

Cancer Pathobiology

Abstract 1

Subcellular Localization of Novel Fluorescent Epidermal Growth Factor Receptor (EGFR) and Hepatocyte Growth Factor Receptor (MET) Targeted Inhibitors in Cancer Cells

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Introduction: Epidermal growth factor receptor (EGFR) inhibitors of the quinazoline class have emerged as potent agents in personalized lung cancer therapy. It is known that EGFR is not only localized on the cell surface, but also inside the cell. However, little attention is paid to the cellular localization of EGFR inhibitors, which could perhaps influence their biological activity. Additionally, EGFR inhibitors can bind to multiple kinases interfering with cellular pathway of some oncogenes, such as MET in EGFR inhibitor-resistant tumors. We hypothesize that the target multiplicity of EGFR inhibitors can be visualized by cell live imaging, reflecting the macromolecular binding profile of these inhibitors.

Methods: One quinazoline-based molecule (anti-EGFR) carrying a red fluorescence marker, called "AB64", and one molecule of crizotinib (anti-MET inhibitor) green-labeled with nitrobenzoxadiazole (NBD), called "AB20", were synthesized. Four cell lines were used to analyze the subcellular distribution of MET and EGFR: A549-wild type, A549-GFP-EGFR, NIH3T3-wild type, and NIH3T3-EGFR. MitoTracker green was used for the colocalization experiment in A549 cells. Visualization was performed using Zeiss LSM780 laser scanning confocal microscope. Observations were made in a monolayer of cells which were treated with AB64 and/or AB20 (5 or 10 μ M) with/without MitoTracker for 15 minutes prior the live imaging. **Results:** The red fluorescence of AB64 (anti-EGFR inhibitor) was observed in the perinuclear region of all cell lines and it was not present on their cellular surface. More interestingly, AB64 co-localized with the mitochondria in A549 cells. In order to determine if AB64 co-localized with MET, the green fluorescent AB20 (anti-MET inhibitor) was co-stained with the red AB64. The co-staining of AB64 and AB20 showed high co-localization. Prior to this double staining, AB20 was observed to be confined to the perinuclear region where it was more largely intracellularly distributed than AB64 in all cell lines. **Conclusions:** We were able to synthesize two new fluorescent molecules showing that the inhibitors of the quinazoline class: (1) has a unique property of colocalization in the mitochondria, which may interfere with the mitochondrial functions like perhaps apoptosis or redox signaling, and (2) can simultaneously bind to MET kinase sites, showing kinase off-targets effects in living cells.

Abstract 2

Targeting Nutrition Via Bioactive Lipids Enhances Immunotherapy in Cancer

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Introduction: Cancer involves a systemic pro-inflammatory and dysregulated immune response. Immunotherapy has emerged as a treatment option in many cancers, although the response rate is low. Dietary intervention provides an opportunity to optimize the host immune response in cancer patients, however, mechanisms of immunonutritional regulation in cancer are poorly understood. Some studies suggest that omega-3 polyunsaturated fatty acids (PUFAs) are beneficial for reducing the risk of cancer, whereas omega-6 PUFAs may stimulate cancer. Cytochrome P450 epoxygenases convert the omega-6 PUFA arachidonic acid into epoxyeicosatrienoic acids, which suppress inflammation. Because the half-life of fatty acid epoxides is rapid, drugs that stabilize their levels by inhibiting soluble epoxide hydrolase (sEH) are in clinical trials for hyperinflammatory diseases. We hypothesized that fatty acid supplementation with sEH would enhance immunotherapy in various cancer types. **Methods:** Mice were fed standard diet, omega-6-rich diet, or omega-3-rich diet for 12 days prior to tumor inoculation and for the duration of the studies. Following tumor cell injection, mice were randomized into treatment groups: control, sEH, immunotherapy, and sEH + immunotherapy. **Results:** We found that dietary supplementation with omega-3 fatty acids improved the

efficacy of immunotherapy. Further, immunotherapy was effective in Fat1 mice, which genetically produce high levels of omega-3 fatty acids. Additionally, we found that sEH synergizes with immunotherapy in mice on a high omega-6 fatty acid diet to transform "cold" unresponsive tumors into "hot" responsive tumors. sEH is alone or in combination with dietary omega-3 supplementation may be a promising new approach to enhance immune checkpoint blockade in cancer. **Conclusion:** These findings identify omega-3 and omega-6 epoxides as regulators of immunotherapy and sEH as a druggable target in cancer.

Abstract 3

Oral Pathogens Regulate Epithelial Cell Behavior Through the Adherens Junction-associated RNAi Machinery

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Introduction: Increasing evidence portrays key roles of host-microbial interactions in the progression of epithelial diseases, including tumorigenesis. A pathogen that has been linked with tumor progression is oral microbe *Fusobacterium nucleatum* (*F. nucleatum*). However, the mechanisms by which *F. nucleatum* may influence epithelial cell behavior are not fully known. Notably, *F. nucleatum* has been associated with loss of epithelial integrity. The adherens junction (AJ) is an essential, E-cadherin-based, cell-cell adhesion complex that is key for epithelial tissue integrity but has also been implicated in regulation of cell behavior. Further, we have previously shown that epithelial AJs through the E-cadherin-p120 catenin partner PLEKHA7, recruit and regulate core components of the RNA interference (RNAi) machinery, such as DROSHA and AGO2, to suppress oncogene expression and pro-tumorigenic cell transformation. Thus, we sought to interrogate the AJ-associated RNAi machinery as a potential mechanism mediating the effects of *F. nucleatum* in epithelial integrity and pro-tumorigenic transformation. **Methods:** We used a well-differentiated colon epithelial cell line model (Caco2), and assessed effects of *F. nucleatum* on AJs and other pro-tumorigenic markers. Caco2 cells were exposed to either heat-inactivated bacteria, or to bacterial supernatant from *F. nucleatum* spp. *nucleatum*, *Streptococcus salivarius* and *Escherichia coli* spp. *Nissle*. Cells were fixed after 8, 16, and 24 hours of incubation, and examined by immunofluorescence and confocal microscopy for changes in the junctional localization of E-cadherin, p120 catenin, PLEKHA7, AGO2, and DROSHA. **Results:** Results showed that localization of PLEKHA7, AGO2, and DROSHA was disrupted by *F. nucleatum*, but not by *Streptococcus salivarius* or *Escherichia coli*. Also, these changes in localization were accompanied by upregulation of oncogenes such as JUN and SNAIL, as shown by western blot of cells treated with *F. nucleatum* supernatant for 24 hours. Oncogenes JUN and SNAIL were previously found to be suppressed by the junctional RNAi. **Conclusion:** This data supports that *F. nucleatum* may be promoting pro-tumorigenic transformation through disruption of the AJ-associated RNAi machinery leading to increased expression of oncogenes, and this can help deepen our understanding of the mechanisms mediating host-pathogen interactions in epithelial homeostasis and disease.

Abstract 4

PIK3CA and cMYC Promote the Expansion of Distinct Ras-initiated, Long-lived Premalignant Clones in a Multistage Murine Breast Cancer Model

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Introduction: A remote carcinogen exposure can predispose to a clinical presentation of breast cancer decades later. Standard multistage carcinogenesis models posit that carcinogen-induced mutations generate long-lived premalignant clones, 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 strategies. In prior work, we showed that a one-time exposure to carcinogen 7,12-dimethylbenzanthracene (DMBA) generates initiated mammary epithelial cell (iMEC) clones bearing signature *Hras*^{Q61L} mutations, which remain subclinical indefinitely until inducible activation of oncogenic Wnt signaling triggers their rapid clonal expansion into malignant outgrowths. **Methods:** Here, we adapted this multistage model by replacing Wnt pathway activation with Doxycycline-inducible expression of either *c-MYC* (iMYC mice) or

PIK3CA^{H1047R} (iPIK mice), recapitulating two of the most prevalent oncogenic events in human breast cancer. Additional cohorts of adult mice also underwent one or two full-term pregnancies to test parity protection against premalignant clones. Mice were monitored for tumor onset, and tumors were subjected to DNA sequence and histological analyses to uncover their mutational and microscopic features. **Results:** Despite using a DMBA exposure identical to that used in our Wnt work, neither inducible *c-MYC* (iMYC) expression nor inducible *PIK3CA*^{H1047R} (iPIK) expression efficiently selected for the outgrowth of *Hras*^{Q61L} iMEC clones. Instead, iMYC and iPIK expression selected for the outgrowth of iMECs bearing activating mutations in distinct Ras family genes, with iMYC expression promoting *Kras*^{mut} and *Nras*^{mut} tumors and iPIK expression prompting the development of *Kras*^{mut} tumors. Selection for these preferred Ras family mutations occurred whether oncogene expression was induced within days of DMBA exposure or months later. Similar to our Wnt work, parity failed to diminish tumor onset in iPIK and iMYC mice. However, our parity-induced protection schemes decreased the frequency of *Kras*^{mut} iMEC clones in iPIK and iMYC mice without affecting *Nras*^{mut} iMEC clones in iMYC mice. **Conclusions:** Together, our findings demonstrate that oncogenes *PIK3CA* and *cMYC* select for the expansion of long-lived, premalignant clones carrying distinct Ras mutations and sensitivity to parity protection. Further investigation of the cellular and molecular mechanisms underlying the differential selection of these premalignant clones may uncover targets for breast cancer chemoprevention.

Abstract 5

The Roles of the Histone Demethylase KDM5A in Breast Cancer and Senescence

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Introduction: Epigenetic alterations including histone methylation have become an increasingly important topic in cancer pathophysiology, with implications in all stages of cancer development and progression. The histone demethylase KDM5A has been found to be upregulated in several cancers including breast cancer, and its associations with cell cycle regulation and senescence make it a promising therapeutic target, however the underlying mechanisms remain largely unclear. Further examination of KDM5A is warranted to probe its therapeutic potential. Using mouse models of breast cancer and *ex vivo* analyses, our group has demonstrated that KDM5A inhibition leads to senescence phenotypes indicating its potential to prevent mammary tumor growth.

Methods: The mouse and cell culture models utilized in our studies are 1) mouse mammary tumor virus expressing polyoma middle T antigen (MMTV-PyMT) mice with genetic knockout of *Kdm5a*, 2) a doxycycline-inducible *Kdm5a* in *Kdm5a*^{-/-} MMTV-PyMT cells, and 3) an orthotopic model established by injection of the metastatic triple negative breast cancer (TNBC) cell line, 4T1, with LentiCRISPR short guide RNA (sgRNA) against *Kdm5a*. Tumor cells from all models were used for quantitative real-time polymerase chain reaction (RT-qPCR), and immunohistochemical (IHC) staining was used to investigate the changes in immune cell infiltration of the 4T1 tumors. **Results:** Genetic deletion of *Kdm5a* in the MMTV-PyMT mouse model resulted in mild change in tumor burden, but had no impact on tumor free or overall survival. Remarkably, however, *Kdm5a*^{-/-} tumor cells from these mice rapidly began to exhibit senescence phenotypes including increased expression of senescence associated secretory phenotype (SASP) markers and hexokinase 2, and an acquired senescent cell morphology with increased media acidification. Consistently, removal of the exogenous KDM5A from the *Kdm5a*^{-/-} MMTV-PyMT cells resulted in a similar phenotype. These changes suggest that KDM5A inhibition leads to tumor cell senescence, but that changes in the tumor microenvironment (TME) *in vivo* are permissive of cancer progression. In fact, *Kdm5a* knockout in the orthotopic 4T1 mouse model significantly increased T cell infiltration and decreased tumor growth. Additionally, KDM5A loss or inhibition resulted in increased expression of interferon stimulated genes and cytokines and chemokines. **Conclusion:** These

studies suggest that KDM5A targeting could be a therapeutic strategy for breast cancer treatment.

Abstract 6

Eicosanoid Regulation of Bladder Cancer

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Introduction: Unresolved inflammation plays a critical role in bladder cancer initiation and progression. Controlling the local and systemic inflammatory response is critical to preventing cancer progression. Chemotherapy, the standard of care for advanced bladder cancer, disrupts the resolution of inflammation and is only partially effective in preventing tumor recurrence. Although immunotherapy induces a durable response in a subset of patients with advanced bladder cancer, a majority of patients do not respond. Thus, there is a critical unmet need to improve chemotherapy and immunotherapy in bladder cancer.

Methods: We have developed dual COX-2/sEH inhibitors (e.g., PTUPB), which target two key enzymes in the arachidonic acid cascade: cyclooxygenase-2 (COX-2) and soluble epoxide hydrolase (sEH). Since chemotherapy and immunotherapy both induce tumor-promoting inflammation via an eicosanoid and cytokine storm, we hypothesized that dual COX-2/sEH inhibition using PTUPB would enhance immunotherapy in experimental bladder cancer via anti-inflammatory and pro-resolution mechanisms. When syngeneic (MB49) bladder tumors reached ~200 mm³ in immunocompetent mice, treatment was initiated with PTUPB, anti-CTLA-4, anti-PD1, gemcitabine, cisplatin, or combinations thereof. **Results:** Dual COX-2/sEH inhibition in combination with chemotherapy (gemcitabine and cisplatin) and immune checkpoint blockade (anti-CTLA-4 or anti-PD1) induced tumor regression via synergistic anti-tumor activities. Chemotherapy and immunotherapy induced the expression of ER stress response genes (e.g., *BiP* and PD1) and the angiogenic factors (e.g. EGF and VEGF-C) in bladder cancer tissue, which was counter-regulated by PTUPB. PTUPB also prevented chemotherapy-induced toxicity. **Conclusion:** Our results demonstrate for the first time that dual COX-2/sEH inhibition is a novel therapeutic approach to enhance immunotherapy in bladder cancer without overt toxicity.

Cardiovascular and Pulmonary Pathobiology

Abstract 7

Increased Ratio of Free LDL/VLDL to Esterified HDL Cholesterol Directly Associates to Advanced Atherosclerosis Development in Mice

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Introduction: Cardiovascular disease (CVD) due to atherosclerosis accounts for over 10 million deaths annually. Increase in low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) or "bad cholesterol" as well as triglycerides (TG) due to exogenous western diet occurs in atherosclerosis. Inversely, low high-density lipoprotein (HDL) or "good cholesterol" results from decrease cholesterol unloading from tissues. Cholesterol in the body exist as either unesterified cholesterol otherwise called free cholesterol and esterified cholesterol. Free cholesterol has bioactive properties and excess accumulation results in lipotoxicity. In humans, increased ratio of unesterified LDL to esterified HDL has been associated to atherosclerosis. Our study aims to evaluate if the increase in unesterified LDL to esterified HDL ratio leads to atherosclerosis preclinically. We will use our novel Triple KO mice aka "TKO" that we developed in the LDL Receptor (LDLR) knockout background. These mice develop extensive atherosclerosis in the

abdominal aorta due to loss of cellular cholesterol efflux in smooth muscle cells. We hypothesize that increased unesterified LDL/VLDL to esterified HDL ratio translates to increase in atherosclerosis development in the abdominal aorta in Triple KO mice compared to controls due to decreased cholesterol efflux in the vascular wall. **Methods:** Triple KO adult male mice were placed on a High Cholesterol Diet (HCD) (Research Diets) at 8 weeks of age with Double KO littermate controls. Samples were taken at baseline and after 16 weeks of HCD, animals were sacrificed, and plasma was isolated. ELISA kits for Triglycerides (Randox Triglycerides TR210), Non-Esterified Fatty Acids (Randox NEFA FA115), HDL and LDL/VLDL (Sigma Aldrich) and Total Cholesterol (Fujifilm Cholesterol E) were used following manufacturers protocol (n of 3-4 per group). **Results:** Our studies show that TKO mice have higher total cholesterol than DKO mice (570 mg/dL \pm 180 vs 240 \pm 100mg/dL) after diet intervention. There is also increase in triglycerides in TKO than DKO mice (1300 \pm 220 mg/dL vs 320 \pm 30 mg/dL). Inversely, after 16 weeks of diet, total HDL (6.8 vs 3.8 mg/dL) and free HDL (6.2 vs 4.6 mg/dL) was higher in DKO mice compared to TKO mice. Total LDL/VLDL (46.6 vs 19.4mg/dL) and free LDL/VLDL (24 vs 24.8 mg/dL) was significantly higher in TKO mice compared with DKO. Importantly, atherogenic risk is 2-fold increase in TKO mice due to high ratio of unesterified non-HDL after 16 weeks was (6.24 vs 3.8 mg/dL). **Conclusion:** Result shows increased unesterified non-HDL cholesterol to esterified HDL has high potential as an atherogenic risk indicator. Our findings in TKO mice correlate to significant atherosclerotic disease progression in our model. Findings strongly indicate that altered cholesterol efflux has a high translational significance to atherosclerosis and CVD.

Abstract 8

PTSD-Induced Alterations of Cardiac Homeostasis

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Introduction: Cardiovascular disease (CVD) is the leading cause of death in the United States. Multiple studies indicate that Post-Traumatic Stress Disorder (PTSD) is a risk factor for CVD. Accordingly, we hypothesize that PTSD-induced chronic inflammation increases macrophage numbers in the heart, leading to increased cardiac fibrosis and the resetting of cardiac homeostasis. **Methods:** To induce experimental PTSD, C57BL/6 male mice (8 months old) were given an intermittent foot-shock (IFS; 1.0 mA, 1 sec duration) 5 times within a 6-minute span. Before each shock, a tone was played to act as the PTSD-associated trigger. Control animals were identical to experimental animals but did not receive the foot shock. At 13-weeks post-IFS, mice underwent open field and fear conditioning chamber behavioral assessments to measure PTSD symptomology. Plasma was collected to determine circulating cardiac troponin I (cTnI) levels. Based on cTnI, mice were separated into 3 groups: no IFS (controls), IFS mice with a slight increase in circulating cTnI (non-responders), and IFS mice that had significantly elevated cTnI (PTSD-like) compared to controls. At tissue sacrifice (13-weeks after IFS), the left ventricle (LV) was collected and perfused with cardioplegic to arrest it in diastole. The LV was fixed in paraformaldehyde and paraffin embedded for picrosirius red (PSR) staining and macrophage immunohistochemistry. **Results:** Thirteen weeks after IFS, mice that displayed an elevation in circulating cTnI also demonstrated symptoms of intrusion (p=0.03) and alterations in arousal (p=0.01) in response to the PTSD trigger, mimicking clinical symptoms of PTSD. Mice that had a more subtle elevation in cTnI did not show significant alterations in behavior, classifying them as non-responders (p=0.31). PTSD-like mice had increased collagen (p=0.03) and macrophages (p=0.02) in the LV compared to controls and non-responders. Non-responders had elevated collagen (p=0.02), but not macrophage numbers (p=0.19) when compared to controls. **Conclusions:** In conclusion, our data suggests that 13 weeks post-IFS, mice that illustrate PTSD-like symptomology have increased macrophages and deposition of cardiac fibrosis indicating increased cardiac remodeling compared to controls and non-responders.

Abstract 9

Effects of Obesity on Angiogenesis and Regenerative Lung Growth

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Introduction: Obesity is associated with the impairment of wound healing and tissue regeneration. Angiogenesis, the formation of new blood capillaries from preexisting blood vessels, plays a key role in organ regeneration and repair. Inhibition of angiogenesis attenuates lung growth after unilateral pneumectomy (PNX) and obesity is accompanied by endothelial cell dysfunction. This project aims to investigate the effects of obesity on post-PNX lung vascular and alveolar morphogenesis. **Methods:** We perform PNX on leptin-deficient (*Lep^{ob/ob}*) mice under a 10% high-fat diet and investigate the effects of obesity on angiogenesis and regenerative lung growth using biochemical and immunohistochemical analysis. **Results:** Post-PNX regenerative lung growth is inhibited in *Lep^{ob/ob}* obese mice compared to *Lep^{ob/+}* mice. The levels of the major angiogenic factor, vascular endothelial growth factor (VEGF) are higher in the serum and the lung tissue collected from post-PNX mice compared to those from sham-operated control mice, while these effects are attenuated in post-PNX *Lep^{ob/ob}* mice. The levels of adiponectin, one of the adipokines that exhibits pro-angiogenic and vascular protective properties, increase in the remaining mouse lungs after unilateral PNX, while these effects are attenuated in *Lep^{ob/ob}* obese mice. Regenerative lung growth, vascular and alveolar morphogenesis, and VEGF levels in the post-PNX mouse lungs are inhibited in adiponectin knockout mice. **Conclusions:** These results suggest that obesity inhibits post-PNX regenerative lung growth through adiponectin-VEGF signaling. Modulation of adiponectin-VEGF signaling may be an efficient strategy to restore lung regeneration and repair in obese people.

Abstract 10

Endothelial Senescence Mediates Hypoxia-induced Vascular Remodeling in the Lung Through TWIST1 Signaling

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Introduction: Pulmonary hypertension (PH) is a fatal pulmonary vascular disease characterized by a sustained elevation of pulmonary arterial (PA) pressure. One of the major characteristics of PH is uncontrolled accumulation of PA smooth muscle cells (SMCs) to normally non-muscularized distal PAs. Cellular senescence contributes to aging and lung diseases associated with PH. We have reported that a transcription factor, TWIST1, in endothelial cells (ECs) mediates hypoxia-induced accumulation of PSMCs to PAs by increasing platelet-derived growth factor (PDGFB) expression. The aim of this study is to examine whether cellular senescence controls vascular remodeling in PH through TWIST1. **Methods:** We utilize PAECs derived from healthy individuals and PH patients to examine EC senescence. We also use a *p16^{INK4Afl/fl}-Cdh5(PAC)-Cre^{ERT2}* mouse model to determine the effects of EC senescence on vascular remodeling in a hypoxia-induced PH model. **Results:** The levels of senescence markers are higher in ECs isolated from PH patients compared to those from healthy individuals. Publicly available microarray data of PH patient lungs reveal the alteration of senescence-related gene expression and their interaction with TWIST1. The levels of PDGFB upregulated in PH patient-derived ECs are inhibited by knocking down *p16^{INK4A}* expression or treatment with senolytic reagent ABT-263. Hypoxia-induced accumulation of α -smooth muscle actin (α SMA)-positive cells to the PAs and TWIST1 expression are attenuated in *p16^{INK4Afl/fl}-Cdh5(PAC)-Cre^{ERT2}* mice after tamoxifen induction. Exosomes derived from hypoxia-treated mouse lung ECs stimulate DNA synthesis and migration of PSMCs, while those derived from *p16^{INK4Afl/fl}-Cdh5(PAC)-Cre^{ERT2}* mouse lung ECs inhibit these effects. **Conclusion:** These results suggest that EC senescence mediates vascular remodeling in PH through TWIST1 signaling.

Cell Injury and Repair

Abstract 11

The Effect of Caspase-11 in Trauma-Induced Coagulopathy (TIC).

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Introduction: Trauma is one of the leading causes of death globally in people 50 years and younger. Hemorrhagic shock following trauma can lead to hypoxia, loss of blood components, and the need for blood transfusion, all of which can lead to trauma-induced coagulopathy (TIC) and organ and tissue damage, resulting in multiple organ dysfunction (MODs). Caspase-4/11 is associated with disseminated intravascular coagulation (DIC) in sepsis by allowing tissue factor binding to cells and initiation of the coagulation cascade. However, its role in TIC is unknown. We sought to investigate the effects of caspase-11 in a murine polytrauma model on hemostasis and coagulation. **Method:** To induce coagulopathy, male C57BL/6J and caspase-11^{-/-} mice, 8-12 weeks old, were subjected to a murine polytrauma model. The model consists of a blind cardiac puncture (25% of total blood volume is taken), laparotomy with liver crush, and bilateral pseudofractures (hindlimb crush injury followed by the injection of crushed bone solution from an age- and weight-matched syngeneic donor). Blood was collected and citrated at 3, 6 and 24 hours. Caspase-11, tissue factor, and fibrin expression were measured by Western blot (WB) of whole liver and lung lysates. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen were measured. **Results:** Polytrauma significantly increased aPTT levels (18.1 ± 0.8 to 42.7 ± 0.8 sec, $p < 0.0001$) and fibrinogen levels (154 ± 0.4 to 433 ± 0.8 mg/dL, $p < 0.0001$) after 24h, indicating TIC. Polytrauma induced expression of caspase-11 in the liver. After 24h of polytrauma, aPTT levels increased in WT mice but did not increase in caspase-11^{-/-} mice (42.7 ± 0.8 vs 10.2 ± 1 sec, $p < 0.0001$). Moreover, fibrinogen levels rose 3 fold in WT and caspase-11^{-/-} mice with lesser post-injury values in caspase-11^{-/-} mice compared to WT mice (331 ± 0.8 vs 433 ± 0.8 mg/dL, respectively). Caspase-11-deficiency increased tissue factor and decreased fibrin levels in both liver and lung at 6h in TIC compared with WT. **Conclusion:** Caspase-11 expression is induced by polytrauma and TIC. Caspase-11 signaling may regulate the coagulation profile in polytrauma as well as the localization of tissue factor. Inhibition of caspase-11 may therefore be a future therapeutic option for TIC.

Fibrosis and Extracellular Matrix

Abstract 12

Interstitial Fibrosis and Macrophages Persist in the Myocardium Following Removal of Left Ventricular Pressure Overload

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Introduction: Aortic stenosis can develop into Left ventricular pressure overload (LVPO). LVPO is associated with increased myocardial collagen content and stiffness. Patients undergoing alleviation of LVPO by surgical aortic valve replacement (SAVR) demonstrate reduced but persistent fibrosis. Little is known regarding the cellular mechanisms that contribute to ECM turnover following hemodynamic unloading of the myocardium. Also, patient biopsies and animal models of LVPO demonstrate an increased level of macrophages and a correlation between myocardial macrophage levels and LVPO-induced interstitial fibrosis. Whether and to what extent myocardial macrophages contribute to ECM turnover after unloading of the myocardium is unexplored. **Methods:** To induce LVPO, transverse aortic constriction (TAC) was performed on 12wk old C57Bl6 mice. After 4wks of LVPO, the TAC band was removed (unTAC) to alleviate pressure overload. Cardiomyocyte cross-sectional area (CSA), Collagen volume fraction (CVF), collagen hybridizing peptide (CHP), and total myocardial macrophage levels were assessed at five time points: Control no TAC, 4wk TAC, 4wk TAC+2wk unTAC (2wk unTAC), and 4wk TAC+4wk unTAC (4wk unTAC), and 4wk TAC+6wk unTAC (6wk unTAC). **Results:** First, at 4wk TAC, CSA increased by 47% compared to control. By 6wk unTAC, CSA returned to control levels. Second, a 204%

increase in CVF was observed at 4wk TAC compared to control. CVF was similar between 4wk TAC and 2wk unTAC, but a significant decrease in CVF was observed at 4wk unTAC although it remained elevated compared to control. CVF remained elevated at 6wk unTAC compared to control. Third, proLOX was significantly increased in 4wk TAC compared to control. proLOX levels significantly decreased in 2wk unTAC and 4wk unTAC compared to 4wk TAC. However, proLOX significantly increased in 6wk unTAC. TIMP1 continued to increase during the unTAC time course. Finally, macrophage area increased by 259% at 4wk TAC compared to control, and early after unloading, a 240% increase was observed at 2wk unTAC compared to 4wk TAC. Macrophages remained elevated at 4wk unTAC and 6wk unTAC compared to control. **Conclusions:** After unloading the myocardium, hypertrophy fully regressed, but persistent fibrosis and myocardial stiffness were observed. Proteins implicated in collagen crosslinking and inhibiting collagen degradation were increased in 6wk unTAC demonstrating a shift in ECM homeostasis that favors persistent fibrosis. Furthermore, macrophage levels remained elevated at all TAC and unTAC time points over control. **Significance:** Our TAC/unTAC murine model mimics the AS/SAVR patient paradigm and emphasizes the importance of investigating new therapies to address persistent cardiac fibrosis. Future studies using this model will focus on elucidating macrophage phenotype and macrophage-dependent mechanisms of collagen turnover to determine if macrophages are a targetable therapy for inhibiting persistent fibrosis.

Gastrointestinal Pathobiology

Abstract 13

Loss of Myo5b Results in Improper Delivery of the Intermicrovillar Adhesion Complex Components

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Background: The elaborate apical membrane of intestinal enterocytes consists of actin rich protrusions called microvilli. Microvilli dramatically increase the surface area of the intestine maximizing absorption. Each enterocyte has about 1000 microvilli lining their apical membrane, collectively known as the brush border. To accommodate so many microvilli on a single cell, microvilli are densely packed and highly organized. This organization is orchestrated by the intermicrovillar adhesion complex (IMAC). IMAC is composed of protocadherins, protocadherin 24 (CDHR2) and mucin like protocadherin (CDHR5), as well as scaffolding proteins USH1C, ANKS4B and Myosin 7b (Myo7b). The IMAC forms physical adhesion links at the tips of microvilli to control packing and microvillar length. Defects in IMAC results in stunted microvilli and has been linked to Crohn's disease. To create an IMAC complex, cells traffic proteins to the apical membrane. Myosin 5b (Myo5b) is a molecular motor that is critical for delivering ion transporters to the apical membrane of enterocytes. Loss of functional Myo5b results in decreased apical expression of transporters and profound diarrhea in animal models and humans. We hypothesized that Myo5b is responsible for IMAC proteins localization. **Methods and Results:** We used 2 different mouse models: neonatal germline Myo5b knockout (Myo5b KO) mice and adult intestinal specific tamoxifen inducible VillinCre^{ERT2};Myo5b^{flox/flox} mice. RNA sequencing showed decreased expression of CDHR2, CDHR5, USH1C, and ANKS4B in germline Myo5b KO mice compared to littermate control mice. In control mice, immunostaining revealed that CDHR2, CDHR5, USH1C, and Myo7b were highly enriched at the microvilli tips. In contrast, neonatal germline and adult Myo5b deficient mice showed loss of apical CDHR2, CDHR5, and Myo7b in the brush border and accumulation in a subapical compartment compared to littermate controls. Co-localization analysis of CDHR2, CDHR5, and the lysosomal marker Lamp1 showed an increase in CDHR2 and CDHR5 in Lamp1-positive lysosomes; suggesting that CDHR2 and CDHR5 may be improperly trafficked for degradation in Myo5b KO mice. In neonatal Myo5b KO mice USH1C was found on the apical membrane of germline Myo5b KO mice, but did not appear enriched at microvilli tips. However, adult inducible Myo5b

deficient mice showed a more complete loss of apical USH1C. Co-localization analysis for the microvilli marker gamma actin and each IMAC component revealed decreased Mander's coefficients in adult inducible Myo5b deficient mice compared to control mice for CDHR2, CDHR5, USH1C, and Myo7b. Fourier's analysis further demonstrated aberrant microvilli packing in adult inducible Myo5b deficient mouse small intestine. **Conclusions:** These data indicate that Myo5b is responsible for the delivery of IMAC components to the apical membrane and thus controls the proper brush border formation and packing of microvilli.

Abstract 14

Rotavirus Infection Elicits Host Responses via P2Y1 Purinergic Signaling

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Introduction: Rotavirus causes life-threatening diarrhea in children, resulting in ~130,000 deaths each year. Rotavirus infects a limited number of cells at the tips of the villi in the small intestine. Yet, rotavirus dysregulates cells far away from the site of infection. We recently identified, using simian (SA11) and rhesus rotavirus (RRV), that infected cells release the purinergic signaling molecule ADP, which binds to the P2Y1 receptor on nearby uninfected cells. Furthermore, using the *in vivo* mouse model, mild rotavirus diarrhea in mouse pups was alleviated by daily treatment with a P2Y1 inhibitor. **Methods:** To elucidate the role of purinergic signaling via P2Y1 receptors during rotavirus infection, we used the mouse-like rotavirus (D6/2) to investigate the effects of purinergic signaling in the context of severe rotavirus infection *in vivo*. C57Black6 mouse pups were orally gavaged D6/2 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 selective P2Y1 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:** Similar to mild rotavirus infection, treatment of D6/2-infected mouse pups with MRS2500 results in decreased severity and incidence of diarrhea. MRS2500 treated pups also exhibit decreased luminal serotonin and chloride content compared to control infected pups. Together, these results confirm that P2Y1 signaling is also involved in the pathogenesis of a homologous murine rotavirus strain. 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. **Conclusion:** Collectively these findings point to the conserved role of purinergic signaling in the pathophysiology of rotavirus infection, and indicate P2Y1 is a new candidate for host-targeted therapeutics that could have both antiviral and antiarrheal effects against rotavirus pathophysiology.

Abstract 15

Acinetobacter calcoaceticus is Adept at Colonizing the Gastrointestinal Tract and Stimulating Inflammation

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Introduction: Inflammatory bowel disease (IBD) is a life-long condition characterized by chronic inflammation of the gastrointestinal tract. IBD consists of two subtypes: Crohn's Disease and Ulcerative Colitis and collectively the disease affects 3 million individuals in the United States. IBD patients have an altered gut microbiota and *Acinetobacter* are one of the groups of microbes that have been shown to be increased in IBD patients. *Acinetobacter* levels significantly correlate with microbial pathways in actively inflamed tissue, suggesting a potential causal relationship between *Acinetobacter* and intestinal inflammation. Analysis of the IBD Transcriptome and Metatranscriptome Meta-Analysis (IBD TaMMA) platform, which houses 3,853 publicly available RNA-Seq datasets from 26 independent studies, revealed that *Acinetobacter calcoaceticus* was one of the top 10 highest elevated bacteria in Crohn's Disease patients. The majority of work with *Acinetobacter* has focused on *Acinetobacter baumannii* and to date no

studies have examined *A. calcoaceticus* in the context of the gut or inflammation. We hypothesized that *A. calcoaceticus* would be capable of withstanding the conditions of the gastrointestinal tract, could colonize with other gut microbes and initiate inflammatory signals. **Methods and Results:** Using 2 commercially available strains and 4 clinical isolates, we found that all *A. calcoaceticus* strains were fairly resistant to high osmolarity (0.1, 0.5, and 1 M NaCl), ethanol (1, 2.5, and 5%) and hydrogen peroxide (0.05, 0.1, and 0.2%). In general, the clinical isolates were more resistant than the commercial strains. All strains were able to grow in pH 7, 6, 5, and 4 media; although a reduction in growth was observed at the lower pHs. Biolog phenotypic microarrays in minimal media lacking glucose revealed that all strains could use the following sugars: glucose, L-arabinose, D-galactose, D-mannose, D-fructose, GluNAC, and trehalose. Additionally, all *A. calcoaceticus* strains could colonize human fecal bioreactors; indicating that these microbes could colonize the setting of a complex gut microbiota. Finally, we sought to examine the interaction between *Acinetobacter* and the gut epithelium. Incubation of live *A. calcoaceticus* strains with inside-out intestinal organoids significantly increased pro-inflammatory cytokines (TNF, KC/IL-8, MCP-1, and IL-1 α) and decreased MUC2 and MUC13 transcripts, without altering tight junctions. **Conclusions:** Collectively, these data demonstrate that *A. calcoaceticus* is well adapted to the gastrointestinal tract and points to the potential for *Acinetobacter* to stimulate inflammation and contribute to the pathogenesis of IBD.

Abstract 16

Restoration of Colonic Barrier Protein Expression following Lipopolysaccharide and Cytokine-Induced Inflammation in Human Colon Organoids

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Introduction: Aquamin is a mineral complex derived from mineralized remains of marine red algae. It is rich in calcium, magnesium and seventy-two minerals and trace elements. Recent studies with Aquamin have demonstrated that it improves gut barrier structure and function in colon organoids (3D tissue culture) derived from colon biopsies of healthy subjects. Colonic barrier dysfunction is a feature of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. Since barrier dysfunction could be a result of toxic insult or inflammatory attacks on epithelial cells that line the colon, strengthening the colonic barrier is critical. Through experimentation in organoids derived from normal colon tissue, these studies will allow us to determine how the pro-inflammatory environment modulates barrier structure proteins and inflammation-related proteins in the colon and to determine if, and to what extent, treatment with Aquamin can mitigate these effects.

Methods: We carried out studies using healthy human colon derived organoids (n=5) that were exposed to a mix of pro-inflammatory cytokines and lipopolysaccharides (LPS) in the absence and presence of Aquamin. Organoids were also cultured in a control medium (LWRN25%) with and without Aquamin. After 7 days in culture, tissue samples were assessed for a proteomic profile by tandem mass tag (TMT) mass-spectrometry. Pathway analyses were conducted using UniProt and Reactome databases. **Results:** On proteomic screen, LPS/cytokines stimulation caused an upregulation of proinflammatory moieties. Aquamin upregulated anti-inflammatory and antimicrobial proteins and increased the expression of proteins involved in barrier structure. LPS/cytokines and Aquamin alone altered 92 and 91 proteins, respectively, while Aquamin treatment in the presence of LPS/cytokines altered 145 proteins with a 1.8-fold-change cutoff. Twenty-six proteins were commonly altered by both Aquamin alone and Aquamin in the presence of LPS/cytokines. These included several keratins, filaggrin-2, trefoil factor 2, protocadherin-1, olfactomedin-4, cadherin-17, desmoglein-2 and glutathione S-transferase A1. The above proteins were all upregulated by Aquamin alone and in the presence of LPS/cytokines and downregulated by LPS/cytokines alone. *Interferon Signaling*, *Cytokine Signaling in Immune system*, *ER-Phagosome pathway* and *TCR signaling* were the top LPS/cytokine-induced upregulated pathways. In contrast, Aquamin (alone and with LPS/cytokines) upregulated *formation of the cornified envelope*, *keratinization*, and *Vitamin D (calciferol) metabolism*; it downregulated *MyD88 deficiency (TLR2/4)*, *IRAK4 deficiency (TLR2/4)*, and *regulation of TLR by endogenous ligand pathways*. **Conclusion:** These data imply that Aquamin can attenuate the inflammatory effects induced by LPS/cytokines. Further investigation may elucidate the beneficial role of Aquamin in mitigating inflammatory bowel diseases and improving the colonic barrier.

Abstract 17

Small Intestinal Responses to *Giardia muris* Infection in Tuft Cell-Deficient Mice

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Introduction: Enteric tuft cells (ETCs) possess the ability to modulate host immune responses to enteric parasitic infections and aid in parasite clearance. They sense luminal signals via various cell surface receptors and secrete the cytokine IL-25, which engages type 2 innate lymphoid cells and other cytokines in the lamina propria for further host responses, such as tuft and goblet cell hyperplasia. Infection with the intestinal protozoan *Giardia* causes intestinal barrier dysfunction and we have previously shown that infection results in altered goblet cell numbers, however the ETC response is unknown during the acute and clearance phases of infection. *Giardia* infection can impact the host in regions of the intestine beyond where active *Giardia* reside in the jejunum. We aim to investigate the role of tuft cells upon *Giardia* infection by assessing both tuft and goblet cell quantities in the small intestine at the site of active colonization and further downstream. **Methods:** A time-series infection study was conducted on 5–7-week-old C57Bl/6 or tuft cell-deficient (*Pou2f3*^{-/-}) mice. Mice were orally infected with *Giardia muris* trophozoites (5 x 10⁴). At days 4, 11, and 21 following infection, parasite burden was measured in the duodenum, and the proximal jejunum and distal ileum were collected for further analysis. Paraffin embedded jejunum and ileum tissue underwent immunofluorescence (IF) staining and periodic acid-Schiff (PAS)/Alcian blue (AB) staining for visualization and quantification of tuft and goblet cells, respectively. Double-cortin like kinase 1 (DCLK1) antibodies were used for IF staining. Quantitative PCR (qPCR) was performed on *Dclk1* (tuft cell marker) and *Atoh1* (transcription factor defining secretory cell types in the intestinal epithelium). **Results:** Tuft cell numbers were elevated in C57Bl/6 *G. muris* infected mice at day 21 post-infection in the jejunum and ileum, while at day 11, tuft cell numbers were decreased in the ileum of infected mice. Goblet cell numbers increased in *Giardia* infected C57Bl/6 mice in the jejunum at days 4 and 21 post-infection and in the ileum at day 21. Goblet cell numbers in *Pou2f3*^{-/-} mice did not differ between *Giardia* infected and uninfected mice in both the jejunum and ileum. *Dclk1* and *Atoh1* gene expression was elevated in *Giardia* infected C57Bl/6 mice at day 4 in the jejunum and day 11 in the ileum. **Conclusions:** *Giardia* infection is associated with tuft and goblet cell hyperplasia in the jejunum and the ileum, demonstrating that *Giardia* can illicit cell hyperplasia downstream of its site of colonization. Elevated gene expression of *Dclk1* and *Atoh1* was present earlier (day 4) in the jejunum and later (day 11) in the ileum, suggesting that signals for gene expression start proximally in the intestine and move distally over time during *Giardia* infection.

Infectious Diseases, Inflammation, and Immunopathology

Abstract 18

Inhibition of LPS-mediated Inflammation by Intravenous Administration of a Cationic Antimicrobial Peptide in Mice

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Introduction: Cationic antimicrobial peptides (AMPs) have long been observed to eliminate pathogenic microorganisms, such as gram positive and negative bacteria, through their ability to disrupt cell membranes and induce bacterial killing. E35, an engineered cationic AMP, is highly effective against multi-drug-resistant bacteria and can also bind lipopolysaccharide (LPS). We examined whether E35 was able to reduce LPS-mediated inflammation in a mouse model *in vivo*. **Methods:** Male C57Bl/6 (WT) mice were randomized to the following experimental groups (n=3-6 per experimental group): (1) intravenous (IV

via tail vein) E35 peptide alone (either 5mg/kg or 10mg/kg); (2) intraperitoneal (IP) LPS (5 mg/kg or 10 mg/kg) alone; (3) IV E35 directly followed by IP LPS; and (4) Control (no treatment). Blood and liver were collected after 4h. Plasma TNF α , IL1 β , and IL6 were measured by ELISA. MAPK (JNK, p38MAPK, ERK) activation/phosphorylation, and caspase-11 expression/activation were measured in liver whole tissue lysates by Western blot. **Results:** LPS significantly increased all measured inflammatory cytokines at 4h at both 5 mg/kg and 10 mg/kg compared with controls. Pretreatment with E35 just prior to LPS injection significantly reduced TNF α (p=0.03) and trended towards reduced IL1 β (p=0.36) compared to LPS alone. When TNF α was measured, LPS induced mice had a mean of 79.02 pg/mL (\pm 16.57) compared to 12.88 pg/mL (\pm 5.51) for mice given E35 treatment before LPS injection. Interestingly, E35 did not reduce IL6 levels in LPS-induced inflammation (p=NS). E35 peptide alone had no effect on systemic cytokine levels, but did significantly increase JNK and ERK1/2 activation/phosphorylation in the liver. However, pretreatment with 10mg/kg E35 in LPS-treated mice did reduce JNK activation. **Conclusions:** These data suggest that E35 peptide is able to reduce systemic LPS-mediated inflammation in a mouse model. The mechanism of liver MAPK activation with E35 administered alone, is interesting and this finding is being explored further. Overall, our results suggest that cationic AMPs may be able to reduce LPS-mediated inflammation, which may be important in regulating response to bacterial sepsis.

Abstract 19

Interaction Between Zika Virus and a Mosquito Axl-like Protein

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Introduction: Although many of the mosquito-borne Flaviviruses are not a major concern in the temperate regions of the world, they should be with the rise of global warming. These viruses could spread into new parts of the world as their mosquito vectors migrate. Similar events may occur with other vector-borne or climate specific viruses and pathogens. Regarding Zika, migration to temperate climates could result in an exponential increase in microcephaly due to the significant proportion of the world population living within the temperate regions (the other major region being the subtropics, where Zika can currently be found). Studying these, and all other viruses will be evermore important given both the changing environment and the recent global pandemic. **Methods and Results:** This study will look at the use of Axl-homologs (particularly Down Cell Adhesion Molecule, or DSCAM) for binding by Zika virus, on both human and mosquito cells. Axl is already a suspected protein used for binding to human cells, however, this protein does not exist within the mosquito genome. Thus, a DSCAM is selected for the study. Currently, we are working on a process known as RLM-RACE (RNA Ligase Mediated-Rapid Amplification of cDNA Ends). This process is helping us to isolate and amplify the very end of the gene we are looking to replicate. The purpose of doing this is to get an exact sequence and determine where the gene starts (there are multiple possible starting points along the predicted sequence). Once this is completed and a sequence has been obtained, custom primers will be designed to replicate the desired gene via PCR (polymerase chain reaction) and will also attach restriction sites on either end of the amplified gene. The purpose of the restriction site is so we can splice the gene into a plasmid. This plasmid will then be transferred to a bacterial culture whose purpose is to clone the plasmid and gene. Once enough genetic material is available, the plasmid will be transferred from the bacterial culture to a mammalian tissue culture (likely a human 293 T Cell) which is void of the Axl protein and the homolog we are studying (DSCAM). The cells will then read the genetic material and express the intended protein. After expression is confirmed, we can then infect the sample with inactive Zika virus and test for binding interactions. **Conclusions:** The overall outcome of this project is identify how Zika binds to its mosquito host. However, future research could involve the use of the receptor in mosquitoes as a potential source to develop preventions or treatments of Zika Virus and similar Flaviviruses. Alternatively, we already know that Axl plays many roles in cancer pathologies, thus Axl-homologs could be studied in oncogenic viral infections, or the potential for Flaviviruses to be used as vectors for cancer treatments.

Abstract 20

Mechanistic Insights into Macrophage Regulation of Neutrophil Transendothelial Migration in Inflamed Mucosa

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Introduction: Rapid neutrophil (PMN) mobilization to sites of insult is critical for host defense and requires crossing of the vascular wall. PMN transendothelial migration (TEM) involves several well-studied sequential adhesive interactions with vascular ECs, however what initiates or terminates this process is not well-understood. **Methods and Results:** Our findings identified a new mechanism where gut interstitial macrophages (M ϕ), which are rapidly recruited towards the vascular wall in response to inflammatory cues, were found to locally prime endothelial cell (ECs) responses to regulate PMN TEM. Using real-time intravital microscopy (IVM) on lipopolysaccharide (LPS)-inflamed intestines in anesthetized CX3CR1-EGFP macrophage-reporter mice, complemented by whole-mount tissue imaging we demonstrate that macrophage presence was critical for the induction of PMN-ECs adhesive interactions and subsequent PMN recruitment and accumulation in the intestinal mucosa. Anti CSFR-1 antibody-based macrophage depletion in the lamina propria and at the vessel wall significantly reduced PMN adhesion and TEM in inflamed intestines. We further observed that macrophages at the vessel wall localized specifically to regions of high ICAM-1 intensity and their removal resulted in elimination of the ICAM-1 "hot spots", overall lowering the ECs ICAM-1 expression. Mechanistically, using murine/human ECs-macrophage and PMN co-cultures we established that activated macrophages elevate PMN adhesion and TEM via TNF α -dependent upregulation of ECs ICAM-1. Antibody-mediated neutralization of TNF α in macrophage co-cultures with ECs and/or PMNs suppressed ICAM-1 upregulation, decreasing PMN TEM. Further *in vivo* imaging studies of inflamed gut revealed high TNF α expression in macrophages and specific expression of TNF α -receptor type II (TNFR II) but not type I in intestinal ECs. Inhibition of intestinal M ϕ -TNF α or ECs-TNFR II reduced intestinal PMN TEM. The use of bone marrow chimeras with TNF α knock-out M ϕ s further confirmed the novel role of M ϕ -TNF α in regulating ECs adhesion molecule expression and PMN TEM. **Conclusions:** Our findings identify a new, clinically relevant mechanism by which macrophages regulate PMN trafficking in inflamed mucosa.

Abstract 21

Klebsiella pneumoniae Isolates From Patients With Inflammatory Bowel Disease Influences *Clostridioides difficile* Pathogenesis

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Introduction: *Clostridioides difficile* is a gram-positive bacterium responsible for half a million infections and associated 29,000 deaths annually in the United States alone. Patients with inflammatory bowel disease (IBD) have increased morbidity and mortality with *C. difficile* infection (CDI). As IBD patients are susceptible to CDI without the use of antibiotics, we sought to determine if bacteria present in the gut microbiota of these patients could be influencing *C. difficile* pathogenesis. We probed the IBD Transcriptome and Metatranscriptome Meta Analysis (TaMMA) database and found that *Klebsiella pneumoniae* was increased in the stool of patients with ulcerative colitis, a subset of IBD. We thus hypothesized that *K. pneumoniae* influences *C. difficile* pathogenesis. **Methods and Results:** We received clinical isolates of *K. pneumoniae* from patients with IBD and utilized those, as well as two commercially available strains of *K. pneumoniae* for experiments. We grew *C. difficile* alone, *K. pneumoniae* alone, or a combination of the 2 species and tested growth with almost 200 nutrient sources using Biolog plates and found that the nutrient sources utilized by the combination of bacteria shifted from both bacteria alone. To test if *K. pneumoniae* was producing a metabolite that was influencing *C. difficile*, we grew *C. difficile* with *K. pneumoniae* conditioned media and measured growth over time. We saw that *C. difficile* growth was enhanced by *K. pneumoniae* conditioned media when compared to unconditioned media. We next tested if *K.*

pneumoniae influences *C. difficile* toxin production utilizing LifeAct expressing Vero cells. We saw that *C. difficile* grown with *K. pneumoniae* conditioned media had enhanced toxin production, as seen by increased Vero cell rounding when compared to Vero cells treated with *C. difficile* grown in unconditioned media. To determine how *C. difficile* and *K. pneumoniae* affects the intestinal epithelium, we treated inside-out colonic organoids with *C. difficile*, *K. pneumoniae*, or the combination of the two bacteria before collecting the organoids for RNA sequencing. We saw that the combination of bacteria caused decreased expression of *Muc2*, as well as increased expression of *Tnf*. **Conclusion:** These results suggest that *K. pneumoniae* influences *C. difficile* growth and pathogenesis. *K. pneumoniae* is found in low levels in the healthy human gut, however, patients with IBD have increased abundance of *K. pneumoniae*, which could potentially help drive *C. difficile* fitness and colonization. Therefore, we believe that clarifying the mechanism by which *K. pneumoniae* is interacting with *C. difficile* will help to better understand CDI in patients with IBD.

Liver Pathobiology

Abstract 22

Autophagy Regulates Hepatic Acetylome

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Introduction: Liver acetylome is a set of protein acetylations whose level reflects cellular metabolic health and is directly linked to intracellular pathways. However, to date, little is known about the cellular pathways that maintain the hepatic acetylome levels. Here, we show that macroautophagy hereafter referred to as autophagy, an intracellular lysosomal degradative pathway, regulates the hepatic acetylome. **Methods and Results:** Examination of total acetylome (nuclear, cytosolic, mitochondrial, membrane) in autophagy-deficient or autophagy-defective liver exhibited remarkably lower levels compared to normal liver. The lower hepatic acetylome was independent of the cellular injury that is commonly seen in autophagy-deficient conditions. In contrast, autophagy activation by fasting or rapamycin treatment increased the level of hepatic acetylome. Moreover, mechanistic studies showed that hepatic autophagy function is essential to maintaining levels of acetyl-CoA, a central intermediate metabolite needed for acetylation of proteins. Autophagy impairment significantly reduced hepatic acetyl-CoA production through transcriptional downregulation of key enzymes involved in the acetyl-CoA synthesis, including *Acy*, *AceCS1*, *AceCS2*, *Mcd*, and *Pdha1*. Notably, replenishing hepatic acetyl-CoA rescued the lowered hepatic acetylome and, interestingly, protected against liver injury in the autophagy-deficient liver. **Conclusion:** Autophagy regulates the hepatic acetylome as an important mechanism for protecting livers against injury and causing liver damage.

Abstract 23

MDR1 as a Potential Therapeutic Target for Progressive Familial Intrahepatic Cholestasis Type II in Children

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Introduction: Progressive Familial Intrahepatic Cholestasis type II (PFIC2) is a rare disease that primarily affects children under the age of two. It is characterized by mutations in the *ABCB11* gene that encodes for the bile salt export pump (BSEP). The loss of BSEP causes bile to be accumulated in the hepatocyte, thus leading to cholestasis and liver injury. The only curable treatment for PFIC2 is a liver transplant. *Abcb11* knockout mouse develops mild cholestasis. It is suggested that Multidrug Resistant Protein 1 (MDR1), another canalicular ABC transporter, may compensate for loss of BSEP. We established the first zebrafish *abcb11b* knockout model and showed that they accumulated bile salts within the liver, and resembled the phenotypes seen in patients with PFIC2. In both human and zebrafish that are deficient in BSEP, we found that MDR1 was mislocalized to the hepatocyte cytoplasm. In *abcb11b* zebrafish mutants treated with rapamycin, an autophagy inducer, MDR1 was localized to the canalicular membrane, coinciding with partial restoration of bile excretion. **Methods:** To understand how

MDR1 is mislocalized within the BSEP deficient zebrafish, we used transgenesis techniques to express MDR1 fluorescent fusion protein in the hepatocytes to visualize MDR1 localization and study the effect of MDR1 overexpression on bile excretion. We generated *abcb11b;mdr1* mutants to determine if MDR1 is necessary for the rescuing mechanism seen when treated with rapamycin, and *abcb11b;cyp7a1* mutants to investigate if bile accumulation effects MDR1 localization. **Results:** We showed that in *abcb11b;mdr1* double mutants the rapamycin treatment failed to restore bile excretion. Meanwhile, overexpression of either the zebrafish or human MDR1 induced a partial rescue of bile excretion in the hepatocytes of *abcb11b* mutants. These studies suggest that MDR1 can be prompted to serve as an alternative bile salt transporter to compensate for BSEP loss. We also showed that in *abcb11b;cyp7a1* double mutants, in which bile synthesis was largely inhibited, the canalicular localization of MDR1 was restored. These results indicate that MDR1 mislocalization within *abcb11b* mutants is due to the bile salt accumulation in hepatocytes. We currently focus on identifying the cellular mechanisms that act downstream of bile salt accumulation to cause MDR1 mislocalization. **Conclusion:** These studies have identified the cellular mechanism causing MDR1 mislocalization within *abcb11b* mutant zebrafish hepatocytes and introduced a new therapeutic target for future PFIC2 treatments.

Abstract 24

Targeted β -catenin Overexpression Mediates Biliary Repair in Murine Model of Intrahepatic Cholestasis

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Introduction: Primary sclerosing cholangitis (PSC) is a chronic progressive cholestatic liver disease with no approved therapies. We and many others have reported the role of β -catenin, a transcriptional coactivator in liver physiology and reprogramming. Hepatocytes (HC) transgenic for Ser-45 mutant β -catenin (TG) show increased expression of biliary markers after short-term exposure to porphyrinogenic biliary toxin 3,5-dihydroxycarbonyl-1,4-dihydrocollidine (DDC), which models some of the phenotypic findings of PSC. However, whether these biliary cell-like hepatocytes fully convert into cholangiocytes (CC) or maintain an intermediate phenotype, remains unclear. Here, we aimed to investigate the role of hepatocyte-specific β -catenin overexpression in mediating hepatocyte-to-biliary cell reprogramming that might help improve intrahepatic cholestasis. **Methods:** Age matched WT control (Con) and TG mice, both containing ROSA26-stop^{fllox/fllox}, were injected with AAV8-TBG-Cre to permanently label hepatocytes with EYFP and then fed 0.1% DDC diet for different time points - 30d, 60d, 90d, 120d, and 150d. Before sacrifice, bile flow was measured for 1h to obtain the flow rate. Liver histology and serum biochemistry were analyzed for fibrosis and parameters of injury. Porphyrin measurement was assessed in the liver tissues. Real-time PCR and immunofluorescence staining were performed to determine the expression of bile transporter genes and biliary markers respectively. **Results:** TG mice showed significantly improved bile flow rate as compared to the Con mice after 150d of DDC diet. Liver histology and porphyrin measurement showed significantly less porphyrin accumulation in TG as compared to Con. This was concomitant with decreased hepatic bile load in TG mice than the Con. Analysis of bile homeostasis and transport genes demonstrated comparable levels of expression in TG and Con mice. This is indicative of a plausible mechanism independent of bile metabolizing genes that contributes to the increased bile flow rate observed in TG mice. HC-derived duct-like structures positive for both EYFP and Sox9 were observed more frequently in TG as compared to Con. We also observed increased expression of hypophosphorylated active β -catenin and phosphorylated Tyr654 β -catenin in TG than the Con. Furthermore, increased number of A6-positive HCs in TG as compared to the Con was observed at 120d of DDC diet administration, indicating intermediate HC phenotype undergoing reprogramming to biliary cell type. **Conclusions:** Mice with β -catenin overexpressing HCs showed significantly improved bile flow rate, reduced porphyrin and decreased hepatic bile load in response to the biliary toxin DDC. Using HC fate-tracing, we observed increased biliary cell markers in TG hepatocytes suggestive of HC-to-biliary cell reprogramming potentially contributing to the enhanced bile flow.

Abstract 25

Cromolyn Sodium Ameliorates Mast Cell(MC)-mediated Hepatic Damage in a Murine Model of Non-alcoholic Steatohepatitis (NASH)

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Introduction: Hepatic inflammation and mast cell (MC) infiltration are major hallmarks of non-alcoholic steatohepatitis (NASH). We have previously shown that MC infiltration occurs in NASH liver as a response to senescence associated secretory phenotype factors (SASPs) from cholangiocytes. These MCs contribute to (i) inflammation, (ii) senescence, and (iii) micro-vesicular steatosis in hepatocytes. However, the effect of MC stabilization on NASH phenotypes and senescent cholangiocytes have not been addressed. Moreover, cromolyn sodium, a MC stabilizer treatment in cholangiopathies have been effective in ameliorating the hepatic damages in a murine model of cholestasis. To evaluate the effect of MC stabilization as a therapeutic approach to ameliorate NASH disease phenotypes in mice fed methionine and choline deficient (MCD) diet. **Methods:** Male C57BL/6J (wild type, WT) mice, 4 weeks of age, were fed MCD diet or control diet (CD) for 5 weeks. To stabilize MCs, a subset of CD and MCD diet fed mice were given cromolyn sodium (crom, 24 mg/kg) intraperitoneally, daily for two weeks before sacrifice. Body and liver weight, liver tissue, serum, isolated hepatocytes and cholangiocytes were collected from all groups. Hepatic damage and steatosis were evaluated by H&E and Oil-Red O staining in liver sections respectively. MC activation in total liver was evaluated by qPCR for chymase, trypsinase, Fc ϵ R1 α and histidine decarboxylase. Serum histamine (HA) level in all groups were measured by enzyme-linked immunoassay. Trypsinase β immunohistochemistry was performed in liver to detect MC infiltration and CK-19 immunostaining and semi-quantification was performed to evaluate DR. Biliary senescence was evaluated by co-immunofluorescence of CK-19 with p16 and γ H2AX. Serum cytokine levels were analyzed by mouse cytokine array EIA. Fibrosis was evaluated by semi-quantification of Fast Green Sirius Red staining. Inflammation was assessed by F4/80 immunostaining in liver. **Results:** MCD diet induced severe hepatic damage and steatosis in WT mice compared to CD, which was reversed with crom injection. Crom treatment (i) stabilized MC activation and infiltration, and (ii) reduced serum HA level in MCD and CD fed mice compared to the respective controls. MCD diet in WT mice increased (i) DR, (ii) biliary senescence, (iii) inflammation, and (iv) fibrosis compared to CD. Crom treatment reduced all these parameters in the MCD diet fed mice compared to the control. Pro-inflammatory cytokine levels in serum increased in MCD mice compared to CD fed mice and crom treatment reduced these cytokine levels. **Conclusion:** MC infiltration is a critical event in progression of advanced NAFLD/NASH phenotypes that drives biliary senescence, hepatic steatosis, inflammation and fibrosis. Administration of cromolyn sodium may be a therapeutic approach for management of NAFLD/ NASH phenotypes.

Abstract 26

Nrf2 Negatively Regulates CMA to Rescue Macroautophagy Deficiency Induced Liver Dysfunction

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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, to meet metabolic needs and to relief stress. Three main forms of autophagy have been identified: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA), which also affect each other by regulation. CMA, a pathway present in all mammalian cells, cytosolic proteins targeted for degradation are identified by the chaperone HSC70 that recognizes a pentapeptide motif (KFERQ) in their amino acid sequence. These KFERQ motif – substrate proteins are transported to, and docks at the surface of lysosomes via interacting with lysosome-associated membrane protein type 2A (LAMP-2A). Translocation of the substrate across the lysosomal membrane also requires the presence of a luminal form of HSC70 (Lys-HSC70), then substrate proteins are rapidly degraded by the lysosomal enzymes. Different from macroautophagy, CMA does not require the ATG molecules and autophagosomes. **Methods and Results:** Our previous study found that macroautophagy deficiency in livers causes severe hepatomegaly and liver injury,

accompanied by inflammation, fibrosis, and tumorigenesis, which can be rescued by *Nrf2* co-deletion. Thus, we hypothesize that CMA plays a key role during this rescue process. Our data showed that the molecular weight of LAMP-2A increased in both *Atg7* knockout and *Atg7/Nrf2* double knockout livers. Meanwhile, the expression level of LAMP-2A was higher and substrate proteins of CMA, including HSC70, α -B₂ and GAPDH, were lower in *Atg7/Nrf2* double knockout livers than those in *Atg7* knockout livers. In the livers of *Atg7/Nrf2* double knockout mice injected with leupeptin, an inhibitor of lysosomal proteases, the expression of LAMP-2A and Annexin I increased significantly. These data indicate that CMA may be enhanced in *Atg7* knockout livers, which is more significant in livers with *Atg7* and *Nrf2* co-deletion. To further confirm this result, we analyzed by immunoblotting the P10 fraction of the livers, which mainly contain the lysosomes and the mitochondria. The data showed that the molecular weight of LAMP-2A and expression level of HSC70 were altered in lysosomes of both *Atg7* knockout and *Atg7/Nrf2* double knockout livers, compared to that in *Nrf2* knockout livers. The substrate proteins, α -B₂ and GAPDH, also decreased significantly. In hepatic lysosomes of *Atg7/Nrf2* double knockout mice injected with leupeptin, the expression level of LAMP-2A and substrate proteins increased significantly, compared to that in liver lysosomes of *Atg7* knockout mice injected with leupeptin. Then we investigated the role of *Nrf2* on CMA by an *in vitro* model. Upon treatment of AML-12 and Huh-7 cells with CDDO-ME, an activator of *Nrf2*, the expression of LAMP-2A decreased, and that of CMA substrate Annexin I was increased, in a dose dependent manner. Thus, *Nrf2* activation seems to inhibit CMA. Conversely, we found that CMA activity could be enhanced by *Nrf2* knockdown in Huh-7 cells. The *in vitro* data showed that *Nrf2* plays a negatively regulatory role on CMA activity. **Conclusion:** Our results demonstrate that CMA activity in *Atg7* and *Nrf2* co-deletion livers is enhanced, which may contribute to the rescue of the liver dysfunction caused by macroautophagy deficiency.

Abstract 27

Bulk RNA Seq Identifies Activation of Oxidative Stress Regulating Glucose/lipid Metabolism as Potential Mechanism of Increased Glucose Sensitivity Under Conditional Knockdown of HMGB1 in a Type-2 Diabetes Model

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Introduction: Type 2 diabetes (T2D) has become one of the most prevalent diseases in the US. T2D is characterized by hyperglycemia, hyperinsulinemia, and systemic inflammation. High mobility group box 1 (HMGB1) is a pro-inflammatory protein that expresses ubiquitously in most tissues. Our previous work has shown that in a Streptozotocin (STZ) induced T2D mouse model, conditional knockdown of HMGB1 significantly decreased hyperglycemia by increasing glucose sensitivity and regulating mitochondria biogenesis through AKT/FoxO1 signaling. However, the metabolic mechanisms that guide how decreased HMGB1 drives this phenomenon remain unknown. We hypothesize that conditional knockdown of HMGB1 will activate genes that regulate oxidative stress in glucose/lipid metabolism under a STZ induced T2D model. **Methods:** Total RNA was extracted using Trizol (Invitrogen) from liver and muscle of inducible (iHMGB1) KO and HMGB1 flox mice that developed T2D after STZ injection (25 mg/kg) following manufacture instructions. Isolated RNA was then reversely transcribed into cDNA using RNA to cDNA EcoDry Premix (TaKaRa). Key markers of glucose and glycogen metabolism including Glycogen synthase kinase (GSK), Phosphoribulokinase (PRK), Insulin Receptor β (IR- β), Protein kinase B (AKT), Forkhead box protein O1 (Foxo1) were quantified by reverse transcriptase PCR (RT-PCR). Genes were then quantified by RNA sequencing measurement of 3 HMGB1 Flox and 3 iHMGB1 KO liver samples. Differential expression analysis was performed comparing KO vs Flox. We identified 84 up and 25 downregulated genes by FDR=5% and fold-change ≥ 1.5 cutoff. These genes were further served as input for pathway analysis. Significant pathways were defined by FDR=5%. **Results:** Liver RT-PCR showed FoxO1, PRK, and GSK to be downregulated in iHMGB1 KO mice with no significant changes in IR- β and AKT. However, in muscle, RT-PCR suggested a more substantial

decrease in expression of Foxo1, PRK, GSK as well as IR- β and AKT. These findings indicated that muscle appears to be more sensitive to insulin and has an important role in maintaining glucose homeostasis under conditional HMGB1 knockdown. Bulk RNA-seq liver analysis showed that conditional HMGB1 knockdown alters metabolic pathways involved in glucose and lipid metabolism, most significantly by NRF2-mediated Oxidative Stress Response (-log(p-value) = 1.00E+01,) FXR/RXR Activation (-log(p-value) = 8.60E+00) and LXR/RXR Activation (-log(p-value) = 5.00E+00.). **Conclusion:** Results from RT-PCR and bulk RNA Seq analysis suggest that conditional knockdown of HMGB1 primarily alters oxidative stress pathways that have roles in enhancing glucose/lipid homeostasis. Future studies will be aimed at using tissue-specific conditional knockout of HMGB1 to further understand the distinct role that HMGB1 plays in each tissue under T2D phenotype.

Abstract 28

ASBT Vivo-Morpholino Reduces Hepatic Inflammation and Fibrosis and Alters Bile Acid Composition In Cholestatic Mice

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Introduction: Primary Sclerosing Cholangitis (PSC) is characterized by increased ductular reaction (DR), biliary senescence, liver fibrosis, and mast cell (MC) infiltration. *Mdr2*^{-/-} mice, model of PSC, mimic some features of PSC. In PSC there is (i) increased total bile acid (TBA) levels, (ii) dysregulation of BA synthesis and (iii) higher cholangiocyte expression of the apical sodium bile acid transporter (ASBT). Hydrophobic BAs enhance cholestasis and ASBT expression. Ileal inhibition of ASBT has been shown to reduce cholestatic damage in *Mdr2*^{-/-} mice, murine model of PSC. No information exists to demonstrate if blocking hepatic and ileal ASBT inhibits subsequent liver damage in *Mdr2*^{-/-} mice. **Methods:** 12 wk male FVB/NJ (WT) and *Mdr2*^{-/-} mice were treated with control or ASBT Vivo-Morpholino (2 injections, 12.5 mg oligo/kg BW via tail vein for 1 wk). Liver damage was determined by H&E and serum enzyme levels. Ductular reaction (DR) and inflammation were evaluated by immunohistochemistry (IHC) for CK-19 and F4/80 in liver. ASBT expression was measured by immunofluorescence (IF) and IHC in liver and ileum. Hepatic fibrosis was determined by Fast Green/Sirius Red staining and qPCR. BA composition was measured in liver and feces in all groups by LCMS. Fecal samples were collected for microbiome composition analysis. Spheroids containing isolated cholangiocytes from healthy and PSC patients, immortalized mast cells, and immortalized hepatic stellate cells were generated. ASBT expression and histamine secretion were measured in spheroids. **Results:** *Mdr2*^{-/-} mice with Control Vivo-Morpholino have increased large DR, hepatic fibrosis, inflammation, and hepatic TBA levels, which decrease in *Mdr2*^{-/-} ASBT Vivo-Morpholino mice. Inhibition of ASBT in WT and *Mdr2*^{-/-} mice reduced biliary and intestinal ASBT expression and fecal TBA levels. PSC spheroids displayed increased ASBT expression and histamine secretion compared to healthy control. **Conclusion:** Elevated TBA levels seen in PSC lead to altered intestinal BA transport and increased biliary ASBT, which alters the BA pool. Vivo-Morpholino inhibition of hepatic and ileal ASBT blocks hepatic damage and alters BA composition. Further, PSC spheroids show increased ASBT expression and histamine secretion indicating crosstalk between mast cells and damaged cholangiocytes during cholestasis. Total ASBT inhibition may provide a novel therapeutic strategy for the management of PSC.

Abstract 29

MicroRNA Drivers for Regenerative Capacity in Liver Transplantation

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Introduction: Liver diseases, including alcoholic liver disease, nonalcoholic fatty liver disease, hepatitis, hepatocellular carcinoma, and other end-stage chronic liver disease complications, often require liver

transplantation. There is an unmet demand for liver transplantation due to the limited availability of deceased-donor livers with adequate regenerative capacity. Current efforts to address this need involve treatments to improve transplant outcomes and utilize livers with diminished regenerative potential. Hence it is imperative to characterize the regenerative potential of donor livers for maximizing the utilization of available organs. We aim to characterize the microRNA profiles of deceased donor livers selected for transplant to those deemed non-transplantable. Further, the study aims to identify microRNAs that can serve as potential markers of the regeneration potential of the deceased donor livers. **Methods:** For this study, flash-frozen wedge-biopsy samples were received from the Thomas Jefferson surgical team collected under the approved consent to research authorization obtained by the Gift of Life Donor Program. We collected NanoString microRNA profiling data from 798 human microRNAs from each sample and analyzed for differential expression between transplant-accepted and deemed non-transplantable liver biopsies. **Results:** Our results show that the accepted and rejected samples did not form distinct sample groups according to microRNA expression, suggesting that some of the samples at the "borderline" may have similar molecular features between accepted and rejected livers. We predicted a microRNA signature that is likely representative of the regenerative potential of the liver, based on similarities of microRNA expression between donor liver samples and regenerating livers in laboratory animal experiments. **Conclusion:** These results suggest a path towards a molecular biomarker to augment the current evaluation for transplantability and thus increase the potential pool of transplantable livers.

Musculoskeletal Disorders

Abstract 30

Platelet-rich Fibrin (PRF) Accelerates the Healing of Achilles Tendon Defect by Promoting the Proliferation and Activation of Tenocytes via FGFR/AKT and TGF- β /SMAD3 Signaling

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Introduction: For orthopedic surgeons, an Achilles tendon defect is challenging to treat, so developing new treatments is desirable. Platelet-rich fibrin (PRF), dense fibrin scaffold composed of a fibrin matrix containing many growth factors. This study aims to investigate whether PRF accelerates the healing of Achilles tendon injuries and to elucidate further the mechanisms involved. **Methods:** To create a rat model for Achilles tendon defect, a 4-mm portion of the right Achilles tendon was wholly resected and filled in the gap with PRF collected from rat blood. We assessed the healing of injured tendons through histology, motor functional recovery, and biomechanical properties. *In vitro*, we assessed the number of viable or proliferative cells and the migration capacity after treatment of PRF using tenocytes isolated from rat Achilles tendon. The extracellular matrix's protein or gene expression level was evaluated by immunofluorescence staining or PCR. In addition, the phosphorylated protein was evaluated by western blotting. Finally, to examine how PRFs signal to tenocytes, we performed inhibition experiments using AKT, FGFR, TGF- β R, and SMAD3 inhibitors. **Results:** In the rat model for Achilles tendon defects, the number of tenocyte-like cells at the injury sites increased in the PRF group compared to the control group. Furthermore, the PRF group also increased proliferating tenocytes (SCXA+Ki-67+) and activating tenocytes (SCXA+ α -SMA+). Rat in the PRF group had more mature and better-aligned collagen deposits and showed an early increase in blood vessels and lymphatic vessels at the injury sites. Consistent with the histological findings, PRF improved motor function (BBB score) and the biomechanical properties of the injured Achilles tendon. *In vitro*, PRF increased the number of viable and proliferative cells and promoted migratory ability in tenocytes. Also, PRF promoted the maturation of tenocytes and increased the protein and gene expression levels of collagen-I, collagen-III, α -SMA, and tenascin-C. Finally, we examined how PRF transduces the signal to tenocytes. PRF induced the phosphorylation of AKT/ FGFR and the nuclear translocation of AKT. Inhibition of AKT or FGF-receptor suppressed the positive effects of PRF on tenocytes. We further found that PRF promoted the phosphorylation of and the nuclear translocation of SMAD3. Furthermore, inhibition of SMAD3 or TGF- β R suppressed the PRF-induced expression levels of the extracellular matrix, whereas the inhibition of the TGF- β R/SMAD did not affect the proliferative effects. Consistent with *in vitro* data, AKT and SMAD3-positive tenocytes were

increased in the PRF group *in vivo*. **Conclusion:** We clarified that PRF accelerated the healing of Achilles tendon defect by promoting the growth and activation of tenocytes. Furthermore, PRF increases the proliferation ability and extracellular matrix protein expression level in tenocytes via the FGFR/AKT and TGF- β R/SMAD3 axis, respectively.

Neuropathology

Abstract 31

2-(4-aminophenyl)benzothiazole Derivatives Modulate Transthyretin Aggregation

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Introduction: Transthyretin (TTR), a protein produced in the liver, plays a key role in thyroxine and retinol transportation. TTR is prone to generate amyloid deposits and has been causally implicated in two fatal, hereditary conditions: familial amyloid polyneuropathy (FAP) and familial amyloid cardiomyopathy (FAC). To provide a diverse portfolio of therapeutic small molecules, we prepared a set of TTR-interacting compounds: 4-(2-benzothiazolyl)aniline (BTA) and its derivatives containing urea (**1**), thiourea (**2**), and sulfonamide (**3**), and triazole (**4**) linker to prevent the formation of transthyretin oligomers and fibrils. This study aims to define the activity of BTA and its derivatives on TTR aggregation by utilizing biophysical methods and relevant TTR fragment peptides. **Methods:** Thioflavin-T (ThT) fluorescence assay was used to monitor fibril formation with synthetic TTR truncated peptides, TTR₆₁₋₁₂₇ and TTR₁₀₁₋₁₂₅, and other neuropeptides treated with BTA and its derivatives. At the end of a time-course study, fibrils were detected and measured by transmission electron microscopy (TEM). Oligomer formation was monitored with photoreactive cross-linking assay. **Results:** As confirmed by TEM, BTA and its derivatives were able to abrogate fibril formation *via* the ThT fluorescence assay. Moreover, BTA inhibited the aggregation of other prone-to-aggregate proteins, such as alpha-synuclein and islet amyloid polypeptides. By photoreactive cross-linking assay, BTA and compound **2** failed to prevent the formation of TTR fragment oligomers. Among twelve newly prepared compounds, two BTA derivatives abrogated the aggregation of alpha-synuclein and other neuropeptides. Compound **15** demonstrated an antifibrillary effect on TTR₁₀₁₋₁₂₅ truncated peptide. **Conclusions:** This study provides an initial platform to generate more potent inhibitors of prone-to-aggregate neuropeptides including TTR fragment peptides.

Abstract 32

A Pathologic Triangle: Herpesviruses, Autophagy Genes, and Alzheimer's Disease

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Introduction: Since the 1960s, experts have speculated that herpesviruses may be a contributing cause of Alzheimer's disease (AD). Previously, the molecular connections between the amyloid precursor protein (APP) and type 1 herpes simplex virus (HSV-1) have been described. Additionally, others have reported molecular connections between HSV-1 and autophagy using samples from four different brain regions taken from post-mortems of people who had and had not had cognitive impairment before they passed away. In 2018, Readhead et al. (*Neuron* 99.1:64-82) discovered molecular-genetic evidence that HSV-1, HSV-2, HHN6, HHN7, VZV, and CMV activity is associated with AD. The most notable of these was the common virus known as HHN6, which causes a slight pediatric disease. A network of potential AD-associated genes was found using a quantitative trait loci (QTL) technique that corresponded with viral activity and load. These ontology networks did not take autophagy genes (ATG) into account specifically. Our hypothesis is that viral infections interfere with cellular membrane functions, changing autophagic activity. People who have genetic variants that guard against this dynamic would either have lower virus loads or would be less susceptible to cognitive impairment. **Method:** In the beginning, we created a comprehensive list of ATG, including 180 that we specifically found through machine learning, using data collected from literature and websites. We used software created by Readhead and made available through Synapse.com to sequence post-mortem brain data taken from publications and open websites provided by brain

banks run by the Alzheimer's Center. Next, we used data mining techniques to search the list of QTL that were associated with elevated viral load and activity for ATG across 300+ brains from the Nun's Study and Mount Sinai Brain Banks. Finally, we compared the expression levels of these ATG in control and preclinical AD with those ATG-associated QTL. From published data, we determined the ATG expression levels that were associated with pre-clinical AD or non-AD and no dementia. When compared to non-AD controls in pre-clinical studies, almost all ATG were downregulated. We discovered a correlation between single nucleotide polymorphisms linked to higher viral load and lower expression of certain ATG in AD. **Results:** Firstly, a comprehensive list of more than 9800 ATG. Moreover, 8 ATG from the comprehensive list have been proposed to support the idea that autophagy is a unique mechanism linking herpesvirus to AD, which may help identify new targets for diagnostic and therapeutic interventions. **Conclusion:** Finding genetic susceptibilities to the survival and advancement of the herpesvirus may be crucial for the future prevention of adult AD because HHV6 infects a huge population throughout childhood.

Abstract 33

Reconfiguration of Adult Brain Responses to Threat After Early Life Adversity

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Introduction: Early life adversity (ELA) correlates with vulnerability to mental disorders later in life. Effects of psychotropic drugs, both pharmacotherapies and drugs of abuse, point to the noradrenergic system (NS) as a contributing factor. How ELA impacts the brain to heighten risk and what roles NS plays, largely remain a mystery. Longitudinal manganese-enhanced magnetic resonance imaging (MEMRI) provides a methodology to investigate impacts of ELA on neural activity in awake-behaving animals. **Methods:** Here, newborn mice are exposed to ELA, fragmented maternal care, then brain activity studied in adulthood. For ELA, dams were deprived of adequate bedding from P2-P9. Separate cohorts with and without ELA were allowed to age to 10 weeks (n=24) in normal housing and then subjected to longitudinal MRI paired with video recordings captured before, immediately and longer times after exposure to acute threat (TMT, 2,3,5-Trimethyl-3-thiazoline). Mn(II) delivered systemically (0.3 mmol/kg) enters neurons via voltage-gated calcium channels. Accumulated Mn(II) thus reports on neural activity retrospectively in T1-weighted MRI. Mice activity is video recorded in our custom arena during Mn(II) accumulation. At conclusion of our longitudinal imaging timeline, mice are sacrificed, brains perfusion fixed, and serial sections stained by immunohistochemistry. MEMRI images were skull-stripped, spatially co-registered, and intensity normalized prior to statistical parametric mapping (SPM), segmentation, and network analysis. Noldus Ethovision XT 15 was used to analyze activity recordings. **Results:** ELA mice showed increased motility and reduced inter-individual variability in baseline exploration. Defensive-avoidance behavior increased for both groups with TMT, attesting to the expected effect of threat. SPM of 3D brain images found heightened neural activity of ELA mice in ventral pallidum prior to threat, in hindbrain nuclei immediately after threat, and in hypothalamus and ventral hippocampus a week later. Additionally, ELA exposure decreased Mn(II)-enhancement in prefrontal cortex and medial thalamic nuclei throughout the timeline as compared to non-ELA mice. Network analysis revealed a decrease of coordinated neural activity between many brain regions and shifts in network structure. Staining for norepinephrine transporter suggested a breakdown of distal tiling in various brain regions. **Conclusions:** Our data find that ELA heightens neural activity of various brain regions basally and in responses to stimuli like predator odor, an ethological threat. Consistently low prefrontal cortical and thalamic activity in ELA mice across the timeline suggests influential roles for these regions in altered brain states after ELA. Disruption of NS anatomy might partially explain these shifts in brain activity and regional coordination after ELA.

Ocular Pathobiology

Abstract 34

Mitochondrial Transplantation as a Novel Inducer of Retinal Pigment Epithelium Epithelial-Mesenchymal-Transition and Fibrosis

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Introduction: Age-related macular degeneration (AMD) is the leading cause of loss of vision of aging populations in developed countries worldwide. Late-stage progression of AMD is characterized by subretinal fibrosis driven by epithelial-mesenchymal-transition (EMT). During EMT, the retinal pigment epithelium (RPE) - the primary support tissue for photoreceptors - lose their epithelialization and transition into a mesenchymal phenotype. This alteration leads to loss of tight-junction proteins, such as E-cadherin, and increased invasiveness of the cells, ultimately resulting in geographic atrophy of the RPE and overlying retina. Further, it is well-reported that production of metalloproteinases in late-stage AMD induces fibrosis causing irreversible vision loss. Recently, mitochondrial transfer has emerged as a critical component in disease pathogenesis such as AMD. Though EMT and fibrosis in AMD has been well-characterized, the role of isolated mitochondria transferred between RPE has never been explored. **Methods:** Here, we characterize the role of isolated mitochondrial transfer as a novel mechanism of metabolic crosstalk and disease induction in the RPE tissue. Cytokine induced-diseased mitochondria were isolated from RPE cells treated with AMD-associated cytokine TGFβ2 (10 ng/mL), administered to recipient RPE cells and the effects of Mito_{TGFβ2} were compared to control Mito_{Ctrl}, purified from healthy, untreated RPE, or positive control directly treated TGFβ2. **Results:** We showed that treatment of healthy RPE with Mito_{TGFβ2}, and not Mito_{Ctrl} induces disruption of the mitochondrial network, reminiscent of the direct effect of TGFβ2 treatment. ELISA assay validated no direct TGFβ2 treatment was carried into the purified mitochondrial fraction. Both assessment of genetic profile via qPCR and bioenergetics via Seahorse XFe96 bioanalyzer validated the ability of Mito_{TGFβ2} to mirror the effects of TGFβ2-induced EMT and fibrosis through suppression of mitochondrial respiration and upregulation of fibronectin 1 (FN1). **Conclusions:** Our results elucidated that mitochondrial transfer induces a context-specific phenocopy that recapitulates the phenotype of donor to host RPE cells. This novel model of disease pathogenesis in RPE may provide insight to the presently nebulous centrifugal expansion of geographic atrophy of RPE in AMD and may provide promising insights to the development of therapeutics for EMT and fibrosis-driven disorders such as in late-stage AMD.

Abstract 35

Protective Role of Resveratrol Against TNFα-induced Inflammation and Mitochondrial Dysfunction in Retinal Pigment Epithelial Cells

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Introduction: Inflammation and oxidative stress are key drivers of retinal pigment epithelium (RPE) dysfunction in the pathogenesis of age-related macular degeneration (AMD), a leading cause of irreversible blindness globally. Tumor necrosis factor-α (TNFα), a pro-inflammatory cytokine involved in AMD, induces defects in mitochondrial health and function in the RPE. Sirtuins, a family of enzymes involved in maintaining metabolic homeostasis, mitochondrial function and longevity, are downregulated in aged RPE cells and AMD retinal specimens. Here we investigated the efficacy of resveratrol, a potent activator of sirtuin1 (SIRT1), in suppressing TNFα-induced inflammation, metabolic dysfunction and oxidative stress in RPE. **Methods:** Matured primary human RPE (H-RPE) cells were treated with TNFα (10 ng/ml) and/or resveratrol (50 μM) or DMSO at equal volume as the vehicle control. Oxidative phosphorylation (OXPHOS) and glycolytic metabolic profiles were determined by the Seahorse XFe96 Mito Stress Test and Glycolytic Stress Test, respectively. Gene expression of metabolic and inflammatory markers was assessed using qPCR. Interleukin-6 (IL-6) secretion was quantified by enzyme-linked immunosorbent assay (ELISA). Intracellular levels of reactive oxygen

species (ROS) were measured using CellROX green reagent. **Results:** TNF α induced elongation and loss of the regular cuboidal cobblestone morphology of H-RPE cells. Concurrent treatment with resveratrol blocked TNF α -induced H-RPE morphological changes. TNF α robustly upregulated IL-6 levels in H-RPE conditioned media with a 19-fold increase while concurrent treatment with resveratrol significantly suppressed TNF α -induced IL-6 secretion by almost 50% to a 10-fold decrease. Moreover, resveratrol suppressed TNF α -induced transcriptional upregulation of proinflammatory genes (IL-6, IL-8, TLR2, and MCP-1) and metabolic genes (ENO1, PFKFB3, HK2). Bioenergetic profiling using the Seahorse Xfe96 showed enhanced maximal mitochondrial respiration, spare respiratory capacity, and basal glycolysis levels in H-RPE treated with resveratrol. While co-treatment with resveratrol and TNF α further increased oxygen consumption rate, resveratrol suppressed TNF α -dependent accumulation induction of the ROS-producing NADPH oxidase NOX4. The protective effect of resveratrol on TNF α was further validated by evidenced reduction of TNF α -dependent accumulation of cytoplasmic ROS. **Conclusions:** RPE cells are profoundly affected by the pro-inflammatory cytokine TNF α . TNF α not only disrupts the structural epithelial morphology of H-RPE cells, it also causes dysfunction of mitochondria and metabolism. Treatment with the natural organic compound, resveratrol, efficiently blocks TNF α -induced proinflammatory activation and bioenergetic reprogramming of RPE. These results reveal a critical interplay between inflammation and metabolic dysfunction in RPE, identifying resveratrol as a potential drug against AMD progression.

Abstract 36

Endomucin Knockout Mice Show Delayed Retinal Vascular Development and Reduced Neovascularization in Oxygen-induced Retinopathy

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Introduction: Endomucin (EMCN), a specific endothelial glycoprotein, has been shown to play a significant role in regulating VEGF-induced VEGFR2 internalization and downstream activities *in vitro*. EMCN knockdown inhibits VEGF165-induced VEGFR2 clathrin-mediated endocytosis and endothelial proliferation, migration, and tube formation. The goal of this study is to characterize the functional role of EMCN *in vivo* normal on retinal vascular development and pathological neovascularization in EMCN knockout mice. **Methods:** Homozygous EMCN knock-out (EMCN^{-/-}) mice were generated by crossing EMCN-floxed mice with the ROSA26-Cre. We collected the eyes from adult EMCN^{-/-} mice (8–16 weeks) and their EMCN^{+/+} control littermates. The retinas and RPE/choroids complex were dissected for RNA extraction and qPCR to measure gene expression. Isolectin-B4 (IB4) was used to stain the retinal vasculature of adult mice (12–16 weeks old) and P5 mice on retinal flat mounts. In the oxygen-induced retinopathy (OIR) model, P7 mice were housed in 75 percent oxygen for five days and then switched back to room air at P12. Eyes were collected at P12 and P17, and the retinal vasculature was stained using IB4, then the avascular and neovascular areas were quantified using photoshop. **Results:** The mRNA level of EMCN in both retinas and RPE/choroids from the EMCN^{-/-} mice was undetectable (n>4, p<0.0001) by qPCR, compared to EMCN^{+/+} mice. The retinal vascular area/retina area ratio at P5 was significantly reduced in the EMCN^{-/-} pups compared to EMCN^{+/+} controls (0.14 ± 0.01 vs 0.2 ± 0.013, p<0.0001, n>10 for both groups). Adult (2-month-old) retinal vascular density remained lower in the EMCN^{-/-} mice compared to EMCN^{+/+} mice (0.135 ± 0.015 vs 0.154 ± 0.017, p<0.05, n=13). In the OIR model, the avascular area P12 shows no significant difference between EMCN^{-/-} and EMCN^{+/+} mice (24.57 ± 1.4% vs 23.18 ± 1.0%, p=0.9, n>6). However, pathological neovascularization at P17 was significantly reduced in the EMCN^{-/-} mice compared to EMCN^{+/+} (8.98 ± 2.9% vs 11.98 ± 1.4%, p<0.05, n>10) while the avascular area at P17 was comparable between the EMCN^{-/-} and controls (9.9 ± 1.5% vs 8.85 ± 1.0%, p>0.5, n>10). **Conclusion:** Deletion of EMCN gene reduces retinal vascularization. As a crucial regulator of retinal angiogenesis under both normal development and pathological conditions, EMCN represents a novel therapeutic target for ocular diseases characterized by abnormal growth of blood vessels.