopted a chemogenetic approach and MR neuroimaging. **Methods:** Brain-wide activity in C57BL/6 mice was imaged by manganese-enhanced MRI (MEMRI) in parallel with video recordings before, during, and 9 days after chemogenetic (hM3Dq) stimulation of LC neurons. After systemic delivery, paramagnetic Mn(II) (0.3 mmol/kg, i.p.) enters active neurons of awake freely moving mice, where accumulation highlights neural activity detected by retrospective T_1-weighted MRI of Mn(II) hyperintense signal. Mice were subjected either to longitudinal MEMRI (n=18) or to open field video recordings (n=8): ±hM3Dq expression, and ±designer drug to activate the transfected neurons (clozapine-N-oxide, CNO). For videos, movements of naïve mice were recorded and tracked by Noldus Ethovision XT15, before and after administration of CNO (5 mg/kg, i.p.). Mice then received intracerebral stereotactic injection into the LC (AP=-5.4, ML=±0.80, DV=-3.80) of viral vector: CAV2-PRS-hM3D(Gq)-mCherry. At 4-8 weeks after transfection the effect of CNO ±hM3Dq on LC, we monitored pupillary light response in live animals. Then pre- and post-CNO recordings of movement were again acquired from hM3Dq expressors. For MEMRI two groups ±hM3Dq were imaged before and after CNO. MEMRI images were analyzed for Mn(II)-dependent intensity increases by statistical parametric mapping (SPM), segment-wise cross-correlation and network analysis. **Results:** Bilateral neuron-specific mCherry expression demonstrated successful transfection of the LC. Our MEMRI images showed little CNO increased brain-wide Mn(II)-enhanced intensity in naïve mice by statistical parametric mapping. CNO heightened activity in limbic regions and in LC of hM3Dq transfected mice. By day 9, activity in these mice persisted within many regions, accompanied by additional signal in the hippocampus. Segmentation and bootstrap resampling cross-correlations of 96 brain regions revealed increased coordination between regions of basal subnetworks immediately after LC stimulation. **Conclusions:** Activation of LC alters distributed patterns of neural activity brain-wide, brain states. These results emphasize the significance of acute LC activation, as it influences both the resting brain state and future physiological state. In neurodegenerative diseases, loss of NS tone could result in the opposite effect, uncoordinated activity, that may represent a pathological brain state. **Acknowledgement:** NIMH RO1MH096093; Harvey Family.

Abstract 004

Diverse Bacteria Possess Histamine Decarboxylase Genes and Are Capable of Generating Histamine

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Introduction: Histamine is an important biogenic amine produced in various environments. Bacteria can generate histamine from the amino acid L-histidine via histidine decarboxylases. Certain bacteria in fish, fermented foods and the gut microbiota have been shown to convert L-histidine to histamine. However, an extensive examination of histamine production in bacteria is lacking. We hypothesized that bacteria from diverse backgrounds would harbor decarboxylases and be able to generate histamine *in vitro.* **Methods and Results**: address this gap in knowledge, we examined 102,018 annotated genomes in the Integrated Microbiome Genome Database and identified 3,679 bacteria which possess the enzymatic machinery to generate histamine. These bacteria belonged to 9 phyla: Firmicutes, Bacteroidetes, Acintobacteria, Proteobacteria, Lentisphaerae, Fusobacteria, Armatimonadetes, Cyanobacteria, and Verrucomicrobia. Of the phyla, Proteobacteria harbored the largest number of histidine decarboxylase possessing microbes. The majority of these identified bacteria were found to be terrestrial or aquatic in origin. In addition to environmental bacteria, we identified 100 bacterial species from the human gut microbiota. We used liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based targeted metabolomics to confirm and quantify L-histidine consumption and histamine production in a cohort of bacterial strains representative of those identified by our extensive genomic survey. Among the terrestrial and aquatic microbes, we found that *Aeromonas hydrophilia, Vibrio parahaemolyticus, Streptomyces griseus, Pseudomonas fluorescens* and *V. harveyi* all generated histamine; with *V. harveyi* producing the highest levels of histamine. Among the gut microbes, we observed *Klebsiella aerogenes, Lactobacillus reuteri, Clostridium perfringens, Fusobacterium varium* and *Morganella morganii* produced substantial histamine. Interestingly, *M. morganii* generated >2x higher levels of histamine than any of the other microbes, suggesting that this strain could be major contributor of histamine. **Conclusions:** This work reveals unique patterns of histamine production among diverse bacterial populations and highlights that ability of genomic analysis to predict histamine production.

Abstract 005

Complete Response of CTNNB1-mutated Tumors to RNAi-mediated β-catenin Inhibition in Aggressive Immunocompetent Mouse Models

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Introduction: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality globally. Approximately 25-35% of HCC patient tumors have mutations in β-catenin (CTNNB1). Several studies have indicated that this HCC subclass is almost entirely resistant to immunotherapy regimens, which are the current standard of care. Preclinical studies have elucidated addiction to β-catenin signaling in β-catenin-mutated HCC, making this target prime for precision medicine therapeutics. In the current study, we investigate the relevance of CTNNB1 suppression in models of β-catenin driven HCC and address possible underlying mechanisms of response. **Methods**: We inhibited β-catenin mRNA levels in the G31A-NFE2L2-T41A-β-catenin and hMet-S45Y-β-catenin mouse models of HCC which each represent around 9-12% of all human HCC. Starting at two different timepoints following hydrodynamic tail vein injection (HDTVi), animals were administered siRNAs formulated in lipid nanoparticles (LNP) targeting either CTNNB1 (LNP-CTNNB1) or scrambled control (LNP-CTRL). **Results**: LNP-CTNNB1 administered once weekly for six weeks starting at 5 weeks post HDTVi, when microscopic tumor foci were present, demonstrated complete tumor responses (CR) compared to LNP-CTRL in both models. Three days following the last dose in each model, the LNP-CTNNB1 group had normal liver weight/body weight ratio (4-4.5%) and histologically normal liver parenchyma compared to significant tumors in the LNP-CTRL group (p<0.001). Additionally, the LNP-CTNNB1 group had durable responses up to 3 weeks following treatment

cessation. Notably, a delayed treatment with LNP-CTNNB1 starting at 8 weeks post HDTVi, when macroscopic tumor nodules were present, delayed disease progression. To investigate the underlying mechanisms, livers were harvested 3 days after a single LNP dose. LNP-CTNNB1 treatment decreased activation of several β-catenin target genes (Glul, Axin2, Lect2), along with decreasing tumor cell proliferation markers (Ki67, Ccnd1), inhibition of mTOR pathway signaling components, and increased tumor cell death observed via TUNEL immunohistochemistry. Strikingly, the LNP-CTNNB1 group revealed dynamic shifts in the tumor microenvironment, including alterations in gene signatures related to type I interferon response, lymphoid progenitor cell differentiation, antigen presentation, and T cell cytokine production. **Conclusions**: Our studies demonstrated the efficacy of RNAi-mediated inhibition of CTNNB1 for β-catenin-mutated HCC and provide strong preclinical evidence to support clinical translation as monotherapy as well as in combination with other treatments.

Engineering the Matrix for Healing

Abstract 006

Regenerating the Pediatric Tympanic Membrane with a Decellularized Cartilage Matrix in an *in vivo* Rat Model

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Introduction: Tympanoplasty is the most common pediatric surgery where a perforation of the tympanic membrane (TM) is patched using an autologous fascia graft. However, pediatric tympanoplasty suffers a 20% failure rate from insufficient graft stiffness, adherence, and integration. Stiffer autologous ear cartilage is then often used to prevent graft retraction, but cartilage harvest is invasive and suffers from poor remodeling. To circumvent these limitations, we propose the use of decellularized porcine meniscal fibrocartilage matrix, which mimics the TM structure. Furthermore, meniscus contains elastin-fibers can be enzymatically removed to create microchannels to support integration. We hypothesized a **men**iscal **d**ecellularized (MEND) matrix will integrate better than autologous cartilage grafts. **Methods:** Meniscus cross-sections were cut to 300-micron thickness, freeze/thawed and digested with pepsin and elastase. Grafts were punched using biopsy-punches and compared histologically to fascia, native meniscus, and ear cartilage. Then, cartilage, MEND, and fascia were used to patch 40 perforated rat eardrums. Healing was monitored via endoscopy over 4 weeks, and via histology and immunochemistry at endpoint. **Results:** Histology of MEND compared to that of cartilage and of the native TM showed robust microchannels with sufficient channel diameters to promote cell invasion. MEND grafts *ex vivo* are thicker than native tissues, potentially providing higher initial mechanical strength. *In vivo*, MEND successfully closed the perforation, integrated into the TM by day 3, and rapidly remodeled by day 28 recreating the tri-layered collagenous structure of the TM. Differently, the cartilage graft, while it adhered to the TM, did not show significant signs of integration or remodeling into the host tissue. This lack of integration is often observed in pediatric patients with autologous grafts, who have a cartilage "island" in their eardrum for the rest of their life. **Conclusions:** Our findings suggest that MEND grafts could significantly improve pediatric tympanoplasty outcomes.

Abstract 007

A Novel Treatment for Scleroderma Fibrosis: The CCN3-based Peptide BLR-200 Impairs Bleomycin-induced Skin Fibrosis

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Introduction: Of the CCN family of matricellular proteins, CCN2 promotes, whereas CCN3 inhibits, in vivo models of fibrosis. A proprietary, synthetic peptide (BLR-200), based on an amino acid sequence identified

within CCN3, mimics CCN3's antifibrotic activity. However, the mechanism underlying BLR-200's antifibrotic activity is only now beginning to be elucidated. Previously, we have shown that collagen-lineage fibroblasts and hippo/YAP1-mediated mechanotransduction are essential for inflammation-induced skin fibrosis. However, the specific subsets of collagen-lineage fibroblasts activated in response to inflammation-induced skin fibrosis are unidentified. Whether BLR-200 affects hippo/YAP and blocks the activation of collagen-lineage fibroblasts is unknown. **Methods:** Three-week-old Col1A2-Cre(ER)T/0; mT/mG C57Bl/6j mice were subcutaneously injected with tamoxifen for 5 consecutive days to label collagen-lineage fibroblasts with green fluorescent protein (GFP) and subsequently injected with PBS or bleomycin daily for 10, 21 or 28 days. Mice were injected every other day with BLR-200 or scrambled sequence control peptide. Intact skin was isolated, and subjected to histological, proteomic, spatial transcriptomic, scRNAseq and bulk RNAseq/real-time polymerase chain reaction (RT-PCR) analyses. **Results:** Histological analysis revealed that BLR-200 broadly impaired the ability of bleomycin to induce skin thickness, collagen production and myofibroblast differentiation ($N=8$, $p<0.05$). Proteomic and RNA analyses confirmed that BLR-200 selectively and subtly impaired pro-fibrotic cohorts of proteins and RNAs induced by bleomycin. BLR-200 impaired the bleomycin-mediated activation of a subset of collagen-lineage fibroblasts, the induction of IL-6 and egr1, but not of IL-1 or AP-1 family members, and the expression of a hippo/YAP signature (including CCN2, YAP1, Smad3, wnt10b, TGFbeta1 and 2). Basal expression of these pro-fibrotic agents was largely unaffected by BLR-200. **Conclusion:** BLR-200 impairs the ability of fibroblasts to respond to inflammatory insults by antagonizing YAP1 and thus may represent a novel, anti-fibrotic therapeutic approach.

Aortic Disease in Marfan Syndrome and Related Conditions

Abstract 008

Fibulin-4 and Fibulin-5 Serve Distinct, Non-overlapping Functions in Elastic Fiber Assembly and Cardiovascular Physiology

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Introduction: Fibulin-4 and -5 (FBLN-4 and -5) are extracellular matrix proteins important for elastic fiber assembly. Mutations in each of the genes lead to cutis laxa, a syndrome characterized by loose skin with variable organ involvement, particularly in the vasculature, lungs and skeleton. We recently characterized the cardiovascular manifestations of a mouse model carrying a disease-causing mutation in Fbln4 (E57K). Fbln4E57K/E57K mice developed ascending aortic aneurysms with elastic fiber fragmentation and increased stiffness in conduit arteries, however, their resistance arteries were structurally intact. Loss of FBLN5 has been shown to lead to elastic fiber fragmentation without aneurysm formation in conduit arteries, but the consequences of its loss on resistance arteries are largely unknown. Herein, our goal was to investigate the individual and combined contribution of FBLN4 and FBLN5 to elastin assembly along the arterial tree and the consequences of their aberration on the cardiovascular system. **Methods:** Fbln4E57K/E57K mice were bred to Fbln5-/- mice and the cardiovascular phenotype of double mutant (Fbln4E57K/E57K; Fbln5-/-) mice was compared to that of wild-type, Fbln4E57K/E57K and Fbln5-/- littermate mice. In addition to determining survival, body and heart weights, the vasculature (conduit, muscular and resistance arteries) was assessed grossly and ultrastructurally via electron microscopy. Central and pulmonary arterial pressures were measured using a Millar catheter and large artery compliance was determined via pressure myography. **Results:** Double mutant mice exhibited increased mortality with nearly 50% lethality by three months of age. Similar to Fbln4E57K/E57K mice, ~70% of double mutant mice developed ascending aortic aneurysms, while none of the Fbln5-/- mice did. Double mutant mice developed cardiac hypertrophy and had worse systolic hypertension and large artery stiffness compared to either Fbln4E57K/E57K or Fbln5-/- mice. Right ventricular pressure of double mutant mice was elevated similar to that of Fbln5-/- mice and was unaffected by mutant FBLN4. Ultrastructurally, conduit arteries including the aorta and common carotid artery of double mutant mice exhibited elastic fiber fragmentation across the entire wall when it was largely limited to the external layers in Fbln5-/- mice. Small arteries, including the renal, saphenous and mesenteric arteries, which were structurally intact in

Fbln4E57K/E57K mice, lacked the external elastic lamina in Fbln5-/- and double mutant mice. **Conclusions:** In summary, our data indicate that FBLN5 is essential for external elastic lamina formation, particularly in small arteries, and it is not compensated for by FBLN4. Furthermore, the worse cardiovascular phenotypes observed by combination of mutant FBLN4 and FBLN5 deficiency compared to each mouse model individually suggests that FBLN4 and FBLN5 serve distinct functions in elastic fiber assembly and cardiovascular physiology.

Abstract 009*

_O_-glucose Modification of Fibrillin Epidermal Growth Factor Repeats is Essential for Mouse Lung Development

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*The authors of this abstract selected not to publish the details of their research

Recent Progress in Osteoarthritis Research

Abstract 010

Type V Collagen is Essential for the Development of Knee Articular Cartilage and Meniscus

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Introduction: Proper knee joint loading is governed by two distinct tissues, collagen II-dominated articular cartilage and collagen I-rich fibrocartilage meniscus. Initial fibrillogenesis of collagens I and II is regulated by minor collagens V and XI, respectively. Little is known about how these two collagens work in concert to regulate the formation of knee joint. This study aims to elucidate the role of collagen V in the development phenotype of articular cartilage and meniscus. **Methods:** IF imaging and RNAScope were applied to assess the distributions of collagens V and XI proteins and gene expressions, respectively, in newborn (post-natal day 0, P0) and adult (P90) wild-type (WT) mice. Knee joints were harvested from joint-specific Col5a1 knockout mice (Col5a1$^{f/f}$/Gdf5Cre, or Col5a1-cKO). Histology was applied to assess joint morphology, cellularity and sulfated glycosaminoglycan (sGAG) staining. AFM-nanoindentation was applied to quantify tissue modulus. MicroCT was applied to additional P240 mice to assess subchondral bone structure. **Results:** In P0 WT mice, collagen V showed a higher gene expression and protein staining than collagen XI in both articular cartilage and meniscus. By P90, collagen V was much reduced, with minor amount in the pericellular domain of both tissues. In contrast, collagen XI was highly concentrated in epiphyseal cartilage. The surprising presence of collagen V in articular cartilage contradicts the classical knowledge that cartilage ECM consists of collagens II/IX/XI, and indicates a potential role of collagen V in the initial joint formation. Indeed, our results highlight a crucial role for collagen V in the development and maintenance of knee joint. In Col5a1-cKO mice, we found reduced modulus in both articular cartilage and meniscus at P0, indicating impaired early ECM assembly. By P90, Col5a1-cKO meniscus showed decreased size, absence of proteoglycan-rich inner zone, and significantly reduced modulus. Meanwhile, articular cartilage exhibited reduced modulus, aberrant cell clustering and flattening of tibial surface. By P240, the knee joint showed excessive osteophyte formation, signifying early onset of osteoarthritis. These changes evidence that collagen V regulates joint formation and maintenance beyond its canonical role of mediating collagen I fibrillogenesis. Recently, collagen V was shown to regulate muscle stem cell niche via Notch-Col V-CACLR axis. It is thus possible for collagen V to mediate the activities of Gdf5-expressing joint progenitors that give rise to both articular cartilage and meniscus, and in turn, determine the formation and maintenance of the joint. **Conclusions:** This study highlights an essential role of collagen V in regulating the initial formation and maintenance of articular cartilage and meniscus. Results could provide a crucial

benchmark for designing new regenerative strategies to repair and restore the function of degenerative joints.

Abstract 011
NG2/CSPG4 Regulates Hypertrophic Differentiation in Mandibular Condylar Cartilage
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Introduction: The growth of the mandible is regulated by the differentiation of the mandibular condylar cartilage (MCC) from a progenitor to hypertrophic fated cell population, promoting bone growth at the osteochondral junction. Type VI collagen is an established regulator of endochondral ossification in the MCC. Neuron-glial antigen 2 (NG2/CSPG4) is the receptor for type VI collagen, is present in the MCC, and engages key biomineralization pathways such as ERK 1/2. The objective of this study is to define the role of NG2/CSPG4 as a regulator of biomineralization in the MCC. **Methods**: To characterize the transcriptional role of NG2/CSPG4 in the MCC, primary mandibular fibrochondrocytes were isolated from 10-14 day c57 BL 6J (control) and NG2/CSPG4 knockout (NG2ko) mice using an overnight type II collagenase digestion and analyzed by bulk RNAseq. To characterize the role of NG2/CSPG4 as a regulator of the transcriptional heterogeneity of MCC cells, a single cell suspension from skeletally mature control and NG2ko mice was generated by overnight collagenase digestion and processed with the 10xGenomics Chromium Single Cell 3' / Illumina NovaSeq 6000 and analyzed with Cell Ranger (version 6.1.1) and the Seurat R Package toolkit (version 4.0.6). Gene ontology enrichment analysis of all differentially expressed genes was done using ShinyGO (v0.77). To mechanistically interrogate the role of NG2/CSPG4 in cartilage biomineralization, control and NG2ko primary cells were treated with and without mineralization media for 14 days. Gene expression changes were quantified by RTqPCR. The mineralization potential of the cells was quantified by alizarin red staining and a fluorescent tagging of calcium chelating compounds (IRDye 800CW BoneTag, Li-COR). **Results**: Gene enrichment analysis of bulk RNAseq data illustrates a significant change in genes related to "ossification" (enrichment score = 4; -log10 FDR = 10*;* N = 4/genotype). SC-RNA-seq of the aggregate sample identified four clusters containing more cells of a particular genotype than predicted by a normal distribution, including clusters 1 and 3 (80% control) and 6 and 7 (~60% knockout). High levels of overlap were identified in clusters 3 and 6 and clusters 1 and 7, suggesting a similar transcriptional profile. Gene enrichment analysis illustrates clusters 3 and 6 are associated with "cartilage development" genes. The control cells in Cluster 1 mapped to "bone mineralization", while the closely associated knockout cluster 7 mapped to "cartilage development". Mineralization media treatment yielded significant upregulation of type X collagen and MMP13 in the control cells but not the NG2ko cells ($p < 0.05$; n=4/genotype). Further, control cells, but not NG2ko cells, had large alizarin positive nodules. **Conclusions**: These findings indicate that NG2/CSPG4 participates in the transcriptional control of differentiation in the cells MCC progressing to a hypertrophic fate.

Higher Order Multi-Analyte Interrogation of Tissue

Abstract 012
Mapping the Interactome of Matrisome Targets Using Extracellular Proximity Labeling (ePL)
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Introduction: Classical methods to investigate protein-protein interactions (PPIs) are generally performed in non-living systems, yet in recent years new technologies utilizing proximity labeling (PL) have given researchers the tools to explore PPIs in living systems. PL has distinct advantages over traditional protein interactome studies, such as the ability to identify weak and transient interactions in vitro and in vivo. Most PL studies are performed on targets within or on the cell membrane. Tissue inhibitors of metalloproteinases (TIMPs) are a family of multi-functional proteins that were initially defined by their ability to inhibit the enzymatic activity of matrix metalloproteinases (MMPs), the major mediators of ECM breakdown and turnover. TIMP2 is a unique family member, with a broad expression profile that is expressed in both normal and diseased tissues, even in those with minimal metalloproteinase activity. Understanding the functional transformation of matrisome regulators, like TIMP2, during the evolution of tissue microenvironments

associated with disease progression is essential to the development of ECM targeted therapeutics. **Methods:** We describe a method to investigate PPIs within the extracellular compartment, using both BioID2 and TurboID, that we term extracellular PL (ePL). To demonstrate the utility of this modified technique, we investigate the interactome of the widely expressed matrisome protein TIMP2 using carboxyl- and amino-terminal fusion peptides of TIMP2 with BioID2 and TurboID. Expanding on this pipeline, we have started to screen the interactomes of new matrisome targets in simple 2D and complex 3D culture conditions. **Results:** We describe the TIMP2 interactome in unique tissue compartments. We also illustrate how the TIMP2 interactome changes in the presence of different stimuli, in different cell lines, and with different reaction kinetics (BioID2 vs. TurboID); demonstrating the power of this technique versus classical PPI methods. Furthermore, we expand this method to interrogate the interactomes of new matrisome targets such as TIMP3 and Thrombospondin-1. **Conclusions:** We propose that the screening of matrisome PPIs in disease models using ePL will reveal new therapeutic targets for further comprehensive studies. Knowledge of disease specific PPIs may also garner understanding of patient-specific therapeutic resistance to conventional and next-generation therapies.

Abstract 013

Expanding the Pool of Transplantable Livers Using Molecular Profiling and Histopathological Analysis

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Introduction: Liver transplantation is the solution for acute or chronic liver failure. There is an unmet demand for liver transplantation, as not all donated organs are transplanted. The clinical selection criteria for donated livers depends on histopathological evaluation and liver function tests. A fraction of the donor livers are rejected for transplantation due to the variability in applying the selection criteria. We applied an integrated transcriptomics and histopathological approach to characterize the donor livers evaluated for transplantation. **Methods:** We obtained liver biopsies from deceased donors during organ collection (n=10 accepted and n= 21 rejected for transplantation). We performed RNA sequencing to characterize the global gene expression profiles. We assessed steatosis, fibrosis, and necrosis via manual histopathological evaluation and a custom artificial intelligence-based image analysis. **Results:** We identified two transcriptomically distinct subsets in the rejected donor liver group (termed rejected-1 and rejected-2), where the rejected-2 subset had a near complete transcriptomic overlap with the accepted livers, suggesting the potential for acceptability from a molecular standpoint. The liver metabolic functional genes were similarly upregulated in the accepted and rejected-2 groups compared to the rejected-1 subset. Expression levels of several extracellular matrix genes were similarly low in the accepted and rejected-2 groups compared to the rejected-1 subset. Filtering the rejected-2 subset based on histopathological evaluation identified the cases with borderline scores and extensive molecular overlap with the accepted group. **Conclusions:** Our integrated molecular and histopathological approach identified a subset of rejected donor livers that may be suitable for transplantation, thereby expanding the pool of transplantable livers. **Acknowledgement:** National Institute of Alcoholism and Alcohol Abuse: R01 AA018873; Gift of Life Foundation.

Abstract 014*

Hyaluronic Acid Production and Matrix Stiffening Promote Biliary Epithelial Cell Proliferation in Early Primary Sclerosing Cholangitis

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*The authors of this abstract selected not to publish the details of their research

Abstract 015

A New Journey for Cancer Research – Targeting Glycosylation: Glysite™ Scout Glycan Screening Kits for Comprehensive Detection of Glycan Expression

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Introduction: Altered glycosylation is a hallmark of cancer. Glycosylation plays critical roles in cell function ranging from controlling tumor cell proliferation to modulating the tumor microenvironment. However, because of the structural complexity of glycan compositions, elucidating the mechanistic details of protein glycosylation in cancer biology remains challenging. Lectins are part of a broader group called glycan-binding proteins that recognize glycan chains and mediate their functionality. Their glycan-specific nature also makes them valuable tools that can help profile, characterize, and capture complex glycans in cancer samples. **Methods:** To explore the complexity of glycan modifications in cancer biology, we adapted the Glysite™ Scout Glycan Screening Kits and applied on a selected panel of normal and cancerous FFPE tissues including colon, pancreas, breast, kidney, brain, and lung. Glysite™ Scout Glycan Screening Kits are fully integrated kits for the detection of glycan expression and applicable in various techniques such as Western blot, tissue-based assay, and flow cytometry. More importantly, the curated lectin selection enables the detection of the major glycan motifs including sialylation, fucosylation and galactosylation, for the evaluation of glycan distribution. We also included several antibodies targeting cancer-specific biomarkers and compared them with our lectins. **Results:** Our lectin histochemical analysis revealed tissue dependent glycan changes. Furthermore, our results demonstrate notable changes in glycosylation between the normal and tumor tissues complementing the antibody biomarker data. This indicates that glycans and/or glycoproteins have the potential to be novel cancer biomarker candidates. Currently, FDA approved glycoprotein antigens as cancer biomarkers are on the rise, including CA 15 3 (mucin 1 as a serum marker for breast cancer, CA 125 (mucin 16 for ovarian cancer and CA 19 9 sialyl Lewis A) for pancreatic cancer. **Conclusion:** Expanding the view of cancer biology by integrating glycosylation will empower the deconvolution of disease biology.

Matrix Degradation: MMPs, ADAMTS, and Proteases

Abstract 016

Collagen Distribution in Mouse Embryo Abdominal Wall

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Introduction: During the developmental process of the mouse embryo, there is a period when the intestine temporarily protrudes outside the body cavity, and then returns into the abdominal cavity (The Atlas of Mouse Development; Academic Press, 1992). This process, involving the retraction of the intestine into the abdominal cavity, is closely associated with the development of the abdominal wall (*Disease Models and Mechanisms* 2018:10.1242/dmm.034611). However, the precise mechanism of gut retraction is not fully understood. The extracellular matrix (ECM) components are known to play a role in the development of the abdominal wall. In this study, we investigated the distribution of collagen in the abdominal wall of mouse embryos. **Methods:** We examined the distribution of collagen in the abdominal wall using picrosirius red (PSR) staining at embryonic stages 14.5, 15.5, and 16.5. We analyzed the changes in PSR-positive signal distribution using a polarizing microscope. **Results:** The dermis of the abdominal wall contains a thin muscle tissue called the panniculus carnosus, which divides the dermis into two layers: the shallow layer and the deep layer. The shallow layer is located between the epidermis and the panniculus carnosus, while the deep layer is located between the panniculus carnosus and the rectus muscle. At E14.5 and E15.5, PSR staining revealed that collagen was localized in the deep layer surrounding the rectus muscle. In contrast, at E16.5, PSR signals extended across the entire dermis. Furthermore, the distribution of red signals observed under the polarizing microscope increased at E16.5. **Conclusions:** The distribution of collagen underwent significant changes during the observed embryonic stages. The expansion of collagen coincided with the period of gut retraction, suggesting that alterations in ECM components are associated with this developmental event. **Acknowledgements**: The authors wish to thank Ms. Morishita and Ms. Monobe at the Central Research Laboratory of Okayama University Medical School for technical assistance. This work was supported in part by a Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (nos.20H00548 to S.H. and 23K16796 to I.S).

Abstract 017*

Conditional Inactivation of *Adamts6* Improves Aortic Wall Structure in Mouse Models of Marfan Syndrome

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Abstract 018

Regulation of TIMPs Through MMP-dependent Cleavage

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Introduction: Tissue Inhibitors of Matrix Metalloproteinases (TIMPs) are a broadly expressed family of matrisome proteins that are the primary regulators of metalloproteinase (MP) proteolytic activity. In addition to their canonically described functions, TIMPs display a broad range of MMP independent functional roles associated with proliferation, apoptosis, migration, and differentiation. MPs are zinc containing endopeptidases that play an essential role in maintaining the extracellular matrix (ECM) through proteolytic turnover; a balancing act maintained (in part) by the local MMP:TIMP ratio. Disturbances in the balance between MPs and TIMPs are associated with various disease states, including cancer, heart disease, arthritis, and cognitive dysfunctions. **Methods**: We performed a series of experiments to interrogate how organomercurial activated MMP9 can cleave TIMP proteins when in a molar excess. Furthermore, we promoted differentiation of myeloid subtypes that support MMP9 expression and activity to assess instances in which TIMP cleavage may occur. **Results**: While TIMPs are classically characterized as regulators of MP proteolytic activity, we describe a novel mode of MP-dependent regulation of TIMP function. We show that active-matrix metalloproteinase 9 (aMMP9) in molar excess can cleave TIMP proteins within their C-terminal domains. As a result of this cleavage, both the MP-dependent and MP-independent functions of the TIMP proteins are altered. MMP9 abundance and activity is often elevated during the pathogenesis of various diseases, and persistent MMP9 activity is often associated with worse prognoses. MMP9 is predominantly produced by stromal cell types, such as fibroblasts, and myeloid cells including neutrophils and macrophages. Importantly, myeloid cell types often display low levels of TIMP production thus may represent a functionally compelling source of TIMP-free MMP9. **Conclusions:** TIMPs proteins are proteolytic targets for activated MMP9. By stimulating myeloid cells into MMP9 secreting and activating subtypes, we interrogate the conditions that may enable TIMP cleavage in vivo. Investigation of the circumstances that modulate the balance between MPs and TIMPs may reveal new therapeutic opportunities in a range of disease models.

Abstract 019*

Unveiling New Immunomodulatory Actions of the Extracellular Protease ADAMTS1: Involvement of Different Substrates at Different Scenarios

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Host-Tumor Interactions

Abstract 020

Modeling the Influence of Stroma in Ovarian Cancer Drug Resistance in a Microvascularized Multiniche Tumor-On-a-Chip

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Introduction: Ovarian cancer (OC) is the leading cause of death among gynecological cancers due to late diagnosis and drug resistance in more than 80% of patients. Therapeutic response is influenced by the tumor microenvironment (TME) composed not only of cancer cells, but also of stromal cancer-associated fibroblasts (CAFs), and endothelial cells, that participate in extracellular matrix (ECM) remodeling. The ECM acts as a physical barrier for drug diffusion into the tumor and generates low levels of oxygen, or hypoxia, a key hallmark of OC, that is correlated with resistance and bad prognosis. Besides, there is an increasing need to develop new preclinical models to predict drug response in cancer patients as more than 95% of drugs fail in clinical trials due to lack of efficacy. Our main goal is to develop a microvascularized multiniche tumor-on-a-chip to recapitulate ECM-remodeling and the hypoxic TME to study the influence of stromal and endothelial cells and cell-cell and cell-ECM interactions on drug resistance in OC. **Methods:** We have developed polydimethylsiloxane (PDMS) tumor-on-a-chip formed by five chambers: one central chamber for seeding OC cells, flanked by two lateral stromal chambers for healthy fibroblasts or CAFs in coculture with endothelial cells and two external circulation chambers for media and drugs to recreate the OC-TME. Confocal microscopy was used for evaluating the cell growth, migration, interactions, drug penetration and uptake, and therapeutic response to standard-of-care treatment with paclitaxel and carboplatin. **Results:** We have validated the microvascularized multiniche assembly in our model and its ability to mimic OC-TME structure by generating the stromal and cancer niche and formation of vessel-like structures. The device was optimized to recapitulate hypoxic gradients from the circulation chamber towards the cancer chamber. We demonstrated that the cancer cells can initiate metastasis-like migration and the stromal components are implicated in collagen I secretion and ECM-remodeling and delay the drug diffusion into the tumor core. Furthermore, we observed increased drug resistance to paclitaxel/carboplatin treatment in cancer cells cocultured with CAFs compared to healthy fibroblasts. These results suggest that ECM remodeling, hypoxia, and tumor-CAFs interactions are the main contributors to OC chemoresistance. Also, we demonstrated that cellular crosstalk recreation and spatial organization through compartmentalization are essential to determining the effect of these physical and biological mechanisms on drug resistance. **Conclusions:** Our results present a functionally characterized microvascularized multiniche tumor-on-a-chip able to recapitulate ECM-remodeling and hypoxic OC-TME to study drug response influenced by CAFs and endothelial cells, that could provide new opportunities for improving chemotherapy and overcome chemoresistance in OC. **Acknowledgment:** This project is supported by NIH/NCI R21CA259158.

Abstract 021

Early Lung Cancer Progression is Driven by Resident Fibroblast-produced Extracellular Matrix Protein Tenascin-C

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Introduction: Activating mutations in upstream components of the RAS→RAF→MEK→ERK pathway initiate pre-cancerous and low-grade lung adenocarcinomas (LUADs). The cellular signals that drive the progression of these early tumors, which lack mutational heterogeneity and reactive tumor microenvironments, are unknown. Here, we investigated how changes in the matrisome of early lung tumors can contribute to progression. **Methods:** We used a combination of bioinformatics, immunohistochemistry of a genetically-engineered mouse model (GEMM) of early RAS-driven LUAD, and immunohistochemistry of patient samples to identify extracellular matrix proteins upregulated in early LUAD. We identified the source of new extracellular matrix using bioinformatics and *in situ* hybridization of mouse and human samples. *In vivo* studies (orthotopic syngeneic tumor transplants and early tumor growth in the GEMMs) tested the significance of the new extracellular matrix. *In vitro* studies in LUAD cell lines dissected the mechanisms of matrisome signaling for tumor cell growth and migration. **Results:** We found that the nonautonomous production of extracellular matrix protein Tenascin-C by resident fibroblasts surrounding early LUADs promotes early lung tumor progression. Tenascin-C signals for early LUAD tumor

cell proliferation and migration without altering the structure of the tumor microenvironment. Instead, we found that the Tenascin-C signals to tumor cells at the tumor-stroma interface through integrins avb1 and avb6 to induce pro-growth and pro-migration pathways. **Conclusions:** The activation of resident fibroblasts to produce Tenascin-C can drive early tumor growth and dissemination, suggesting that Tenascin-C status could distinguish early LUADs likely to progress and needing therapeutic intervention from benign LUADs whose patients don't need to suffer therapeutic side effects.

Abstract 022

Microbial-host Co-metabolite Hippurate Reduces Breast Cancer Growth and Modulates Tumor Fibrosis

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Introduction: Obesity increases the risk of developing postmenopausal breast cancer by up to 50%. The obesity-associated increase in both breast cancer risk and progression is thought to be regulated in part by several systemic and localized factors. These mechanisms include increased inflammation and immunosuppression, hypoxia of breast tissue, increased aromatase-mediated estrogen conversion, perturbations in the adipokines leptin and adiponectin, and changes in insulin signaling. Intriguingly, all these potential risk factors are also reportedly influenced by the microbiome, suggesting a critical relationship between the microbiome and obesity-driven breast cancer burden. Hippurate is a microbial–host co-metabolite considered thus due to the production by bacterial-mediated phenylalanine metabolism and/or by host-mediated glycine conjugation of benzoic acid. Previous metabolomic studies of plasma samples from germ-free or conventionally housed mice demonstrated 17-fold higher hippurate concentrations in conventional housed mice compared with germ-free animals, demonstrating the importance of the bacterial microbiome on the generation of hippurate. **Methods and Results:** We now show through untargeted metabolomics of murine mammary glands, non-human primate (NHP) breast tissue, and human breast tissue that diet and obesity are associated with reduced breast hippurate concentrations. In our NHP model, we demonstrated breast-specific modulation of hippurate concentrations, which were not observed systemically in plasma samples, suggesting tissue localized regulation. Breast tissue displays its own microbiota that can be regulated by diet and adiposity. We show that breast tissue Lactobacillus abundance positively correlates with breast hippurate concentrations. Furthermore, using a MMTV-PyMT genetic model of mammary carcinogenesis, we show that intra-nipple injection of Lactobacillus reduces tumor incidence and growth with a corresponding increase in tissue hippurate concentration implicating Lactobacillus metabolism in generation of hippurate and potential anti-cancer signaling activities. Using a syngeneic 4T1.2ER+ breast cancer model, we show that exogenous administration of hippurate (via drinking water) reduced tumor volume and tumor weight. Immunohistochemistry analysis of FFPE tumor tissue shows that hippurate administration reduced tumor fibrosis with no significant effect on tumor proliferation. **Conclusion:** Taken together, we demonstrate that obesity modulates breast tissue microbiota resulting in decreased hippurate metabolite levels. Hippurate may represent a novel anti-cancer, anti-fibrotic microbial-produced metabolite for obesity-mediated breast cancer prevention.

Abstract 023

Deciphering the Dual Role of ADAMTS2 on Cancer Progression: From ECM to Immunity

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Introduction: ADAMTS2 is an extracellular metalloproteinase required for the maturation of fibrillar collagens. Tumours forming after subcutaneous injection of cancer cells grow faster in ADAMTS2-KO (TS2-KO) mice as compared to WT mice, identifying ADAMTS2 as a potential anti-tumour proteinase. However, unexpectedly, high expression of ADAMTS2 is a poor prognosis marker for most types of human cancers. Our goal was to identify the specific roles of ADAMTS2 at different stages of cancer progression in order to reconcile these seemingly contradictory data. **Methods:** Experiments were performed *in vitro* to determine whether recombinant ADAMTS2 can directly affect the phenotype of cancer cells. Mouse models

were also developed in order to evaluate the impact of ADAMTS2 expression on tumour growth and metastasis spreading. **Results:** Survival, proliferation and migration of cancer cells *in vitro* are not affected by recombinant ADAMTS2, suggesting that the *in vivo* effects on tumour growth are mediated by the environment. Using the MMTV-PyMT model, we demonstrated that spontaneous primary mammary tumours grow faster in absence of ADAMTS2 (MMTV-PyMT/TS2-KO versus MMTV-PyMT/WT). Surprisingly in view of these data, a strong reduction of lung metastases was observed in these mice, suggesting a dual effect of ADAMTS2 during cancer progression. In a model of subcutaneous injection of cancer cells, we confirm that tumour grow faster in TS2-KO, as compared to WT, and we further demonstrated that expression of recombinant ADAMTS2 by cancer cells inhibits tumour growth. The profile of immune cells in these tumours has been established, evidencing an increase in $CD206^{high}$ tumour macrophages in TS2-KO mice. We have also shown that bone marrow derived macrophages display an increase M2-like polarisation when they are co-cultured with TS2-KO cancer associated fibroblasts (CAFs), as compared to WT CAFs. **Conclusions:** ADAMTS2 was identified as a metalloproteinase with dual functions in cancer progression, repressing the growth of the primary tumour but favouring the establishment of lung metastasis. We showed, through *in vivo* and *in vitro* models, that ADAMTS2 can control the growth of the primary tumours through regulation of the immune system, and that its absence drives macrophages to a M2-like phenotype. Further studies are ongoing to unravel how ADAMTS2 regulates macrophages polarisation in the tumour microenvironment.

Matrix Resources, Big Data, and Data Integration

Abstract 024

Investigating the Regulatory Impact of Collagen Type XI N-terminus Domain (NTD) Variants on Collagen Self-assembly Kinetics and Insights on the Molecular Interactions

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Introduction: Collagen α1(XI) is a minor fibrillar collagen that plays a critical role in the regulation of collagen fibril assembly, with its amino-terminal domain (NTD) central to maintaining this regulatory behavior. In this study, we aimed to examine the regulatory impact of spliced variants of the collagen type XI NTD on the self-assembly kinetics of collagen type I, employing a turbidity-time assay. **Methods:** To gain deeper insights into the underlying molecular interactions governing the regulatory function of the collagen type XI NTD, we utilized molecular dynamics simulations and protein-protein docking studies. **Results:** Our findings reveal a positive influence of the collagen type XI NTD on the growth phase, particularly in the formation of collagen bundles through the assembly of collagen chain trimers. Notably, our comprehensive molecular dynamics simulations and protein-protein docking analyses emphasize the contribution of electrostatic interactions, along with hydrogen bonds and van der Waals interactions, in driving these essential molecular interactions. **Conclusion:** By elucidating the role of collagen type XI NTD variants in modulating collagen fibril assembly, our study provides valuable insights into the intricate regulatory mechanisms at play. These findings contribute to our understanding of collagen biology and may have implications for therapeutic interventions targeting collagen-related disorders.

Abstract 025*

Mechanical Stiffness Regulates Epigenetic Control of Cardiac Fibroblast Gene Expression

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Abstract 026

Multi-omics Characterization of Matrisome Dynamics During IPF Pathogenesis

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a complex, lethal disease with limited treatment options. Changes to the extracellular matrix (ECM) are known to drive this disease; however, current methodologies for analyzing ECM are minimal and require improvement. Given limited IPF human tissue availability, developing enhanced cell-based strategies will be imperative for accelerating research in this field. By advancing methodologies to study ECM, a more mechanistic understanding of IPF disease biology can be established which will be crucial for improving patient care. **Methods:** We performed a multi-omic investigation of IPF integrating data from patient tissue and *in vitro* models. We generated bulk RNA-seq data from TGFβ-treated normal healthy lung fibroblasts (NHLF) and compared to fibroblasts identified in published sc-RNA-seq from IPF patient tissue. Building on findings from these data highlighting ECM-level changes, we developed methodology to investigate ECM dynamics at the protein level in a primary human lung fibroblast culture model. We applied a compartmental proteomic strategy to assess evolution of ECM from intracellular space to supernatant to a deposited form. Given the importance of not only protein composition but also ECM crosslinking, we further evaluated crosslinked species of key ECM proteins generated in culture. **Results:** Despite variability in differentially expressed genes across datasets, functional enrichment analysis of sc-RNA-seq datasets from patient tissue highlighted the prevalence of ECM dysregulation in IPF. Supporting this, bulk RNA-seq from TGFβ-treated NHLF's also showed strong differential expression of ECM-related genes. Molecular function analysis identified 'extracellular matrix structural constituent' across all three datasets analyzed. To expand on the transcriptomics data, we successfully applied novel sample preparation and mass-spectrometry proteomics methodologies to characterize ECM proteins from multiple compartments of primary human lung fibroblast culture samples. We identified and quantified matrisome proteins across intracellular, secreted, and deposited ECM compartments, deepening our understanding of ECM dynamics related to IPF pathogenesis. In addition, we analyzed crosslinked ECM protein species from the fibroblast culture model and human tissue. We successfully quantified desmosine/isodesmosine, pyridinoline, deoxypyridinoline, lysinenorleucine, dihydroxylysinonorleucine, and hydroxylsylnonorleucine. **Conclusions**: Analysis of tissue-derived and *in vitro* transcriptomics emphasized the importance of ECM as a driver of IPF pathophysiology. To facilitate future investigations into these changes, we produced robust strategies for proteomic analysis of ECM in multiple compartments from fibroblasts grown under various culture conditions. By applying innovative, multi-omic approaches continuation of this work will contribute to building a comprehensive understanding of IPF. **Disclosure Statement:** All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Abstract 027

MatrisomeDB: An ECM Proteomics Tool to Facilitate Biomarker Discovery

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Introduction: The extracellular matrix (ECM) is a complex meshwork of proteins forming the structural scaffold of tissues. Alterations in ECM composition are hallmarks of disease, including cancer. Hence, the ECM contains a vast array of exploitable biomarkers and therapeutic targets. Profiling the composition of the ECM is challenging due to its insolubility and heavy post-translational-modifications (PTMs). The development of different sequential extraction and decellularization methods has enhanced ECM enrichment. Using these techniques, researchers can employ mass spectrometry (MS)-based proteomics to characterize the ECM, or matrisome, of healthy and diseased tissues. Data from these studies are available in public repositories, but difficult to use without specific expertise. To help non-specialist researchers access this rich source of data, we developed MatrisomeDB (https://matrisomedb.org), a user-friendly database compiling ECM proteomics data. **Methods:** We curated over 200 studies on ECM proteomics and manually screened them for inclusion in MatrisomeDB. We identified studies with sufficient ECM enrichment and similar experimental pipelines (tryptic digestion, data acquired via LC-MS/MS, etc). Raw data files from these studies were obtained and reprocessed using unified search parameters, allowing

for quantitative analysis and inter-study comparisons. **Results:** The updated release of MatrisomeDB includes 42 studies profiling the ECM of 39 organs across physiological systems, ages, and diseased states, including 8 cancer types (breast, colon, lung, pancreas, ovary, stomach, insulinoma, melanoma). Aggregation of these studies resulted in experimental detection of 97.8% of the predicted matrisome, a 23.4% increase from the previous release of MatrisomeDB. Upon query, users will access heat maps displaying confidence scores and relative abundance of protein(s) across tissues and studies. A word cloud depicting enriched terms in the abstracts containing the protein of interest is also generated. These tools can help point users to a given protein's role in certain physiological contexts. Users can also view peptide and PTM mapping onto 2D domain-based schematics and 3D AlphaFold predicted models of ECM proteins. Additionally, we provide references to external sources to help users access validated assays or design their own for targeted MS experiments, enabling accurate ECM protein quantification. **Conclusions:** MS-based proteomics has become the method of choice for unbiased characterization of the ECM protein signature of tissues. Hence, we plan to periodically update MatrisomeDB and encourage researchers to submit their datasets to our submission interface for consideration. ECM proteomics has made the discovery of biomarkers achievable. MatrisomeDB has the potential to advance this effort by facilitating hypothesis-driven discovery of proteins that play key roles in pathophysiological contexts and can be exploited as treatment targets.

Cancer: Imaging Immuno-Oncology

Abstract 028

Immune Checkpoint Protein Expression Patterns in Gastric Cancer: Unraveling the Immunological Landscape

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Introduction: Gastric cancer (GC), ranking as the fifth most prevalent malignancy worldwide and the third leading cause of cancer-related mortality, remains a formidable health challenge. In the context of tumor eradication, cytotoxic T cells (CTCs) display their potent arsenal by selectively obliterating malignant cells, contingent upon the expression of major histocompatibility I molecules on antigen-presenting cells. Regrettably, the efficacy of CTCs in combating cancer falls short of expectations, and a contributing factor to this disparity lies in the orchestrated activation of immune checkpoint proteins (ICPs), which wield suppressive capabilities to impede the anti-tumor immune response orchestrated by CTCs. To shed light on this intricate interplay, the present investigation delved into the expression patterns of immune checkpoint proteins in gastric cancer. **Methods:** With meticulous adherence to ethical regulations, we obtained a collection of normal (n = 50) and malignant (n = 50) formalin fixed paraffin embedded gastric biopsies, facilitating a comprehensive assessment of ICP profiles. Employing immunohistochemical analytical techniques, we determined the expression levels of crucial clusters of differentiation (CD) markers, including CD3, CD5, CD80, CD86, CD117 and PDL1 within the sampled tissues. **Results:** We observed a remarkable overexpression of CD3, CD5, CD80, CD86, CD117, and PDL-1 within the GC microenvironment when compared to their expression levels in normal tissues. This compelling evidence highlights the substantial upregulation of immune checkpoint proteins in gastric cancer. **Conclusion:** This study contributes vital insights into the intricate landscape of immune checkpoint protein expression within gastric cancer. Such knowledge stands to shape future endeavors aimed at devising therapeutic strategies tailored to counteract the suppressive impact of immune checkpoint proteins, ultimately fostering improved treatment outcomes for individuals affected by gastric cancer.

Abstract 029

Fluorescence Resonance Energy Transfer Based Molecular Beacon Probe for *in situ* Hybridization

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Introduction: *In situ* hybridization (ISH) is a powerful method for detecting specific ribonucleic acids (RNAs) at the cellular level. Although conventional ISH using hapten-labeled probes are useful for detecting multiple RNAs, the detection procedures are still complex and required longer time. Therefore, we introduced a new application of fluorescence resonance energy transfer-based molecular beacon (MB) probes for ISH (*Acta Histochem Cytochem* 2022;**55**:119-128). **Methods:** MCF-7, a breast cancer cell line and C57BL/6J mouse uterus were used for ISH. MB probes for estrogen receptor alpha (ERα) mRNA and 28S rRNA were labeled with Cy3/BHQ-2 and 6-FAM/DABCYL, and conventional probes were labeled with digoxigenin. The sensitivity of the MB probe was evaluated by dot blot hybridization. The specificity of MB probe was confirmed by various control experiments, including complementary and homologous sequences, RNase treatment, competition and neutralization assays. The mismatch discrimination ability of MB probe was examined both in medium and tissue sections. Super resolution microscopy was used to detect subcellular localization of 28S rRNA and ERα mRNA in MCF-7 cells. **Results:** Fluorescence spectroscopy measurements revealed that MB probes had more-rapid hybridization kinetics compared to conventional probes. Dot blot hybridization revealed that 10 pg of oligo-DNA could be detectable, that indicates the MB probes are highly sensitive probe similar with conventional probes. In MCF-7 cells, 28S rRNA was detected in nucleolus and cytoplasm of all cells, whereas ERα mRNA was detected in some nucleolus. In the uterus, 28S rRNA was clearly detected using complementary MB probe, but there were no signals in control slides. Moreover, 28S rRNA was detected in all cells, whereas ERα mRNA was detected mainly in the epithelium. These results indicated that the differential subcellular localization of target RNAs can be determined by MB probes. Super resolution microscopy confirmed that the localization of 28S rRNA and ERα mRNA were independently expressed in the cell nuclei. Mismatch discrimination experiments revealed that significantly decreased fluorescence intensity was detected in 1 or 2 base-mismatched sequences, confirming that highly specific detection of MB probes in medium and tissue sections. **Conclusions:** Our results demonstrate that MB probes are very useful for ISH, providing high sensitivity, specificity and simpler compared to conventional probes for ISH. MB probe could discriminate target sequence based on 1 or 2 base-mismatches in the tissue sections. **Acknowledgements:** This study was supported in part by a Grant-in-Aid for Scientific Research from the JSPS (16K08471, 19K16477).

Tissue Clearing and Expansion

Abstract 030*
Rac1 Promotes Kidney Collecting Duct Repair by Mechanically Coupling Cell Morphology to Mitotic Entry
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*The authors of this abstract selected not to publish the details of their research

Abstract 031
Quantitative Digital Image Analysis of Whole Slide Images to Investigate White Matter Rarefaction in Alzheimer's Disease
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Introduction: Alzheimer's 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), regions of myelin rarefaction, precede cognitive symptoms in AD, are predictive of disease onset and progression, and can be identified non-invasively by magnetic resonance imaging (MRI). However, little is known about the composition of WMH due to the difficulty locating these regions postmortem. **Methods:** We have overcome this challenge by aligning premortem MRI, with postmortem MRI and histology. The goal of this study was to develop a quantitative digital image analysis pipeline to

analyze the composition of these pathologies, specifically, to understand the relationship between vessel size and integrity with histopathologic signatures in WMH human AD tissue. Immunohistochemistry (IHC) and *in situ* hybridization (ISH) was used identify axonal density, myelin quantity, oligodendrocyte and immune cell density, vascular markers, and markers of blood brain barrier leakage. **Results:** Machine learning algorithms in the QuPath software were used for cell and pixel classification to allow for automated and high-throughput data collection of whole slide images. We developed algorithms to investigate cell and protein abundance in perivascular regions radiating from vessels, measure vessel wall thickness and lumen/vessel wall ratio, measure cortical thickness, identify tangentially cut regions of cortex, and measure cell/protein abundance in areas of increasing white matter depth. **Conclusion:** These data will allow us to use supervised and unsupervised machine learning algorithms to understand the clinical relevance of histopathologic signatures of WMH to Alzheimer's Disease onset and progression.

Abstract 032

Non-invasive Electric Stimulation Suppresses Microglia Activation and Angiogenesis Through the Mediation of Mitochondrial Potential and Ca2+ Signaling in Microglia and Endothelial Cells

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Introduction: Age-related macular degeneration (AMD) is characterized by the progressive loss of central vision, associated with microglial activation and abnormal blood vessel growth. Electrical stimulation (ES) using a low-intensity current has emerged as a potential non-invasive therapeutic modality. This study investigates ES's effects on microglia and endothelial cells (EC). **Methods:** In adult C57BL/6 mice, CNV was induced using a 532 nm laser. ES was applied transpalpebraly (biphasic, 300 µA, 20 Hz, 4 min, daily). *In vivo,* assessments conducted 7 days post-laser CNV induction, including fluorescein angiography (FA) and optic coherence tomography (OCT). Retinal pigment epithelial/choroidal/scleral complexes (RCSC) were labeled to detect endothelial cells, IBA-1, COX-2 and VEGF. *In vitro* experiments utilized primary microglia and human retinal endothelial cells (HREC). Both cell types were subjected to ES (biphasic, 300 µA, 20 Hz, 1 h, once). The calcium flux in response to ATP stimulation was measured using Fura-2. The mitochondrial function was assessed by high-resolution respirometry (Seahorse) and JC-1 staining, FluoVolt was employed to investigate membrane potential. Protein expression of VEGFa, ATP2A2 and electron transport chain complexes (OXPHOS) were studied by western blotting. The HREC's angiogenesis was evaluated through tube formation and choroidal explants. **Results**: In the *in vivo* experiments, ES significantly reduced the FA leakage area and the size of laser-induced CNV lesions as measured by OCT compared to the sham-treated mice. Immunolabeling of RCSC revealed significant reductions in the lectin-positive area, the number of infiltrating IBA-1-positive cells, the number of COX-2-positive cells, and the VEGFa. In the *in vitro* experiments, ES exhibited inhibitory effects on ATP-induced calcium flux in microglia and HREC. The seahorse analysis results demonstrated reduced basal respiration, maximum respiration, and ATP production in microglia following ES; the proton leak, however, was not significantly changed. The OXPHOS complexes and VEGFa were reduced, whereas ATP2A2 was dramatically upregulated. The JC-1 staining indicated reduced mitochondrial polarization, and FluoVolt demonstrated decreased membrane potential upon the ES. Further *in vitro* experiments revealed suppressed tube formation, choroidal explant outgrowth, and VEGF expression in HREC after ES. **Conclusions:** ES inhibits microglial activation and EC angiogenic responses by reversibly depolarising mitochondrial potential and ATP production without significant mitochondria damage. The reduced ATP results in a compensatory increase in the expression of ATP2A2, which hinders its function as endoplasmic reticulum calcium pumps, leading to the reduction of cellular calcium stores and a reduction in Ca2+ flux when exposed to pro-inflammatory and pro-angiogenic stimuli. These findings present the possibility of non-invasive therapeutic intervention for AMD patients.
Acknowledgement: This study was supported by The Norwegian Research Council; Department of Ophthalmology, Oslo University Hospital, Oslo, Norway (T.P.U); Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway (T.P.U); The Norwegian Association for the Blind and Partially Sighted

(T.P.U); The Ministry of Science and Technology of Taiwan MOST 105-2917-I-002-031, MOST 109-2917-I-564-032 (K.C.); National Eye Institute Grant EY031696 (D.F.C); Harvard NeuroDiscovery Center Grant (D.F.C); Core Grant for Vision Research from NIH/NEI to the Schepens Eye Research Institute (P30EY003790).

Abstract 033

A New View: Quantifying Cellular Spatial Relationships in CLARITY-Processed Tissue from the Rhesus Monkey Brain

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Introduction: Tissue clearing techniques have revolutionized our ability to visualize complex biological structures in three dimensions (3D) by rendering tissues transparent, primarily by removing lipid-based barriers. These techniques allow deep imaging of cleared brain tissues with minimal scattering and distortion. Tissue clearing is proving to be instrumental in elucidating the intricate organization of neural circuits and studying the underlying mechanisms of brain function. Additionally, the integration of quantitative analytic methods are providing important insights into the functional and anatomical aspects of the brain. In this study, we highlight the significance of tissue clearing and quantitative analysis for neuroscience and neuroanatomical research by demonstrating its application in brain tissue from the rhesus monkey. Rhesus monkeys have long been recognized as a valuable non-human primate model due to their similarities with humans in terms of neuroanatomy, behavior, and cognitive abilities. These animals also show a high degree of similarity in terms of cytoarchitecture and functional organization of the brain. Here, we leverage tissue clearing methods in conjunction with 3D quantitative analysis to better understand the cellular spatial relationships and organization in prefrontal, primary motor, and visual as opposed to the traditional methods of using single label immunohistochemistry and analysis in 2D. **Methods:** Blocks of brain tissue (5x5x10mm) containing both the grey and subcortical white matter from the monkey brain were dissected and processed with Fast-Passive CLARITY followed by fluorescent immunohistochemistry to label neurons (NeuN), dendrites (MAP2), and axons (NF-H). This approach enabled the 3D visualization of intricate neural connections, neuronal populations, and morphological variations of the cortices. **Results:** Spatial relationships were compared in prefrontal, primary motor, and visual cortices in the rhesus monkey brain. Advanced image analysis algorithms, including automated cell counting, morphological profiling, and orientation analysis using Fiji and Imaris, were used for extraction of quantitative data from large-scale imaging datasets. These approaches provide a novel means to characterize spatial relationships in neuronal populations, investigate neuronal morphology, and explore connectivity patterns in the rhesus monkey brain. **Conclusions:** The integration of tissue-clearing and 3D quantitative analysis methodologies show significant promise for unlocking new avenues of research, deepening our understanding of the primate brain, and ultimately contributing to the development of novel therapeutic strategies for neurological disorders in both monkeys and humans. Further, novel 3D analysis techniques shed light on the traditional interpretation of 2D measurements, highlighting the importance of the third dimension for a robust description of neuroanatomical correlates.

Fibronectin and the Developmental Biology of Extracellular Matrix

Abstract 034

Versican Mediates the Crosstalk between Keratinocytes and Dermal Papilla Cells in Hair Follicles

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Introduction: Versican, a large proteoglycan of the extracellular matrix has been recognized as a biomarker for dermal papilla cells in the hair follicles. However, its function in hair follicles is poorly understood. **Methods and Results:** Here, we found that the transcription of the versican gene (*Vcan*) is strongly correlated with that of the ciliary hedgehog signaling pathway, an instrumental molecular signaling mechanism for the dermal papilla cells. Disrupting *Vcan* in DP cells severely impaired hair follicle morphogenesis and regeneration in mice, mimicking what were observed in cilia and hedgehog mutant mice. These findings suggested that versican is a crucial target gene of the hedgehog signaling pathway in

dermal papilla cells. To determine how versican in the dermal papilla cells could eventually affect keratinocytes in the hair follicles, we focused on integrin signaling because versican can interact with beta 1 integrin. Indeed, disrupting *Vcan* in the dermal papilla cells was correlated with decreased integrin signaling in keratinocytes, accompanied by diminished proliferation and increased differentiation and apoptosis. Consistent with these findings, genetically overexpressing *Vcan* in mice could offer resistance to hedgehog inhibitor-induced hair loss characterized by reduced proliferation and increased apoptosis in follicular keratinocytes. **Conclusion:** This study indicates that versican is a key component of a previously unknown paracrine signaling mechanism between the dermal papilla cells and keratinocytes in the hair follicles.

Abstract 035

Development of a Novel Mouse Strain with a Multifunctional and Switchable Tagged Allele of COL4A1

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Introduction: Collagen $\alpha1\alpha1\alpha2$(IV) heterotrimers are fundamental components of basement membranes and are highly conserved across species. *COL4A1* and *COL4A2* mutations cause a multi-system disorder known as Gould syndrome that is characterized by variable cerebrovascular, ocular, renal, and neuromuscular manifestations. Despite the importance of collagen a1a1a2(IV), much is unclear about their biological functions, partially due to lack of models to visualize and quantify protein dynamics and tools to study their biochemical and biophysical properties in tissues. **Methods:** We developed a novel versatile mouse model (COL4A1^{Tag}) in which COL4A1 is fused with switchable tandem fluorescent and biochemical tags for isoform-specific visualization and differential affinity purification, respectively. At baseline, a FLAG/mCherry tag is fused near the COL4A1 amino terminus. CRE-mediated recombination switches the FLAG/mCherry–COL4A1 isoform to an HA/eGFP–COL4A1 isoform which allows visualization of dynamic collagen $\alpha1\alpha1\alpha2$(IV) deposition and replacement. Alternatively, FLP-mediated recombination leads to replacement of COL4A1 with a FLAG/mCherry tag that is not secreted allowing for intracellular labeling of *Col4a1*-expressing cells while creating a null *Col4a1* allele. **Results:** Mice heterozygous and homozygous for the COL4A1^{Tag} allele are viable and fertile and we demonstrated that the tagged isoforms correctly label the basement membranes in a variety of organs. Moreover, used a tamoxifen inducible CRE line to generate dual-colored basement membranes and validate the isoform switching function of the construct *in vivo*. **Conclusions:** This novel mouse strain enables generation of heterozygous *Col4a1* null mutant mice and represents a valuable genetic resource to characterize the spatial, temporal, biochemical, and biophysical parameters of collagen $\alpha1\alpha1\alpha2$(IV) in normal and pathological conditions.

Abstract 036

Subendothelial Fibronectin Disrupts Endothelial Cell Monolayer Integrity

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Introduction: Fibronectin (FN) is a large glycoprotein that is polymerized by cells into a 3D extracellular matrix (ECM) and serves as a scaffold for the deposition of other matrix proteins. In normal blood vessels, endothelial cells sit on a thin, laminar basement membrane (BM) that is rich in laminin, collagen IV, nidogen and perlecan, but contains very little FN. The BM is essential for forming cell-cell and cell-substrate contacts and maintaining apical-basal polarity. In numerous diseases such as diabetic retinopathy and cancer, FN builds up beneath endothelial cells, and is correlated with vascular leak and new vessel growth. Given FN's role in wound healing and embryonic vasculogenesis, we hypothesized FN is more than a marker of fibrosis in the vessels and is likely playing a role in coordinating vascular dysfunction. Here we report the effects of FN accumulation on endothelial cell monolayer integrity and provide evidence for an active role of FN in vascular fibrosis. **Methods:** HUVECs were grown on a Matrigel-coated Transwell membrane for 3 days to establish a uniform monolayer with cell-cell junctions and few Ki-67-positive dividing cells. FN was then added to the basal compartment and allowed to diffuse through the membrane to simulate leak of stromally-

produced FN. Cell layers were analyzed at timepoints ranging from 2-48 h by immunocytochemistry and immunoblotting for ECM and cell junction proteins. **Results:** Subendothelial accumulation of FN was detected as early as 4 h and increased for at least 24 h. Significant increases in Ki67-positive nuclei correlated with the accumulation of FN in the endothelial monolayer and clustered where subendothelial FN staining was brightest. Cells on these FN patches had larger areas and dimmer junctional VE-cadherin staining than surrounding endothelial cells. Quantitative analysis of the relationship between subendothelial FN, Ki67 staining, and cell area suggests that FN accumulation precedes progression through the cell cycle, and that cell size and junctional changes are later events in the phenotypic response to FN. Functionally, exposure to FN affects monolayer permeability as demonstrated by the higher rate of basal to apical transit of albumin compared to cells without basal FN addition. **Conclusion:** Endothelial cell monolayers are affected in numerous ways by exposure to exogenous, basally-localized FN. Alterations in proliferation, cell morphology, and cadherin localization implicate FN as a causative agent in vascular dysfunction in fibrotic disease, and suggest FN as a potential target for modifying vascular dysfunction. **Acknowledgments:** NJ Commission on Cancer Research Predoctoral Fellowship (COCR23PRF009), R01 AR073236, NJ Health Foundation award.

Abstract 037

Longitudinal Mechanical, Histological, and Functional Analysis of Myxomatous Mitral Valve Disease in Marfan Syndrome Mice

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Introduction: Heart valve structural and extracellular matrix (ECM) remodeling starts at late embryonic stages and continues postnatally. Marfan syndrome (MFS), caused by a dominant mutation in Fibrillin1 (Fbn1), can lead to congenital heart valve abnormalities including myxomatous valve disease (MVD). Progressive MVD is characterized by collagen fiber fragmentation and leaflet thickening, however, the mechanistic links between mechanical forces and biological changes in valve degeneration remain unknown. *We hypothesize that congenital MVD results from an initial increase in proteoglycans, leading to lower ECM stiffness, which results in biomechanical and functional abnormalities, as exemplified by deficient maturation of the MFS valves.* **Methods:** To determine the functional and mechanical valve characteristics in healthy and *Fbn1$^{C1039G/+}$* MFS mouse mitral valve leaflets, we performed echocardiography (ECHOs) in parallel with uniaxial mechanical testing at 2-, 6- and 12-months-of-age. ECM remodeling was quantified histologically by Movat's Pentachrome staining as a proteoglycan: collagen ratio. Initiation of valve leaflet maturation and gene expression was examined using RNA scope in situ hybridization to detect ECM markers *versican (Vcan)*, *collagen 1a1 (Col1a1)* and *elastin* (*Eln*) as well as maturation markers, including *chondroadherin (Chad)* and *angiopoietin like 7 (Angptl7)* postnatally. **Results:** Functional data from ECHOs showed that *Fbn1$^{C1039G/+}$* MFS mice develop regurgitation/stenosis in both mitral and aortic valves by 2 months-of-age. Mechanical testing demonstrated a reduction in the average stiffness of the mitral valves in the *Fbn1$^{C1039G/+}$* MFS mice at all timepoints. During postnatal remodeling, the *Fbn1$^{C1039G/+}$* MFS mitral valves already have dysregulation of ECM compartmentalization indicated by disorganized Col1a1, Vcan, and Eln. Furthermore, *Fbn1$^{C1039G/+}$* MFS mitral valves at p7 have decreased expression of the mature collagen marker Chad. Interestingly, expression of Angptl7, potentially involved in ECM organization and inflammation was, increased. **Conclusions:** In *Fbn1$^{C1039G/+}$* MFS mice, changes in function and mechanics of the mitral valve, coincident with higher ECM ratio and lower stiffness in the valve leaflets, can be detected by 2 months-of-age. During the initial stages of postnatal valve leaflet remodeling, dysregulation of ECM gene expression and patterning are apparent providing evidence for valve maturation defects at p7. Therefore, understanding mechanisms of valve dysfunction and progression of disease needs to start at developmental stages. Together, these data provide evidence that aberrant ECM gene expression and valve leaflet remodeling precede cardiac functional deficits and compromised biomechanics in adult MFS mice mitral valves.

Biomaterials

Abstract 038

Vascular Elastic Lamellae Fabricated In Vitro by a Layer-by-layer Method

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Introduction: Elastic lamellae are required for integrity of the large vessels. Although a recent advance in the research of elastic fibers, the fabrication of elastic lamellae in vitro is still challenging. We have reported that a layer-by-layer technique using fibronectin and gelatin enabled to fabrication of multiple layered vascular smooth muscle cell (SMC) sheets containing elastic fibers (*Atherosclerosis* 2014;**233**:590-600). Based on these studies, we aimed to fabricate a three-dimensional vascular model containing elastic lamellae. **Methods:** We performed repeated cell seeding using rat neonatal aortic SMCs by a layer-by-layer technique to fabricate ten-layered SMC sheets, followed by incubation with ascorbic acid-containing medium for 14 days. Elastica van Gieson stain and transmission electron microscopic analysis were performed to evaluate elastic lamellae. Desmosine/isodesmosine concentrations were measured using an isotope dilution LC-MS/MS method. The SMC sheets were used for tensile strength measurement and implantation into the rat abdominal aorta. Microarray analysis was performed to examine the gene expression profiles of the SMC sheets. **Results:** Elastic fibers became visible between SMCs during the repeated cell seeding and continuous elastic lamellae, which were partially connected to SMCs, were formed in the SMC sheets 14 days after incubation. The concentrations of desmosine/isodesmosine in the SMC sheets without additional incubation were 10.4±7.2/9.4±5.7 μg/mg (n=7), and that in the SMC sheets incubated for 14 days were 26.2±5.8/24.3±5.3 μg/mg (n=5) which is approximately three-fold greater than that in the rat neonate aorta. The tensile strength of the SMC sheets was 1874±255 mmHg and were subjected to implantation in the rat aorta. The SMC sheets withstood arterial blood pressure and did not form aneurysms even 5 months after implantation. Microarray analysis demonstrated that the SMC sheet had a similar gene expression pattern to the native aortic tissues rather than to planar cultured SMCs. Gene ontology and pathway enrichment analyses revealed that the gene sets of blood vessel morphogenesis, extracellular matrix assembly, and ECM-receptor interaction were positively associated with the SMC sheets. The expression of 17 elastic fiber-related genes was increased more than 4-fold in SMC sheets compared to adult aortic tissues which have almost no regenerative capacity of elastic fibers. In particular, *Thbs1*, *Thbs2*, *Thbs4*, and *Comp* were highly expressed in the SMC sheets compared to the adult rat aorta. **Conclusions:** Thrombospondin family may play a role in elastic lamellae formation, and the SMC sheets may be useful as a new experimental model for the study of vascular elastic fibers.

Abstract 039

Magnetic Extracellular Vesicle Delivery System for Matrix Synthesis for Abdominal Aortic Aneurysm Repair

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Introduction: Abdominal aortic aneurysm (AAA) can be characterized by loss of vascular wall integrity caused by elastic fiber degradation (*Acta Biomaterialia* 2020;**113**:267-278). Restoration of these elastic fibers can help restore strength to the vascular wall. Our group (*Bioengineering Basel* 2021;**8**:51) and others (*Acta Biomaterialia* 2020;**113**:267-278) have investigated the use of extracellular vesicles (EVs) derived from mesenchymal stem cells as a therapeutic to stimulate elastic fiber synthesis for AAA repair. To improve delivery of these EVs, our group has developed a delivery system that can magnetically localize and release EVs to stimulate matrix production. **Methods:** Regenerated silk fibroin (*ACS Applied Nano Materials* 2018;**1**:5441-5450; *Bio-Materials and Prototyping Applications in Medicine* 2021;Chapter 3:35-57) was chemically conjugated to iron oxide nanoparticles (IONPs) (*Nanoscale* 2012;**4**:837-844) and mixed with a potassium phosphate buffer (KPB) to generate silk-iron microparticles (SIMPs) (*PLoS One* 2019;**14**:e0219790). The characterization of SIMPs was performed using scanning electron microscopy,

energy-dispersive x-ray spectroscopy (EDS), and Fourier transform infrared spectroscopy (FTIR) to evaluate morphology, chemical composition, and silk secondary structure. IONP concentration in SIMPs was optimized using magnetic movement through solution, and this optimal concentration (20 mg/mL) was used to test magnetic movement through a hydrogel. The SIMPs were tested for biodegradation in a proteolytic environment to model AAA (*Acta Biomater* 2020;**105**:146-158) and tested for cytotoxicity. EVs were isolated, resuspended in KPB, and mixed with silk-iron solution to create silk-iron packaged extracellular vesicles (SIPEs). To evaluate EV release, SIPEs and SIMPs were incubated over 7 days and analyzed with flow cytometry for presence of CD63 (*J Extracell Vesicles* 2016;**5**:31803). SIPEs and SIMPs were co-cultured with smooth muscle cell-seeded fibrin gel constructs (*Bioengineering Basel* 2021;**8**:51; *Matrix Biol Plus* 2019;**4**:100014) for 28 days to test for an effect on matrix production. **Results:** Microparticles demonstrated diameters ranging from 0.5-5 µm and internal porosity. EDS confirmed the presence of both iron and protein in the SIMPs. FTIR showed a peak at 1623 cm^{-1}, indicating a shift toward β-sheet formation in SIMPs compared to silk microparticles without iron (Silk MPs). Following 1 day of proteolysis, SIMPs were completely degraded, in contrast to their long-term stability in water. SIMPs showed better magnetic movement through solution (SIMPs: 0.578±0.126 magnetic protein/non-magnetic protein vs. Silk MPs: 0.028±0.007, p=0.0002, n=3) and hydrogel compared to Silk MPs. SIMPs showed low cytotoxicity compared to cell death controls (94.7±5.3% live cells, p=0.0010 vs. 73.3±5.9). Incubation of SIPEs showed release of CD63 over 7 days compared to SIMPs. Co-culture of SIPEs with fibrin gel constructs showed a trend toward an increase in elastin (SIPEs: 0.790±0.73% elastin vs. SIMPs: 0.268±0.120, p=0.211, n=4) compared to SIMP controls. **Conclusions:** Silk and iron can be chemically conjugated and formed into microparticles. EVs can be encapsulated in these SIMPs during the precipitation phase. A magnetic silk delivery system is a novel approach to localizing and releasing EVs. Slow release of EVs via SIMPs may improve matrix production in fibrin gel constructs.
Acknowledgements: The authors would like to thank Maria Montoya for her assistance in fabricating and washing the microparticles and counting cells, David Maestas for his assistance in capturing stained fibrin gel images, Matthew Poskus for his assistance in LIVE/DEAD imaging, Kamali Charles for his assistance in SMC culture, and Mark DeAngelis for his assistance with flow cytometry. This work was supported by AHA Predoctoral Fellowship and NIH T32HL076124.

Abstract 040

A Decellularized Cartilage Matrix Approach to Pediatric Airway Reconstruction

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Introduction: Severe subglottic stenosis (SGS), the narrowing of the airway just below the vocal folds, develops in 8.3% of ICU neonates following prolonged intubation and directly leads to debilitating comorbidities. In the most severe cases, laryngotracheal reconstruction (LTR) surgery is required to expand the airway and restore airflow. During LTR, autologous costal cartilage is harvested, shaped, and implanted into the child's airway. However, a major obstacle is that young children often lack sufficient costal cartilage to ensure a successful graft surgery and suffer from donor site morbidity. Our objective is to utilize xenogeneic decellularized meniscal cartilage (MEND) to engineer an implant populated with host's cells to circumvent the limitations of pediatric autologous cartilage. **Methods:** *Meniscus decellularization, channel formation, and recellularization:* Meniscal porcine cartilage was freeze-thawed and washed with pepsin and elastase to decellularize and create microchannels. Meniscus decellularized (MEND) cylinders were placed in a transwell insert and $2x10^5$ cartilage progenitor cells (CPC) were seeded on each MEND cylinder. A 1% to 20% FBS gradient was created to drive cell recellularization. After 3 days, constructs were fixed, sectioned, and stained. *Differentiation:* CPC-seeded MEND were cultured in chondrogenic medium under gentle agitation for 3 weeks with medium renewed twice/week. The differentiated constructs were examined for gene expression, mechanics, and histology. *Rabbit Laryngotracheal Reconstruction:* Acellular, re-cellularized, or differentiated MEND constructs were implanted in an anterior cricoid split LTR and compared to a costal graft standard. After 3 months, the rabbits were euthanized and the construct phenotype, airway expansion, and histomorphometry quantified. **Results:** H&E sections of MEND clearly showed aligned channels post-decellularization and following a serum gradient migration, CPCs penetrated

the entire depth of the construct in 3 days. After 3 weeks of differentiation, cells were viable, presented robust chondrogenic gene expression and had remodelled the matrix resulting in increased bulk mechanical strength and GAG content. Finally, a 38 rabbit study was conducted and MEND constructs successfully expanded and integrated into the native hyaline cartilage over the course of the 3 months as seen from endoscopy, histology, and micro-CT, with substantial neo-cartilage formation. **Conclusions:** The successful creation of micro channels within fibro-elastic cartilage circumvented many of the issues plaguing decellularized cartilage therapies. The channels fully supported eCPC recellularization to the same density as native cartilage, and when exposed to chondrogenic medium, remodeled the matrix to resemble hyaline cartilage's biochemical, mechanical and organizational structure. Finally, eCPC-MEND constructs integrated with the hyaline airway cartilage indicating its superiority to current therapies.

Abstract 041
A Regulatory Role for Macrophages in Fibroblast Circadian Rhythm and Collagen-I Deposition
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Introduction: Fibrosis is associated with 45% of deaths in the developed world and yet no effective treatments exist. Dysregulated collagen production drives fibrosis, thus it is of clinical importance to determine how collagen regulation is achieved in homeostasis and what causes pathological collagen production. Fibroblasts are the predominant collagen producers and macrophages have been characterised as collagen regulators, either through direct degradation or through production of cytokines that modulate collagen synthesis in fibroblasts. A recent discovery showed macrophages themselves can deposit collagen in wound sites (*Nature Commun* 2020;**11**:600). Another breakthrough study in the collagen field showed certain proteins responsible for collagen homeostasis are controlled by the circadian clock (*Nature Cell Biol* 2020;**22**:74-86). We hypothesise that macrophages contribute to collagen production, which may be through circadian control. **Methods:** To record circadian rhythms in different cells, bioluminescence recording of PER2-luciferase was utilised where the luciferase signal reflects expression of the clock gene, PER2. Immunofluorescence (IF) imaging was used to investigate fibril deposition. **Results:** Macrophages induce a circadian rhythm in PER2-luciferase fibroblasts but not vice-versa. Addition of conditioned media from macrophages also induced a circadian rhythm in fibroblasts, suggesting macrophages secrete a rhythm-inducing soluble factor. IF imaging of mono- and co-cultures showed that macrophages deposit collagen alone and increase collagen fibril deposition by fibroblasts. Disruption of the circadian rhythm led to disrupted collagen fibril deposition. These data begin to elucidate the role that macrophages play in collagen regulation. We showed that macrophages themselves deposit collagen fibrils in vitro in the absence of external stimuli, whereas previously it was thought that only fibroblasts possessed this function. Co-culture experiments show interactions between macrophages and fibroblasts result in increased collagen deposition by fibroblasts, demonstrating that macrophages can be a positive regulator of collagen production by fibroblasts, rather than the traditional degradation role. We showed the interaction between macrophages and fibroblasts has a circadian clock element. Macrophages also induce the synchronisation of circadian rhythm in fibroblasts and we have shown that these clock-altering interactions are cell-contact independent: cultured media released by macrophages can induce the rhythm in fibroblasts. **Conclusions:** Macrophage:fibroblast communication plays a role in collagen regulation. The involvement of macrophages could provide a new target when designing therapies for collagen dysregulation disorders.

Genetics of Matrix Disorders

Abstract 042*
Effects of Fibrillin-1 Loss on Osteoarthritis Development.
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*The authors of this abstract selected not to publish the details of their research

Abstract 043
Using Suppressor Genetics to Identify Therapeutic Targets for Thoracic Aortic Aneurysm in a Mouse Model of Neonatal Marfan Syndrome (nMFS)

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Introduction: Mutations in *FBN1* clustering in exons 23-32 predispose individuals to neonatal Marfan Syndrome (nMFS), a rare variety of MFS characterized by severe clinical manifestations, such as thoracic aortic aneurysm/rupture (TAA/TAAD) and heart failure during infancy. The life-threatening nature of the cardiovascular conditions in nMFS creates a clinical need for therapeutics. To identify therapeutic targets, we initiated a suppressor screen in mice homozygous null for *Fbn1* (Fbn1 KO), which phenocopies nMFS. Candidate suppressor genes were selected based on our current understanding of TAA/TAAD pathophysiology, which involves TGFβ signaling, ECM remodeling, and intima/media dysfunctions. **Methods and Results:** Using CRISPR/Cas9, candidate genes were targeted in FBN1 KO ES cells, which were then injected into 8-cell stage WT embryos to generate 100% ES-derived double KO F0 mice. Double mutant F0 mice were scored for enhanced survival, extent of aortic aneurysm and nMFS comorbidities (heart and lung abnormalities). Our screen showed that loss of function of *Ltbp3* overcame the complete lack of fibrillin-1 microfibrils and strongly protected the mice from TAA/TAAD and nMFS-associated comorbidities, resulting in a significant improvement of survival. Interestingly, loss of function of *Thrombospondin-1*, a TGFβ activator, resulted in a worsening of both the aortic phenotype and survival of Fbn1KO nMFS, highlighting the complexity of TGF signaling in the pathophysiology of MFS. In addition to *Ltbp3*, a moderate improvement of TAA/TAAD was also observed with loss of function of iNos (but not eNos) and *Adamts4,* confirming published observation and supporting alternative therapeutic approaches for nMFS. **Conclusion:** In summary, the highly penetrant and rapidly developing aortic phenotype of Fbn1 KO mice combined with the genetic strategy for double mutant mouse generation empower our screen for reliable and fast selection of therapeutic targets in TAA/TAAD in nMFS.

Abstract 044*
Expanding the Mutational and Phenotypic Spectrum in Elastin-driven Disease Through a Gene-first Approach

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*The authors of this abstract selected not to publish the details of their research

Abstract 045
The Role of Fibronectin in Mediating the Progression of TGF-β1-Induced Renal Fibrosis in a 3D Culture Model

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Introduction: The cytokine transforming growth factor beta 1 (TGF-β1) is a core regulator of renal fibrosis through the induction of cell processes like epithelial-mesenchymal transition, as well as increased extracellular matrix (ECM) production and assembly. Previous research has shown that TGF-β1 and the extracellular matrix (ECM) protein fibronectin (FN) form a positive feedback loop, where increased TGF-β1 activity results in increased FN synthesis and assembly. This increase in FN fibril formation promotes the activation of latent TGF-β1 that further promotes fibrogenesis. We hypothesize that inhibiting FN fibril assembly will inhibit TGF-β1 signaling and decrease ECM assembly, thus preventing the progression of fibrosis. We will use the protein fragment FUD (functional upstream domain of the bacterial protein Adhesin F1) to disrupt FN fibril formation. **Methods**: Experiments were conducted using the Madin-Darby canine kidney (MDCK) epithelial cell line. Renal epithelial spheroids were cultured in rat tail-derived Collagen I gel, then treated with a combination of FUD (500 nM) and/or TGF-β1 (4 ng/mL). We analyzed changes in spheroid morphology and functionality through immunofluorescence staining of renal markers and TGF-β1 signaling markers (FN, integrin α_v, aquaporin 2 (AQ2)). Z-stack images were taken with a 2 µm step width using a Zeiss LSM 710 laser scanning confocal microscope. Relative fluorescence intensity and morphological features were quantified using FIJI. Gene expression of TGF-β1 signaling markers (CDH1, CDH2, FN1, SNAI1, ZEB1) was assessed using RT-qPCR. **Results**: Quantification of total and lumen

volume showed that inhibiting FN fibril assembly prevented spheroid dilation. Observing the relative intensity of FN and integrin α_v showed decreased surface intensity in FUD + TGF-β1 conditions. Further investigation of polarity markers AQ2 and Snail showed the prevention of spheroid depolarization in response to TGF-β1 in the FUD + TGF-β1 condition when compared to those treated with TGF-β1 alone. RT-qPCR results prevented the upregulation of TGF-β1 markers SNAI1 and ZEB1 in FUD + TGF-β1 conditions. **Conclusions**: FN fibril assembly is a major regulator of TGF-β1 activation. Inhibition of this interaction as observed through IF images, prevented spheroid dilation and depolarization that characterizes the progression of renal fibrosis, further suggesting that TGF-β1-FN interactions are critical drivers of fibrogenesis. **Acknowledgements**: Research was supported by the IGNITE KUH Training Program (NIH U2C/TL1 DK132771-1).

Neuropathology and Neuro-inflammation

Abstract 046
Brain Endothelial Translatome in Response to Endotoxemia
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Introduction: Shock is the main cause of delirium in intensive care units. The mechanisms for this acute response, however, remain unknown. We hypothesized that transient changes in the brain microvasculature may lead to acute brain injury and delirium. We have previously shown that mice challenged with lipopolysaccharide (LPS) display brain microvascular leak, which was more severe in mice lacking endothelial SOCS3 (SOCS3iEKO), the main regulator of the interleukin 6 (IL-6) pathway. **Method:** To determine the changes in gene expression in the brain microvasculature, we performed cdh5-CreERT2-driven translating ribosomal affinity purification and RNA sequencing (TRAP-seq) from brains of control or SOCS3iEKO mice challenged or not with LPS for 15 h. Bioinformatics was performed in R. Cognitive abilities were measured by Y-maze, open field, novel object recognition, and nest building tests. **Results:** Brain TRAP-Seq revealed a significant endothelial transcriptional response, with upregulated expression of 446 genes and downregulated expression of 285 genes, including >100-fold upregulation of genes associated with brain injury such as LCN2, MMP3 and CXCL5. The response was distinct from that of whole brain RNA-seq (R=0.55), underscoring the specific changes in the microvascular translatome. Gene ontology analysis demonstrates a strong activation of innate immunity and proinflammatory signaling. Consistent with a role for IL-6 signaling, IL6 expression was upregulated 106-fold, and SOCS3 was upregulated 78-fold. Loss of endothelial SOCS3 exacerbated these responses (882 genes upregulated, 593 downregulated), highlighting the role of the STAT3/SOCS3 signaling axis in the acute endothelial response to LPS. Delirium is commonly transient and subsides upon shock resolution. Brain TRAP-Seq data from mice that recovered from LPS (8 days post-challenge) demonstrate the transient nature of the majority of the endothelial responses. However, a limited inflammatory response to LPS remains, with increased levels of several proinflammatory genes, such as IL-1β, CCL12, the complement factor C3, the pattern recognition receptor LRRC19 and multiple IFN-induced genes that form part of a tight cluster in mouse chromosome 1q (IFI204/205/207/208). Consistent with an acute response that resolves within days, we did not identify any significant impairments of the cognitive abilities of mice upon recovery from LPS. **Conclusions:** We described the brain microvascular transcriptional response to systemic inflammation, identified genes with possible links to brain damage, and demonstrated a key role for endothelial SOCS3 in limiting the response to proinflammatory signaling. These changes quickly resolved, underscoring the transient nature of endotoxin challenges and suggesting that acute sterile inflammatory processes may not be sufficient to induce lasting cognitive impairment.

Abstract 047
Microglia Transcriptome Suggests Persistent Activation and Impaired Oxidative Phosphorylation in Alzheimer's Disease
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Introduction: Alzheimer's disease (AD) is currently estimated to impact more than 6 million Americans, with late-onset AD comprising ~90% of cases. The etiology of AD is unknown, however, the increased risk

for AD in the context of pro-inflammatory conditions hints at chronic brain inflammation as a key driver of disease development and progression. As the principal and only resident immune cell in the brain, microglia protect the brain from pathogenic entities through immune surveillance, responses to foreign substances, and clearance of cellular debris and toxic materials, such as amyloid beta (Aβ). Additionally, microglia effect learning and memory through synaptic pruning and refining neuronal connectivity to optimize brain circuitry throughout life. As such chronic immune activation of microglia can be detrimental to the brain, not only through production of pro-inflammatory factors and metabolites, but also through impaired functions that are critical for maintaining brain homeostasis. **Methods:** We performed bulk RNAseq analysis on laser-capture microdissected (LCM) Iba-1+ microglia from formalin-fixed paraffin-embedded (FFPE) brain, Brodmann area (BA) 9, of patients who died with AD (n=13) and age-matched controls (n=7). Approximately 2,500 microglia were captured from each case. RNA was extracted by Trizol and underwent nonbiased amplification for whole transcriptome amplification before entering the Illumina RNAseq workflow. Brain tissues from the same subjects were interrogated for select antigens of interest by immunohistochemistry (IHC) and nonbiased quantitation. All tissues were acquired de-identified through the NIH NeuroBioBank. **Results:** Multiple changes in microglial transcription in AD were revealed. Of particular interest to our hypothesis that microglia are persistently activated in AD, we found two microglial off-signal receptor transcripts of interest, CD200R and CX3CR1, are downregulated in AD, as compared to age-matched controls. This was also seen at the protein level by IHC. Additionally, dysfunction in oxidative phosphorylation is suggested by downregulation of several mitochondria-encoded subunits of the electron transport chain in microglia from AD brain. Interestingly, estrogen receptor β and G protein-coupled estrogen receptor 1 transcripts were also reduced in AD, which may impact the immunosuppressive impact of estrogen on activated microglia. **Conclusions:** The use of LCM allowed for the microglia transcriptome to be interrogated in its "natural" environment in human brain. Our findings suggest significant mitochondrial injury in AD that may be caused by chronic microglial activation with increased energy demands and subsequent oxidative stress. This may further impair microglial transcription and function, including reduced capacity for clearing the brain of toxic factors. Additional studies are needed to elucidate the role of persistent microglial activation and impaired function in the etiopathogenesis of AD.

Abstract 048

Reduction in the Expression of Tight Junction Proteins at 4-Weeks Post-SARS-CoV-2 Infection in Non-Human Primates

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Introduction: Persistent neurological symptoms, including impairments in memory, concentration, and fatigue have been reported from individuals after initial infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (*Neurol Neuroimmunol Neuroinflamm* 2023;**10:**e200097). The mechanisms behind the neurological symptoms of coronavirus disease of 2019 (COVID-19) are still unknown and the investigation into the underlying pathophysiological features are essential for identifying potential targets for therapeutic intervention. Previously, we reported an increase in microhemorrhages and significant inflammation (*Nat Commun* 2022;**13**:1745) in the brains of SARS-CoV-2 infected African green monkeys (AGMs) and rhesus macaques (RMs) at 4-weeks post-infection, as compared to non-infected controls. The absence of detectable thrombi in the region of the microbleeds suggested leakiness of the blood brain barrier (BBB) as a result of infection. Endothelial cells of the BBB are tightly adhered with tight junction (TJ) proteins such as claudin-5 and zonula occludens-1 (ZO-1). Endothelial dysfunction and decreased expression of these TJ proteins have been observed in neuroinflammation, aging, and neurodegenerative diseases (*Free Radic Biol Med* 2012;**53**:1213-1221; *Immun Ageing* 2015;**12**:2; *Brain* 2019;**142**:1077-1092). We propose that in the context of infection, alterations in ZO-1 and claudin-5 contribute to BBB leakage, facilitating the infiltration of red blood cells and other factors that would typically be limited from accessing the brain parenchyma. **Methods:** To investigate our hypothesis, we performed immunohistochemistry on formalin-fixed paraffin-embedded tissue to identify claudin-5 and ZO-1 protein expression intensity in

brainstem, basal ganglia, and cerebellum from the same animals as in our previous report (*Nat Commun* 2022;**13**:1745). This included SARS-CoV-2 infected AGMs (n=4) and RMs (n=4) and age-matched AGM (n=2) and RM (n=2) seronegative controls. The brain regions were selected because they showed the most injury in the context of infection in previous studies. **Results:** Our study revealed lower claudin-5 and ZO-1 expression in infected RM and AGMs, as compared to the species-specific controls, suggesting increased permeability of the BBB in the context of COVID-19. **Conclusions:** This may contribute to the observed increased frequency of microhemorrhages, as well as contribute to neuroinflammation by allowing the passage of pro-inflammatory factors from the vasculature and/or peripheral blood into the brain parenchyma. Additional exploration is needed to uncover the mechanisms that underlie decreased TJ expression at the BBB in SARS-CoV-2 infection. **Acknowledgements:** Histochemical Society's Graduate Medical Trainee and Graduate Student Cornerstone Grant, NIH/ORIP P51OD011104, Tulane startup funds and Emergent Ventures at the Mercatus Center, George Mason University Fast Grants for COVID-19

Abstract 049

The Effects of a Closed Head Injury on Anxiety-like Behaviors in Male Rats

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Introduction: The relationship between concussions and behaviors related to emotional stress remains unclear. Recent reports suggest that concussion increases fear-behaviors in paradigms of Pavlovian fear conditioning. Here, freezing behavior is used as an index of fear. Of note, other ethologically relevant behaviors such as grooming and rearing can also be utilized as indicators of emotional stress. Studies have introduced them as an index of anxiety. For this reason, we hypothesized that a closed head injury would alter rearing and grooming. **Methods:** To help us determine this, rats were anesthetized and delivered either closed head injury via weight drop, or sham injury for controls. After recovery, rats were exposed to an open field space. Spontaneous activity was recorded to quantify rearing and grooming behaviors in the open field. Data collection was performed by observers blinded to experimental manipulations. Specifically, the number of rears, time spent rearing, grooming bouts, and time spent grooming were quantified manually. **Results:** Preliminary results suggest that closed head injury *(n=10)* versus sham *(n=9)* does not affect either the number of rears (p=0.16), time spent rearing (p=0.32), the number of grooming bouts (p=0.96), nor the time spent grooming (p=0.75). Next, we will assess rearing and grooming in other contexts. **Conclusion:** Together, these data suggest that concussive brain injury does not affect these specific behaviors related to anxiety. Currently, activity in brain regions involved in anxiety, namely the ventral hippocampus, is being quantified using c-Fos immunohistochemistry.

Liver Pathobiology I

Abstract 050

Metabolomics-based Serum Signature of Hepatic HNF4alpha Downregulation: A Novel Diagnostic and Prognostic Tool for Liver Diseases

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Introduction: Loss of expression and function of HNF4α, the master regulator of hepatic differentiation is a key feature of majority of the chronic liver diseases. Previous studies from our group have identified and validated a HNF4α target gene signature that can accurately identify HNF4α activity in the liver as prognostic marker in liver diseases. However, broad use of such gene signature is limited because it requires good quality patient biopsies that can generate enough RNA for analysis. The focus of current studies was to develop a serum-based signature of hepatic HNF4α decline to overcome logistic challenges presented by the gene signature. **Methods and Results:** Serum metabolomics analysis was performed on male WT and hepatocyte specific HNF4α knockout (HNF4α-KO) mice (n=10 per genotype), which identified a total of 792 biochemicals out of which, 447 exhibited statistically significant change ($p<0.05$). This included

241 downregulated and 206 upregulated biochemicals in HNF4α-KO mice. PCA and correlation analysis showed clear segregation of WT and HNF4α-KO groups. HCA analysis showed lipids and amino acids as the most affected categories of metabolites in the HNF4α-KO mice. Major changes were observed in unsaturated fatty acids, amino acid, nucleotide metabolism and bile acid metabolism following hepatic HNF4α deletion. The top metabolites increased in HNF4α-KO mouse serum included several bile acids, and C16-C18 lipids. The top metabolites downregulated included nucleotides such as adenosine, inosine and xanthine, and several sphingolipids, especially sphingomyelins. Next, we performed similar studies in WT and HNF4α-KO mice treated with diethylnitrosamine (DEN), a murine model of hepatocellular carcinoma (HCC). PCA analysis showed clear segregation of WT+DEN and KO+DEN groups. A total of 352 metabolites were detected in HCC samples, out of which metabolites were significantly 241 downregulated and 111 metabolites were significantly upregulated. The major classes of metabolites affected in HNF4α-KO HCC as compared to WT HCC were similar to non-HCC samples and included unsaturated fatty acids, amino acids, nucleotides and bile acids. The top metabolites increased in HNF4α-KO HCC included bile acids and immune active tryptophan metabolites such as kynurenine. The top downregulated metabolites in HNF4α-KO HCC were similar to non-HCC samples and included nucleotides, sphingolipids. Finally, a comparative analysis of top 30 metabolites from non-HCC and HCC data from WT and HNF4α-KO mice identified a set of 9 common metabolites that show similar pattern of change when hepatic HNF4α is downregulated. **Conclusion:** This set of 9 metabolites could serve as a serum diagnostic test to determine loss of HNF4α function in the liver during chronic liver diseases.

Abstract 051
Overexpression of Atg7 in Mouse Liver Paradoxically Suppresses Autophagy
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Introduction: Autophagy plays roles in diverse physiological and pathological processes through degradation of macromolecules and subcellular organelles. Atg7, an essential autophagy gene, functions in the two ubiquitin-like conjugation cascades, which are critical for the formation of autophagosomes. In murine livers deletion of Atg7 leads to defective hepatic autophagy. On the other hand, overexpression of two other essential autophagy genes, Atg5 [Whole body] or Beclin 1 [Lung/heart] in mice was found to enhance autophagy. However, overexpression of an autophagy gene specifically in the liver has not been examined before. Surprisingly, we found that the transient overexpression of Atg7 in HEK293T cells resulted in increased p62 protein level and decreased lipidated LC3, which shows an autophagy suppression. **Methods:** To investigate the Atg7 functions in murine liver, we generated a transgenic mice model, which carries an atg7 gene inactivated by a floxed stop codon (FSF-Atg7). Atg7 overexpression can be induced by injection of AAV8 virus carrying Cre recombinase. We then examined the autophagic flux in the liver, which reflects the level of the degradation of autophagosome through the lysosome, in mice after a short-term [10days] or long-term [7-10weeks] induction of Atg7 overexpression with AAV8-Cre. The flux is quantified based on the accumulation of the lipidated LC3 (LC3-II) in the presence of a lysosome inhibitor, leupeptin. **Results:** Mice with liver Atg7 overexpression for 1 week showed reduced LC3-II level and increasing p62. After 7 weeks of overexpression induction, they remained a reduced basal autophagic flux. These results indicate a suppression of autophagic function. Mice over-expressing hepatic Atg7 also developed a moderate liver injury with increased ALT level after 7 weeks of induction, but no morphology changes were observed. These results indicate that Atg7 might be different from other autophagy related genes, such as Atg5 and Beclin 1, in that excessive Atg7 is not beneficial to autophagy function, but rather negatively impacts autophagy. **Conclusion:** Future studies will need to confirm these findings by crossing the FSF-Atg7 mice to transgenic Alb-ERT2 mice for a more consistent, and stable expression of Atg7 across the entire liver, and to study the molecular mechanism by which over-expressed Atg7 acts in a negatively fashion.

Abstract 052
Acetaminophen-Induced Acute Liver Injury is Differentially Regulated by Diploid and Polyploid Hepatocytes
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Introduction: The liver contains diploid and polyploid hepatocytes, with polyploids comprising nearly 50% of human and 90% of mouse hepatocytes. The functional differences between diploid and polyploid hepatocytes are poorly understood, but emerging data suggest that each ploidy population promotes regeneration in an injury-specific manner. We hypothesize that diploid hepatocytes drive rapid regeneration in acute liver injury induced by acetaminophen (APAP), a common analgesic that causes liver injury and failure when taken in excess. **Methods:** To study ploidy populations *in vivo,* we utilized mice with a lifelong liver-specific knockout of *E2f7* and *E2f8* (LKO) that are functionally normal but are depleted of polyploid hepatocytes (LKO livers are >70% diploid). To determine the effects of *E2f7/E2f8* deficiency when ploidy is unchanged, *E2f7/E2f8* was deleted in adult hepatocytes using AAV8-TBG-Cre, generating hepatocyte-specific knockouts (HKO). LKO, HKO, and control mice were dosed with 300 mg/kg APAP and evaluated over 0-96 hours. Liver injury, necrosis, proliferation, and gene expression were assessed. To evaluate ploidy effects in a wild-type (WT) model, the response to APAP by diploid and polyploid hepatocytes was investigated *in vitro*. **Results:** Elevated liver enzymes, necrosis, and DNA fragmentation in hepatocytes revealed that while APAP damaged both treatment groups, LKO livers were less damaged than controls. Reduced damage and accelerated liver healing in the LKO model could be caused by gene expression effects associated with *E2f7/E2f8* loss or by enrichment of diploid hepatocytes. To discriminate between these possibilities, we first analyzed gene expression by bulk RNA-sequencing after APAP injury. Minimal gene expression differences were observed between LKO and control at baseline, but differences emerged over the APAP time course. Pathways controlling resistance (Sirtuin Signaling Pathway) and proliferation (WNT/β-Catenin Signaling) were activated after APAP, which could contribute to APAP sensitivity. Second, to focus on gene expression differences only, we evaluated the APAP response in HKO mice and found that HKO and controls responded equivalently. Finally, the role of WT diploid and polyploid hepatocytes was determined. Both populations were equally damaged, but diploid hepatocytes showed enhanced proliferation. **Conclusions:** Together, the data suggest that the response to APAP overdose in the LKO model is controlled by variations in gene expression and the enrichment of diploid hepatocytes. Future work will elucidate mechanisms of resistance and proliferation that allow diploid hepatocytes to drive rapid compensatory regeneration in APAP-induced acute liver injury. These data contribute to the understanding of polyploidy in liver homeostasis and disease, underscoring novel roles for hepatic ploidy populations.

Abstract 053

Inhibition of Wnt/beta-catenin Signaling as a Novel Therapeutic Strategy for Porphyria

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Introduction: The porphyrias are a group of metabolic disorders caused by inherited or acquired deficiency of any of the eight enzymes involved in heme biosynthesis, resulting in abnormal accumulation of heme precursors and their intermediates in organs such as the liver. Porphyrias are incurable, so novel therapies that have pleiotropic effects on disease progression would significantly improve patient quality of life. Because the liver is either a source or sink for accumulation of heme precursors, or porphyrins, we investigated the role of Wnt/beta-catenin signaling, a pathway that contributes to unique hepatic attributes such as zonation, autophagy, and metabolic regulation. Our earlier reports indicate that loss of hepatocyte beta-catenin protects mice from experimental porphyrin-induced liver injury. This was due to downregulation of key heme enzymes, fewer porphyrin-protein aggregates, and increased autophagy. In this study, we investigated the hypothesis that increased clearance was due to loss of glutamine synthetase (GS), a target of beta-catenin that regulates mTORC1 activation and thus autophagy. **Methods**: 3,5-Diethoxycarbonyl-1,4-dihydrocollidine (DDC), a xenobiotic compound that causes accumulation of porphyrin plugs in bile ducts, was fed to *Glul*$^{fl/fl}$ mice subjected to global Wnt inhibition using a PORCN inhibitor that blocks secretion of Wnt ligands, AAV8-Cre-mediated recombination to delete hepatic GS, or both. Half of these mice were also subjected to treatment with leupeptin to measure autophagic flux. Porphyrin accumulation, protein aggregation, autophagic vacuoles, mitochondria morphology, single cell spatial transcriptomics, mRNA expression, and mass-to-charge ratios of heme enzymes were assessed. **Results**: Our findings indicate that Cre-mediated deletion of GLUL (GS KO) or global Wnt inhibition results in defective GS activity in the liver. Phospho-mTOR-Serine2448, an indicator of active mTORC1 and

autophagy in zone-3 hepatocytes, was decreased in both groups. A surplus amount of autophagic vacuoles engulfing defective mitochondria and porphyrin accumulates were observed via Transmission Electron Microscopy in GS KO as well as a decrease in heme biosynthesis and autophagy proteins. **Conclusions**: Inhibiting hepatic Wnt signaling results in lower levels of glutamine and mTOR activity, prompting an increase in autophagy that clears the accumulated porphyrin-protein complexes and helps alleviate porphyria. These observations collectively offer a novel opportunity to treat porphyria by targeting the Wnt/beta-catenin signaling pathway. **Acknowledgement:** Research reported herein is supported by R01DK124412.

Abstract 054

Dual Loss of β- and γ-catenins from Cholangiocytes Leads to Acute Cholestasis and Mortality due to Hepatobiliary Injury and Maladaptation as Revealed by Single-Nucleus RNA Sequencing

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Introduction: β-catenin as a key effector of the canonical Wnt signaling is vital for normal liver functioning. However, β-catenin is also a component of adherens junctions (AJ), where its loss is compensated by γ-catenin, a homologous desmosomal protein. Neither the roles of β-catenin in cholangiocytes, nor the importance of AJ maintenance in bile ducts have been characterized in detail. In the present study we inducibly and specifically delete β- and γ-catenins from biliary epithelium to study pathophysiological implications of dual elimination. **Methods:** We utilized *Opn-iCreERT2$^{+/-}$; Ctnnb $^{fl/fl}$; Jup $^{fl/fl}$* mice (DKO), in which both β-catenin (*Ctnnb1*) and γ-catenin (*Jup*) are acutely deleted from biliary epithelium by Tamoxifen-inducible, cholangiocyte-specific osteopontin-driven expression of Cre-recombinase. 4 doses of Tamoxifen (100mg/kg) were administered to adult animals intraperitoneally. Tamoxifen-treated *Opn-iCreERT2$^{-/-}$* sex-matched littermates were used as controls. **Results:** DKOs showed morbidity and mortality with probability of survival dropping to 50% within 3 weeks and reaching 100% by 5 weeks after the induction of recombination. DKO mice exhibit decreased body weight and size, jaundice, and lethargy. Severe liver injury in DKOs was also manifested by elevated serum levels of ALT, AST, and alkaline phosphatase, in addition to increased bilirubin, reduced blood glucose and high cholesterol. A 10-fold decrease in bile flow rate was observed in DKOs following bile duct cannulation. Bile acid (BA) quantification by mass spectrometry revealed total bile acid levels to be increased 10^3- fold in the serum and 10-fold in the liver tissue of DKOs, with majority of BAs represented by hydrophilic conjugated species. Histology revealed multiple biliary infarcts, inflammation, stellate cell activation and severe portal fibrosis in DKOs. These findings were corroborated by bulk-RNA sequencing/IPA analysis, which also indicated changes in bile acid metabolism. Significant decrease in mRNA levels of select basolateral influx transporters and components of BA synthesis pathway was also evident from qRT-PCR. CK19-positive bile ducts in DKOs were malformed, irregular, and showed decreased or absent lumen by both IHC and electron microscopy. Ink injections into the common bile duct showed abundant blebbing and leakages along the entire intrahepatic biliary tree. Single-nucleus RNA sequencing (snRNA-seq) revealed differential clustering of hepatocyte as well as presence of a distinct population of cholangiocytes in DKO livers. DKO-associated genetic signature included 381 cholangiocyte-specific DEGs and indicated remodeling of the cell-cell junctions and upregulation of necroptosis. **Conclusions:** Dual loss of β- and γ-catenins from cholangiocytes causes severe intrahepatic cholestatic injury in mice. Our model demonstrates critical role of cell-cell junctions in cholangiocytes and provides unique insights into cholestatic disease pathogenesis.

Abstract 055

Maternal Obesogenic Diets Alter the Microbiome-Dependent Weaning Reaction and Variably Promote Non-Alcoholic Fatty Liver Disease in Offspring

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Introduction: Recent studies have found that maternal obesity increases an offspring's susceptibility to non-alcoholic fatty liver disease (NAFLD). The mechanism behind this phenomenon is unclear, but a critical time window in early life, known as the weaning reaction, has been established to impact the development of pathological inflammation. Weaning reaction is dependent on the early microbiome and is essential for the establishment of the intestinal immune system. Given that the microbiome is altered following maternal obesogenic diet exposure (MODE), we hypothesized that the weaning reaction is blunted as a result, thus promoting NAFLD in offspring. **Methods:** Female mice were fed chow (CON), high fat diet (HFD), high fat/high sucrose (HF/HS) diet or high fat-fructose-cholesterol (HFFC) diet for 6 weeks and bred with lean males. Terminal ileum was collected from offspring at 2 to 5 weeks of age to evaluate the expressions of the weaning reaction biomarkers, tumor necrosis factor-alpha (*Tnfa*) and interferon gamma (*Ifng*), via real-time qPCR. Cecum from offspring at 3 weeks of age was collected for 16srRNA sequencing. Expression of tight junction proteins (*Tjp1, Tjp2* and *Ocln*) was also measured in 3-week-old offspring. To examine the progression of NAFLD, another group of offspring was weaned to HFFC for 7 weeks and their liver was collected for histopathologic analysis. The direct impact of the early microbiome was measured in a group of offspring mice that were cross-fostered with delivery matched CON and HFFC dams. **Results:** The expected spike of ileal expression of *Tnfa* and *Ifng* was observed in CON offspring. However, peak expression of ileal *Tnfa* and *Ifng* was attenuated following MODE. MODE induced shifts in the offspring microbiome at 3 weeks with a reduction in alpha-diversity in HFFC and HFD offspring. Changes in abundance of multiple genera of bacteria were identified in MODE offspring. Decreased expression of intestinal barrier genes (*Tjp2* and *Ocln*) were also observed in MODE offspring. When weaned to HFFC, MODE offspring exhibited increased steatosis, inflammation and fibrosis. Preliminary data from our cross-foster study demonstrated that fostering HFFC offspring with a CON mother induced a weaning reaction and decreased NAFLD associated inflammation and fibrosis. Conversely, fostering CON offspring with HFFC dam resulted in attenuation of the weaning reaction and increased NAFLD associated inflammation and fibrosis. **Conclusion:** These findings provide that MODE shifts the microbiome and attenuates the weaning reaction, with an associated increase in susceptibility to NAFLD. Our preliminary data indicates that the development of this phenotype is dependent on the early microbiome. **Acknowledgements**: Washington University School of Medicine in St. Louis Pediatric Student Research Program (PSRP).

Update on Collagen Biology

Abstract 056
The Role of Microvilli in the Organization of Apical Extracellular Matrix
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Introduction: The tectorial membrane (TM) is an apical extracellular matrix (ECM) in the inner ear mediating auditory transduction (*Curr Top Dev Biol* 2018;**130**:217-244). The developing TM exhibits a highly ordered architecture composed of parallel collagen fibrils that are attached to the tip of the microvilli of the cochlear supporting cells. We previously showed that the attachment of elongating collagen fibrils to the tip membrane is crucial for collagen arrangement (*Sci Adv* 2019;**5:**eaay6300). We identified that both surface-tethering and the release of alpha-tectorin (TECTA), a GPI-anchored collagen binding protein, play critical roles in TM morphogenesis by preventing the diffusion of secreted collagens and mediating matrix elongation. However, the mechanisms underlying the recruitment of collagen fibrils to specific membrane compartments and the release of TECTA from the cell surface remain unknown. We have discovered that TECTA is released from the cell surface membrane through proteolytic shedding by transmembrane serine proteases (TMPRSSs) and extracellular vesicle (EV)-mediated release. To uncover the contributions of each release mode to ECM organization, we generated Tecta knock-in mouse lines, which specifically block either the proteolytic shedding or EV-mediated release of TECTA, and characterized the ultrastructure of the TM. **Methods:** We employed CRISPR/Cas-9 gene editing to create the knock-in mouse lines. We generated the following mutations: (1) Tecta-R2061S missense mutation, which hampers TMPRSS-mediated shedding of TECTA, and (2) Tecta-ZP3 swapping mutation, where the GPI-anchor of TECTA is

substituted with the transmembrane domain of the ZP3 protein. We characterized the TM's structure using Airyscan fluorescence microscopy and Transmission electron microscopy (TEM). **Results:** In Tecta-R2061S mice, collagen fibrils deviated from their normal attachment to the tip membrane in wildtype mice and instead connected to the lateral and base membranes of the microvilli. Additionally, we observed a dramatic increase in the microvilli's length and the density of extracellular vesicles (EVs). Conversely, in Tecta-ZP3 mice, the release of TECTA and EV density significantly diminished. Consequently, collagen fibrils lacked crosslinking fibers and exhibited denser packing. **Conclusion:** Our findings revealed novel roles of microvilli in matrix organization. The protrusion of microvilli restricts the proteolytic shedding of a collagen-binding protein from the tip membrane, leading to the specific attachment of collagen fibrils to the microvillus tip. Moreover, the EV-mediated release of TECTA from the microvillus tip contributes to organizing crosslinking fibers between collagen fibrils, thereby maintaining the spacing and arrangement of the collagen fibrils. **Acknowledgement:** This work is supported by R01DC018814 to S.P.

Abstract 057

Fused in Sarcoma: An RNA-DNA Binding Protein with Pro-fibrotic Activity

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Introduction: Integrin α1β1 (Itgα1β1) is a collagen IV receptor that downregulates extracellular matrix production. Itgα1β1 plays an anti-fibrotic action by negatively regulating the tyrosine phosphorylation of the ribonucleoprotein Fused in Sarcoma (FUS) thus preventing its nuclear translocation mediated by transportin/karyopherin β2. In cells lacking Itgα1β1, FUS is tyrosine phosphorylated and translocates to the nucleus where it promotes collagen IV transcription by forming a complex with its promoter. Thus, the goal of this study was to determine the role of FUS in promoting fibrotic responses *in vivo* and to explore the potentially beneficial effects of preventing FUS nuclear translocation in two different preclinical models of organ fibrosis. **Methods:** We treated wild-type mice with adriamycin (ADR) or carbon tetrachloride (CCl4) to induce kidney and liver injury/fibrosis, respectively. The direct role of FUS nuclear translocation in driving fibrosis was evaluated in FUS-R521G mice that carry a mutation in the FUS nuclear localization sequence. Alternatively, wild-type mice were treated with the cell penetrating peptide (CP-FUS-NLS) that binds to transportin/karyopherin β2 and prevents FUS nuclear translocation (*J Cell Biol* 2020;**219**:e202001120). Peptide treatment started at the time of organ injury and mice were sacrificed two weeks after ADR or six weeks after CCl4 treatment. Kidneys were evaluated for markers of fibrosis and nuclear FUS. **Results:** Both kidney and liver cells had significantly higher levels of nuclear FUS after ADR or CCl4 treatment compared to vehicle-treated mice. Injured mice developed robust fibrosis in both models. In contrast, FUS-R521G mice or wild-type mice treated with CP-FUS-NLS showed reduced FUS nuclear translocation and diminished development of fibrosis following ADR treatment. Moreover, pharmacological inhibition of FUS nuclear translocation also ameliorated CCl4-induced liver fibrosis. Finally, differential gene expression analysis of published datasets from tissues of patients with either focal segmental glomerulosclerosis or nonalcoholic steatohepatitis revealed FUS, COL1A1, COL1A2, COLLIVA2 significantly upregulated in diseased organs. **Conclusions:** Nuclear translocation of FUS occurs after kidney and liver injury and promotes fibrosis. The novel finding that inhibition of FUS nuclear translocation decreases matrix synthesis and ameliorates fibrosis, suggests that FUS can be viewed as a targetable molecule in organ fibrosis. **Acknowledgements:** This work was supported by a VA Merit review and R01-DK119212. AP is the recipient of a Senior Research Career Scientist award.

Abstract 058*

A Bidirectional Cross-talk Between the Adherens Junction-associated RNAi Machinery and the Extracellular Matrix Regulates Colon Epithelial Cell Behavior

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Abstract 059
Collagen Degradative Pathways Ensure Cervical Remodeling During Pregnancy
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Introduction: Dynamic changes in the composition of the extracellular matrix (ECM) modulate cervical function during pregnancy. Our prior proteome and transcriptome studies identified a remarkably high turnover rate of collagen 1 in the mouse cervix during non-pregnancy and pregnancy. To date, collagen degradation pathways that ensure continuous collagen turnover during cervical remodeling are unknown. Collagen degradation can be achieved via both the extracellular and the intracellular pathways. The focus of this study is to determine if both the intracellular and extracellular collagen-degradative pathways are utilized in the cervix during term remodeling and whether dysregulation of one or both pathways may lead to cervical insufficiency and subsequently into preterm birth. **Methods**: Expression and activity of extracellular collagen-degrading proteases was evaluated via transcriptomic, immunohistochemistry (IHC), in-situ zymography (ISZ), and activity assays in the cervix from non-pregnant (NP) and pregnant (d6-d18) mouse. Components of the intracellular pathway were assessed by Western blot. Assessment of collagen internalization was conducted by immunofluorescence (IF) and flow cytometry in human cervical fibroblasts. Mice lacking the collagen receptor, MRC2 were used to explore functionality of extracellular pathway in absence of receptor-mediated intracellular pathway. **Results:** Components involved in both pathways were identified in mouse cervical transcriptomic analysis. Of interest, expression of 1) collagen degrading proteases such as MMP14, MMP2, MMP9, and FAP and 2) receptors and proteases required for collagen uptake and degradation such as MRC2 and lysosomal cathepsins were evident in the dataset. Animal and cell studies identify expression and activity of molecules required for collagen degradation in the cervix. Continuous MMP14 collagenolytic activity was observed in the NP and throughout pregnancy. FAP, MMP2 and MMP9 activity was temporally regulated through pregnancy. MRC2 was expressed in the mouse and human cervix. IF revealed internalization of collagen by the human cervical fibroblasts. Knockdown of MRC2 resulted in a significant decrease in intracellular-mediated collagen uptake. Gelatinase activity was detected in the cervix from NP and pregnant MRC2 deficient mice suggesting functional compensation of the extracellular pathway in the absence of the intracellular pathway. **Conclusion**: Constant collagen turnover in the cervical ECM is critical to maintain the structural and mechanical changes that occur during pregnancy. Our studies suggest that the intracellular pathway for collagen degradation in conjunction with the temporally-specific activity of extracellular proteases ensures collagen homeostasis. These findings provide insights into mechanisms by which collagen turnover is achieved in physiologic cervical remodeling and suggest fibroblast-mediated proteases are drivers of ECM homeostasis in the mouse cervix.

Elastic Fibers

Abstract 060
Fibulin-1 Supports BM Stretching During Ovulation in *C. elegans*
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Introduction: All tissues are enwrapped and structurally supported by basement membrane (BM), a thin, dense extracellular matrix (ECM). A critical but understudied BM property is its ability to stretch to support mechanically active tissues such as muscles and vasculature. To elucidate the mechanism underlying BM plasticity, we are developing *C. elegans* ovulation as an *in vivo* experimental system to investigate how BMs dynamically expand and retract. During ovulation, the *C. elegans* spermathecal BM stretches ~2-fold in area to accommodate an oocyte for fertilization. Once fertilized, the oocyte moves into the uterus and the spermathecal BM rapidly relaxes. **Methods and Results:** To establish the spermathecal BM composition, a localization screen was performed using 58 BM-associated proteins that have been endogenously tagged with mNeonGreen, including all core components, BM associated receptors and most

known matricellular proteins, enzymes, and proteases (*Dev Cell* 2020;**54**:60-74.e7)[1]. We discovered that the spermathecal BM is enriched with the sole *C. elegans* fibulin, fibulin-1. Notably, vertebrate fibulin-1 promotes BM and vasculature integrity, however, the underlying mechanism remains unclear (*Mol Cell Biol* 2001;**21**:7025-7034). Fibulin-1 depletion in adult animals led to BM rupture and deformed spermatheca organs, suggesting that fibulin is required to maintain BM and tissue integrity. Consistent with a specific role in BM stretching, the defect in BM rupture and tissue shape increased with repeated ovulations. Strikingly, we also discovered that fibulin-1 is present at high levels in the oocyte specifically during ovulation, suggesting that fibulin-1 is delivered to the spermathecal BM during ovulation to support stretching. **Conclusion:** Together, our findings establish a new model to elucidate mechanisms underlying BM stretching and reveal a key role for fibulin-1 in maintaining BM integrity during tissue stretching.

Abstract 061

Elastic Fiber Defects Facilitate Transmural Fluid Flux and Macrophage Migration Across the Aortic Wall: An Ex Vivo Study

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Introduction: Thoracic aortic aneurysms (TAAs) exhibit degraded elastic fibers and immune cell infiltration (*Curr Opin Biomed Engineering* 2018;**5**:50-57; *J Thorac Cardiovasc Surg* 2008;**136**:922-929). This study explores the role of elastic fiber defects in facilitating transmural fluid flux and macrophage migration, which may contribute to TAA progression or be leveraged for TAA treatment. **Methods:** *Transmural fluid transport:* The ascending thoracic aorta was removed from 3-4 month-old female and male wild-type mice. The aorta was sutured into a pressure myograph (110P, Danish Myo Technology), pressurized to 100mmHg, and the outlet of the myograph was closed so transport occurred through the aortic wall. A bubble was added and its position over time was recorded (*J Biomech* 2022;**145**:111360; *Am J Physiol Heart Circ Physiol* 2023;**325**:H113-H124). Elastase (7.5 U/mL, Worthington Biochemical) was injected into the pressurized aorta for 1 minute (ELA-1) or 6 minutes (ELA-6) followed by 100 mM NaCl for 10 minutes to stop the enzymatic digestion (*Ann Biomed Eng* 2023;**51**:806-819). The aorta was repressurized and the bubble position over time was recorded. Aortic dimensions were measured at each step. Fluid flux and hydraulic conductance were calculated from the bubble velocity using 1-D advection-diffusion mass transport equations (*J Biomech* 2022;**145**:111360; *Am J Physiol Heart Circ Physiol* 2023;**325**:H113-H124). *Transmural cell migration:* 1 million Raw 264.7 macrophages (ATCC) stained with cell tracker green CMFDA (Invitrogen) were then injected into the pressurized aorta. Samples were taken from the outer bath every 10 minutes for 60 minutes and cell concentration in the outer bath was determined using a fluorescence standards curve. **Results:** Elastase treatment for 6 minutes increases transmural fluid transport. Before elastase treatment, bubble velocity was comparable for all groups. After elastase treatment, bubble velocity for ELA-1 aortas was unchanged from untreated (UNT) aortas, while bubble velocity for ELA-6 aortas was significantly increased. This increase in bubble velocity was reflected in an increased fluid flux and hydraulic conductance in ELA-6 aortas compared to ELA-1 and UNT aortas. Elastase treatment facilitates macrophage migration across the wall. The UNT aortas had the lowest macrophage migration rate. The ELA-1 aortas showed a modest increase ($P>0.05$), while the ELA-6 aortas showed a significant ($P<0.0001$) increase in macrophage migration compared to UNT aortas. **Conclusion:** We demonstrated that elastase treatment causes increased fluid flux and macrophage migration across the aortic wall. Future work will investigate microstructural differences to understand how elastic fiber defects facilitate transmural macrophage migration. **Acknowledgements:** Funding partially provided by AHA 19TPA-34910047 and NIH HL 164800.

Abstract 062

Spatio−temporal Expression of EP4 Regulates Vascular Intimal Hyperplasia Through Fibulin-1

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Introduction: Activation of the prostaglandin E2 (PGE_2)-producing enzymes cyclooxygenase-2 (COX2) or microsomal PGE_2 synthase-1 exacerbate injury-induced vascular intimal hyperplasia (IH) (*Circ Res* 2013;**113**:104-114; *Circulation* 2011;**123**:631-639). However, the downstream signaling of PGE_2 receptors is not fully elucidated. We aimed to investigate the expression and roles of EP4 in vascular IH. **Methods:** We generated EP4 reporter mice (*Ptger4*-IRES-nlsLacZ), vascular smooth muscle-specific EP4 heterozygous deficient and control mice (*Ptger4*$^{fl/+}$;*SM22*-Cre and *Ptger4*$^{+/+}$;*SM22*-Cre), fibulin-1 deficient and control mice (*Fbln1*$^{fl/fl}$;*SM22*-Cre and *Fbln1*$^{fl/fl}$), and EP4 overexpressing and control mice (*Ptger4*-Tg and Non-Tg). Vascular injury was induced by inserting a wire into the femoral artery. Vascular smooth muscle cells (VSMCs) isolated from these mice were used to assess cell proliferation by a cell counting kit-8. X-gal stain, immunofluorescence, and western blotting were performed to evaluate protein expressions. **Results:** X-gal stain using EP4 reporter mice demonstrated that prominent EP4 expression was detected in the part of neointima at 2 weeks after injury in which proliferating cell nuclear antigen (PCNA) was highly expressed, but not in uninjured arteries and in the arteries 4 weeks after injury. Injury-induced IH was diminished in *Ptger4*$^{fl/+}$;*SM22*-Cre mice at 2 and 4 weeks (0.2-and 0.5-fold, $p<0.01$) compared to controls, whereas IH was enhanced in *Ptger4*-Tg at 2 and 4 weeks (2.5-and 1.4-fold, $p<0.01$). Stimulation of *Ptger4*-Tg VSMCs with an EP4 agonist increased fibulin-1C and 1D mRNAs (2.3-and 2.4-fold, $p<0.05$), and fibulin-1 protein (4.3-fold, $p<0.05$). Cell proliferation was increased in Non-Tg VSMCs treated with full-length fibulin-1C or 1D recombinant proteins (2.0-and 1.6-fold, $p<0.05$), while proliferation was decreased in fibulin-1-deficient VSMCs. Epidermal growth factor-like (EG) module 2-8 deletion mutant of fibulin-1 abolished full-length fibulin-1-induced proliferation. Extracellular matrix protein 1 (ECM1) which binds to fibulin-1 was upregulated by EP4 stimulation and ECM1 expression pattern during injury was similar to that of EP4 and fibulin-1. Administration of ECM1 proteins enhanced fibulin-1-mediated proliferation in Non-Tg VSMCs. IH was reduced in *Fbln1*$^{fl/fl}$;*SM22*-Cre compared to controls at 2 and 4 weeks after injury (0.5-and 0.4-fold, $p<0.01$). Oral administration of EP4 antagonists attenuated injury-induced IH in wild-type mice (0.5-fold, $p<0.01$). **Conclusions:** These data suggested that PGE_2-EP4 promotes IH via fibulin-1 together with ECM1. EP4 inhibition may be a therapeutic strategy to reduce injury-induced IH.

Abstract 063*

MFAP4 Forms Octamers Required for Functional Interactions with Elastogenic Proteins

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*The authors of this abstract selected not to publish the details of their research

Matrix and Receptors in Development

Abstract 064

Multiple Cell Types Influence Extracellular Matrix Dynamics During Planarian Regeneration

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Introduction: While the adult vertebrate restricts its tissue regeneration to particular organs, the invertebrate planarian, *Schmidtea mediterranea,* has the remarkable ability to regenerate its entire body from a tiny fragment. The vast amount of adult pluripotent stem cells in this animal makes it an excellent model to study how extreme tissue regeneration programs launch in an adult animal. Our goal is to understand the role of extracellular matrix (ECM) in creating a favorable niche for the stem cell to support whole body regeneration. While fibroblasts are the predominant source of ECM production in mammals, planarian fibroblasts are not clearly identified. Therefore, we hypothesize that planarians must use one or more different cell types to produce and remodel the ECM. **Methods and Results:** To test this hypothesis, we first empirically defined the protein components of the planarian ECM during regeneration via mass spectrometry of decellularized animals. While we identified many canonical ECM components, such as collagen family members, we were able to identify many ECM proteins that have no detectable homology.

Afterward, we compared ECM protein dynamics with transcriptional changes in bulk RNA-seq data. We uncovered that ECM undergoes extensive remodeling at both the mRNA and protein levels in response to regeneration. Furthermore, single-cell RNA-seq data suggested that the epidermis, intestinal, and parenchymal cell types are responsible for ECM transcription during the early phase of tissue regeneration, while muscle cells are responsible during the latter phase. Nevertheless, it appears that most cell types secrete ECM proteins at all time points. Gene ontology enrichment indicates that both early ECM mRNA and protein are necessary for proteolysis as the animals digest pre-existing ECM components while the latter phase is essential for ECM assembly as animals produce new tissue. Functional roles for specific ECM proteins were investigated using RNA interference. We find both positive and negative regulators of stem cell proliferation: Periostin, Trig-1, and Netrin 4, promote stem cell proliferation, while Collagen alpha 2(IV), Carboxypeptidase, Laminin subunit alpha 2 have an opposing effect. **Conclusion:** In conclusion, our current evidence suggests that planarians rely on cells from all three germ layers to remodel their ECM during tissue regeneration. Our data may help inform how non-fibroblast cells can collectively create proper tissue microenvironments, enable powerful tissue regeneration in planarians.

Abstract 065

Delineating the Timeline of SNED1 Fibrillogenesis and Assembly in the Extracellular Matrix

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Introduction: The extracellular matrix (ECM) is a complex meshwork of proteins that plays a vital role in many cellular processes. Many of these proteins are fibrillar in nature and their assembly into the ECM takes place over time via fibrillogenesis. This process involves protein-protein interactions, and has been well-studied for ECM proteins such as fibronectin, collagen I, and fibrillins. For example, both collagen I and fibrillins require fibronectin to be present in the ECM prior to their assembly. Fibrillogenesis remains critically unstudied for dozens of other ECM proteins, one of which is SNED1 (Sushi, Nidogen, and EGF-like Domain 1). We found SNED1 to be highly upregulated in metastatic breast cancer and to act as a metastasis promoter. We generated a *Sned1* knockout (KO) mouse model to further investigate SNED1's mechanisms. The KO mice showed distinct phenotypes, showing that SNED1 plays a crucial role in craniofacial development and is essential for survival. Additionally, we observed that SNED1 forms fibers in the ECM *in vitro*. However, the timeline of SNED1's fibrillogenesis as well as whether it requires protein-protein interactions for its assembly have not yet been investigated. **Methods:** We have isolated mouse embryonic fibroblasts (MEFs) from the *Sned1* KO mice our lab generated to create a blank slate into which we can introduce SNED1 constructs. We used *Sned1* KO MEFs engineered to overexpress GFP-tagged SNED1 to obtain cell-derived decellularized matrices (CDMs), a widely-used technique allowing us to study the ECM *in vitro*. CDMs were decellularized and fixed at 3, 5, and 7 days post-seeding, then imaged using immunofluorescence microscopy. **Results:** We observed the formation of SNED1 fibrils in the ECM as early as 3 days post-seeding. Fibrils observed at the earliest timepoint were short and thin but thickened, lengthened, and increased in number in later timepoints. Fibronectin and collagen I fibrils were also present at 3 days post-seeding, and were notably longer and formed a denser meshwork than SNED1 fibrils. At 7 days post-seeding, we observed a partial colocalization between SNED1 and collagen I and fibronectin fibers, which suggests SNED1 may require interactions with these abundant proteins for its assembly into the ECM. **Conclusions:** Knowing when an ECM protein appears in the ECM allows us to begin investigating how it is assembled into the matrix. We recently published an *in-silico* prediction of the SNED1 interactome and found that it could potentially bind to 33 ECM proteins including fibronectin, and 11 integrin subunits. These predicted interactions are yet to be demonstrated experimentally. Leveraging these data, we will continue our investigation of protein-protein interactions involving SNED1 and leading to its fibrillar assembly. Learning how SNED1 forms fibers is of great interest due to its implication in development and breast cancer metastasis, as well as its function in ECM homeostasis.

Abstract 066*

Fibronectin-mediated Physiological and Pathological Mechanisms in Skeletal Development

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Abstract 067
Temporally-Restricted Patterns of Endothelial Cell Collagen IV Expression Determined with a Novel Knockin Col4a1-GFP Mouse Line
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Introduction: Collagen IV is the principal type of collagen synthesized by endothelial cells (EC) and is a major constituent of vascular basement membranes. Classical type IV collagen comprises of a heterotrimer of two collagen IV alpha 1 chains and 1 collagen IV alpha 2 chain. Mutations in genes encoding collagen IV alpha 1 and alpha 2 result in vascular dysfunction in both mice and man. Additionally, mutations in genes that encode the Ephrin receptor B4 (EPHB4) and the p120 Ras GTPase-activating protein (RASA1) result in vascular dysfunction as a consequence of impaired export and accumulation of collagen IV in EC. Of note, mutations in EPHB4 and RASA1 genes cause increased activation of the Ras mitogen-activated protein kinase (MAPK) signaling pathway in EC. Thus, it is crucial to identify at which specific times during development and in postnatal life, collagen IV is actively synthesized by EC. This helps to understand the pathogenesis of vascular diseases and phenotypes related to collagen IV. **Methods:** We used CRISPR/Cas9 targeting in mice to insert a sequence that encodes a self-cleaving P2A peptide followed by enhanced green fluorescent protein (eGFP) immediately after the terminal *Col4a1* codon. Timed matings were then set up to generate embryos at various stages of development from E8.5 to E18.5. Also, various organs from 3-month-old Col4a1-eGFP mice were recovered. Sections (5 µm) of formalin-fixed paraffin-embedded tissues were stained by immunofluorescence and GFP signals were detected and quantified in blood vessels. **Results:** Analysis of eGFP expression as an index of Collagen IV alpha 1 synthesis in *Col4a1-P2A-eGFP* mice revealed active embryonic EC synthesis of collagen IV alpha 1 through mid to late gestation followed by a sharp decline before birth. There was no eGFP in various organs including the heart, brain, lung, and liver of adult 3-month-old *Col4a1-P2A-eGFP* mice. **Conclusion:** These novel findings help understand the basis for the different vascular defects seen due to the disturbances in the expression and secretion of functional collagen IV.

Basement Memberanes: Structure and Function

Abstract 068
Non-Cell-Autonomous Deposition of Ectopic Laminin α2 in the Glomerular Basement Membrane in Alport Syndrome
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Introduction: The glomerular basement membrane (GBM) is a key component of the glomerular filtration unit that filters blood to produce primary urine. The GBM is mainly composed of laminin (LM)-521 (α5β2γ1), type IV collagen (COL4)-α3α4α5, nidogen and agrin. Mutations in the COL4-α3α4α5 genes cause Alport syndrome, which is characterized by an abnormal GBM structure and progressive kidney disease. Compositional GBM defects due to COL4A3/4/5 mutations include ectopic deposition of LMα2 (the major skeletal muscle LM) in the Alport GBM, reported to be part of a LM trimer secreted by glomerular mesangial cells. LMα2 is considered pathogenic in the GBM, which usually contains only LM-521. We therefore attempted to reduce LM α2 levels in the GBM of Alport mice to investigate its therapeutic impact on kidney disease. **Methods:** To manipulate *Lama2* expression in mouse tissues, we used *in vivo* CRISPR/Cas9 platforms. Briefly, we screened guide (g)RNAs in cultured cells with Cas9 for CRISPR-knockout (CRISPR-KO) and dCas9-KRAB for CRISPR interference (CRISPRi; transcriptional repression) approaches. We then generated transgenic mice ubiquitously expressing Lama2-gRNAs appropriate for both CRISPR-KO and

CRISPRi systems. Using ubiquitous Cas9 and dCas9-KRAB mice as well as Cre-inducible Cas9 mice, we investigated the functionality of Lama2-gRNAs for reducing *Lama2* expression. **Results:** Both ubiquitous Cas9 and dCas9-KRAB mice expressing Lama2-gRNAs showed reduced LMα2 protein throughout the kidney as well as in most other tissues (including skeletal muscle), resulting in a variable congenital muscular dystrophy-like phenotype. Using Pax3-Cre to activate Cas9 expression (and thus reduce *Lama2* expression) in diverse cell types in the kidney, including podocytes, mesangial cells, tubular epithelial cells, and fibroblasts, LMα2 was decreased in the interstitium but not in the mesangial matrix of wild-type mice and not in the mesangial matrix or GBM of Alport mice. This result led us to hypothesize that the ectopic LAMA2 in Alport GBM is derived from external tissues via the circulation. To investigate this, we administered recombinant LM-211 (α2β1γ1) protein intravenously to Alport and non-Alport mice unable to express LMα2. Administered LM-211 localized to the mesangial matrix in non-Alport mice and to both the mesangial matrix and GBM in Alport mice. **Conclusions:** Our results suggest that much or all of the ectopic LMα2 deposition in Alport GBM is derived not from glomerular cells, but from laminin shedding by external tissues, perhaps skeletal muscle. In support of this, a public proteomics database that includes human plasma samples shows LAMA2 is detected in the blood. A non-cell autonomous mechanism for ectopic LAMA2 deposition in Alport GBM is evidence for a novel mechanism of basement membrane dynamics in mammals that could be active in other tissue contexts.

Abstract 069

Antibody Mediated Binding of Amyloid Beta Causes Degradation of Collagen IV in the Basement Membrane of Viable Microvessels Isolated from Brains with Alzheimer's Disease Neuropathology

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Introduction: Brain microvascular changes occur early in Alzheimer's disease (AD), often predating significant amyloid beta protein (Aβ) deposition (*Nature Med* 2019;**25**:270-276). Loss of the integrity of the basement membrane (BM) from brain microvessels (MVs) due to alterations in Collagen IV (Col IV), a major component of the BM, can predispose the MV to complications such as edema and microhemorrhages (*Stroke Vasc Neurol* 2019;**4**:78-82). In the MVs of both AD and the related condition of congophilic amyloid angiopathy (CAA), Col IV and Aβ are in close spatial proximity. We wished to determine the effect of antibody-mediated removal of Aβ using the Aβ binding monoclonal antibody lecanemab, on Col IV in the BM of isolated viable human brain MVs (*Neurotherapeutics* 2023;**20**:195-206). **Methods:** Viable human MV were isolated from the parietal region of brains obtained from rapid autopsies of a 78-year-old and 88-year-old females and cryostored as previously described (*Microvasc Res* 2022;**140**:104282). Subsequent neuropathology identified these specimens as having a total ADNC score of at least intermediate and a CAA score of at least moderate (*Acta Neuropathol* 2012;**123**:1-11*). APOE* genotype was ε3/ε3 for both donors. MV were thawed, placed in human endothelial cell serum free media with B27 (Gibco) and treated once with 0, 3.3, 6.6 or 10µg/ml of lecanemab (Proteogenix). After 4 days, MV were collected, centrifuged, rinsed with PBS, and lysed in buffer with protease inhibitors. Equal amounts of protein from each donor and condition were loaded on gradient gels, transferred and probed with antibodies to Aβ (6E10 Biolegend), Col IV (ab6586 ABCAM), MMP-9 (AB19016 Sigma), or pan-laminin (L9393 Sigma). **Results:** MV retained their morphology with no evidence of degradation over the 4-day period. Western blotting for Aβ showed complete loss of Aβ at 10µg/ml lecanemab, and decreases of Aβ at 6.6 and 3.3 µg/ml of lecanemab. All 3 doses of lecanemab caused degradation of MV Col IV as indicated by multiple lower MW bands that were detected with the Col IV antibody in the MV lysates from both donors. There was a detectable increase in MMP9 levels in MV treated with lecanemab from one donor. There was no evidence of laminin degradation in MV treated with lecanemab from either donor. **Conclusion:** Exposure of viable human brain MV to the Aβ binding monoclonal antibody, lecanemab, induced degradation of Col IV in MV from the brains of 2 human subjects with AD neuropathology. There was also evidence of increased MMP9 and no effect on laminin. This suggests that breakdown of Col IV represents a potential mechanism for Aβ binding antibody-

mediated MV disruption, which could contribute to clinical manifestations including edema and microhemorrhages. **Acknowledgements:** NIH R03AG051071 (MJR), R21AG073676 (MJR), P30AG066509 (UW ADRC), and the Nancy and Buster Alvord Endowment (CDK).

Abstract 070

Dual Linker Protein Repair of the Lama2-null Dystrophic Mouse by AAV Somatic Gene Therapy

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Introduction: Complete absence of laminin-α2 expression results in a severe non-ambulatory congenital neuromuscular dystrophy (MDC1A, LAMA2-RD). The dy^{3K}/dy^{3K} (laminin α2-null) mouse model is characterized by a short life span, reduced weight, severe weakness, limited mobility, and hindlimb paresis. These mice express increased levels of α4-laminins in muscle and peripheral nerve basement membranes, replacing the absent α2-laminins. **Methods:** Neonatal dy^{3K}/dy^{3K} mouse pups (P1) were treated i.v. with adeno-associated virus (AAV_9) ubiquitously expressing a laminin-binding linker protein, αLNNdΔG2', alone or in combination with AAV_9 expressing miniagrin. The two proteins act by modifying α4-laminins such that they gain the polymerization and α-dystroglycan receptor-binding activities normally present in α2-laminin. Mice were evaluated for ambulatory activity, weight, grip strength and histology. **Results:** Treatment with AAV-αLNNdΔG2' alone resulted in correction of the hindlimb paresis and extension of survival, but little improvement in strength or weight. Adding AAV-miniagrin to that of AAV-αLNNdΔG2' resulted in substantial increases in weight, survival, and ambulation along with a modest increase in grip strength. AAV-αLNNdΔG2 alone prevented sciatic nerve amyelination with a greater improvement of muscle histology seen with double AAV treatment. **Conclusions:** These findings support the approach of employing dual AAV-delivery of genes coding for small laminin-binding proteins that provide missing functions as a treatment of a devastating disease.

Abstract 071

Cryo-EM Reveals the Molecular Basis of Laminin Polymerization and LN-lamininopathies

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Introduction: Laminin (Lm) polymerization is the major step in basement membranes (BM) assembly. The Lm polymer node, which constitutes the repeating unit of the Lm lattice, is a flexible heterotrimer consisting of Lm alpha, betta and gamma subunits that join together through their N-termini. Genetic alternations of Lm subunits result in failures of Lm polymerization manifesting as a wide spectrum of human disorders, which we collectively define as LN-lamininopathies. Understanding the process of Lm polymerization and its defects requires the knowledge of the Lm polymer node structure. We've employed cryo-EM to determine a 3.7 Å structure of the functional Lm polymer node. **Methods:** Cryo-EM samples were prepared by mixing 56 kDa alpha 1, 64 kDa beta 1 and 52 kDa gamma 1. We collected 44,743 movies using a 300 kV Titan Krios/K3. The structure (PDB ID: 8DMK, EMD-27542) was calculated using a combination of cryoSPARC, Scipion, and Relion. The atomic model of the complex was derived from the EM map using a combination of Phenix and COOT. **Results:** The asymmetric cryo-EM structure of the Lm polymer node resembles a triskelion with centrally located LN domains and three rod-like structures projecting outwards, each containing one or two LE domains. The Principal Components Analysis reveals the planar and rotational mobility of LE rods. The inter-subunit interfaces are formed by two sets of interacting regions. The interface between alpha 1 and gamma 1 differs from other inter-subunit interfaces, because it involves a calcium-binding site, the fact with implications for the mechanism of sequential assembly of Lm trimer. The cryo-EM structure reveals that the interface involves a loop from gamma 1 consisting of residues critical

for coordination of a calcium ion. We propose that in the absence of a calcium ion the loop in gamma 1 is not structured, hence the alpha 1 - gamma1 interface cannot be formed, explaining the calcium dependence for the assembly of a trimeric Lm node. The structure provides insight into polymerization defects manifesting in human disease. For example, a subset of Pierson syndrome results from mutations in the human LAMB2 gene. The structure provides detailed explanation how reported to date mutations in LAMB2 lead to failures in Lm polymerization. **Conclusions:** We determined a cryo-EM structure of the Lm polymer node. The structure reveals fundamental molecular mechanisms governing formation of extracellular Lm matrix. Importantly, the structure unveils the molecular basis underlying Pierson syndrome and other related LN-lamininopathies. The structure offers to facilitate rational drug design aiming in the treatment of Lm deficiencies, and can foster development of biomimetic BMs for tissue implants.

New Approaches to Modelling Matrix Changes in Human Development and Disease

Abstract 072

Pgp-1 Deficiency Results in Intestinal Defects in *C. elegans*, Modeling Human Disease

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Introduction: Inflammatory Bowel Disease (IBD) is characterized by chronic inflammation of the intestinal tract. IBD is an autoimmune reaction with an unknown cause. Although there are treatments to relieve symptoms, there is no current cure. *MDR1* codes for a protein called P-glycoprotein (Pgp). Pgp is known to be expressed in intestinal epithelial cells in several organisms, including humans. The relationship between Pgp and IBD is unknown, however, *MDR1* polymorphisms have been associated with human inflammatory bowel disease. Our studies utilize *Caenorhabditis elegans* (*C. elegans*) to investigate intestinal function and development in the absence of a p-glycoprotein molecule. Using *Pgp1*-deficient (*Pgp1*$^{-/-}$) *C. elegans*, we have conducted several measures of intestinal and digestive function. **Methods:** *C. elegans* Culture: Control and *Pgp1*$^{-/-}$ *C. elegans* were obtained from the *Caenorhabditis* Genetics Center at the University of Minnesota and cultured on Nematode Growth Medium at 20°C using the standard OP50-1 *E. coli* food source. Age synchronization was done via bleaching of gravid adults to obtain embryos, which were allowed to grow to young adults (4 days). All imaging was done using a Keyence BZ-X800 microscope. Growth Analysis: Control and *Pgp1*$^{-/-}$ *C. elegans* were imaged every 24 hours for 4 days, and lengths measured using ImageJ. Mean Defecation: Young adult worms were monitored for defecation behaviors for 5 minutes. The average time between defecation was calculated. Staining: Young adult worms were moved to NGM plates with 4μM LysoTracker Red or 1mg/ml Nile Red mixed with OP50-1 food overnight. Worms were then washed, anesthetized with 10mM levamisole and imaged. Brightness per worm was established using ImageJ. Immunohistochemistry was performed by first dissecting the intestine, freeze cracking, and staining using primary antibodies obtained from the Developmental Studies Hybridoma Bank. **Results:** *Pgp1*$^{-/-}$ *C. elegans* displayed slower growth and mean defecation behavior length relative to control. Pgp 1$^{-/-}$ worms were also found to have a decreased life span. Lysosomal staining and Nile Red staining decreased density of lysosomal granule and fat storage granules in the intestine of *Pgp1*$^{-/-}$ *C. elegans*. Lastly, immunohistochemistry showed decreased expression of apical junction related proteins in *Pgp1*$^{-/-}$ *C. elegans*. **Conclusions:** Our results suggest that there are physical changes in the digestive process of Pgp 1 deficient *C. elegans*. Decreased growth rate and defecation rate could result from decreased nutritional uptake. This is supported through decreased detection of digestive lysosomal granule and fat storage granules. Changes in apical junction proteins suggest structural changes in the *Pgp1*$^{-/-}$ intestine. The data helps to support that *C. elegans* are potential models for human diseases, and may provide a model for determining the molecular link between MDR/P-gp and IBD.

Abstract 073

Human 3D Organotypic Co-culture Model of Pleura

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Introduction: Pleural mesothelial cells (PMCs) lead the pleural immune response and serve as progenitor cells after pleural or lung damage. They therefore play a key role in pleural diseases such as bacterial infections, malignant pleural effusion, pleural carcinosis or pleural mesothelioma. All these diseases prove a significant healthcare burden; however, its scientific consideration is quite low. The aim of the present study was to develop a three-dimensional (3D) in vitro organotypic model for investigation of pathological conditions of the pleural mesothelium. 3D organotypic models are a promising approach to gain an in vivo like understanding of molecular disease development. While these models are well established for a variety of organs, such as lung, colon, intestine, liver, pancreas, esophagus, prostate, or omentum majus, a 3D organoid model of the pleura is still missing. **Methods:** To construct a 3D organotypic model, primary human PMCs and fibroblasts were isolated from human pleura biopsies. Purification of primary human PMCs and fibroblasts was verified by immunofluorescence staining. The 3D collagen gel culture was assembled by plating of human pleural fibroblasts inside the gel, followed by seeding of PMCs on the gel to construct the normal pleura. **Results:** Isolated human PMCs showed cobblestone appearance and expressed mesenchymal characteristics: αSMA, vimentin but not prolylhydroxylase 1 (PHD1). The extracted fibroblasts maintained their spindle cell appearance and were positive for PHD1 (fibroblast marker). Grown on top of matrix-embedded fibroblasts, the primary human PMCs establish a monolayer and have direct contact with the underlying fibroblasts. Forty-eight hours after attachment, PMCs had cobblestone appearance and intercellular junctions were present between the mesothelial cells as shown by immunostaining for ZO-1. The structural and functional phenotype of the PMCs in our 3D organotypic culture was preserved over six days of culture, as evidenced by the expression of mesenchymal (vimentin, α-SMA, ZO-1) and proliferation marker (Ki67). **Conclusions:** The presented 3D organotypic model of pleura functions as a robust assay for pleural research serving as a precise reproduction of the in vivo morphology and microenvironment and presents a novel tool for development of preventive and therapeutic enhancement of various pleural diseases.

Abstract 074

A Comparative Analysis of Pulmonary Arterial Hypertension in Human Subjects and in Experimental Rodent Models

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Introduction: Pulmonary arterial hypertension (PAH) is a life-threatening disease, characterized by pulmonary vascular remodeling and right ventricle (RV) dysfunction. Chronic hypoxia (CH) and monocrotaline (MCT) induced PAH are the two popular rodent models that have been used for decades to replicate the pathology of PAH in humans. However, the two models provoke a milder course of PAH. Multiple-pathological insult (MPI) animal models may be increasingly preferred, especially the CH + Sugen 5416 (SuHx), because it causes more significant pulmonary artery remodeling and may reflect the later onset in life of most disease presentations. Although many other forms of experimental PAH exist, there is currently no perfect animal model that recapitulates all the features of human PAH. **Methods:** Our group previously developed a SU5416-treated athymic nude rat PAH model (athymic), which confers increased vascular remodeling risk and has significant hemodynamic consequences based on an absence of regulatory T cell immune regulation. To systematically evaluate the efficacy of the four experimental PAH models, namely CH, MCT, SuHx, and athymic, we carried out an unbiased whole-transcriptome network analysis to measure and assess associations among animal models and humans. Lung and RV tissues were harvested from a total of eight athymic-PAH and euthymic rats, and subsequent to RNA extraction and sequencing. RV hypertrophy was measured by the ratio of right ventricular to left ventricular and septum weight (RV/LV+S). Publicly available transcriptome and clinical data for human and animal models of PAH were collected and analyzed. Unsupervised machine learning and cross-species network comparisons were carried out to investigate and determine their associations. **Results:** The RV/LV+S ratio was significantly increased in athymic PAH rats (0.32±0.05) compared with euthymic controls (0.23±0.01). Differentially expressed genes and signaling pathways between athymic and euthymic animals were identified, including previously reported and novel pathogenic and therapeutic candidates of PAH. Whole transcriptome-wide expression data for each animal and human PAH sample was employed for machine learning and network analysis to infer their associations. At the level of the transcriptome, the athymic PAH exhibited the animal model of disease most proximate to human PAH. CH is closer to human PAH than the

SuHx and MCT models, and high similarities were observed between the SuHx and MCT lungs. **Conclusions:** We conducted, for the first time, a meta-cohort and cross-species comparative molecular analysis of PAH in humans and experimental rodent models. High-throughput lung transcriptomics suggests a possible advantage of athymic rat model, and could provide some novel insights into new therapeutic avenues in PAH, including potential cell-based therapies with regulatory T cells.

Abstract 075

MET and Fibroblast Growth Factor 19 Co-expression in Hepatocellular Carcinoma: Molecular Biology and Clinical Relevance

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Introduction: Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths worldwide, with limited therapeutic options. Currently, there are no precision medicine-based therapies to guide patient treatment selection. We hypothesize that specific molecular drivers potentiate response to different therapeutic agents. Developing clinically relevant animal models which recapitulate human HCC biology may provide a platform to screen potential therapeutic targets. Met is frequently overexpressed in HCC, yet Met inhibitors do not have optimal response rates, necessitating the need to explore how other driver oncogenes cooperate in the pathogenesis of Met-activated HCC. Here, we investigate cooperation between Met and fibroblast growth factor 19 (FGF19) signaling in HCC. **Methods**: The Cancer Genome Atlas (TCGA) was queried for patients with overexpression of Met and FGF19 in HCC. We used the hydrodynamic tail vein injection delivery with sleeping beauty transposon/transposase (HDTVi-SBTT) system to induce HCC in mice through co-expression of Met and FGF19. Lastly, we utilized bulk RNA-sequencing to compare our mouse Met/FGF19 model to normal mouse liver and compare gene expression signatures between our Met/FGF19 mouse model to human HCC. **Results**: We identified 43/377 (11%) HCC patients in TCGA with high co-expression of Met/FGF19. We performed bulk RNA-sequencing and Ingenuity Pathway Analysis (IPA) on differentially expressed genes (DEGs) comparing TCGA HCC patients with co-expression of Met/FGF19 to adjacent normal tissue and observed alterations in several unique (B cell development & receptor signaling, Tumor Microenvironment, and hepatic stellate cell activation) pathways. Co-expression of both Met and FGF19 via HDTVi-SBTT yielded well differentiated HCC development and mortality by 13-15 weeks. These tumor nodules were simultaneously positive for V5-tag (present on the Met plasmid) and Flag-tag (present on the FGF19 plasmid) via immunohistochemistry (IHC). IHC and Western Blot also revealed these tumors to be positive for the FGF19 receptor, FGFR4, along with mammalian target of rapamycin (mTOR) pathway targets, including p-mTOR-S2448, p-S6-235/236, and p-4EBP1-T37/46, and PI3K/AKT pathway targets, including p-AKT-S473 and p-AKT-T308. Bulk RNA-sequencing and pathway analysis on DEGs comparing Met/FGF19 mouse tumors to normal mouse liver revealed activation of pathways involved in T and B cell activation, proliferation, and lineage commitment. This was confirmed with IHC demonstrating increased tumoral infiltration of $CD4^+$, $CD8^+$, and $CD20^+$ immune cells. Lastly, we observed a high concordance (74.9% transcriptome similarity and 48 common IPA pathways) of this preclinical Met/FGF19 model to human HCC. **Conclusions**: We developed a murine model which recapitulates the biology of Met and FGF19 co-activation in patients. This preclinical model provides a platform to test various targeted agents and immunotherapies.

Matricellular Proteins

Abstract 076

***In Situ* Engineering of Stromal Cell-Derived Extracellular Matrix to Restore Functionality of the Aging Bone Marrow Niche**

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Introduction: Aging-related skeletal degeneration is driven by changes in the regulation of bone marrow (BM) progenitors by cues in the microenvironment (niche). Previously, we compared ECMs produced from "elderly" (≥60 y/o) and "young" (≤25 y/o) human bone marrow stromal cells (hBMSCs). We reported that the composition of Cyr61/CCN1, a matricellular protein containing binding motifs for osteogenic growth factors, was significantly reduced in ECM produced by the elderly hBMSCs. We confirmed this observation in young (9-11 m/o) and aged (21-33 m/o) mice, showing that that bone mineral density and Cyr61-content both decline with age. In the present study, we develop *in vitro* ECM models for Cyr61-definicency, investigate its mechanistic role in directing osteogenic differentiation and describe novel bioengineering methods to evaluate the therapeutic potential of restoring Cyr61-content in the BM niche. **Methods:** Cyr61-deficient ECMs were produced *in vitro* from primary elderly hBMSCs, as well as an immortalized hBMSC line (HS-5). To isolate the effect of Cyr61 ablation from broader, aging-related changes, ECM was produced from gene-edited HS-5 cells, treated with a CRISPR-SpyCas9 system to delete *CYR61*. The therapeutic restoration of Cyr61 in deficient ECMs was modeled by employing fibrillar carbon nanocarriers (FCNs) to deliver the protein. Nanocarriers loaded with recombinant human (rh) Cyr61 demonstrated effectiveness for dose-dependent enrichment of rhCyr61 to matrices. The osteogenic functionality of Cyr61-deficient and -enriched ECMs was compared by assessing their ability to direct the responsiveness of cultured BM progenitors to osteogenic growth factors (BMP-2 & IGF-1). **Results:** Knock-down of Cyr61 in young ECM abrogated responsiveness of cultured progenitors to both BMP-2 and IGF-1, while elderly ECM, enriched with exogenous rhCyr61, significantly restored responsiveness to both growth factors. Importantly, we showed that ablation of Cyr61, in matrix produced by HS-5 cells, resulted in a similar abrogation of BMP-2 & IGF-1 responsiveness, indicating that Cyr61 deficiency independently reduces osteogenic signaling in the elderly BM matrix. **Conclusions:** These results distinguish Cyr61 as a critical ECM component for maintaining regulatory function of the BM niche and directing responsiveness of BM progenitors to osteogenic growth factors. Additionally, we demonstrated the therapeutic potential of enriching Cyr61-content within ECM and promote osteogenic signaling in the aging bone marrow microenvironment. **Acknowledgement:** VA Career Development Award (1IK2BX005694-01) to MM and VA Merit Review (1I01BX002145-01) to XDC. MM received pilot funding from the San Antonio Nathan Shock Center of Excellence in the Basic Biology of Aging.

Abstract 077

Elucidating the Role of Anti-aging Matrix Fibulin 7 in Skin Inflammatory Disease Psoriasis

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Introduction: Skin inflammation is a complex process regulated by numerous players. Psoriasis is a skin inflammatory condition mainly attributed to hyperactivation of the immune system, however, emerging evidence has suggested the involvement of keratinocytes as well. Fibulin 7 is a matricellular protein belonging to the short fibulin family without any elastogenic function. We recently reported that fibulin 7 is expressed by epidermal stem cells and localizes in the basement membrane and positively regulates the long-term self-renewal ability of the fast-cycling epidermal stem cell population which correlates with promotion of wound healing. Fibulin 7 loss of function in 1-year-old mice is associated with an increased inflammatory gene expression profile. **Methods:** 5% imiquimod cream was applied topically for 5 days to induce psoriasis-like lesions in the mouse dorsal and tail skin prior to euthanasia and skin sampling. Wholemount tail and back skin section were subsequently performed for markers analysis. **Results:** From a public database, we observed that *FBLN7* mRNA expression was decreased in human psoriatic skin compared to unaffected areas or healthy controls. Here, we aim to explore role of fibulin 7 in psoriasis. Immunostaining revealed fibulin 7 localizes at the rete ridges in the adult human skin, which coincides with the fast-cycling stem cell area. After application of imiquimod in the dorsal skin of *Fbln7* wild type (WT) or knock-out (KO) mice, we observed that the loss of *Fbln7* in the 1-year-old mice led to thicker epidermis,

higher vascularization and a tendency for higher phosphorylation of JNK, an inflammatory marker, in epidermal basal cells. Intriguingly, application of imiquimod to the tail skin of 1-year-old Fbln7 KO mice showed a trend of interscale expansion marked by keratin 10 (K10), similar to the chronologically aged 2-year-old skin. **Conclusions:** Fibulin 7 may suppress the imiquimod-induced inflammation through controlling proliferation of keratinocytes. The interscale expansion upon loss of *Fbln7* may indicate that imiquimod preferentially induces the proliferation of the slow-cycling epidermal stem cells which regenerate the interscale area. Future studies will further characterize the effects of fibulin 7 and its mechanism of action in psoriasis, with emphasis on the epidermis and epidermal stem cell heterogeneity.

Abstract 078

Next-Generation Formulation of Curcumin, Ameliorates Growth Plate Chondrocyte Stress and Increases Limb Growth in a Mouse Model of Pseudoachondroplasia

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Introduction: Mutations in cartilage oligomeric matrix protein (COMP) causes protein misfolding and accumulation in chondrocytes that compromises skeletal growth and joint health in pseudoachondroplasia (PSACH), a severe dwarfing condition (*Matrix Biol* 2023;**119**:101-111). **Methods and Results:** Using the MT-COMP mice, a murine model of PSACH, we showed that pathological autophagy blockage was key to the intracellular accumulation of mutant-COMP. Elevated mTORC1 signaling, driven by tumor necrosis alpha inflammation and endoplasmic reticulum stress, blocks autophagy, preventing ER clearance and ensuring chondrocyte death (*Am J Pathol* 2019;**189**:132-146). Resveratrol was found to reduce growth plate pathology by relieving the autophagy blockage and allowing the ER clearance of mutant-COMP, which partially rescues limb length. To expand potential PSACH treatment options, CurQ+, a uniquely absorbable formulation of curcumin, was tested in MT-COMP mice at doses of 82.3 (1X) and 164.6 mg/kg (2X) (*Int J Mol Sci* 2023;**24**:3845). Chondrocyte stress was evaluated by the presence of markers of the mutant-COMP pathology and microCT was used to measure femur lengths. CurQ+ treatment of MT-COMP mice from 1 to 4 weeks postnatally decreased mutant COMP intracellular retention, and inflammation, restoring both autophagy and chondrocyte proliferation (*Int J Mol Sci* 2023;**24**:3845). This dramatically reduced chondrocyte death, normalized femur length at 2X and recovered 60% of lost limb growth at 1X dosage (*Int J Mol Sci* 2023;**24**:3845). **Conclusion:** These results indicate that CurQ+ is a potential therapy for PSACH-associated lost limb growth, joint degeneration, and other conditions involving persistent inflammation, oxidative stress, and a block of autophagy.

Abstract 079

Thrombospondin-1 Regulates Skeletal Muscle Mass in a TGF-β-Smad2/3-ATF4-Dependent Manner

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Introduction. Skeletal muscle atrophy contributes to the morbidity and mortality of a wide array of catabolic conditions, including myopathies, starvation, cancer, heart failure and age-related sarcopenia. Thrombospondins (Thbs) are a family of five stress- and injury-induced matricellular proteins. Recently, we identified that Thbs1 regulates cardiomyocyte size in the stressed heart. Nevertheless, its potentially conserved role in skeletal muscle remains unknown. **Methods and Results.** To investigate the potential causal role of Thbs1 during sarcopenia, we generated skeletal muscle specific Thbs1 overexpressing mice (Thbs1-Tg). These mice displayed profound muscle atrophy with age-dependent decrease in exercise capacity and premature lethality. Mechanistically, we show that Thbs1 expression directly mediates transforming growth factor (TGF)-β activity, downstream Smad2/3 signaling, and the induction of pro-atrophic activating transcription factor 4 (ATF4) signaling. ATF4 then enhances autophagy and the ubiquitin-proteasome system (UPS) to facilitate protein degradation in the muscle. Indeed, *in vitro* analysis confirmed that TGF-β and Smad3 were sufficient to induce ATF4. Furthermore, myofiber-specific inhibition of TGF-β-receptor signaling or deletion of *Smad2 and 3* not only repressed the induction of ATF4,

autophagy, and the UPS, but significantly restored body weight and muscle mass in the Thbs1-Tg animals. Similarly, myofiber-specific deletion of the *Atf4* gene was sufficient to antagonize the autophagy, UPS activity, and the atrophic phenotype observed in Thbs1-Tg animals. Finally, Thbs1-null mice exhibited significant resistance to denervation- and caloric restriction-induced muscle atrophy along with blunted TGFβ-Smad3-ATF4 signaling. **Conclusions.** Overall, our study uncovers a novel mechanism whereby Thbs1-mediated TGFβ-Smad3-ATF4 signaling in the myofiber is a required pathway underlying muscle atrophy, and thus unveils an attractive novel target to blunt muscle atrophy due to a variety of underlying causes.

Liver Fibrosis: Mechanisms and Signaling Pathways

Abstract 080

Dose-dependent Changes in Hepatic Fibrin(ogen) Deposition Reveals a Pathological Role of Fibrin Polymerization in Acetaminophen-induced Acute Liver Failure

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Introduction: Coagulation activation and hepatic deposition of fibrin(ogen) are hallmarks of acetaminophen (APAP) overdose in mice, a standard challenge (i.e, 300 mg/kg) that produces acute hepatotoxicity followed by liver repair. Comparatively large APAP doses (e.g., 600 mg/kg) cause acute liver failure-like features in mice, including a consumptive coagulopathy. We sought to define the impact of APAP dose on hepatic fibrin(ogen) deposition in mice. **Methods:** Wild-type mice were challenged with APAP (300 mg/kg or 600 mg/kg) or saline, and livers and plasma were collected 6 hours after APAP challenge. **Results:** Plasma alanine aminotransferase (ALT) activity increased equivalently at both doses, but sinusoidal congestion was evident primarily in mice challenged with 600 mg/kg. Biomarkers of coagulation activation were significantly increased in mice challenged with 600 mg/kg compared to mice given 300 mg/kg APAP. Immunohistochemistry revealed a distinct pattern of hepatic fibrin(ogen) deposition in mice challenged with 600 mg/kg APAP typified by sinusoidal fibrin(ogen) accumulation within and bordering necrotic lesions. Insoluble fibrin(ogen) levels and the relative extent of fibrin(ogen) cross-linking were increased in mice challenged with 600 mg/kg APAP compared to 300 mg/kg APAP. Hepatotoxicity induced by 300 mg/kg APAP was similar in wild-type mice and mice expressing fibrinogen that does not clot in response to thrombin (Fib^{AEK} mice). In striking contrast, liver injury induced by 600 mg/kg APAP challenge was significantly reduced in Fib^{AEK} mice. **Conclusion:** The results suggest that fibrin polymer emerges as a pathologic driver of liver damage in experimental APAP-induced acute liver failure owing to unique exacerbation of coagulation and fibrin formation.

Mucosal Pathobiology

Abstract 081

Intestinal Epithelial Recruitment of Neutrophils to Sites of Colonic Mucosal Injury

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Introduction: Inflammatory bowel disease (IBD) is a chronic inflammatory condition that waxes and wanes, with active symptomatic disease characterized by infiltration of the intestinal mucosa by large numbers of neutrophils in association with poorly healing mucosal wounds. While normal mucosal wound healing is dependent on coordinated recruitment of neutrophils, precise mechanisms regulating recruitment of neutrophils at different stages of wound healing and contributions of intestinal epithelial cells (IEC) to this process are unclear. Leukotriene B4 (LTB_4) is a potent chemoattractant that stimulates recruitment of neutrophils to sites of injury by binding to its receptor BLT1. LTB_4 is derived from arachidonic acid (AA) by

the successive action of the enzyme 5-lipoxygenase (5-LOX) and LTA4 hydrolase (LTA4H). Additionally, the enzyme 5-lipoxygenase-activating protein (FLAP) is required for 5-LOX activity. Here, we investigated the role of IEC in recruitment of neutrophils to promote mucosal wound repair. **Methods and Results:** We hypothesized that LTB_4 produced by IEC and PMN is a critical autocrine and paracrine mediator of leukocyte recruitment and mucosal epithelial repair. To test the hypothesis, we aimed to (i) Determine the relative contributions of leukocyte derived versus epithelial derived LTB_4 on PMN recruitment and repair of mucosal wounds, and (ii) Determine the machinery by which epithelial cells synthesize LTB_4 during mucosal injury and repair. Recent single cell transcriptomics data from our lab revealed that, in addition to PMN, IECs express the enzymatic machinery to produce LTB_4. Here, we show that LTA4H expression is upregulated in colonic wound associated epithelial cells following acute mucosal injury. In addition, by ELISA we show that mucosal wounds isolated from mice lacking PMN produce LTB_4 at very early stages of wound healing. Because PMNs are the first immune cells responding to sites of injury, our results suggest that IECs are a major contributor of LTB_4 at early stages of wound healing. Finally, we show that wounding induced hypoxia via Hypoxia-inducible factor-1α (HIF-1α) stabilization leads to increased LTB_4 production in human colonoids. **Conclusion:** Overall, these findings reveal a novel pro-repair function of LTB_4-BLT1 signaling in epithelial cells and help pave the way towards developing targeted treatments for IBD patients.

Abstract 082

Thrombospondin-1 Enhances Epithelial Migration During Colonic Wound Repair

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Introduction: Thrombospondin-1 (TSP1) is a matricellular protein that regulates cell migration through binding to receptors that affect integrin function such as CD47 and CD36 as well as binding to integrins directly. Our previous work revealed an important role for the TSP1 receptor CD47 in driving intestinal epithelial wound repair following colonic mucosal injury. Despite this, the specific contribution of TSP1 to intestinal mucosal repair processes remains poorly understood. **Methods:** To determine how thrombospondin-1 regulates the intestinal epithelial response to injury, we utilized total TSP1 knockout (KO) mice and mice with IEC-specific TSP1 depletion (Villin-Cre; *Thbs1*$^{f/f}$). Colonic wound repair was assessed in TSP1-deficient mice and control mice using an endoscopic biopsy injury model. Effects of TSP1 depletion on recovery from DSS-induced colitis was also assessed. Finally, primary intestinal epithelial cells (IECs) were isolated from TSP1 expressing and KO mice before assessment of epithelial wound closure *in vitro*. **Results:** Here we demonstrate that TSP1 expression is upregulated following mucosal damage in the intestine with release of TSP1 observed locally at sites of wounding. Importantly, we show that mice with IEC-specific loss of TSP1 (Villin-Cre; *Thbs1*$^{f/f}$) exhibit defective intestinal mucosal wound healing *in vivo* after biopsy-induced colonic wounding or DSS induced colitis. *In vitro* analysis of primary murine colonoids revealed that deficiency in TSP1 results in defective collective epithelial migration responses that were rescued by addition of exogenous TSP1. Furthermore, exogenous TSP1 was found to enhance epithelial β1 integrin upregulation and focal adhesion dynamic turnover while TSP1 deficiency resulted in higher levels of actin stress fiber formation. **Conclusions:** Here we report, for the first time, that epithelial-produced TSP1 plays an important role in regulating mucosal wound repair in the colon. Mechanistically we show that autocrine signaling through TSP1 regulates intestinal epithelial cell migration through actin cytoskeletal rearrangement. Increased understanding of how TSP1 regulates epithelial repair has the potential to inform rational therapies that promote resolution of chronic injury/inflammation in mucosal tissues such as the gut.

Abstract 083

Spatially Separated Intraepithelial and Lamina Propria Neutrophils Present Distinct Functional Identities in Inflamed Colonic Mucosa

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Introduction: Polymorphonuclear neutrophils (PMNs) play a crucial role in protecting the host against invading pathogens. However, dysregulated PMN trafficking and activity in tissue can also lead to exacerbated inflammation and damage, as in the case of Inflammatory Bowel Disease (IBD). IBD encompassing Crohn's Disease and Ulcerative Colitis (UC), is a chronic inflammatory disorder featuring *en masse* PMN infiltration of intestinal mucosa. Notably, spatial localization of PMNs is an important clinical feature of UC, where the presence of intraepithelial neutrophils (IENs) in addition to lamina propria neutrophils (LPNs) underlies a more severe condition than the presence of LPNs only. Furthermore, elevated presence of IENs has been negatively correlated with response to biologics. Despite this clinical relevance, the difference between IENs and LPNs has been understudied. **Methods:** To profile PMN localization in inflamed colon tissue, we performed Elastase and S100A9 IHC staining on human UC biopsied tissue and murine dextran sulfate sodium (DSS)-treated colon Swiss roll preparations, respectively. DSS-induced epithelial injury model was used as it resembles inflammatory injury in UC patients. To compare functional programs between IENs and LPNs, we optimized an EDTA- and Collagenase-mediated digestion method that allowed separation of the epithelial compartment from the LP compartment for downstream flow cytometry-based assays. We measured functional outputs, such as reactive oxygen species (ROS) production, cytokine production, surface integrin expression and survival (AnnexinV/SytoxRed) on IENs and LPNs from isolated epithelial- and LP-compartments. **Results:** Elastase and S100A9 IHC staining confirmed increased LPN and importantly IEN presence in UC and in murine DSS-colitis colon tissue, respectively. In murine inflamed colon tissue, IENs were found to express higher levels of active CD11b b2-integrin and to have elevated ROS levels compared to LPNs, indicating hyperactivation. IENs were also found to express higher levels of inflammatory cytokines TNFα and IL1β, and appeared more apoptotic/necrotic, suggesting having a detrimental impact on surrounding epithelial cells. **Conclusions:** Our findings indicate that colon IENs and LPNs have distinct functional identities; notably IENs have a hyperactivated and potentially pathogenic phenotype, likely driving epithelial dysfunction. This supports clinical observations where UC patients with elevated IEN numbers present with more severe disease and impaired response to therapy. Further transcriptomic analysis of IENs and LPNs (in progress) could help define distinct phenotypic signatures, allowing for specific targeting of IENs to improve epithelial healing and to sustain clinical remission in IBD patients.

Abstract 084

Neutrophil-derived Myeloperoxidase Disrupts Epithelial Tight Junctions by Chlorination of Occludin Tyrosines

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Introduction: A hallmark of mucosal inflammation is the accumulation of neutrophils (PMN) at sites of injury. Myeloperoxidase (MPO) is a primary antimicrobial enzyme in PMN. MPO generates hypochlorous acid via a reaction between hydrogen peroxide and chloride. Hypochlorous acid diffuses through the tissue and can indiscriminately react with the phenol moiety on tyrosine to generate chlorinated tyrosine. It is unknown what impact tyrosine chlorination might have on cellular function. We hypothesized that targeting MPO attenuates acute colitis and prevents the development of chronic colitis by limiting bystander tissue damage. **Methods and Results:** To define the role of MPO in murine colitis, we employed wild-type and MPO-deficient mice in combination with acute and chronic DSS-induced colitis. Initial studies revealed that MPO-KO mice experience less inflammation and more rapidly recover from acute colitis compared to wild-type controls. Next, we evaluated the impact of MPO on the development of chronic colitis. MPO KO mice exhibited significantly less inflammation when compared to WT mice. MPO mice had longer colons, lower cytokine levels, and decreased tissue damage as determined by histological scores. To better understand the mechanism(s) involved in MPO associated tissue damage and inflammation we extended these studies to include *in vitro* models. Analysis of the extracellular loops of occludin revealed a disproportionately high number of tyrosine residues (24% and 15% of amino acids in extracellular loops 1 and 2, respectively). Given the observation that MPO activation has the potential to chlorinate tyrosine, we examined tyrosine chlorination by HPLC analysis of occludin immunoprecipitants. Following PMN transepithelial migration or exposure to activated recombinant MPO, we observed prominent 3-chlorotyrosine in occludin. We further examined the functional role of tyrosine chlorination within the binding domain of occludin. Utilizing tyrosine

chlorinated and non-chlorinated occludin blocking peptides in combination with functional and morphological analysis, in intestinal epithelial cells (IEC), we demonstrated that chlorination of occludin tyrosines results in abnormal tight junction morphology and barrier dysfunction. **Conclusions:** These results support a pathological role for the enzymatic action of MPO in bystander tissue damage during acute and chronic colonic inflammation. Activated MPO not only damages tissue and inhibits wound healing, but also disrupts IEC barrier function. This work suggests that barrier dysfunction associated with MPO activation results in tyrosine chlorination in the functional domain of occludin. Taken together these studies highlight the need to further study the impact of MPO on the inflammatory microenvironment during active mucosal inflammation.

Abstract 085

Rotavirus Infection Elicits Host Responses Via P2Y1 Purinergic Signaling

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Introduction: Rotavirus causes life-threatening diarrhea in children, resulting in ~200,000 deaths each year. Rotavirus infects a limited number of cells at the tips of the villi in the small intestine; however, rotavirus is known to dysregulate cells far away from the site of infection through paracrine signaling. We recently identified that rotavirus infected cells release the purinergic signaling molecule ADP, which binds to the P2Y1 receptor on nearby uninfected cells; causing a signaling cascade that affects distant uninfected cells. *In vitro* P2Y1 was found to contribute to chloride and serotonin secretion; suggesting P2Y1 may activate pathways responsible for diarrhea. We hypothesize that P2Y1 orchestrates cell-cell communication involved in diarrhea and infection severity. **Methods:** To elucidate the role of purinergic signaling via P2Y1 receptors during rotavirus infection, we used the mouse-like rotavirus (rD6/2) to investigate the effects of purinergic signaling in the context of homologous murine rotavirus infection *in vivo*. C57Black6 mouse pups were orally gavaged rD6/2 rotavirus at day 4-6 of age and assessed over the course of 5 days. Beginning at day 1 post infection, infected pups were treated daily by oral gavage with saline or 4mg/kg MRS2500, a P2Y1-selective, competitive antagonist. Stool was collected and scored for diarrhea daily prior to each treatment. Pups were euthanized and small intestine tissue was collected at 3- and 5-days post infection for immunostaining, qRT-PCR and luminal contents. **Results:** Rotavirus-infected pups exhibited significant diarrhea incidence and high diarrhea scores on days 2, 3, and 4 post infection. Treatment of rotavirus-infected mouse pups with MRS2500 resulted in decreased severity and incidence of diarrhea compared to vehicle treated controls. Consistent with limited diarrhea, MRS2500 treated pups also exhibited decreased luminal serotonin and chloride content compared to control infected pups. Viral stool shedding, assessed by qRT-PCR for rotavirus gene 11 levels, revealed that MRS2500 treated pups had significantly lower viral shedding starting at day 4 post infection compared to saline treated pups, which suggests P2Y1 signaling may amplify rotavirus replication. Preliminary experiments in litters with infected and pretreated PBS or MRS2500 uninfected pups show a decreased incidence in diarrhea suggesting that P2Y1 inhibition has a potential protective role in reducing the effects of transmission. **Conclusion**: Collectively these findings establish a conserved role of purinergic signaling in the pathophysiology of rotavirus infection. Additionally, our data indicates P2Y1 as a new candidate for host-targeted therapeutics that could have both antiviral and antidiarrheal effects against rotavirus pathophysiology.

Abstract 086

Coactosin-Like Protein 1 is a Novel Regulator of the Intestinal Epithelial Barrier Integrity and Repair

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Introduction: The actin cytoskeleton plays critical roles in regulating integrity and repair of the intestinal epithelial barriers by mediating assembly of tight junctions (TJs) and adherens junctions (AJs) and controlling epithelial wound healing. Actin filaments that associate with epithelial junctions and drive collective cell migration are highly dynamic and undergo a constant turnover guided by numerous actin-binding proteins. The roles of actin filament turnover in regulating intestinal epithelial barrier integrity and repair remain poorly understood. Coactosin-like protein 1 (COTL1) is an enigmatic molecule with dual activity as an F-actin-binding protein and a regulator of leukotriene biosynthesis. However, its functions in

the intestinal epithelium have not been previously addressed. The goal of this study is to investigate the roles of COTL1 in regulating the structure and remodeling of TJs and AJs in model human intestinal epithelial cells (IEC). **Methods:** The study was performed using well-differentiated SK-CO15, DLD1 and Caco-2 human IEC lines. COTL1 activity was probed using loss-of-function and gain-of-function approaches. The former approach involved siRNA-mediated knock-down of COTL1, whereas the latter approach involved overexpression of the wild-type COTL1 and its actin binding-deficient mutant. **Results:** COTL-1 was found to be enriched at apical junctions in polarized IEC monolayers. The knockdown of COTL1 significantly increased paracellular permeability of IEC monolayers, according to trans-epithelial electrical resistance and trans-monolayer dextran flux measurements, and impaired a steady state integrity of both TJs and AJs. Additionally, COTL1 depletion significantly attenuated junctional reassembly in a calcium-switch model. The observed TJ and AJ abnormalities in COTL1-depleted IEC monolayers were accompanied by the impaired assembly of the perijunctional actomyosin cytoskeleton. In addition, the knockdown of COTL1 inhibited formation of polarized 3-D epithelial cysts by IEC embedded into Matrigel. Finally, COTL1 depletion attenuated collective IEC migration in a wound healing model. **Conclusion:** Our findings highlight COTL1 as a novel regulator of the intestinal epithelial barrier integrity and repair that controls assembly of cytoskeletal structures associated with epithelial apical junctions. **Acknowledgement:** Supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health grant R01DK126702 to A.I.I.

Immunomatrix and Fibrosis

Abstract 087

Antimicrobial Peptides Reduce Bacterial-Induced Myofibroblast Differentiation Abrogating Subglottic Stenosis in Mice

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Introduction: Pediatric subglottic stenosis (SGS) is characterized by the formation of aberrant fibrotic scar tissue from prolonged endotracheal tube (ET) intubation, leading to breathing difficulties and impaired phonation that often requires invasive surgery (*Laryngoscope* 2021;**132**:1356-1363; *Adv Drug Deliv Rev* 2021;**174**:168-189). We have recently discovered that changing the laryngotracheal microbiome in SGS-induced mice with antibiotic cocktails has immunomodulatory effects, resulting in decreased SGS. During airway injury, the epithelial barrier is damaged permitting bacteria to enter the lamina propria, inducing in an inflammatory cascade that promotes myofibroblast differentiation and scar tissue formation. Therefore, modulating the airway bacterial communities represents a novel avenue for a preventative SGS treatment. In this work, we demonstrate that the antimicrobial peptide (AMP) Lasioglossin-III can modulate the upper airway microbiome when locally delivered on a coated ET and mitigate inflammation. We then show that decreased airway bacteria abundance leads to reduced fibrosis with negligible scar matrix deposition, and altogether abrogates SGS. **Methods:** We fabricated mouse-sized ETs coated with a polymeric AMP-eluting layer, quantified AMP release over 10 days, and validated bactericidal activity for both planktonic and biofilm-resident bacteria against *S. aureus* and *P. aeruginosa*. *Ex vivo* testing: We inserted AMP-ETs and ET controls into excised laryngotracheal complexes (LTC) of C57BL/6 mice and assessed biofilm formation after 24 hours. *In vivo* testing: AMP-ETs and ET controls were inserted in sham or SGS-induced LTCs (*Otolaryngol Head Neck Surg* 2011;**144**:927-933), which were then implanted subcutaneously in receptor mice, and assessed for immune response and SGS severity after 7 days by histology and immunohistochemistry. **Results:** We achieved reproducible, linear AMP release from mouse-sized ETs at 1.16µg/day resulting in strong bacterial inhibition *in vitro* and *ex vivo*. *In vivo*, SGS-induced LTCs exhibited a thickened scar tissue typical of stenosis, while the use of AMP-ET abrogated stenosis. Notably, SGS airways exhibited high infiltration of T cells and macrophages, which was reversed with AMP-ET treatment. Additionally, AMP-ET treated airways exhibited decreased myofibroblast differentiation indicated by fewer α-SMA positive fibroblasts and decreased collagen and fibronectin deposition. **Conclusions:** We developed an AMP-ET platform that modulates the laryngotracheal microbiome and upon treatment during

SGS injury, reduces macrophage infltration and activation, antigen specific T cell responses, and myofibroblast differentiation resolving stenosis progression.

Abstract 088

A Biomarker of Collagen Type-I Degradation is Associated with Cardiac Fibrosis and Inflammation in Patients with Liver Cirrhosis

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Introduction: Cirrhosis is the most advanced stage of liver fibrosis and has been associated with reduced cardiac function, known as cirrhotic cardiomyopathy (CCM) (*Am J Physiol Gastro Liver Physiol* 2019;**317**:G253-G263). Fibrosis is characterised by the accumulation of collagens resulting in tissue remodelling and abnormal organ function (*Liver Int* 2020;**40**:736-750). As the primary fibrillar collagen, we sought to determine collagen type-I (COL1) turnover and its' association to inflammatory markers and cardiac function in late-stage fibrosis patients. **Methods**: The C1M enzyme-linked immunosorbent assay (ELISA) was used to quantify COL1 degradation in serum from liver cirrhosis patients. Sixty-three cirrhosis patients were included in a study of CCM (Hvidovre Hospital, Denmark) (*Am J Physiol Gastro Liver Physiol* 2019;**317**:G253-G263). The COL1 biomarker was measured at baseline in 57 out of 63 patients from the study and compared with 46 sex-matched healthy controls (sex: 65% male). Six patients were excluded due to low sample volume. Student's t-tests were used to determine statistical significance unless otherwise stated and analysis is reported as mean [95% CI] on log-transformed data. **Results**: C1M levels were significantly higher in patients with cirrhosis compared to healthy controls ($p<0.0001$, cirrhosis 47.2 [41.9-52.4], healthy 29.7 [27.0-32.4]). Linear regression showed a moderate association between the C-reactive protein (CRP) inflammatory marker and C1M levels ($r = 0.54$, slope = 1.44, $p<0.001$, linear regression). C1M levels were increased in cirrhosis patients with high myocardial extracellular volume (ECV) (50.9 [42.6-60.7]), a marker for myocardial tissue remodelling, compared to those with low myocardial ECV (40.9 [35.8-46.6]) ($p=0.041$, $n = 47$). No association was found between C1M, and any of the other magnetic resonance and echocardiographic cardiac measurements. **Conclusion**: Elevated C1M in cirrhosis patients as compared to healthy controls suggests a dysregulated turnover of COL1 in the diseased population. As both CRP and C1M are released as part of inflammatory processes (*BMC Rheumatol* 2019;**3**:3), it is not surprising that we see a moderate correlation between these two markers. C1M was associated to an elevated myocardial ECV, which is an accepted surrogate marker of cardiac fibrosis. C1M may be a useful marker to identify cirrhotic patients with a higher inflammatory and fibrotic burden. **Acknowledgements**: We would like to acknowledge the work completed by the team at Hvidovre Hospital. The CIRCACOURSE study was supported by the Novo Nordisk Foundation. The biomarker research was funded by the European Union's Horizon Europe Marie Skłodowska-Curie Actions Doctoral Networks—Industrial Doctorates Programme (HORIZON—MSCA—2021—DN-ID), grant number 101072828.

Abstract 089

Lung Mesenchymal Cell Apoptosis and Plasticity Revealed by a BCL2 Overexpression Mouse Model

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Introduction: Resident mesenchymal cells (MCs) are a heterogeneous group that acts as important homeostasis regulators in lung development and repair. These cells remodel the extracellular matrix and regulate the differentiation and architecture of the air-blood barrier. However, lung MCs differ in molecular profile, location, and shape, and understanding their cell fate determination, function, and regulation remains elusive. In this study, we aim to characterize the pathophysiological relevance of one type of MCs

in the distal lung called alveolar myofibroblasts (AMFs). AMFs are contractile cells involved in the septation of the alveoli during alveologenesis and are associated with pulmonary developmental disorders. However, unlike other MCs, AMFs are transient residents in the lung and undergo apoptosis upon the completion of lung maturation. The differentiation and proliferation of AMFs are known to be essential for alveologenesis. Nevertheless, the relevance of apoptosis has yet to be established. **Methods:** We generated an inducible mouse model that expresses the pro-survival gene BCL2 using myofibroblasts-specific drivers *PdgfraCreER* and Myh11-CreER. We asses lung physiology and mechanics by force oscillation technique and atomic force microscopy respectively. The morphological and molecular analysis were performed using single cell sequencing, together with immunostaining and confocal microscopy in wholemount tissue and cryosections. **Results:** Based on morphological and molecular profiling, our results showed that BCL2 is sufficient to reduce AMF apoptosis. Consequently, persistent AMFs change the lung parenchyma's morphology, the organization of elastic fibers, and the distribution and fate of MCs in the distal lung. Additionally, we found that persistent AMFs transdifferentiate into a proximal contractile subpopulation called ductal myofibroblast, believed to suppress lymphocytic inflammation and regulate the stress response to factors such as oxidative stress and inorganic substances. Finally, our preliminary data suggest that AMF persistence alters the mechanical properties of the alveolar parenchyma and reduces pulmonary function in mice, resembling a fibrotic-like phenotype. **Conclusion:** The need for more information on instructive signals and complex cell interactions to build and repair the lung remains a challenge in regenerative therapies. However, our model contributes to understanding the role of AMF apoptosis as a regulator of alveologenesis and reveals novel insights into MCs' dynamics and plasticity in the lung. These findings on mesenchymal cell heterogeneity and its differential activity in maintaining lung homeostasis have potential implications for improving and developing therapeutic strategies for pulmonary pathologies. **Acknowledgements**: We thank the University of Texas MD Anderson Cancer Center DNA Analysis Facility and Flow Cytometry and Cellular Imaging Core Facility.

Abstract 090

Fibro-inflammatory Changes in the Aging Ovarian Matrisome Inform Potential Ovarian Cancer Pathways

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Introduction: High-grade serous ovarian cancer (HGSOC) is the most lethal cancer of the female reproductive system and is frequently diagnosed post-menopause, suggesting that the aging ovary may provide a unique niche for tumor progression. Our lab and others have previously shown that the ovary becomes fibrotic with advanced reproductive age, accumulating collagens I and III, which in turn increases tissue stiffness. Ovarian cancer precursor cells preferentially adhere to collagen matrices and cancer cell proliferation is increased on stiffer substrates. Thus, we hypothesize that the fibrotic and stiff properties of the aging ovary enhance HGSOC adhesion and expansion. **Methods:** To test this hypothesis, we examined adhesion of fluorescently labelled ovarian cancer cells to ex vivo cultured ovaries and monolayers of isolated ovarian stromal cells from reproductively young (6-12 weeks) and old (10-12 months) mice. Moreover, we performed label-free, quantitative proteomics to obtain an unbiased profile of age-associated changes to the mouse ovary and identify candidates which may impact HGSOC pathogenesis. **Results:** Interestingly, cancer cell adhesion to ovaries, but not stromal cells, from reproductively old mice was increased two-fold compared to young counterparts, suggesting that age-dependent alterations to the ECM may drive increased adhesion. To specifically determine how aging alters the ovarian matrisome, we treated contralateral ovaries from each mouse with 0.1% SDS for 12.5 hours to enrich for the ECM prior to proteomic analysis. We identified 383 proteins that were significantly altered with age, of which 74 were matrisome components. Biological processes upregulated with age were involved in immune function and ECM remodeling. Periostin, a protein that has been implicated in cardiopulmonary fibrosis, in addition to being highly expressed in HGSOC, was significantly upregulated with age in mouse ovaries. Proteins that induce periostin expression, including those involved in TGF-β and Wnt signaling, as well as periostin cofactors, including tenascin, were also upregulated with age. Periostin may serve as a driver of age-

associated fibrosis in the ovarian stroma, while also increasing the invasiveness of cancer cells through direct signaling. The role of periostin on age-dependent HGSOC pathogenesis is currently being investigated using a novel murine organoid model of the ovarian stroma. Organoids have the capacity to interact with cancer cells in vitro. Moreover, treatment of organoids with recombinant TGF-β significantly increases expression of collagen I and periostin, suggesting that increased TGF-β signaling with age may contribute to increased periostin expression and fibrosis. **Conclusions:** Taken together, these results demonstrate that age-related changes to the ovarian ECM may create a niche conducive to cancer cells and identify key candidates whose involvement in HGSOC and fibrosis will be interrogated in future studies.
Acknowledgement: This research was supported by the National Institute of Child Health and Human Development (R01HD093726 to F.E.D. and T32HD094699 to S.S.D), the National Cancer Institute (P20CA233304 to F.E.D. and F31CA257300 to S.S.D), NIH Office of the Director (U54 AG075932 to Campisi and B.S.), and startup funds from the Department of Obstetrics and Gynecology (to F.E.D).

Tissue Injury, Regeneration, and Intervention

Abstract 091

Treating Vocal Fold Scarring: Leveraging Decorin as a Regulator of Vocal Fold Extracellular Matrix Structure in Development and Repair

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Introduction: Vocal fold scarring (VFS) is the leading cause of dysphonia (*Stem Cell Res Ther* 2020;**11**:128) and is characterized by dysregulated collagen deposition from vocal fold myofibroblasts (VFMs), resulting in altered tissue mechanics. Current therapies include steroid and growth factor injections but have limited efficacy due to their poor regenerative capacity and rapid drug clearance (*Adv Drug Deliv Rev* 2021;**174**:168-189). Herein, we show that decorin (DCN) is directly associated with vocal fold extracellular matrix (VF ECM) organization and inversely associated with fibrotic response to injury *in vivo* in a rat model of VFS. We establish DCN as a strong anti-fibrotic agent *in vitro* in human cells and engineer DCN-loaded hyaluronic acid microgels (MGs) for local drug delivery of DCN. **Methods:** *DCN in VF development:* VFs were harvested from neonatal, sexually mature, and skeletally mature male rats then examined by histology, immunofluorescence (IF), and electron microscopy for organization of ECM components. Rats were chosen for their VF structure, similar to humans'. *DCN is down regulated in VFS:* Human VFMs were compared to VF fibroblasts (VFFs) via bulk RNA sequencing for differentially expressed genes. Findings were confirmed *in vivo* in scarred rat VFs by histology and IF at 3-, 7-, and 21-days post-injury. *DCN anti-fibrotic properties:* We compared the effect of DCN and the clinical standard bFGF on VFMs by RT-qPCR and IF in 2D and 3D by seeding in methacrylated type I collagen gels (5 mg/ml, 0.1% LAP). For 3D studies, unconfined compression tests were performed to quantify modulus. *Microgels:* Methacrylated HA MGs were fabricated via extrusion fragmentation, lyophilized, and loaded with DCN. Size was determined via microscopy. Drug loading and release in PBS and 5U hyaluronidase were quantified via protein assay. VFMs were treated with the releasate from DCN-loaded MGs to confirm efficacy. **Results:** Skeletally mature VFs showed increased DCN, collagen fiber homogeneity, and ECM organization, correlating DCN with healthy VF structures. DCN gene expression was downregulated in VFMs, and scarred VFs showed decreased DCN. Exogenous treatment of VFMs with DCN decreased the gene expression of alpha-smooth muscle actin (α-SMA) and collagen type I as well as reduced collagen gel contraction. Protein levels were also decreased in both 2D and 3D. Anti-fibrotic effects of DCN were superior to bFGF. DCN was loaded into MGs and a sustained release profile over 21 days was achieved. 2.49μg/day and 4.02μg/mL were released in PBS and 5U hyaluronidase, respectively, both within the therapeutic range identified for DCN. Bioactivity was confirmed after drug delivery. **Conclusion:** DCN correlates with high VF ECM organization and is downregulated in VFS. Exogenous DCN is strongly anti-fibrotic in 2D and 3D, and DCN-loaded MGs were engineered for a local, sustained delivery system to treat VFS.

Abstract 092

Migrational Decision Making in Response to Multiple Directional Extracellular Matrix Cues

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Introduction: Directional cell migration drives development, immune response, tissue repair and regeneration and cancer metastasis. Cells directionally migrate in response to collagen fibril alignment and stiffness gradients within the extracellular matrix (ECM), processes called contact guidance and durotaxis, respectively. How cells integrate multiple directional cues is completely unclear. In this study, we leverage a unique approach by which to assemble aligned collagen fibrils and transfer them to flexible substrates with gradients of stiffness. These substrates allow us to orient contact guidance and durotactic cues in parallel or orthogonally to understand how they cooperate or compete to direct cells towards targets. **Methods:** Aligned collagen fibrils were assembled on the surface of the mica. These fibrils can be transferred to glutaraldehyde-functionalized glass or Sulfo-SANPAH-functionalized polyacrylamide gels by pouring 30% warm gelatin onto the fibers, allowing it to solidify, stamping it to the new substrate and melting and washing the gelatin away. Human foreskin fibroblasts (HFFs), human epidermal keratinocytes (HaCats) and breast carcinoma cells (MDA-MB-231) were fixed and stained for F-actin and migration was assessed using live cell microscopy. **Results:** Assembling collagen on mica results in aligned collagen fibrils that can be successfully transferred to other substrates. Different cells sense aligned collagen fibril networks with different fidelities. HFFs and MDA-MB-231s align well, but HFFs are so contractile that they delaminate the collagen fibrils. Chemically functionalizing glass and transferring the collagen fibrils to these surfaces blocks delamination. HaCats also align with the collagen fibril network. However, there are two populations of HaCats. The first moves perpendicular to the fibrils and the second moves parallel to the fibrils. Finally, we generated gradients of stiffness in polyacrylamide and transferred aligned collagen fibers onto these gradients. HFF alignment fidelity and aspect ratio increase at low stiffness, although cells can align at high stiffness. The direction of cell alignment is controlled by collagen fibril alignment direction and not stiffness gradient. We intend to probe how formins and Arp2/3, F-actin regulatory proteins that control linear and branched F-actin networks, reset the preference of contact guidance over durotaxis. **Conclusions:** These results outline the rich differences in contact guidance behavior among cells and show the potency of contact guidance cues above durotactic gradients in driving cell migration. Understanding how different cells respond to multiple cues is critical in understanding complex physiology involving many cell types and in designing artificial tissues with directional migration cues encoded within the biomaterial, orchestrating migration of different cells to different targets. **Acknowledgement:** Work was supported by R01GM143302.

Abstract 093

Cardiac Fibroblast-MHCII Contributes to Cardiac Pathophysiology in Doxorubicin-Induced Cardiomyopathy

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Introduction: Cancer patients receiving Doxorubicin (Dox), one of the most widely used chemotherapy agents, often develop cardiomyopathy and heart failure (HF) because of the cardiomyocyte toxicity of Dox. Prominent hallmarks include the development of cardiac fibrosis, cardiomyocyte atrophy and cardiomyocyte death, and contractile dysfunction. In other etiologies of HF, cardiac T cell inflammation and fibrosis co-exist, and cardiac fibroblasts (CFBs) expressing the major histocompatibility complex II (MHC-II) in response to T cell released IFNγ contribute to cardiac fibrosis and systolic dysfunction. We hypothesized that CFB-MHC-II expression is induced by Dox in a T cell dependent manner and contributes to cardiac pathophysiology. **Methods:** We treated wild type (WT) and T cell-deficient mice (*Tcra*$^{-/-}$) with PBS or Dox intraperitoneally (i.p.) (5 mg/kg/week) for 8 weeks. We investigated the surface expression of MHC-II on CFBs from enzymatically digested hearts on CD31^{-}/CD45^{-}/MEFSK4^{+} cells using flow cytometry. **Results:** We found enhanced MHC-II expression in CFBs from Dox treated WT mice compared to those from PBS treated WT controls. In contrast, CFBs from *Tcra*$^{-/-}$ mice treated with Dox did not express MHC-II, and echocardiography determined that they developed less systolic dysfunction than Dox treated WT mice. 3-day *in vitro* treatment of primary cultured CFBs with Dox (0.1ug/mL) did not induce expression of MHC-II surface protein or gene expression by flow cytometry and qPCR, respectively, compared with IFNγ treated CFB, used as a positive control. To further investigate the functional role of CFB-MHC-II in Dox

induced cardiomyopathy, we used *Tcf21*$^{iCre/+}$ *MhcII*$^{fl/fl}$ mice and treated them with tamoxifen (TMX) (75mg/kg/daily, or vehicle for 5 days, i.p), prior to starting Dox treatment. TMX treated mice selectively lacked MHC-II expression in CFBs (CFB-*MhcII*$^{-/-}$) and were compared with vehicle-treated littermate controls (CFB-*MhcII*$^{+/+}$). After 8 weeks of Dox treatment, echocardiography, whole heart gene expression by qPCR, and histological analyses were performed. Wheat-germ agglutinin (WGA) staining determined larger cardiomyocyte size in CFB-*MhcII*$^{-/-}$ mice compared with CFB-*MhcII*$^{+/+}$ littermate controls receiving Dox. Strikingly, Dox treated CFB-*MhcII*$^{-/-}$ mice showed decreased collagen deposition determined by picrosirius red staining and preserved systolic function compared with Dox treated CFB-*MhcII*$^{+/+}$ controls. **Conclusion:** Together, these data demonstrate that Dox induces MHC-II expression on CFBs in a T cell dependent manner and not through the direct actions of Dox on CFBs. Our results also identify a novel contributing role for CFB-MHC-II to the development of cardiac fibrosis, cardiomyocyte atrophy and systolic dysfunction in Dox cardiomyopathy.

Abstract 094

Examining the Effects of Nanoscale Patterning of TGFβ Receptors and Viscoelastic Material Properties on Regulating TGFβ Signaling

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Introduction: Mechanotransduction is the cell's ability to sense and respond to mechanical signals. Previously, our group described the use of microgel thin films with controllable loss tangents to investigate the influence of viscoelastic properties on pro-fibrotic cellular properties. However, it is currently unknown how viscoelastic properties influence cell surface receptor clustering, which is likely upstream of these observed events. Here, we describe the development of a material platform combining molecularly self-assembled DNA nanostructures (DNA origami) and viscoelastic microgel thin films to probe transforming growth factor beta (TGF-β) receptor clustering and downstream signaling. Chester et al. displayed by altering intraparticle cross-linking density, the microgel particles will display varying degrees of loss tangent. On microgel thin films with decreasing loss tangent, dermal fibroblasts increased cellular velocity, cell area, αSMA stress fibers, and CTGF expression. The DNA origami allows for precise nanopatterning of TGF-β receptors through the presentation of peptide nucleic acids (PNA) that binds to TGF-β receptors. Prior work showed that pre-patterning of the PNA peptide on the DNA origami clusters TGF-β receptors and enhances TGF-β signaling. In this work, we constructed randomly patterned or pre-patterned peptide on DNA origami on low or high loss tangent viscoelastic microgel thin films. This combinatorial platform allows multiscale probing of influence of receptor patterning (nanoscale) and material viscoelasticity (microscale) on TGF-β signaling and downstream cellular outcomes. PNA peptide distribution, following fluorescent analysis, was equivalent across all samples. **Methods:** To probe TGF-β signaling and downstream effects, alpha-smooth muscle actin (αSMA) expression and phosphorylated SMAD3 (pSMAD3) were analyzed. Human dermal fibroblasts (HDFn) will be cultured on low and high loss tangent pNIPAM based films with randomly distributed or pre-patterned TGFβ receptor binding peptide. Films will be coated with human fibronectin, allowing for cell culture and attachment for 24 hrs. Fibroblasts will be fixed and stained for α-SMA and nuclei, mounted, and then imaged using an EVOS FL Auto at 10X magnification. **Results:** Neonatal dermal fibroblasts (HDFn) seeded on high loss tangent films with randomly distributed peptide displayed high levels of αSMA expression and αSMA stress fiber positive cells. Additionally, high loss tangent films with randomly distributed peptide displayed high levels of total pSMAD3 in comparison to low loss tangent films with pre-patterned peptide. **Conclusion:** Utilizing viscoelastic microgel thin films and random or pre-patterned (via DNA origami) presentation of TGF-β receptor binding peptides, our lab has developed a platform to understand the relationship of chemical and mechanical signaling in TGF-β activation and myofibroblastic differentiation.

Matrix on Diet

Abstract 095*

Evaluating the Therapeutic Potential of Antibiotics and Gut Microbiota Modulation for Intrahepatic Cholestasis using a Murine Model

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*The authors of this abstract selected not to publish the details of their research

Abstract 096

Cullin 3-NRF2 Axis Links Reductive Stress to Hepatic Metabolic Inflexibility in Obesity

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Introduction: Members of the Cullin RING E3 ligase (CRLs) family have recently emerged as regulators of disease modifying pathways and promising therapeutic targets. Cullin 3 (Cul3)-containing CRL (CRL3) has been shown to regulate hepatic insulin sensitivity and oxidative stress, but how modulating hepatic CRL3 activity impacts liver pathophysiology is largely unknown. **Methods:** We used hepatocyte-specific Cul3 KO mice and hepatocyte-specific Cul3/NRF2 DKO mice fed Western diet as models to study the role and mechanisms of Cul3 regulation of liver metabolism. We used global metabolomics approach to identify the metabolic pathways mediating the pathological changes in Cul3 deficient livers. **Results:** We report that genetic deletion of hepatocyte Cul3, despite enhancing antioxidant capacity, led to reductive stress, a redox imbalance caused by excessive cellular reducing equivalent NADH, rendering liver maladaptive to obesity. Hepatocyte Cul3 ablation in obese mice rapidly reduced hepatic steatosis but paradoxically caused lipotoxicity and systemic insulin resistance. Mechanistically, we identified that NRF2-mediated transcriptome changes resulted in NADH reductive stress in Cul3 deficient livers, which led to hepatic metabolic reprogramming to impose a strong repression on lipogenesis, diverting glycolytic intermediates to anaplerosis support of gluconeogenesis and fatty acids to synthesis of ceramides. Such hepatic metabolic inflexibility also increased circulating fatty acids and worsened systemic insulin resistance. These metabolic dysregulations were significantly attenuated when hepatic NRF2 activation and elevated $NADH/NAD^+$ ratio were abolished in Cul3 deficient livers. **Conclusions:** This study revealed important roles of hepatic CRL3-NRF2 axis in linking cellular redox imbalance to metabolic deregulation. The findings also demonstrate that heightened hepatic reductive stress in obesity reprograms liver metabolism to deleteriously impact whole body metabolic homeostasis.

Abstract 097

Extracellular Matrix Viscoelasticity Promotes Liver Cancer Progression in Pre-cirrhotic NASH

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Introduction: Type 2 diabetes mellitus (T2DM) is a risk factor for HCC in Nonalcoholic steatohepatitis (NASH). 30% NASH HCCs arise in non-cirrhotic livers when the matrix stiffness is still low. We hypothesize that in T2DM, advanced glycation end products (AGEs) in the extracellular matrix (ECM) create profound collagen architectural changes increasing viscoelasticity and activating mechano-signaling to promote HCC progression. **Methods:** Samples from healthy or NASH patients with/without T2DM were analyzed for AGEs-modified collagen networks by Second-Harmonic Generation (SHG) microscopy, matrix characteristics by Atomic Force Microscopy (AFM) and rheometry. Mice fed chow, fast food (FFD) or a diet high in AGEs (HiAD), with/without inhibitors of AGEs formation (pyridoxamine, PM) or crosslinking (ALT711), were studied, as well as the receptor for AGEs (RAGE) hepatocyte KO mice ($RAGE^{hepKo}$). To clear AGEs, the clearance receptor (AGER1, downregulated in NASH) was restored by AAV8-AGER1 prior to hydrodynamic injection (HD). To induce HCC, we HD-injected human hMet/mutant-β-catenin vs. control plasmids in the above models. Atomic force Micrscopy and Rheometry, and RNAseq were performed to identify mechano-sensitive pathways. AFM, Rheometry, SHG microscopy, and computational modeling were used to analyze matrix architectural and mechanical changes in AGEs-modified collagen 3D hydrogels,. with tunable viscoelasticity. Invadopodia formation and proliferation were studied after modulating mechano-sensitive signals. **Results:** Patients with T2DM/NASH had higher viscoelasticity, AGEs, and similar stiffness. SHG microscopy/AFM revealed that AGEs-collagen crosslinks promote higher viscoelasticity. Mice fed HiAD accumulated more AGEs and had higher viscoelasticity thanthose on high fat diet. In the HCC model, earlier tumor foci and faster progression (more foci $p<0.001$) were seen on the HiAD background. AGEs or crosslink inhibitors (PM, ALT711), $RAGE^{hepKo}$ or restoring AGER1 lowered viscoelasticity and reduced HCC progression in HiAD-fed mice ($p<0.001$). Matrix analysis and computational modeling demonstrate that lower interconnectivity of AGEs-bundled collagen matrix, marked by shorter fiber length and greater heterogeneity, enhanced viscoelasticity. RNAseq revealed induction of YAP1 pathways in an AGEs/viscoelasticity-dependent way. HCC cells had YAP nuclear translocation, invadopodia and proliferation in more viscoelastic hydrogels through Integrin β1 and tensin 1-meditated mechanosensitive signals. **Conclusion:** AGEs accumulation in the liver ECM creates a viscoelastic niche to activate mechano-signaling pathways and promote HCC progression in pre-cirrhotic NASH/T2DM. **Acknowledgement:** This research was supported by funding from the NIDDK R01 2DK083283 (to NJT), and I01 BX002418 (NJT).

Abstract 098

Understanding the Role of EGFR and PPARγ in NAFLD Utilizing Hepatocyte-specific Deletion in Adult Mice

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Introduction: Our previous study indicated a role of epidermal growth factor receptor (EGFR) in non-alcoholic fatty liver disease (NAFLD) via regulating peroxisome proliferator-activated receptor γ (PPARγ) signaling in liver. In our previous study, treatment with EGFR inhibitor suppressed NAFLD phenotype, which was associated with decreased PPARγ expression and its decreased binding to enhancers of steatosis-regulating genes. Here, we directly studied hepatocyte-specific role of EGFR and PPARγ in NAFLD utilizing specific gene deletion strategy in combination with fast-food diet (FFD) model. **Methods**: Hepatocyte-specific deletion of EGFR or PPARγ in adult mice was achieved by injecting adeno-associated virus 8 (AAV-8) expressing Cre recombinase driven by hepatocyte-specific promoter (thyroxin binding globulin, TBG) into $EGFR^{flox/flox}$ or $PPARγ^{flox/flox}$ mice, respectively, which was followed by two months of FFD feeding. **Results:** The FFD-fed hepatocyte-specific EGFR KO mice exhibited reduced serum triglyceride levels with mild decrease in steatosis in the periportal liver areas compared to WT mice. Surprisingly, total liver triglycerides levels were not significantly altered in EGFR KO mice and the overall effect on NAFLD phenotype was much weaker compared to systemic EGFR inhibition. Compensatory increase in expression of HER2 and HER3 was observed in response to EGFR loss in hepatocytes. Nevertheless, EGFR KO mice did show substantial decrease in PPARγ protein expression, although, lesser compared to chemical EGFR inhibition. Interestingly, hepatocyte-specific PPARγ deletion *per se* decreased serum cholesterol levels and significantly reduced steatosis, as evident by more than 2-fold decrease in liver triglyceride levels. Both, EGFR or PPARγ deletion decreased gene expression of several key fatty acid synthesis

enzymes/transcription factors and increased expression of lipolysis genes. Bulk RNA sequencing followed by Ingenuity Pathway Analysis revealed consistent inhibition of TGFβ1 signaling and hepatic fibrosis signaling pathways in both EGFR and PPARγ KO mice. Lastly, our single-cell RNA sequencing study revealed association of EGFR expression with key lipid metabolism genes in specific hepatocytes clusters. **Conclusion:** The hepatocyte-specific deletion strategy revealed potential role of EGFR and PPARγ in lipid metabolism/NAFLD and uncovered differences with regards to systemic EGFR inhibition approach in a short-term (2 months) FFD study. In future, this will be further confirmed in a long-term (5 months) FFD study. **Acknowledgements:** NIH Project Number 5R01DK122990-03

Interorganelle Communication in Health and Disease

Abstract 099

SIRPα Expression is Associated with Breast-to-brain Metastasis Through Modulation of Fibronectin in the Tumor Microenvironment

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Introduction: Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer characterized by the lack of specific targets and an incidence of brain metastasis from the primary site of approximately 35%. There is no standard treatment for managing brain metastasis associated with TNBC; therefore, new strategies are urgently needed to overcome disease mortality. The extracellular matrix protein fibronectin is released to tumor microenvironment by multiple cell types, including the cancer cells. However, whether fibronectin benefits cancer invasion and metastasis through escaping the immune surveillance is still unclear. The CD47/SIRPα signaling pathway is implicated in tumor progression due to bypassing innate and adaptive immune surveillance. Most strategies targeting this pathway focus on targeting the receptor CD47; however, targeting SIRPα as a potential strategy to mitigate metastatic burden remains understudied. **Methods:** Immunohistochemistry (IHC) staining and flow cytometry were used to examine the expression of SIRPα in tumor lesions and metastatic cell lines. SIRPα antibody and antisense morpholino were applied for treatment options. *In vitro* cell migration rate was measured by Agilent xCELLigence Real-Time Cell Analysis. The intracardiac injection model with IVIS was used to investigate survival and brain metastasis lesions *in vivo*. Fibronectin expression and immune cell population were measured by Nanostring GeoMX digital spatial profiling, western blotting, and IHC. Bulk RNA-Seq data were applied to examine gene expression change between control and SIRPα blockade treatment. **Results:** Breast cancer patient biopsies shows a 3.5-fold increase in SIRPα expression in metastatic lesions compared to the primary tumor (n=19; p≤0.01). Besides the myeloid cells, SIRPα is also expressed on triple-negative cancer cells (4T1). The protein expression of SIRPα in 4T1 brain-trophic 4T1-Br3 cells is 84% higher than the 4T1 parental cells (n=3; p≤0.05). *In vitro*, SIRPα knockdown by antisense morpholino inhibits TNBC brain-trophic 4T1br3 cell migration (n=4; p≤0.01). *In vivo*, SIRPα blockade by antibody reduced metastatic brain lesion formation by approximately 90% (n=4-7; p≤0.05). In addition, SIRPα blockade shows a 5-fold increase of pro-inflammatory microglia in TNBC brain lesions (n=3-7; p≤0.01). Interestingly, fibronectin was reduced in SIRPα treated brain lesions (70%, n=3-6; p≤0.05) and in SIRPα knockdown 4T1br3 cells. Fibronectin was studied to affect the innate immune signaling. The decrease of fibronectin expression was potentially associated with a 66% reduction in protein expression of the calcium-binding protein B (S100b) in brain lesions of mice treated with SIRPα blockade (n=3-6; p≤0.05). S100b is known to promote fibronectin expression, thus providing a potential mechanism for promoting brain metastasis. **Conclusions:** SIRPα blockade reduces the synthesis of fibronectin in tumor microenvironment to limit brain metastatic breast cancer growth and enhance survival. **Acknowledgement:** This work is supported by NCI-R21 (5R21CA249349-02) and ASTRO-BCRF Career Development Award.

Abstract 100

DMT1-Dependent Endosome-Mitochondria Interactions Regulate Mitochondrial Iron Translocation and Metastatic Outgrowth

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Introduction: In cancer cells, the accumulation of iron into the mitochondria, the main cellular iron sink, can rewire iron metabolism homeostasis toward adaptation to malignant features, including enhancement of growth, invasion, and metastasis. Transient early endosome (EE)-mitochondria interactions can mediate mitochondrial iron translocation, but the associated regulatory mechanisms in cancer progression and metastasis are still elusive. **Methods and Results:** In the present work, we first evaluated the role of endosomal DMT1 in transient "kiss-and-run" EE-mitochondria interactions, mitochondrial iron translocation, and cytoplasmic iron regulation in different cell lines representative of the breast cancer subtypes triple-negative (MDA-MB-231) and luminal A (T47D). We found that DMT1 can be present in EE as well as in association with the OMM, acting as a bridge between EE and mitochondria organelles. These DMT1-mediated bridging events are more frequent in MDA-MB-231 than in T47D breast cancer cells. In agreement, DMT1 also regulates mitochondrial iron translocation in MDA-MB-231 but not in T47D cells. Labile iron pool (LIP) levels, as well as mitochondrial ROS generation, increase upon DMT1 silencing in MDA-MB-231 breast cancer cells. Mitochondrial bioenergetics was severely impaired upon DMT1 silencing in MDA-MB-231 cells. Moreover, PINK1/Parkin-dependent mitophagy is regulated by DMT1 via the association of PINK1 at the OMM with the DMT1 interactor PMPCB, a peptidase regulating PINK1 turnover, in MDA-MB-231 cells. Interestingly, transcriptome analysis revealed significant differences upon DMT1 ablation between 2D and 3D cell culture conditions. Furthermore, we observed that DMT1 silencing induced lower invasive migration in 2D cell culture conditions but conversely increased MDA-MB-231 invasive capacity in 3D cell culture (spheroids). Concurringly, *in vivo*, DMT1 silencing increases the metastatic outgrowth of MDA-MB-231 cells upon lung metastatic colonization. Immunofluorescence analysis of lung metastases shows that DMT1 regulates the EE-mitochondria inter-organelle association similarly to 2D cell culture conditions. Furthermore, analyses of triple-negative MDA-MB-231 derived from metastases *in vivo* showed increases in LIP and significant delay in mitochondrial iron translocation compared to parental cells, like DMT1 KO cells. **Conclusions:** We showed that Divalent Metal Transporter 1 (DMT1) sustains mitochondrial iron translocation via EE-mitochondria interactions in triple-negative MDA-MB-231, but not in luminal A T47D breast cancer cells. Importantly, *in vivo* lung metastasis assay revealed that DMT1 silencing promoted the outgrowth of lung metastatic nodules. These findings reveal a DMT1-dependent pathway connecting EE-mitochondria interactions to mitochondrial iron translocation, mitochondrial metabolism, and metastatic fitness of triple-negative breast cancer cells.

Extracellular Matrix Mechanosensing and Cell Behavior

Abstract 101

Decorin Maintains Cartilage Surface Integrity and Chondrocyte Mechanotransduction During Aging

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Introduction: Aging is the leading factor of osteoarthritis, which afflicts > 10% of adults over 60 years of age, and is marked by irreversible breakdown of cartilage extracellular matrix (ECM). The impact of aging on cartilage ECM at the molecular level is poorly understood. Our recent study found decorin regulates the integrity of aggrecan and its sulfated glycosaminoglycans (sGAGs) in cartilage during post-natal growth. In both young and old human cartilage, decorin is actively expressed. Thus, this study tests whether decorin also mediates cartilage ECM maintenance during aging. **Methods:** Knockout of *Dcn* was induced in 3-month-old cartilage-specific decorin knockout mice ($Dcn^{f/f}$/AcanCreER, or Dcn-cKO) via tamoxifen. At 9 and

18 months (9M, 18M), cartilage phenotype was evaluated by histology for sGAG staining, collagen I IF imaging and SEM for surface fibril structure. IF-guided AFM nanomechanical mapping was applied to unfixed cartilage cryo-sections to quantify the micromodulus of both ECM bulk and the pericellular matrix (PCM), a distinct, ~2-4 μm-thick layer surrounding chondrocytes. Following the labeling with Calbryte-520, confocal imaging was applied to tibial cartilage to quantify in situ chondrocyte intracellular calcium signaling transients in DMEM media. **Results:** In 9M Dcn-cKO cartilage, loss of decorin led to an overall reduction of sGAGs and the formation of aligned collagen I-rich fibrils on the surface. This was in stark contrast to the transversely isotropic mesh of thin collagen II fibrils on normal cartilage surface. At 18M, these changes became more pronounced, with nearly full depletion of sGAGs and salient surface fissures. This surface fibrillation may be attributed to several mechanisms. First, loss of sGAGs indicates aggrecan depletion as a result of decorin loss. Second, decorin also directly regulates collagen interfibrillar spacing. Thus, the loss of both decorin and aggrecan together could result in reduced biophysical resistance against collagen fibril fusion and alignment under joint loading, thus accelerating surface fibrillation. We observed progressive reduction of cartilage micromodulus at both 9M and 18M ages, for both ECM bulk and PCM. The impairment of PCM indicates perturbed cell mechanotransduction due to decorin loss. Indeed, in both age groups, Dcn-cKO chondrocytes showed demoted in situ calcium signaling, as marked by a lower percentage of responsive cells. This impact could be attributed to the fact that the loss of decorin reduces the retention of aggrecan in the PCM, and therefore impairs chondrocyte osmo-microenvironment, supporting decorin as an indispensable constituent of cartilage throughout lifespan. **Conclusions:** This study highlights the crucial role of decorin in regulating cartilage surface integrity and chondrocyte mechanotransduction during aging. Results provide a basis for using decorin as a new target in molecular therapy to enhance cartilage maintenance during aging.

Abstract 102

Effects of Collagen V on Fibril Morphology and Fibroblast Adhesion and Contractility

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Introduction: Ehlers-Danlos Syndrome classical type (cEDS) is a hereditary connective tissue disorder causing soft fragile skin with poor wound healing, joint hypermobility, and instability. cEDS is caused by mutations in COL5A1 resulting in haploinsufficiency for collagen V, or more rarely missense mutations in COL5A2. Collagen V is a fibrillar type of collagen commonly found alongside collagen I and regulates collagen fibril diameter. However, how the reduced production of collagen V in cEDS leads to softer connective tissue and poor wound healing is unclear. The lack of integrins specific to collagen V points to the cause lying in different morphology in the extracellular matrix, (ECM) instead of a pathway that directly involves the lack of substrate for a specific integrin to bind to. **Methods:** Here, to investigate how collagen V induces different cell-ECM interactions, we measured the traction force of fibroblasts by seeding them on soft substrates coated with collagen I and collagen V using our TFM experiments and software (*Nat Methods* 2015;**12**:653-656; *Curr Protoc* 2021;**1**:e233). To discover the mechanical factors that could contribute to this traction and adhesion responses, we measured the collagen morphology containing varying ratios of collagen I to collagen V using scanning electron microscopy (SEM) and subsequent image analysis. **Results:** We found that fibroblast traction per adhesion was increased at 4 hours after seeding on collagen V, which was ~3 times larger than ones cultured on collagen I-coated substrates. Adhesion density, measured by endogenously expressed paxillin-mCherry, was smaller on collagen V than on collagen I, but they were more prominent along the edge of the cells. We also found that adhesions were more dynamic on collagen V by showing an adhesion assembly rate higher on collagen V than on collagen I. Through SEM imaging, we found that the proportion of collagen V decreases fibril diameter and also its variance, consistent with previous findings. Via computer vision analysis of the SEM images, we also found that the proportion of collagen V decreases fibril curvature as well. **Conclusion:** Our data suggest that the reduced production of collagen V in cEDS leads to increased collagen fibril diameter and curvature. We hypothesize that these will contribute to reduced ECM stiffness, which could hamper cell migration response. Our ongoing work includes measuring the rheological properties of the collagen V-containing gels on macro- and micro-scale. We also plan to measure fibroblast migration performance as both adhesion and contractility regulate cell migration.

Abstract 103

P-glycoprotein Trafficking is Dependent on Myosin Vb in Intestinal Cells

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Introduction: The intestinal epithelium is constantly exposed to luminal contents that can contain harmful compounds. Absorption of toxins and noxious substances in intestinal enterocytes is limited by the efflux transporter, P-glycoprotein (P-gp, also known as ABCB1 or MDR1). P-gp actively exports a wide range of xenobiotics and endogenous compounds out of enterocytes and back into the intestinal lumen thereby limiting their systemic exposure. Diverse types of cancer cells can develop resistance to chemotherapy drugs, which represents a major obstacle to successful cancer chemotherapy. While cancer cells can upregulate P-gp to avoid cell death in response to drugs, decreased expression of P-gp is linked to intestinal disorders such as inflammatory bowel diseases. Given the importance of P-gp in the gastrointestinal tract and in cancer a better understanding of the mechanisms regulating P-gp trafficking and function is needed. The molecular motor Myosin Vb (Myo5b) traffics diverse cargo to the apical membrane of intestinal epithelial cells. We hypothesized that P-gp was trafficked to the apical membrane of enterocytes by Myo5b. **Methods:** We used two mouse models that lack Myo5b, a germline full body knockout (KO) of Myo5b that is a neonatal model and an adult intestinal specific tamoxifen inducible Myo5b KO model (VillinCre^{ERT2};Myo5b$^{flox/flox}$). Cancer-derived human intestinal cells were treated with a Myosin V inhibitor (MyoVin-1) and the efflux of rhodamine 123 was examined via cell fluorescence to determine P-gp function. **Results:** In control mice P-gp localized to the brush border of enterocytes showing overlapping expression with the actin cytoskeleton marker gamma actin. In contrast, mice lacking Myo5b had P-gp restricted to the subapical compartment in enterocytes. Similarly, loss of Myo5b in differentiated intestinal organoids showed an accumulation of subapical P-gp compared to control organoids. Human intestinal organoids transduced to express a Myo5b tail domain harboring a GFP tag showed co-localization between P-gp and Myo5btail-GFP demonstrating that P-gp serves as a cargo for Myo5b in human enterocytes. Staining for the Myo5b binding partner Rab11a showed co-localization in control and Myo5b KO mice suggesting that Myo5b trafficking of P-gp is mediated by Rab11a. Immunostaining of intestinal specific Rab11a KO mice for P-gp showed loss of apical P-gp in enterocytes of Rab11a KO mice compared to apical localization in control mice. Treatment of cancer cells (T84 and Caco2$_{BBE}$) with the Myosin V inhibitor significantly decreased the ability of cells to efflux rhodamine 123, a molecule transported by P-gp demonstrating altered function of P-gp. Finally, exposure of intestinal cancer cells to the chemotherapeutic drug doxorubicin resulted in increased cell death in cells treated with a Myo5 inhibitor. **Conclusion:** Collectively, these data demonstrate that Myo5b and Rab11a are responsible for the apical delivery and function of P-gp.

Imaging the Matrix

Abstract 104*

Type III Collagen Regulates Matrix Architecture and Mechanoperception During Cutaneous Wound Healing

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*The authors of this abstract selected not to publish the details of their research

Abstract 105

TGFβ2 Induced Ultrastructure and Morphometric Changes in the Mouse Trabecular Meshwork

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Introduction: The trabecular meshwork (TM) and Schlemm's canal (SC) regulate aqueous humor outflow and intraocular pressure (IOP). TGFβ2 is well known to be involved in regulating both the extracellular

matrix in the TM as well as ocular hypertension. Adenovirus 5 (Ad5) is known to have select tropism to the TM and overexpression of a bioactivated form of TGFβ2 using Ad5 is known to induce ocular hypertension. Here, we aimed to analyze TGFβ2-induced ultrastructure and morphometric changes to the outflow pathway. **Methods:** A/J mice were injected intravitreally with $2.5x10^7$ pfu Ad5.TGFβ2 or Ad5.null. IOP was measured weekly with a TonoLab tonometer. Eight weeks post-injection mice were sacrificed, and eyes dissected and processed for assessment of changes in ultrastructure using electron microscopy and immunohistochemistry (IHC). Segmental flow was also assessed by perfusion of fluorescent microbeads into the anterior chamber. Anterior segment flat mounts were then analyzed for high and low flow regions of outflow by fluorescent microscopy. **Results:** IOP was significantly elevated in Ad5.TGFβ2 injected eyes compared to Ad5.null injected eyes (n=6, $p<0.01$). Quantification of electron microscopic images of the entire area of the TM from anterior to posterior demonstrated Ad5.TGFβ2 injected eyes had significantly more area occupied by ECM compared to Ad5.null injected and uninjected eyes, (n=5, $p<0.0001$). IHC analysis showed a significant increase of collagen I and fibronectin expression in the TM of Ad5.TGFβ2 treated eyes compared to control (n=6, $p<0.05$). In addition, an increase in the presence of macrophages was detected in Ad5.TGFβ2 treated eyes in the outflow region compared to Ad5.null treated eyes (n=6, $p<0.05$). Ad5.TGFβ2 treated eyes had significantly less high outflow regions compared to Ad5.null treated eyes (n=6, $p<0.05$). **Conclusions:** TGFβ2 alters the ultrastructure of the TM leading to a decrease in aqueous humor outflow. These data fully characterize the Ad5.TGFβ2 model of inducible ocular hypertension at both a structural and physiological level, providing a reliable method to study ocular hypertension and identify potential new targets to lower IOP.

Abstract 106*
The Development of the Extracellular Matrix of the Brain
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*The authors of this abstract selected not to publish the details of their research

Abstract 107
Collagen Fibers within Dystrophic Skeletal Muscle Extracellular Matrix Dynamically Re-align and Resist Enzymatic Digestion in a Strain Dependent Fashion
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Introduction: In Duchenne muscular dystrophy, there is chronic damage that leads to fibrosis, an accumulation of extracellular matrix (ECM) tissue. Previous research has demonstrated that the alignment of collagen fibers is increased in fibrotic skeletal muscle and relates to muscle stiffness. Further, it has been hypothesized that collagen fibers in skeletal muscle ECM alter their alignment as the muscle is stretched or shortened. Because the alignment of collagen fibers is related to their incurred load, we hypothesized that the degree of re-alignment that collagen fibers undergo during a muscular stretch drives muscle passive stiffness. Additionally, *in vitro* studies show that collagen fibers under load exhibit resistance to enzymatic degradation. Thus, we also hypothesized that the aligned collagen matrix within stretched muscle exhibits greater resistance to enzymatic digestion than the unaligned matrix in slack muscle. **Methods:** We collected extensor digitorum longus (EDL), soleus, and diaphragm (DP) muscles from D2 (wildtype) and D2.*mdx* (dystrophic) mice of 47-50 weeks of age. Diaphragm muscles were cut into strips along the myofiber angle. Left and right limb muscles and two strips of diaphragm were treated with a sham or collagenase treatment and then decellularized to isolate the muscle ECM. Decellularized ECMs (DCMs) were then mechanically tested for tensile stiffness from 85% to 115% of optimum muscle length. DCMs were subsequently imaged under second harmonic generation microscopy to analyze collagen alignment index across the range of muscle lengths corresponding to the mechanical testing. Four separate strips of diaphragm were mechanically tested before and after a sham or collagenase digestion while at a slack or strained length to simulate muscle stretching. **Results:** We found that collagenase significantly reduced the stiffness of all muscle DCMs. Additionally, we found that collagen alignment index increased in EDL and soleus DCMs in response to muscle stretching (EDL 85%: 0.6356±0.1523, EDL 115%: 0.7776±0.07327; Soleus 85%: 0.6363±0.1048, Soleus 115%: 0.7059±0.1801), but the increase in collagen alignment was blunted by collagenase. We also found that diaphragm strips that were digested at a strained length maintained more elastic stiffness than strips that were digested at a slack length (DP slack:

109.2kPa±99.98, DP strained: 171.8kPa±94.34). **Conclusions:** Our results demonstrate that collagen fibers within dystrophic muscle ECM re-align as the muscle is stretched. Additionally, we found that in dystrophic diaphragm muscles, a greater proportion of passive stiffness was maintained in muscles that were collagenase digested at strained lengths versus slack lengths. Altogether, our data provide evidence that collagen fibers within the muscle ECM undergo dynamic re-alignment during muscle length changes, and this alignment is related to the susceptibility of the collagen matrix to enzymatic digestion.

Organoids and Three-dimensional Models

Abstract 108

Fibrotic Fibro-Adipogenic Progenitors Impair Myogenesis Through Extracellular Matrix Deposition

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Introduction: Fibrosis is the pathological accumulation of extracellular matrix (ECM) components, impairing tissue function and increasing passive stiffness. In skeletal muscle, the main source of ECM deposition is fibro-adipogenic progenitors (FAPs). FAPs can signal to muscle satellite cells (MuSCs) through soluble factors and ECM deposition. FAPs are more highly activated into myofibroblasts in fibrotic conditions, but how this affects their signaling to MuSCs is unknown. Cell-derived matrices (CDMs) can be synthesized from FAPs to investigate their ECM deposition. We hypothesized that FAPs isolated from *mdx* mice, a model for skeletal muscle fibrosis, would have increased collagen deposition compared to *wt* FAPs and would produce a matrix that reduces myogenesis. **Methods:** FAPs were isolated from the lower limb muscles of *wt* and *mdx* mice. FAPs and muscle satellite cells (MuSCs) were isolated using magnetic-activated cell sorting. To create CDMs, FAPs were plated in media containing 50μM ascorbic acid to promote collagen deposition for 2 weeks. FAPs were removed while keeping the matrix intact. CDMs were imaged using second harmonic generation (SHG) imaging to visualize collagen organization. Some CDMs were solubilized and replated to create a flat protein coating of matrix. MuSCs were then plated on the CDMs, solubilized matrix, in a transwell co-culture with FAPs, and on plastic in differentiation media for 5 days. Cells were then fixed and stained with MF20 (MuSCs) or αSMA (FAPs), F-actin, and Hoescht. Cells were imaged using an inverted Leica DMi8. Images were captured with a dry 20X/0.40 objective using a Leica DFC9000 GTC camera and LAS X software. Images were analyzed using FIJI: ImageJ. **Results:** There was a significant negative correlation between the level of FAP activation into myofibroblasts and the MuSC differentiation index, independent of if the FAPs were isolated from *wt* or *mdx* mice. SHG images revealed CDMs derived from *mdx* FAPs had less aligned collagen fibrils compared to *wt* CDMs. **Conclusions:** High activation into myofibroblasts is often an indicator of a fibrotic FAP phenotype. Here, the negative correlation between myofibroblasts and MuSC differentiation suggests this phenotype impairs myogenesis. FAPs in transwell co-culture did not have a significant effect on MuSC differentiation, highlighting the importance of ECM deposition rather than soluble signaling is important in FAP signaling to MuSCs. Targeting FAPs activation into myofibroblasts provides a therapeutic target to attenuate fibrosis and improve regeneration in skeletal muscle. **Acknowledgement:** Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number F31AR082700. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abstract 109

LPS and Cytokines-Induced Pro-Inflammatory Insult: Upregulation of Barrier Proteomic Expression in Human Colonoids

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Introduction: Ulcerative colitis is a chronic inflammatory condition, and continuous inflammatory stimulus may lead to barrier dysfunction. The goal of this study was to assess barrier proteomic expression by a marine red algae-derived multi-mineral intervention in the absence or presence of a pro-inflammatory insult, which can produce a milieu that resembles the inflamed human colonic mucosa. **Methods:** Human colonoids (colon organoids) were maintained in a control culture medium alone or exposed to lipopolysaccharide with a combination of three pro-inflammatory cytokines [tumor necrosis factor-α,

interleukin-1β and interferon-γ (LPS-cytokines)] to mimic the environment in the inflamed colon. Untreated organoids and those exposed to LPS-cytokines were concomitantly treated for 2 weeks with calcium-, magnesium-, multiple trace elements-rich, multi-mineral product (Aquamin®) that has previously been shown to improve barrier structure and function. The colon organoids were subjected to proteomic analysis to obtain a broad view of the protein changes induced by the two interventions alone and in combination. In parallel, confocal fluorescence microscopy, tissue cohesion and transepithelial electrical resistance (TEER) measurements were used to assess barrier structure and function. **Results:** The LPS-cytokine mix altered the expression of multiple proteins that influence innate immunity and promote inflammation. Several of these were significantly decreased with Aquamin® alone but only a modest decrease in a subset of these proteins was detected by Aquamin® in the presence of LPS-cytokines. Among these, a subset of inflammation-related proteins including fibrinogen-β and -γ chains (FGB and FGG), phospholipase A2 (PLA2G2A) and SPARC was significantly downregulated in the presence of Aquamin® (alone and in combination with LPS-cytokines); another subset of proteins with anti-inflammatory, antioxidant or anti-microbial activity was upregulated by Aquamin® treatment. When provided alone, Aquamin® strongly upregulated proteins that contribute to barrier formation and tissue strength. Concomitant treatment with LPS-cytokines did not inhibit barrier formation in response to Aquamin®. Confocal microscopy also displayed increased expression of desmoglein-2 (DSG2) and cadherin-17 (CDH17) with Aquamin®, either alone or in the presence of the pro-inflammatory stimulus. Increased cohesion and TEER with Aquamin® (alone or in the presence of LPS-cytokines) indicates improved barrier function. **Conclusion:** Taken together, these findings suggest that multi-mineral intervention (Aquamin®) may provide a novel approach to combating inflammation in the colon by improving barrier structure and function as well as by directly altering the expression of pro-inflammatory proteins.

Abstract 110

Loss of Myosin 5b Results in Mislocalization of Intestinal Alkaline Phosphatase and a More Susceptible Epithelium

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Introduction: The intestinal epithelium serves as a crucial barrier that distances the immune system from harmful luminal contents and bacteria. The barrier comprises mucins and microvilli on the apical membrane of epithelial cells. Disorders that arise from the dysfunction of this barrier include celiac disease, microbial infections, inflammatory bowel disease (IBD), food allergies, and metabolic syndromes. Recent research has shown significant changes in microvilli and decreased levels of intestinal alkaline phosphatase (IAP) in patients with inflammatory bowel disease. IAP is a microvilli component that detoxifies inflammatory bacteria and stimuli. Myosin 5b, a molecular motor that delivers critical proteins from the Golgi to microvilli has been shown to have decreased levels in IBD patient tissue. We hypothesize that decreased Myosin 5b expression results in IAP mislocalization contributing to increased epithelial susceptibility to damage. **Methods:** To test our hypothesis we used neonatal control and littermate germline Myosin 5b knockout mice as well as adult intestinal specific tamoxifen-inducible VillinCre^{ERT2};Myo5b$^{flox/flox}$ mice. We performed immunostaining on intestinal sections from mice to determine alterations in IAP that result from loss of Myosin 5b *in vivo*. We also generated intestinal organoids from control and mice lacking Myosin 5b to analyze IAP localization and function in a reductionist model. Human IBD tissue was stained to determine levels of Myosin 5b and IAP compared to healthy tissue. Human intestinal cells were treated with a Myosin 5 inhibitor and analyzed for changes in pro-inflammatory genes. **Results:** Staining of IAP in control mice showed IAP localized to microvilli on the apical membrane of intestinal enterocytes. In contrast, mice lacking Myosin 5b were observed to have altered localization of IAP, with predominantly cytoplasmic localization. Interestingly, bacterial encroachment was exclusively observed in mice lacking Myosin 5b. Intestinal organoids generated from control mice showed apical distribution of IAP. Intestinal organoids derived from Myosin 5b knockout mice exhibited decreased apical IAP compared to control organoids. Inhibition of Myosin 5 *in vitro* in HT29 cells in the presence of the pro-inflammatory cytokine IL-1a resulted in a significant increase in IL-8 gene expression compared to vehicle-treated cells. This suggests that alterations in Myosin 5b levels or function could result in an increase in inflammation. Staining of human tissue samples showed reduced Myosin 5b in IBD patients compared to healthy controls. **Conclusions:** Our findings demonstrate

that Myosin 5b is necessary for the delivery of IAP, and altered levels of Myosin 5b during inflammation could contribute to the decreased IAP expression reported in IBD. Our research sheds light on the importance of Myosin 5b in maintaining the intestinal barrier and indicates that Myosin 5b could play a role in regulating inflammation.

Abstract 111

Biofabricated Microcapsules Facilitate Formation of Matrigel-free 3D Tumor Model Alternatives

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Introduction: The development of improved targeted therapeutics to combat breast cancer requires a deeper understanding of how these tumors develop, grow, and metastasize. To address this, many researchers have turned to benchtop 3D *in vitro* tumor models to study the key processes of tumor progression *in vivo*. Multicellular tumor spheroids (MCTSs) have grown increasingly popular because they can mimic the 3D structure and pathophysiologic gradients seen in *in vivo* solid tumors. However, traditional techniques for spheroid fabrication have been shown to yield a variety of model morphologies depending on the cell line used, with particular emphasis placed on the cell type's innate ability to self-aggregate. Spheroid morphologies display a variety of different shapes and sizes, which are expected to result in functional behavior differences. Further challenges are related to our inability to assess these mesoscopic models in 3D, leading to poor model characterization and incorrect geometric assumptions being drawn from 2D assessments. **Methods:** Herein, we studied two well-known triple negative and HER2+ breast cancer cell lines with different aggregation properties (i.e., MDA-MB-231, AU565) and assessed the resulting tumor models formed via "gold-standard" liquid overlay (L.O.) using Matrigel and grown within biofabricated core-shelled microcapsules. We characterize aggregate development (i.e., morphology, cellular density), transcriptome and pathophysiologic gradient formation, drug penetration, and drug uptake. **Results and Conclusions:** Herein, we use a variety of advanced imaging techniques to characterize 3D constructs made from multiple cancer cell lines using traditional liquid overlay and bioprinted microcapsules. The aim of this study was to investigate the efficacy of core-shelled microcapsules for producing physiologically-relevant tumor spheroids in cell lines possessing a range of inherent self-aggregation abilities. We benchmark our results against gold standard liquid overlay models prepared with and without Matrigel, hypothesizing that capsules would enable consistent formation of 3D aggregates with spherical morphology and gradient formation even in cell lines with poor self-aggregation abilities. We characterize differences in aggregate development (i.e., morphology, cellular density) and correlate these results with gene expression signatures identified by RNAseq. We also investigate differences in drug penetration, drug-target engagement, and overall viability post-drug administration between the fabricated models and identify differences in drug uptake between the models. Overall, these results highlight the power of microcapsule-based techniques for producing size- and shape-controlled spheroids, regardless of a cell type's inherent ability to self-aggregate, and without requiring the addition of exogenous additives.

Cardiac Fibrosis

Abstract 112

Novel Role of Endothelial Cell Stimulator of Interferon Genes (STING) in Systolic Dysfunction and Adverse Cardiac Remodeling Induced by Cardiac Pressure Overload

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Introduction: Cardiac inflammation, cardiomyocyte (CM) hypertrophy and endothelial cell (EC) capillary density loss are hallmarks of heart failure (HF). The protein STING is expressed in immune and non-immune cells and promotes inflammation by inducing Type I Interferons (IFN-I). IFN-I are detected in the heart at the onset of cardiac inflammation, but the cell source of STING activation, IFN-I release and how it modulates HF remains unknown. We previously reported that EC STING contributes to T cell transendothelial migration in sterile inflammation, yet whether this mechanism aids cardiac inflammation and systolic dysfunction in HF remains unknown. We hypothesized that EC STING contributes to adverse cardiac remodeling and systolic dysfunction in experimental HF. **Methods**: Male wildtype (WT), global deficient (STING$^{-/-}$) and inducible EC STING$^{-/-}$ mice or WT littermate controls (*Cad5*$^{ERTCre2+/-}$*Sting*$^{fl/fl}$ treated intraperitoneally with 85mg/kg Tamoxifen or oil, respectively) were subjected to transverse aortic constriction (TAC), a well-established model of HF, and compared to mice subjected to Sham surgery for 2, 4 and 8 weeks. Cardiac function was analyzed by echocardiography and invasive hemodynamics. Left ventricular (LV) sections were stained with wheat germ agglutinin, picrosirius red, and isolectin to analyze CM hypertrophy, fibrosis, and capillary density, respectively, by histology. Cardiac CD4+ T cells and CD11b+ myeloid cells were analyzed by Immunohistochemistry, and flow cytometry was used to investigate CD4+CD62^{lo}CD44^{hi} effector T cells in the mediastinal lymph nodes (MdLN). **Results**: In contrast to WT mice, STING$^{-/-}$ mice did not show a decline in fractional shortening (FS) in response to TAC as compared to Sham controls. LV hypertrophy was decreased in STING$^{-/-}$ TAC mice compared to WT TAC mice, assessed by organ weight and CM size. In response to TAC, effector T cells expanded in WT mice, but only mildly expanded in STING$^{-/-}$ mice, and both cardiac CD4+ T cells and CD11b+ myeloid cells decreased in STING$^{-/-}$ TAC mice compared to WT TAC. Capillary density was also preserved in STING$^{-/-}$ mice in response to TAC as compared to WT mice. We next explored whether EC STING was responsible for the observed systolic protection. Thus, we ran similar analyses in *Cad5*$^{ERTCre2+/-}$*Sting*$^{fl/fl}$ mice and found that EC STING$^{-/-}$ mice had a blunted decline in FS at 4 weeks of TAC that persisted into chronic stages of HF at 8 weeks post-TAC, in contrast to the progressive FS decline in WT controls. Strikingly, EC STING$^{-/-}$ mice had ameliorated CM hypertrophy and preserved capillary density at 8 weeks of TAC, yet cardiac infiltration of CD4+ and CD11b+ cells and MdLN effector T cell expansion were similar to WT controls post-TAC. **Conclusions**: Our data support that EC STING contributes to systolic dysfunction by modulating CM hypertrophy and capillary density independent of cardiac immune cell infiltration, whereas STING in non-ECs modulates cardiac inflammation.

Abstract 113

Regulating Senescence via PADI Inhibition in Cardiac Myofibroblasts, May Help the Fibrosis in Dilated Cardiomyopathy

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Introduction: Dilated cardiomyopathy (DCM) develop during injury repair and aging, resulted in chronic fibrosis, a leading cause of heart failure. Cardiac myofibroblasts particularly found in areas of injury, are responsible to secrete extracellular matrix (ECM) components during repair phase, and dysregulation results in cardiac fibrosis. Underlying cellular mechanisms behind dysregulated process hasn't been fully uncovered yet. Cellular senescence is an irreversible cell cycle arrest accompanied by various cellular functional changes, primarily affects the aged cardiac fibroblasts. Senescent cells promote fibrosis through increased ECM production, remodeling, and deposition. ECM modify through citrullination by Peptidyl arginine Deiminase (PADI) enzymes and are known to influence the fibrogenic pathways. However, the specific interactions/association or underlying mechanisms between PADI, and senescence in cardiac myofibroblasts is not extensively studied, and not well-established. Therefore, we aim to explore the PADI and ECM- senescence biology of aged cardiac myofibroblasts, to understand their potential contribution in cardiac fibrosis. **Methods and Results:** Primary cardiac myofibroblasts from Dilated cardiomyopathy (CM, N=5) and healthy (HCF, N=3) participants (40-60 years), represents aged and irreversible mature phenotype. Senescence-associated β-galactosidase (SA-β-gal) assay showed 87% Senescent cells (SnCs) exhibited flattened and enlarged morphology in CM in comparison to HCF (47%). Increased expression of ECM proteins, Fibronectin and Vimentin including a-SMA defined the dysregulated senescent

phenotype in CM compared to HCF, despite aging. Blocking the PADIs enzyme by treating the TGFB1 activated cardiac myofibroblasts (CM), with PAD inhibitor (BB-Cl-Amidine) (TGFB1+BB-Cl), resulted in significantly decreased senescence (67%), indicating the role of PADI enzyme linked with senescence. Western blot analysis also showed significantly upregulated expression of p53 in CM, which is decreased after treatment with PAD inhibitor. These results further accompanied by flow cytometer analysis, showing decreased expression of citrullinated proteins (10%) in TGFB1+BB-Cl group verses untreated CM group (14%). These results inspired to study protein and gene expression of PADI enzymes via Flow cytometer, Western Blot, and q-RT-PCR techniques. **Conclusions:** Results showed that cardiac myofibroblasts expressed four PADI isoforms (1, 2, 3 and 4) but PADI 2 is most abundant and significantly high in CM. Key findings about increased senescence, ECM-citrullination and PADI 2 expression in CM compared to HCF suggesting the possible association to fibrotic behavior of cardiac myofibroblasts derived from Dilated cardiomyopathy. Additionally, these studies also suggested a potential link between p53 (key senescence regulator) and citrullination. Therefore, we conclude that inhibition/alteration of PADI derived senescence, may attenuate the senescence pathway, which could help to reverse the fibrosis.

Cancer Pathobiology I

Abstract 114

Rapid Region-Based Detection AI Accurately Localizes Oral Squamous Cell Carcinoma Tumors

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Introduction: The diagnosis and treatment of cancer depends on extensive analysis of histopathology images. Due to the size and complexity of these images, there is a significant burden on pathologists to manually find and grade lesions. To aid pathologists and alleviate this burden, computational tools based on artificial intelligence (AI) have emerged to automatically analyze digitized histopathology images to find and classify lesions (*npj Precis Oncol* 2020;**4**:14; *Nat Med* 2018;**24**:1559-1567). Despite reporting classification accuracy rivaling that of human experts, state-of-the-art AI models are prohibitively resource intensive and slow. Further, analyzing images on a purely cellular level fails to capture the contextual cues between lesion sites and their surrounding tissues, which have great prognostic value (*Cell Commun Signal* 2010;**8**:22; *Genes Basel* 2021;**12**:538). To overcome these challenges, we developed a lightweight region-based tumor detection AI capable of identifying tumor regions of interest (ROI). This tool presents a thousand-fold speed-up over existing automated methods and can run on consumer-grade equipment, which is unprecedented and necessary for widespread clinical deployment. **Methods and Results:** Our AI consists of a processing pipeline which predicts neoplastic ROI bounding boxes from digital pathology whole-slide images (WSI). Our AI simulates a pathologist's workflow by examining each WSI at low to high magnifications to progressively analyze lesions at the slide, tissue, and cellular levels. Our region-based tumor detection AI is drastically more efficient than most commonly used tile-based AI. We pretrained our model on a diverse set of cancer histopathology images including prostate, breast, and skin cancers (n=4211), then fine-tuned on a primary and metastatic oral squamous cell carcinoma (OSCC) dataset. Predictions were considered correct if they covered more than 50% of the tumor in each image. In a validation study (n=202), 90% of images were classified correctly, with 76% sensitivity and 93% specificity. **Conclusion:** Our model demonstrates the potential for rapid region-based detection in digital pathology. We are currently optimizing the AI to improve its sensitivity and specificity, as well as enhancing its capability to detect neoplastic cells from their nuclei. Further, we are incorporating semi-supervised training to take advantage of the vast unlabeled data available on public repositories to boost performance without requiring exhaustive manual labelling. **Acknowledgements:** Special thanks to pathologists Dr. Tra Truong (University of Toronto) and Dr. Catherine Poh (University of British Columbia) for labeling of OSCC images and feedback on AI design.

Abstract 115

Promoting Immune Cell Activation and Recruitment via the Interferon Response Factor-2 (IRF2) Reduces Disease Burden in β-catenin-mutated HCC Models

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Introduction: Immune checkpoint inhibition (ICI) is the current standard of care for inoperable HCC. This began with the IMbrave150 trial and has expanded to additional clinical trials and other methods of ICI since not all patients respond equally. Specific molecular aberrations are being investigated to determine their relationship to ICI efficacy. For example, 30% of patients that harbor mutations in *CTNNB1*, the gene that encodes β-catenin, seem to respond poorly to ICI intervention. However, understanding if *CTNNB1* mutations directly contribute to immune escape in clinical HCC is still in need of investigation. Interferon therapy has been applied in other neoplasms to induce an activated immune response, so we investigate here whether inducing an inflammatory response through the interferon pathway is a sufficient intervention in *CTNNB1*-mutated HCC. **Methods:** Bioinformatic analysis was performed on β-catenin loss and gain of function livers to identify mechanisms of immune cell regulation. Next, HCCs generated in mice by co-delivering mutant-β-catenin-(S45Y) and the MET tyrosine kinase (B+M model) that represent 11% subset of HCCs with a 69% genetic accuracy to patients was used to validate the *in-silico* findings. The interferon response transcription factor, IRF2, was delivered alongside oncogenes in the B+M model. HCC burden was assessed in controls and +IRF2 condition. IRF2 activity was also measured by immunoblot and immunostaining. Intrahepatic and peripheral immune populations were characterized by FACS and by immunostaining to identify shifts in the immune microenvironment and exhaustion. **Results:** Overexpression of IRF2 was detectable in HCC nodules by 4 weeks post-HCC induction, which led to a significant reduction in overall tumor burden 7.5 weeks after HCC induction. We also found a significant accumulation of intratumoral $CD45^+$ cells after IRF2 expression in the B+M model. FACS identified significant shifts towards a less exhausted immune microenvironment, and a decreased accumulation of immunosuppressive FoxP3+ T-regulatory cells validated by immunostaining. Bulk RNA-Seq data identified a significant increase in activated inflammatory pathways and an upregulation of pro-inflammatory genes. We also asked if endogenous IRF2 stimulation is sufficient to drive a functional inflammatory response. Three weeks after HCC induction, B+M animals were treated daily with 1×10^4 IU IFNy for one week, off for one week, then treated again for one more week prior to harvest 2.5 weeks after last treatment resulting in a modest 10% reduction in overall disease burden. **Conclusion:** Activating the interferon response in *CTNNB1*-mutated HCC is sufficient to reduce overall tumor burden and disease. This is driven by an inflammatory response characterized by less exhausted cytotoxic T-cells and immunosuppressive T-regs. Our data indicates inducing cytotoxic inflammation in β-catenin-mutated HCCs may be an effective method to produce an anti-tumor response.

Abstract 116

NF-κB Activation in HCCs with *CTNNB1* Mutations Reduces Immune Escape and Inhibits Disease Pathogenesis

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Introduction: The current standard of care for unresectable HCC is combination immune checkpoint inhibitors (ICIs), whose foundation was laid by the IMbrave150 trial. Because of imperfect response rates to ICIs, specific molecular aberrations have been implicated in diverse responses to ICI therapy. 30% of patients that harbor mutations in *CTNNB1*, the gene that encodes β-catenin, seem to respond poorly to ICI intervention. However, understanding if *CTNNB1* mutations directly contribute to immune escape in clinical HCC is still in need of investigation. Here, we investigate whether promoting an inflammatory response in HCCs with *CTNNB1* mutations is sufficient to prevent HCC progression. **Methods:** Bioinformatic analysis was performed on β-catenin loss and gain of function livers to identify mechanisms of immune cell regulation. Data from a computational analysis of differentially expressed genes (DEGs) were used in a transcription factor enrichment analysis which identified NF-κB as a contributor of DEGs. Next, HCC was induced by co-delivering mutant-β-catenin-(S45Y) and the MET tyrosine kinase (B+M model) – a model that represents an 11% subset of HCCs with a 69% genetic accuracy to patients. The p65 component of

NF-κB or a control plasmid were co-expressed alongside the oncogenes in the B+M model. HCC burden was compared in the two groups. NF-κB activity was also measured by immunoblot and immunostaining. Intrahepatic and peripheral immune populations were characterized by FACS and by immunostaining to identify shifts in the immune microenvironment and exhaustion. **Results:** Overexpression of p65 was detectable in HCC nodules by 4 weeks post-HCC induction and resulted in a significant reduction in overall tumor burden 7.5 weeks after HCC induction. We also found a significant accumulation of intratumoral $CD45^+$ and $F4/80^+$ cells after p65 expression as compared to the control group. Flow cytometry identified significant shifts towards a less exhausted immune microenvironment, and increased accumulation of cytotoxic NKT cells and a depletion of FoxP3+ T-regulatory cells validated by immunostaining. RNA-Seq analysis of bulk tissue identified significant shifts in inflammatory pathways and activated immune response in the +p65 group compared to controls. **Conclusion:** Activating NF-κB in *CTNNB1*-mutated HCC is sufficient to reduce overall tumor burden and disease. This is driven by an inflammatory response characterized by less exhausted cytotoxic T-cells and an increased accumulation of NKT cells. Our data indicates inducing inflammation in β-catenin-mutated HCCs may be an effective method to produce an anti-tumor response. Investigating how to effectively induce cytotoxic inflammation in HCCs can potentially inform how to enhance the efficacy of currently applied ICI therapies.

Abstract 117

Nuclear Factor Erythroid 2-related Factor 2 and MET Co-activation Induces Hepatocellular Carcinoma in Mice

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Introduction: Hepatocellular carcinoma (HCC) is the most common primary liver cancer with poor clinical outcomes. There is a need for improved understanding of the heterogeneity of response to current HCC therapeutics. We hypothesize that patient response is influenced in part by specific oncogenic drivers. Developing animal models which represent clinically relevant molecular subclasses of HCC will improve our understanding of HCC pathogenesis, along with providing a platform for testing novel therapeutics. Here, we investigate whether co-overexpression of mutated NFE2L2 and MET cooperate to induce HCC in mice, and the oncogenic signaling pathways activated. **Methods:** We utilized the TCGA to investigate patients with NFE2L2 and MET co-expression. Next, we followed HCC development in mice through co-overexpression of NFE2L2 and MET via hydrodynamic tail vein injection delivery with sleeping beauty transposon/transposase (HDTVi-SBTT) system. Lastly, we performed bulk RNA-sequencing to assess transcriptional differences between tumor and normal mouse liver. **Results:** Overall, ~3% of HCC patients in TCGA show high co-expression of NFE2L2 and MET. Co-overexpression of G31A-NFE2L2 and hMET in mice via HDTVi-SBTT led to well differentiated HCC development by 14 weeks with average liver weight/body weight ratio of 10%. Immunohistochemistry (IHC) demonstrated these tumor nodules to be positive for NFE2L2 targets, such as NAD(P)H quinone dehydrogenase 1 (Nqo1) and V5-tag (present on the hMET plasmid). IHC also showed these nodules to be negative for β-catenin targets, such as glutamine synthetase (GS) and cyclin-D1. Additionally, IHC revealed these nodules to be negative for mTOR pathway markers, including p-mTOR-S2248, p-4EBP1-T37/46, p-S6-S235/236, and p-S6-240/244. Bulk RNA-sequencing revealed the G31A-NFE2L2-hMET mouse model to cluster distinctly from three different β-catenin-driven mouse models using principal component analysis. Ingenuity Pathway Analysis (IPA) comparing the G31A-NFE2L2-hMET mouse model to normal liver demonstrated activation of expected (NRF2-mediated Oxidative Stress Response) and unique (Pathogen Induced Cytokine Storm Signaling Pathway, S100 Family Signaling Pathway, Pyroptosis Signaling Pathway, and Neutrophil Extracellular Trap Signaling Pathway) pathways. **Conclusions:** Our study demonstrates that Nrf2 and Met may cooperate in subset of human HCC. Co-overexpression of G31A-NFE2L2 and hMET induces formation of HCC in mice and represents a non Wnt/β-catenin activated mouse model of HCC.

Abstract 118

Distinct Cooperating Oncogenes and Reproductive History Promote the Progression of Specific *Ras*-mutant Premalignant Clones in Multistage Murine Breast Cancer Models

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Introduction: A remote carcinogen exposure can predispose to a clinical presentation of breast cancer decades later. Standard multi-stage rodent carcinogenesis models posit that carcinogen-induced mutations generate long-lived premalignant cells, which acquire additional oncogenic mutations as they evolve toward invasive cancers. However, the biological features of the earliest clones "initiated" on the path to cancer by carcinogen-induced mutations remain obscure, hindering the rational design of chemoprevention and cancer interception strategies. **Methods:** We administered a well-studied carcinogen, 7,12-dimethylbenzanthracene (DMBA), to introduce stereotyped *Ras* mutations in mice engineered for doxycycline-inducible expression of clinically-relevant mammary oncogenes, *MYC* (iMYC) or *PIK3CA*^{H1047R} (iPIK). To determine whether DMBA-initiated premalignant cells are irreversible and remain competent to undergo *MYC*- or *PIK3CA*^{H1047R}-driven progression despite a delay in the subsequent transgene expression, we compared the tumor latency and multiplicity parameters between those with a 4-day or 12-week interval between DMBA and doxycycline administration. To determine whether parity could block the progression of DMBA-generated premalignant cells, additional iMYC and iPIK cohorts underwent one or two rounds of pregnancies after DMBA treatment and prior to doxycycline administration. Tumors were collected and analyzed for *Ras* mutations using Sanger sequencing. Histological analysis was also performed using immunohistochemical and hematoxylin/eosin staining. **Results:** In prior work, we showed that DMBA exposure generated premalignant clones bearing *Hras*^{Q61L} mutations, which remained subclinical until the subsequent induction of *Wnt1* oncogene expression. However, despite using an identical DMBA exposure, here, we show that neither expression of inducible *MYC* nor *PIK3CA*^{H1047R} efficiently drove the progression of *Hras*^{Q61L}-mutant mammary cells. Instead, each oncogene promoted the outgrowth of premalignant cells bearing hotspot mutations in distinct *Ras* family genes. Specifically, *MYC* drove cancer progression from both *Kras*^{mut} and *Nras*^{mut} premalignant cells, whereas *PIK3CA*^{H1047R} promoted progression only from *Kras*^{mut} cells. Selection for these preferred Ras family mutations was maintained whether oncogene expression was induced within days of DMBA exposure or several months later. Moreover, although our parity protection schemes did not diminish the overall tumor onset in DMBA-treated iPIK and iMYC mice, parity was associated with the progression of fewer *Kras*^{mut} premalignant cells in the iPIK and iMYC groups. In contrast, the progression of *Nras*^{mut} cells was preserved in parous DMBA-treated iMYC mice regardless of the number of completed pregnancies. **Conclusion:** Together, our findings suggest that the progression of distinct premalignant clones may depend on the cooperating oncogenic pathway and reproductive history of our murine breast cancer models.

Abstract 119

Lenvatinib Monotherapy in a Novel Acral Melanoma Patient-derived Xenograft (PDX) Cohort

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Introduction: Acral melanoma (AM) is an aggressive melanoma variant that arises from palmar, plantar and nail unit melanocytes. Compared to non-acral cutaneous melanoma (CM), AM is biologically distinct, constitutes the majority of melanomas in non-Caucasians, typically presents in advanced stage disease, is less responsive to modern therapeutics, and has an overall worse prognosis. The lack of reliable and reproducible tumor models is a major barrier to understanding the unique biology of AM and in developing tailored therapeutics. **Methods and Results:** To overcome this limitation, a panel of 6 AM and 2 CM patient derived xenograft (PDX) tumors has been successfully established and passaged in mice. These tumors have been clinically characterized and undergone targeted genome and transcriptome analysis, for the goal of providing the PDX tumors as a resource to the field. In a proof-of-concept study, PDX tumors were screened for response to several small molecule inhibitors, which were selected based on a combination of transcriptomic data and literature review. Of the tested agents, Barasertib and Sunitinib demonstrated tumor regression in approximately half of the AM cohort, while treatment with Lenvatinib resulted in tumor regression in all AM and slowed growth kinetics in 1 of 2 CM. **Conclusion:** In summary, we have

demonstrated that AM form viable PDX tumors, developed a stable and reproducible AM tumor model for the field, and have acquired preclinical evidence for the efficacy of Lenvatinib monotherapy in AM.

Pathobiology of Inflammation

Abstract 120

The Effect of Noncanonical Inflammasome Caspase-11 on Tissue Factor Release in Trauma-Induced Coagulopathy and Organ Damage

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Introduction: Trauma-induced coagulopathy (TIC) is a life-threatening complication following severe injury that results in impaired hemostasis. Endothelial cells (ECs) and platelets play key roles in managing inflammation and facilitating clotting, respectively, after injury. Previous studies have suggested that the noncanonical inflammasome, caspase-11 (casp-11), is activated in trauma-induced coagulopathy (TIC), but its specific role remains unclear. This study aims to elucidate the effect of casp-11 on tissue factor release, ECs, and platelets in a murine model of TIC. **Methods:** Male C57BL/6J (WT), casp-11 knockout ($casp11^{-/-}$), and endothelial cell-specific and platelets cell-specific casp-11 knockout ($casp11^{EC-/-}$, $casp11^{plt-/-}$) mice, were subjected to polytrauma (n=3-4/gp), consisting of a blind cardiac puncture (25% of total blood volume taken), liver crush, and bilateral pseudofractures (hindlimb crush injury and injection of crushed bone solution from an age- and weight-matched syngeneic donor). Six-hours post polytrauma, lung and liver lobes were perfused in situ, and 6-8µm thick sections were stained with CD31 endothelium and tissue factor markers. Sections were imaged and quantified using fluorescent microscopy. To assess hemostasis and liver damage, circulating tissue factor (TF) and ALT levels in plasma were measured 6h post-trauma, respectively. Baseline hemostasis in mice was assessed using a tail vein transection model (1cm tail tip cut followed by submersion in 20mL of lactated Ringer's solution). Time to the cessation of bleeding was measured in seconds and recorded as bleeding time (n=4-5/gp). **Results:** Six-hours following trauma, both WT and $casp11^{-/-}$ mice exhibited a significant increase in TF, with $casp11^{-/-}$ mice demonstrating a higher degree of TF co-localization with lung endothelium ($p<0.05$). Circulating TF in plasma showed an increasing trend in $casp11^{-/-}$ mice post-trauma. $Casp11^{-/-}$ and $casp11^{plt-/-}$ mice recorded significantly lower ALT levels compared to WT mice (775±0.0 & 510.0±0.0 vs. 2297±0.0 IU/L, $p<0.05$). However, ALT levels in $casp11^{EC-/-}$ mice were similar to WT mice. Moreover, WT and $casp11^{plt-/-}$ mice demonstrated significantly prolonged bleeding times relative to $casp11^{-/-}$ mice (739.8±121.1 & 990±148 vs. 265.8±27.7 seconds, $p<0.05$ and $p<0.005$, respectively). Similar to ALT, $casp11^{EC-/-}$ and WT had comparable bleeding times. This finding suggests that inhibiting casp11 improves clotting potential. **Conclusion:** Our data suggests that casp-11 plays a key role in TIC and impaired hemostasis through tissue factor. Casp-11 also plays role in liver damage post-injury. Casp-11 inhibition appears to enhance clotting potential, independent of ECs and platelets. The cell type expressing casp-11 may significantly impact these effects, highlighting the complexity of inflammation and coagulation interplay in trauma. These findings shed light on a potential therapeutic role for casp-11 inhibition in the treatment of TIC. **Acknowledgment:** This work was supported by NIGMS R01-GM102146.

Abstract 218

The Role of CCR2+ Myeloid Cell Transmigration in Non-proliferative Diabetic Retinopathy

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Introduction: Diabetic retinopathy (DR) is a leading cause of blindness in working-aged adults (*JAMA* 2010;**304**:649-656). DR is classified into (1) non-proliferative DR (NPDR), where capillary dropout causes ischemia and microaneurysms, and (2) proliferative DR, where significant ischemia stimulates neovascularization (III, D.W.P., 2016, How to Classify the Diabetic Eye). There are no treatments for NPDR due to lack of understanding of its pathophysiology. Leukostasis is an early phenotype of DR and is associated with capillary dropout, a clinical hallmark of the disease (*Am J Pathol* 2001;**158**:147-152). CCL2 is the most consistently elevated intraocular chemokine in patients with all stages of DR ((*Retina Phila Pa* **18**, 546–550 (1998); *Retina Phila Pa* 2010;**30**:1412-1419; *J Diabetes Complications* 2020;**34**:107641;

Diabet Med J Br Diabet Assoc 2004;**21**:1292-1297; *Diabetologia* 2023;**66**:590-602). *Ccr2*$^{-/-}$ mice show reduced leukostasis and endothelial cell (EC) degeneration, highlighting the importance of myeloid cells in disease (*Diabetologia* 2023;**66**:590-602). However, how leukocytes promote capillary degeneration, where in the retina this occurs, and whether transmigration contributes to disease progression is not known. To address these knowledge gaps, we are investigating leukocyte and EC dynamics in a CCL2-induced acute model of inflammation and streptozotocin (STZ)-induced model of diabetes. **Methods:** To induce acute inflammation, *cx3cr1*^{GFP}*/ccr2*^{RFP} and *catchup*^{RFP} (Ly6G neutrophil-labeled) mice are intravitreally (ivt) injected with 5 ng/uL CCL2. Alternatively, diabetes is induced by i.p. injection of STZ or citrate buffer (sham). Hyperglycemia (>300 mg/dL) is confirmed seven days post injection via tail prick and glucometer. For both models, wholemount retinas are prepared for immunofluorescence imaging. For intravital microscopy (IVM), mice receive non-blocking anti-PECAM-1 i.v. to label vessels, dilating drops, and ketamine/xylazine for anesthesia. Eyes are immersed in hydrogel and retinas imaged with a Leica multiphoton scope equipped with 10x water objective. **Results:** CCR2+ monocytes are first seen within the retina vasculature at 6 hours post-CCL2 ivt injection but do not extravasate until 24 hours post-injection. In addition to monocytes, there is evidence that neutrophils respond to CCL2. In STZ-induced diabetic mice, CCR2+ leukocytes are seen within the vasculature at 2 weeks and have extravasated by four weeks. S100A9+ cells, presumably neutrophils, also extravasate by 4 weeks. We are narrowing down the earliest time point of extravasation in both models. Ongoing experiments include studying the effect of blocking extravasation on downstream DR phenotypes, such as vascular permeability and EC death. We are also studying leukocyte dynamics with IVM to determine whether leukocyte extravasation or merely leukostasis causes EC damage. **Conclusions:** Our studies using both endpoint assays and IVM will provide an understanding of when and where leukocytes extravasate in the retina in response to CCL2, a known contributor to early DR pathogenesis. In a model of early-stage DR we will investigate how blocking leukocyte adhesion and transmigration affects disease progression. These experiments could aid in development of novel therapy for NPDR.

Abstract 122

Identifying Mechanisms Regulating Transendothelial Migration of Neutrophils in the Lungs During Acute Airspace Disease

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Introduction: Inflammation is critical in the body's response to insults such as infection or tissue damage. Leukocytes are generally recruited to sites of inflammation via an inflammatory cascade in which selectins and their ligands control leukocyte rolling, and leukocyte integrins interacting with molecules of the Ig Superfamily regulate leukocyte adhesion and locomotion on the endothelial surface. The step where leukocytes migrate out of the blood through which they've traveled and across the endothelium into the tissue is known as transendothelial migration (TEM) and occurs via interactions between leukocyte and endothelial PECAM and CD99 in the systemic vasculature. However, the inflammatory response in the lung is known to be different in several ways: 1) TEM occurs primarily across capillaries rather than postcapillary venules, 2) leukocyte homing is selectin-independent, and 3) the requirement for leukocyte β2 integrins is stimulus-specific. Therefore, we are investigating whether TEM in the pulmonary vasculature is regulated by the same molecules as in the systemic circulation. Specifically, in this study we have examined the requirement of PECAM and CD99 in transmigration of leukocytes in the lungs and the role of neutrophils during aspiration pneumonia (sterile) and bacterial bronchopneumonia (nonsterile). **Methods:** We have utilized a novel method of intravital microscopy (IVM) of the live murine lung to visualize neutrophil movement in real time, as well as automated analysis of histopathological sections, tissue clearing and light sheet fluorescence microscopy of whole lung lobes, flow cytometry, and RNA-sequencing of neutrophils during disease over time. **Results:** (i) Response to sterile injury is PECAM-dependent in FVB/n mice. (ii) Response to sterile injury is PECAM-independent in C57Bl/6 mice. (iii) We found that in both aspiration pneumonia and Pseudomonas aeruginosa induced pneumonia, PECAM and CD99 were necessary for migration of neutrophils. (iv) Interestingly, blocking neutrophil migration to the lungs simultaneously with administering sterile or nonsterile inflammatory insult showed no meaningful benefit in survival. This is likely because neutrophils are required for the initial response to injury. We are currently examining the timing and efficacy of blocking neutrophil TEM therapeutically. **Conclusions:** Based on our results thus far, neutrophil TEM in the pulmonary vascular bed is PECAM and CD99-dependent. Understanding the roles

of neutrophils early and late in airspace diseases caused by different stimuli is critical for informing therapies to treat these diseases effectively with drugs that block TEM. These therapies could be particularly critical for patients with steroid-resistant pneumonia who present with severe disease but don't respond to steroids.

Abstract 123

HES1 Loss and Counteracting ATOH1 Up-regulation Promotes Intestinal Stem Cell Regeneration of Ulcerative Colitis

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Introduction: Ulcerative colitis (UC) is associated with a higher risk of colorectal cancer (CRC) and impaired colonic epithelial regeneration. The latter is regulated by the interaction of hairy and enhancer of split-1 (HES1) and atonal homolog 1 (ATOH1). HES1 promotes intestinal progenitor proliferation and absorptive lineage differentiation while also inhibiting ATOH1 from activating secretory lineage differentiation. ATOH1^{+} secretory progenitor cells can exhibit stem cell properties following severe tissue injury (*EMBO J* 2019;**38**:e99984). We reported that HES1 loss is frequent in serrated adenocarcinomas and colorectal cancers with KRAS mutation (*Am J Surg Pathol* 2016;**40**:113-119). We also established a mouse model of colitis and colitis-associated CRC (*Fx*$^{-/-}$ mouse) to recapitulate the loss of Notch/Hes1 signaling in colonic epithelium (*Gastroenterology* 2017;**152**:193-205). However, the role of ATOH1 and HES1 in UC remains unknown. **Methods:** We evaluated HES1 and ATOH1 expression in the colonic epithelium of paired UC tissues collected from the quiescent phase and active inflammation by immunostaining. The patients were further grouped into the non-relapsing and the relapsing group to investigate the association between HES1 and ATOH1 with the risk of relapse. The expression of stem cell markers and lineage markers was examined by RT-qPCR. Deletion of *Atoh1* in the intestinal epithelium of *Fx* mice was generated by crossing *CDX2-Cre*/*Atoh1*$^{+/flox}$ mice with *Fx* mice[3]. The effect of *Atoh1* deletion in mice colonic epithelium was assessed with colon histology and ex vivo culture of colonic organoids. **Results:** In the colonic epithelium from the quiescent phase of UC, the expression of HES1 and ATOH1 was like that from the healthy control. HES1 displayed a strong nuclear expression in crypts of quiescent UC but showed attenuated expression during active inflammation. In contrast, ATOH1 expression was low in crypts of quiescent UC but increased by 2.5-fold during active inflammation. After treatment, HES1 expression recovered while ATOH1 returned to a lower level. Further, HES1 showed a near-complete loss in the non-relapsing group during active inflammation but had a much higher expression in the relapsing group. However, ATOH1 expression was similar between the two groups. The upregulation of ATOH1 during active inflammation was accompanied with the upregulation of the stem cell genes but not the secretory lineage genes downstream of ATOH1. *Atoh1*$^{-/-}$*Fx*$^{-/-}$ mice colon displayed decreased goblet cell expansion compared to that of *Fx*$^{-/-}$ mice. They showed significantly improved gut barrier integrity and inflammation-associated dysplasia progression (inflammation and dysplasia score both decreased by ~38%). Colonic organoids from *Atoh1*$^{-/-}$*Fx*$^{-/-}$ mice, however, had severely impaired development and regeneration. **Conclusions:** In all, we have shown that attenuated HES1 expression is a prominent feature of active UC while upregulation of ATOH1^{+} secretory progenitors in active inflammation is critical for intestinal stem cell regeneration but it may also promote inflammation-associated transformation. **Acknowledgements:** This work was supported in part by research funding from NCI CA222064, Case GI SPORE Research Development Award, CCCCC VeloSano Pilot Award, and NIDDK DDRCC Pilot/Feasibility Award (to LZ), and by the Department of Pathology Case Western Reserve University faculty startup fund to WX and LZ. SPORE Pilot grants (to LZ).

Abstract 124

Deletion of MyD88 in T-cells Improves Anti-Tumor Immunity in Melanoma

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Introduction: Downregulation of T-cell immunity by tumor cells is associated with cancer progression. In response to tumor damage-associated molecular patterns (DAMPs), antigen presenting cells (APCs) enhance antigen presentation to T-cells through signals that converge onto the protein "Myeloid differentiation response 88" (MyD88). T-cell antigen recognition is regulated by immune checkpoint molecules such as programmed death ligand-1 (PD-1) that limit T-cell activation, and inhibitors of these checkpoints can be used to enhance T-cell anti-tumor activity. However, these inhibitors are not effective in all patients, suggesting other mechanisms exist to limit cytotoxic T-cell activity. We recently reported that T-cell MyD88 limits $CD4^+$ T-cell activation by tuning down T-cell receptor signaling in the context of cardiac inflammation. Whether this mechanism is similar in cytotoxic T-cells and could be harnessed for anti-tumor immunity is unknown. We hypothesized that MyD88 restrains $CD8^+$ T-cell activation and that deletion of MyD88 in T-cells enhances T-cell cytotoxicity in melanoma. **Methods:** We implanted SM1-750 melanoma cells into *Tcra*$^{-/-}$ mice, and once tumors were palpable, we adoptively transferred WT or MyD88$^{-/-}$ $CD8^+$ Type 1 cytotoxic T-cells (Tc1 cells). Tumor growth was measured 3 times weekly, and 4 weeks later tumors were harvested and leukocyte tumor infiltration was assessed by flow cytometry and immunostaining. We performed similar experiments using B16-OVA melanomas and WT or MyD88$^{-/-}$ Tc1 cells generated from OVA-antigen restricted OTI mice. We also implanted B16-OVA tumors on T-cell specific MyD88 knockout mice ($CD4^{Cre}MyD88^{fl/fl}$ – T-MyD88$^{-/-}$) and Cre^- littermate controls (T-MyD88$^{+/+}$). T-cell activation, survival, and cytotoxic capacity were assessed *in vitro.* **Results:** We found that mice receiving MyD88$^{-/-}$ Tc1 or OTI cells had slowed melanoma growth with or without the presence of OVA-antigen, compared to mice receiving WT Tc1 or OTI cells. In both models, mice receiving MyD88$^{-/-}$ cells also had greater tumor infiltrating $CD8^+$ T-cells and increased tumor cell death compared to those receiving WT cells. MyD88$^{-/-}$ Tc1 cells expressed higher phospho-ZAP70 and surface CD69 *in vitro*, both markers of T-cell activation. MyD88$^{-/-}$ T-cells also exhibited improved survival *in vitro* compared to WT cells as measured by propidium iodide incorporation using live cell imaging. Lastly, *in vitro* killing assays demonstrated MyD88$^{-/-}$ T-cells had higher cytotoxicity than WT cells against both melanoma cell lines. The adoptive transfer results were replicated in melanoma bearing T-MyD88$^{-/-}$ mice, which exhibited slower melanoma growth compared to T-MyD88$^{+/+}$ controls, as well as increased T-cell activation in draining lymph nodes and more tumor infiltrating T-cells. **Conclusions:** Together, these data demonstrate MyD88 regulates $CD8^+$ T-cell activation, cytotoxic ability, and survival, and that deletion of T-cell MyD88 might improve T-cell therapies in melanoma.

Abstract 125

Spatial Transcriptomics Identifies Epithelial Cell Control of Neutrophil Chemotaxis in the Human Female Genital Mucosa

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Introduction: The mucosal surface in the female reproductive tract (FRT) has evolved to meet the unique requirements that combine reproduction and protection against infection. The first barrier of protection is formed by the epithelial cell barrier, mucus and secreted immune mediators. In addition, neutrophil recruitment is crucial to sustain homeostasis, reproductive function and fight pathogens, being first-responder cells to sites of injury and initiators of the wound healing process. However, tissue location and regulation of neutrophils in the FRT during homeostasis remain undefined. In this context, we hypothesize that the epithelium regulates neutrophil chemotaxis in the FRT mucosa. **Methods:** We performed immunofluorescent (IF) staining of human endocervical tissue sections using neutrophil elastase (neutrophils) and pancytokeratin (epithelium) antibodies. Using spatial transcriptomics, neutrophils or epithelial cells were selected in regions of interest (ROIs) with a segmentation mask and RNA extracted from each area for sequencing. Whole transcriptome analysis was performed to identify differentially expressed genes (DEGs) between areas of interest and to execute gene ontology (GO) enrichment analysis. **Results:** Under homeostasis, neutrophils were located close to and in contact with the epithelium, and displayed transcriptional profiles related to chemotaxis. Subepithelial neutrophils showed a specific signature of enriched GO terms compared to neutrophils located deeper in the tissue, including Granulocyte chemotaxis (DEGs: *CXCL1, S100A9, ANXA1*), chemokine receptor activity (*CXCR6, CCR1, CCR5, CCR6*) and binding of specific ligands, including CCL5 (*CCR1, CCR5*), CCL7 (*CCR1*), and complement peptides C5a and C5L2 (*C3*). In addition, we observed by IF that subepithelial neutrophils did not show a homogenous distribution, but clustered beneath the epithelium in some areas, some of them attached to the basal layer of the epithelium or with interepithelial location, suggesting transepithelial migration. Based

on this observation, we analyzed spatially resolved transcriptome data of several ROIs containing epithelial cells, either with low number of neutrophils beneath (referred as “immunologically non-active”) or with clusters of neutrophils (“immunologically active”). We observed 243 upregulated DEGs in “active” epithelium, and 384 downregulated DEGs (“non-active” epithelium). Epithelial ROIs with clusters of neutrophils close to them showed enriched GO terms related to migration and chemotaxis of neutrophils, in addition to T cells, DCs, NK cells and monocytes. Conversely, epithelial cells with a limited number of neutrophils beneath them showed enriched GO terms inhibiting leukocyte migration, including neutrophils and mononuclear cells, and negatively regulating cell adhesion. **Conclusion:** These results evidence that the epithelium controls cell migration and exert a *pull effect* of immune cells for first-line barrier protection in the FRT. **Acknowledgement:** Funding: NIH grants R01-AG06801 and R21-AI172065

Basement Membranes

Abstract 126*
Laminin α5_CD239_Spectrin Is a Compensatory Linkage between Basement Membrane and Cytoskeleton in Skeletal Muscle Fibers
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Abstract 127*
Factors Involved in the Biosynthesis of Type IV Collagen (α1α1α2) in CHO cells
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*The authors of this abstract selected not to publish the details of their research

Abstract 128
Strategy for Making Functional Collagen Fragments: A Window into Collagen Biology and Therapy
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Introduction: Defects in collagens underlie the cause of nearly 40 human genetic diseases in millions of people worldwide. Pathogenesis typically involves genetic alterations of the triple helix, a hallmark structural feature that bestows exceptional mechanical resistance to tensile forces and a capacity to bind a plethora

of macromolecules. Yet, there is a paramount knowledge gap in understanding the functionality of distinct sites along the triple helix. Heterotrimeric types of collagen are challenging to make as they require approaches to ensure correct composition. The problem further escalates with a requirement to control a chain register (staggering), a unique structural feature of the collagen triple helix. Three identical (homotrimer) or different (heterotrimer) chains of GXY-repeated sequences form a superhelix with every third Gly placed near the axial center of the molecule. Such packing and inter-chain hydrogen bonding between backbones are only possible with staggering of three chains. **Methods:** To overcome these problems, we developed a hetero-trimeric cassette specific for collagenous sequences. The experimental strategy utilizes the unique property of the noncollagenous (NC) 2 heterotrimerization domain of collagen IX, which controls composition and staggering of collagenous sequences. **Results:** We produced collagen IV CB3 fragments of certain composition, demonstrated formation of thermally stable triple helical conformation, confirmed collagen-specific post-translational modifications and ability to bind α1β1, α2β1, and α11β1 integrin receptors. **Conclusions:** This recombinant technique is suitable for production of heterotrimeric collagenous fragments with or without interruptions of any type of collagen for structural studies, mapping functional sites, determining coding sequences of binding sites, elucidating pathogenicity and pathogenic mechanisms of genetic mutations, and production of fragments for protein replacement therapy.

Abstract 129

Compression Regulates Molecular Permeability of the Glomerular Basement Membrane

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Introduction: The glomerular basement membrane (GBM) provides a size selective barrier to passage of macromolecules such as albumin from the circulation into the glomerular filtrate. Loss of molecular selectivity and development of proteinuria is a key indicator of progressive kidney damage. Protein transport across the GBM is regulated by physical forces including filtration pressure. We evaluated the effects of pressure dependent gel compression on GBM molecular permeability. Kidney disease is associated with upregulation of matrix crosslinking enzymes including transglutaminases that may regulate GBM compression. We further determined the effects of transglutaminase crosslinking on pressure dependent molecular transport of the GBM. **Methods:** Glomeruli were isolated from porcine kidneys and decellularized by detergent extraction with deoxycholate. Decellularized glomeruli were compacted onto a permeable support to conduct molecular transport studies under applied pressure using Ficoll, a broad molecular weight tracer. Convective Ficoll transport studies were performed at varying transmembrane pressures to evaluate effects of GBM compression on molecular transport across the GBM. GBM was treated with the crosslinking enzyme transglutaminase to determine how increased GBM stiffness regulates pressure dependent molecular transport. **Results:** We found that native GBM exhibited pressure dependent compression and more stringent size selectivity at higher transmembrane pressures based on reduction in the Ficoll sieving coefficient with increasing pressure. This was attributed to a decrease in porosity of the GBM due to gel compression. Mathematical modeling of the GBM sieving coefficient showed that accounting for membrane compression effects was necessary to properly simulate the transport properties of the GBM. Transglutaminase mediated stiffening of the GBM mitigated compression effects on molecular transport resulting in an increased sieving coefficient in crosslinked GBM compared to native at higher transmembrane pressures. **Conclusions:** These data show that GBM has enhanced molecular size selectivity under applied pressure. Crosslinking and stiffening of the GBM effectively eliminates compression effects and renders the GBM more permeable to macromolecules under applied pressure. This suggests that disease-mediated increases in GBM crosslinking may contribute to increased molecular permeability in the setting of chronic kidney disease.

Abstract 130*

Using Zebrafish as a Novel Model for COL4A1-associated Cerebral Small Vessel Disease

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*The authors of this abstract selected not to publish the details of their research

Abstract 131

Determining the Collagen IV Biosynthetic Interactome and the Differential Roles of Collagen Modifying Enzymes

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Introduction: Collagen biosynthesis is a complex process relying on a series of highly orchestrated events mediated by a collection of endoplasmic reticulum resident proteins referred to as the collagen molecular ensemble. The molecular ensemble comprises a group of over twenty proteins that participate in collagen post-translational modification, folding and secretion, and mutations of genes encoding the collagen molecular ensemble can cause connective tissue disorders like those resulting from mutations in collagens, including osteogenesis imperfecta and Ehlers-Danlos syndrome. The proteins involved can differ between collagen types, and the molecular ensemble for fibrillar collagens, particularly collagen I, is the most investigated and well-understood. In contrast, relatively little is known about the molecular ensemble of collagen IV. **Methods:** In this study, we performed mass spectrometry analysis combined with an affinity pull-down assay to define the biosynthetic interactome of collagen a1a1a2(IV). **Results:** We used biochemical characterization of collagen a1a1a2(IV) from genetically engineered cells to validate and determine the functional roles of three key ensemble proteins – P4HA2, GLT25D1 and LH3. **Conclusions:** This work represents the first characterization of the collagen a1a1a2(IV) molecular ensemble and provides a platform to further define the essential steps of collagen a1a1a2(IV) biosynthesis and quality control.

Abstract 132

The Laminin β2 Chain Regulates the Selective Routing of Retinal Ganglion Cell Axons

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Introduction: Retinal ganglion cells (RGCs) are the output neurons of the retina. RGCs collect visual information and transmit it to the brain through their axons that collectively form the optic nerves and tracts. During formation of the visual pathway, RGC axons are selectively routed to the correct side of the brain. Routing occurs at the optic chiasm and is directed by the radial glia and midline neurons that reside therein (*Ann Rev Neurosci* 2008;**31**:295-315; *J Neurosci* 1995;**15**:3716-3729). These specialized glia and neurons adhere to the underlying pial basement membrane (PBM) that is rich in β2-containing laminins. Here we asked if β2-containing laminins present in the PBM control the selective routing of RGC axons. **Methods:** The proportional size of the retinal projections was determined by anterograde labeling of RGC axons with DiI in wild-type (WT) and Lamb2$^{-/-}$ mice at P4. The disruption in the radial glia and the PBM were determined by immunolabeling sections from WT and Lamb2$^{-/-}$ optic chiasms at the peak and late phases of axonal routing. Lastly, expression of EphrinB2, α-Dystroglycan, and GPR56 was determined by measuring their relative fluorescent intensities following immunolabeling of chiasm sections. **Results:** At P4, the selective routing of retinal axons is disrupted, with a larger proportion of ipsilateral axons entering the ipsilateral tract in β2$^{-/-}$ (46%) than in wild-type (WT) (40%) mice ($p<0.0001$). Analysis of the embryonic chiasm in β2$^{-/-}$ mice showed that the radial glia basal processes are disrupted with poorly defined endfeet that in some areas lose contact with the PBM. Concurrent with these changes, the expression of the laminin receptor α-DG is lost from the radial glia endfeet and processes (55%, $p=0.01$). Also, the expression of the collagen III receptor GPR56 is lost from the endfeet and the total cellular expression is reduced (76%, $p=0.014$). Interestingly, the expression of the ipsilateral cue EphrinB2 is abnormally increased in the radial glia during the late phase of axon routing, when ipsilateral axon routing has ceased (70%, $p=0.0004$). **Conclusions:** These data suggest that β2-containing laminins regulate RGC selective routing by providing cues that confer polarity to the radial glia and that decrease EphrinB2 expression to terminate the growth of axons into the ipsilateral optic tract. **Acknowledgements:** This work was supported by a Research to Prevent

Blindness (RPB) Career Development Award (RIMDL), an unrestricted RPB grant to the Department of Ophthalmology and Visual Sciences, and The Lion's Club of Central New York.

Abstract 133

Proteomics Analysis of the Pkd1nl/nl Mouse at P28 Identifies Novel Insights into Matrix Pathology Associated with ADPKD

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Introduction: >90% of cases of Autosomal Dominant Polycystic Kidney Disease (ADPKD) are caused by mutations in the PKD1 and PKD2 genes, which encode for polycystin-1 (PC1) and PC2, respectively. The polycystins form an ion channel that is important for proper ciliary function. Much effort has been made to investigate the molecular mechanisms driving pathology in ADPKD. However, a detailed analysis of the proteome in ADPKD, using up-to-date cutting-edge mass spectrometers, has not been performed. **Methods:** Using the latest mass spectrometry approaches, proteomics analysis was performed on kidneys taken at P28 from wild-type and Pkd1nl/nl mice. ThePkd1nl/nl is a Pkd1 hypomorphic mouse and recapitulates the pathology associated with ADPKD. 4 biological replicates were assessed and data processing was performed using MaxQuant and msqrob2 differential analysis. **Results:** Differential protein intensity analysis showed a number of previously identified markers of ADPKD are up-regulated in the Pkd1nl/nl model. This includes Lcn2, Acta2, multiple claudins, markers of leukocytes (such as Itgam), downstream effectors of the Yap/Taz Hippo pathway (eg Col12a1), and multiple markers of fibrosis (TgfB, Col1a1, Postn). Novel proteins were also detected that are likely to be important in pathogenesis, including multiple RhoGEFs, Thy1, Grb2 and CD44. We have a particular interest in cell-matrix interactions and how these underpin disease. Of note, we find multiple integrins are more highly expressed in ADPKD, with integrin alpha1, alpha3, beta1 and beta5 particularly up-regulated. Given loss of integrin beta1 is known to preclude cystogenesis, we are currently investigating further the roles of integrin alpha subunits in ADPKD, with the specific aim to generate therapeutics against these more desirable targets. **Conclusion:** Improved proteomics analyses have enabled greater understanding of ADPKD kidneys and highlights the power of the modern and cutting-edge mass spectrometers available to researchers. We have found multiple molecules that are likely to be important in disease progression in ADPKD and are developing ways to disrupt the function of these proteins with the aim of slowing down disease progression.

Cancer Pathobiology II

Abstract 134

PEGylated Functional Upstream Domain (PEG-FUD): An Anti-cancer Therapy for Breast Cancer

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Introduction: Breast cancer (BC) is the most common cancer diagnosed in women. In breast cancers, the extracellular matrix (ECM) is known to play a key role in disease progression by mediating cell signaling processes that drive cancer cell proliferation, migration and invasion. Work by our group identified that aligned collagen fibers perpendicular to the tumor boundary are prognostic of poor patient prognosis. Further, through matrix targeted proteomics we determined that the ECM protein, Fibronectin (FN), organizes with aligned collagen fibers in BC tissues. FN is known as the master regulator of ECM assembly because FN deposition precedes and regulates the deposition of several other ECM proteins. Clinical evidence shows that high expression of FN levels in BC patients correlates with an increased mortality risk. Due to the abundant expression of FN in the tumor microenvironment, FN has been a useful biomarker for cancer imaging and therapy. Despite multiple efforts in developing therapies that target the ECM, currently there are no effective ECM treatments available due to toxicity and lack of specificity. **Methods:** To target abnormal FN deposition, we used a FN binding peptide, Functional Upstream Domain (FUD), derived from the F1 adhesin from *Streptococcus pyogenes*. PEGylated-FUD (PEG-FUD) is a potent inhibitor of FN assembly which binds the 70 kDa N-terminal region of FN with high affinity. We hypothesize that PEG-FUD will localize to the tumor site and that targeted disruption of FN assembly with PEG-FUD will reduce ECM

deposition, thus block tumor progression in-vivo. In this study, we used an in vivo imaging system to assess the biodistribution of 20-kDa PEG-FUD following subcutaneous injection of Cy5 labeled peptide in 4T1 mammary tumor bearing mice. Additionally, in a second therapeutic experiment, we treated 4T1 mammary tumor bearing mice with 20-kDa PEG-FUD every 48 hr for a total of 10 treatments and measured tumor volume as an indicator of primary tumor burden. **Results:** As anticipated, we observed PEG-FUD's accumulation and localization in 4T1 tumors. Additionally, PEG-FUD treatment significantly reduced tumor growth compared to saline treatment. There were no observed changes in standard toxicity assessments such as body weight and spleen weight among treatment groups. After PEG-FUD's therapeutic treatment, we observed a significant reduction in the deposition of FN matrix and an increase in cleaved caspase-3, a known marker for apoptosis by western blotting providing a potential mechanism of action of PEG-FUD inducing changes in tumor growth. **Conclusions:** PEG-FUD's localization in tumors suggests its utility as a solo cancer imaging agent while providing indications that PEG-FUD can be used to deliver other therapeutics to the tumor site as a conjugate in the future. In addition, therapeutic treatment of PEG-FUD inhibited tumor growth providing preliminary evidence for PEG-FUD's use as a tumor targeting anti-cancer agent.

Abstract 135

Effects of Biological Sex on Tumor-Mediated Muscle Dysfunction and Wasting

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Introduction: Cancer cachexia is an untreatable disease characterized by the excessive catabolism of fat and muscle for energy. Emerging evidence indicates that cancer cachexia also presents in a sexually dimorphic manner. Therefore, the purpose of this study was to determine the underlying biological sex-mediated mechanisms of cancer cachexia. **Methods and Results:** Male (M) and female (F) LC3 Tg+ mice and wildtype littermates were implanted with tumors (T; 1x10^6 LLC cells in flank) or remained non-tumor (NT) for 21 days. In this dual tagged LC3 Tg+ autophagy reporter mouse model, early phase autophagosomes appear yellow (GFP+RFP) while late phase autolysosomes appear red (RFP only). Cardiac autophagy analyses showed M+T resulted in a significant increase in both autophagosomes ($P<0.05$) and autolysosomes ($P<0.05$) compared to M+NT, and significantly greater early phase autophagosomes compared to F+T ($P<0.05$). In vivo cardiac function (echocardiography) assessment revealed that tumor bearing (T) resulted in cardiac dysfunction in both males and females, and males exhibited a significantly greater decline in fractional shortening compared to females ($P<0.01$). Both M+T and F+T exhibited significantly increased cardiac p-NF-kB protein expression compared to respective NT controls. Interestingly, only cardiac protein IL-6 was significantly increased and p-FOXO3a was decreased in F+T vs F+NT ($P<0.05$). Only cardiac IGF-1beta was significantly decreased in M+T vs M+NT ($P<0.01$). In skeletal muscle, autophagy analyses showed M+T exhibited significantly greater autophagosomes ($P<0.05$) and autolysosomes ($P<0.05$) compared to M+NT, and M+T showed significantly greater late phase autolysosomes compared to F+T ($P<0.05$). Additionally, only skeletal muscle p-NF-kB and IL-1beta protein expression was significantly increased in M+T vs M+NT ($P<0.05$). While tumor-bearing resulted in significant muscle weakness in males and females, M+T showed a significantly greater decline in muscle strength compared to F+T ($P<0.05$). This coincided with M+T growing significantly larger tumors compared to F+T ($P<0.05$). **Conclusions:** These data support the notion that tumor-mediated muscle wasting and dysfunction present in a sexually dimorphic manner. While tumor bearing imposed cardiac and skeletal muscle dysfunction in both males and females, males exhibit a worse phenotype and larger tumors. Tumor-mediated cardiac atrophy appears to be slightly more metabolically driven, while male cardiac atrophy may be slightly more driven by changes in growth hormone signaling. Tumor-mediated skeletal muscle dysfunction appears to be largely driven by inflammation in males, leading to elevated levels of autophagy and muscle atrophy. These data are critical to understanding how muscle wasting develops differently in males and females, and help illuminate therapeutic targets aimed at cancer cachexia.

Abstract 136

Tumor Bearing Results in Metabolic Dysfunction and Skeletal Muscle Wasting in Apc(min/+) Mice

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Introduction: Cancer cachexia is a metabolic wasting syndrome that affects up to 80% of cancer patients and leads to death in up to one third of these patients. While research is growing in the field, there are currently no clear diagnostic criteria nor effective treatments. Most patients progress undetected to late stages of cancer cachexia and become unresponsive to traditional treatment. The purpose of this study was to determine the effect of spontaneous tumor growth on skeletal muscle function at different time points and identify the underlying molecular pathways associated with cancer-induced muscle wasting. **Methods:** To determine the effect of tumor burden on cancer cachexia progression and skeletal muscle function, body weight and grip strength measurements of Apc(min/+) and litter-matched non-carrier mice (WT) were collected at 12 weeks, 16 weeks, 20 weeks, and 24 weeks of age. At 24 weeks of age, gastrocnemius tissue was collected, weighed, and normalized to body weight at sacrifice. To determine underlying molecular pathways associated with skeletal muscle wasting and cancer cachexia, gastrocnemius tissue was homogenized and analyzed via Western Blotting. **Results:** Tumor bearing resulted in a significant decline in body mass overtime ($P<0.05$) indicating progressive cancer cachexia. While there were no significant differences at baseline, body mass of Apc(min/+) mice was significantly lower ($P<0.05$) compared to WT mice at sacrifice. Similarly, tumor bearing resulted in a significant decline in grip strength ($P<0.05$) from the 12-week to 24-week timepoints. Grip strength normalized to body weight at sacrifice was significantly ($P<0.05$) lower in Apc(min/+) mice compared to WT mice. Cancer-induced body mass and grip strength loss coincided with significantly lower P-Akt/Akt and P-FoxO1/FoxO1 protein expression and upregulated MuRF1 protein expression in Apc(min/+) compared to WT mice. **Conclusion:** These data shed light on the metabolic alterations and skeletal muscle wasting associated with cancer cachexia that leads to skeletal muscle dysfunction and atrophy. Such data is crucial to understand the metabolic consequences and underlying molecular pathways of cancer cachexia to identify potential therapeutic targets.

Abstract 137

Identification of the Roles of ADAMTS12 Secreted by Stellate Cells during Tumor Progression in Cholangiocarcinoma

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Introduction: Cholangiocarcinoma (CCA) is the second most prevalent liver cancer. The stroma of CCA is characterized by an abundance of cancer associated fibroblasts (CAFs) deriving mainly from the activation of hepatic stellate cells (HSCs). We have recently demonstrated that the metalloproteinase ADAMTS12 is overexpressed by activated HSCs present in the tumor stroma, while preliminary data suggest that its expression could regulate the activation levels of HSCs. Our main goal is therefore to unravel the role of ADAMTS12 in the dialog establishing in the tumor microenvironment between HSCs and cancer cells. **Methods:** Through a Crispr/Cas9 approach followed by the use of lentiviral vectors, we have generated LX2 cells (human HSC line) deficient in endogenous ADAMTS12 expression, but able to conditionally express recombinant ADAMTS12 upon induction with doxycycline. As an *in vitro* model of cancer, we use precancerous organoids derived from mouse cholangiocytes carrying the *Kras*^{G12D} mutation specifically in cholangiocytes (Di-Luoffo M et al., Hepatology, 2021;74(3):1445-1460). These two models, alone or combined, are being used to evaluate the effect of ADAMTS12 expression on the activation of HSCs (transcriptomic and proteomic analyses), and to characterize the role of ADAMTS12 in the dialog between HSCs and cancer cells. Mice deficient in Adamts12 (TS12$^{-/-}$) are also available for the study of cancer progression *in vivo*. **Results:** *Kras*^{G12D} organoids, but not wild-type organoids, initiate tumor formation when injected subcutaneously in mice. The tumors consist of desmoplastic cysts-like cholangiocarcinoma in which the epithelium is predominantly monolayered. The epithelium also displays transitional areas with loss of polarity, multilayer formation, loss of epithelialization and invasion through the basal lamina, mimicking the precancerous lesions found *in vivo*. *Kras*^{G12D} organoids cultured *in vitro* show modulation in several EMT markers (Krt19, vimentin, N-cadherin) when HSC are present in the surrounding matrix, showing their impact on the organoid phenotype and demonstrating the relevance of our model. We are currently evaluating these regulations in the presence and absence of ADAMTS12 (+ or – doxycycline). An

additional model was used to evaluate the consequences of ADAMTS12 expression on tumor growth *in vivo*. We have shown that Lewis Lung Carcinoma cells form tumors growing slower in ADAMTS12-KO mice, compared to "wild-type" mice, confirming that ADAMTS12 can regulate tumor progression. **Conclusion:** We have developed original models to study the implication of ADAMTS12 in different stages of tumor progression. Our current data suggest that ADAMTS12 is overexpressed in the tumor microenvironment, where it could potentially display a "pro-tumor" function. Future work will focus on the characterization of the involved mechanisms, notably by identifying the factors whose cleavage by ADAMTS12 could be involved in the observed regulations.

Abstract 138

Dissecting the Role of miR-146a in Metabolic Dysfunction-Associated Steatohepatitis and Hepatocellular Carcinoma

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Introduction: The incidence of hepatocellular carcinoma (HCC) in the United States is on the rise and is expected to continue to increase by 2030. Chronic liver disease as a result of metabolic dysfunction-associated steatohepatitis (MASH) is a major contributor to HCC. MASH is characterized by hepatic inflammation and fibrosis that may progress to cirrhosis and/or HCC Activating mutations in the *CTNNB1* gene encoding β-catenin are found in ~30% of patients with HCC. Our lab has characterized a transgenic zebrafish model of activated β-catenin-driven HCC (ABC-HCC), characterized by liver hyperplasia and increased liver size at 6 days post fertilization (6 dpf) and HCC within months. MicroRNAs (miRNA) are small RNA molecules of 20-22 nucleotides that negatively regulate the translation of mRNA into protein, are readily taken up by the liver, and have shown therapeutic promise in treating diseases of the liver. miRNA dysregulation plays an important role in liver pathologies such as MASH and HCC, and has potential for targeted treatment. One miR of interest is *miR-146a* with anti-inflammatory and tumor suppressor properties. *miR-146a* has been characterized broadly in HCC, but not in MASH or MASH-HCC. **Methods:** To characterize miRNAs in MASH, MASH-HCC, and ABC-HCC zebrafish, we quantified miRNA levels in liver tissues from patients with MASH (without HCC) and tumor/non-tumor liver tissues of patients with MASH-HCC. We analyzed these data alongside HCC miRNAseq data from The Cancer Genome Atlas (TCGA). To characterize the role of *miR-146a* in HCC, we developed two transgenic lines of zebrafish to overexpress (*miR-146aOE)* or to sponge/knock-down (*miR-146aSP) miR-146a* specifically in hepatocytes. We then crossed these to wild-type or ABC zebrafish to look at the effects of *miR-146a* dysregulation on liver size at 6 dpf. **Results:** We identified significant overlap of 21 miRNAs between clinical samples of MASH, MASH-HCC, TCGA and ABC-HCC zebrafish. *miR-146a* was upregulated in MASH and downregulated in MASH-HCC, ABC-HCC zebrafish, and TCGA. At 6 dpf *miR-146aOE* zebrafish had no change in liver size, but at 6 dpf *miR-146aSP* had significantly larger livers compared to non-transgenic control siblings. Conversely, we found that ABC;*miR-146aOE* zebrafish had significantly smaller livers, whereas ABC;*miR-146aSP* zebrafish had significantly larger livers than ABC control siblings. **Conclusions:** We found conserved changes in miRNA expression during zebrafish and human hepatocarcinogenesis. The finding that *miR-146a* overexpression suppresses β-catenin-driven liver overgrowth, while sponging *miR-146a* enhances liver overgrowth, supports the hypothesis that *miR-146a* plays a protective role in HCC. Future directions include dissecting the protective mechanism(s) of *miR-146a* such as its effect on inflammation, proliferation, and metabolism.

Abstract 139

Gp78 Expression Activates Breast Cancer Tumor Growth Through Immunogenetic, Lipogenetic, and Endoplasmic Reticulum Stress Relief Pathways

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Introduction: Breast cancer - a leading cause of death among women in the United States - is deadliest among women of African descent. Although a variety of determinants are believed to contribute to racial disparities in breast cancer survival, emerging cancer pathology research demonstrates the significant role of tumor biology in predicting breast cancer survival outcomes based on race. More specifically, the expression of glycoprotein 78 - an E3 ubiquitin ligase that inhibits the accumulation of unfolded proteins in

the endoplasmic reticulum - has been discovered to be an independent predictor of poor survival in breast cancer patients of African ancestry. **Methods and Results:** This study investigates the role of gp78 expression in breast cancer tumor growth through its activation of immunogenetic, lipogenetic, and endoplasmic reticulum stress relief pathways. An analysis of in vitro 4T1 cell lines, in vivo syngeneic allograft murine modeling, protein immunoblotting, lipidomic profiling, and immunohistochemistry staining experiments implicates gp78 expression as a catalyst for tumor growth through the stimulation of lipogenesis, the induction of adaptive immunosuppression, and the mitigation of tumor cell autophagy through endoplasmic reticulum stress relief. **Conclusion:** These insights offer new targets for therapeutic interventions that have the potential to reduce racial disparities in breast cancer survival outcomes.

Abstract 140

P120 Expression Mitigates Kaiso Expression-Associated Survival Risk in Breast Cancer Patients of African Ancestry

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Introduction: Breast cancer - a leading cause of death among American women - is especially deadly among American women of African descent. Although a multitude of factors are understood to play a role in survival outcome disparities between African-descended and European-descended breast cancer patients, advancements in cancer pathology research reveal the significant role of protein expression and protein-protein interactions in the tumor microenvironment. Two proteins of interest include Kaiso - a transcription regulator associated with poor breast cancer survival - and P120 - a catenin that facilitates cell-cell adhesion and signaling pathways. **Methods and Results:** This study investigates the population-specific role of P120 expression in attenuating Kaiso expression-associated survival risk among African-descended breast cancer patients. Survival analysis of data generated through immunohistochemistry experiments implicates P120 expression as a disproportionately greater protective agent against both nuclear and cytoplasmic Kaiso expression in African-descended breast cancer patients when compared to their European-descended counterparts. **Conclusion:** While additional research is needed to determine the specific causation of this phenomenon, this unanticipated discovery offers novel insight into population-specific breast cancer survival outcome disparities, and can serve as an impetus for future population-specific predictive, prognostic, and therapeutic clinical approaches.

Abstract 141

Inhibiting Melanoma-Associated Axonogenesis Using Semaphorin-3F

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Introduction: Tumor cells secrete growth factors and cytokines which stimulate surrounding blood vessels to sprout towards the tumor. This process of neovascularization supplies increased oxygen and nutrients to the tumor as well as provides a route for tumor cell escape or spread to distant organs. Similarly, tumor cells stimulate neo-neurogenesis from peri-tumoral nerves. Increased axonal density in and around solid tumors correlates with cancer aggressiveness and dissemination. Our overall goal is to uncover molecular mediators of tumor-induced axonogenesis in order to target these pathways to inhibit cancer progression and improve patient prognosis. During embryonic development, neuronal guidance and angiogenesis are mediated, in part, by cell surface receptors called Neuropilin-1 (NRP1) and Neuropilin-2 (NRP2) that bind to secreted class 3 semaphorin (SEMA3) proteins. Tissue patterning of nerves and vessels is governed by the directionality of the chemorepulsive SEMA3 ligand and the localization and specificity of the NRP receptor. Our hypothesis is that tumor neurogenesis can be regulated by the SEMA3/NRP signaling pathways and that SEMA3 proteins can be used therapeutically to prevent or diminish axon density in the tumor microenvironment to decrease cancer metastasis and improve survival. **Methods:** To test our hypothesis, immunohistochemistry was used to detect neurons in human melanoma xenografts overexpressing SEMA3A or SEMA3F compared to parental metastatic melanomas implanted in immunodeficient mice. Alternately, axonal density was also compared in syngeneic melanoma isografts implanted in *Nrp2*-deficient mice compared to *Nrp2*-wildtype mice. *In vitro*, repulsion assays were performed using neuronal cell lines (PC12, SH-SY5Y) and cells stably transfected with SEMA3A or SEMA3F. Immunoblotting and immunocytochemistry were used to examine the expression of NRP1 and

NRP2 in the neuronal cell lines. **Results:** Our results from SEMA3A or SEMA3F chemorepulsion *in vitro* and *in vivo* reveal that tumor-associated neurons behave similarly to endothelial cells expressing NRP1 or NRP2. Specifically, SEMA3A repels cells expressing NRP1 while SEMA3F repels NRP2-expressing cells. **Conclusions:** Overall, our conclusions suggest that SEMA3F treatment may be a potent therapy to inhibit multiple processes in the tumor environment that contribute toward metastasis including angiogenesis, lymphangiogenesis, and neurogenesis.

Abstract 142

Exploring the Role of Obesity-Induced Extracellular Matrix Remodeling in the Progression of Breast Cancer

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Introduction: Breast cancer is the most prevalent invasive cancer in women globally, with obesity being a major risk factor associated with its incidence and mortality. Obesity, prevalent in over 15% of women worldwide and more so in developed countries, has been implicated in the progression from Ductal Carcinoma In Situ (DCIS) to Invasive Ductal Carcinoma (IDC). However, a comprehensive understanding of how obesity impacts breast cancer progression, particularly the early stages of invasion, is constrained by the dearth of pertinent models. In a retrospective study on clinical breast specimens, we measured gene expression profiles of DCIS and IDC in women with normal or high body mass index (BMI). IDC in normal weight women had similar gene expression to DCIS in women with obesity, suggesting an obesity-induced, accelerated progression towards invasive disease, and involved key genes related to extracellular matrix (ECM) remodeling and epithelial-to-mesenchymal transition. We hypothesize that tumor extrinsic mechanisms, particularly ECM alterations due to obesity, contribute to this accelerated progression. **Methods:** We developed a novel 3D in vitro model by isolating adipose ECM from both lean and obese environments to mimic the in vivo microenvironment of the tumor. We cultured human breast cancer cells in ECM from lean or obese rats or mice and measured spheroid size and growth rates. We used untargeted mass spectrometry to measure the matrix proteome from adipose tissue and performed pathway analysis of enriched networks in lean and obese conditions. Immunoblot and PCR analyses were used to measure cellular changes in signaling molecules and genes associated with breast cancer progression. **Results:** Preliminary findings from the study demonstrated a significant increase in breast cancer cell proliferation when cultured in obese ECM over two days indicative of an obesity-induced ECM shift fostering tumor cell proliferation. Gene expression analysis of human tumors revealed a distinct upregulation of ECM-related genes that corresponded to features of aggressive breast tumors only in the context of obesity. Additionally, there was a greater abundance of ECM related proteins in obese adipose thus providing a plausible mechanism for enhanced proliferation of MCF7 parental and MCF7 tamoxifen resistant cells in the obese ECM environment. **Conclusion:** Our study highlights that obesity-induced ECM remodeling is an imperative factor in the progression of DCIS to IDC in breast cancer. These findings present promising implications for the development of therapeutic strategies that target the ECM in obese breast cancer patients, aiming to attenuate obesity-induced breast cancer progression and resistance to treatment. **Acknowledgements:** This work was supported by R01 CA241156 to EAW.

Abstract 143

Novel Multianalyte Biomarker Panel for Early Detection of Ovarian Cancer Leveraging the Matrisome

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Introduction: Ovarian cancer (OC) is a deadly disease, with the 3rd highest mortality to incidence ratio of all cancers. OC's asymptomatic nature early in the disease causes identification at advanced stage in more than 70% of patients, at which point the 5-year survival rate can be as low as 20%. Thus far, no single

biomarker has been able to detect early OC with high sensitivity and specificity. While elevated levels of CA125 have been used preoperatively to monitor patients, CA125 sensitivity is suboptimal. Therefore, there is an essential need for the development of sensitive and specific biomarkers for early diagnosis of OC in order to identify women at high risk for the presence of a malignancy. Furthermore, biomarkers to differentiate between OC and other forms of cancer as well as benign pelvic masses are critically needed in the clinical setting. **Methods**: 288 plasma samples from women with OC (at various stages), benign pelvic conditions, other cancer types, and healthy non-cancer controls were analyzed with a curated panel of biomarkers that included the following matrisome-associated secreted factors – Growth Differentiation Factor 15 (GDF-15), Interleukin-6 (IL-6), Prolactin (PRL), Leukemia Inhibitory Factor (LIF), Hepatocyte Growth Factor (HGF) – and established ovarian cancer biomarkers – Cancer Antigen 125 (CA-125), Human Epididymis Protein 4 (HE-4), and Osteopontin (OPN). Receiver-operating characteristic (ROC) analysis was performed to assess sensitivity and specificity of single and multi-biomarkers in the different subgroups. **Results**: Univariate ROC analysis identified HE-4 and GDF-15 as having highest area under the curve (AUC) (AUC=0.868, AUC=0.850, respectively) when comparing all stages of OC to healthy control subjects, a value higher than that of CA125 (AUC=0.7621). GDF-15 also had the highest AUC when differentiating between early OC and healthy controls (AUC=0.772), a value significantly greater than CA-125 (AUC=0.607). Among the univariate markers, LIF was able to significantly differentiate between several other types of cancer (peritoneal, pancreatic, lung, and stomach) and OC better than any other marker. Multivariate analysis identified the panel containing CA125, GDF-15, HE-4, IL-6, OPN, and PRL as having highest AUC when comparing OC to healthy cancer controls (AUC=0.956 for all stages) and to benign conditions (AUC=0.826 for all stages). Adding LIF to this model allowed for best differentiation between OC and other types of cancer (AUC=0.805 for all stages). **Conclusion**: These results validate a novel biomarker panel for early detection of OC as well as distinction of benign masses and other malignancies from OC, which includes matrisome biomarkers not previously extensively studied in OC (GDF-15, IL-6, PRL and LIF). Our results are expected to have an important positive impact by accurately detecting premalignant changes or early-stage OC in asymptomatic women. **Acknowledgments:** This project was supported by NIH/NIGMS Center for Cancer Biology 5P20GM103548 and NIH/NCI R21CA259158.

Abstract 144

Unraveling the Molecular Basis of SNED1-Mediated Cell Adhesion

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Introduction: The extracellular matrix (ECM) is a complex meshwork of proteins that plays critical roles in health and disease. My mentor, Dr. Naba, developed proteomic approaches to understand the ill-studied complexity of ECM. This led to the identification of the novel ECM protein, SNED1, and, for the first time, established its role as breast cancer metastasis promoter. Interested in determining the physiological roles of this novel ECM protein, our lab generated a *Sned1* knockout mouse and found that *Sned1* is an essential gene since its deletion led to early peri-natal lethality due to craniofacial malformations resulting from altered neural crest cells (NCC) migration. Interestingly, cancer cells and NCCs share common features including high migratory and invasive potentials, two processes highly dependent on cell-ECM adhesions. However, the molecular mechanisms behind SNED1-mediated breast cancer metastasis and craniofacial development are unknown. Integrins are known receptors mediating cell-ECM adhesions. Our *in-silico* sequence analysis revealed that SNED1 contains two putative integrin-binding motifs: RGD and LDV. In addition, using molecular modeling, we predicted that SNED1 can interact with 7 integrin heterodimers including α5β1 and α4β1, known interactors of the RGD and LDV motifs, respectively. This led us to hypothesize that SNED1 may facilitate cell-ECM adhesion through integrins. **Methods:** We engineered 293T cells to stably express His-tagged SNED1 and designed a pipeline to purify SNED1-His secreted into the cell-culture medium using affinity chromatography. Using site-directed mutagenesis, we disrupted the putative integrin binding sites of SNED1 alone (RGD>RGE, LDV>LAV) or in combination. We then used purified SNED1 to coat 96-well plates and tested cells' ability to adhere to wild-type or mutant forms of SNED1 using the gold standard crystal-violet-based colorimetric assay. **Results:** Our results show that SNED1 promotes both breast cancer and neural crest cell adhesion in a dose-dependent manner. Mutation of the RGD motif of SNED1 led to reduced cell adhesion. However, we did not observe a decrease in cell adhesion when the LDV motif was mutated. This suggests that SNED1 mediates cell adhesion through its

RGD motif. In addition, using function-blocking antibody against β1 integrin, we observed a decrease in cell adhesion further strengthening our hypothesis that SNED1-mediated cell adhesion occurs via integrin binding. **Conclusion:** We report here, for the first time, that SNED1 interacts with β1 integrin to mediate cell adhesion. Cell adhesions provides traction forces and organizes signaling networks to regulate migration of cancer cells during metastasis and of neural crest cells during craniofacial development and both are shown to be regulated by SNED1. Future work will focus on identifying the integrin heterodimer/s that interact with the RGD motif of SNED1 and elucidate the downstream molecular signaling events.

Abstract 145

TIMP2 as an Anti-tumor Homeostatic Regulator in a Lewis Lung Carcinoma Mouse Model

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Introduction: Tissue Inhibitor of Metalloproteinase 2 (TIMP2) is one of four paralogous mammalian TIMPs that are classically defined as inhibitors of metalloproteinase (MP) activity. TIMP2 also has a number of MP inhibition-independent functions that include regulatory control over cell growth, invasion, and gene expression. Furthermore, TIMP2 is widely expressed in normal tissues. Mounting evidence suggests that forced expression of TIMP2, or treatment with recombinant TIMP2 (rTIMP2), can reduce tumor burden and metastasis. Recently, we demonstrated and published that daily administration of rTIMP2 slows primary tumor growth and significantly reduces pulmonary metastasis in a murine triple-negative breast cancer model. The aim of the study is to address the role which TIMP2 plays in primary tumor growth and metastasis in Lewis lung carcinoma (LLC). **Methods:** We utilized an orthotopic, syngeneic murine model of LLC in wild-type (WT), and mutant TIMP2 (mT2) mice lacking exons 2 and 3 of the mature TIMP2 protein. Using the LL/2 cell line, we compared tumor growth and metastasis in WT and mT2 mice with and without daily rTIMP2 treatment following intratracheal instillation of LL/2 cells. Tumor volumes were measured using bioluminescence imaging (BLI) and caliper measurements. RNA sequencing, immunoblotting, and immunohistochemistry were performed to assess the biological outcomes of these studies. **Results:** Our investigation demonstrated a marked increase in tumor growth in mT2 mice compared with WT controls. Tumor bearing mT2 mice also show a significant increase in mortality. Additionally, analysis of CD31 staining reveals a considerable increase in intratumor vasculature, consistent with enhanced tumor angiogenesis and diminished TIMP2 inhibition of tumor neovascularization in mT2 mice, likely contributing to enhanced primary tumor growth. Furthermore, mT2 mice also exhibit elevated levels of myeloid cell subtypes compared to WT mice. Daily treatment with rTIMP2 (200ug/kg/day) reduces primary tumor growth and metastasis in WT and mT2 mice, supporting the idea that TIMP2 displays anti-tumor functions in LLC. RNA sequencing analysis comparing (1) tumor-free lungs, (2) tumor-bearing lungs, and (3) rTIMP2 treated tumor-bearing lungs reveals that rTIMP2 treatment can effectively normalize the transcriptome of tumor-bearing lungs. The rTIMP2 treated tumor-bearing lungs more closely resemble healthy lung tissue, with observations confirmed by immunoblotting, supporting the notion that TIMP2 displays homeostatic functions in diseased tissues. **Conclusions:** In summary, we describe how TIMP2 displays anti-tumor effects in LLC in both WT and mT2 mice. rTIMP2 treatment restores homeostasis and supports a normalizing effect within tumor-bearing lungs that may represent a novel means of therapeutically targeting tumors in concert with other concurrent treatment options.

Abstract 146

Integrin α3β1 Regulates *Mmp9* mRNA Alternative Polyadenylation in Skin Tumors and Wounds

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Introduction: Tumors have been described as wounds that do not heal due, in part, to similarities in signaling mechanisms that promote cell proliferation, migration, and extracellular matrix remodeling. These mechanisms are tightly regulated in wounds, however, dysregulated in tumor cells. The laminin-binding integrin, α3β1, regulates diverse cellular functions and gene expression in epidermal keratinocytes, and it is upregulated in skin tumors and wounds. A large portion of the keratinocyte secretome is regulated by α3β1, including matrix metalloproteinase-9 (MMP-9). Mmp9 promotes invasion, metastasis, and

angiogenesis in many cancers, and it is upregulated in early stages of wound healing. We have shown that, in immortalized mouse keratinocytes (IMK), α3β1 regulates *Mmp9* mRNA expression through alternative polyadenylation (APA) that determines mRNA stability. Presence of α3β1 promotes a shorter and more stable *Mmp9* mRNA transcript that is amenable to translation. Conversely, absence of α3β1 causes a switch to a longer transcript that contains destabilizing AU-rich elements in the 3'UTR, priming the transcript for degradation. However, it has not been known whether this α3β1-dependent switch occurs *in vivo* or the extent to which α3β1 regulates APA of other genes in skin tumors and/or wound keratinocytes. **Methods:** To investigate α3β1-dependent APA of *Mmp9* mRNA *in vivo*, we used our inducible, epidermis-specific α3 knockout mouse model in combination with established models of (1) two-step skin tumorigenesis or (2) cutaneous wound healing. Using RNAscope probe sets that target either both *Mmp9* mRNA variants (total *Mmp9*) or only the long variant, we determined the percentage of the total transcript that is comprised of the long variant. Furthermore, we performed RNA-Seq of IMK cell lines that express or lack α3β1 and applied bioinformatics (DaPars2) to identify the α3β1-dependent effect on APA of other genes. **Results:** Upon α3β1 ablation in skin tumors, we observed a decrease in total *Mmp9*, as seen previously. Importantly, ablation of α3β1 promoted a switch to the generation of the long, unstable transcript that correlated with a decrease in *Mmp9* mRNA and protein. Our preliminary study in cutaneous wounds similarly revealed an increase in the long transcript following ablation of α3β1 by 3 days post-wounding, although we did not detect a statistically significant difference in total *Mmp9* at this time point. RNA-seq analysis identified several genes with alternative mRNAs that appeared to be generated through α3β1-dependent APA, which we will validate in our cell culture and *in vivo* models. **Conclusions:** Our findings support a novel model of integrin-mediated regulation of APA in both skin tumors and wounds, and they suggest that the regulation of APA may represent an α3β1-dependent mechanism that extends to the regulation of many genes.

Abstract 147

β-catenin Negatively Influences B Cell Recruitment to the β-catenin-mutated Hepatocellular Carcinoma Microenvironment Correlating to Patient Outcomes

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Introduction: Current immune checkpoint blockade (ICB) regimens for hepatocellular carcinoma (HCC) are principally based on blocking T cell and tumor cell interactions, which have shown improved survival outcomes for subsets of HCC patients. β-catenin-mutated HCC patients, which are 25-35% of all HCC patients, demonstrate limited benefit to ICB. The mechanisms contributing to lack of ICB response in β-catenin-mutated HCC patients remains poorly understood. Recently, investigations into other solid tumors have demonstrated that B cells aggregated in tertiary lymphoid structures (TLS) are observed in patients with strong ICB response. Here, we investigate the role of B cells in β-catenin-mutated HCC using mouse models and clinical datasets. **Methods:** HCC patients with and without CTNNB1 (encoding β-catenin) mutation were queried from The Cancer Genome Atlas (TCGA) dataset. We performed immunohistochemistry (IHC) for B cell markers in our HCC mouse models of T41A-CTNNB1-G31A-NFE2L2, S45Y-CTNNB1-hMET, cMYC-hMET, and FGF19-hMET. Also, β-catenin-mutated models were treated with an antisense oligonucleotide targeting β-catenin to test influence of β-catenin on immune cell infiltration. Lastly, bulk RNA-sequencing was performed on β-catenin knockout (β-KO) and β-catenin-mutated livers to address potential upstream regulators which are influencing β-catenin-driven B cell recruitment. **Results:** We identified 97 β-catenin-mutated HCC patients, which had low expression of 101/200 genes from a previously reported B cell gene signature. We also observed that β-catenin-mutated HCC patients who had increased expression of multiple B cell surface markers (CD20, CD37, CD38, and CD79A) had improved disease-free survival. In our HCC mouse models, we observed decreased $CD20^+$ and $CD79A^+$ immune infiltration and fewer lymphoid aggregates via IHC in the β-catenin-mutated HCC mouse models compared to both the non-β-catenin mutated HCC mouse models. Using antisense

oligonucleotide to suppress β-catenin in our β-catenin-mutated HCC mouse models, resulted in an influx of B cells to the tumor microenvironment (TME) and correlated with reduced tumor burden, as evident by reduced liver weight/body weight. Analysis on the DEGs comparing mouse livers with and without antisense oligonucleotide demonstrated alterations in pathways in B cell activation, proliferation, and lineage commitment using the Molecular Signatures Database. To address mechanism of β-catenin driving a B cell excluded TME, we performed promoter enrichment analysis on the DEGs comparing β-KO and β-catenin-mutated livers and found low expression of the transcription factor POU2F1, which is a known regulator of CXCL13 (a known chemokine which promotes B cell recruitment and TLS formation). **Conclusions:** β-catenin-mutated HCCs drive an immune excluded TME, including absence of B cells. Future studies aim to investigate the functional relevance of the β-catenin/POU2F1/CXCL13 axis in driving ICB response.

Abstract 148

Evidence that Tumor/Fibroblast Crosstalk Potentiates ESR1 Mutant BC Cell Malignancy

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Introduction: Breast cancer (BC) is the most common diagnosed cancer in women worldwide. Approximately, 70% of all BC express the estrogen receptor alpha (ERα), thus the gold standard treatment for BC is endocrine therapies (ET) aiming to interfere with estrogen-mediated effects. However, tumor cells may do not initially respond (de novo resistance) or may later develop resistance to ET (acquired resistance) leading to metastatic BC. Particularly, acquisition of somatic mutations within the hormone-binding domain (HBD) in the gene encoding for ERα, *ESR1*, is one of the major contributor to the resistance and poor clinical outcome in metastatic BC. Besides, a growing amount of evidence also demonstrated that components of tumor microenvironment (TME) might additionally interfere with therapy response. The aim of the present study was to investigate how the major component of the TME, the fibroblasts, may functionally interact with BC cell expressing the most common mutation of HBD-*ESR1*, the Y537S, and cooperate in the onset of ET resistance. **Methods:** We employed co-culture systems to create the in vitro conditions that can mimic the complex in vivo microenvironment, between BC cells CRISPR-engineered to express the Y537S mutation and normal/cancer associated fibroblasts (NFs/CAFs). We collected conditioned media (CM) from all experimental models and we assessed several biological assays (Trypan blue and soft agar assays, boyden chamber transmigration assays). We performed genomic and proteomic analysis to explore specific genes/proteins involved in the potential crosstalk between BC and fibroblasts. We orthotopically co-implanted BCs and CAFs in xenograft models. **Results:** We demonstrated that the exposure to the CM-derived from BC cells induced an increase of proliferation and migration of both NFs and CAFs although in a markedly higher extent in the presence of mutant CM. On the other hand, we found that CM-derived from NFs and CAFs, in a higher extent, sustained the aggressive behavior of mutant clones in terms of proliferation, growth, migration and invasion. Moreover, we found Insulin Growth Factor 1 Receptor (IGF1-R) as the major up-regulated gene in microarray analysis and furthermore displayed by proteomic analysis as a central hub in direct interaction network in mutant clones. The latter finding gave us the rational to investigate the potential involvement of this receptor in the functional interaction between BC cells and fibroblasts. Finally, we found that pharmacological inhibition of IGF1-R blocked, both in “in vitro” and in “in vivo” conditions, the reciprocal effects between fibroblasts and BCs that sustained tumor growth and progression. **Conclusions:** Our study addresses how disconnecting tumor-fibroblast crosstalk may represent a novel therapeutic strategy engaged against breast tumor growth and progression. **Acknowledgements:** This work is supported by the AIRC IG GRANT#26246 and the PON Salute ARS01_00568 SI.F.I.PA.CRO.DE.

Abstract 149

ARF6 Dictates the Size and Quantity of Small and Intermediate-Large Extracellular Vesicles in Melanoma

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Introduction: Extracellular vesicles (EVs) mediate intercellular communication and influence disease trajectory and therapeutic efficacy in melanoma (*Cells* 2021;**10**:2740; *Front Cell Dev Biol* 2023;10:1061982). EV cargo includes a myriad of proteins, nucleic acids, and lipids instrumental in tumor progression, immune suppression, and metastasis (*Front Cell Dev Biol* 2023;10:1061982; *Cancers* 2022;**14**:3086). ADP-ribosylation factor 6 (ARF6), a member of the RAS superfamily of small GTPases, is critical for endomembrane trafficking and cooperates with RAB proteins to enhance microvesicle (MV) biogenesis (*Nat Rev Mol Cell Biol* 2006;**7**:347-358; *Methods Mol Biol* 2021;**2174**:143-170). Furthermore, ARF6 coordinates with Exportin-5 to deliver pre-miRNA cargo, and miRNA processing enzymes, to tumor microvesicles (TMVs). Thus, ARF6 promotes both the formation and cargo of TMVs (*Nat Cell Biol* 2019;**21**:856-866). Early data also suggest that ARF6 is essential for exosome biogenesis and release (*Nat Commun* 2014;**5**:3477). We employed a novel inducible, genetically-engineered mouse melanoma model to interrogate ARF6 in tumor progression. Tumor-specific *Arf6* deletion in this model reduced tumor incidence, delayed onset, curtailed growth, and improved survival. Given these findings, we set out to determine if ARF6-dependent EV biogenesis, might impact tumor progression in our model. We hypothesized that EV generation from ARF6^{NULL} neoplastic cells is compromised *in vivo*. To this end, we quantified ARF6-dependent shedding of EVs in the tumor microenvironment. **Methods:** EVs were harvested from dissociated tumor supernatants (DTS) of ARF6 wild type (ARF6^{WT}) (*Dct::TVA; Braf^{V600E}; Cdkn2a$^{f/f}$*) and ARF6^{NULL} (*Dct::TVA; Braf^{V600E}; Cdkn2a$^{f/f}$; Arf6$^{f/f}$*) melanomas through differential ultracentrifugation. After eliminating cells and debris with centrifugation at 1000g and 2000g for 10 minutes each, intermediate-large vesicles were pelleted at 10,000g for 30 minutes, while small vesicles were separated at 100,000g for 70 minutes. These pelleted vesicles were then separated with a 10- 30% iodixanol gradient, spun at 268,000g for 50 minutes. After the top ten fractions were collected, the gradients were spun at 100,000g for 70 minutes. The resultant pellet was harvested, serving as a source of enriched EVs. Enriched EVs were assessed for size, concentration, and morphology via Nanoparticle Tracking Analysis (NTA) and Transmission Electron Microscopy (TEM). Western blot analysis was used to verify the successful enrichment of EVs. **Results:** ARF6^{WT} and ARF6^{NULL} tumors show disparities in the quantity and size range of EVs. ARF6^{NULL} tumors produce fewer overall EVs, and among the vesicles detected, there are fewer small EVs (<150nm) with diameters, ranging from 40nm to over 100nm, and fewer intermediate-large EVs (200-900nm) spanning from 200nm to 799nm. **Conclusion:** Our preliminary data suggest that ARF6 is critical for the biogenesis of a variety of EV species *in vivo*, including both small (<150nm) and intermediate-large (200-900nm) EVs. More work is needed to understand if ARF6 controls the cargo content of tumor-derived EVs and whether ARF6-dependent EV biogenesis controls tumor progression.

Abstract 150

The Small GTPase ADP-Ribosylation Factor 6 (ARF6) Alters the Intratumoral Metabolic Landscape

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Introduction: ARF6 controls endocytic trafficking and recycling and localizes at the plasma membrane (*Inter J Mol Sci* 2019;**20**:2209). Using an inducible, genetically engineered murine model, melanoma-specific deletion of *Arf6* leads to a delay in tumor onset, and tumor growth rate restriction when compared with tumors expressing wild-type ARF6. We have shown previously that ARF6 plays a role in cancer cell invasion and metastasis (*Science Signal* 2013;**6**:ra14; *Cancer Res* 2019;**79**:2892-2908). ARF6 has been demonstrated to: control energy metabolism in neutrophils (*Free Rad Biol Med* 2021;**172**:550-561), regulate cholesterol homeostasis through phosphoinositide signaling (*Nature Commun* 2016;**7**:11919), and be activated by AMPK (a regulator of catabolism during cell starvation) (*J Cell Sci* 2022;**135**:jcs259609), among other metabolic processes. Based on these preliminary reports of ARF6 in cellular metabolic

activities, we hypothesized that tumor-specific deletion of *Arf6* would have an effect on the intratumoral metabolome. **Methods:** A genetically engineered melanoma mouse model was used to generate tumors by subcutaneous injection of RCAS/Cre-expressing DF-1 cells inducing, via a *Dct::TVA* transgene, melanocyte-specific constitutive activation of BRAF (*BRAF$^{V600E/V600E}$*), deletion of *Cdkn2a*, and +/- deletion of *Arf6* {*Dct::TVA; BRAF$^{V600E/V600E}$; Cdkn2a$^{flox/flox}$* (ARF6^{WT}) n=3; and *Dct::TVA; BRAF$^{V600E/V600E}$; Cdkn2a$^{flox/flox}$; Arf6$^{flox/flox}$* (ARF6$^{f/f}$) n=3.}. Murine melanomas were harvested at 2 cm and tumor interstitial fluid was extracted by centrifugation. We utilized Liquid Chromatography-Mass Spectrometry (LC-MS) to assay metabolite content. Final readouts were obtained by normalizing metabolite counts to the tumor weight in grams, and comparing relative differences between ARF6^{WT} and ARF6$^{f/f}$ groups. **Results:** ARF6$^{f/f}$ tumor interstitial fluid, compared with the ARF6^{WT}, showed an enrichment of 4-aminobutyrate (GABA), d-2-aminobutyric acid, and b-aminoisobutyric acid, compared to ARF6^{WT} tumors. These three molecules are isoforms of each other and could not be distinguished in these data. Next, ARF6^{WT} tumor interstitial fluid, compared with the ARF6$^{f/f}$, showed an enrichment of pantothenate (Vitamin B5). **Conclusions:** ARF6 may control the intratumoral metabolic landscape in melanoma. The GABA receptor, in its many isoforms, has been associated with cancer progression, cell proliferation, and invasion (*Cancer Med* 2023;doi.org/10.1002/cam4.6102). Additionally, Vitamin B5 has been associated with improved response to immunotherapy (*OncoImmunology* 2022;**11**:2031500). Further investigation is required to delineate whether these ARF6-dependent metabolic signatures are related to tumor progression.

Abstract 151

Developing Animal Models to Study the Impacts of Bone Microenvironment on Cancer Bone Metastasis

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Introduction: Cancer metastasis is a major cause of patient mortality and bone is a common site for metastatic cancer cells to home and reside. However, the mechanisms driving cancer metastasis to bone remain poorly understood. We have recently demonstrated that in many bone-metastatic cancers, including multiple myeloma (MM), osteoblastogenesis and bone formation are inhibited via the suppression of Runx2 expression in osteoblasts. In addition, increased numbers of apoptotic osteocytes are found in MM. Importantly, our studies have revealed that enhanced suppression of osteoblast-derived Runx2 (OB-Runx2) and increased osteocyte apoptosis also occur at distant bone sites, prior to the detection of metastatic tumor cells. **Methods and Results:** To study whether these changes in distant bones regulate MM dissemination to and progression in these areas, we developed two syngenic models of MM, the OB-Runx2$^{-/-}$ and DTR (diphtheria toxin receptor) mouse models. In OB-Runx2$^{-/-}$ mice, Runx2 is specifically deleted in osteoblasts of C57BL6/KaLwRij mice, whereas in DTR mice, osteocyte apoptosis can be induced through targeted expression of human diphtheria toxin (DT) receptor (DTR) in osteocytes and administration of DT (DTR+DT). The injection of 5TGM1-GFP-Luc MM cells ($2x10^6$) via tail vein in these two models showed that MM cells home to bone significantly faster and grow larger tumors in the bone of OB-Runx2$^{-/-}$ and DTR+DT mice than in respective control mice. BM cell flow cytometry and BM cytokine array revealed that several classes of immune suppressor cells (e.g., myeloid-derived suppressor cells, regulatory T cells and regulatory B cells), as well as multiple metastasis-related cytokines are significantly increased in the BM of both OB-Runx2$^{-/-}$ and DTR+DT mice. Interestingly, OB-Runx2 suppression also significantly increases the numbers of adipocytes in the BM while osteocyte apoptosis also induces a hypoxic condition in BM. **Conclusion:** These data suggest that the loss of Runx2 expression in OBs and increased apoptosis of osteocytes in new bone sites of MM can transform the normal bone microenvironment into an environment that actively attracts MM cells, protects tumor cells from immune-mediated killing, and actively supports the survival and growth of MM cells in these new bone sites. Our study also provides a new stratagem to study the mechanisms of cancer bone metastasis.

Abstract 152

The Use of the Three-Dimensional Spherical Invasion Assay to Measure the Invasive Activity of Human Cancer Cells

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Introduction: The invasion of tumor cells into the neighboring blood vessels and lymph nodes is a vital step for distant metastasis. Traditionally, the invasive activity of growth factors (or the anti-invasive activity of drugs) is measured with the Boyden chamber assay. It would be advantageous to develop another assay that could validate the results of the Boyden chamber assay. With this in mind, our laboratory developed the spherical invasion assay (SIA) to measure the pro-invasive activity of human cancer cells. Here we illustrate the use of the SIA for measuring the anti-invasive activity of the Src kinase inhibitor PP2 in A549 human non-small cell lung carcinoma (NSCLC) cells. **Methods:** The SIA protocol is comprised of two steps. In the first step, A549 human NSCLC cells (treated or not with PP2) were mixed with phenol-red-free Matrigel and seeded in the middle of an eight-well chamber slide. After 24 h, a second layer of phenol-red containing Matrigel was overlaid over the first layer. Over the course of the next 24 h, the A549 cells invade from the primary to the secondary Matrigel layer. Subsequently, the cells are visualized by phase-contrast microscopy and the images obtained are quantified using ImageJ to calculate the anti-invasive activity of PP2 in A549 cells. **Results**: The results of the SIA correlate well with Boyden chamber invasion assays. **Conclusions**: The SIA may be adapted for multiple experimental designs, such as drug screening (to combat invasion and metastasis), measuring the pro-invasive activity of growth factors, and elucidating the signaling pathways underlying the pro-invasive/anti-invasive activity of biological modifiers. We hope that the SIA will be valuable tool for researchers in the field of cancer invasion and metastasis. **Acknowledgement:** Funding for our study was supported by the NIH R15-AREA Grant (2R15CA161491-02 and 2R15CA161491-03) to PD and MAV. Furthermore, this study was supported in part by the West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) grant (NIH grant P20GM103434; PI: Dr. G. Rankin), the National Institute of General Medical Sciences of the National Institutes of Health under the award number P30GM122733.

Abstract 153

Overall Survival and Epithelial-Mesenchymal Transition (EMT) Genes Enrichment Analysis in Renal Papillary Carcinoma

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Introduction: As cells undergo EMT, they adjust metabolic pathways to support growth and adapt to the new surroundings. As important as this process is, there is a relative dearth of information about EMT in papillary renal cell carcinoma (pRCC), the second most common type of renal cancers, approximately a fifth of all cases. This project utilizes publicly available datasets to elucidate the role of EMT-related genes in pRCC progression and prognosis. **Methods:** dbEMT 2.0, a database curated for focus on EMT-related genes, was mined for 1184 distinct genes. Kaplan Meir plots were generated through the R2 platform using the TCGA dataset for "Renal Papillary Cancer," *n=288*. The built-in "KaplanScan" algorithm, was used to divide mRNA gene expression into "high" versus "low" categories. Gene Enrichment Analysis through Cytoscape and Metascape was employed to show which biological pathways the highlighted EMT genes were involved in and to find any statistically overrepresented transcription factors linked to these genes. The EMT-related gene fingerprint profile of the pRCC TCGA dataset was compared to various disease processes using the DisGeNET database. Furthermore, network analysis was performed using established genetic and physical interaction data, along with pathway information. This approach utilized the mentioned list of genes to create an additional catalog of non-EMT related genes that might also play a role in mediating cancer survival. **Results**: From the 1184 genes analyzed, 60 showed a significant change in gene expression in regards to survival outcomes post-Bonferroni correction ($p<0.05$). Enrichment analysis showed 47 distinct biological processes significantly overexpressed in the aforementioned subset of EMT genes, examples of which include the MYC activation pathway, cellular response to growth factor stimuli, and response to hypoxia. Network analysis highlighted 20 different genes involved through physical interactions or co-localization and expression such as Cyclin B1 and DNA topoisomerase II alpha. **Conclusions:** Overall, this study highlights the importance of EMT-related genes in pRCC prognosis. While a small subset of the 1184 genes were linked to significantly worse outcomes, the subset highlighted were shown to be significant involved in canonical EMT pathways such as positive regulation of angiogenesis

and fibroblast activity, as well as more novel processes such as the Aurora-A pathway, involved in centrosome-related changes often seen in cancer. In addition to genes such as CDK1 and MKI67 well established in the cancer literature, other genes noted in network analysis include less known proteins such as KIF23 and HMMR, suggesting possible launching points for further wet-lab experiments and analysis. As renal papillary is less studied than its clear cell counterpart, this sort of preliminary *in-silico* analysis allows for more targeted studies and speeds up the hypothesis formation process.

Abstract 263

Impact of Intraductal Ablation of Mammary Epithelium by 70% Ethanol in MNU Rat Models for Breast Cancer Prevention

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Introduction: Breast carcinoma (BC) is the leading diagnosis of cancer and the second leading cause of cancer-related deaths in women in the United States. Current approved BC prevention methods for high-risk individuals include prophylactic mastectomy and systemic hormonal therapy. Prophylactic mastectomy is a highly invasive surgery that removes the entire breast, including the epithelial-lined ductal tree where BC arises but also uninvolved stroma. Even with a 90% risk reduction, many women are deterred from prophylactic mastectomy due to its associated risk of infection, lymphedema, painful recovery time, and mental health toll. Hormonal therapies, tamoxifen and raloxifene, reduce BC risk by upwards of 50% but also increase the risk of uterine cancer and stroke. Thus, new interventions to reduce the risk of BC and minimize deterrents are necessary. Intraductal (ID) injection is a minimally invasive technique that avoids systemic toxicity by inserting a needle directly into the ductal tree opening to administer a solution to the targeted epithelial cells. We study BC prevention using 70% ethanol (EtOH) as a cell-killing solution for ID injections and have shown its effectiveness in an aggressive genetically engineered mouse model of BC with minimum collateral tissue damage. Here, we study ID infusion of 70% EtOH in a Methyl-N-Nitrosourea (MNU) chemically induced rat model of BC to improve scalability towards clinical trials, increase infusion volume, and evaluate the impact of full-volume infusion on non-injected adjacent glands. **Methods:** Two weeks after intraperitoneal MNU injection, rats received ID injections of a solution containing 70% EtOH to ablate the mammary epithelium, ethyl cellulose (EC) to minimize collateral tissue damage, and Tantalum Oxide (TaOx) to visualize ductal tree filling post-injection via micro-CT imaging. Mammary glands were palpated weekly to establish tumor latency and collected upon reaching euthanasia criteria to record tumor incidence. Kaplan-Meier curves for tumor-free survival were constructed and log-rank tests were used to compare experimental groups. **Results:** Full-volume injected glands had the highest tumor latency increase by 5.86 months (273 days, $p < 0.0001$) compared to non-injected controls (97 days). A protective effect was noted in adjacent untreated glands as shown by a tumor latency increase of 2.56 months (174 days, $p < 0.001$) when compared to non-injected controls. No iatrogenic effects of EtOH were observed in either non-transgenic or cancer-prone rats. No statistically significant differences were noted in epithelial ablation or collateral tissue damage between treatment groups receiving ID injections of 70% EtOH with varying concentrations of EC, but there was a trend to lower collateral damage with EC-containing solutions. Local retention of TaOx in injected mammary glands did not interfere with post-procedure MRI imaging after 15 d of ID infusion. **Conclusion:** This preclinical study shows that ID injection of a refined solution containing 70% EtOH and TaOx is an efficacious and scalable technique for primary prevention of BC.

Cardiovascular Biology

Abstract 154

Duration of SARS-CoV-2 mRNA Vaccine Persistence in Recently Vaccinated Patients and Factors Associated with Involvement of the Myocardium

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Introduction: Years of mRNA vaccine development culminated to an immediate response to the COVID-19 pandemic such that the BNT162b2 (BioNTech-Pfizer) and mRNA-1273 (Moderna) mRNA vaccines were expediently designed, mass-produced, and administered worldwide. Both vaccines produce the full-length SARS-CoV-2 spike protein for gain of immunity and have greatly reduced hospital burden and morbidity. The distribution and duration of SARS-CoV-2 mRNA vaccine persistence in human tissues is unclear. **Methods:** Here, we developed two specific TaqMan RT-qPCR assays to detect each mRNA vaccine. Fresh tissue was placed in TRIzol for RNA extraction. Lymph nodes, liver, spleen, and myocardium from 20 recently vaccinated deceased patients were screened for the presence of vaccine RNA. Immunohistochemistry was used to quantify macrophage infiltration. **Results:** SARS-CoV-2 vaccine was detected in the axillary lymph nodes in the majority of patients dying within 30 days of vaccination, but not in patients dying more than 30 days from vaccination. Vaccine was not detected in the mediastinal lymph nodes, spleen, or liver. Vaccine was detected in the myocardium in a subset of patients vaccinated within 30 days of death. The presence of vaccine in the heart correlated with the presence of healing myocardial injury at the time of vaccination and with the density of myocardial macrophages. **Conclusions:** These results suggest that SARS-CoV-2 mRNA vaccines routinely persist up to 30 days from vaccination and can distribute to internal organs that have ongoing healing injury at the time of vaccination.

Abstract 155*

Myocardial CD34^{+} Stromal Cells/Telocytes and CD68^{+} Macrophages Reveal a Dynamic Pattern of Interactions During Development of Post-Myocardial Infarction Scar

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*The authors of this abstract selected not to publish the details of their research

Cardiovascular Biology and the Extracellular Matrix

Abstract 156

Collagen Fibril Structure, Surface Charge and Vascular Calcification

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Introduction: Soft tissue calcification is a pathological hallmark of vascular diseases such as abdominal aortic aneurysms (AAA). While a number of studies have identified how vascular calcification is modulated by cells, the role of extracellular matrix (ECM) in this process is less well understood. This is especially important in diseases such as AAA where both elastin and collagen (the major constituents of the vascular ECM) undergo extensive remodeling and are characterized by macromolecular structural changes. In our recent studies (*Acta Biomater* 2020;**110**:129-140), we demonstrated the presence of collagen fibrils with ultrastructural abnormalities in human AAA. These fibrils exhibited reduced D-periodicity, smaller diameters, and increased local curvature. **Methods:** This project aims to investigate how structural changes in the collagen fibrils in AAA correlate with changes in their surface charge density and vascular calcification. Excised AAA tissue from patients undergoing surgical interventions was obtained via an approved IRB protocol. Aortic tissue from non-AAA subjects were used as a control. Multimodal histological staining was performed to assess (i) degraded collagen via Collagen Hybridizing Peptide (CHP) staining (ii) mineral location via Von Kossa staining and (iii) surface charge via Hematoxylin and Eosin (H&E) staining. We also developed an atomic force microscopy (AFM) approach to spatially map the relative surface charge in tissue sections at nanoscale level. **Results:** Our results indicate that degraded collagen in AAA tissue could be identified via CHP staining and co-localized with calcific deposits. Further, a significant difference in surface charge density of the ECM was observed between control and AAA tissue. **Conclusions:** Understanding how the altered ECM affects vascular calcification can provide novel insights into the functional characteristics of AAA, its susceptibility to rupture, and the propensity of thrombus

formation. Insights from this project can help advance our understanding of the mechanisms of vascular calcifications and promote the early diagnosis and treatment of the underlying pathology.

Abstract 157

Type XXVIII Collagen Formation as a Prognostic Marker of Mortality Risk in Patients with Atherosclerosis

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Introduction: Atherosclerosis has been linked to imbalances in extracellular matrix composition. Several collagen types are contained in the atherosclerotic lesions affecting plaque stability (*Curr Opin Lipidology* 2021;**32**:277-285). This study aimed to investigate if a marker reflecting collagen type XXVIII formation is associated with cardiovascular events in advanced atherosclerotic patients (*J Biol Chem* 2006;**281**:3494-3504; *J Pharm Biomed Anal* 2021;**204**:114272). **Methods:** Collagen type XXVIII formation was quantified in serum from 195 patients that underwent carotid endarterectomy (Carotid Plaque Imaging Project, CPIP in Sweden) using the enzyme-linked immunosorbent assay PRO-C28. The PRO-C28 assay quantifies the C-terminal fragment of collagen type XXVIII (*J Pharm Biomed Anal* 2021;**204**:114272). Detailed clinical data and outcomes were recorded during an average follow-up period of 90 months. The association of circulating PRO-C28 levels to risk of cardiovascular and all-cause mortality was investigated with Kaplan-Meier survival analysis with log rank test followed by multivariate Cox regression using a continuous variable. **Results:** High levels (above median) of circulating PRO-C28 were associated with an increased risk of both future cardiovascular death (p=0.018) and all-cause death (p<0.001), but not cardiovascular events. PRO-C28 was significantly associated with increased risk for both cardiovascular mortality (HR = 1.505, 95% CI: 1.063 to 2.132, p= 0.021) and all-cause mortality (HR = 1.502, 95% CI: 1.178 to 1.915 p=0.001) even after adjustment for relevant confounders (age, sex, symptoms, hypertension, diabetes and hsCRP). **Conclusions:** PRO-C28 levels predicted cardiovascular mortality and all-cause mortality in atherosclerotic patients undergoing carotid endarterectomy. Further studies will be needed, but PRO-C28 might be used in the future to enhance risk stratification in atherosclerosis. **Acknowledgements:** This project was supported by the Swedish Research Council, the Swedish Heart and Lung Foundation and the Danish Research Foundation (“Den danske forskningsfond”).

Abstract 158*

Long-Read Transcriptomics Reveals Tissue- and Age-Specific Differences in Elastin Isoform Expression

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*The authors of this abstract selected not to publish the details of their research

Abstract 159*

Fibulin-4 and LTBP-4 Interact with Syndecans to Regulate Elastogenesis

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Abstract 160

Inhibition of the Histone Methyltransferase EZH2 with GSK126 Induces Vascular Stiffness in Mouse Aorta and Human Aortic Smooth Muscle Cells

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Introduction: Vascular stiffness increases with age and predicts cardiovascular risk. We previously showed that vascular expression of the histone methyltransferase EZH2 decreases with age, reducing H3K27 methylation and driving aging-associated vascular stiffness by inducing transcription of profibrotic genes. Since EZH2 inhibitors are in clinical trials for cancer, we tested the hypothesis that EZH2 inhibition with GSK126 would promote the vascular aging phenotype with increased vascular stiffness and profibrotic gene expression. **Methods:** Young (3 months) and old (12 months) mice were treated with GSK126 for 2 months. Aortic stiffness was measured by pulse wave velocity (PWV)) and vascular fibrosis and remodeling quantified by histology and protein expression. Primary human aortic smooth muscle cells (HASMCs) from young (28 years) and old (63 years) donors were treated with GSK126 for 24 and 48 hours. Fibrosis and extracellular matrix (ECM) gene regulation was studied by immunoblotting, zymography, and chromatin immunoprecipitation (ChIP). Cell stiffness and morphology were analyzed by atomic force microscopy (AFM) and phalloidin staining. **Results:** GSK126 treatment significant decrease in aortic H3K27me and increased H3K27ac in mice and in HASMCs. In young and in old mice, vascular stiffness increased after 4-5 weeks of treatment. However, vascular fibrosis and expression of profibrotic proteins (CTGF, Collagen 1, and Integrin□5) were not changed by EZH2 inhibition. Rather, GSK126 treatment enhanced aortic elastin fiber degradation by histology in association with increased MMP2 protein. EZH2 inhibition in primary HASMCs similarly increased MMP2 protein expression and activity by zymography. This was associated with enrichment of H3K27ac at the MMP2 promoter by ChIP. Finally, GSK126 treatment significantly increased intrinsic stiffness of HASMCs stiffness measured by AFM with actin fibers staining showing changes in cytoskeletal structure with loss of the elongated SMC morphology. **Conclusions:** These data support a model in which EZH2 inhibition with GSK126 induces vascular stiffness via increased MMP2 activity to promote elastin degradation and changes in SMC cytoskeletal stiffness, without inducing fibrosis. These findings suggest that EZH2 inhibitors developed to treat cancer could negatively impact the vasculature by enhancing stiffness and merits examination in human trials.

Abstract 161

Investigating the Impact of Near Complete Estrogen Deprivation on Cardiac Remodeling in Preclinical Models

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Background: Breast cancer is the most frequently diagnosed cancer in women. Based upon receptor expression, 75% of invasive cases are hormone-receptor positive (HR+). Premenopausal women with intact ovarian function need to undergo ovarian suppression before being prescribed aromatase inhibitors (AIs) as part of their treatment regimen. The long-term survival of breast cancer patients prescribed AIs improve outcomes; however, the long-term negative impact on cardiovascular health is a growing concern. **Methods:** In this study, we used a murine model exposed to different diets (modeling obesity), ovariectomy (OVX) surgery (modeling ovarian suppression), and/or letrozole treatment (an AI) to explore cardiovascular health outcomes of near-complete estrogen deprivation. Female 10-week-old BALB/c mice were fed either a low-fat control diet or high-fat Western diet. Subgroups of mice underwent ovariectomy surgery at 11-weeks of age. Subgroups of mice were administered letrozole at 4.6 ppm in diets until 26-weeks of age. Study groups include young (8-week-old) intact control diet (n=5), aged (26-week-old) intact control diet (n=5), aged-intact Western diet (n=5), aged-intact Western diet + AI (n=8), aged-OVX Western diet (n=5), and aged-OVX Western diet + AI (n=8). At the end of the study, transthoracic echocardiography was performed using a Vevo 2100 LAZR ultrasound system and arterial stiffness was calculated via pulse wave velocity (PWV). To analyze cardiac fibrosis and damage markers, 5μm cross-sections of FFPE hearts were stained using a Picrosirius red collagen protocol to determine perivascular and interstitial cardiac tissue

fibrosis. Vimentin (fibroblast marker) staining was also performed using a DAB protocol. The hearts were imaged using a Mantra/inform at 40x and 5 images were taken per section. Correlate summation clustering was utilized to establish a clear clustering pattern. **Results:** Immunohistochemical (IHC) analysis of FFPE cardiac tissue indicates a trend for elevated interstitial fibrosis in OVX animals. Perivascular fibrosis was significantly elevated in the OVX+AI animals, which was not observed in the other groups. Cardiac vimentin was significantly elevated in animals administered AIs regardless of ovary status, suggesting potential drug effects modulating cardiac fibroblast content. Arterial stiffness, as determined by pulse wave velocity, was significantly increased in the OVX +AI animals compared with all other groups. **Conclusions:** Taken together, these data indicate that OVX+AI in Western diet-fed mice induce vascular stiffness. IHC analysis suggests OVX status and/or AI administration differentially modifies cardiac remodeling markers, as indicated by collagen deposition and fibroblast content. This study establishes a model of cardiac dysfunction induced by near complete estrogen deprivation and will allow future interventional approaches to prevent breast cancer therapy-induced cardiotoxicities.

Collagens

Abstract 162*

Molecular Magnetic Resonance Imaging of Prostate Cancer with a Collagen-specific Probe

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Abstract 163

Collagen Optical Characteristics Vary Between Low Stage and Muscle-invasive Bladder Cancers

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Introduction: Invasion of the detrusor muscle by bladder cancer has important staging, prognostic and treatment implications. Unfortunately, the determination of muscle invasion is not always possible in transurethral biopsies of the tumor by conventional methods. Since collagen fibrils change an altered morphology in several types of cancer, this project addresses the hypothesis that even in the absence of detrusor in the biopsy it would be possible to determine that the tumor is muscle-invasive. **Methods:** Tissue microarrays (TMAs) from tissue samples of confirmed stage T1 (non-muscle invasive) and stage T2 (muscle-invasive) papillary urothelial carcinomas were built. TMA sections were stained by picrosirius red to show collagen, visualized and whole slide images scanned (WSI) by fluorescence. CT-FIRE software was used for computer vision analysis comparing T1 vs. T2 TMA cores. **Results:** A total of 10,870 collagen fibers were identified and analyzed across the 18 WSIs of our cohort. Collagen fibers developed a considerably more rectilinear profile as tumors progress from stage T1 to stage T2, as evidenced by a statistically significant decrease in the standard deviation (SD) of fiber straightness in the T2 group compared to the T1 group (*t*-test; p-value=0.000015). An optimal cut-off value of 0.084 was determined using a univariate logistic regression analysis, which resulted in classifying stage T1 vs. stage T2 with perfect accuracy. **Conclusion:** To the best of our knowledge, this study is the first of its kind to create a computational pipeline for distinguishing between stages T1 and T2 in bladder cancer using fibrillary

collagen morphology. If externally validated our computer vision-based approach could offer an alternative way for differential diagnosis between these two stages when muscle invasion cannot be ascertained from biopsy.

Abstract 164

Long-term Exposure to Organic Dust Promotes Inflammation, Oxidative Stress and Collagen Deposition in the Airways

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Introduction: Occupational exposure to organic dust in swine production facilities is associated with development of a spectrum of lung diseases, including chronic bronchitis and chronic obstructive pulmonary disease. Noxious substances, including reactive oxygen and nitrogen species (RONS), can promote oxidative stress and inflammation of the airway epithelium. Overproduction of RONS can also disrupt extracellular matrix (ECM) composition. Together, accumulation of RONS, chronic inflammation and disruption of ECM homeostasis can contribute to development of chronic bronchitis. However, it is unknown whether exposure to organic dust is associated with modulation of ECM components. Our goal was to evaluate whether organic dust exposure promotes collagen deposition, smooth muscle hypertrophy and excess mucus production in the lung. **Methods:** Porcine tracheobronchial epithelial tissue, blood, and bronchoalveolar lavage fluid (BALF) of commercial pigs were analyzed for levels of collagens, inflammatory and oxidative stress. Porcine tracheobronchial epithelial (PTBE) cells were pretreated (or not) with antioxidant specific inhibitors followed by exposure to organic dust extract (DE) or lipopolysaccharide (LPS) and assessed for oxidative stress and inflammation. Differences among means were determined by performing T-test or two-way analysis of variance ($p<0.05$). **Results:** There was evidence of early goblet cell hyperplasia and mucus accumulation in the airways of indoor compared to outdoor reared pigs. Protein expression analysis revealed higher levels of collagens (I and III) in the airway tissue, alpha smooth muscle actin and tumor necrosis factor alpha in lung tissue of indoor compared to outdoor pigs ($p<0.05$). Total antioxidant capacity (TAC) was lower in tracheobronchial tissues, serum and BALF of indoor reared pigs compared to outdoor pigs ($p\leq 0.0001$). Compared to outdoor reared pigs, all enzymatic antioxidant activity levels were significantly lower in tracheobronchial tissue of indoor reared pigs ($p<0.05$). Compared to media, exposure to environmental pollutants (DE and LPS) caused enhanced oxidative stress in PTBE cells ($p<0.05$). Compared to normal PTBE cells (media, DE, and LPS treated) there was a significant increase in oxidant accumulation in antioxidant inhibited PTBE cells ($p< 0.001$). **Conclusions:** Oxidative stress associated with organic dust exposure promotes collagen accumulation, smooth muscle hypertrophy and goblet cell hyperplasia in the lung. These findings could help to explain the livestock production conditions responsible for development of chronic bronchitis. More studies are needed to understand the role of oxidative stress pathways in agriculture-related lung disease. **Acknowledgements:** NIH-National Heart, Lung, and Blood Institute (Award No. SC1HL150742) and USDA-National Institute of Food and Agriculture Capacity Building Grant (Project No. 2020-38821-31106).

Abstract 165

Insights into the Genetic and Molecular Mechanisms of Hypermobile Ehlers Danlos Syndrome

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Introduction: The Ehlers-Danlos Syndromes are a group of 14 heritable connective tissue disorders. While the genetic etiology of many EDS subtypes is known and involve collagen and extracellular matrix (ECM) modulating genes, the genetic underpinnings of the hypermobile subtype of EDS (hEDS) remain unknown. hEDS is the most common type of EDS affecting 1 in 500 individuals and is characterized by joint hypermobility, musculoskeletal defects, and systemic co-morbidities. The discovery of genetic causes and molecular mechanisms will provide novel insight into disease pathogenesis and allow for the development of diagnostic tools and therapies. **Methods:** Whole exome sequencing (WES) was performed on a large

family and 198 hEDS patients was and combined with gene burden testing to evaluate the enrichment of genetic variants in this hEDS cohort. Expression analyses and *in vitro* assays were performed to test mutation pathogenicity. CRISPR-Cas9 generated knock-in mice were generated for the lead variant and tested for connective tissue defects through mechanical testing, transmission electron microscopy, and histopathology. **Results:** A heterozygous single nucleotide polymorphism (SNP) was identified in a novel gene from a multigenerational family with hEDS. All affected family members shared the missense change, and a second family was identified with the same mutation. WES on a cohort of 198 hEDS patients revealed statistical enrichment of variants within the same gene and the broader gene family. *In vitro* assays revealed that the variant caused a dominant-negative effect resulting in impaired secretion of both wildtype and mutant proteins into the ECM. Mice generated with the corresponding human mutation resulted in smaller collagen fibrils, increased laxity of connective tissues, as well as functional and histological features in multiple organ systems that are consistent with an hEDS phenotype. **Conclusions:** These findings demonstrate a biologically supported genetic discovery for hEDS. The extension of this genetic discovery to a murine model provides the first animal model for hEDS, poised to enhance our understanding of disease pathogenesis. These studies provide an important first toward earlier, improved diagnostic capabilities and better clinical outcomes.

Abstract 166*

Impairment in Processing of Collagen I in a Novel Murine Model of Dermatosporaxis Ehlers Danlos Syndrome

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*The authors of this abstract selected not to publish the details of their research

Abstract 167*

Interactions Between ECM Proteins Reveal Insights into the Mechanism Behind Hypermobile Ehlers-Danlos Syndrome

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Abstract 168*

Evaluation of Fibrosis Phenotypes with Novel Digital Pathology Methods in the NOD/ShiLtj Mouse Model of Sjögren's Disease Following Treatment with Nintedanib

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Abstract 169

Dissecting Collagen Type I Regulation by the RNA-Binding Protein Larp6

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Introduction: As collagen represents the primary component of the extracellular matrix, and the most abundant protein in the human body, understanding collagen regulation is of critical importance. Dysregulation of collagen is key to the development of a wide range fibroproliferative disorders, and the pathology of countless diseases; from superficial scarring to pulmonary fibrosis and cardiovascular disease.

La-related protein 6 (Larp6) is an RNA binding protein which has been identified as a key regulator of collagen type I mRNA. Early work has shown Larp6 binds a conserved 5' stem-loop (5'SL) on the collagen 1a1 and 1a2 mRNA and regulates collagen synthesis through a yet to be defined mechanism. This 5'SL includes the translation start site, and Larp6 interaction seems to be critical to efficient localization of the mRNA to the rough ER for translation initiation, likely mediated through additional trans-acting factors. Observations of subtle changes to the Larp6/5'SL show substantial consequences to binding and collagen synthesis, informing our hypothesis that: Larp6 utilizes conformational selection in the recognition and discrimination of collagen mRNAs and that tertiary interactions dictate mRNA fate. **Methods:** By analyzing the RNA chaperone kinetics of Larp6 on 5'SL mimic RNAs via FRET, and RNA structural variation by NMR we are gathering new information regarding the dynamics of Larp6 interaction with collagen mRNA. Additionally, we are investigating the biochemical pathway by which Larp6 regulates collagen mRNA through *in vitro* translation assays, immunoprecipitation, and fluorescent microscopy. **Results:** Preliminary results indicate Larp6 increased, specific chaperone activity toward the 5'SL of col1a1, which is abrogated by key single point mutations. These mutations additionally diminish binding in electromagnetic shift assays. Ongoing work is examining the consequences of this chaperone activity on mRNA fate. **Conclusions:** Insights into this regulatory pathway could inform the development of novel therapeutics for a wide range of fibroproliferative diseases. **Acknowledgement:** P20GM109095, R15GM141770.

Abstract 170

MUSC Ehlers Danlos Syndrome Biorepository: A Gateway to Understanding Genetic Connective Tissue Diseases

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Introduction: The Ehlers-Danlos syndromes (EDS) are a group of heritable, connective tissue disorders characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. There is phenotypic and genetic variation among the 14 subtypes. Genetic causes for the various subtypes have been uncovered for 13 of the 14 subtypes, leaving the most common subtype, hypermobile EDS, still without a definitive genetic marker. Although gene variants have been identified for the rare EDS subtypes, research into the fundamental mechanisms of action of altered genes has been scant. In order to accelerate research into the rarer forms of EDS, MUSC has established a biorepository in which animal models will be generated for every gene variant that has been previously associated with EDS subtypes. By generating these models and offering them free to research and clinical groups around the world, we intend to grow knowledge and awareness on this disease while informing new mechanisms of connective tissue biology. **Methods/Results:** The presentation will focus on genetic variants based on human genetic discoveries that were chosen for CRISPR-Cas9 murine construction, preliminary phenotyping data, and methods for registering interest. **Conclusions:** By developing this important resource, new information will be revealed on Ehlers Danlos Syndromes as well as connective tissue development and disease pathways.

Abstract 171

LaRP-6 Post Transcriptional Regulation of Fibrosis

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Introduction: Fibrosis is a pathological process that is an outcome for many chronic inflammatory diseases. Fibrosis can affect most organs and tissues of the body and is defined by the accumulation of extracellular matrix molecules with collagen I being the most abundant. When fibrosis becomes dysregulated the overproduction of collagen synthesis usually occurs. This dysregulation can further progress and cause serious health consequences like organ failure, even death. Unfortunately, there does not exist a specific drug for the treatment of dysregulated fibrosis. Understanding the molecular mechanisms of dysregulated pathological fibrosis is important to better guide possible solutions for creating a cure. In this research, we specifically look at the protein LaRP-6, which has been shown to regulate collagen synthesis. This RNA binding protein, from the La-related family of proteins, is known to associate with the 5' untranslated region (UTR) of collagen mRNAs, specifically to the 5' stem loop (SL) in the 5' UTR

of some collagens. Interestingly, the 5'UTRs of collagens 1a1, 1a2, 3a1, and 5a2 contain the start codon, which is predicted to reside in the base-pairing portion of the stem loop. The binding of LaRP-6 upregulates collagen synthesis and is important for directing collagen mRNA to the rough endoplasmic reticulum for translation initiation. It is our hypothesis that the 5' UTR is a post-transcriptional control element, sequestering the AUG start codon and restricting polymerase action and that LaRP-6 acts as an RNA chaperone to melt the stem loop, allowing access to the AUG. **Methods:** Plasmid constructs of the collagens to test the 5'SLs were created along with the purification of the LaRP-6 protein. Different combinations of those collagens and LaRP-6 interactions will be measured through luciferase assays, specifically through *in vitro*. **Results:** We have observed that pNLF1-C plasmid provides robust translation and luciferase signal. The 5'stem loops of COL1A1 and COL3A1 have been cloned in and confirmed by sequencing. Additionally, there are point mutations of the COL1A1 stem loop that have been attributed to altering the sequestering of the start codon. These mutations are currently pending along with the COL5A2 5' stem loop cloning. **Conclusion:** Here, we describe the design and initial results of a luciferase assay that was developed to directly report on the effect of specific interactions of LaRP-6 with the 5'UTRs.

Abstract 172
Comparison of Vitreous Collagen Fiber Network between Humans, Marmosets, Pigs, and Rabbits
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Introduction: The structure of mammalian vitreous is provided by collagen, which assembles into fibers on the micron scale. A comparison of vitreous collagen fiber structure between species can provide insight into why humans develop vitreous liquefaction and posterior vitreous detachment, whereas non-primate species do not. **Methods:** Eyes from adult humans, marmosets, rabbits, and pigs were obtained post-mortem. The eyes fixed with paraformaldehyde and imaged by confocal reflectance microscopy in three locations: the anterior, mid, and posterior vitreous. To determine fiber density and width for each image, automated fiber segmentation with the Fiji ridge detection plugin was used. The results from the three locations within each eye were averaged to obtain a single value of density and width per eye for comparison with one-way ANOVA. **Results:** Results were obtained from 3 adult animals of each species (humans, marmosets, rabbits, and pigs). Fiber density was highest in the pigs (0.040 ± 0.009 fibers/μm^2; mean $\pm$ SD), lowest in the marmosets (0.011 ± 0.004 fibers/μm^2), and intermediate in rabbits (0.018 ± 0.007 fibers/μm^2) and humans (0.020 ± 0.010 fibers/μm^2). The variation among species of fiber density was statistically significant ($p = 0.027$). In contrast, fiber width did not differ significantly among species ($p = 0.10$). The mean width was 1.1 ± 0.1 μm in the humans, 1.0 ± 0.1 μm in the marmosets, 1.2 ± 0.04 μm in the pigs, and 1.1 ± 0.05 μm in the rabbits. **Conclusions:** Between the three non-human species studied, the characteristics of the collagen fiber network were most similar in rabbits compared to humans. This suggests that rabbits may be a better animal model for studying human vitreous structure. However, rabbits do not naturally undergo vitreous liquefaction or posterior vitreous detachment, suggesting that factors other than fiber density and width are involved in the etiology of human vitreous pathology.

Abstract 173*
Effect of the Ratio of Type I to Type III Collagen on Cell Behavior in Three-dimensional Culture
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Extracellular Matrix Biology

Abstract 174*
Piezo Initiates Transient Production of Collagen IV to Repair Damaged Basement Membranes
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Abstract 175
Heterogeneous Tendon Composition Dictates Location-dependent Material Properties

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Introduction: Tendon extracellular matrix (ECM), comprised primarily of load-bearing collagen and elastic fibers surrounded by proteoglycans (PGs) and glycosaminoglycans (GAGs), provides the structure necessary to transmit forces from muscle to bone. The role of PGs and GAGs has been studied in tendon development, but the mechanical contribution of these ECM to healthy adult tendon is understudied, particularly given compositional changes of PGs and GAGs in aging and disease are associated with compromised mechanical properties. Previous mechanics research on PGs and GAGs has been limited to the bulk tissue scale, governed chiefly by type I collagen, where smaller fiber-scale interactions at which cells sense and respond to mechanical stimuli may not be resolved. Specifically, the interaction of hyaluronic acid (HA) and collagen fibers has not been studied in tendon despite its prevalence in connective tissues and as an endogenous lubricant. **Methods:** Our newly developed methods capture collagen fiber-scale mechanics and fiber interactions with surrounding ECM. Adult murine tendons were harvested and incubated with either buffer (control) or hyaluronidase (HA-digest) prior to testing. Each tendon was loaded uniaxially in tension to 80 mN. Segments of individual collagen fibers below the tendon surface were laser-ablated and imaged using second harmonic generation (SHG) to view recoil. Ablation was performed at five x-positions from the proximal to distal end of each tendon. Image sequences were used to calculate instantaneous, or elastic, strain recovery and time dependent, or viscoelastic, strain recovery. Tendons were fixed, stained with wheat germ agglutinin (WGA) and hyaluronic acid binding protein (HABP), and imaged using a confocal microscope. **Results and Conclusions:** Both control and HA-digest tendons exhibited a gradient in elastic and viscoelastic strain recovery, where fibers near the bone insertion showed the least strain recovery, or reduction in length after being cut, and fibers near the muscle insertion showed the greatest strain recovery. WGA, which binds to select PGs and GAGs, indicated a gradient in ECM distribution along the length of tendon, with higher levels of staining correlated with lower amounts of collagen fiber strain recovery, suggesting a role of PGs and GAGs in modulating fiber strain transfer. HA-digest tendons significantly reduced the amount of elastic strain recovery across all x-positions, correlating with HABP that showed a homogenous distribution in controls. These results indicate that HA may facilitate fiber sliding but does not contribute to the tendon mechanical gradient. Ongoing work aims to characterize regional ECM differences in tendon using liquid-chromatography tandem mass spectrometry. These results demonstrate a compositionally driven, region-specific, mechanical response in tendon at a scale relevant to cell mechanotransduction and can inform location-dependent therapies and regenerative constructs.

Abstract 176

A Novel Mutation in the Hybrid1 Domain of the Fibrillin 1 Gene Causes an Abnormal Skin Phenotype

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Introduction: In a familial case of aortic dissection in Japan, a novel pathological variant was identified in the first hybrid domain of the FBN1 gene. This gene encodes fibrillin 1, an extracellular matrix (ECM) protein. Notably, the clinical presentation associated with this variant was distinct from Marfan syndrome. To investigate its effects, the corresponding missense mutation was introduced into mice, resulting in aortic dissection and skin abnormalities in the homozygous Fbn1 mutant (mutant) mice. **Methods**: To study the skin abnormalities in mutant mice, we collected dorsal skin from mice of different age groups. Immunohistochemical staining was performed to observe ECM changes compared to wild-type (WT). Skin stiffness was measured as the pulling force needed to stretch the skin 0-10% and 20-30% from its initial

resting state. Bulk RNAseq was performed using neonatal dermal fibroblasts to observe differentially expressed genes. Additionally, secretome analysis using mass spectrometry was conducted on these fibroblasts to further investigate secreted proteins in WT and mutant mice. **Results**: The mutant mice exhibited a tighter skin phenotype in their dorsal skin, although it did not display characteristics of stiffness or fibrosis as indicated by skin tensile strength measurements and collagen staining, respectively. Further analysis revealed that the dermal expression of fibrillin 1 and elastin was decreased in the mutant mice, leading to compromised elastic fibers in the dermis and fascia, compared to WT mice. Additionally, the mutant mice showed irregularities in the hair cycle. At 4 weeks of age, WT mice exhibited features of the anagen phase, while the mutant mice tended to be in telogen or catagen phases. This delay in the hair cycle was supported by reduced expression of α-smooth muscle actin (a dermal sheath marker) and CD31 (an endothelial cell marker). The ECM surrounding the hair follicles, including periostin, which may affect hair follicle stem cell regulation and the attachment of the arrector pili muscle was also reduced in the mutant skin compared to WT skin. Furthermore, mutant epidermal basal cell proliferation was significantly reduced at 3 weeks of age. RNAseq analysis of newborn dermal fibroblasts revealed upregulation of genes related to protein transport and protein metabolism, along with downregulation of cell cycle-regulating genes. In addition, secretome analysis of primary cultured dermal fibroblast showed significantly upregulated ECM components, particularly proteoglycans which could impact collagen structural arrangement and elastin-associated protein. **Conclusion**: Future investigations will focus on further understanding the skin and hair phenotypes resulting from the Fbn1 mutation, as well as elucidating the underlying mechanisms behind the tight skin phenotype.

Abstract 177

The Selective Removal of Gram-negative Bacterial Communities Prevents Subglottic Stenosis in a Mouse Model

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Introduction: Pediatric subglottic stenosis (SGS) is characterized by airway narrowing and occurs when injury-induced myofibroblasts aberrantly deposit extracellular matrix (ECM) that forms fibrous scar tissue and reduces airway patency (*Laryngoscope* 2021;**132**:1356-1363; *Adv Drug Deliv Rev* 2021;**174**:168-189). We have recently discovered that there are increased proportions of gram-negative bacteria composing the laryngotracheal microbiome during active SGS, compared to control airways. Therefore, we hypothesized that the removal of gram-negative bacteria could prevent SGS progression and act as a prophylactic therapy in the clinic before intubation. Here, we selectively remove gram-negative bacterial populations with the administration of an antibiotic cocktail and compare the degree of stenosis after injury to airways with native, gram-positive dominated, or absent bacterial communities. We show that removing gram-negative bacteria prevents lamina propria thickening (i.e., stenosis), reduces immune cell infiltration, and mitigates myofibroblast differentiation leading to minimal scar tissue formation. **Methods:** 8-week-old C57BL/6 mice were treated with antibiotic cocktails intranasally and in their drinking water *ad libitum* for 2 weeks. Then, SGS was induced by tracheal brushing and allowed to develop for 7 or 21 days. Mice were euthanized and tracheas stained to assess stenosis progression by H&E, extracellular matrix deposition by Masson's Trichrome, and gene expression with RT-qPCR. Immunophenotyping was achieved by flow cytometry and immunostaining while collagen maturation was assessed by polarized light microscopy after picrosirius red staining. **Results:** Native and gram-negative dominated airway microbiomes exhibited robust stenosis indicated by a significantly thicker lamina propria and upon removal of the gram-negative bacteria, however, stenosis was abrogated and returned thickness similar to that of healthy controls. Within the lamina propria, there were significantly fewer infiltrating T cells and macrophages as observed with immunofluorescence and an increase in α-SMA positive fibroblasts. Furthermore, there is more collagen deposited in the lamina propria in the stenotic airway seen with Masson's trichrome and picrosirius red staining, as well as increased lysyl oxidase responsible for stiffening the ECM by crosslinking collagens. **Conclusions:** Overall, we demonstrate that the selective elimination of gram-negative bacteria from the murine upper airway reduces SGS likely by attenuating immune cell infiltration and myofibroblast differentiation thus resulting in the discovery of a potential preventative treatment for SGS.

Abstract 178

A Novel Role for the PI3 Kinase Delta Isoform in Normal Hepatocyte Proliferation

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Introduction: The phosphatidylinositol-4,5-bisphosphate 3-kinase delta isoform (PIK3CD), usually considered to be specific for immune cells, was unexpectedly identified as a gene potentially related to either regeneration and/or differentiation in animals lacking hepatocellular Integrin Linked kinase (ILK). Since a specific inhibitor (Idelalisib, or CAL101) for the catalytic subunit encoded by PIK3CD (PIK3Cδ) has reported hepatotoxicity when used for treating Chronic Lymphocytic Leukemia and certain types of lymphoma, we aimed to elucidate PIK3Cδ's role in normal liver. **Methods and Results**: To determine the effect on normal liver regeneration, partial hepatectomy (PHx) was performed using mice in which PIK3Cδ was first inhibited using CAL101. Inhibition led to over a 50% decrease in proliferating hepatocytes in the first two days after PHx as determined by Ki67 staining. This difference correlated with changes in the HGF and EGF receptors (MET and EGFR, respectively) and NF-κB signaling, as shown by immunohistochemistry and/or western blot analyses. Ingenuity Pathway Analyses (IPA) implicated C/EBPβ, HGF and the EGFR heterodimeric partner, ERBB2, as three of the top 20 regulators downstream of PIK3Cδ as their pathways were suppressed in the presence of CAL101 at one day following PHx. A regulatory role for PIK3Cδ signaling in rat hepatocytes through MET and EGFR was further verified using hepatocyte primary cultures stimulated with either HGF or EGF, in the presence or absence of CAL101. **Conclusion:** Combined, this data supports a role for PIK3Cδ as a downstream regulator of normal hepatocytes when they are stimulated to proliferate.

Abstract 179

The Matrigel Duplex Assay: A Sensitive Method to Measure Retinal Angiogenesis

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Introduction: Neovascular diseases of the retina like diabetic retinopathy (DR) and age-related macular degeneration (ARMD) are proliferative retinopathies involving the growth of new blood vessels in the retina, which in turn causes impairment/loss of vision. A drawback of "conventional angiogenesis assays" is that they are not representative of the angiogenic processes in the retina. In the retina, the new blood vessels grow (from pre-existing blood vessels) and migrate into a region (previously devoid of blood vessels) like the inner limiting membrane of the retina and the vitreous, both of which contribute to vision loss. The Matrigel duplex assay measures the migration of angiogenic capillaries from a primary Matrigel layer to a secondary Matrigel layer which recapitulates the pathological angiogenesis in ARMD and DR. Furthermore, these retinal endothelial sprouts can be characterized and quantified by phase-contrast- by confocal- and transmission electron microscopy. **Methods:** Human retinal microvascular endothelial cells (HRMECs) were serum starved in EGM-R for 24 hours. The cell suspension was treated with/without 10micromolar of the Src inhibitor PP2, diluted 1:1 in phenol-red containing Matrigel. A 6-microliter aliquot of this mixture was pipetted as a drop onto the center of each well of an eight-well chamber slide. These spots of cells allowed to polymerize at 37°C (for 1 hour), after which they were bathed in 200 microliters EGM-R medium and incubated for 24 hours at 37°C. After 24 hours, the medium was then gently aspirated. A second phenol-red free Matrigel solution was prepared by diluting Matrigel with EGM-R (1:1). Two hundred microliters of this diluted solution was pipetted over the drops and incubated for 1 hour at 37°C. The cells were then bathed in 300 microliters EGM-R and incubated for 24 hours at 37°C. Subsequently, three independent fields of these duplex cultures were photographed by phase-contrast microscopy (DMIL LED; Leica). The images were processed using Adobe Illustrator and Photoshop and quantified using Image J software. **Results:** The Src inhibitor PP2 robustly inhibited the migration of angiogenic capillary tube-like structures from the primary Matrigel layer to the secondary Matrigel layer. **Conclusion:** The Matrigel duplex assay is a powerful technique to measure retinal angiogenesis in normal and pathological conditions. **Acknowledgement:** Funding for our study was supported by the NIH R15-AREA Grant (2R15CA161491-02 and 2R15CA161491-03) to PD and MAV. Furthermore, this study was supported in part by the West

Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) grant (NIH grant P20GM103434; PI: Dr. G. Rankin).

Abstract 180

Calreticulin Electrospun into Biomimetic Extracellular Matrix Nanofibers Functionally Synergize for Cellular Responses that Enable Tissue Regeneration

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Introduction: Calreticulin (CALR), an ER chaperone protein that directs protein folding and calcium homeostasis intracellularly, also functions exogenously in in vivo and in vitro cellular activities. CALR potently enhances the rate and quality of wound healing in porcine and diabetic murine wound models and uniquely, heals full thickness excisional wounds by a tissue regenerative process marked by epidermal appendage neogenesis and no scarring. Cellular mechanisms involved are chemoattraction of the wound healing cells, keratinocytes, fibroblasts, and macrophages, proliferation, and induction of extracellular matrix proteins (e.g., collagen, fibronectin, elastin) and integrins of these cells to enable migration. Electrospun nanofibers (NFs) have been topographically engineered as tunable biomimetic extracellular matrix (ECM) scaffolds that provide biophysical and biochemical cues to elicit cellular responses important for wound repair. We hypothesized that the incorporation of CALR into NFs would synergize their biological and physicochemical cues for as an improved biotherapeutic for cutaneous tissue regeneration. **Methods**: Fluoresceinated CALR (CALR-FITC) was electrospun into polycaprolactone/collagen 1 (PCL/Col1) NF and CALR release kinetics, protection from proteolytic digestion, and retention of previously shown in vitro biological functions, were determined. **Results:** 50% of CALR-FITC was released in 24h, 77% by 96h; CALR-FITC electrospun into NFs was protected from proteolytic digestion by elastase, subtilisin, and proteinase K compared to CALR-FITC in solution. Remarkably, CALR-NFs retained full biological activities and potency in induction of migration, proliferation and the expression of TGF-β1 &3, fibronectin, collagen 1, and integrins by human keratinocytes and fibroblasts seeded onto the CALR-NFs. Compared to NFs alone CALR-NFs promoted cell polarization and an elongated cell migratory phenotype including F-actin and vinculin capping at the periphery of fibroblasts and p-FAK expression, which was also observed in trailing migrasomes. In addition, laminin-5 induction by keratinocytes and activation of macrophages expressing CD68 was observed. **Conclusions**: The synergistic activities of CALR-NFs have potential as a novel biotherapeutic for tissue regeneration of acute and chronic wounds, such as diabetic foot ulcers.

Abstract 181

Anisotropic Force Transmission within Aligned Collagen Networks

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Introduction: Cellular mechanical forces are crucial in mechanobiology**.** These forces are generated by actin polymerization and actomyosin contraction. The force transmission between different F-actin cytoskeletal networks (linear and branched) and the surroundings drives a variety of cell behavior including contact guidance or directed migration along aligned fibers. Contact guidance controls development, tissue repair and regeneration and cancer metastasis. Formins, a group of protein associated in actin polymerization, are critically important organizers of different contractile networks of F-actin. Interestingly, formins also selectively control contact guidance through the regulation of transmitted anisotropic traction forces. While much work has been done in understanding how forces are transmitted during random cell migration, how these forces act during contact guidance, are poorly understood. In this study, we report the role of formin in force transmission during contact guidance. For this, we leverage a unique approach by which to assemble aligned collagen fibrils and transfer them to flexible substrates. These substrates allow us to both control the stiffness of the contact guidance cue and measure traction forces in response to altered formin function. **Methods:** Aligned collagen fibrils were assembled on the surface of the mica. These fibrils can be transferred to Sulfo-SANPAH-functionalized polyacrylamide gels by pouring 30% warm gelatin

onto the fibers, allowing it to solidify, stamping it to the new substrate and melting and washing the gelatin away. Traction force microscopy was used to examine anisotropic forces on these substrates. Cell migration was assessed using live cell microscopy. Cells were fixed and stained for F-actin. **Results:** Assembling collagen on mica results in aligned collagen fibrils that can be successfully transferred to flexible substrates. Different cells sense aligned collagen fibril networks with different fidelities. Mesenchymal cells (MDA-MB-231) align well and migrate directionally. Epidermal cells (HaCats) also align with the collagen fibril network, but consistently show migration parallel and perpendicular to the alignment direction. Directional alignment (not migration speed itself) in some cell lines is optimized at intermediate stiffness. Finally, deformations and forces on aligned collagen fibers are organized differently across mesenchymal, amoeboid and epithelial motility modes. **Conclusions:** These results outline the rich differences in contact guidance behavior among cells and indicate force organization differences amongst cells with different motility modes. While current traction force microscopy approaches allow for understanding force anisotropy other methods for sensing not just force magnitude, but direction are needed. Furthermore, we are excited to leverage these forces to release drugs to treat diseases driven by the underlying mechanobiology. **Acknowledgement:** Work was supported by R01GM143302.

Abstract 182*
Quantification of C-pp Processing and Collagen Secretion by Visualized Type I Procollagen Iα1
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*The authors of this abstract selected not to publish the details of their research

Abstract 183
Collagen XVIII Long Form Might be a New Type of Membrane Bound Collagen
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Introduction: Collagen XVIII is a component of basement membranes and exists in three isoforms (long, medium and short). In glomerular basement membranes, collagen XVIII is known to have different molecular orientations among the isoforms. The frizzled domain, which is unique to the long isoform, has been reported to be involved in the regulation of Wnt signaling, but its function in tissue orientation is unknown. In this study, we aimed to clarify the structure and function of the frizzled domain in the long isoform of collagen XVIII. **Methods and Results:** The medium and short isoforms were secreted extracellularly without any problems, but not the long isoform. Subcellular localization was examined using cell fractionation and Western blotting. The long isoform of collagen XVIII was detected in the membrane fraction but not in the cytoplasmic fraction. By immunofluorescence analysis under non-permeabilized conditions, the long isoform was detected at the plasma membrane surface. Analysis using the membrane protein prediction system SOSUI predicted two transmembrane domains within the frizzled domain. The single transmembrane domain-deficient mutants were not secreted out of the cell, while the two transmembrane domain-deficient mutants were secreted out of the cell. **Conclusion:** These results indicate that the long isoform of collagen XVIII overexpressed in CHO cells is localized at the plasma membrane, and its localization to the plasma membrane may involve a predicted transmembrane domain within the Frizzled domain. Type XVIII collagen long isoform may be a novel type of membrane collagen.

Abstract 184
Development of Human Decellularized Extracellular Matrix Hydrogel Biomaterial for Applications in Tissue Engineering for Investigating Pulmonary Fibrosis
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Introduction: Idiopathic pulmonary fibrosis (IPF) is a condition characterized by a progressive decline in lung function, and, outside of lung transplant, is fatal with a median survival of ~3.8 years after diagnosis. Treatment discovery for this condition meets with a critical need for models that can reflect the pathobiological processes of the disease. Tissue engineering offers a possible avenue for fibrosis modeling. Our team has developed techniques in alveolar lung 3D culturing (organoid) models. We have previously developed a lung alveolar tissue hybrid suspension organoid model that uses a low adhesion environment supplemented with non-gelling concentrations of basement membrane hydrogel (BME) with a triculture of three fluorescent cell lines. We named this model the human fluorescent lung organoid model (hFLO). hFLO displays organotypic patterning with lumen lung-like structures and characteristics. Our goal is to investigate how extracellular matrix (ECM) environment in fibrotic lung diseases influences disease progression. Our hypothesis is that using a hydrogel derived from whole lung (ECM) will mimic the interstitium for a more accurate lung model compared to the use of BME alone, and that ECM derived from fibrotic tissue will recapitulate a fibrotic microenvironment, creating an organoid that models fibrotic tissue. We believe by optimizing this model, we will understand IPF disease progression and provide a modeling system with wide applications in the field of pulmonary therapeutic research. **Methods:** We optimized reported methods for decellularizing pulmonary tissue (DT) and hydrogel formation. Gelled materials and decellularization were measured using histology, immunohistochemistry (IHC), DNA contents, western blotting, and kinetic measurements. The hFLO model was used for all organoid experiments. Bleomycin or supplementation of fibrotic ECM hydrogel was used for fibrotic induction in hFLO cultures. Analysis of the fibrosis was accomplished using immunohistochemical methods and western blotting. Confocal microscopy (Leica TCS-SP8-HyVolution) was used to visualize the organoids from both the DT and organoid experiments. **Results:** We successfully created a hydrogel from decellularized lung tissue, both from non-fibrotic donor and end stage IPF patient lungs. Hydrogels from non-fibrotic versus fibrotic lungs have different biochemical and physical characteristics. In the hFLO model human DT hydrogels induce lung like characteristics. Bleomycin fibrotic induction and supplementation with hydrogel derived from fibrotic ECM caused a measurable disease state in the organoids. **Conclusion:** Our data suggests that our novel organoid culture method with modulatory ECM can be used to investigate fibrotic disease progression. **Acknowledgements:** Wooley Innovations in Research Award, Brigham Young University, University of Utah, Donnor Connect.

Abstract 185

Novel Organotypic Lung Triculture Method Paving the Way to Personalized Medicine

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Introduction: Despite currently available antifibrotic agents, idiopathic pulmonary fibrosis (IPF) and other fibrotic lung diseases are characterized by progressive decline in lung function with a median survival of ~3.8 years in IPF patients. The need to abort disease progression or revert the fibrotic process is unmet. A limiting factor to accomplish this goal is that induced fibrosis in lungs of rodents do not accurately mimic human lung disease and most treatment strategies for IPF and progressive pulmonary fibrosis (PF) are extrapolated from experimental models in rodents. While numerous studies document that the extracellular matrix (ECM) components modulate disease progression, the recent advances in tissue culture techniques have allowed us to develop organotypic structures from stable cells in tissue culture mimicking distal lung through ECM modulation. We utilized the evolving knowledge in developing a human lung organoid model to investigate the relationship between ECM concentration, composition and cell-tissue-specific

molecular/cellular maintenance of healthy and diseased 3D architecture. **Methods:** Our human Fluorescent Lung Organoid (hFLO) model consists of three fluorescently labeled stable cell lines representing the three major alveolar cell lineages: endothelial, lung epithelial type II-like cells, and fibroblasts. These cell lines were cultured in 300 μg/mL mouse basement membrane (BM). The hFLO model was evaluated using a variety of microscopy of both organoid sections and 3D whole-mount images along with cytokine arrays and proteomics. Images were quantified for organoid lumen size, count, growth and presence of alveolar-like characteristics and comparted mammalian lung. Cell survival was calculated using imaging flow cytometry. Fibrotic phenotype was achieved using bleomycin and the addition of fibrotic hydrogel originating from lungs of patient with advanced stages of IPF. **Results:** Our hFLO model resulted in organotypic patterning with lumen-airspace-like structures, formation of branching, perfusable vasculature, and long-term maintenance of the gas exchange units and lumen structure. Our data show that by substituting whole decellularized porcine lung in the hFLO media, organoids were significantly larger. We successfully mimicked PF in our hFLO organoids with blyeomycin induction or the replacement of BM with hydrogel from lung slices obtained from patients with advanced stages of IPF. Bleomycin fibrotic induction was alleviated fibrosis using anti-fibrotic agents, Fasudil and an αvβ6 inhibitor; both of these agents decreased disease histopathologylogy in our organoid model. **Conclusions:** We demonstrate the importance of ECM in organotypic patterning with the emergence of lumen airspace-like structures and perfusable vasculature. Our hFLO model mimics human distal alveolar structure and provides opportunities to further understand pathogenesis of PF with treatment strategies to abort or reverse pulmonary fibrosis in human. **Acknowledgement:** BYU Woolley Innovations in Research Award.

Abstract 186

Elucidating Ovarian Cancer Extracellular Matrix Spatiotemporal Dynamics Linked to Platinum Chemoresistance

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Introduction: Seventy percent of patients treated with neoadjuvant chemotherapy for high-grade serous ovarian carcinoma (HGSOC), one of the most common and deadly forms of gynecological cancers, will develop chemoresistance within 5 years. Chemoresistance in HGSOC is associated with dysregulation of the tumor extracellular matrix (ECM) composition and structure. Although the effects of ECM composition and structure on therapeutic response have been well described, knowledge of single-cell spatiotemporal ECM changes during the course of therapy remains limited. There is, therefore, a critical need for HGSOC technologies to determine spatiotemporal ECM dynamics to chemotherapy on a single-cell basis in HGSOC. **Methods**: HGSOC cell lines (Kuramochi and SKOV3) were incubated with cancer-associated fibroblast (CAFs) in a 3D ECM (collagen/fibrinogen hybrid) matrix and co-cultured for 14 days in the absence or presence of cisplatin (10uM), recapitulating standard of care treatment with platinum-based chemotherapy. Spatiotemporal evolution of newly synthesized or nascent ECM in response to chemotherapy was visualized and quantified using metabolic labeling. Azidohomoalanine (AHA), a methionine analog, is incorporated into all deposited nascent ECM proteins and fluorescently labeled via bio-orthogonal click chemistry. Moreover, cell-ECM interactions via mechanosignaling through ECM-dependent integrin activation were assessed by visualization and quantification of the phosphorylation of focal adhesion kinase (pFAK). High-throughput drug screening of cisplatin in combination with ECM targeting agents was performed in order to identify whether a platinum resistant cell line can be re-sensitized to platinum therapy. **Results**: Bio-orthogonal metabolic labeling revealed that nascent ECM deposition was significantly enhanced (5-fold) in response to cisplatin treatment. Cisplatin-treated 3D-ECM hydrogels showed significantly higher nascent ECM deposition when compared to PBS hydrogel controls, as well as higher matrix thickness in both HGSOC cell lines. Moreover, pFAK expression per cell was significantly increased after cisplatin treatment when compared to the PBS control in 3D-ECM in both HGSOC cell lines. Finally, we identified that ECM targeting in combination with cisplatin restores efficacy of cisplatin overcoming platinum resistance. **Conclusions**: Critically, these results suggest that chemotherapy itself induces changes in the ECM, and those ECM-derived changes further promote chemoresistance but can be reversed by targeting them. Moreover, our results are expected to have an important positive impact because they will provide strong-evidence for further development of therapeutics

that possess potentiality and specificity towards the chemotherapy-induced ECM changes, defining the best therapeutic window of opportunity, and ultimately providing new opportunities for improving chemotherapy effectiveness and mitigating chemoresistance in HGSOC. **Acknowledgments:** This project was supported by NIH/NIGMS Center for Cancer Biology 5P20GM103548 and NIH/NCI R21CA259158.

Abstract 187

A Novel Reagent for Probing Fibronectin Matrix Assembly

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Introduction: The dysregulated accumulation of extracellular matrix (ECM) is a hallmark feature of fibrotic pathologies, many of which are incurable. Matrix deposition is an elusive pharmacological target, complicated by the ubiquity of ECM proteins and the numerous entities that regulate and deposit ECM. A key regulator of ECM deposition is fibronectin (FN), a large glycoprotein that is polymerized into an insoluble matrix by cells and that supports the deposition of other matrix proteins. FN matrix is prominently assembled at sites of fibrotic disease and cancer, as well as during wound healing, making it a potentially useful target for drug delivery or diagnostic imaging. Here we report the generation of a novel probe that associates with FN matrix, enabling live imaging of matrix formation. **Methods:** Peptides with FN-binding activity were identified by phage display using a commercially available peptide library and panning on a surface coated with a fragment of human FN. Positive phage clones were amplified and confirmed for FN binding by ELISA. Phages were also screened for co-localization with FN matrix fibrils. Positive peptides were inserted into a fusion protein expression vector and purified fusion proteins were used for subsequence experiments. **Results:** After three rounds of panning, 50 phages were selected at random for sequencing and from those, 25 unique peptide sequences were identified. 22 of the 25 unique peptide sequences showed positive ELISA signals for phage binding to the FN fragment. A subset of those 22 phages co-aligned with FN in fibrils assembled by both NIH 3T3 and WI-38 cells. Further analyses of positive phage peptides were facilitated by cloning each peptide cDNA sequence into a GFP expression vector. One of the peptide fusion proteins (S2) shows extensive co-localization with FN fibrils when incubated with cells in culture. The GFP peptide fusion allows for live imaging of FN matrix, and can be used in pulse-chase experiments to label fibrils at different times during assembly. **Conclusions:** Peptide S2, which localizes phage to FN fibrils in vitro, can also strongly associate fusion proteins with the FN matrix. This FN-binding peptide GFP fusion protein is a useful tool for live imaging of FN matrix assembly. **Acknowledgments:** NJ Commission on Cancer Research Predoctoral Fellowship (COCR23PRF009), R01 AR073236, NJ Health Foundation award.

Abstract 188

Versican Distribution and Its Cleavage in Adamts1 Knockout Mouse Embryos

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Introduction: During the embryonic development of mice, transient omphalocele of the ventral body wall has been observed. The mechanism behind this transient omphalocele during development remains unclear. Versican, a large chondroitin sulfate proteoglycan, is a significant component of the extracellular matrix (ECM) and contributes to the provisional matrix. Versican undergoes degradation by metalloproteinases during development, leading to the formation of a mature ECM rich in collagen (*J Biol Chem* 2013;**288**:1907-1917). ADAMTS1 (a disintegrin-like and metalloproteinase with thrombospondin motifs 1), a member of the matrix metalloproteinase family, is capable of degrading versican (*J Histochem Cytochem* 2011;**59**:463-473). In this study, we investigated the distribution of versican degradation in Adamts1 knockout (KO) mouse embryos at different embryonic stages, along with the observation of abdominal muscle structure. **Methods:** Embryos were harvested on embryonic days 14.5, 15.5, and 16.5. Immunofluorescent staining using anti-versican (GAG-β) and anti-DPEAAE (neo-epitope) antibodies was performed to determine the localization and degradation of versican, respectively. Desmin immunohistochemical staining was conducted to analyze muscle development, including the panniculus

carnosus structure. **Results:** Immunofluorescent staining revealed that versican was widely distributed in and around the panniculus carnosus and the rectus abdominis in both wild type (WT) and Adamts1 knockout (KO) mice. Versican expression peaked at E15.5 and remained high in KO mice at E16.5. However, versican accumulation was reduced in E16.5 WT mice. The cleaved versican fragment was lower in KO mice. PC elongation was hindered in KO mice, accompanied by less versican degradation. The ventral tip of the PC was located ahead of the rectus muscle in WT mice but not in KO mice. **Conclusions:** The period between E14.5 and E16.5 is a critical stage for PC extension in the mouse abdominal wall. PC extension may be associated with versican degradation by Adamts1, resulting in omphalocele formation in Adamts1 KO mice.

Abstract 189

Fibulin-5 is Essential for Elastogenesis and Preserved Vascular Function in the Mesentery

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Introduction: Elastin, a crucial component of the extracellular matrix, imparts resilience and elasticity to various tissues. Elastic fiber formation is a regulated process involving multiple components with tissue-specific requirements as exemplified by our previous work demonstrating that fibulin-4 is essential for elastic fiber assembly in large conduit, but not small muscular vessels. Given the described role of fibulin-5 in elastic fiber assembly in large vessels, in this study, we sought to investigate its role in small vessel elastogenesis and function, focusing on mesenteric arteries and the surrounding mesentery. **Methods**: The mesentery and mesenteric arteries from *Fibulin-5*$^{-/-}$ and *Fibulin-5*$^{+/+}$ mice were used. RNA in situ hybridization (RNAScope), immunofluorescence, and electron microscopy were used to visualize the expression of elastin, fibulin-5, and fibulin-4 in whole-mount preparations of mesenteric tissue. Pressure myography was used to assess myogenic tone and contractile and vasodilatory responses of resistance arteries to the vasoactive agents, phenylephrine, acetylcholine, and angiotensin II. **Results**: At postnatal day 0, all three molecules, elastin, fibulin-4, and fibulin-5 were expressed in cells of the mesentery and mesenteric arteries with fibulin-5 and elastin showing higher expression levels than fibulin-4. The loose connective tissue and arterial adventitia of *Fibulin-5* $^{-/-}$ mice lacked elastic fibers, however, the internal elastic lamina was intact. Functional studies in adult mice showed that the absence of fibulin-5 led to increased vascular smooth muscle contractility to pressure and angiotensin II, but not to phenylephrine, and reduced vasodilatory response to acetylcholine in mesenteric arteries. **Conclusions**: Collectively, these findings highlight distinct spatial molecular requirements for elastinogenesis with fibulin-5 being a requisite for its assembly within the mesentery and arterial adventitia. Additionally, our studies reveal a novel role for fibulin-5 in regulating myogenic tone and reactivity of resistance arteries in a pathway-specific manner.

Abstract 190

LOXL1 Deficiency Reduces TGFβ1-induced Fibrosis in Trabecular Meshwork Cells/Tissues

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Introduction: Pseudoexfoliation syndrome (PEX) is a systemic, age-related disorder characterized by the deposition of fibrillar material in body tissue (PMID27845061). Pseudoexfoliation glaucoma (PEXG), the common ocular manifestation of PEX, presents with fibrillary material in the trabecular meshwork (TM) and elevated intraocular pressure (IOP) (PMID21572731). Genetic risk variants for PEXG are found in lysyl oxidase-like 1 (LOXL1), an enzyme that crosslinks collagen/elastin and is critical for extracellular matrix (ECM) formation/repair (PMID17690259). In addition, TGFβ1, a pro-fibrotic factor, is elevated in the aqueous humor of PEXG patients (PMID11846508). The aim of this study is to investigate the interplay between LOXL1 and TGFβ1 in TM biology, which regulates IOP. **Methods:** Adenovirus encoding TGFβ1$^{223-225}$ Ad5-CMV-TGFβ1(Ad-TGFβ1), or Ad5-CMV-GFP (Ad-GFP) as control were intravitreally injected to

induce expression of TGFβ1 active form in TM tissues of C57BL/6 wild type mice. Also, 2-month-old C57BL/6xBalbC (CB6F1) and 4-month-old 129S1/SvImJ (129S) *Loxl1*$^{-/-}$ (and *Loxl1*$^{+/+}$) mice were treated with Ad-TGFβ1 or Ad-GFP. IOP was measured twice a week and outflow facility (F, inverse of outflow resistance) determined by iPerfusion. In parallel studies LOXL1 was knocked down (KD) in primary human TM cells using small interference RNA, and then treated with TGFβ1 (5ng/mL). TGFβ1 signaling and ECM remodeling/deposition modulators were determined by qPCR and western blot. **Results:** For 2-month-old C57BL/6 mice, IOP increased (ΔIOP=5.36±3.01mmHg, n=11) 7 days after injection with Ad-TGFβ1 and F were reduced compared to Ad-GFP (1.85±0.77 vs 2.33±1.20 nl/min/mmHg). For 6-month-old C57BL/6 mice, IOP increased (ΔIOP=5.29±1.60 mmHg, n=7) 12 days after treatment and F were reduced (2.16±0.64 vs 2.59±0.63 nl/min/mmHg). In the CB6F1 *Loxl1*$^{+/+}$ mice, Ad-TGFβ1 caused IOP to dramatically increase (ΔIOP=10.00±7.68 mmHg, n=5), while *Loxl1*$^{-/-}$ mice appeared resistant (ΔIOP= -0.67 ±4.16 mmHg, n=3); although variability in responses of control mice to TGFβ1 and limited numbers of available healthy *Loxl1*$^{-/-}$ mice prevented statistical significance (p=0.24). In 129S *Loxl1*$^{+/+}$ mice, IOP was also increased (ΔIOP=4.00±1.23 mmHg, n=5) while also reduced in *Loxl1*$^{-/-}$ in Ad-TGFβ1 treated mice (ΔIOP= 0.83 ±1.94 mmHg, n=6) (p=0.025). In vitro, TGFβ1 stimulated mRNA expression of LOXL1 (p=0.04), TGFβRI (p<0.0001), BMP1 (p=0.006), and protein expression of FN (p=0.006) and α-SMA (p=0.03) in TM cells. Significantly, KD of LOXL1 in TM cells inhibited all of these effects (p<0.01), aside from BMP1 upregulation. **Conclusions:** These results suggest that LOXL1 expression levels in conventional outflow tissues determine IOP responses to active TGFβ1, play a role in the regulation of fibrosis and have implications for ocular hypertension in PEXG. **Acknowledgments:** 5P30EY005722-34, 1R01EY030124-01, 1R01EY030617-01 SUB#1, Research to Prevent Blindness, The Glaucoma Foundation.

Abstract 191

The *Triptryium wilfordii* Derivative Celastrol, a YAP Inhibitor, has Anti-fibrotic Effects in Systemic Sclerosis by Suppressing Activation of Reticular Fibroblasts

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Introduction: Systemic sclerosis (SSc) is characterized by extensive tissue fibrosis maintained by mechanotranductive/pro-adhesive signaling. Drugs targeting this pathway are therefore of likely therapeutic benefit. The mechanosensitive transcriptional co-activator, yes activated protein-1 (YAP1), is activated in SSc fibroblasts. The terpenoid celastrol is a YAP1 inhibitor: however, if celastrol can alleviate SSc fibrosis is unknown. Moreover, the cell niches required for skin fibrosis are unknown. **Methods:** Human dermal fibroblasts from healthy individuals and patients with diffuse cutaneous SSc were treated with or without transforming growth factor β1 (TGFβ1), with or without celastrol. Mice were subjected to the bleomycin-induced model of skin SSc, in the presence or absence of celastrol. Fibrosis was assessed using RNAseq, real-time polymerase chain reaction, spatial transcriptomic analyses, Western blot, enzyme-linked immunosorbent assay and histological analyses. **Results:** In dermal fibroblasts, celastrol impaired the ability of TGFβ1 to induce an SSc-like pattern of gene expression, including that of cellular communication network factor 2 (CCN2), collagen I and TGFβ1. Celastrol alleviated the persistent fibrotic phenotype of dermal fibroblasts cultured from lesions of SSc patients. In the bleomycin-induced model of skin SSc, increased expression of genes associated with reticular fibroblast and hippo/YAP clusters was observed; conversely, celastrol inhibited these bleomycin-induced changes, and blocked nuclear localization of YAP. **Conclusions:** Our data clarify niches within the skin activated in fibrosis and suggest that compounds, such as celastrol, that antagonize the YAP pathway may be potential treatments for SSc skin fibrosis.

Abstract 192

Nanostring Gene Expression Analysis of Normal and Glaucomatous Trabecular Meshwork Cells With and Without the N700S Thrombospondin-1 Polymorphism.

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Introduction: Glaucoma is a common eye disease that is associated with increased intraocular pressure (IOP). IOP elevation results from dysfunction in an anterior eye tissue called the trabecular meshwork (TM), which functions to regulate IOP. Glaucoma TM tissue has excess, disorganized extracellular matrix (ECM), but the cause is elusive. We recently identified a single nucleotide polymorphism (SNP), rs2228262, in *THBS1* to be significantly associated with glaucoma in a large Oregon family. This SNP causes N700S in thrombospondin-1 (TSP-1). TSP-1 is a matricellular protein that binds and organizes many ECM molecules including several cell surface receptors. Therefore, we hypothesized that TSP-1 N700S may alter gene expression of other ECM-related genes. **Methods:** The *THBS1* SNP is relatively common in the Caucasian population (~11%) so DNA sequencing was performed on primary TM cells cultured from normal (n=36) or glaucomatous (n=22) cadaver eyes. Aged-matched TM cell strains were cultured at identical passage number in the presence of 0.1 mM ascorbate. RNA was isolated and Nanostring technology was used to profile gene expression changes. Unique fluorescent barcodes were hybridized to gene targets in each sample. The hybridized target-probe complexes were immobilized on human fibrosis v2 cartridges (Nanostring) containing 770 gene targets. The cartridges were scanned using a nCounter Pro and fluorescently-labeled barcodes were digitally counted. After normalization to housekeeping genes, Rosalind software identified differentially expressed genes. **Results:** DNA sequencing showed that the SNP was detected in 13.8% of TM cells derived from normal individuals, while it had higher prevalence (36%) in TM cells derived from glaucoma donors. To identify differentially expressed genes, we compared two groups. First, we compared normal wild-type (n=4) versus glaucoma wild-type cells (n=4) to investigate gene changes associated with glaucoma. Three genes were up-regulated and 32 genes were down-regulated in glaucoma TM cells. These included 11 genes related to ECM organization (*COL1A2, COL5A1, COL6A3, COL14A1, CTSB, CSTL, ELN, MMP2, MMP14, NID2, VCAM1*). Next, we analyzed glaucoma wild-type (n=4) versus glaucoma heterozygous (n=4) TM cells. Twenty-seven genes were up-regulated in heterozygous *THBS1* glaucoma cells, while 4 were down-regulated. Nine genes were related to ECM organization (*COL6A3, COL14A1, CSTL, ELN, MMP2, MMP16, PRKCA, TIMP1, VCAM1*). Gene Ontology analyses identified extracellular structure and organization being affected in glaucoma TM cells with the *THBS1* SNP. **Conclusions:** Gene expression analyses of TM cells harboring an endogenous *THBS1* SNP suggest that a single amino acid change in TSP-1 has major effects on ECM gene expression. Our results could have broader significance because the same *THBS1* SNP has been genetically linked to myocardial infarction. Ongoing studies are using additional functional assays to confirm these results. **Acknowledgements:** NIH/NEI grants R01-EY032590, R01-EY019643, P30-EY010572, the Malcolm M. Marquis, MD Endowed Fund for Innovation, and an unrestricted grant from Research to Prevent Blindness.

Abstract 193

Leukemia Inhibitory Factor (LIF): A Matrisome-Associated Secreted Factor Inducing Tumorigenesis and Chemoresistance in High Grade Serous Ovarian Cancer

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Introduction: High grade serous ovarian carcinoma (HGSOC) is the deadliest form of gynecological cancer and 70% patients develop recurrence within 5 years. The poor survival and high recurrence rates are attributable to late-stage diagnosis and chemoresistance due to the hypoxic tumor microenvironment of HGSOC. Matrisome dysregulation is emerging as a cause of cancer cell chemoresistance. Leukemia inhibitory factor (LIF) is a matrisome-associated secreted factor and IL-6–type cytokine with pro-tumor or tumor-suppressor actions seen depending on the cancer type. Our main goal is to elucidate the roles, drivers, and downstream effects of LIF overexpression within HGSOC. **Methods**: HGSOC cell lines (KURAMOCHI, OVCAR3) and ovarian cancer associated fibroblasts (CAFs) are co-cultured in a human plasma-derived 3D culture model that has been engineered to recreate physiological oxygen levels of the normal ovary (physoxia) or HGSOC tumors (hypoxia). Genomic data analysis, primary tissue RNAscope, ELISA, flow cytometry, cell viability assays, and migration assays have been used to characterize the implication of LIF in HGSOC. Standard of care chemotherapeutics (Carboplatin and Paclitaxel) were used

to assess HGSOC cell drug resistance. Genetic perturbations of LIF and the LIF receptor (LIFR) were established by shRNA and CRISPR and pharmacological inhibition using a LIF neutralizing antibody and a small molecule inhibitor of the LIFR, EC330. **Results**: Genetic analysis confirmed that LIF is overexpressed in HGSOC compared to healthy ovary and it correlates with worst clinical outcome. We validated that LIF is primarily secreted by CAFs, and its secretion is increased both by cellular crosstalk and hypoxia. Hypoxia was identified as a key driver of LIF overexpression at both the protein and mRNA level. Genetic and pharmacological inhibition of LIF/LIFR axis revealed that LIF has pro-tumor effects in HGSOC and significantly impacts cell migration and hypoxia aggravates these effects. Inhibition of LIF/LIFR signaling in combination with standard of care chemotherapy significantly reduces cell survival in hypoxic co-cultures of HGSOC with CAFs. *Critically, these results suggest that LIF is pro-tumor matrisome cytokine driving HGSOC tumorigenesis and chemoresistance through tumor-stroma crosstalk.* **Conclusions**: Our results reveal that LIF, a matrisome-associated secreted factor, is overexpressed in HGSOC and drives tumorigenesis and chemoresistance. Moreover, we have further identified hypoxia as a key driver of LIF overexpression. Our results are expected to have an important positive impact because they highlight the LIF/LIFR axis as a potential candidate target for adjuvant treatment in HGSOC. **Acknowledgement:** This project is supported by NIH/NIGMS Center for Cancer Biology 5P20GM103548 and NIH/NCI R21CA259158.

Abstract 194

Role of Integrin Alpha 4 and Alpha 9 in the Development of Elevated IOP and Trabecular Meshwork Damage

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Introduction: The extracellular matrix (ECM) of the trabecular meshwork (TM) is known to play an important role in intraocular pressure (IOP) regulation. The integrins comprise a family of transmembrane glycoproteins that mediate cell-cell adhesion and cell-extracellular matrix adhesion. Here, we evaluate the expression of α4 and α9 integrin subunits in TM cells and their possible functions in development of ocular hypertension. **Methods:** Human TM cells obtained from donors were characterized by treating the cells with dexamethasone for 1 week and measuring the expression of myocilin and CLAN formation in response to dexamethasone. The expression of integrin α4 and α9 in primary human TM cells were visualized by immunocytochemical staining using integrin-specific antibodies. Primary human TM cells were treated with TGFβ2 and the expression of integrin α4 and α9 were evaluated by western blot. In addition, primary human TM cells were treated with cFN (containing the EDA domain), TGFβ2, or cFN and TGFβ2 together for 48 hours and total FN and FN+EDA gene and protein expression levels evaluated using qRT-PCR and western blotting, respectively. Conditional knockdown of integrin α4 and α9 were performed by injecting Ad5.Cre in one eye of $EDA^{+/+}\alpha4^{fl/fl}$ and $EDA^{+/+}$ $\alpha9^{fl/fl}$ mice (constitutively express the EDA isoform of fibronectin and contain α4 and α9 specific LoxP sites, respectively) at 12 weeks of age. Contralateral eyes were injected with Ad5.null virus as controls. The knockdown was confirmed by in situ hybridization. IOP was measured up to 8 weeks after injection. **Results:** Immunostaining of integrin α4 and α9 confirmed the presence of these two proteins in TM cells. In addition, protein expression of these two proteins was increased with TGFβ2 treatment (n=6, $p<0.05$). TM cells treated with cFN, TGFβ2, or cFN and TGFβ2 together for 48 hours revealed significant increase in FN+EDA expression for each treatment compared to controls (n=3, $p<0.002$) and increase in total FN expression for TGFβ2, or cFN and TGFβ2 combined treatment (n=3, $p<0.003$). Knockdown of integrin α9 in $EDA^{+/+}\alpha9^{fl/fl}$ mice showed significantly decreased IOP compared to the contralateral control eyes, which are known to develop elevated IOP, for 8 weeks post injection (n=11). Knockdown of integrin α4 in $EDA^{+/+}\alpha4^{fl/fl}$ mice showed a significant decrease in IOP compared to the contralateral control eyes for 3 weeks post injection (n=11). As expected, $EDA^{+/+}$ mice developed elevated IOP compared to wildtype controls. **Conclusion:** Overall, these data suggest that integrin α4 and α9 may play important roles in IOP regulation and the development of ocular hypertension.

Abstract 195

Chromosomal Translocation Converts a Laminin Gene, LAMC2, Into an Oncogene in Ovarian Carcinoma

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Introduction: Laminins are heterotrimeric extracellular matrix (ECM) proteins composed of α, β, and γ chains. One of these chains, γ2 (Lm-γ2) chain, is frequently expressed as a monomer and its expression is closely associated with cancer progression. Lm-γ2 chain contains an EGF-like domain in its domain III (DIII), and matrix metalloproteinases (MMPs) can cleave the DIII region of Lm-γ2 chain that acts as the EGFR ligand (*Int J Mol Sci* 2019;**20**:226). **Materials and Methods:** The materials are as follows. Human ovarian cancer SKOV3 cells were obtained from the Japanese Collection of Research Bioresources Cell Bank. Cells were cultured in RPMI1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and maintained at 5% CO_2 at 37℃. Formalin-fixed paraffin-embedded tissues and frozen cancer tissue specimens surgically removed from ovarian carcinoma patients were used in Kanagawa Cancer Center Biobank. The detail methods were shown in our previous paper (*Cancer Sci* 2021;**112**:4957-4967). **Results:** We have recently reported that a novel short form of Lm-γ2 (termed Lm-γ2F) chain containing DIII is generated without requiring MMP and chromosomal translocation between LAMC2 on chromosome 1 and the NR6A1 gene locus on chromosome 9 in human ovarian cancer SKOV3 cells (*Cancer Sci* 2021;**112**:4957-4967). Lm-γ2F chain, which contains an EGF ligand-like domain (DIII), is expressed as a truncated form lacking domains I/II, which are required for its association with Lm-α3 and -β3 chains of Lm-332. Secreted Lm-γ2F can act as an EGFR ligand without being processed by proteases and activates EGFR/AKT signaling pathways more efficiently than Lm-γ2 chain itself, which in turn promotes the proliferation, survival, and motility of ovarian cancer cells. The LAMC2-NR6A1 fusion gene was detected by fluorescence *in situ* hybridization in 3 of 6 ovarian cancer tissues (50%) and the transcripts of the fusion gene were expressed in 11 of 16 ovarian cancer tissues (68%). Overexpression and suppression of LAMC2-NR6A fusion gene transcripts significantly increased and decreased cell tumorigenic growth in mouse models, respectively. **Conclusions:** Together, this is the first report that the ECM gene, LAMC2 translocation, plays a crucial role in malignant growth and progression of ovarian cancer cells, and the consequent product is a promising therapeutic target for ovarian cancers.

Abstract 196

A Novel Interaction of CD47 with Filamin A Identified Using Single Vesicle Imaging and Mass Spectrometry Analyses of Extracellular Vesicles released from T Lymphoblast and Prostate Carcinoma Cells

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Introduction: CD47 is a ubiquitously expressed membrane protein that functions as a receptor for thrombospondin-1 and the counter receptor for signal regulatory protein-α on phagocytes. CD47 is widely expressed on extracellular vesicles (EV), and identifies a distinct population of EV from those that express the traditional EV markers. **Methods:** Mass-spectrometry, immunoprecipitation-western blotting (IP/WB), flow cytometry, confocal image analyses and single particle interferometric reflectance imaging sensing (SP-IRIS) via ExoView® imaging platform were used. **Results:** Targeted mass spectrometry analysis of proteins captured on anti-CD47 beads from wild type (WT) but not $CD47^-$ mutant Jurkat T lymphoblast cells identified filamin A as a novel interacting protein partner of CD47. Filamin A, YBX1, and the CD47 cytoplasmic adapter ubiquilin-1 were uniquely present in EV secreted from WT but not $CD47^-$ mutant Jurkat cells. Specific association of CD47 with filamin A in cells was further confirmed from WT and $CD47^-$ mutant Jurkat and PC3 prostate carcinoma cells using IP/WB. SP-IRIS analysis showed less filamin A and α4β1 integrin sort into EV in CD47- Jurkat and PC3 cells. 3D visualization via Z-stack images showed colocalization of filamin A with CD47 on Jurkat and PC3 WT cells and altered filamin A localization in the respective $CD47^-$ mutants. Our findings suggest that CD47 indirectly binds to filamin A via ubiquilin-1 and/or β Integrin. Filamin A may thereby play an important role in CD47-dependent sorting of protein cargoes into specific subsets of EV. **Conclusions:** CD47 and ubiquilin-1 interact with filamin A, which is known to interact with the cytoplasmic domain of β1 integrins to regulate integrin function. Less filamin A and α4β1 integrin sort into EV in the absence of CD47, suggesting that CD47 promotes filamin A and integrin sorting into EV, mediated through ubiquilin-1.

Abstract 197

Toll-Like Receptor 4 Dependent Optic Nerve Head Damage

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Introduction: The cell-signaling mechanisms inducing damage during glaucoma disease progression are still unknown. We have identified DAMP (damage associated molecular pattern) activation of toll-like receptor 4 (TLR4) signaling as a novel pathway involved in extracellular matrix (ECM) regulation in the ONH. Here we examined whether TGFβ2 and TLR4 signaling crosstalk is essential for increased ECM deposition by primary ONH astrocytes and lamina cribrosa (LC) cells. We also examined whether increased activation of TLR4 signaling by the DAMP fibronectin extra domain A (FN+EDA) leads to a progressive glaucoma phenotype in mice. **Methods**: Human primary ONH astrocytes and LC cells (*n*=3) were isolated, characterized, and exposed to exogenous TGFβ2 or cFN containing the EDA isoform, with or without the selective TLR4 inhibitor TAK-242. Changes in ECM production were quantified by western blot and immunocytochemistry. We also used our novel mouse model of glaucoma, B6.EDA$^{+/+}$, which constitutively expresses the EDA isoform of fibronectin, and assessed intraocular pressure via rebound tonometry, RGC loss and ONH damage via immunohistochemistry, and ON damage by PPD staining at one year and two years (*n*=6-7) of age. **Results:** In human ONH primary astrocytes, TGFβ2 treatment induced elevated ECM protein expression ($p<0.05$), but concurrent treatment with the selective TLR4 inhibitor TAK-242 blocked this effect ($p>0.05$) compared to controls. Utilizing a co-culture system with primary human LC cells seeded on a cFN coated plate and primary human ONH astrocytes seeded on the co-culture insert, cFN treatment of the LC cells led to an increase in ECM protein expression ($p<0.05$) in the co-cultured human ONH primary astrocytes. In contrast, astrocyte pre-treatment with the selective TLR4 inhibitor blocked this effect ($p>0.05$). IOP was significantly elevated ($p<0.001$) in B6.EDA$^{+/+}$ mice and RGC loss and ON damage was significant at both 1 year and two years of age ($p<0.05$, $p<0.01$) compared to age-matched controls. **Conclusions:** Here we show TLR4 signaling is necessary for DAMP-dependent increases in ECM expression in ONH astrocytes, LC cells and ONH astrocytes undergo paracrine signaling, and constitutive activation of FN+EDA leads to a glaucomatous phenotype in our mouse model system. These results implicate TLR4 as an important regulator of glaucomatous damage.

Abstract 198

Regulation of Macrophage Gene Expression by Matrix Components

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive disease characterized by excess accumulation of extracellular matrix (ECM) proteins that leads to increased lung stiffness (*N Engl J Med* 2018;**378**:1811-1823). Macrophages are implicated in progression of fibrosis, and an emergent profibrotic macrophage has been identified in IPF lung (*Eur Resp J* 2019;**54**:1802441). Additionally, single-cell RNAsequencing reveals that *SPP1* and *APOE* is highly upregulated in human IPF lungs and mouse models of the disease (*Eur Resp J* 2019;**54**:1802441; *J Exp Med* 2017;**214**:2387-2404). Despite elaboration of abundant ECM and observed profibrotic role of macrophages in IPF, it remains unclear how interaction between ECM and macrophages may influence macrophage phenotypes and gene expression. In this study, we utilize a small peptide termed the functional upstream domain (FUD) to inhibit fibronectin (FN) matrix formation *in vivo*. We also used *in vitro* models of matrix proteins, stiffness and collagen structure to test the hypothesis that matrix components may influence macrophage gene expression. **Methods:** A single-dose intratracheal bleomycin (bleo, 1U/kg) or normal saline control was administered to wild-type C57/Bl6 mice to induce fibrosis. To inhibit FN, we subcutaneously injected 12.5mg/kg/day of PEGylated FUD (PEG-FUD), or PEG-mFUD control, 3-13d post-bleo. We assessed collagen content and macrophage numbers by hydroxyproline assay and immunohistochemistry (IHC), respectively. Human monocytes were purified from peripheral blood mononuclear cells (PBMCs) using DynaBeads Isolation kit. To evaluate macrophage gene-expression in response to FN and matrix stiffness, THP-1 human macrophages or PBMCs were plated on 12-well plates coated with human plasma FN (pFN) or collagen coated Matrigen plates of various stiffnesses, and stimulated with PMA or M-CSF, respectively. mRNA expression was assessed by RT-qPCR. Normal and IPF human lung collagen was imaged using second harmonic

generation microscopy to create scaffolds via multi-photon excited polymerization. THP-1 cells were plated on these scaffolds and imaged over the course of 24h to track movement and displacement. **Results:** Total macrophage population and subpopulations increased in the lung 14d post-bleo as measured by CD68, F4/80, CD11b, and CD206. Treatment of bleo-injured mice with PEG-FUD decreased lung collagen content and the number of $CD11b^+$ and $CD206^+$ cells at d14, suggesting decreased macrophage presence. THP-1 cells plated on IPF collagen scaffolds increased displacement and moved at higher speeds than cells on a normal collagen scaffold. PBMCs demonstrate a bi-phasic response to matrix stiffness. From 1kPa to 2kPa *SPP1* and *APOE* gene expression increased and decreased at 25kPa while *IL1β* expression decreased at 2kPa. **Conclusions:** Our results support a role for ECM components in modifying macrophage behavior both *in vitro* and *in vivo* in the context of altered matrix cues found in lung fibrosis. Future studies will assess how loss of FN-ECM may impact macrophage influx over time.

Abstract 199

Shock Drives a STAT3 and JunB-mediated Coordinated Transcriptional and DNA Methylation Response in the Endothelium

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Introduction: Endothelial dysfunction is a critical factor in promoting organ failure during septic shock. Organ dysfunction during shock increases survivors' risk of long-term sequelae through unknown mechanisms. Interleukin 6 (IL-6), a major mediator of the cytokine storm during shock, activates JAK kinases to phosphorylate the transcription factor STAT3, promoting SOCS3 expression for negative pathway regulation. The aim of this study is to determine the transcriptional and epigenetic changes that occur in endothelial cells in response to proinflammatory stimuli initiated by endotoxin of mice lacking SOCS3, specifically in the endothelial cells (SOCS3iEKO). **Methods:** Two weeks after tamoxifen-induced SOCS3 deletion, we induced severe acute inflammation by a single IP injection of LPS. We performed cross-omics analyses of transcriptome (RNA-seq) and DNA methylome (Epic DNA methylation array) data obtained from isolated kidney endothelium of wild type and SOCS3iEKO mice challenged with LPS. **Results:** Our results revealed that kidney injury after LPS leads to strong endothelial transcriptional and DNA methylation responses. Further, SOCS3 loss leads to an aggravation of the responses, demonstrating a causal role for the STAT3/SOCS3 signaling axis in the acute endothelial response to LPS. Performing Homer Motif analysis, we found that increased STAT and AP1 (JunB, BATF, and cFos) transcriptional activity was associated with DNA methylation changes, suggesting a potential mechanism driving transcription-induced gene-specific methylation changes. In vitro, HUVEC were treated with recombinant IL-6 and sIL-6Rα, and genomic DNA and total RNA were extracted 72 hours post-treatment. We detected similar changes in DNA methylation induced by IL-6, and a significant overlap was observed between the gene subsets with altered methylation and expression. Specifically, the genes SERPINA3, NOSTRIN, and PLCE1 exhibited hypomethylated CpGs in response to IL-6+R challenge, accompanied by upregulated expression at 72 hours. Conversely, TNFSF4 and NAV2 showed hypermethylated CpGs, respectively, and their expression was downregulated after 72 hours of IL-6+R treatment. Motif enrichment analysis implicated the involvement of STAT3, JunB, BATF, and cFos in both epigenetic and transcriptional changes. Functional experiments using siRNA-mediated depletion of STAT3 and JunB confirmed their roles in mediating these changes. Furthermore, treatment of HUVEC with the methyltransferase inhibitor 5-AZA for 72 hours resulted in a significant increase in SERPINA3, NOSTRIN, PLCE1, and TNSF4 expression. In conclusion, endothelial cells respond with a coordinated response that depends on overactivated IL-6 signaling via STAT3, JunB, and possibly other transcription factors. **Conclusion:** Our findings provide evidence for a critical role of IL-6 signaling in regulating shock-induced epigenetic changes and sustained endothelial activation, offering a new therapeutic target to limit vascular dysfunction.

Abstract 200

Matrix Proteoglycans Regulate $CD4^+$ T cell Functions in Secondary Lymphoid Organs

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Introduction: The extracellular matrix (ECM) in secondary lymphoid organs (SLO) provides a complex compartmentalized environment for antigen presentation and development of antigen-specific T cells that shapes the adaptive immune response. Yet, beyond collagen fibrils this ECM milieu is poorly defined at the molecular level. Small leucine-rich repeat proteoglycans (SLRPs) lumican (Lum), biglycan (Bgn) and decorin (Dcn) are expressed by stromal cells in SLO. We detected paracrine regulation of innate immune signals by Lum in antigen presenting cells (*Proceed Natl Acad Sci USA* 2021;**118**:e2100999118). We hypothesize that SLRPs may regulate antigen presentation to T cells in SLO to modulate adaptive immunity. Here we used *in vitro* and *in vivo* inflammation models to investigate regulations of $CD4^+$T cell mediated adaptive immune by SLRPs. **Methods:** Splenic $CD4^+$T cells activated in vitro (anti-CD3/CD28) in the presence of recombinant (r) SLRPs were assayed for proliferation (CFSE) and activation (anti-CD69) by flow cytometry. Cell proliferation and activation was also assayed after pretreatment with function blocking anti-β2 integrin antibody. In a dermal inflammation model, $Lum^{-/-}$, $Bgn^{-/-}$ and WT mice (n=8/genotype) were sensitized with 0.5% dinitrofluoro benzene (DNFB) and elicited by 0.25% DNFB application on the ear. On day 5, $CD4^+$T cells were analyzed in the ear tissues and draining lymph node (dLN) by flow cytometry, immunohistochemistry (IHC) and confocal microscopy. **Results:** IHC of dLN showed Lum in the subcapsular sinus and B cell region, whereas Bgn and Dcn more prominently in the T cell zone. All three rSLRPs suppressed in vitro proliferation of $CD4^+$ T cells in a dose-dependent manner. All three rSLRPs at 2.5µg/ml, the dosage of 70% inhibition of proliferation, also suppressed $CD4^+$T activation. rDcn had the most inhibitory effect on T cell activation which is consistent with the presence of Dcn in the T cell zone. The rSLRPs are known to interact with β2 integrin subunits of the $CD4^+$ T cell surface receptor LFA-1. Anti-β2 antibody treatment lifted lumican and biglycan-mediated inhibition, suggesting their function to be integrin-dependent. Our DFNB inflammation model substantiates our *in vitro* findings further. Histology of sections through the DNFB-sensitized ear shows significantly more ear swelling of the $Lum^{-/-}$ and $Bgn^{-/-}$ compared to WT mice. By flow cytometry, we found higher proliferating ($PCNA^+$) $CD4^+$ T cells and higher Th1 cells in the dLN of the sensitized $Lum^{-/-}$ and $Bgn^{-/-}$ mice compared to WTs. **Conclusions:** Stromal SLRPs may function as a natural brake for $CD4^+$T cells by suppressing their proliferation and activation. Therefore, these SLRPs may be manipulated to modulate $CD4^+$ T cells in homeostasis and disease settings.

Abstract 201

Identification of ADAMTS9 and ADAMTS20 Basement Membrane Substrates using N-Terminomics

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Introduction: Proteolysis is an important irreversible post-translational modification which can either activate or inactivate protein substrates, or generate fragments with new functions. The homologous secreted metalloproteases ADAMTS9 and ADAMTS20 appear to have dual roles in extracellular matrix (ECM) turnover and biogenesis of the primary cilium during mouse embryogenesis. They are genetically associated with several human traits and disorders, but have few known substrates. On the basis of observed angiogenesis, eye and renal anomalies, they may have a potential role in basement membrane remodeling. Our objective was to identify their substrates via an unbiased proteome-wide strategy and determine potential overlap in their activity. **Methods:** We applied the mass spectrometry-based N-terminomics strategy Terminal Amine Isotopic Labeling of Substrates (TAILS) to ADAMTS9 and ADAMTS20 digests of the matrisome of a basement membrane producing mouse cell line, PFHR9. HEK 293F cells were transfected with plasmids encoding ADAMTS9, ADAMTS20, or catalytically inactive ADAMTS9 and ADAMTS20 EQ as negative controls and were co-cultured with PFHR 9 cells in a 20:80 ratio. Peptides from the serum-free culture medium were analyzed using a Bruker TimsTof Pro2 Q-Tof mass spectrometer. Data was analyzed using MSFragger 19 and Perseus for visualization and statistical analysis. Only high confidence peptides (i.e., false discovery rate < 1%) were included. The N-terminally labeled/blocked and positionally internal peptides were considered as potentially proteolytically cleaved peptides. **Results:** This N-terminomics strategy identified several autoproteolytic cleavage sites in ADAMTS9 and ADAMTS20 and 779 proteolytically cleaved peptides (from 187 proteins) for ADAMTS9 and 1256 proteolytically cleaved peptides (from 262 proteins) for ADAMTS20. Laminin subunit alpha-1, laminin subunit gamma-1, SPARC, collagen alpha-2(IV) chain and serpin H1 were top 5 substrates for both ADAMTS9 and ADAMTS20 but not necessarily in the same order. 211 cleavage sites corresponding to 143 protein substrates overlapped between these proteases. We further orthogonally validated SPARC as an

ADAMTS9 substrate. **Conclusions:** Our study has identified several novel ADAMTS9 and ADAMTS20 substrates and demonstrated the overlapping activity of these proteases, consistent with their previously demonstrated cooperative roles in mouse development. Cleavage of SPARC may be relevant to the observed roles of ADAMTS9 in eye development and angiogenesis.

Abstract 202*
Expansion of Extracellular Proximity Labeling Technique to Novel Matrisome Targets and Culture Conditions
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*The authors of this abstract selected not to publish the details of their research

Abstract 203
The Matrisome Project: A One-Stop Shop for ECM-Omic Research
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Introduction: The extracellular matrix (ECM) is a complex meshwork of molecules that provides structural, mechanical, and chemical signals to cells of multicellular organisms. Due to the ECM's integral and diverse roles, changes in its composition or architecture have been linked to many diseases such as cancer and fibrosis. The Naba lab has developed novel proteomic and computational methods to study, with high throughput, the composition of the ECM and strives to help others do the same. This has led to the development of an interactive platform: the Matrisome Project: https://matrisome.org. **Methods:** The Matrisome Project is organized around three main sections. The Matrisome Annotations section provides matrisome gene lists for multiple organisms and proposed classifications of those genes based on the structure and functions of the core ECM and ECM-associated proteins they encode. We also provide disease-specific matrisome signatures derived from our proteomic datasets. The Resource section points to external portals hosting our experimental protocols (protocols.io), codes (GitHub), and lists of validated anti-ECM antibodies. Last, the Tools section provides access to web-based applications including MatrisomeDB, a searchable repository of curated ECM proteomic datasets, and the new version of Matrisome AnalyzeR. **Results:** We are presenting here examples of how the Matrisome Project can be used by novices and experts alike. Using the annotations of matrisome components allows for a comprehensive classification of ECM components and their interconnected functionalities in big datasets while utilization of proteomic matrisome signatures can uncover commonalities between seemingly distinct processes to gain deeper insights into user datasets. The matrisome lists of various species and validated antibodies can be used to design targeted experiments to study the ECM. The protocols.io matrisome repository enables users to adopt and adapt our protocols to perform reproducible experiments. The latest addition to the Matrisome Project is Matrisome AnalyzeR, a user-friendly web-based application that allows the annotations, classification, and tabulations of ECM components in -omic datasets. **Conclusion:** The multiple freely available protocols, open-access codes, and web-based tools provided by The Matrisome Project constitutes a unique resource for researchers in the ECM field biology and beyond. We hope our efforts will help advance our understanding of the roles of the ECM in health and diseases and pave the road to important discoveries.

Fibroblasts

Abstract 204
Human Upper-Airway Fibroblasts from Patients with Subglottic Stenosis Retain Transcriptional Memory of Fibrotic Niche
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Introduction: Subglottic stenosis (SGS) is the narrowing of the upper airway resulting from prolonged intubation with an endotracheal tube (ET) (*Laryngoscope* 2021;**132**:1356-1363; *Adv Drug Deliv Rev* 2021;**174**:168-189. Iatrogenic ET injury leads to disrupted epithelium causing exposure of the vulnerable fibroblast-resident lamina propria to the airway lumen leading to inflammation, myofibroblast differentiation, and stenosis. Recently, transcriptomic and functional assays have stratified skin fibroblast populations based on their secretome, transcriptome, and proteome (*Nat Commun* 2023;**12**:3709; *Cells* 2021;**10**:2840). In this work, we examine the phenotypic differences between isolated fibroblasts originating from the stenosed upper airway and a distal, internal control at baseline. We also correlated the differential response of fibroblast subpopulations to TGF-β1 stimulation by assessing surface markers, cytokine and chemokine secretion, matrix deposition, and gene expression to define potential targets for novel treatments of SGS. **Methods:** Laryngeal biopsies were obtained from a consented patient with grade 4 SGS at the Children's Hospital of Philadelphia and isolated via standard culturing techniques. Then, fibroblasts from passage 1 and passage 5 were stimulated with 10ng/mL TGF-β1 for 5 days and assessed for inflammation and fibrosis by flow cytometry for fibroblast specific surface markers, atomic force microscopy for matrix stiffness, RT-qPCR for fibrotic and inflammatory gene expression, and ELISA for cytokine and chemokine secretion. **Results:** Subcultured fibroblasts exhibited surface negativity for leukocyte and epithelial markers CD45, CD31, and CD326, and positivity for PDGFR-α and Thy-1 indicating correct isolation of airway fibroblasts. Further cytometry revealed increases in α-smooth muscle actin (α-SMA) and decreases in platelet-derived growth factor receptor-α (PDGFR-α) and Thy-1 between SGS and internal control fibroblasts. Immunofluorescence of TGF-β1 stimulated fibroblasts showed increased abundance of α-SMA and p-SMAD2/3 in fibrotic myofibroblasts compared to controls. Gene expression for inflammatory cytokines IL-6 and IL-8 was greater in SGS fibroblasts and confirmed with ELISA. Fibrotic markers also showed significantly greater expression in SGS fibroblasts than controls after TGF-β1 stimulation. **Conclusions:** Overall, we demonstrate that SGS fibroblasts are phenotypically matrix-depositing and pro-inflammatory fibroblasts that respond strongly to TGF-β1, while those from the internal control region are more matrix-remodeling and resist fibrosis. This indicates that targeted therapeutic approaches accounting for the distinct fibroblast populations are necessary to achieve superior SGS treatment.

Abstract 205

Doxorubicin-Induced Modulation of TGF-β Signaling Cascade in Mouse Fibroblasts: Insights into Cardiotoxicity Mechanisms

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Introduction: Doxorubicin (DOX)-induced cardiotoxicity has been widely observed, yet the specific impact on cardiac fibroblasts is not fully understood. Additionally, the modulation of the transforming growth factor beta (TGF-β) signaling pathway by DOX remains to be fully elucidated. This study investigated doxorubicin's ability to modulate the expression of genes and proteins involved in the TGF-β signaling cascade in mouse fibroblasts from two sources by assessing the impact of DOX treatment on TGF-β inducible expression of pivotal genes and proteins within fibroblasts. **Methods and Results:** Mouse embryonic fibroblasts (NIH3T3) and mouse primary cardiac fibroblasts (CFs) were treated with DOX in the presence of TGF-β1 to assess changes in protein levels by western blot and changes in mRNA levels by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Our results revealed a dose-dependent reduction in cellular communication network factor 2 (CCN2) protein levels upon DOX treatment in both NIH3T3 and CFs. Moreover, we observed that DOX inhibited the TGF-β1 induced expression of BMP-1 in NIH3T3 cells, while BMP-1 levels remained high in CFs and that TGF-β1 induces the phosphorylation of SMAD2 in both NIH3T3 cells and CFs. While DOX treatment diminished the extent of phosphorylation, the reduction did not reach statistical significance. DOX also inhibited the TGF-β1 induced expression of COL1 in NIH3T3 cells and CFs. Finally, DOX inhibited the TGF-β1 induced expression of ATF4 and increased the expression of Cdkn1a, Id1, Id2, Runx1, Tgfb1, Inhba, Thbs1, Bmp1, and Stat1 in NIH3T3 cells but not CFs, indicating the potential for cell-specific responses to DOX and its modulation of the TGF-β signaling pathway. **Conclusion:** Understanding the underlying mechanisms of the ability of DOX to modulate gene expression and signaling pathways in fibroblasts holds promise for future development

of targeted therapeutic strategies to mitigate DOX-induced cardiotoxicity specifically affecting cardiac fibroblasts.

Abstract 206

Transcription Factor HOXA5 is Mechanically Regulated in Pulmonary Fibroblasts via Integrin αV Signaling

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Introduction: Appropriate regulation of integrin activation and engagement with the surrounding extracellular matrix (ECM) is pivotal to maintaining tissue homeostasis and mobilizing fibroblasts in response to tissue insult. Failure of these endogenous regulatory mechanisms can skew normal wound healing behaviors towards pathological ECM remodeling and fibrosis. Our group has previously shown that the cell surface protein THY-1 functions as a stiffness-dependent inhibitor of αVβ3 integrin activation. When THY-1 expression is silenced, the inhibitory effect on αVβ3 is lifted and lung fibroblasts assume a contractile, activated phenotype independent of substrate stiffness. Yet, the genetic mechanisms by which this pathological state is achieved remain unknown. **Methods:** Assay for transposase accessibility of chromatin with sequencing (ATAC-seq) and RNA-seq were performed in parallel to investigate changes in chromatin accessibility and transcription in THY-1 KO cell human lung fibroblasts relative to wild-type (WT) counterparts. Samples were collected along a range of substrate stiffnesses and time points to catalogue a mechanoregulatory genomic profile of the THY-1 negative fibroblast. A suite of molecular biology approaches were utilized to confirm sequencing findings as well as investigate the mechanistic roles of targets identified from sequencing. **Results:** Our sequencing data showed a clear segregation of all samples on the basis of THY-1 expression. Pathway enrichment analysis of RNA-seq data showed a downregulation of pathways related to lung development, ECM organization, and embryonic axis patterning in THY-1 KO fibroblasts. Analysis of principal component loadings revealed homeobox A (HOXA) cluster genes were critical drivers of the observed transcriptional variance between WT and THY-1 KO cells. Investigation of ATAC-seq chromatin accessibility revealed a potent epigenetic silencing of the HOXA gene cluster after THY-1 loss. Given the well described role of HOXA5 in lung development, we performed *in vitro* follow-up studies on HOXA5 function. We confirmed HOXA5 expression, at the protein and RNA levels, was suppressed in THY-1 KO fibroblasts. Further, HOXA5 was mechanically regulated in WT fibroblasts with increasing ECM stiffness leading to HOXA5 downregulation. Culturing WT fibroblasts on fibronectin fragments that engage αV integrin binding abrogated HOXA5 levels on soft ECM entirely. Downstream analysis of HOXA5 loss demonstrated an enhanced proliferative capacity and resistance to apoptosis in *Hoxa5* KO mouse lung fibroblasts. **Conclusions:** We propose that THY-1 loss leads to excess levels of integrin αV activation on soft ECM and downregulation of HOXA5 which, in turn, leads to a more proliferative, anti-apoptotic phenotype. Thus, HOXA5 appears to be a key mediator of cell survival and apoptosis sensitivity in the soft ECM environment. HOXA5 could be a central target to induce myofibroblast clearance in the fibrotic environment.

Abstract 207

Fibroblast Mechanobiology Governs the Progression of Vocal Fold Scarring via the MRTF/SRF Axis

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Introduction: Vocal folds (VFs) vibrate at a frequency of 20Hz-3kHz during phonation (*J Cytol Mol Biol* 2013;**1**:001), and resident VF fibroblasts (VFFs) experience one of the most dynamic biomechanical environments. Increased tissue stiffness resulting from injury and subsequent vocal fold scarring (VFS) causes VFFs to differentiate in VF myofibroblasts (VFMs) (*Dis Model Mech* 2015;**8**:311-321) that excessively secrete disorganized collagen, further affecting VF architecture. To develop next-generation therapeutics, we need to elucidate the mechanisms involved in VFS progression and the positive mechanobiological feedback loop between VFMs and their microenvironment. Herein, we combined RNA

sequencing of human cells with an *in vivo* VFS rat model to identify the role of the mechanoresponsive MRTF/SRF axis in VFS. **Methods:** Six human VFF cell lines were treated with TGF-β1 for 5 days to induce a VFM phenotype and then compared to controls via bulk RNA sequencing. RT-qPCR and immunofluorescence (IF) were performed to validate changes in gene and protein expression. To confirm findings in 3D, VFFs were seeded into methacrylated type I collagen hydrogels (5 mg/ml, 0.1% LAP) engineered to match the mechanical properties of native VFs. Elastic modulus was determined by unconfined compression testing. Mechanobiology in VFS *in vivo*: VFS was induced by injuring the VF of male rats, chosen for the similarity of their VF ECM structure to that of humans. Histology and IF confirmed VFS and identified differentially expressed proteins at 3-, 7-, and 21-days post-injury. **Results:** Human VFFs and VFMs exhibit distinct transcriptional phenotypes with 989 significantly down-regulated and 1073 significantly up-regulated genes in VFMs. Gene set enrichment analysis revealed positive enrichment in pathways associated with ECM stiffening such as collagen synthesis and collagen fibril crosslinking as well as with fibroblast-ECM interactions such as integrin, ECM-receptor, and focal adhesion kinase (FAK) signaling. By analyzing downstream targets of transcription factors (TFs), we identified serum response factor (SRF) as the most highly up-regulated TF in VFMs, along with MRTF-A. The MRTF/SRF axis is mechanoresponsive and is upstream of alpha-smooth muscle actin (α-SMA), CTGF, and COL1A1, all key players in VFS. RT-qPCR and immunostaining confirmed increased gene expression of MRTF/SRF targets in 2D and 3D, and nuclear translocation of MRTF-A in VFMs was confirmed. In a rat model of VFS, MRTF/SRF targets were increased as well as FAK, an upstream regulator of the MRTF/SRF axis. **Conclusion:** VFMs have uniquely enriched mechanoregulated pathways, such as the MRTF/SRF axis. Both downstream targets and upstream regulators of this signaling pathway were also up regulated, which we confirmed in an *in vivo* model of VFS. These data suggest that the targeted inhibition of the MRTF/SRF axis may be a potential therapeutic target for VFS.

Abstract 208

Reprograming Ovarian Cancer-Associated Fibroblasts Using Tumor Conditioned Media

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Introduction: Ovarian cancer (OC) is one of the deadliest forms of cancer in women, where more than 80% of patients develop chemotherapy resistance. Cancer-associated fibroblasts (CAFs) are key contributors to this therapeutic resistance through dysregulation of the tumor extracellular matrix (ECM) composition and structure. CAFs may be derived from several cell types through activation and reprogramming by cancer cells, recruitment or epithelial to mesenchymal transition. While primary CAFs can be isolated from tumors and cultured *in vitro*, their lifespan is finite, and they have limited expansion capacity. Therefore, *there is a need to establish protocols that allow for the reprogramming of normal fibroblasts*. In the absence of reprogrammed CAFs, the development of effective anticancer therapies that mitigate CAF-mediated chemotherapy resistance in OC will likely remain difficult. **Methods:** Human primary uterine fibroblasts (HUFs) were reprogrammed into CAFs using tumor-derived conditioned media from two OC cell lines (Kuramochi and SKOV3). Tumor-derived conditioned media was characterized at the chemokine level. HUFs, conditioned CAFs, and primary CAFs were functionally and phenotypically characterized, as well as their effects on tumor promotion, enhancement of drug resistance, and tumor spheroid formation in 3D cultures elucidated. Retrieval of tumor-derived conditioned media was also investigated. **Results:** HUFs and primary CAFs have limited expansion capacity and short lifespans (about 10 doublings), while conditioned CAFs have a higher expansion rate of about 3-4-fold and were able to be kept in culture for longer passages. Phenotypically, conditioned CAFs were positive for stromal and CAF markers and negative for epithelial markers. Cytokine secretion capabilities of conditioned CAFs were similar to primary CAFs. Functionally, conditioned CAFs have significantly higher collagen contractility compared to HUFs. Moreover, conditioned CAFs significantly enhanced tumor growth about 2.5-3-fold, enhanced drug resistance by reduced cell killing, and promoted better spheroid formation with larger spheroids that were more intact in shape when compared to HUFs, as expected in a CAF-like phenotype. Retrieval of conditioned media did not alter either their phenotype or functionality. *Critically, these results suggest that uterine fibroblast can be reprogramed into CAFs using tumor-derived conditioned media*. **Conclusion:** Our results present a reproducible and functionally characterized protocol to reprogram HUFs

into CAFs using ovarian tumor-derived conditioned media. Moreover, our results are expected to have an important positive impact because they will provide strong evidence for further development of therapeutics that possess potentiality and specificity towards CAF-mediated chemotherapy resistance in OC as well as a translatable-based engineering approach that could be applied to different cancer cell types. **Acknowledgement:** This project is supported by NIH/NIGMS Center for Cancer Biology 5P20GM103548 and NIH/NCI R21CA259158.

Abstract 209

Fibroblasts Repair Blood-brain Barrier Damage and Hemorrhagic Brain Injury Via TIMP2

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Introduction: Fibroblasts are found in the perivascular space of large but not small blood vessels in brain parenchyma. Their functions in brain homeostasis and disease pathogenesis remain largely unknown due to the lack of fibroblast-specific markers. A recent single-cell RNA-sequencing study identified Col1α1 as a fibroblast-specific marker. **Methods:** We generated fibroblast-ablated mice using the Col1α1-Cre line and characterized the phenotypes of these mutants in both homeostatic conditions and after intracerebral hemorrhage (ICH). Specifically, ICH outcomes were assessed by neurological functions, hematoma volume, and blood-brain barrier (BBB) leakage. The paracellular and transcellular mechanisms of BBB disruption were investigated by immunohistochemistry and transmission electron microscopy (TEM). In addition, the molecules that mediate the neuroprotective role of fibroblasts were screened using a proteomic approach in an *in vitro* ICH model. The functions of identified molecules were investigated *in vitro* and *in vivo* using both pharmacological and genetic approaches. **Results:** Fibroblast-ablated mice failed to exhibit any gross defects under normal conditions. After ICH, however, these mutants showed worse neurological function and enlarged injury volume. In addition, we showed that fibroblasts migrated to and accumulated around capillaries after ICH, indicating a possible role in BBB repair. Consistent with this hypothesis, fibroblast-ablated mice displayed exacerbated BBB damage after ICH and fibroblasts significantly decreased endothelial permeability in an *in vitro* ICH model. Subsequent mechanistic studies revealed that fibroblasts repaired BBB damage mainly via up-regulating tight junction proteins without affecting transcytosis. Using LC-MS/MS, we identified ~20 fibroblast-secreted proteins that may mediate this neuroprotective effect. Further studies demonstrated that blockage or knockdown of TIMP2 in fibroblasts exacerbated BBB disruption in the *in vitro* ICH model. More importantly, we found that exogenous TIMP2 was able to attenuate BBB disruption in fibroblast-ablated mice after ICH. **Conclusion:** These results suggest that Col1α1^{+} fibroblasts repair BBB damage in ICH via the paracellular pathway in a TIMP2-dependent manner, and that fibroblasts and TIMP2 may be targeted in the treatment of ICH.

Infectious Diseases

Abstract 210

Commensal Microbes Limit *Fusobacterium nucleatum* Colonization of Human Bacterial Bioreactors

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Introduction: Inflammatory bowel disease (IBD) affects more than 1 million people per year and is increasing in prevalence worldwide. The pathophysiology of IBD is characterized by patterns of acute and chronic inflammation of the gastrointestinal tract. It has been speculated that in addition to environmental and genetic factors, intestinal bacteria may also contribute to the development and progression of IBD. 16S sequencing has revealed that the gut microbiota of IBD patients are enriched with oral-derived microbes compared with that of healthy individuals. One of the striking features of IBD patients is an increase in the genus *Fusobacterium,* a known inhabitant of the oral cavity. Of the *Fusobacterium* species**,** *F. nucleatum* has recently emerged as a compelling candidate for IBD exacerbation given its prevalence in biopsy specimens. We previously found that *F. nucleatum* could not colonize and cause inflammation in mice harboring a complete gut microbiota. As a result, we hypothesized that the gut microbiota of IBD patients is permissive to *F. nucleatum* colonization and subsequent inflammation. **Methods and Results**: To address whether the IBD microbiome was more permissive to *F. nucleatum* colonization, we created

bioreactors that resembled the intestinal environment of IBD patients or healthy individuals. To model the healthy gut, we grew a cohort of human *Bacteroides, Prevotella, Clostridium, Blautia, Lactobacillus, Escherchia, Bifidobacterium, Streptococcus, Roseburia, Akkermansia* and *Lactococcus* strains. To model the IBD gut microbiota we grew different strains of human *Bacteroides, Clostridium Streptococcus, Escherchia* and *Bifidobacterium,* and included *Parabacteroides, Veillonella, Enterobacter, Enterococcus, Pseudomonas, Staphylococcus, Morganella* and *Alistipes* strains. These microbial communities were grown together in a chemically defined medium designed to grow all microbes for 48 hrs. When we added *F. nucleatum* ATCC 25586 to healthy bioreactors, we observed low levels of *F. nucleatum* by qPCR. However, when we added *F. nucleatum* to IBD bioreactors, we observed a robust expansion of *F. nucleatum*; suggesting that IBD microbes are permissive to *F. nucleatum* colonization. Secreted factors from bioreactors were used to stimulate human intestinal epithelial cells. We found that the healthy communities elicited moderate levels of NFkB as measured by an NFkB luciferase reporter, while IBD communities stimulated significantly higher levels of NFkB. Addition of *F. nucleatum* to healthy communities, didn't influence the NFkB levels, but the addition of *F. nucleatum* to IBD communities robustly increased the ability of these communities to stimulate NFkB. We likewise observed a significant increase in the pro-inflammatory cytokines IL-8 and TNF in IBD communities, particularly those harboring *F. nucleatum*. **Conclusions**: These data indicate that the presence of *F. nucleatum* significantly elevates the pro-inflammatory capability of IBD microbial communities.

Abstract 211

Interleukin 6 Induces an Antiviral Response Independent of Interferons Through Mitochondrial DNA Release and Direct STAT1 Activation

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Introduction: Interleukin 6 (IL-6) promotes vascular dysfunction, leading to organ failure in response to severe inflammatory conditions. Consistently, we have shown that mice lacking the main negative regulator of IL-6 signaling, SOCS3, in the endothelium (SOCS3iEKO) displayed vascular leak, kidney failure, and fast mortality upon a single challenge with LPS. These responses were associated with increased expression of antiviral response genes in the failing organs. In this study, we aim to elucidate the endothelial transcriptional profile within the failing endothelium and the signaling mechanisms regulating this transcriptional response. **Methods:** We performed cdh5-CreERT2-driven translating ribosomal affinity purification (TRAP) and RNA-seq from kidneys of control or SOCS3iEKO mice challenged or not with LPS. In vitro experiments in cultured human umbilical vein endothelial cells (HUVEC) involved RT-qPCR, subcellular fractionation, Western blotting, and super-resolution imaging after immunofluorescence. **Results:** LPS induced a strong increase in multiple interferon stimulated genes (ISG), a response that was further increased by loss of endothelial SOCS3. We have previously shown that IL-6 leads to an increase in expression of several ISGs without inducing the expression of interferon (IFN) themselves. Thus, we sought to determine how IL-6 promotes ISG expression. We found that this transcriptional response was directly induced by IL-6 activated pathways because it did not require de novo protein synthesis or STAT3 expression. Instead, this response was inhibited by PI3K inhibitor or STAT1 knockdown. Immunofluorescence of IL-6 treated cells showed nuclear localization of STAT3 and STAT1, but not of STAT2. In contrast, STAT2 quickly translocated to the nucleus in response to an IFNβ challenge. Surprisingly, we found that IL-6 induced changes in mitochondrial localization minutes after treatment. Consistent with acute mitochondrial stress, IL-6 led to a quick and transient release of mitochondrial DNA to the cytoplasm that did not lead to apoptosis. Pharmacological inhibition of the cytosolic DNA sensor cGAS, or knockdown of the downstream mediator STING, reduced ISG expression. Subcellular fractionation assays show that interferon regulatory factors (IRF) 1,2,3 and 4 are present in the nucleus of IL-6-treated cells, whereas IRF9, the canonical partner of STAT1 and STAT2, was not. **Conclusions:** We demonstrated that IL-6 induces ISG expression through a non-canonical pathway that involves STAT1, PI3K and cGAS/STING, but not the release of IFN or the activation of the classical STAT1/STAT2/IRF9 trimeric transcription factor. A similar expression profile was detected in the endothelium of inflamed kidneys, suggesting that this pathway occurs upon shock. Given the deleterious effects of interferon signaling in the absence of a viral infection, this pathway represents a novel therapeutic target to limit organ dysfunction during bacteria-induced shock.

Abstract 212

Clostridioides difficile Enzymatically Degrades the Outer Wall of _Candida_ Species to Support its Growth

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Introduction: *Clostridioides difficile* is a spore forming, gram positive enteric pathogen that causes diarrhea and colitis. According to the Center for Disease Control, *C. difficile* infects approximately half a million Americans each year. *C. difficile* infection commonly occurs after the use of broad-spectrum antibiotics that disrupt the normal gut microbiota. This disruption in the bacterial communities allows *C. difficile* to colonize the large intestine, where it produces toxins that cause the symptoms of disease. In addition to altered bacterial populations, recent evidence suggests that there is a shift in gut fungi in *C. difficile* infection. *Candida* species are the dominant fungi in the intestine, and these organisms are covered with an outer layer of mannan consisting of multiple α- and β-linked mannose residues. Analysis of *C. difficile* genomes revealed that *C. difficile* harbors the glycosyl hydrolase enzymes required to cleave mannose of fungal mannan. As a result, we hypothesized that *C. difficile* could enzymatically remove mannose from *Candida* strains and use it as a substrate for growth. **Methods and Results:** Analysis of 391 *C. difficile* genomes in the Integrated Microbial Genome (IMG) database revealed that all *C. difficile* genomes contained α-mannosidases (GH38, EC 3.2.1.24), and 387 genomes contained mannosylglycerate hydrolase (GH38, EC 3.2.1.170), suggesting that *C. difficile* could enzymatically remove α-linked mannose residues. We found that multiple strains of *C. difficile* (R20291, 630, M68 and 37) exhibited robust growth in a chemically defined minimal media, CDMM, which lacked glucose but was supplemented with mannose and yeast extract. To confirm that fungal mannan could specifically be degraded by *C. difficile*, we purified mannan from *C. albicans*, *C. glabrata* and *C. tropicalis*. We found that all *C. difficile* strains grew in media containing purified mannan, indicating that *C. difficile* could use fungal mannan as a fuel source. Aggregation assays and microscopy indicated that *C. difficile* did not bind to *Candida* strains. However, they did form biofilms. Additionally, we observed that *Candida* strains quenched oxygen, resulting in localization of an anaerobic environment. We confirmed that *C. difficile* could grow in aerobic environments when co-cultured with *Candida* species but was unable to grow in aerobic environments when alone. **Conclusions**: These data demonstrate that *C. difficile* participates in an interkingdom interaction with *Candida* species and provides insight into another pathway by which *C. difficile* can gain a competitive advantage in the gut.

Abstract 213

Pathogenicity of _Acinetobacter calcoaceticus_

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Introduction: Although *Acinetobacter* species are commonly thought of as a hospital acquired infection, *Acinetobacter* occupy several ecological niches, including the mammalian intestine. *Acinetobacter* species are found in the human fecal microbiota, and it has been reasoned that the gut could serve as a potential reservoir for infection. The importance of *Acinetobacter* in the gut is highlighted by the fact that *Acinetobacter* species are elevated in patients with Crohn's disease, a subset of inflammatory bowel disease (IBD) as confirmed by the database IBD Tamma. Little is known about the interactions between *Acinetobacter* and the gut and its effects on gut health. To fill this knowledge gap and determine the pathogenicity of *A. calcoaceticus*, we are utilizing *Caenorhabditis elegans* as a model. *C. elegans* consume bacteria as their normal food source allowing bacteria to colonize their gut like the mammalian intestinal microbiota. Other groups have shown that *A. baumannii* is lethal to *C. elegans*, but no information exists on *A. calcoaceticus*. We hypothesize that *A. calcoaceticus* can infect *C. elegans* and secrete compounds which damage the gut. **Methods**: To determine if *A. calcoaceticus* can colonize the gut of *C. elegans*, we fluorescently tagged commercially available strains and clinical isolates of *A. calcoaceticus* and allowed *C. elegans* to consume these microbes over 3 days. As a control, we fed *C. elegans* fluorescently tagged *A.*

baumannii ATCC 19606 or *E. coli* OP50. By microscopy and plate reader, we observed similar levels of bacteria inside the *C. elegans* after ingestion. To examine survival, we reared *C. elegans* on plates with *A. calcoaceticus* strains, *A. baumannii* and *E. coli* at 20°C and examined survival over time. **Results:** Interestingly, we observed a similar survival curve for worms fed all microbes, suggesting that *Acinetobacter* does not cause significant lethality on solid plates. We reasoned that *Acinetobacter* may secrete compounds at 37°C which are not observed in the worm at 20°C. To address this, we grew *Acinetobacter* strains and *E. coli* in LB at 37°C and added the cell-free supernatant to *C. elegans* as a liquid culture. Liquid survival assays revealed that *A. calcoaceticus* strains were highly lethal to *C. elegans*. Proteinase K treatment and filtering of the supernatant through centrifuge columns diminished the lethality, indicating that *A. calcoaceticus* secretes a protein which is lethal to *C. elegans*. **Conclusions**: Our data suggests that *A. calcoaceticus* is capable of colonizing the *C. elegans* gut and in its natural environment it can secrete proteins which stimulate inflammation and cause death in *C. elegans*. These findings point to a causative role for *A. calcoaceticus* in IBD and suggests that *A. calcoaceticus* could be a target for future therapeutic approaches. **Acknowledgement:** Funding was provided in part by the National Institutes of Health T32-GM132055 (JSG) and R00-ES029552 (JHH).

Abstract 214*

Blood Group Positive Microbes Stimulate the Development of Anti-blood Group Antibody Formation

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*The authors of this abstract selected not to publish the details of their research

Abstract 215

Integrating Genomics, Transcriptomics and Virulence to Study *Streptococcus dysgalactiae* subspecies equisimilis (SDSE) Strains Causing Severe Infections in Humans

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Introduction: *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) is a Gram-positive pathogen that can infect humans and several animals; it is closely related to group A streptococcus (GAS). Much less is known about SDSE strains compared to GAS. One SDSE *emm* type (*stG62647*) can cause severe infections, including necrotizing soft-tissue infections (NSTIs), endocarditis, and osteoarticular infections (OAI). We combined genomics, transcriptomics, and virulence to begin to study the molecular pathogenesis of *stG62647* SDSE strains causing human infections. **Methods**: We sequenced to closure the genomes of 125 SDSE *emm* type *stG62647* isolates using Illumina and ONT platforms. Genomes were annotated and shared genes identified bioinformatically. We performed mouse virulence assays for these 125 strains and analyzed transcriptome profiles (RNA-seq) for 43 strains grown *in vitro*, including the least and most virulent strains. We also used whole genome sequence data to perform polymorphism analysis. **Results**: The genomes of these strains varied between 2.1 and 2.24 Mega base pairs. The largest source of genomic diversity was differences in regions of difference, including putative mobile genetic elements. The 125 *stG62647* strains were tested for virulence with a mouse model of necrotizing myositis. The strains had a wide range of virulence, as assessed by mouse survival curves. To begin to provide a molecular explanation for these virulence differences, we performed transcriptome analysis on the most and least virulent strains. Due to the inherent genetic diversity, RNA-seq data were mapped to each closed genome. The results identified certain SDSE virulence factors as potential contributors to differences in transcriptomes and virulence. **Conclusions:** Increased rates of invasive SDSE infections in humans have recently been reported in many countries worldwide. By integrating genomics, RNA-seq and virulence data generated with a mouse model of necrotizing myositis we have identified several molecular targets for future studies. Taken together our study provides new information about SDSE infection pathogenesis and highlights the need for expanded studies on the genomics and molecular mechanisms of virulence in this bacterial pathogen.

Abstract 216

Population Genomic Analysis of 499 *Streptococcus dysgalactiae subsp. equisimilis* Clinical Isolates Collected in French Brittany, 2010-2018

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Introduction: *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) is an increasingly recognized cause of severe invasive human infections. Recently for reasons unknown, SDSE strains of *emm* type *stG62647* have emerged in multiple countries as a prevalent cause of invasive human infections in association with severe clinical manifestations. At the species level, SDSE is most closely genetically related to the human specific pathogen *S. pyogenes*. However, compared to *S. pyogenes*, much less is known about the pathogenomics of SDSE in human infectious disease. To address these knowledge deficits, we conducted whole genome sequencing of 499 SDSE clinical isolates collected in French Brittany, from Sep. 2010 to Dec. 2018. **Methods:** Isolated genomes were sequenced with Illumina paired-end short read methods. Genomes were assembled denovo using SPAdes. Isolate genomes were characterized as to the common epidemiological markers of streptococcal group antigen, *emm* type and multi-locus sequence type by BLAST search against public reference databases. Polymorphisms (SNPs and indels) were identified genome-wide for the cohort using MUMmer, and core genome SNPs were used to define genetic linages by density-based spatial clustering using PopPUNK and to infer phylogeny using SplitsTree4. Genome assemblies were annotated using PROKKA. Core, accessory and pangenome gene content was defined using Panaroo. **Results:** Genome assemblies ranged in size from 1.96 to 2.30 Mbp with an average of 2.10 Mbp. The isolates differed in streptococcal group antigen, with 71% being G, 28% C and 1% A. The isolates were of 28 *emm* types, with the emergent high-virulence associated type *stG62647* (*n*=125) isolates being the most abundant accounting for 25% of the cohort. Whereas the 2nd through 5th most prevalent *emm* types were present throughout the 9-year period of the survey, *stG62647* isolates were only present from 2013 and after. The isolates are of 38(+) MLSTs with ST20 (*n*=124) and ST17 (*n*=86) being most abundant. A total of 144,712 core SNPs were identified among the genomes, with any 2 core genomes differing on average by 12,932 SNPs. Density-based spatial clustering of the strain-to-strain core SNP differences parsed the 499 isolates into 88 distinct genetic lineages. The top 10 most abundant lineages accounted for the bulk of the isolates (*n*=347) while 50 of the lineages were unique to a single isolate. The average genome had 1,946 protein encoding genes (range = 1,779 to 2,124). The 499 isolates have a pangenome of 4,906 genes, and a common core genome of 1,525 genes (present in >490). **Conclusions:** The cohort of 499 SDSE clinical isolates collected in French Brittany encompasses substantial genetic diversity as exemplified by differences in genome size, gene content, core SNPs, and common epidemiological markers. The abundance and temporal distribution of the s*tG62647* isolates is consistent with their emergence in 2013 as the most prevalent cause of SDSE infections.

Abstract 217

Regulation of the Adherens Junction-Associated RNAi Machinery by Oral Pathogens

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Introduction: Increasing evidence portrays key roles of host-microbial interactions in epithelial cancer progression. The pathogen *Fusobacterium nucleatum* has been strongly linked with colorectal tumors. However, the mechanisms by which *F. nucleatum* may promote tumorigenesis are still debated. Bacterial pathogens notoriously disrupt epithelial integrity. The adherens junction (AJ) is an essential, E-cadherin–based, cell-cell adhesion complex that is key for epithelial tissue integrity. We have previously shown that epithelial AJs recruit and regulate the core complexes of the RNA interference (RNAi) machinery, including the microprocessor and its main components DROSHA and DGCR8, to suppress pro-tumorigenic cell transformation through miRNA-mediated suppression of oncogenes. We have also found that this mechanism is extensively disrupted in colon tumors. Thus, we asked whether *F. nucleatum* may be

disrupting the AJ-associated RNAi, as a mechanism mediating its pro-tumorigenic effects. **Methods:** Confluent monolayers of the well-differentiated colon epithelial Caco2 cells were cocultured with *F. nucleatum* bacterial supernatant, heat-inactivated bacteria, and isolated LPS. Cells were examined after 24 hours of coculture: a) by immunofluorescence and confocal microscopy for AJ and microprocessor component localization; b) qRT-PCR for miRNA/mRNA expression; c) western blotting for oncogene expression. **Results:** Co-culturing Caco2 cells with *F. nucleatum* resulted in loss of DROSHA localization from bicellular junctions and its strong accumulation at tricellular junctions. Instead, DGCR8, essential for DROSHA's miRNA processing function, remained unaffected at bicellular junctions. This suggests that *F. nucleatum* disrupts the junctional microprocessor, which is necessary for processing tumor-suppressing miRNAs. Indeed, this was accompanied by increased expression of SNAI1, a key oncogenic target of the junctional microprocessor. Interestingly, the degree of both the altered DROSHA localization and SNAI1 upregulation were proportional to increasing bacterial load. Overall, the core adherens junction components E-cadherin and p120 remained unaltered by *F. nucleatum*, indicating that the effects on the microprocessor are downstream of them, in agreement with our previous findings that show retainment of E-cadherin in tumors, but the loss of junctional RNAi. Tricellular junctions are under high actomyosin tension, implying that this translocation of DROSHA may be through actomyosin mechanotransduction, which we are currently investigating as the mechanism mediating these effects. **Conclusions:** This data supports the notion that *F. nucleatum* promotes pro-tumorigenic transformation through disruption of the AJ-associated RNAi, leading to oncogene upregulation, through miRNA disruption. Once completed, these studies may deepen our understanding of the mechanisms mediating host-pathogen interactions in tumorigenesis.

Inflammation

Abstract 121

Loss of Glutamine Synthetase Results in Hyper-progressive β-catenin-mutated Hepatocellular Carcinomas Driven by Enhanced Myeloid Cell Induced Immunosuppression

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Introduction: Around 30% of HCC cases harbor mutations in *CTNNB1*, the gene that encodes β-catenin. Glutamine Synthetase (GS), encoded by *Glul*, a well-known β-catenin target gene, can be used with high sensitivity and specificity on histology to identify these tumors. Previously, the β-catenin-GS-glutamine-mTORC1 axis has been examined in *CTNNB1*-mutated HCCs and demonstrated high susceptibility to mTOR inhibitors in preclinical models. Here, we investigate this axis further by targeting GS in β-catenin-mutated HCCs with a major focus on tumor immune environment. **Methods:** HCCs were induced in *Glul*-floxed mice by co-delivering mutant-β-catenin-(T41A) and mutant nuclear factor erythroid 2–related factor 2 (Nrf2-G31A) (B+N model). *Glul* elimination following tumorigenesis was achieved by delivering AAV8 carrying Cre-recombinase. HCC burden was assessed in *Glul*-KO and controls. We preformed 10x Genomics Spatial Gene Expression profiling to measure mRNA with fluorescent immunostaining in tandem, as well as single-cell RNA-sequencing (scRNA-Seq) to identify changes in the immune microenvironment in *CTNNB1*-mutated HCCs ± GS. All results were checked by immunostaining and immunoblot. **Results:** *Glul* was sufficiently deleted by two weeks from HCCs in the B+N model using AAV8-Cre. This was associated by decreased intratumoral glutamine levels. Interestingly, 4.5-weeks post *Glul* elimination, a significant increase in LW/BW ratio, indicating a greater tumor burden, was observed and persisted to 7-weeks. A counter-intuitive mTORC1 activation was evident due to GS-independent mechanisms in the *Glul*-KO tumors. While spatial expression analysis showed no transcriptomic differences between *Glul*-KO tumors and control, we identified reduced *Mrc1*, *Adgre1*, *Cd68*, *Vsig4*, and *Cxcr4,* myeloid cell markers, in *Glul*-KO HCCs which was validated by immunostain. Sc-RNA-Seq data identified an increase in the numbers of myeloid cells expressing immunosuppressive patterns in GS-KO HCCs. We also found that *Glul*-KO HCCs are still unresponsive to αPD-1 ICI likely due to a combination of myeloid-driven immunosuppression and immune escape in *CTNNB1*-mutated HCCs. Finally, since targeting GS promotes immunosuppressive myeloid function in β-catenin-mutated HCCs, we tested the consequences of depleting myeloid cells in B+N control animals and found no difference in overall disease burden. **Conclusion:** Our data indicate that GS-loss increases the immunosuppressive function of myeloid cells thus contributing to

the increase in disease burden in *Glul*-KO animals. Since myeloid cells are still found in these tissues, our data suggests intrinsic *Glul* expression and in turn tumoral glutamine in β-catenin-mutated HCCs alter resident macrophage function and physiology. Current studies are addressing if tumor associated macrophages in HCCs are dysfunctional Kupffer cells or originate from other myeloid populations and may provide insight into HCC disease pathogenesis and information regarding ICI efficacy.

Abstract 219

Intestinal Protein Sorting Nexin 27 Preserves Epithelial Barrier and Inhibits Inflammation

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Introduction: The sorting nexin (SNX) 27 belongs to a family crucial in protein trafficking and endocytosis. SNX27 contains an additional PDZ domain and recycles transmembrane proteins. *PDZ includes the* first letters of the first three proteins discovered to share the domain — post synaptic density (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 (zo-1). SNX27 has been explored in neurological disorders, however its impact on intestinal homeostasis and inflammation is unknown. Here, we aim to determine the tissue-specific role of SNX27 in intestinal barriers and inflammation. **Methods:** We generated a novel model of intestinal epithelial cells (IECs) SNX27 conditional knockout and analyzed the basal level changes in the $SNX27^{\Delta IEC}$ mice. We then investigated the role of SNX27 in intestinal chronic inflammation using colitis models. Furthermore, we analyzed the changes of SNX27 in patients with inflammatory bowel disease using human datasets. Loss of function studies of SNX27 and its function in inhibiting inflammatory pathways was also investigated *in vitro*. **Results:** The $SNX27^{\Delta IEC}$ mice had shorter colon and higher intestinal permeability without any treatment, compared to the $SNX27^{loxp}$ mice. We analyzed junction proteins in the colon and observed downregulation of Claudin10, upregulation of Claudin15, and rearranged distribution of ZO-1 and E-cadherin in $SNX27^{\Delta IEC}$ mice. We observed that $SNX27^{\Delta IEC}$ mice were more susceptible to DSS-induced colitis as they lost more body weight, had shorter colon and small intestine, and significantly increased intestinal permeability. Dysregulated ZO-1 and E-cadherin were observed by immunostaining and western blots. Cytokines, such as TNF-alpha, significantly increased in the $SNX27^{\Delta IEC}$ mice compared to $SNX27^{Loxp}$ mice after challenging DSS. Moreover, $SNX27^{\Delta IEC}$ mice were more susceptible to AOM/DSS with a higher mortality rate. AOM/DSS-$SNX27^{\Delta IEC}$ mice had earlier-onset of tumorigenesis, larger small intestine and spleen, smaller cecum, and higher intestinal permeability. The overall tumor burden was higher in $SNX27^{\Delta IEC}$ mice with larger tumors, compared to the $SNX27^{loxp}$ mice. We compared human datasets GSE102134, -9452, -10616, and -1710 to analyze SNX27 expression in Crohn's disease (CD) and ulcerative colitis (UC) patients. We showed a trend of downregulated SNX27 mRNA expression in human CD, and significant reduced SNX27 in UC patients. *In vitro* SNX27 siRNA knockdown study showed elevated levels of phosphorylated p65 subunit of NF-kB, the proinflammatory regulator. In contrast, the total p65 level remained unchanged with respect to SNX27 deletion. **Conclusion:** Loss of SNX27 leads to a disrupted epithelial barrier integrity and increased inflammation. There was downregulated SNX27 mRNA in human IBD. Functional studies will be done using human samples and organoids. We will provide insights into intestinal homeostasis and pathophysiology of barrier dysfunction and inflammation.

Liver Pathobiology II

Abstract 220

Targeted Hepatocyte-Specific β-catenin Overexpression Facilitates Improved Biliary Repair During Intrahepatic Cholestasis

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Introduction: Cholestasis is a progressive hepatobiliary disease characterized by chronically impaired biliary system. Only a few effective medical therapies to halt disease progression are available, and so cholestasis represents a major unmet need in clinical hepatology. β-catenin has previously been shown to enhance hepatocyte (HC) reprogramming to biliary cells (BC) when the latter gets impaired due to chronic cholestatic injury. Owing to this, we aimed to investigate the role of HC-specific β-catenin overexpression in mediating HC-to-BC reprogramming during intrahepatic cholestasis. We hypothesized that livers with HC-specific β-catenin overexpression will have more HC-derived BCs than wild type and contribute to functional de novo biliary branches that will help improve intrahepatic cholestasis. **Methods:** Age matched WT control (Con) and Ser-45 mutant β-catenin (TG) mice, both containing ROSA26-stop$^{flox/flox}$, were injected with AAV8-TBG-Cre to permanently label HCs with EYFP. Following a two-weeks washing period, mice were fed 0.1% of porphyrinogenic biliary toxin 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet for up to 150d. Bile duct cannulation was done following anesthesia to monitor bile flow for 60 mins. Liver and blood were collected for analyzing fibrosis and parameters of injury. Porphyrin was measured in the liver tissues and bile. Real-time PCR and staining techniques were utilized to determine the expression of bile transporter genes and biliary markers respectively. **Results:** TG livers showed significantly less porphyrin accumulation in comparison to the Con after DDC diet as depicted by histological staining and biochemical assay. In contrast, porphyrin levels were elevated in TG bile likely to facilitate porphyrin clearance through the bile in TG than the Con. Interestingly, TG showed significantly increased bile flow rate (BFR) as compared to the Con after DDC diet which was concomitant with decreased bile accumulation within the TG livers as compared to the Con. Analyzing the bile homeostasis and transport genes depicted comparable levels of expression in both groups, suggesting that the increased BFR observed in TG is independent of bile metabolizing genes. TG showed an increased number of HC-derived duct-like structures with biliary marker expression as compared to Con. These HC-derived BC showed CFTR expression, indicative of BC functionality. In addition, TG had an increased number of A6-positive HCs than the Con – suggestive of an increased number of intermediate oval cells or HCs undergoing reprogramming. **Conclusions:** TG showed significantly reduced porphyrin, improved BFR, and decreased hepatic bile load in response to the biliary toxin DDC. Utilizing HC fate-tracing, we showed increased BC markers in TG hepatocytes that describes HC-to-BC reprogramming. **Acknowledgements:** This study was supported by NIH grants R01DK103775, R01DK119435, R01DK124412 and Pittsburgh Liver Research Center grant P30DK120531 to KNB.

Abstract 221

TET1 Antagonizes Ferroptosis in Alcohol Associated Liver Disease

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Introduction: Alcoholic Liver Disease (ALD) is significantly correlated with hepatic-associated death. Ferroptosis is a form of iron-dependent cell death that has been shown to be exasperated in ALD. Hepatic cell death has also been linked to downregulated TET1 and 5-hydroxymethylcytosine (5hmC) in ALD. DNA demethylation is associated with 5hmC formation and catalyzed by the TET family proteins. This study aimed to reveal how TET1 protects against ferroptosis in hepatocytes in the presence of alcohol. **Methods:** Liver specific TET1 knock (TET-1 LKO) out mice were fed with a 5% ethanol liquid diet to induce ALD and compared with WT mice fed with an isocaloric diet. Experiments, including western blot, 5hmC dot blot, real-time quantitative PCR, and histological assays (Iron staining) were used to determine TET1's activity and its effects on ferroptosis. **Results:** It was found that TET1-LKO was associated with an increase in ferroptosis in ALD mice. This was demonstrated through the upregulation of 4HNE, a lipid peroxide derivative involved in ferroptosis, in the LKO mice. Further, liver tissue samples revealed evidence of ferric deposits in the TET1 LKO mice. 5hmc dot blot was utilized to determine the activity of TET1 and a decrease was apparent in TET1 LKO mice. **Conclusion:** In conclusion, these findings begin to reveal the defensive role of TET1 against ferroptosis in ALD. In the future, activation or upregulation of TET1 could serve as a potential therapeutic to combat ferroptosis in ALD.

Abstract 222

Determination of the MicroRNA Profile of the EVs in the Context of Autophagy Deficiency

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Introduction: As an evolutionarily conserved metabolic process autophagy functions in transporting intracellular components by the autophagosome to the lysosome for degradation. Autophagy deficiency or inhibition results in increased production of extracellular vesicles (EVs). EVs are defined as membrane-surrounded, nanometer-sized vesicles released by cells into the extracellular space in a highly regulated manner. They play critical roles in cell-cell communications and in pathogenesis of autophagy deficiency and in that of many other diseases. The impact of EVs is likely dependent on the cargo they carry, which can be different in the disease context from that in the normal condition. However, the profile of EVs cargo in specific disease scenario varies and needs to be specifically defined. **Methods:** Here we use a mouse model of liver injury caused by autophagy deficiency to investigate the signature of EVs. **Results:** Nanoparticle tracking analysis (NTA) data showed that there was an increased serum presence of liver-specific EVs when hepatic autophagy function was disabled. These EVs had an average size of 100 nm and expressed hepatocyte-derived ASGR1 and CYP2E1. We decided to profile the microRNA content in these EVs as microRNAs have been shown to account for a large number of the function of EVs. The sequencing results showed that expression of 105 miRNAs were altered significantly in EVs isolated from *Atg7*$^{\Delta Hep}$ mouse serum, compared to *Atg7*$^{flox/flox}$ mouse serum. 47 miRNAs were up-regulated, and 58 miRNAs were down-regulated. Based on the enrichment score, the top five items are in biological process, cellular component, molecular function, and specific pathways. We then focused on the inflammatory process where we were able to identify microRNA that regulates inflammation. In vitro analysis verified the role of these microRNA, which in turn suggests that EVs generated by autophagy deficient livers can promote inflammation, thus promoting liver pathology. **Conclusion:** In summary, our work has revealed microRNA profile of EVs under autophagy deficient condition, which provides the information in detail for further study the liver diseases related to autophagy inhibition.

Abstract 223

Inhibition of β-catenin Attenuates Lithocholic Acid-Induced Hepatotoxicity

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Introduction: Lithocholic acid (LCA) is a toxic hydrophobic bile acid associated with hepatotoxicity and adverse clinical outcomes in primary sclerosing cholangitis patients. We previously reported that conditional loss of β-catenin decreases bile acid synthesis and prevents the development of cholestatic liver injury after bile duct ligation, wherein obstructive bile flow is the primary cause of injury. To ascertain if loss of β-catenin can attenuate injury in models of toxic bile acids, we aimed to investigate the role of β-catenin inhibition in modulating liver injury against LCA. **Methods:** Age-matched wild-type control (Con) and liver-specific β-catenin knockout (KO) mice were fed LCA (0.6%) for a week. Mice were euthanized and liver tissues analyzed by histology, qPCR, RNAseq, immunostaining, Western blot and liquid chromatography-mass spectrometry. Liver-infiltrating mononuclear cells were isolated and analyzed by flow cytometry. Serum biochemistry was measured to assess liver injury parameters. **Results:** β-catenin KO livers displayed fewer and smaller bile infarcts as compared to Con livers after LCA administration. Serum biochemistry showed significant decrease in hepatic and biliary injury in KOs compared to Cons. Gene expression analysis revealed decreased bile acid uptake transporters, increased apical and basolateral efflux transporters, and increased expression of detoxifying cytochrome P450 enzymes in the KO group with the net result of decreased accumulation of toxic bile acid in KO liver and hence less hepatic injury. Pan-cytokeratin immunostaining showed increased ductular response in KOs, likely as a defense mechanism for mediating enhanced BA clearance. RNA-seq analysis demonstrated decreased proinflammatory genes IL-33, IL-1β and target receptors IL-1R, and TNFR in the KO livers compared to Con. Immunostaining analysis further confirmed significant activation of IL-33 in the hepatocytes of Con group but not in KOs. Interestingly, liver-resident innate lymphoid cells (ILC2) were downregulated in KOs during LCA-induced liver pathology as compared to the Cons. BA composition was slightly altered in KO after LCA, with a predominance of TbMCA

over TMDCA. **Conclusions:** As observed after BDL, loss of β-catenin offers protection from LCA-induced hepatotoxicity and biliary injury through increased transport and hydroxylation of toxic BA. IL-33 seems to be an important target of β-catenin and its secretion from hepatocytes may contribute to hepatic injury induced by the toxic bile LCA. Detailed mechanistic pathway(s) will be assessed in future studies. **Acknowledgements:** This study was supported by NIH grants R01DK103775, R01DK119435, R01DK124412 and Pittsburgh Liver Research Center grant P30DK120531 to KNB.

Abstract 224

Pivotal Role of MET in Promoting Liver Regeneration Following Acetaminophen Hepatotoxicity Identified Using Liver-Specific Knock-Out and Pharmacological Inhibition Strategies in Mice

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Introduction: MET is the receptor for hepatocyte growth factor (HGF), which plays a vital role in driving hepatocyte proliferation in liver following partial hepatectomy (PH). The involvement of MET in acetaminophen (APAP)-induced liver injury (AILI) and compensatory liver regeneration is not well understood, which is clinically relevant as APAP overdose is the foremost cause of acute liver failure in the western world. The role of MET in AILI should not be presumed identical to its role in PH of a healthy liver, as the regenerative response in AILI is uniquely shaped by the presence of massive liver injury and inflammation, setting it apart from the PH model. Interestingly, in our previous study, we observed a distinct dose-dependent activation pattern of MET in mice following APAP overdose, with higher MET activation after severely toxic (non-regenerative dose) of APAP. **Methods:** Here, we investigated a causal role of MET in AILI and compensatory regeneration, utilizing two different strategies. One involved systemic administration of the c-MET inhibitor, Capmatinib (15 mg/kg), while the other utilized specific deletion of MET in the liver using an albumin-CRE system. All mice were administered 300 mg/kg of APAP to examine liver injury, regeneration parameters, and associated signaling cascade at multiple time points. **Results:** Systemic administration of MET inhibitor did not alter initial liver injury but suppressed hepatocyte proliferation following APAP overdose in mice. To substantiate, liver-specific MET KO mice also displayed profound inhibition of hepatocyte proliferation compared to WT mice. The phenotype of impaired liver regeneration was much severe in MET KO mice, leading to uncontrolled progression of liver injury and significant mortality. As expected, WT mice exhibited spontaneous compensatory regenerative response and achieved complete recovery. The mechanisms underlying initiation of AILI, including metabolic activation of APAP, formation of APAP-protein adducts, and activation of JNK, remained unchanged after MET inhibition or deletion. However, ERK signaling was consistently inhibited in both MET inhibitor and MET KO mice, which was associated with suppressed induction of cyclin D1 and failed activation of core cell cycle machinery, resulting in impaired liver regeneration. Lastly, RNA sequencing followed by Ingenuity Pathway Analysis revealed failed activation of several key regulators of hepatocyte proliferation upon MET signaling disruption. **Conclusion:** Pharmacological inhibition of MET or liver-specific MET deletion impaired hepatocyte proliferation and impeded liver regeneration following APAP overdose. Taken together, our studies highlighted a crucial role of MET in the recovery process following APAP-induced acute liver failure. **Acknowledgement:** The authors gratefully acknowledge the financial support provided by the National Institutes of Health (1R01DK135566-01) awarded to Bharat Bhushan.

Abstract 225

Stellate Cell Wnts Regulate Endothelial Cell-Hepatocyte Zonation to Maintain Hepatic Metabolic and Proliferative Homeostasis

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Introduction: The liver, a main regulator of homeostasis, is histologically organized into metabolic zones defined by distinct gene expression along the portal-central axis for functional division of labor. Current

understanding of the maintenance of zonation defines endothelial cells (ECs) in pericentral zones as the source of Wnt2 and Wnt9b, which regulate hepatocyte ß-catenin activity and gene expression in this zone. However, ECs are not the sole source of Wnts in the liver. Here, we investigate if there is any role of Wnts from hepatic stellate cells (HSCs) by characterizing mice incapable of secreting Wnts from these cells. **Methods**: Male and female HSC-specific Wntless (Wls) knockout (KO) mice were generated by interbreeding Wls-floxed mice and lecithin retinol acyl-transferase-driven Cre transgenic mice. Single nuclei RNA sequencing (snRNA), single cell spatial transcriptomics (scST), and immunohistochemistry (IHC) were performed to identify changes in gene expression and zonation between KO and littermate controls. Metabolism was assessed by bile acid composition and Oroboros. Proliferation was assessed after partial hepatectomy (PH) and acetaminophen (APAP) challenge. **Results**: No difference in baseline liver functions were evident in HSC-Wls KO versus controls. KO mice were overall smaller and had a greater lean-to-fat mass body composition than controls ($p<0.05$). scST, validated with IHC, revealed expanded expression of normally pericentral genes like Cyp2e1, Cyp7a1, and Cyp1a2 to mid- and periportal zones. Additionally, the increase in expression of pericentral markers occurred without expending the midzonal and periportal marker zonation. These zonation changes in the KOs were further corroborated by snRNA sequencing. Ingenuity Pathway Analysis on snRNA sequencing identified significant upregulation of FXR/RXR activation, fatty acid oxidation, cholesterol biosynthesis, and bile acid metabolic pathways in hepatocytes. Bile acid analysis confirmed increased bile acid synthesis in KOs. Oroboros O2k Respirometry on mitochondria from KO livers demonstrated increased fatty acid metabolism and efficiency. Intriguingly, the observed zonation changes were not due to altered HSC zonation but rather pericentralization of sinusoidal ECs throughout the hepatic lobule as seen by altered Fabp4, c-Kit, and Wnt2 expression. Analysis of liver regeneration following insult by APAP toxicity or PH revealed impaired hepatocyte regeneration measured by Ccnd1 and Ki67 positive hepatocytes. **Conclusions**: HSC-Wnts regulate zonation of ECs and hepatocytes, to serve as the master regulator of metabolism and proliferation balance in the liver.

Abstract 226*

PPARα-SMPD3 Axis Regulates Hepatic Lipid Accumulation and Inflammation in NAFLD/NASH

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*The authors of this abstract selected not to publish the details of their research

Abstract 227

Co-activation of β-catenin with Nuclear Factor Erythroid 2-related Factor 2 and MET: Biological and Therapeutic Implications

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Introduction: Liver cancer is the third-leading cause of cancer-related mortality globally, with hepatocellular carcinoma (HCC) comprising 90% of these cases. These outcomes are in part due to lack of animal models which fully recapitulate heterogenous biology of human HCC. We have previously shown that expression of activating mutations in CTNNB1 (encoding β-catenin) with either nuclear factor erythroid 2-related factor 2 (NFE2L2) or MET result in development of HCC in mice, which represents 12% and 10%, respectively, of all human HCC cases with >70% transcriptional similarity. Here, we investigate whether combined co-overexpression of mutated β-catenin, NFE2L2 and MET recapitulates tumor heterogeneity seen in HCC patients, and whether these tumors are addicted to β-catenin oncogenic signaling. **Methods:** We monitored HCC development in mice following co-overexpression of mutant CTNNB1, NFE2L2, and MET via hydrodynamic tail vein injection delivery with sleeping beauty transposon/transposase (HDTVi-SBTT) system. We performed bulk RNA-sequencing to assess transcriptional differences between various combinations of oncogenes. Lastly, we inhibited β-catenin mRNA levels in this G31A-NFE2L2-hMet-S45Y-β-catenin mouse model of HCC through administration of siRNAs formulated in lipid nanoparticles (LNP) targeting either CTNNB1 (LNP-CTNNB1) or scrambled control (LNP-CTRL). **Results:** Co-overexpression

of mutated CTNNB1 with mutant NFE2L2 and hMet led to HCC development in mice and mortality by 4.5 weeks. Gross pathology showed these tumors to be similar to the hMet-S45Y-β-catenin mouse model. These HCCs contained tumor nodules which shared histological features of both the G31A-NFE2L2-S45Y-β-catenin and hMet-S45Y-β-catenin mouse models. Additionally, all tumor nodules were positive for β-catenin downstream targets, such as glutamine synthetase (GS) and cyclin-D1. However, some Myc-tag positive tumor nodules (β-catenin active) were only positive for NFE2L2 targets, like NAD(P)H quinone dehydrogenase 1 (Nqo1) or V5-tag (present on the hMet plasmid), representing the increased tumor heterogeneity seen in this model. Bulk RNA-sequencing revealed the G31A-NFE2L2-hMet-S45Y-β-catenin mouse model to cluster similarly to the G31A-NFE2L2-S45Y-β-catenin mouse model using principal component analysis. Lastly, the G31A-NFE2L2-hMet-S45Y-β-catenin model was administered LNP-CTNNB1 once weekly for five weeks starting at 3 weeks post HDTVi, and demonstrated complete tumor responses, as evident via normalized liver weight/body weight (p<0.001) compared to LNP-CTRL treated animals. **Conclusions:** Our study demonstrates that co-overexpression of G31A-NFE2L2, hMet, and S45Y-β-catenin represents a CTNNB1-mutated HCC mouse model to study tumor heterogeneity. Additionally, we provide direct evidence to support RNAi-mediated inhibition of CTNNB1 for β-catenin-mutated HCC, irrespective of the second or third hit oncogene.

Abstract 228

Lipid Dysregulation in Beta-catenin-driven Hepatocellular Carcinoma

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Introduction: Hepatocellular Carcinoma (HCC) is one of the most common types of primary liver cancer worldwide, with an estimated 1 million people being affected by 2025 per year (*Nature Rev Dis Primer* 2021;**7**:1-28). This study focuses on elucidating the role of lipid metabolism in HCC. Metabolic reprogramming is essential in cancer as it enables a healthy normal cell to transform into a malignant one. Several clinical data suggest that essential lipids such as Phosphatidylcholine (PC) are commonly dysregulated and are found to be elevated in human HCC patient samples (*JHEP Rep* 2022;**4**:100479). PC is one of the significant constituents of biological membranes and is synthesized via the phosphatidylethanolamine N-methyltransferase (PEMT) and cytidine diphosphate (CDP)-choline pathways in the liver (https://www.ncbi.nlm.nih.gov/books/NBK26871/). However, the role of PC in HCC has yet to be fully explored. **Methods and Results:** Our lab developed a transgenic zebrafish HCC model expressing hepatocyte-specific activated beta-catenin (ABC), which shows liver overgrowth as early as 6 days post fertilization (dpf) and develops HCC, histologically and transcriptomically similar to human HCC as early as 2 months post fertilization(mpf) (*PLoS Genet* 2015;**11**:e1005305). We performed a lipidomic analysis of male and female transgenic ABC HCC zebrafish and non-transgenic sibling control zebrafish livers using LC-MS and found significant differences in numerous lipid species, including acylcarnitines, ceramides, and PCs. We also performed isotope tracing in these HCC and non-HCC zebrafish liver tissues to quantify the metabolic pathways contributing to these changes. We discovered that the PC flux was downregulated in zebrafish HCC via sex-specific mechanisms. Ongoing studies focus on the effects of manipulating lipid metabolism on zebrafish hepatocarcinogenesis using genetic tools. Using CRISPR-Cas9, we generated chpt-1 deletion mutants, gene involved in PC synthesis. Our preliminary data show that loss of chpt-1 leads to enhanced tumorigenesis in transgenic ABC HCC zebrafish. We are currently generating and characterizing other mutant and transgenic zebrafish lines with alterations in lipid metabolic genes. **Conclusions:** Overall, our results support a role for lipid metabolism in hepatocarcinogenesis.

Abstract 229

Maternal Obesogenic Diet Exposure Increases Post-natal Hepatocyte Proliferation and Shifts Liver Regeneration in Offspring Following Partial Hepatectomy

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Introduction: Obesity is a complex metabolic syndrome associated with a wide range of abnormalities in liver known as non-alcoholic fatty liver diseases (NAFLD). Evidence supports that maternal

obesity/obesogenic diet exposure programs increased NAFLD in offspring. The liver responds to lipotoxic hepatocyte injury through hepatocyte proliferation. It is not clear whether maternal obesogenic diet exposure (MODE) affects the proliferative capacity of offspring hepatocytes. We hypothesized that MODE enhances hepatocyte proliferation in the post-natal period and during liver regeneration in offspring. **Methods:** Female mice were fed chow or high fat-fructose-cholesterol (HFFC) diet for 6 weeks and bred with lean males. Liver and serum samples were collected at different postnatal time periods 5, 10, 15, 20, 25, and 30 days to analyze histological and molecular markers for liver proliferation. In addition, a subset of offspring were weaned to the chow diet and underwent two-thirds partial hepatectomy at 8 weeks of age to analyze the hepatocyte proliferation during regeneration. **Results:** No significant difference between neonatal body weight and liver weight was observed; however, the liver weight/body weight was increased at day 20 in HFFC offspring compared to chow offspring. The number of ki-67 positive hepatocytes peaked in both groups at day 15; however, an increase in ki-67 positive hepatocytes was observed at 15, 20, and 30 days in HFFC offspring compared to chow offspring. Following partial hepatectomy (PH), peak proliferation in HFFC offspring occurred at 36 hours compared to 48 hours in chow offspring. HFFC offspring had sustained proliferation at 72 hours with an increase in ki67 positive hepatocytes compared to chow offspring. **Conclusion:** MODE increases post-natal hepatocyte proliferation in offspring and induces a left-shift in the proliferation curve during liver regeneration. These findings suggest a shift in the intrinsic hepatocyte proliferation capacity following MODE and could have implications for the potential for development of hepatic cancer in the setting of sustained liver injury.

Mechanisms of Fibrosis

Abstract 230
Role of DEK in Chronic Liver Disease Development
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Introduction: Chronic liver disease (CLD) from progressive injury is associated with local or systemic inflammation, and fibrosis that can lead to multiorgan failure & death. During CLD development, stressed hepatocytes release several hepatokines, including HMGB1. The hepatokines impact liver disease locally as well as other organs in the body. In continuation of our HMGB1 study, we examined the role of a HMGB1 related nuclear protein- DEK in CLD development. DEK is an abundantly expressed DNA-binding chromatin factor that stimulates hematopoiesis and directly contributes to tissue inflammation. The rationale for the study is that the role of DEK in liver pathophysiology is unknown. Specifically, the role of DEK in chronic liver injury, inflammation, and fibrosis related to CLD is particularly unclear. We hypothesize that DEK is actively released by stressed hepatocytes and contributes to liver injury and injury associated hepatic inflammation in CLD. **Materials and Methods:** Expression of DEK in hepatic and non-hepatic tissues was analyzed by western blot, immunofluorescence (IFC) staining, and quantitative polymerase chain reaction. Genetic models (hepAtg7-/- or hepAtg5-/-) or dietary models (3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) hepatotoxin-containing diet) of CLD were examined for DEK release and its impact on CLD development. DEK-/- mice and wild type mice were fed DDC for a period of one or two weeks. **Results:** A single-cell transcriptomics dataset and human protein atlas revealed DEK expression by all hepatic cells. Using a normal liver, we confirmed that DEK was expressed by all hepatic cells. IFC staining showed DEK in the nuclei of all hepatic cells (hepatocyte, cholangiocyte, Kupfer cells, stellate cells, and endothelial cells). Likewise, the expression of DEK was solely nuclear in non-hepatic tissues, like the spleen, kidney, lungs, and skeletal muscles. DEK, however, was also expressed in the cytosol of normal hepatocytes, but not in non-parenchymal cells and other tissues. Interestingly, stressed hepatocytes due to Atg7- or Atg5 deletion or DDC toxin exposure decreased both hepatic nuclear and cytosolic DEK protein without affecting DEK mRNA levels, indicating extracellular release of DEK. Moreover, genetic deletion of DEK significantly reduced liver injury (serum ALT and AST levels). Paradoxically, levels of total bile acid, total bilirubin, and direct bilirubin were elevated in DEK-/- mice, suggesting a non-cholestatic nature of liver injury. Histological analysis of liver pathologies revealed no remarkable differences in the level of infiltration of F4/80, MPO, CD11b, inflammatory cells between control and DEK-/- mice exposed to the DDC diet. Interestingly, DEK deletion significantly reduced hepatic fibrosis indicating the fibrogenic role of DEK in CLD. **Conclusions:** During the CLD development, DEK is released by stressed hepatocytes and could be involved in liver injury

and fibrosis. **Acknowledgments:** This work was supported in part by Louisiana Board of Regents grant R & D, RCS LEQSF (2021-24)-RD-A-17, LA CaTS Pilot Award, and TU SOM Pilot grant (to BK).

Abstract 231

The Potential of Long Noncoding RNAs to Regulate Conjunctival Fibrosis

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Introduction: Ocular fibrosis remains one of the largest areas of unmet need in ophthalmology and it is one of the major causes of vision loss after severe injury or surgery. Glaucoma filtration surgery (GFS), also known as Trabeculectomy, can alleviate intraocular pressure (IOP) by providing an alternative path for the flow of the aqueous humor. However, this surgery's success rate is only 55%, representing a severe limitation. This complication is due to fibrosis of the ocular tissue during the wound healing process. Recently, noncoding RNA gene therapies, using long-noncoding RNAs (LncRNAs), show potential therapeutic value for fibrotic diseases including ocular fibrosis. The primary objective of this project is to investigate the potential anti- and pro- fibrotic molecular mechanisms of long non-coding RNAs following glaucoma filtration surgery (Trabeculectomy) or injury, specifically in conjunctival epithelial cells. Previous studies have identified specific LncRNAs for their potential involvement in ocular diseases, including MALAT1, H19, LncNR003923, LINC00028, NR033585, LINC00963 as modulators of fibrosis in ocular diseases, however, further studies are needed to elucidate the mechanism by which these regulators work in conjunctival wound healing or alternatively, fibrosis. To our knowledge, none have been investigated specifically in conjunctival epithelial fibrosis. Previous studies have reported that the TGF-β signaling pathway and associated biomarkers (α-SMA, TGF-β, CTGF, ROCK, TNF-α, VEGF, and Collagens I, III, and XI) play a role in the Epithelial to Mesenchymal Transition (EMT) in ocular fibrosis and are associated with long non-coding RNAs expression levels. However, the association between TGF-β signaling pathway, EMT and LncRNAs remains unclear. **Methods and Results:** We address this gap in knowledge by first defining selected LncRNAs (MALAT1, H19, LncNR003923, LINC00028, NR033585, LINC00963) expression levels in normal conjunctival epithelial cells vs fibrotic conjunctival epithelial cell model. Then determine the effect of overexpression and silencing of these LncRNAs in the same models. Resulting changes will be assessed by cell morphology, cytoskeletal structure, and expression of EMT biomarkers α-SMA, TGF-β, CTGF, ROCK, TNF-α, and VEGF expression as well as Collagens I, III, and XI deposition compared to controls. **Conclusions:** We hypothesize that LncRNAs H19, NR003923, and LINC00028 overexpression will result in an increase of EMT and LncRNAs MALAT1, NR0033585, and LINC00963 overexpression will result in a decrease of EMT. We hypothesize that the candidate LncRNAs will be modulated by TGF-β-induced fibrosis in conjunctival epithelial fibrosis.

Abstract 232

Hydrogel-mediated Delivery of Thy-1 to Alleviate Fibrosis

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Introduction: Biomaterial-mediated fibrosis is a significant challenge in biomedical engineering and healthcare. Foreign materials introduced into the body, such as implants, medical devices, or scaffolds, often trigger complex biological responses and an inflammatory response that eventually leads to fibrotic encapsulation which compromise the functionality of the implanted biomaterial. Earlier research has shown that during fibrosis across multiple organ system, Thy-1 expression has anti-fibrotic characteristics where Thy1 expressing fibroblast has been associated with less activation thus resulting in less deposition of collagen. Consequently, it has been shown that Thy-1 plays a role in regulation of fibroblast activation by mediating integrin signaling. However, it has not been determined whether Thy-1 expression effects biomaterial-mediated fibrosis. The microporous annealed particle (MAP) hydrogel system has demonstrated success in variety of regenerative medicine applications and delivering Thy- to the fibrotic area might help mitigate fibrosis. Therefore, we hypothesize that by tethering Thy-1(CD90) on the surface of hydrogels as a delivery system can help mitigate fibrosis. **Methods:** We have attached Thy-1 to 4-arm PEG hydrogel in both MAP gels and bulk gels and confirmed attachment via flow cytometry. We have cultured Thy-1 KO fibroblasts on PEG hydrogels with Thy-1 attached and flow cytometry will be used to analyze the availability of Thy-1 on the cells collected indirectly confirming their availability. The goal of

performing flow cytometry is to determine transfer of Thy-1 onto fibroblast MAP gels. After confirming Thy-1 presence we will go ahead and perform an in vivo study on where we will subcutaneously implant tethered bulk PEG hydrogel that is traditionally fibrotic. After a week we will euthanize our mice, collect the sample, and freeze them in oct for histology. **Results:** Using flow cytometry, we were able to confirm Thy-1 presence on our MAP hydrogels. Attaching Thy-1 has no effect on microparticle size or geometry. Earlier research showed that when implanted in Thy-1 KO mice both MAP and hydrogels yield fibrotic encapsulation that are indistinct from each other. In this scenario, we expect tethering Thy-1 to bulk hydrogels will reduce fibrotic capsule formation and, foreign body giants cell formation that leads to fibrosis. **Conclusion:** Finally, this work strongly suggests that we are able to deliver Thy-1 as a therapeutic using a hydrogel system into fibrotic regions and successive delivery of Thy-1 into fibrotic areas can aid in mitigating biomaterial-mediated fibrosis.

Abstract 233

Exercise in Chronic Kidney Disease Patients Does Not Significantly Alter Fibrosis in Quadriceps Muscle

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Introduction: Chronic kidney disease (CKD) is commonly associated with decreased physical activity and muscle dysfunction. Muscles in CKD become fibrotic, containing excess extracellular matrix (ECM), particularly collagen. Collagen is cross-linked in the ECM, and increased cross-linking is often associated with fibrosis, stiffness, and reduced ECM remodeling. However, it is not known how exercise in patients with CKD may impact the extent of collagen cross-linking or fibrosis. Additionally, muscle fibrosis may impair muscle adaptations to exercise including, fiber size, fiber type, or vasculature. We predicted that collagen content and cross-linking would decrease, and there would be more Type I fibers and larger fiber size in the patients that were in the exercise group. **Methods:** Participants were randomized to exercise (n=15) or no exercise (n=5) groups in the Exercise Study Testing Enhanced Energetics of Mitochondria (ESTEEM) clinical trial of non-dialysis patients with CKD. The exercise program consisted of 35 min sessions, 3 times a week for 12 weeks consisting of highintensity interval training, strength training, and power walking. Vastus lateralis biopsies were obtained before and after the 12 weeks of exercise. A bundle of muscle tissue was powdered and separated into a pepsin soluble and insoluble fraction before applying a hydroxyproline assay for collagen content, with the insoluble fraction representing more cross-linked collagen. For histological analysis 10 μm thick cryosections were stained with Picrosirius Red, or immunofluorescent stains to investigate satellite cells, vasculature, fibro-adipogenic progenitors (FAPs), and muscle fiber types. Picrosirius red sections were imaged under polarized light to look at birefringence as a measure of collagen density. Sections that were stained for fiber type and vasculature were analyzed using semi-automatic muscle analysis using segmentation of histology (SMASH). **Results:** The overall amount of collagen and collagen cross-linking were not altered in either the exercise or no exercise groups. Picrosirius red stained sections did not reveal a change in ECM area or collagen packing density with exercise. Surprisingly minimum ferret diameter and percentage of type I (slow twitch) fibers decreased in most patients that underwent the exercise program. Type I and type IIa (fast oxidative) fiber size also significantly decreased, while there was no change in type IIx (fast glycolytic) fiber size. **Conclusion:** This study shows that exercise does not dramatically reduce ECM content or muscle fibrosis in patients with CKD. Surprisingly, the response to exercise was opposite of that anticipated in healthy subject with exercise in CKD producing smaller muscle fibers and a shift away from more oxidative type I fibers. This shows that exercise may have limited capacity to enhance function in CKD muscle, potentially due to irreversible fibrosis. **Acknowledgments:** This work is supported by NIH R03DK114502.

Abstract 234

Osteopontin and CCR2 Are Involved in the Inflammatory Cells in Non-alcoholic Steatohepatitis Model Rats

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Introduction: Non-alcoholic steatohepatitis (NASH), characterized by fatty liver and fibrosis due to inflammation, can progress to cirrhosis. The number of NASH patients has been increasing with rising obesity rates, highlighting the importance of understanding the pathogenesis of NASH and the mechanisms of fibrosis. Osteopontin (OPN) and C-C Chemokine Receptor 2 (CCR2) are known to act in a pro-fibrotic manner and are expressed in NASH livers. In this study, we focused on macrophages, which play a central role in fibrosis progression, and analyzed the induction of OPN and CCR2 using Stroke-prone spontaneously hypertensive (SHRSP5/Dmcr) rats, which develop NASH through a high-fat and high-cholesterol (HFC) diet. Additionally, we examined OPN and CCR2 expression using mouse bone marrow-derived macrophages (BMDM). **Methods:** SHRSP5/Dmcr rats (10 weeks of age) were fed an HFC diet for 4, 6, and 8 weeks, respectively. Immunohistochemical staining and fluorescent immunostaining were conducted to examine OPN, CCR2, and macrophage markers. BMDMs were collected from the femur and tibia of C57BL/6J mice and cultured in MCS-F containing medium for 3 days. The cultured cells were stimulated with lipopolysaccharide (LPS) and interferon-gamma (IFN-γ) and then analyzed by flow cytometry and quantitative RT-PCR. **Results:** The livers of SHRSP5/Dmcr rats became markedly whitish and hypertrophic with HFC diet loading. Hepatic fibrotic areas expanded with an increasing duration of HFC diet loading. In the fluorescent immunostaining, it was suggested that macrophages expressed OPN and CCR2, as indicated by CD-68. Interestingly, OPN-positive macrophages and CCR2-positive macrophages were clearly distinguished. OPN-positive areas increased, while CCR2-positive areas decreased with NASH progression. LPS and IFN-γ stimulation induced BMDMs to differentiate into inflammatory (M1) macrophages. The proportion of CCR2-positive cells increased during M1 differentiation, as analyzed by flow cytometry. Conversely, quantitative RT-PCR revealed a decrease in OPN mRNA with M1 macrophage polarization. **Conclusions**: OPN and CCR2 are expressed in infiltrating macrophages in NASH livers. Moreover, it is suggested that OPN-positive macrophages and CCR2-positive macrophages are distinct entities, with each playing a different role in NASH progression.

Abstract 235

***Trypanosoma cruzi* Induces the Expression of Host piwi-interacting RNAs Targeting *TGFB1* to Facilitate Cellular Infection**

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Introduction: *Trypanosoma cruzi*, the etiological agent of Chagas Disease, causes cardiac fibrosis, severe morbidity, mortality, and economic burden. The disease is now present in all industrialized countries due to modern globalization. About 40% of infected individuals will develop severe cardiovascular, neurological, or gastrointestinal pathologies. Transforming growth factor beta (TGF-ß) and the intact active signaling pathway are essential for *T. cruzi* infection and pathogenesis. *T. cruzi and* the cytosolic components activate host TGF-ß to promote cell invasion and pathology. However, the mechanism by which *T. cruzi* infection increases *TGFB1* expression and fibrogenic responses remains elusive. Specifically, the influence of regulatory, small non-coding RNA (sncRNA) on *TGFB1* expression during infection remain unknown. PIWI-interacting RNAs (piRNAs) are a class of sncRNA with several regulatory roles in germ and somatic cells. We identified piRNAs dysregulated by *T. cruzi* in primary human cardiac myocytes (PHCM) and predicted their interactions with *TGFB1*. **Methods:** We challenged PHCM with *T. cruzi* trypomastigotes for 1 and 2 hours and the purified small RNA was subjected to RNA-Seq. Piano was used to identify piRNAs and NOISeq was used for differential expression analysis. RNA22, miRanda, and TarPmiR were used to predict piRNA interaction sites on *TGFB1*. A TGF-β biological interaction network was constructed with GeneMANIA and visualized with Gephi. **Results:** Eight differentially expressed piRNAs were computationally predicted to target *TGFB1*. The piRNA binding sites were located in exon 1 and the 5' UTR. Furthermore, two of the piRNAs, npiR_587 and npiR_573 could also target *TGFBR3*, *SAR1A*, *FSTL1*, *TBX21*, and *DAXX*, which are within one degree of biological interaction of TGF-β. **Conclusions:** *T. cruzi* induced differential expression of host piRNAs during PHCM challenge. The interaction of these

piRNAs with *TGFB1* and its related genes suggests that they could play critical roles in the regulation of *TGFB1* gene expression during *T. cruzi* infection and pathogenesis including cardiac fibrosis. The antagomirs of these piRNAs could be developed as RNA therapeutics against *T. cruzi* infection, and pathogenesis and potentially other related fibrotic diseases. **Acknowledgement:** This work is supported by the following NIH grants: SC1AI127352, F31AI167579, CA163069, S10RR025497, U54MD007586, 2T32AI007281-31, 2T32HL007737-26.

Abstract 236

Higher Urine LG3 Levels are Associated with Risk of Cardiovascular Events and Chronic Kidney Disease Progression in Individuals with Type 2 Diabetes and Microalbuminuria

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Introduction: LG3 is an antiangiogenic and autophagic signaling domain released by BMP-1 from the extracellular matrix (ECM) basement membrane protein perlecan. Perlecan is important for heart and kidney tissue development and function, whereas LG3 has been linked to different fibrotic diseases contributing to microvascular damage (*J Biol Chem* 2005;**280**:7080-7087). Increased release of ECM protein fragments into the circulation and urine is an underlying feature in many pathologies. Type 2 diabetes (T2D) is a well-known risk factor for developing chronic kidney disease (CKD) and cardiovascular disease (CVD) (*BMC Med* 2022;**20**:63). We investigated whether increased urine LG3 (uLG3) levels were associated with the risk of experiencing cardiovascular (CV) events, CKD progression, and all-cause mortality in people with T2D. **Methods**: uLG3 levels were measured by the LG3 competitive enzyme-linked immunosorbent assay in 193 participants with T2D and microalbuminuria without symptoms or history of cardiac disease. uLG3 levels were corrected for urinary creatinine. Participants were included in an observational, prospective cohort at Steno Diabetes Center Copenhagen in Denmark from 2007-2008. Endpoints were all-cause mortality, CV events (CV mortality, stroke, ischemic CVD, and heart failure) and CKD progression (>30% decline in estimated glomerular filtration rate (eGFR)). Cox proportional hazard regression analysis was used to generate unadjusted hazard ratios (HRs) and HRs adjusted for sex, baseline age, body mass index, LDL cholesterol, smoking, HbA1c, plasma creatinine, systolic blood pressure and urinary albumin excretion rate (UAER), using a forward selection method. **Results**: Median (IQR) of uLG3 was 0.098 (0.025 to 0.218) ng/mg, median (IQR) age of the cohort was 60 (55-65) years, mean baseline eGFR was 91 (± SD 17.5) ml/min/1.73m^2, median (IQR) UAER was 103 (41-235) mg/24-h and 75 % were males. Median (range) follow-up time was 5.0 (0.3–6.8) years, and 25 deaths, 39 CV events, and 41 CKD events were recorded. A doubling of uLG3 was independently associated with a higher risk of experiencing a CV event (HR: 1.2 [95% CI: 1.00-1.43], p=0.047). In crude analyses, higher levels of uLG3 were associated with an increased risk of CKD progression (HR: 1.17 [95% CI: 1.01-1.34], p=0.04), but not after adjustment. The uLG3 levels were not associated with all-cause mortality (p= 0.19). **Conclusion**: In people with T2D and microalbuminuria, higher uLG3 was an independent risk marker for CV events. Higher uLG3 levels were associated with CKD progression, but not independent of conventional risk factors. These findings suggest that uLG3, measuring the release of the LG3 fragment from perlecan, is a relevant risk marker of complications related to diabetes. **Acknowledgements:** This work was supported by the Danish Research Foundation.

Abstract 237

Determining the Role of the Extracellular Matrix in the Pathogenesis of Polycystic Kidney Disease

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Introduction: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common, potentially life-threatening hereditary disease, affecting 1 in 1000 individuals. 50% of patients require renal replacement therapy before the age of 60, and 1 in 10 kidney transplants performed are in patients with ADPKD. Therefore, the burden of this disease on healthcare services and patients is significant. Despite

this, only one drug targeting ADPKD has reached the market, tolvaptan. However, tolvaptan is not available to all patients and users report side effects, including polyuria and, in the most severe cases, liver damage. As such, additional treatments are urgently needed to improve the lives of people with ADPKD and to also reduce the burden on healthcare systems. >93% of ADPKD cases are caused by variants in the PKD1 and PKD2 genes, which encode for polycystin-1 (PC1) and PC2. PC1/2 form a calcium ion channel that is located at the primary cilium and other cellular compartments (cell-cell junctions, focal adhesions, and the ER). When depleted, local apical concentrations of Ca2+ are reduced, leading to an accumulation of the cAMP second messenger in kidney tubule/duct cells. When combined with a "third hit" signal (such as kidney injury), cystogenesis is initiated. Over time, multiple large cysts form in the kidneys of patients with ADPKD, resulting in an immune response, fibroblast transdifferentiation, and interstitial fibrosis that results in kidney failure. Previous studies have suggested a role for the extracellular matrix in ADPKD pathogenesis. We have found that signals emanating from the extracellular space are important in cyst formation. Specifically, Integrin and RhoGEF signals are altered in ADPKD and lead to the epithelial-to-mesenchymal transition, hyper proliferation, and fibrotic phenotypes. **Methods:** We will present our proteomics analysis of zebrafish and mouse animal models of ADPKD, as well as in vitro data testing the candidate molecules we have found important in ADPKD. **Results:** Our analyses highlight the tumour suppressor Vwa5a, the receptor integrin a1b1, and numerous RhoGEFs as important regulators of ADPKD. **Conclusions:** Taken together, these results highlight a range of significant extracellular signals are present and are likely to be useful candidates for future bio markers and therapies targeting ADPKD.

Abstract 238

Loss of Neuropilin-1 in Vascular Smooth Muscle Results in Hypertension

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Introduction: Hypertension affects nearly one billion people worldwide and is a significant global health concern as it is the leading risk factor for heart failure. Microarray data comparing mRNA expression in aortas from spontaneously hypotensive or hypertensive strains of mice show an inverse relationship between *Semaphorin-3A* (*Sema3A*) levels and blood pressure. Moreover, vascular smooth muscle cells (VaSMC) robustly express the Sema3A transmembrane receptor, Neuropilin-1 (Nrp1). We hypothesize that Sema3A/Nrp1 signaling decreases blood pressure by relaxing VaSMC. **Methods and Results:** *In vitro*, treatment of primary mouse VaSMC with angiotensin-II induces activation of the small G protein RhoA, a major regulator of blood pressure, while addition of Sema3A reverses RhoA activation. Silencing of *Nrp1* with siRNA has the opposite effect in VaSMC as it increases contractility in collagen gel contraction assays compared to siControl cells. *In vivo*, *SM22□cre^{ERT2};Nrp1$^{f/f}$* transgenic mice show increased systolic blood pressure using tail cuff air plethysmography following 4-hydroxytamoxifen induction. Evoked smooth muscle contraction in response to chemical vasoconstrictors such as phenylephrine, thromboxane A2 receptor agonist (U46619), and potassium chloride in *Nrp1*-deficient aortas surpasses that of *Nrp1*-intact aortas in *ex vivo* isometric tension analysis. **Conclusion:** Taken together, our results demonstrate that the Sema3A/Nrp1 signaling axis is a novel regulator of vascular tone. Sema3A induces a relaxant signal to SMC thus lowering blood pressure, while loss of Nrp1 signaling results in elevated contractility and hypertension. Targeting this pathway may provide new therapies for cardiovascular diseases.

Neuropathology

Abstract 239

Placing Tumor Cells in the Organ-Specific Context: 3D in Vitro Models of Glioblastoma on Acellular Brain Matrices

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Introduction: Glioblastoma is the deadliest type of malignant tumor of the brain. Currently, the treatment options for the patients diagnosed with glioblastoma are very limited and the survival rates are poor. The low availability of the tumor biopsy material is one of the very important obstacles for the development of new diagnostic and treatment approaches for glioblastoma. Here, we report on our results in application of tissue engineering methodology that is recognized in regenerative medicine as a way to reconstruct living human tissues outside the body for the creation of three-dimensional (3D) macroscale (10 mm^3) in vitro models of glioblastoma. The particular focus of this study was placed on the exploration of the role of brain extracellular matrix (ECM) in controlling the biology of tumor cells. **Methods:** First, we obtained 3D brain tissue matrices (scaffolds) by decellularizing lamb brains, following our previously published immersion protocol with minor modifications. We subsequently seeded these scaffolds with glioblastoma cells (human lines U251 and U87) to get 3D tissue engineering constructs (TECs). The cells were cultured in TECs for 1-28 days. The most pronounced formation of the tissue-like structures was observed in 14 days after seeding of the scaffolds with glioblastoma cells. The brain scaffolds and TECs were analyzed using histology, scanning electron microscopy, atomic force microscopy, digital image analysis, cell viability assays and transcriptomics. The scaffolds were additionally characterized by mass-spectrometry-based proteomics to reveal their extracellular matrix components. Next, we also applied the glioblastoma TECs as optical phantoms for the development of the method of fluorescence-guided surgery of glioblastoma. **Results:** The project resulted in better understanding of the brain ECM and its effects on the proliferation, metabolism, and invasiveness of glioblastoma cells. It revealed a complex protein composition (matrisome) and region-specific spatial structure (matritechture) of brain ECM. The organ-specific ECM composition and architecture significantly modified the behavior of glioblastoma cells, compared with 2D cell cultures and Matrigel-based cultures. **Conclusion:** We proved that the glioblastoma TECs created by combining acellular 3D brain matrices and human tumor cells can serve as reliable and accessible 3D tissue-like in vitro models for quantitative biological studies and cancer biology research, providing a new pre-clinical alternative to animal models and traditional in vitro cell cultures.

Abstract 240

Role of ICI-182,780 in Antagonize Angiotensin II-Stimulatory Effects in Glioblastoma Stemness

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Introduction: Malignant gliomas are aggressive primary brain tumors characterized by high morbidity and mortality. Glioblastoma multiforme (GBM) is the most common tumor of the central nervous system, accounting for 16% of all gliomas. Despite the improvements in the therapeutic approaches the prognosis remains very poor, with an average survival of 14 to 15 months after diagnosis, making GBM a crucial public health issue. Growing evidence recognized a subpopulation of cancer stem cells (CSCs) from GMB as the leading cause of tumor progression and resistance to conventional therapy. Recently, it has been evidenced an important role of the renin-angiotensin system in extracellular matrix reorganization in GBM associated with genes involved in pro-tumoral functions. Moreover, we demonstrated that ANGII induces an increase of aromatase gene expression, leading to an enhanced local estrogen production that through the activation of non-genomic signaling, promote tumor progression. Thus, in the present study we aimed to investigate how ANGII/AGTR1 and estrogen signaling may cooperate in sustaining GBM tumor growth and potential resistance to conventional therapy. **Methods:** U87MG cells were treated with ANGII (5μM), anti-estrogen ICI-182,780 (ICI) (1μM) and LY294002 (10μM) alone or in combination. Transcriptomic profiling was analyzed to identify genes involved in tumor growth and progression and uploaded into MetaCore database to explore potential gene networks and signaling pathways. qRT-PCR and western blotting (WB) assay were performed to evaluate mRNA levels and protein expression. Neurosphere formation (NF) and flow cytometry analysis were carried out to investigate GBM stemness. **Results:** KEGG pathway analysis of up- and down-regulated genes from U87MG cells treated with ANGII and ICI alone or in combination highlighted PI3K/Akt signaling as one of the most significant enriched pathways. Moreover, MetaCore analysis showed a significant association of Hedgehog pathway (Hh) with GBM development and progression upon ANGII exposure, as well as an inhibition of PI3K/Akt signaling in the presence of ICI, recalling the link existing between ERα and Hh pathway. These data were corroborated by WB and qRT-

PCR analysis which revealed an enhanced Akt phosphorilation, concomitant with a strong upregulation of GLI1, the terminal transcriptional effector of the Hh signaling pathway, upon ANGII treatment. The reduction of pAkt and GLI1 expression in the presence of specific inhibitors of PI3K/Akt signaling or ICI revealed an estrogen-dependent crosstalk between PI3K/Akt and Hh pathways. Interestingly, the reduction of NF upon anti-estrogen exposure suggests the estrogen involvement in GBM stemness and may be reasonably linked to ICI-induced downregulation of GLI1. **Conclusions:** Based on these findings, it is reasonable to candidate ICI as a repurpose drug to improve GBM patient outcomes, offering several advantages, such as lower costs and faster development timelines.

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Brainstem CircRNAs Regulation in Neonatal Sepsis

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Introduction: Neonatal sepsis remains one of the leading causes of mortality in newborns (*Lancet Respir Med* 2018;**6**:223-230; *Arch Dis Child* 2021;**106**:745-752). Systemic inflammation caused by pathogens triggers autonomic nervous system response manifesting as changes in respiration and temperature homeostasis (*Pediatr Res* 2022;**91**:273-282). Our prior studies revealed disrupted brainstem neural circuits and physiological functions during neonatal sepsis. Circular RNAs (circRNAs), a class of closed long non-coding RNAs, presents a dynamic regulation throughout development and neuronal activity (*Nat Neurosci* 2015;**18**:603-610). Given circRNAs abundance in the brain, a better understanding of their potential functional role in health and disease represents an emerging need. We sought to study total and epitranscriptomic modifications neonatal sepsis evoke on brainstem circRNAs. **Methods**: CD1 mice were I.P. treated at postnatal day 5 (PD5) with LPS, or PAM3CSK4 (PAM), or saline (control). Following 3-hours, PD5 were euthanized, brainstem was dissected out, and kept in -80C until further processing. Total RNAs were digested with Rnase R to remove linear RNAs. Using random prime, fluorescent cRNAs were amplified and transcribed. Labeled cRNAs were hybridized onto the Arraystar Mouse circRNA Array V2 and scanned by the Agilent Scanner G2505C. Arraystar Mouse circRNA Epitranscriptomic Arrays utilized total RNAs immunoprecipitated with anti-5-methylcytosine (m5C) or anti-N6-methyadenosine(m6A) antibodies. Enriched and not-enriched m5c or m6A circRNAs were labeled with Cy5 and Cy3, respectively. All data analyses were performed in RStudio Version 2022. **Results**: Gene expression analysis reveals detection of approximately 13890 total circRNAs in the brainstem of PD5 pups. Neonatal sepsis results in modulation ~741 circRNAs. Our analysis shows that relative to controls, LPS induced upregulation of 02 circRNAs whereas PAM3CSK4 resulted in 184 differentially expressed circRNAs. Gene ontology (GO) analysis demonstrated enrichment of metabolic processes and mitochondrial gene expression and translation due to LPS-induced inflammation. Nervous system development, generation of neurons and neurogenesis were GOs exclusively enriched in PAM3CSK4. KEGG pathways such as GABAergic, dopaminergic and glutamatergic synapses, as well as, synapses vesicles cycle, calcium signaling, and long-term potentiation were modulated by these circRNAs in PAM3CSK4 m6A and m5c circRNA modifications were found in LPS and PAM3CSK4 brainstem. LPS induced a robust hypermethylation of m5c brainstem circRNAs. Similar m6A circRNA modification were modulated by systemic inflammation. **Conclusions**: Neonatal sepsis increases susceptibility of circRNAs modulation in neuronal processes of the brainstem. Distinct transcriptional networks were found unique to the inflammatory stimuli. Our data indicate that circRNAs are positioned to regulate brainstem-related functions. **Acknowledgements**: This work was supported by: NIH/NHLBI R01HL132355 for JJO.

Abstract 242

Evaluation of the Alpha-synuclein and Tau Anti-fibrillary Activity of 2-Amino-4-methoxybenzothiazole Derivatives

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Introduction: Alzheimer's disease (AD) is one of the most prevailing neurodegenerative diseases emanating from the accumulation of extracellular amyloid-beta (Aβ) peptide-containing senile plaques and

intraneuronal neurofibrillary tangles (NFTs) comprised of aggregated hyperphosphorylated tau protein (p-tau), among other factors. Tau is an intraneuronal protein essential for the stability of microtubules and contributing to the axonal transport. In AD, tau protein becomes hyperphosphorylated, leading to its misfolding and aggregation (i.e. oligomer and fibril formation). The assembly and aggregation of this protein have a damaging effect on neuronal health and function, most especially the disruption of the microtubule, leading to eventual loss of memory. However, more than half of AD cases also display the presence of aggregated α-synuclein (α-syn)-containing Lewy bodies (LB). Conversely, LB disorders have been reported to have concomitant Aβ plaques and NFTs. Our drug discovery program focuses on the synthesis of multitarget-directed ligands to abrogate aberrant α-syn, tau, and p-tau aggregation and to slow down the progression of AD and related dementias. Very few efforts have been made toward tau, p-tau, and α-syn-linked neurodegenerative drugs, especially the prevention of oligomer and fibril formation. The main objective of the study was to explore the anti-aggregation activities of more than 65 synthesized molecules based on the 2-amino-4-methoxybenzothiazole moiety with different central functional groups such as urea, amide, and sulfonamide. **Methods:** The anti-aggregation effect of the compounds was determined using the Thioflavin-T (ThT) fluorescence assay. Through this, we were able to monitor the formation of fibrils with vehicle and in conjugation with tested compounds. Also, transmission electron microscopy (TEM) was employed in detecting fibrils upon the completion of a time-course study with the ThT assay. Additionally, the photo-induced crosslinking of unmodified protein (PICUP) assay was used to monitor the formation of oligomers. **Results:** ThT fluorescence assay showed four compounds to be the most effective to inhibit fibril formation. All the best compounds exhibited high anti-fibrillary activity on α-syn and tau 0N4R, as confirmed by TEM. No compound stopped the formation of α-syn, tau (0N4R), and p-tau (0N4R) oligomers. **Conclusions:** Four of the newly synthesized compounds were identified as inhibitors of α-syn and tau fibril formation. Chemical refinement is ongoing in order to inhibit oligomer formation. **Acknowledgements:** The authors would like to thank the NIH NIA (AG070447-01A1, 1K08AG071985-01A1) for funding this research.

Abstract 243

A Symmetric Molecule with Anti-oligomer, Anti-seeding, and Disaggregation Activities

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Introduction: Alzheimer's disease (AD) is a neurodegenerative disease characterized by the accumulation of amyloid plaques and tau neurofibrillary tangles (NFTs), which causes cognitive impairments and dementia. A cure or prevention method for AD has yet to be identified despite extensive research. In recent years, attention has shifted to targeting tau pathology after previous therapeutic approaches failed in clinical trials. The potential for tau-directed therapies has been highlighted by studies linking tau hyperphosphorylation to memory deficits. During AD, tau becomes hyperphosphorylated (p-tau) and aggregates into NFTs. It is believed that this abnormal self-assembly of p-tau is a crucial step in the progression of AD, which makes it an attractive therapeutic target. Our lab focuses on the design and synthesis of compounds with p-tau anti-aggregation and disaggregation activities. **Methods:** Thioflavin-T (ThT) fluorescence assay was used to evaluate the level of fibril formation and monitor the anti-aggregation effect of the different compounds. We opted for transmission electron microscopy (TEM) as a direct mean to confirm the anti-fibrillar effect. The oligomer formation was monitored via the photo-induced cross-linking of unmodified proteins (PICUP). Disaggregation experiments were performed with amyloid plaques and NFTs extracted from AD brains. The anti-seeding and anti-inclusion activities of the best compound were evaluated using biosensor cells and M17D intracellular inclusion cell-based assays, respectively. **Results:** As assessed by ThT assays and TEM, our best compound exhibited anti-fibrillary activity against p-tau isoform 1N4R. Our lead molecule reduced oligomerization dose-dependently based on the PICUP assays. This novel compound abrogated completely tau seeding in biosensor cells. The anti-inclusion effect was confirmed using the M17D neuroblastoma cell model. In contrast to the control, disaggregation experiments

showed smaller Aβ-plaques and less paired helical filaments with our lead molecule. **Conclusions:** Our lead compound represents a crucial step toward the discovery of effective therapeutics that can mitigate tau and p-tau aggregation. This research highlights the urgent need for disease-modifying treatments in AD and underscores the importance of targeting tau pathology. Further optimization and investigation of our best compound(s) could lead to the development of novel and effective interventions against AD. **Acknowledgements:** The authors would like to thank the support received from the NIH NIA (AG070447-01A1, 1K08AG071985-01A1).

Abstract 244

Exploring the Impact of Signal Peptide Region on Amylin Misfolding as Potential Target for Alzheimer's Disease Treatment

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Introduction: Signal peptides (SPs) are short peptides situated at the N-terminus of proteins, serving as indicators for the protein secretion pathway and determining target destinations. Consisting of N-, H-, and C-regions, the positive charge associated with the N-region influences the orientation of translocation. SPs have been identified as influential factors in protein misfolding. Earlier studies revealed that the SP modulates the tertiary conformation and stability of SAA1.1, a protein linked to amyloid A (AA) amyloidosis. Moreover, the presence of the SP has been found to provide protection prior its cleavage against misfolding events and the formation of amyloid fibrils. In order to evaluate the propensity for misfolding, we examined the effect of the amylin SP region for its misfolding and seeding of the full-length protein. Importantly, amylin is potentially involved in the seeding of amyloid-beta and tau in specific cases of Alzheimer's disease associated with insulin resistance. We also aimed to identify potential small molecules to inhibit the deleterious conformational changes of amylin SP. **Methods:** the synthetic amylin SP and full-length amylin peptide were obtained from GenScript and AnaSpec respectively. The amyloidogenic potential of each peptide was validated through *in silico* analysis, thioflavin T fluorescence assays, transmission electron microscopy, and seeding experiments employing misfolded human amylin SP. **Results:** Notably, the human amylin SP region exhibited the capability to misfold. The heterogenous cross seeding of amylin SP as well as the anti-seeding effect of different compounds are currently being investigated. **Conclusions:** Studying the specific properties of fibrillar conformations enhances our understanding of the mechanisms underlying protein misfolding in the context of endocrine and neurodegenerative diseases. This research underscores the critical role of the SP region in proteins prone to aggregation such as amylin. Discovery of compounds with anti-seeding activity may be relevant for subset of AD patients with insulin resistance (i.e. type 3 diabetes).

Abstract 245

Examination of Autophagy, Vascular Integrity, and Cognitive Decline

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Introduction: Vascular and autophagy factors are likely contributors to Alzheimer's (AD) and cerebrovascular diseases. Our UNM Brain Bank contains over 265 consented post-mortem brains from New Mexico, an ethnically diverse population, with 61% Hispanics. Hispanics have a high incidence of AD and vascular disease mixed dementias and pure vascular cognitive impairment and dementia (VCID). White matter changes from vascular injury alone may present with cognitive impairment, and when coupled with protein processing defects may potentiate harm. MMP1 and 10 in CSF may distinguish between these types of dementia, yet the origin of these proteins is unknown. **Methods:** We selected biomarkers for autophagy (ATG) and for small vessel brain injury (μVBI) and applied them to histopathology of brains sections from specimens in our Brain Bank. For ATG, we used machine learning to identify other unknown ATG, are developing a comprehensive annotated ATG list. For vascular markers, we selected two matrix-metalloproteinases, MMP1 and 10, that appear in CSF of subjects with mixed but not pure AD dementias. We performed immunohistochemistry for MMP1 and 10 on 9 cases +/- AD taken from our archival post-mortem brain bank. We scored AD cases in histopathology with either Bielschowsky-Hirano silver for Amyloid/Braak/CERAD (ABC) on superior and medial temporal gyrus, inferior parietal lobule (IPL), and

middle frontal gyrus, or with p-tau by immunohistochemistry on IPL, and also examined cerebral vasculature in H&E-stained slides for evidence of vascular disease and/or microhemorrhages in these regions. We further developed double chromogen staining for p-tau and the MMPs. **Results:** For ATG, we created a comprehensive annotated list to scan genomic databases for allelic variations associated with AD. For VCID, MMP1 stained arterial smooth muscle and pericytes, and was not visible in venous structures. Capillaries stained only part-way along their length, suggesting that pericytes do not uniformly surround them. MMP10 stained perivascular macrophages were prevalent in cases with evidence of µVCID, such a microbleeds. and found no colocalizations. We are developing new MRI-pathology techniques to correlate abnormalities found by ante-mortem imaging in life with post-mortem cellular and molecular pathologies. **Conclusion:** We developed a comprehensive list of ATG genes and a subset with AD-associated allelic variations differentially expressed in AD. MMP1 and 10 originate from the vasculature. MMP1 is useful for evaluating arterial smooth muscle and pericyte integrity, and MMP10 informs on perivascular inflammation and neuronal injury in histopathology of VCID, AD and mixed dementias. **Acknowledgements:** Supported by NIH NIA P20AG068077 Harvey Family Endowment.

Nutrition and Disease

Abstract 246

Intermittent Fasting Improves Colonic Immune Response in TNBS-induced Colitis Experimental Model

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Introduction: Intermittent fasting, dietary pattern with periodic energy restriction, has been shown to provide potential health benefits against aging, cancer, neurodegenerative diseases and to improve the immune response studied in both animal models and clinical settings. Its known that the intestinal compartment, including mucosa, immune cells and microbiota, are affected by diet quality, timing and food intake. The objective of this investigation was to assess the effects of intermittent fasting on colonic architecture and immune response to *inflammation induced by* 2,4,6-trinitrobenzene sulfonic acid (TNBS). **Methods:** Twenty-four adult male Wistar albino rats were assigned into 4 groups (n=6/group): group I (control), group II (intermittent fasted group, 14h:10h for 4 weeks), group III (Control +TNBS) and group IV (intermittent fasted group +TNBS). The animals were sacrificed 48h after intraluminal instillation of 1% TNBS in 50% ethanol. Colonic morphological and histopathological changes were individually assessed at the macroscopic and microscopic levels. Colon paraffin sections were stained by hematoxylin-eosin and morphometrical measurements were performed by using the Axiovison software. Blood samples were collected for hematology counts and the inflammation-associated marker C-reactive protein (CRP) and nitric oxide (NO) were also investigated. **Results:** Intermittent fasting reduced the score of disease activity index and reversed TNBS-mediated shortening of colon length. The histological analyses of the colon structure have shown that intermittent fasting ameliorated TNBS-induced colitis-associated symptoms, by decreasing tissue damage and inflammatory cell infiltration in the crypt of colon. **Conclusions:** Our results have shown that intermittent fasting ameliorates the colonic immune response in TNBS-induced colitis group by decreasing CRP and NO plasma levels. Furthermore, intermittent fasting lowered the percentage WBC in the peripheral blood. Our results suggest that intermittent fasting boost colon immune function, and show promise effect to ameliorate the intestinal bowel disease-associated inflammation in humans.

Abstract 247

Use of Structure-Activity Relationship (SAR) Studies to Design Region B Capsaicin Analogs with Robust Anti-cancer Activity

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Introduction: Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the hot and spicy component of chili peppers (which are plants belonging to the genus *Capsicum*). Conventionally, capsaicin is recognized as a powerful pain-relieving agent which is present in many over-the counter creams and lotions. Several published reports have shown that capsaicin suppresses the growth and progression of several types of human cancers. However, the clinical applications of capsaicin as a viable anti-cancer drug are hindered by its adverse side effects, such as gastric irritation, stomach cramps and burning sensation. This has led to extensive research focused on the identification and rational design of second-generation non-pungent capsaicin analogs, with comparable/greater bioactivity than capsaicin. The structure of capsaicin may be divided into three regions: Region A (the aromatic ring), Region B (the amide group) and Region C (the hydrophobic side chain). The present poster the design of Region B capsaicin analogs and their growth suppressive activity in human SCLCs. **Methods**: As a first step, we synthesized a panel of compounds with variations in Region C of capsaicin. This was accomplished by adding unsaturated fatty acyl chains (with 0-4 double bonds) to the Region C of capsaicin. These uN-AVAMs were assessed for their growth-suppressive activity using a MTT based screening assay. The results of the MTT assay were confirmed by measuring the pro-apoptotic activity of these compounds. Based on our findings, out hit compound was "ARVANIL". We investigated the anti-tumor activity of Arvanil in *SCID* mouse tumor xenograft models and patient-derived organoid models of SCLC. Using our hit compound Arvanil as the template, we then sought to design Arvanil analogs which would have different chemical groups in the Region B of the molecule. The growth-suppressive activity of these Region B analogs was measured by a MTT assay in human SCLC cells. **Results**: SAR studies led to the identification of a panel of synthetic Region B capsaicin analogs with varying growth-inhibitory activity in human SCLC cells. **Conclusions**: The Region B and Region C capsaicin analogs may have applications in the therapy of human SCLC. Our future studies will examine the anti-cancer activity of selected Region B capsaicin analogs *in vivo*. **Acknowledgement:** Funding for our study was supported by the NIH R15-AREA Grant (2R15CA161491-02 and 2R15CA161491-03) to PD and MAV. Furthermore, this study was supported in part by the West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) grant (NIH grant P20GM103434; PI: Dr. G. Rankin), the National Institute of General Medical Sciences of the National Institutes of Health under the award number P30GM122733.

Abstract 248

Anti-cancer Activity of Non-Pungent Region C Capsaicin Analogs

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Introduction: Capsaicin is the spicy pungent ingredient of chili peppers. Although traditionally associated with analgesic activity, recent studies have shown that capsaicin has profound anti-neoplastic effects in several types of human cancers. However, the applications of capsaicin as a clinically viable drug are limited by its unpleasant side effects, such as gastric irritation, stomach cramps and burning sensation. This has led to extensive research focused on the identification and rational design of second-generation capsaicin analogs, which possess greater bioactivity than capsaicin. The pharmacophore of capsaicin can be divided into three regions Region A, B and C. Published reports show that the addition of long-chain unsaturated fatty acyl groups in the Region C of capsaicin generated non-pungent compounds (called unsaturated N-acylvanillamides, uN-AVAMs) which retained the pain-relieving activity of capsaicin. However, the anti-cancer activity of these compounds is yet to be studied. **Methods:** We synthesized a panel of uN-AVAMs with 0-4 double bonds in the side chain of capsaicin. We investigated the growth-inhibitory activity of these Region C capsaicin analogs with an MTT-based screening assay. Based on the results of the screening assay, we selected ARVANIL as our "hit compound". The apoptotic activity of Arvanil was compared to capsaicin (in a panel of human small cell lung cancer cells) using the Caspase-3 activity assay and the Cell Death ELISA assay. The anti-tumor activity of Arvanil *in vivo* was determined using *SCID* mice tumor xenograft model. In addition, the growth-suppressive activity of Arvanil was also determined in patient-derived organoids of small cell lung cancer (SCLC). **Results:** We observed that the non-pungent capsaicin-analog Arvanil displayed greater pro-apoptotic activity than capsaicin. Most

interestingly, Arvanil did not impact the growth of normal lung epithelial cells. The dietary administration of Arvanil robustly decreased the growth rate of human SCLC tumors xenografted on *SCID* mice. Moreover, Arvanil displayed greater growth-suppressive activity (than capsaicin) in patient-derived organoids of SCLC. **Conclusions**: The non-pungent uN-AVAM compound Arvanil may be a promising agent for the treatment of SCLC. **Acknowledgement:** Funding for our study was supported by the NIH R15-AREA Grant (2R15CA161491-02 and 2R15CA161491-03) to PD and MAV. Furthermore, this study was supported in part by the West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) grant (NIH grant P20GM103434; PI: Dr. G. Rankin), the National Institute of General Medical Sciences of the National Institutes of Health under the award number P30GM122733.

Tissue-based Analysis

Abstract 249
Insights into the Distribution of P-glycoproteins in Adult *Toxocara canis* using RNASCOPE
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Introduction: *Toxocara canis* is a zoonotic, cosmopolitan helminth of dogs. While dogs are primary hosts, larval stages can infect humans and cause serious disease. The clinical presentation of infections in humans includes visceral or ocular larva migrans, resulting in fever, fatigue, stomach pain, and eye damage. Evidence of antiparasitic drug tolerance in *T. canis* prevents successful eradication efforts and subsequently increases the likelihood of zoonotic spillover events. ATP-binding cassette (ABC) transporters like P-glycoproteins (Pgps) may be responsible for drug tolerance through drug expulsion before compounds are able to reach their molecular targets. Pgps have been implicated in antiparasitic drug resistance in several other helminth species. In this study, we aimed to assess the distribution of three *T. canis* Pgp genes (*Pgp- 9, Pgp-13, and Pgp-16*) in relation to the targeted site of macrocyclic lactones (*glc-2*) throughout the body of adult worms using an mRNA in situ hybridization technique (RNASCOPE). The use of RNASCOPE allows for a more accurate and reliable visual detection method of Pgp expression in relation to *glc-2* within various tissues of the worm, providing insight into the role of Pgps in drug tolerance. **Methods:** Ten worms, consisting of five males and five females, were opportunistically collected from a naturally infected puppy, and preserved using formalin fixation. Subsequently, the worms were divided into anterior, mid, and posterior segments and paraffin embedded. In triplicate, consecutive sections were cut from the formalin-fixed paraffin-embedded (FFPE) samples, and RNASCOPE was performed on each worm using probes specifically designed by ACDBio. The probes targeted *T. canis* genes *Pgp-9, Pgp-13, Pgp-16*, and *glc-2*. For controls, *T. canis* beta-tubulin *ttb4* (positive) and *Bacillus subtilis DapB* (negative) were used. Tissue samples, including intestinal, reproductive, body wall, nerve, and lateral cords, were photographed for each probe at the anterior, middle, and posterior segments of both male and female worms. The spatial relationships between the locations of Pgp and *glc-2* genes were assessed. **Results and Conclusions:** This is an ongoing study and results are pending. The localization data obtained from this study is expected to inform the development of targeted drug delivery strategies, aiming to enhance the efficacy of antiparasitic treatments in dogs and reduce the risk of zoonotic infections.

Abstract 250*
Clearing the Way: Non-toxic Clearing and Labelling with Fluorescent REAfinity Antibodies for the Enhanced 3D Visualization of Tissues and Organs
Kevin Bigott, Johanna Werheid, Daniel Barleben, Kira Gerhold, Lea Bornneman, Florian Kuster, Diogo Bessa-Neto, Simon Merz, Dmytro Yushchenko, Jonathan Fauerbach, Lutz Haalck, Gernolt Elbel, Christian Dose, Andreas Bosio, Melanie Jungblut, Dominik Eckardt, Lena Nolte, and Kyle Kern
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*The authors of this abstract selected not to publish the details of their research

Vascular Biology

Abstract 251
Glypican 1 and Intracellular Calcium Levels in Lung Endothelial Cells

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Introduction: Glypican 1 is a heparan sulfate proteoglycan with putative mechano-sensing properties that has recently emerged as a regulator of cardiovascular function. Previous work from our group has shown that glypican 1 determines calcium storage in vascular smooth muscle cells. Whether glypican 1 plays a role in calcium handling in endothelial cells is unclear. Herein we sought to investigate if glypican 1 regulates intracellular calcium levels in lung endothelial cells. **Methods:** Male and Female wild type (WT) and glypican 1 knockout mice (Gpc1KO), with 6-10 weeks of age were used. Mouse lung endothelial cells (MLEC) were isolated and cultured up to passage 5. The presence of the glycocalyx components glypican 1, syndecan 1, syndecan 4 and heparan sulfate was assessed by immunofluorescence. Cells were exposed to either a chemical (hydrogen peroxide, H2O2, 50uM) or a mechanical (45cm of hydrostatic pressure for 30 minutes) stimulus known to cause increase in intracellular calcium levels in endothelial cells. To investigate if the effects of pressure on intracellular calcium levels are associated with the production of reactive oxygen species (ROS), WT MLEC were exposed to high pressure in the presence of PEG-SOD and PEG-catalase, ROS scavengers. Intracellular calcium levels were determined by plate fluorometry using Fluo-4 AM. **Results:** Primarily isolated MLEC from WT and Gpc1KO mice showed the presence of glycocalyx as indicated by the presence of heparan sulfate, syndecan 1 and syndecan 4. Glypican 1 was absent in Gpc1KO MLEC, validating the global knockout model. Hydrogen peroxide increased intracellular calcium levels by 3-fold in WT MLEC but had no effect on Gpc1KO MLEC. High hydrostatic pressure increased intracellular calcium levels by 27% in WT MLEC and by 13% in Gpc1KO MLEC. PEG-SOD and PEG-Catalase mitigated the effects of high hydrostatic pressure in MLEC intracellular calcium levels. **Conclusion:** Glypican plays a role in intracellular calcium dynamics in lung endothelial cells. This may be important for lung endothelial barrier stability and function.

Abstract 252

The Histone Modification H3K4me2 and the DNA Demethylase TET2 Coordinately Regulates Microvascular SMC Recruitment and Coverage During Hindlimb Ischemia-induced Angiogenesis

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Introduction: Patients with Peripheral Artery Disease (PAD) experience cramping, chronic pain and eventually limb loss due to atherosclerosis and partial obstruction in their limb arteries leading to reduced tissue blood perfusion. The decrease in limb perfusion is associated with a deficiency in compensatory microvascular remodeling (angiogenesis, arteriogenesis). During vascular remodeling, vascular smooth muscle cells (SMCs) can reversibly switch between a contractile and a synthetic, migrative phenotype. This process relies on transcriptional and epigenetic mechanisms, including Ten Eleven Translocation 2 (TET2) which promote SMC differentiation through active DNA demethylation of SMC contractile genes (Acta2, Myh11). Recent studies from our lab identified the histone modification H3K4 di-methylation (H3K4me2) as a physical partner of TET2 mediating its selective recruitment on the SMC gene repertoire. Epigenome editing of H3K4me2 on the SMC gene repertoire unexpectedly led to a profound inhibition of SMC migration capacities and participation to vascular remodeling. Together, these observations raised the question of the role of the H3K4me2/TET2 complex in ischemia-induced vascular remodeling. **Methods:** Gastrocnemius and soleus tissue sections from PAD and control patients were immunostained to assess microvascular remodeling and DNA methylation. H3K4me2/TET2 function during hindlimb ischemia was tested in a model of femoral ligation/excision using two SMC lineage tracing strains: Myh11-CreERT2 YFP TET2 flox (TET2$^{SMC-/-}$) and Myh11-CreERT2 YFP Myocd-LSD1 (Myocd-LSD1$^{SMC+/-}$) for SMC-specific lineage tracing and TET2 knockout or H3K4me2 editing on the SMC contractile genes, respectively. Vascular remodeling, SMC abundance, distribution, and phenotype was assessed by immunofluorescent staining combined with confocal microscopy. In vitro, dye-loaded SMC and endothelial cells (EC) were cocultured in pro-angiogenic conditions and live-imaged for evaluation of angiogenesis and SMC/EC

interaction after SMC TET2 KD or H3K4me2 editing. **Results:** We found an increase in DNA methylation in the calf muscle of patients with PAD compared with controls associated with a decreased expression in smooth muscle a-actin (ACTA2), suggesting a defect in TET2 activity. We conducted neovascularization analysis after femoral artery ligation-excision using a newly generated SMC-specific Myocd-LSD1 transgenic mouse. Impaired blood reperfusion was observed in Myocd-LSD1 $^{SMC+/-}$ and TET2 $^{SMC-/-}$ mice compared with controls. Lineage tracing analysis revealed that this defect was due to a loss of SMC-mediated perivascular coverage on neovessels. In vitro, we found that H3K4me2 editing and TET2 knockdown both led to reduced SMC migration and impairment of EC/SMC interaction and vascular tube formation. **Conclusions:** Our data provide evidence of the central role of H3K4me2/TET2 in regulating SMC ability to interact with EC and remodel the microvasculature during hindlimb ischemia.

Abstract 253

PECAM-1 Blockade Modulates Leukocyte Extravasation into the Subcortex after Ischemic Stroke and Reperfusion

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Introduction: Neutrophils are the earliest responders to ischemia/reperfusion injury; however, in stroke when and where neutrophils enter the parenchyma to exert their effects is controversial. Advancement of therapies that address the acute inflammatory response in reperfusion injury requires a precise understanding of the spatiotemporal underpinnings of leukocyte extravasation. We describe the evolution of neutrophil recruitment and extravasation following stroke in a mouse model. **Methods**: The transient Middle Cerebral Artery Occlusion model (tMCAO, 90 minutes of ischemia followed by reperfusion) was used to simulate large vessel occlusion stroke and recanalization. In vivo labeling of neutrophils with 5-Ethynyl-2′-deoxyuridine (*EdU*) followed by wide field immunofluorescence microscopy was used to track neutrophil persistence and location with regard to the brain vasculature and the perivascular space. Flow cytometry of single cell suspensions was used to track the percentage of EdU+ neutrophils at different time points post-stroke. **Results**: Ischemic strokes involving over 20% of cerebral volume were induced in mice. At 24h, a majority of infiltrating neutrophils are near the cortical surface. At 12h and 24h, leukocyte recruitment and extravasation were primarily localized to the cortical surface. This contrasts with other organs where there is considerable migration of neutrophils deep into the inflamed tissue by 24h. Over 48 to 72h, neutrophils were found increasingly deeper into the subcortex. Throughout the infarct (determined with triphenyl tetrazolium chloride staining), leukocyte recruitment was not uniform but rather organized in clusters. tMCAO surgery was timed with EdU+ disappearance from circulating neutrophils so that the last EdU+ neutrophils would be in the blood only in the first 24h post stroke. Heat maps show distribution of EdU+ neutrophils throughout the infarct at 72 hours. Disrupting leukocyte diapedesis with PECAM function-blocking monoclonal antibody restricted leukocytes to within 500 microns of the surface when compared to control; and this was still evident at 72 hours (51% compared to 69%, at 72h, n=5, $p<0.05$). High-resolution wide-field microscopy confirmed inhibition of TEM by PECAM-1 blockade at 24h. Flow cytometry showed approximately equal numbers of monocytes and neutrophils at 72h. **Conclusions**: Our findings demonstrate that leukocyte infiltration into a stroke evolves over several days following reperfusion. The distribution of EdU+ neutrophils suggests neutrophils are recruited to the infarct within the first 24 hours, enter the infarct at the cortical surface, distribute throughout the infarct and persist over 72 hours. The use of PECAM blockade modulates the natural progression of leukocytes into the infarcted stroke bed. A better understanding of leukocyte spatiotemporal infiltration and its regulators could help inform the next generation of therapeutic interventions.

Abstract 254

Characterizing the Relationship Between Hypoxia, Inflammatory Cell Infiltrate, and Angiogenesis in a Murine Model of Type II Diabetic Wound Healing

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Introduction: Impaired cutaneous healing is a significant and growing complication associated with type II diabetes. Currently, there is a lack of effective and reproducible clinical strategies to induce wound resolution (http://blog.mediligence.com/2013/01/29/wound-prevalence-and-wound-management-2012-2020/). In association with hypoxia, neutrophil persistence has been described in human wounds even after healing is complete (Rosalind J Butterworth, 1992, MD Thesis, Leicester University). Hypoxia and neutrophils interact bi-directionally, with hypoxia postulated to be able to bolster neutrophil survival via an autophagy like mechanism (*J Leukocyte Biol* 2019;**106**:1367-1379), and neutrophils being able to deplete the molecular oxygen level in the wound via a process termed "respiratory burst" (*J Biol Chem* 2002;**277**:30463–30468). Depletion of available molecular oxygen in the context of healing can negatively impact a wide array of necessary healing milestones such as cell proliferation, collagen deposition and timely resolution of inflammation (*Br J Dermatol* 2010;**163**:257-268). Angiogenesis is also a critical milestone during healing as the re-establishment of vascular supply and adequate oxygenation of tissues is a necessary milestone in cutaneous healing. The current project aims at investigating the relationship between hypoxia, neutrophil recruitment to the wound, and angiogenesis in a murine model of diabetic healing. **Methods:** Based on previously reported data (*Matrix Biol* 2015;**43**:71-84), we hypothesized that db/db diabetic mice would exhibit delayed wound closure, increased neutrophil persistence and reduced angiogenesis in comparison with wild-type mice. Selected timepoints for histology were day 3 and day 7, corresponding to the peak of acute inflammation and the peak of proliferation/beginning of angiogenic phase respectively. **Results:** Db/db mice exhibited a significant delay in closure at days 3, 7 and 12 post-wounding. No significant differences were observed in the relative amount of hypoxia in the epithelium at day 7 assessed through HypoxyProbe. A significant difference was observed in the amount of hypoxic cells in the wound beds of wild-type animals at day 7. However, no significant differences were observed in neutrophil numbers between phenotypes at day 7. Finally, a significant increase in the number of blood vessels at the wound edges of wild-type mice at day 7 was detected, but not in granulation tissue. **Conclusion:** We conclude that wild-type mice show a minor increase in hypoxia at day 7 post-wounding compared to db/db mice. Our current evidence suggests that the db/db diabetic model may not be optimal as a model of hypoxic healing, and may further suggest that delayed hypoxia resolution is a necessary stimulus for normal physiological healing to occur.

Abstract 255

The Inhibition of the Alpha7-nicotinic Acetylcholine Receptor Blocks Retinal Angiogenesis: Potential Applications in ARMD and Diabetic Retinopathy

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Introduction: Neovascular diseases of the retina like diabetic retinopathy (DR) and age-related macular degeneration (ARMD) constitute the leading cause of blindness in developed countries. These proliferative retinopathies involve the pathological growth of new blood vessels as a result of hypoxic stimuli like ischemia or inflammation. Cigarette smoking is a risk factor in the development of ARMD and DR. Nicotine (the active component of cigarette smoke) has been known to stimulate angiogenesis in cancer and heart disease via the alpha7-nicotinic acetylcholine receptor (α7-nAChRs) on target cells. The central hypothesis of our study was two-fold: (i) Nicotine accelerates retinal angiogenesis, and (ii) Disruption of nicotine-induced angiogenesis could pave the way to novel treatments for this constellation of retinal diseases. **Methods:** ELISA assays showed that a diverse array of nicotinic acetylcholine receptor (nAChR) subtypes were expressed on primary human retinal microvascular endothelial cells (HRMECs). The angiogenic effect of nicotine in the retina was evaluated by the Matrigel duplex assay (MDA). The results obtained from MDA were confirmed using the rat retinal explant angiogenesis assay. ELISA assays were used to measure the levels of VEGF in cell culture supernatants. Similarly, the expression of MMP-2, MMP-9 and MMP-13 levels in HRMEC culture supernatants was analyzed by ELISA. The role of α7-nAChRs in nicotine-induced angiogenesis was examined by siRNA techniques. **Results**: Nicotine-induced angiogenesis required nAChR function and was associated increase in VEGF levels which in turn caused the upregulation of MMP-2 and MMP-9 in HRMECs. Specifically, α7-nAChRs mediated the stimulatory effects of nicotine on

retinal angiogenesis and MMP levels. Treatment of HRMECs with α7-nAChR antagonists ablated nicotine-induced angiogenesis. The inhibitory actions of α7-nAChR antagonists correlated with the suppression of VEGF, MMP-2 and MMP-9 levels in HRMECs. **Conclusions:** The α7-nAChR is vital for the pro-angiogenic activity of nicotine. The α7-nAChRs expressed on HRMECs upregulate VEGF levels in HRMECs. Such increase in VEGF leads to the increase of MMP-2 and MMP-9, which stimulate retinal angiogenesis. Our data suggest that α7-nAChR antagonists may be useful for the therapy of angiogenesis-related retinal diseases. **Acknowledgement:** Funding for our study was supported by a grant from the PhRMA foundation and American Retina Foundation to PD. Furthermore, this study was supported in part by the West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) grant (NIH grant P20GM103434; PI: Dr. G. Rankin).

Abstract 256

Endocrine Disrupting Chemical Bisphenol A Exposure Induced Testicular Toxicity in Gerbils : Histopathological Evaluation

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Introduction: Bisphenol A (BPA) is a well-known endocrine disruptor chemical that affects reproductive function. Recently, BPA has been receiving increased attention due to its link to various health problems that develop after direct or indirect exposure. However, the main biological events through which BPA affects testicular function remain to be identified. **Methods:** Adult male gerbils were treated by oral administration of BPA (50 mg/kg and 100mg/kg, respectively) for five weeks. Testes were collected for histological and immunohistochemical analysis. Epididymal spermatozoa were collected for sperm quality evaluation. Plasma was collected for detection of testosterone and estradiol levels. Germ cell proliferation was examined in BPA-treated and control gerbil testes. Ki-67 antigen assay was used to detect the effect of BPA on the proliferation of male germ cells. **Results:** The BPA-treated gerbils were characterized by decreased sperm quality, plasma testosterone levels and reduced fertilization efficiency. Histological examination of seminiferous tubules demonstrated vacuoles, atrophy, and separation of the germinal epithelium in BPA-administrated gerbils as compared to the control. In the lower BPA (50 mg/kg) treatment group, Ki-67 antigen was down-expressed that indicated impaired germ cell proliferation. **Conclusion:** Low doses BPA exposure reduced sperm quality mainly by impairing germ cell proliferation, leading to reduced male fertility. The study would provide relevant information for investigation on molecular mechanisms and protective strategy on male reproduction.

Abstract 257

Dimethyl Fumarate Inhibits VEGF-Driven Angiogenesis of Human Retinal Endothelial Cells

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Introduction: Wet age-related macular degeneration (AMD) is characterized by uncontrolled angiogenesis disrupting the intricately organized choroidal vasculature. The newly formed vessels are prone to leakage, leading to edema, further inflammation, and ultimate tissue dysfunction. Our prior study showed the anti-inflammatory effects of dimethyl fumarate (DMFu) in retinal pigment epithelial cells. Here we investigated the anti-angiogenic potential of DMFu, utilizing human retinal microvascular endothelial cells (HRECs) and mouse choroidal explant models. Already approved by the FDA for psoriasis, DMFu emerges as a prime candidate for drug repurposing with the potential for swift clinical translation for AMD treatment. **Methods:** HRECs were cultured in Endothelial Basal Media-2 (EBM-2) at 37°C with 5% CO_2. Culture plates were coated in 0.2% porcine-derived gelatin in PBS. The migratory capacity of HRECs was examined using the scratch wound assay. Confluent HRECs were mechanically scratched with a p200 pipette tip in EBM-2 with/without VEGF (10 ng/ml) and DMFu (80 μM). Changes in gene expression of the pro-inflammatory

cytokine, interleukin-6 (IL-6), was assessed using qPCR. Tube formation was assessed by seeding HRECs at 20k cells/well in a 96-well plate coated in Cultrex BME. Tubes were visualized using fluorescence calcein staining, imaged on the EVOS M7000, and evaluated using the Image J Angiogenesis Analyzer plugin. Glycolysis and oxidative phosphorylation (OXPHOS) were examined by high-resolution respirometry on the Seahorse XFe96. Protein expression of electron transport chain (ETC) complexes was assessed by western blotting with the OXPHOS Human WB Cocktail Antibody. For the choroidal sprouting assay, 1 mm^2 punch biopsies of RPE/choroid/sclera were collected from 3-week-old C57BL/6J mice and embedded in 30 µL of Cultrex BME. **Results:** Concurrent addition of DMFu significantly reduced cell migration with or without VEGF, highlighting the anti-angiogenic potential of DMFu in HRECs. While VEGF did not change IL-6 levels compared to untreated HRECs, DMFu suppressed basal levels of IL-6, supporting its anti-inflammatory activity. DMFu significantly reduced protein expression of Complex II of the ETC compared to control, which was accompanied by a suppression in mitochondrial OXPHOS and a shift towards increased glycolytic capacity. No change in vascular tube formation was observed between VEGF and/or DMFu was observed. Concurrent addition of DMFu robustly inhibited VEGF-induced choroidal sprouting, and DMFu alone also inhibited choroidal sprouting. **Conclusions:** Our data demonstrate that the metabolic drug, DMFu, has both anti-inflammatory and anti-angiogenic effects on human retinal endothelial cells. DMFu appears to reduce OXPHOS capacity through the reduction of Complex II expression, thus rewiring the metabolic profile towards a glycolytic phenotype. The inhibitory effect of DMFu against VEGF highlights its role as a potential therapeutic for wet AMD.

Abstract 258

Endomucin Deletion Leads to Reduced Pathological Retinal Neovascularization

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Introduction: Endomucin (EMCN) is an endothelium-specific glycoprotein expressed by venous and capillary endothelial cells. We reported that EMCN knockdown in vitro significantly inhibits VEGF165-stimulated VEGFR2 internalization and subsequent cell proliferation, migration, and tube formation. This study aimed to elucidate the role of EMCN in normal retinal vascular development and its impact on pathological neovascularization using EMCN knockout mice in oxygen-induced retinopathy (OIR) and laser-induced choroidal neovascularization (CNV) models. **Methods:** $EMCN^{-/-}$ mice were generated by crossing EMCN-floxed mice with ROSA26-Cre mice. Tissues from the choroid, retina, kidney, lung, spleen, liver, and thyroid were collected RNA extraction. Retinal flat mounts were prepared from postnatal day(P) 5 mice and adult mice (12-16 weeks old) for isolectin-B4 (IB4) staining to visualize the retinal vasculature. Eyes from adult $EMCN^{-/-}$ mice and $EMCN^{+/+}$ control littermates (4-6 months old) were fixed for transmission electron microscopy. For the OIR model, P7 mice were exposed to 75% oxygen for five days, and the retinal vasculature was examined at P12 and P17 using IB4 staining. CNV was induced in 6-8-week-old $EMCN^{+/+}$ and $EMCN^{-/-}$ mice using laser photocoagulation. Lesion size and vascular leakage were quantified using in vivo optical coherence tomography (OCT) and fluorescein angiography (FA) seven days after laser treatment. Immunohistochemistry and SP8 confocal microscopy were employed to examine CNV lesions by IB4 staining. **Results:** Of the tissues examined, EMCN mRNA levels were the highest in the choroid of EMCN wildtype mice and undetectable in retinas and RPE/choroid of $EMCN^{-/-}$ mice ($n \geq 4$, $p < 0.0001$). $EMCN^{-/-}$ pups showed a significantly reduced area of retinal vascularization at P5 (0.14±0.01 vs 0.2±0.013, $p < 0.0001$, $n \geq 10$). Ultrastructural analysis of the choriocapillaris $EMCN^{-/-}$ mice ($n=3$) revealed disorganized fenestrations and thickened endothelial cells. In the OIR model, the avascular area in $EMCN^{-/-}$ and $EMCN^{+/+}$ mice at P12 was similar (24.57±1.4% vs 23.18±1.0%, $p=0.9$, $n \geq 6$). In contrast, $EMCN^{-/-}$ mice had significantly reduced area of pathological neovascularization at P17 compared to controls (8.98±2.9% vs 11.98±1.4%, $p < 0.05$, $n \geq 10$). In the CNV model, lesions of $EMCN^{-/-}$ mice exhibited reduced fluorescein leakage ($22.2 \times 10^4 \pm 2.2 \times 10^4$ pixels vs $41.06 \times 10^4 \pm 3.6 \times 10^4$ pixels, $n=24$, $p < 0.001$) and smaller lesion

areas visualized by OCT (2.7 x 10^4±841.2 um^2 vs 3.5 x 10^4±1119 um^2, n=24, p<0.0001) and IB4 staining (2.2 x 10^4±1639 um^2 vs 3.2 x 10^4±2226 um^2, n=23, p<0.001) compared to $EMCN^{+/+}$ mice. **Conclusion:** Lack of EMCN in vivo leads to delayed developmental retinal vascularization and disorganized choriocapillaris fenestrations in adult mice. Moreover, the absence of EMCN interferes with pathological angiogenesis and permeability processes, driven by VEGF signaling, pointing to EMCN as an alternative endothelial-specific therapeutic target.

Wound Healing

Abstract 259
Increased Wound Healing Rates in P-glycoprotein Deficient Intestinal Cells
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Introduction: Inflammatory bowel disease (IBD) is an autoimmune disease of unknown cause and includes conditions such as Crohn's disease and ulcerative colitis. With no cure and only palliative therapies available, many patients with these conditions struggle with diarrhea, abdominal pain, and other chronic symptoms. This study is interested in investigating the multidrug resistance gene (*MDR*) which encodes the small molecule pump P-glycoprotein (P-gp). This gene is responsible for regulating drug absorption and accumulation in various parts of the body including the intestines. Polymorphisms of the *MDR1* gene (encoding p-glycoprotein) have been linked to IBD in humans. We hypothesized that $MDR^{-/-}$ Caco-2 intestinal cells would heal wounds slower than control Caco-2 cells, resulting in increased leakage between the tight junctions of the intestines and an increase in disease progression. **Methods:** Control and $MDR^{-/-}$ Caco-2 cells were cultured in DMEM supplemented with 10% serum. Cells were grown to confluency and wounded using a 10μl pipette tip. All imaging was done using a Keyence BZ-X800 microscope. Wound healing was measured by capturing images of wounds at various time points, and measuring wound area using ImageJ. Proliferation was determined using EdU staining (ThermoFisher Click-iT EdU staining kit), and also assessed using CellTrace Violet labeled cells analyzed via flow cytometry. Membranes were stained using CellMask plasma membrane stain, and cell size determined with ImageJ. Lastly, the tight junction proteins occludin and ZO-1 were detected using immunofluorescent staining of confluent cells after wounding. **Results:** Surprisingly, The *MDR* deficient cells have shown increased wound healing compared to control cells. To determine the mechanism by which the $MDR^{-/-}$ cells were healing wounds faster, we investigated cellular proliferation and analyzed the cellular shapes and sizes of the *MDR* deficient cells relative to control cells. Increased proliferation rates were observed via EdU staining and flow cytometry. Membrane staining revealed increased cell size along the wound front in $MDR^{-/-}$ cells. Little change was observed when staining for tight junction proteins (occludin and ZO-1). **Conclusions:** This data revealed changes in proliferation, cell size, and cell shape in post-wound the $MDR^{-/-}$ cells when compared to the control cells. Because the role of *MDR* deficiency in the development in IBD is currently unknown, this data suggests altered wound healing may play a role in eliciting a chronic immune response after damage in the intestine. Overall, the changes we detected suggest that the wound healing mechanism differs when *MDR* is missing from cells, which, in the intestine, could lead to the development of inflammatory bowel disease.

Abstract 260
The Extracellular Microenvironment Modulates Fibronectin Matrix Formation by Cortical Astrocytes
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Introduction: Recovery from injury depends on the formation of the appropriate extracellular matrix (ECM). In the central nervous system (CNS), astrocytes play essential roles in supporting neuronal connections and communication and blood-brain barrier integrity. Astrocytes reportedly secrete ECM molecules, including fibronectin (FN), an essential ECM protein that supports the assembly of many other ECM molecules and is a critical component of the matrices that form throughout the tissue repair process. It remains unclear whether astrocytes' role in nervous tissue repair includes production and assembly of FN matrix. **Methods:** We isolated cortical astrocytes from E16.5-17.5 rat embryos and characterized them for expression of the astrocyte markers glial fibrillar acidic protein (GFAP) and aquaporin 4 (AQP4) using flow cytometry. Cultures were about 80% positive for GFAP and AQP4. FN production and matrix assembly

were analyzed by immunofluorescence staining and immunoblotting. **Results:** Permeabilized GFAP-positive cells showed staining for intracellular FN localized in a perinuclear compartment and FN was detected in astrocyte conditioned medium. Astrocyte cultures grown under standard conditions assembled very few FN fibrils. However, when astrocytes were grown on a FN-coated surface, the amount of matrix was significantly higher with a FN fibril mean fluorescence intensity about 2-fold higher on FN compared to no coat. Culturing astrocytes on 3D decellularized FN matrix or co-culturing astrocytes with NIH 3T3 mouse fibroblasts also appeared to increase the assembly of rat FN fibrils suggesting that astrocyte fibril formation might be stimulated when cells are in a FN-rich environment. Two different populations of astrocytes were observed in our cultures based on the level of GFAP expression. FN assembly primarily co-localized with dim GFAP+ astrocytes rather than bright GFAP+ astrocytes. **Conclusions:** Our results show that astrocytes are a source of FN matrix and indicate that their ability to assemble FN depends on the microenvironment and may correlate with GFAP levels. At sites of CNS injury, FN in the blood clot and provisional matrix could provide a stimulatory environment to promote astrocyte ECM assembly required for neural repair. **Acknowledgements**: Sud Cook '39 Fund and NIAMS R01 AR073236.

Abstract 261

Age-related Deterioration of the Dermal Extracellular Matrix Microenvironment Promotes Skin Cancer Development

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Introduction: Keratinocyte skin cancer affects a significant portion of the elderly, yet the pathophysiological mechanisms remain incompletely understood (*J Am Acad Dermatol* 2021;**85**:388-395). Fragmentation, disorganization, and depletion of the collagen-rich dermal extracellular matrix (ECM) are characteristic features of aged human skin dermis (*Gerontology* 2015;**61**:427-434). These deleterious alterations critically mediate many of the prominent clinical attributes of aged skin including thinning, fragility, impaired wound healing, and propensity for carcinoma. As aging has long been recognized as a major risk factor for cancer, we investigated the impact of age-related dermal ECM microenvironment on keratinocyte skin cancer formation using humanized transgenic mouse models of accelerated dermal aging. **Methods:** Two mouse models of dermal aging were generated by selective expression of human matrix metalloproteinase-1 (MMP-1) or CCN1, a matricellular protein, in dermal fibroblasts driven by the fibroblast-specific collagen1A2 (*Col1a2*) promoter and upstream enhancer. We generated these mouse models of dermal aging based on data demonstrating that both MMP-1 and CCN1 are significantly elevated in dermal fibroblasts in aged human skin and mediate deleterious alterations in the dermal ECM microenvironment (*Gerontology* 2015;**61**:427-434). **Results:** At six months of age, both *Col1a2;hMMP1* (*J Invest Dermatol* 2023;**143**:1700-1707) and *Col1a2-hCCN1* (*J Invest Dermatol* 2021;**141**:1007-1016) mice display many of the hallmarks of aged human skin, including fragmentation, disorganization, and reduced density of dermal collagen fibrils; increased expression of multiple endogenous MMPs, and proinflammatory mediators Il-1β, IL-6, and IL-8. RNA-seq analysis revealed the upregulation of the age-associated secretory proteins genes and downregulation of core matrisome genes in both *Col1a2;hMMP1* and *Col1a2-CCN1* mice versus control mice. Importantly, *Col1a2;hMMP1 and Col1a2-hCCN1* mice exhibit an enhanced preponderance of keratinocyte cancer in two mouse models of skin carcinogenesis: two-stage chemical carcinogenesis, and inducible expression of oncogenic HRas. RNA-seq and Gene Ontology analyses identified that pathways in cancer, such as mitogenic and inflammatory signaling pathways, were specifically enriched in SCC-like tumors in HRas-oncogene-driven tumors. **Conclusion:** Our data demonstrate that age-related alterations of the dermal ECM microenvironment mediate the development of keratinocyte cancer, partially explaining skin cancer's prevalence in the elderly. **Acknowledgements:** NIH R01AG054835, U01AG077924, and R01AG081805.

Abstract 262

Investigating Oxidative Stress in Human Chronic Wounds

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Introduction: Dermal wound healing is an essential process that allows skin to maintain its ability protect against environmental stressors. Impairment of the healing process, associated with co-morbidities including diabetes mellitus, critical limb ischemia, and immobility, leads to the development of chronic wounds, classified as diabetic, venous, and pressure ulcers. Standard treatment for these wounds in the early stages is mostly limited to debridement and appropriate dressings of the wound to maintain moisture and offload pressure. Overall, many experimental therapies targeting aspects of wound chronicity have been unsuccessful at promoting wound closure leaving chronic skin wounds in a perpetual pro-inflammatory state. Many of the molecular mechanisms responsible for the chronic inflammation and subsequent impairment of healing processes are still not well understood. Although oxidative stress and hypoxia are considered a central part of the pathology, an analysis of human chronic wound tissue with respect to cellular markers has not yet been performed. We hypothesized that the transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (pro-oxidant) would be upregulated and active in keratinocytes on the edge of non-healing wounds, that oxidative damage will be detected in keratinocytes and surrounding matrix through the presence of 8-hydroxy-2'-deoxyguanosine (8-OHG) (nucleic acid damage), 3-nitrotyrosine (3-NT) (protein damage), and 4-hydroxynoneal (4-HNE) (lipid damage), and that the transcription factor nuclear factor erythroid 2–related factor 2 (Nrf2) (anti-oxidant) would not be upregulated or active in keratinocytes on the edge of non-healing wounds. **Methods:** Using antibodies specific to phosphorylated (activated) NF-kB, phosphorylated (activated) Nrf2, 8-OHG, 3-NT, and 4-HNE, immunohistochemistry was performed on samples of end stage human chronic wounds from lower limb amputations and debridement tissue from early presentation human chronic wounds. Staining of NF-kB, Nrf2, and 8-OHG was evaluated based on nuclear localisation, while 3-NT and 4-HNE staining was evaluated based on cytoplasmic and extracellular localisation. **Results:** Nuclear localisation of activated NF-kB and 8-OHG was detected in wound edge keratinocytes and dermal cells from both groups. 3-NT and 4-HNE were also detected both intracellularly and in the extracellular matrix at the wound edge of both groups. This evidence of oxidative stress and damage was also present up to ten centimeters away from the wound edge in lower limb amputations. Activated Nrf2 was also prominently detected within the nuclei of wound edge keratinocytes and dermal cells from both groups. **Conclusion:** These observations suggest that dermal wound chronicity could be associated with oxidative tissue damage, as well as keratinocytes receiving signals from multiple antagonistic pathways resulting in impaired cellular functions necessary for wound closure.