ABSTRACTS

SYMPOSIUM 1: THE IMMUNE MICROENVIRONMENT IN LIVER DISEASE PROGRESSION AND RESOLUTION

#1. THE ROLE OF ALCOHOL-INDUCED HEPATOCYTE EPIGENETIC CHANGES IN ALCOHOL-ASSOCIATED LIVER DISEASE RESOLUTION.

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Abstinence is an important therapeutic intervention for patients with ALD. However, fibrosis improvement after cessation is not uniform and some patients progress to cirrhosis even while abstinent. We aimed to study the epigenetic mechanisms of ALD resolution. **Results:** We showed that alcohol induced liver steatosis rapidly resolved after alcohol cessation. In contrast, fibrosis persisted in the liver for up to 8 weeks after the end of alcohol exposure. Defects in fibrosis resolution were in part due to alcohol-induced KDM5B and KDM5C-dependent epigenetic changes in hepatocytes. We found that alcohol induced Kdm5b and Kdm5c activation in hepatocytes in IL-17A - C/EBPβ-dependent manner and resulted in persistent H3K4 methylation changes in hepatocytes. We found that AAV mediated knockout of KDM5B, KDM5C or C/EBPβ in hepatocytes at the time of alcohol withdrawal promoted fibrosis resolution. scATAC-seq analysis showed that during ALD resolution there are unique epigenetic cell states distinct from both control and alcohol states and identified associated transcriptional regulators including LXRα. In vitro and in vivo analysis confirmed that knockout of KDM5B and KDM5C demethylases promoted LXRα activity, through regulation of 27-hydroxycholesterol biosynthesis, and this activity was critical for the fibrosis resolution process. Reduced LXR activity by small molecule inhibitors prevented fibrosis resolution in KDM5-deficient mice. In patients with ALD, serum 27-hydroxycholesterol levels negatively correlated with disease severity and transplant-free survival, suggesting that this mechanism is relevant in humans. **Conclusion:** In summary, KDM5 demethylases prevent liver fibrosis resolution after alcohol cessation in part through suppression of LXR activity.

#2. LIVER MACROPHAGE CHANGES INDUCED BY CESSATION OF ALCOHOL DRINKING IN A MOUSE MODEL OF ALCOHOL-ASSOCIATED LIVER DISEASE.

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During the development of alcohol-associated liver disease (ALD), embryonic Kupffer cells (emKCs) are partially replaced by several subtypes of monocyte-derived KCs (moKCs) and novel non-KC macrophages (IMs) appear in the liver as well. However, how KCs and IMs change during the resolution phase of alcohol-associated liver disease (ALD) is unknown. The aim of this study was to determine the changes in both KCs and IMs that occur upon the cessation of alcohol consumption. Mice were fed a high fat "western diet" (WD) with 10%-20% alcohol in the drinking water for 16 weeks (WDA

model). These mice developed steatohepatitis with zone 3 pericelluar fibrosis (Brunt stages 1A, 1B or 2). At the end of WDA feeding, KCs consisted of CD163+ emKC and several subtypes of CD163- moKCs. Mice were then suddenly changed to a chow diet without alcohol and the time course of histological resolution and macrophage population changes were determined. Fat resolved quickly but fibrosis did not appreciably resolve by 4 weeks. There was an increase in moKC by one week and large numbers of macrophage crown-like structures formed surrounding the remaining lipid droplets. Selective diphtheria toxin mediated ablation of KCs during the recovery phase increased the numbers of crown-like structures and caused fibrosis to increase without affecting steatosis resolution. In conclusion, KC and IM populations change rapidly during the process of alcohol cessation with an increase in moKCs. KCs appear to play a role in limiting fibrosis while non-KC macrophages form crown-like structures associated with steatosis resolution.

#3. RESOLVIN D1 MITIGATES INFLAMMATION AND APOPTOSIS IN MASH AND LEADS TO HEPATIC FIBROSIS RESOLUTION.

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Background: Resolvin D1 (RvD1), a DHA-derived specialized pro-resolving mediator, is defective in metabolic dysfunction-associated steatohepatitis (MASH), but its role in MASH is not well understood. Our study aims to determine the effect of RvD1 on MASH and the underlying mechanism. **Methods:** RvD1 in MASH livers was detected by LC-MS/MS. Bulk-/sc-RNA-seq were performed in MASH and MASH+RvD1 mouse livers. In vitro experimentation was conducted in bone marrow-derived macrophages (BMDMs), primary hepatocytes, and T cells. **Results:** Liver levels of RvD1 were decreased in MASH-patients and -mice, likely due to an increase of pro-inflammatory M1-like macrophages that have compromised capacity to produce RvD1. MASH-induced liver inflammation, apoptosis, and fibrosis were attenuated in RvD1-treated mice, assessed by histology and qPCR. Reduced pro-inflammatory cytokine gene-expression (Cxcl10, Ccl2, Infg) and down-regulation of inflammatory response pathway were observed by bulk-RNA-seq in MASH+RvD1 livers. In vivo and in vitro, RvD1 decreased MASH-induced Cxcl10 in macrophages by preventing T cell activation. MASH+RvD1 livers showed lower hepatocyte apoptosis, evidenced by reduced cleaved-caspase-3 and TUNEL+cells, by preventing ROS/ER-stress. We studied downstream effects of RvD1-mediated reduction in inflammation and apoptosis on MASH using scRNA-seq and found down-regulation of inflammatory pathway in liver macrophages and less T-cell/HSC interaction in MASH+RvD1 livers. Moreover, we noted a corresponding increase of RvD1-induced fibrosis resolving metalloproteinases: Mmp2 in HSCs; Mmp9 and Mmp12 in macrophages. **Conclusion:** RvD1 reduced inflammation by modulating T-cell/macrophage-crosstalk without compromising host defense and mitigated ER stress-induced apoptosis, leading to resolution of liver fibrosis. Thus, RvD1 represents a novel anti-fibrotic therapy in MASH.

#4. THE GENOME STRUCTURE OF CLUSTERED IMMUNE GENES IN MONOCYTES IN ALCOHOL-ASSOCIATED HEPATITIS.

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Many inflammatory genes in the immune system are clustered in the genome. The genome structure of these clustered genes likely plays a critical role in their regulation and contribute to diseases where inflammation is poorly controlled. Alcohol-associated hepatitis (AH) is a severe inflammatory disease that contributes significantly to morbidity in alcohol-associated liver disease. Monocytes in AH are hyper-responsive to inflammatory stimuli and contribute significantly to systemic and liver inflammation. Using single-cell RNA-seq (scRNA-seq), we found that clustered immune genes, like the CXC-chemokines, have highly coordinated expression in response to lipopolysaccharide (LPS), and the correlation is higher in AH. Here, we use high throughput chromatin conformation capture (Hi-C) technology to better understand how genome structure is altered in AH and the effect on expression of clustered genes. We performed Hi-C on monocytes isolated from 4 AH patients and 4 healthy controls. Using an algorithm to assess the similarity of a chromosome between two individuals, we found AH and healthy controls were significantly dissimilar from each other. Looking at immune gene clusters, AH monocytes had a combination of altered chromatin loops, movement between topologically associated domains (TADs), and changes in long range interactions. Finally, we compare these results to scRNA-seq data from patients with AH challenged with LPS to predict how chromatin conformation impacts transcription of clustered immune genes. Together, these results reveal changes in the chromatin structure of monocytes from AH patients that perturb expression of highly clustered proinflammatory genes.

#5. DISSECTING THE MECHANISMS SHAPING LIVER MACROPHAGES IN METABOLIC LIVER DISEASE.

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Metabolic dysfunction-associated steatohepatitis (MASH), previously known as NASH (Non-alcoholic steatohepatitis), represents a severe stage of steatotic liver disease characterized by hepatocyte injury, inflammation, and liver fibrosis. Innate immune cells, such as macrophages play an important role in disease pathogenesis. Recent work has demonstrated that TREM2+ NASH-associated macrophages (NAMs) are metabolically active and contribute to disease progression. Despite this, the mechanisms underlying NAM induction and their role in MASH pathogenesis remain poorly defined. We recently identified TGFBR1 as a NAM-enriched membrane receptor that stimulates NAM gene expression. Here we generated myeloid-specific Tgfbr1 knockout mice and demonstrated TGF-β signaling deficiency in macrophages exacerbated diet-induced MASH pathologies, including hepatocyte injury and liver fibrosis, and promoted MASH-

associated hepatocellular carcinoma (MASH-HCC). Mechanistically, liver macrophages lacking Tgfbr1 exhibited decreased NAM signatures, but elevated expression of NOD-like receptor pathway components, and pro-inflammatory cytokines. In MASH livers, flow cytometry and single-nucleus RNA sequencing revealed a shift of macrophage populations characterized by diminished NAMs, increased Kupffer cells, and emergence of a new macrophage population. Together, these studies uncover TGF-β-TGFBR1 as a crucial upstream signal within the liver microenvironment that shapes macrophage heterogeneity and inflammatory properties during diet-induced MASH.

#6. LIVER INJURY UPREGULATES MACROPHAGE RECEPTOR WITH COLLAGEN STRUCTURE (MARCO) IN A MACROPHAGE SUBSET.

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Background. The scavenger receptor MARCO (Macrophage Receptor with Collagen Structure) is expressed on healthy tissue-resident macrophages. While macrophages can exhibit anti- or pro-inflammatory properties and modulate inflammation, the role of MARCO in liver macrophages during fibrosis remains unknown. This study aims to analyze the expression of MARCO in response to liver injury and its role during liver fibrosis. **Methods.** Single-cell RNA sequencing, immunostaining, western blot, and qPCR were performed on livers from carbon tetrachloride (CCl4)-treated mice or livers from lipopolysaccharide (LPS) injected mice. In vitro functional studies were conducted on RAW264.7 cell line and Bone Marrow Derived Macrophages. **Results.** Single-cell RNA sequencing data revealed two macrophage subsets distinguishable by MARCO expression. MARCO was upregulated in CCL4 mice (Log2FC 3.6, p=3.55e-17). Immunostaining demonstrated increase in MARCO-expressing macrophages in CCL4-treated mice within the non-fibrotic areas. Similarly, MARCO was upregulated in livers of LPS treated mice, showed by flow cytometry and immunostaining. In vitro, overexpression of Marco showed downregulation of pro-inflammatory genes (Tnf, FC 0.46, p=0.02; Ccl5, FC 0.4, p=0.03; Cxcr3, FC 0.41, p=0.05), upregulation of metalloproteinases (Mmp2, FC 149, p=0.02; Mmp3, FC 57, p=0.03; Mmp12, FC 6.03, p=0.003), and functional differences like reduced migration capacity (FC 0.025, p<0.0001) and higher phagocytic capacity (FC 1.87, p<0.0001) suggesting a protective role for MARCO. **Conclusion.** MARCO-expressing macrophages are upregulated in response to chronic and acute liver injury. Marco-positive macrophages phenotypic characteristics suggest immunomodulatory effects. Ongoing studies are investigating its role during liver inflammation as well as therapeutic potential of MARCO macrophages for liver diseases.

#7. A DEFICIENCY OF OSTEOPONTIN IN HEPATOCYTES AND BILIARY EPITHELIAL CELLS ACCELERATES BILIARY ATRESIA PROGRESSION.

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Background: Biliary atresia (BA) is a severe neonatal cholangiopathy that leads to cholestasis and progressive hepatic failure. The limited understanding of BA's onset and progression results in compromised transplant-free survival. Osteopontin (OPN), encoded by the secreted phosphoprotein-1 (SPP1) gene and highly expressed in biliary epithelial cells (BECs), plays a role in chronic liver disease. However, its involvement in BA remains unclear. Our study aims to investigate OPN's role in BA's onset and progression. **Methods:** We analyzed *SPP1* gene expression in BA patients using publicly available datasets of single-cell RNA sequencing (scRNA-seq), RNA sequencing (RNA-seq), and microarrays. We examined OPN protein expression in BA livers by immunohistochemistry and measured serum OPN concentration by ELISA. To induce BA in mice, we injected newborn BALB/c pups and pups with hepatocyte and BEC-specific OPN depletion with rhesus rotavirus (RRV) within 24 hours of birth. We monitored BA symptoms post-RRV inoculation and sacrificed the mice on day 11. We evaluated growth rate, serum alanine aminotransaminase activity, total bilirubin level, and histopathological changes. **Results:** in humans, BECs predominantly express *SPP1* in healthy individuals, with increased expression in cirrhosis cases. *SPP1* gene expression is notably higher in BA patients' livers than those with non-BA cholestatic liver diseases or control pediatric liver donors (GSE122340, GSE46960). *SPP1* gene expression correlates strongly with BEC markers KRT7, KRT19, and MMP7. BA patients with fibrosis show higher *SPP1* expression than those with only inflammation (GSE15235). OPN expression is prominent in BA patients' liver tissue. Serum OPN levels are significantly higher in BA patients than those with other cholestatic liver diseases. BALB/c mice with BA exhibit delayed development, jaundice, and acholic stools, alongside increased serum alanine aminotransaminase activity and total bilirubin level. Histological analysis by H&E reveals extensive ductular reaction and hepatic inflammation. OPN levels in serum and urine and hepatic OPN expression are significantly elevated in BA mice compared to controls. Mice lacking *Spp1* in hepatocytes and BECs showed higher transaminase activity, reduced body weight, increased inflammatory response, and lower survival rates than control littermates. **Conclusions:** *SPP1* gene expression in the liver and serum OPN levels distinguish BA from other cholestatic liver diseases. OPN is abundantly present in the serum, urine, and liver tissue in the mouse model of BA. *SPP1* depletion from hepatocytes and BECs leads to BA progression.

#8. GUARDIANS OF GRAFTS: UNVEILING THE PROTECTIVE ROLE OF RECIPIENT NEUTROPHIL-DERIVED HMGB1 IN EARLY ALLOGRAFT DYSFUNCTION AFTER LIVER TRANSPLANTATION.

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Background: Early allograft dysfunction (EAD) is a severe event that leads to graft failure after a liver transplant (LT). Extracellular high-mobility group box-1 (HMGB1) is a damage-associated molecular pattern (DAMP) that contributes to hepatic ischemia-

reperfusion injury (IRI). However, the contribution of intracellular HMGB1 to LT graft injury remains elusive. Our aim was to investigate the role of intracellular HMGB1 in hepatocytes and myeloid cells in post-LT EAD. **Methods:** We generated mice with conditional ablation or overexpression of *Hmgb1* in hepatocytes, myeloid cells, or both. We performed LTs and injected lipopolysaccharide (LPS) to evaluate the effect of intracellular HMGB1 on EAD. RNA sequencing was performed on LPS-treated neutrophils to elucidate the mechanisms. **Results:** Ablation of *Hmgb1* in hepatocytes and myeloid cells of donors and recipients exacerbated early allograft injury after LT. Ablation of *Hmgb1* from liver grafts did not affect graft injury; however, lacking *Hmgb1* from recipient myeloid cells increased reactive oxygen species (ROS) and inflammation in liver grafts, exacerbating injury. Neutrophils lacking *Hmgb1* were more activated, showed enhanced pro-oxidant and pro-inflammatory signatures, and reduced biosynthesis and metabolism of inositol polyphosphates (InsPs). Other myeloid cells did not play a distinct role when *Hmgb1* was ablated. Upon LT reperfusion or LPS treatment, there was significant neutrophil mobilization and infiltration into the liver, with enhanced production of ROS and pro-inflammatory cytokines when intracellular *Hmgb1* was absent. Depletion of neutrophils using anti-Ly6G antibody (Ab) attenuated graft injury in recipients with myeloid cell *Hmgb1* ablation. **Conclusions:** neutrophil HMGB1 is essential in regulating their activation, limiting the production of ROS and pro-inflammatory cytokines, and protecting from early liver allograft injury.

#9. MYELOID CELL-DERIVED OSTEOPONTIN AMELIORATES ALCOHOL-INDUCED LIVER INJURY IN MICE BY REDUCING INFLAMMATION.

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Aim: Alcohol use disorder (AUD) often leads to alcohol-associated liver disease (AALD). This condition starts as hepatic steatosis and can progress to alcoholic steatohepatitis (ASH). Present treatments are limited, including corticosteroid therapy and, for severe cases, liver transplantation. Osteopontin (*SPP1*), a matricellular protein, has been found to mitigate hepatic steatosis in AALD by reducing liver inflammation and intestinal permeability. This study explores the impact of OPN produced by myeloid cells on AALD. **Methods:** We analyzed the human alcoholic hepatitis dataset (GSE135285) for OPN expression. To simulate early AALD, we fed *Spp1*$^{KI\ Mye}$, *Spp1*$^{\Delta Mye}$, and wild-type (WT) mice either control or ethanol Lieber-DeCarli (LDC) diet for six weeks. We then evaluated liver histopathology and serum markers of liver injury (AST & ALT). We measured serum and hepatic levels of triglycerides, cholesterol, and TBARS to determine the severity of steatosis and oxidative stress. **Results:** Increased expression of OPN in hepatic myeloid cells in human ASH was confirmed by immunohistochemistry (IHC). *Spp1*$^{\Delta Mye}$ mice, when fed with ethanol, exhibited significantly higher levels of hepatic steatosis and inflammation compared to *Spp1*$^{KI\ Mye}$ mice. The latter showed fewer pathological changes. Serum oxidative stress markers were also lower in *Spp1*$^{KI\ Mye}$ mice on an ethanol diet than in

Spp1$^{\Delta Mye}$ and WT mice. **Conclusion:** overexpression of myeloid cell-derived OPN offers protection against developing AALD by reducing liver inflammation.

#10. ROLE OF MYELOID CELL-DERIVED HMGB1 IN THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA.

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Background: High mobility group box 1 (HMGB1) is a non-histone chromatin-associated protein involved in the pathogenesis of chronic liver disease. HMGB1 is expressed in myeloid cells, including conventional dendritic cells (cDC) and tumor-associated macrophages (TAMs), which play a major role in the tumor microenvironment. However, whether intracellular myeloid cell-derived HMGB1 is involved in HCC is unknown. Our hypothesis is that intracellular HMGB1 drives cDC maturation towards LAMP3+ DCs and decreases immunosuppressive TAMs in the tumor, hence allowing effective cytotoxic CD8+ T cell responses to reduce HCC. **Methods:** We analyzed publicly available scRNA-seq datasets from human HCC for the expression of HMGB1 in all subsets of DCs and TAMs in HCC tumor and non-tumor tissue and in hepatic draining lymph nodes (dLNs). We generated mice with conditional ablation or overexpression of *Hmgb1* in myeloid cells (*Hmgb1*$^{\Delta Mye}$ and *Hmgb1*$^{KI\ Mye}$). To induce HCC, 14-day-old male mice were injected i.p. with diethylnitrosamine (DEN) and were sacrificed at 5, 6, 8, and 10 months. Histopathological analysis of livers was performed using H&E staining. Liver tumor and non-tumor tissues were analyzed for the immune cell populations using flowcytometry. TAMs and angiogenic markers were measured by Immunohistochemistry and gene expression analysis. **Results:** Mature LAMP3+ DCs increase in human and mouse HCC tumor tissue and hepatic dLNs. TAMs, with low expression of HMGB1, are proangiogenic and immunosuppressive and increase in human and mouse HCC tumor tissue. *Hmgb1*$^{KI\ Mye}$ mice are protected from HCC, whereas control mice develop HCC after 8 months and *Hmgb1*$^{\Delta Mye}$ mice start developing HCC at 5 months. Macroscopic analysis and H&E staining of the livers from *Hmgb1*$^{\Delta Mye}$ mice show more tumors and higher tumor volume than control and *Hmgb1*$^{KI\ Mye}$ mice. Immunohistochemistry of HCC tumor sections reveals that *Hmgb1*$^{\Delta Mye}$ mice have increased infiltration of TAMs. Analysis of immune cell populations by flow cytometry shows that *Hmgb1*$^{\Delta Mye}$ mice have less mature LAMP3+ DCs in liver and hepatic dLNs compared to control and *Hmgb1*$^{KI\ Mye}$ mice, suggesting less CD8+ T cell activation. In addition, there is enhanced CD8+ T cell apoptosis in the HCC tumor tissue from *Hmgb1*$^{\Delta Mye}$ mice. **Conclusion:** ablation of myeloid-derived HMGB1 accelerates HCC development in mice. Therefore, increasing HMGB1 expression in specific myeloid cell subsets (cDCs and TAMs) could be a therapeutic approach to protect from HCC.

#11. STING-MEDIATED NEUTROPHIL INFILTRATION PROMOTES INFLAMMATION IN PRIMARY SCLEROSING CHOLANGITIS.

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Background: Primary Sclerosing Cholangitis (PSC) is an immune-mediated cholestatic liver disease. The inflammatory milieu observed in PSC recruits immune cells, including neutrophils, to infiltrate the peri-biliary region. The cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS-STING) signaling pathway is a key mediator of inflammatory gene expression in response to cellular stress but its role in PSC is unclear. Our goal is to investigate the role of STING in neutrophil homing to the biliary tree and the downstream signaling. **Results:** Immunofluorescence (IF) on human PSC liver tissues and mouse models of PSC (3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC)-fed mice and Mdr2-/- mice) revealed increased presence of peri-biliary neutrophils compared to controls (6.7±1.74-fold, $p<0.0005$, $n=5$). Congruently, oxidative stress was also increased in these liver tissues as marked by 8-OHDG immunohistochemistry. RNA-sequencing on primary cholangiocytes isolated from diseased mice demonstrated genes enriched in proinflammatory signaling, neutrophil chemotaxis, and degranulation. IF on PSC liver tissues and organoids derived from diseased mice cholangiocytes showed an increase in the DNA damage marker, 53BP1, compared to controls. We also observed activation of the cGAS-STING pathway by IF for phospho-STING. Cholangiocytes treated with LPS with genetic or pharmacologic intervention for STING showed reduced proinflammatory chemokine expression. Consequently, increased neutrophil chemotaxis was observed in the presence of LPS-treated cholangiocyte media, which was attenuated with STING inhibition. **Conclusion:** Our findings suggest that cGAS-STING pathway activation in cholestatic liver disease triggers an immune response resulting in increased expression of proinflammatory chemokines. This induces peri-biliary neutrophil infiltration which propagates oxidative stress and perpetuates the biliary inflammation in PSC.

#12. THERAPEUTIC TARGETING OF T CELL CLONAL EXPANSION FOR THE PREVENTION OF MASH.

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Background and Aims: A key feature of metabolic dysfunction-associated steatohepatitis (MASH) is the accumulation of T cells in the liver, however, the cause and consequences of T cell accumulation in the liver remain undefined. Here we aimed to test the hypothesis that antigen-driven clonal expansion is the mechanism by which T cells are accumulating in the liver during MASH. **Approach and Results:** Using single cell sequencing and multiplexed imaging technology we discovered that both MASH induced cirrhosis in humans and diet-induced MASH in mice resulted in the accumulation of activated and clonally expanded T cells in the liver. Furthermore, using unbiased antigen discovery. we identified novel antigens that stimulate clonally expanded T cells found in the liver of mice with MASH. Importantly, immunization of obese mice with these stimulatory peptides enhanced MASH pathology. Finally, when investigating the cellular interactions leading to T cell clonal expansion in MASH, we found that depletion of

conventional dendritic cell subset 1 (cDC1), the primary subset of DCs that induces CD8+ T cell clonal expansion through antigen presentation, resulted in decreased T cell accumulation in the liver and reduced fibrosis. **Conclusions:** Overall, our data provides exhaustive documentation that during progression of MASH, T cells undergo antigen-dependent T cell clonal expansion and implicate an active role for T cell clonal expansion in disease progression. Furthermore, disruption of DC:T cell interactions prevents the progression of MASH. These studies could lead to identification of potential targets to manipulate antigen-specific T cell responses in the setting of human MASH.

#13. EXERCISE THERAPY DRIVES METABOLIC BENEFITS ON MASH VIA MODULATING HEPATIC REGULATORY T-CELLS.

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Background: Metabolic dysfunction-associated steatohepatitis (MASH) is one of the leading causes of chronic liver diseases worldwide. While exercise therapy is known to alleviate MASH, the mechanisms remain unclear. Regulatory T cells (Tregs) play a crucial role in modulating inflammatory responses and immune suppression. However, Treg involvement in exercise-mediated MASH improvement requires further exploration. **Methods:** Male 6-week-old mice were fed a Western Diet (WD) for 26 weeks, with normal diet (ND) fed age-matched mice as controls. Both groups were to sedentariness (Sed) or a 4-week treadmill running regimen (Ex). Whole-body metabolism changes were assessed by body weight (BW), liver weight to body weight ratio (LW/BW), and liver ultrasound imaging. MASH progression was determined by ALT, cholesterol, and liver triglyceride levels. Treg populations in the liver were analyzed by flow cytometry. The cytokine profiles were evaluated by ELISA. **Results:** MASH mice exhibited increased BW and LW/BW, and a diffuse hyperechoic pattern in ultrasound compared to ND. WD induced liver injury, steatosis, and Treg accumulation. Compared with Sed, Ex considerably reduced BW gain and LW/BW, and ameliorated MASH progression in both ND and WD groups. This was evidenced by significantly decreased ALT, cholesterol, and hepatic triglyceride levels in WD mice. Ex also substantially reduced Treg infiltration in the liver. Pro-inflammatory cytokines associated with MASH pathogenesis were significantly reduced. **Summary:** This study suggested a potential link between exercise therapy-modulated Treg attenuation and improvements in hepatic inflammation during MASH, offering a rationale of Ex for improving the outcomes of MASH patients.

#14. THERAPEUTIC POTENTIAL OF IL-27 SIGNALING BLOCKADE IN NASH DEVELOPMENT.

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Nonalcoholic steatohepatitis (NASH) is a progressive liver disorder associated with obesity and metabolic disorders, leading to severe complications such as cirrhosis and hepatocellular carcinoma (HCC). Interleukin-27 (IL-27), an anti-inflammatory cytokine, has been implicated in NASH-driven HCC, presenting a potential target for intervention. In this study, the impact of IL-27 blockade on NASH was explored. Contrary to the strong inhibitory effect observed on HCC development, IL-27 blockade demonstrated limited efficacy in suppressing NASH. Macroscopic and microscopic examinations revealed no significant differences between IgG2a and anti-IL27 treated cohorts, with both exhibiting similar steatosis patterns. Histological analyses showed no alterations in fibrosis or cell proliferation. Transcriptional analyses, however, unveiled heightened expression of IL-1β and CCND1 in response to IL-27 neutralization, suggesting a potential pro-inflammatory and proliferative influence. Overall, while IL-27 blockade exhibited a robust impact on HCC development, its limited effect on NASH suggests complex interactions within the IL-27 signaling pathway in the context of liver disorders. Further investigations are warranted to decipher the intricate role of IL-27 in NASH progression and its potential as a therapeutic target.

#15. CELL CYCLE SIGNATURE-ENRICHED PROLIFERATION-COMPETENT T CELLS PREDICT LIVER FIBROSIS REGRESSION DURING VIRAL CLEARANCE IN PATIENTS WITH CHRONIC HBV INFECTION.

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Background & Aims: Clinical evidence indicates that suppressing or eliminating the hepatitis B virus (HBV) does not consistently lead to liver fibrosis remission for all patients, posing challenges for patients undergoing long-term anti-HBV treatment. Therefore, it is crucial to identify the underlying factors contributing to diverse outcomes in liver fibrosis reversal after HBV control. **Approach & Results:** Liver fibrosis patients with chronic HBV infection (CHB) underwent sequential biopsies during antiviral treatment. Fibrosis regression was evaluated using the Ishak score and P-I-R system. Transcriptomic analyses of liver biopsies revealed accelerated gene expression and molecular function restoration in regressing cases using antiviral treatment. A cell cycle gene signature (CC signature) was discovered using Orthogonal Partial Least Squares Discriminant Analysis (OPLA-DA) and bioinformatics, stratifying treatment-naïve patients into CClow and CChigh subgroups and predicting liver fibrosis regression following antiviral therapy in both the discovery and validation cohorts. Analyses of single-cell RNA sequencing (scRNA-seq) data and multiplex immunohistochemical (mIHC) staining confirmed that the CC signature was primarily enriched in a subset of T cells with heightened proliferative, tissue-resident memory, and cytotoxicitic capacities, termed proliferation-competent T cells (Tpc cells). A greater abundance of Tpc cells was found in CChigh patients and regressing cases, associated with reduced low-level viremia (LLV) and increased regression susceptibility during long-term antiviral treatment. **Conclusions:** CC

signature-enriched liver Tpc cells possess the potential to facilitate liver fibrosis regression during viral clearance in CHB patients, providing a cautionary signal for non-regressing cases during nucleos(t)ide analogue-based anti-HBV treatments and offering novel cellular targets for combined immunotherapy with antiviral therapy.

#16. MFAP2 ABLATION RETARDS HEPATIC FIBROSIS REGRESSION BY ORCHESTRATING EXTRACELLULAR MATRIX AND INFLAMMATION.

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Background & Aims: Microfiber-associated glycoprotein 1 (MAGP1) is a glycoprotein associated with extracellular matrix (ECM) remodeling, yet its association with liver fibrosis remains poorly understood. This study aimed to investigate the underlying roles and mechanisms of MAGP1 in liver fibrosis. Approach & Results: Analysis of publicly available transcriptomic profiles, human tissue microarrays, and liver fibrosis mouse models revealed MAGP1 was primarily expressed in activated hepatic stellate cells (HSCs), upregulated in advanced liver fibrosis, and decreased during regression. The role of MAGP1 in liver fibrosis was studied using *Mfap2* (MAGP1 gene) knockout mice (*Mfap2*$^{-/-}$) and littermates undergoing carbon tetrachloride (CCl_4) injection or cessation. *Mfap2* knockout had minimal effects on liver fibrosis progression but significantly impeded spontaneous fibrosis regression. Transcriptomic analyses, pathological assessments, and molecular measurements demonstrated that *Mfap2* ablation exacerbated intrahepatic inflammatory infiltration of lobular areas. Matrisome analysis and immunofluorescence revealed *Mfap2* deficiency accelerated ECM remodeling, as evidenced by upregulation of insoluble collagens, lysyl oxidase-like 1 (LOXL1), and fibrillin 1 (FBN1) in the ECM. In vitro experiments using LX-2 cells further confirmed that inhibiting MFAP2 promoted ECM production, while MFAP2 overexpression suppressed it. Moreover, single-cell RNA sequencing (scRNA-seq) and cellchat analyses showed that *Mfap2* ablation enhanced cell communications within HSCs and between HSCs and Kupffer cells. Finally, *Mfap2* overexpression via adeno-associated virus vectors (serotype 6, AAV6) reduced insoluble collagens, LOXL1, and FBN1, and alleviated lobular inflammation in CCl4-induced liver fibrosis mouse models. **Conclusion:** This study highlights the role of MAGP1 in the progression and regression of liver fibrosis, likely through its involvement in orchestrating extracellular matrix remodeling and inflammation. These findings suggest that increasing MAGP1 may benefit patients with liver fibrosis.

#17. MACROPHAGE HETEROGENEITY AND REDUCED INFLAMMATORY POTENTIAL IN HUMAN HEPATOCELLULAR CARCINOMA REVEALED BY SINGLE CELL AND SPATIAL TRANSCRIPTOMICS.

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Hepatocellular carcinoma (HCC) ranks sixth in global cancer incidence and is the third most lethal reflecting the inadequacy of its current treatments. Furthermore, the sequential changes to the immune microenvironment within the liver during its transition from health to chronic liver disease to HCC remains poorly understood. Here we report a human transcriptomic cell atlas of 237,938 cells from healthy human livers (n=13; 69,494 cells), chronically HCV-infected livers (n=5; 48,765 cells), and matched treatment-naive HCC resection samples from the tumor-core (n=12; 53,731 cells), the tumor-margin (n=12; 49,765 cells), and adjacent-normal liver tissues (n=12; 27,008 cells) of varying HCC-etiologies. We identified a progressive T-cell exhaustion and regulatory T cell gradient that correlates with tumor proximity. The tumor microenvironment (TME) is infiltrated with immunosuppressive and angiogenic myeloid cell phenotypes and lacks memory CD8+ T cells. Myeloid cells exhibit reduced responsiveness to inflammatory stimuli in vitro with increased proximity to the tumor and TME macrophage-T cell interactions occur through immunoregulatory pathways. Further, we describe a periportal-liver sinusoidal endothelial cell signature present within the TME and a novel LGALS1+ cancer associated fibroblast signature that correlates with poor HCC patient outcomes. We validate these populations using spatial transcriptomics revealing distinct endothelial cell and fibroblast niches and key myeloid-T cell interactions at the HCC tumor margin. Furthermore, we functionally validate key macrophage activating (e.g. cGAS-STING) signals in vitro to potentially reinvigorate local T cell mediated anti-tumor immunity. Overall, we describe targetable etiology-independent pathways that drive an immunoregulatory macrophage phenotype in the human HCC TME.

SYMPOSIUM 2: ENDOTHELIAL CELL SIGNALING IN LIVER DISEASE

#18. MECHANOSENSITIVE SELF-FUELING VASCULAR WNT CONTROLS LIVER FUNCTION.

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The vascular endothelium forms a systemically disseminated organ that translates micromilieu factors into instructive cues, designated as angiocrine signaling. While vascular control of organ function mechanisms have during the last decade been discovered in essentially all major organs, the mechanisms of translating milieu factors into angiocrine signaling mechanisms remain largely elusive. Here, focussing on the well-established angiocrine Wnt signaling-regulated liver metabolic zonation paradigm, we identified blood flow-induced mechanical shear stress as a biophysical sensor that governs the angiocrine expression profile of liver sinusoidal endothelial cells (LSECs). Surgically imposed elevation of blood flow in the liver led to increased vascular Wnt

expression, whereas chronically reduced shear stress in a vascular malformation model phenocopied the genetic ablation of vascular Wnt signaling at the organ level. Combining single-cell RNA sequencing with spatial proteomics, we generated a high-resolution crosstalk map of LSECs and hepatocytes from Wnt-deficient mutant mice and littermate controls. Angiocrine Wnt ligands acted synergistically with the Wnt signaling enhancer Rspo3 to establish the spatial division of labor in hepatocytes. Intriguingly, vascular Wnt receptors, in parallel with angiocrine Wnt ligands, were specifically enriched in pericentral LSECs, creating a zonated self-feeding autocrine Wnt loop. Autocrine Wnt signaling regulated the expression of channel-forming junctional molecules and the shear sensing machinery, thereby controlling vascular tone. Conversely, changes in vascular tone directly affected the expression and secretion of angiocrine Wnt, highlighting vascular Wnt-signaling as a crucial mechanosensing rheostat of liver function. Taken together, the data define LSECs as dynamic cellular decoders that translate biophysical forces into instructive angiocrine signals through an autocrine canonical Wnt circuit.

#19. CHARACTERIZATION OF HEPATIC LYMPHATIC ENDOTHELIAL CELLS IN LIVER REGENERATION.

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Aim: Contribution of hepatic lymphatic endothelial cells (LyECs) towards liver regeneration remains unexplored. We characterized LyECs in physiological and pathophysiological rat models of liver regeneration. **Methods:** For physiological regeneration, 70% partial hepatectomy (PHx) was used and for pathophysiological regeneration, we used 2-Acetyaminoflourene followed by 70% PHx (AAF-PHx). LyECs were imaged using Podoplanin and CD31 by microscopy. Liver endothelial cells were isolated by percoll-gradient and LyECs were sorted by flow cytometry. Proteomics was performed on LyECs by LC-MS/MS. LyECs were cultured and effect of their CM was studied on hepatocytes and hepatic progenitor cells (HPCs). **Results:** In PHx, proliferating hepatocytes were seen post-PHx and in AAF-PHx, we observed damaged hepatocytes with fibrosis and increased HPCs near periportal areas post-PHx. In comparison to shams, there were increased percentage of dilated LyECs in portal tracts in both PHx and AAF-PHx. More than 1500 total proteins were identified in LyECs, of them 627 were differentially expressed proteins (DEPs) with 318 up and 309 downregulated in PHx compared to PHx-sham while 452 DEPs with 200 up and 252 downregulated in AAF-PHx compared to AAF-sham. Many secreted proteins like Wnt2, R-spondin-2, Reelin were significantly elevated in LyECs of PHx while R-spondin2 was also higher in AAF-PHx. In co-cultures, there was increase in number of hepatocytes and HPC organoids in presence of CM of LyECs from PHx as compared to untreated controls. **Conclusion:** Our study identifies specific lymphangiocrine factors that might be playing a crucial role in governing proliferation of hepatocytes and HPCs during liver regeneration.

#20. HEPATIC LSECTIN REDUCTION CONTRIBUTES TO PROINFLAMMATORY T HELPER 17 CELL EXPANSION IN PATIENTS WITH DECOMPENSATED CIRRHOSIS.

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Background: Liver Sinusoidal Endothelial Cells (LSECs) express C-type lectin LSECtin, which negatively regulates hepatic T-cell immune response. Our aim was to elucidate the mechanisms of LSECtin-dependent T cell expansion during liver damage. **Methods:** Cirrhotic liver tissue was obtained from patients with alcohol/non-alcoholic steatohepatitis who underwent liver transplantation and non-lesioned tissue used as control. Proinflammatory/profibrogenic markers, LSECtin, Tbet, RORγT and Foxp3 expression were analyzed by IHC. Isolated RNA was subjected to RNAseq. Splenic CD3+ lymphocytes were incubated with CD3 and CD28 +/-LSECtin during 72h. Activation/exhaustion markers were studied by flow cytometry (FC). Control lymphocytic populations were studied on an LSECtin knock-in model by FC. Results: LSECtin was reduced in tissue from cirrhotic patients shown by IHC and Western Blot. RORγT (Th17) and Foxp3 (Treg) were progressively increased in hepatic tissue from compensated to decompensated disease. RORγT and Foxp3 transcripts levels were significantly increased in patients vs controls. An inverse significant correlation was observed for LSECtin with RORγT and FoxP3, further supported by reduced IL-17+ population (1.89% vs 1.12%; p=0.043). Also, CD4+ T lymphocytes stimulated in vitro with rLSECtin reduced expression of activation markers, like CD69 (5.26% vs 2.29% controls; p=0.048), also T CD4+ lymphocytes of the KI model. **Conclusion:** Th17 cell expansion is inversely associated with LSECtin expression in hepatic tissue of cirrhotic patients. Moreover, LSECtin promotes lymphocyte inhibition in vitro and in vivo restricting the expansion of Th17 cells. Thus, the recovery of LSECtin could ameliorate the unbalanced inflammatory milieu and contribute to restore homeostasis in patients with cirrhosis.

#21. A PRELIMINARY INVESTIGATION OF LIVER'S ROLE IN THE ELIMINATION OF AB40.

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The liver has been identified as being involved in the elimination of beta amyloid (Aβ) from the peripheral circulation and further, that this function may reduce with aging contributing to the pathogenesis of Alzheimer's disease (AD). On this basis, restoration of this function using liver directed therapies could offer therapeutic potential for the reduction of Aβ load, and prevent the development of AD. To characterize the liver's role and any age-related changes in Aβ40 uptake we performed multiple indicator dilution

technique (MID) experiments in 3-month-old and 24-month-old rats and cellular and whole of body experiments in 3 month and 24-month-old mice. Our preliminary findings indicate there was no significant uptake of 3H-Aβ40 by primary LSEC and hepatocyte cultures, that there was no substantial uptake of 3H-Aβ40 by the liver as assessed by western blots of livers following large doses of peripherally injected Aβ40, nor any significant immunofluorescent staining of primary hepatocyte culture following incubation with fluorescently labelled Aβ40. Finally, MID experiments were unable to confirm any of the interactions between Aβ40 and mouse liver cells that had been previously observed. This work suggests that the liver's role in the elimination of Aβ40 was not substantiated in this study and suggests it would not be an effective target for the treatment of Alzheimer's disease.

#22. ENDOSOMAL ESCAPE OF ASOS INTERNALIZED BY STABILIN-1 AND STABILIN-2.

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Liver sinusoidal endothelial cells (LSECs) have a high endocytic capacity and accumulate synthetic pharmacologically active phosphorothioate-based antisense oligonucleotides (ASOs) from blood circulation. Our previous work has shown non-DNA binding Stabilin/CLEVER-1 and Stabilin-2/HARE receptors expressed by LSECs specifically binds to and internalize several classes of ASO. ASOs are typically small (18-30 nucleotides) polymers, single stranded, chemically modified RNA/DNA oligos specifically designed to hybridize with mRNAs of interest to modulate their expression. ASOs may be designed for any disease related gene in which expression may be reduced or corrected in splicing errors. Continual modification of ASO chemistry and sequence over the past 25 years have resulted in the enhancement of their pharmacokinetic profile. However, Stabilin-mediated endocytosis results in over 98% of the ASO trafficked to and destruction in the lysosome which is an impediment in the drug efficacy process. Our previous studies have identified the role of endosomal proteins (EEA1, Rab5c, Rab7) in facilitating trafficking and escape of ASOs from endosomes. Here, through a variable time point study, we traced the endosomal path of ASOs after being internalized by Stabilin-1 or Stabilin-2 using confocal microscopy. We quantified ASO knock-down efficacy after treatment with endosomal membrane destabilizing agent, Chloroquine, using qPCR. We also analyzed the trafficking role of known endosomal markers after chloroquine treatment involved with ASO escape. Lastly, we discovered that knockdown of Galectin-1 enhances ASO efficacy, which has so far been used as a reporter gene for imaging ASO escape.

#23. UNRAVELING THE INNATE IMMUNE FUNCTIONS OF LIVER SINUSOIDAL ENDOTHELIAL CELLS: IMPLICATION FOR DISEASES.

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Liver sinusoidal endothelial cells (LSEC) are crucial cell types of liver innate immunity, influencing diseases like HIV and endotoxemia. Following are two significant discoveries from my laboratory: 1. Role of LSEC Stabilin Receptors in TLR4-Mediated Immune Response to LPS: Lipopolysaccharide (LPS), a major constituent of Gram-negative bacterial cell wall, is a potent microbial ligand that induces intense systemic inflammation via TLR4 receptor. LPS in blood circulation (endotoxemia) affects multiple organs and can cause life-threatening inflammatory reactions. As a proactive host defense mechanism, the liver clears LPS from blood circulation. However, the innate immune mechanisms of the liver cells and associated receptors involved in this process are not known. We identified that LSEC efficiently clear (LPS) from circulation via Stabilin-1 (Stab1) and Stabilin-2 (Stab2) receptors through endocytosis and lysosomal degradation. Stabilin double knockout mice exhibit reduced LPS clearance, heightened systemic inflammation, and increased mortality. Stab1, and to a lesser extent Stab2, contribute to host defense against LPS. Additionally, Stabilins and TLR4 act as functionally opposite receptors in LPS immune response, and LSEC Stabilin regulate TLR4 mediated systemic inflammation. 2. Role of LSEC FcγRIIb in HIV infection and immunotherapy: Neutralizing anti-HIV antibodies suppress HIV infection by accelerating viral clearance from blood circulation, in addition to neutralization. The elimination mechanism is largely unknown. We identified that FcγRIIb expressed in LSEC, facilitates the rapid clearance of antibody-opsonized HIV pseudoviruses from circulation. The mechanism involved was identified to be FcγRIIb-mediated endocytosis followed by lysosomal degradation, providing insights for HIV vaccine and antibody therapy development.

#24. MIRNAS EMBEDDED IN HEPATOCYTE-DERIVED EXTRACELLULAR VESICLES PROMOTE ENDOTHELIAL CAPILLARIZATION IN CHRONIC LIVER DISEASE.

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Background: Liver cells paracrinally affect neighboring cells through the release of extracellular vesicles (EVs). We investigated the role of miRNAs enclosed in hepatocyte-derived EVs (hepEVs) as modulators of endothelial dedifferentiation in cirrhosis. **Methods:** EVs were purified from the supernatant of healthy (CT) or cirrhotic (CH) primary hepatocytes from human and rat livers. In vivo: CT-rats were intravenously treated with fluorescence-labelled hepEVs-CT or hepEVs-CH to evaluate their biodistribution and LSECs phenotype. In vitro: miRNAs profile in human hepEVs-CH was validated in rat. Commonly deregulated miRNAs were over-expressed in CT-LSECs and RNAseq and pathway analysis were performed. The results obtained were analysed in primary CH-LSECs, in rat and human cirrhotic liver tissue, and in CT-LSECs treated with hepEVs containing miRNAs specific up-regulation (n= 3-10 per experiment). **Results:** In vivo: hepEVs-CH predominantly accumulated in liver endothelium triggering fibrosis, inflammation, and cell-death processes. In vitro: human hepEVs-CH revealed

significant deregulation of 37 miRNAs, of which miR-A and miR-B were validated in rat hepEVs-CH. Transcriptome analysis of CT-LSECs transfected with miR-B mimic showed significant deregulation of 771 genes, exhibiting 51% of homology with the transcriptome of CH-LSECs. Pathway analysis demonstrated detrimental effects of miR-B, promoting cell death processes, particularly pyroptosis. Up-regulation of pyroptosis was validated in CT-LSECs overexpressing miR-B, in rat CH-LSECs and in rat and human cirrhotic liver tissue. Importantly, up-regulation of pyroptosis mediated by hepEVs-mir-B in LSECs was demonstrated. **Conclusion:** hepEVs actively contribute to endothelial dedifferentiation through activation of miR-B-pyroptosis pathway, suggesting it as a new therapeutic approach for endothelial dysfunction in cirrhosis.

#25. ENGINEERING A PERFUSABLE BIOMIMETIC VASCULARIZED LIVER ON A CHIP.

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The liver is one of the largest internal organs in the body. It conducts hundreds of biological functions such as protein synthesis, carbohydrate and lipid metabolism, xenobiotic metabolism, and toxin removal. Although small animal models have proven to be valuable for drug toxicity screening, the physiology and biology of these models do not phenocopy human physiology. As such, there is a tremendous interest in creating in vitro human liver models that recapitulate human liver biology and physiology. However, current 2D and microfluidic-based models have fallen short of incorporating a vascular bed (a network of capillary vessels), thereby missing a crucial architectural component of the liver. In fact, liver endothelial cells (ECs) are known to regulate the functional and structural organization of the liver. Here, in this study, we present a biomimetic perfusable vascularized human liver-on-chip where we incorporate capillary vessels to better model liver functions and probe the cellular communication between hepatocytes and endothelial cells. To engineer a vascularized human liver-on-chip, we utilized engineered endothelial cells which we re-expressed a vasculogenic transcription factor ETV2. Upon re-expression of ETV2, the engineered ECs were intermingled with primary human hepatocytes in a microfluidic device. After the device was connected to a peristaltic pump to simulate blood circulation from arteriole to capillary to venule, we used this system to investigate the long-term function of hepatocytes in both homeostasis and under toxin exposure. We utilized single cell sequencing to investigate how human hepatocytes re-educate endothelial cells in the vascularized human liver-on-chip.

#26. VASCULAR ENDOTHELIAL PGC1A SUPPRESSES METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE.

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Background: The liver plays a central role in whole-body energy metabolism by regulating glucose, lipid, and protein metabolism. Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells possessing unique features of high endocytosis capacity, presence of fenestrae, and lack of basement membrane making them the most permeable endothelial cells in the human body. LSECs regulate hepatic vascular tone, hepatic stellate cell quiescence, and initiation and progression of chronic liver diseases. Elucidating the intercellular communication between LSECs and hepatocytes is pivotal in understanding the initiation and progression of hepatocyte dysfunction in chronic liver diseases. PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) was initially found as a transcriptional coactivator. PGC1α regulates oxidative energy metabolism as well as glucose and fatty acid metabolism in the heart, skeletal muscle, liver, and brain. However, it is completely unknown whether endothelial PGC1α has unique roles in the liver. In this study, we hypothesize that endothelial PGC1α is essential for hepatocyte health to maintain whole-body metabolism. **Methods and Results:** We found that PGC1α depleted endothelial cells (ECs) impaired angiogenic activity such as sprouting, capillary-like network formation and cell migration compared to control ECs. To define whether endothelial PGC1α regulates tissue-specific functions, we generated tamoxifen-inducible endothelial specific PGC1α knock-out mice (PGC1α fl/fl: Cdh5-CreERT2, PGC1αEC-/-). Unexpectedly, we found that PGC1αEC-/- mice had significantly increased total fat mass by nuclear magnetic resonance (NMR) analysis, and impaired glucose tolerance but normal insulin tolerance compared to control mice. Under a high-fat diet (HFD) feeding condition, the PGC1αEC-/- mice significantly increased liver mass (g/mm, tibial length) compared to control mice. Interestingly, although the liver mass did not alter by EC-PGC1α depletion in normal diet-fed mice, the liver of PGC1αEC-/- mice significantly increased mRNA expression levels of inflammation-related cytokines by RT-qPCR analysis, fibrosis regions by Masson trichrome staining, and highly accumulated lipid droplets by Oil-Red-O staining compared to control mice. **Conclusion:** The PGC1αEC-/- mice show pre-diabetic syndromes and fatty liver pathology. Our study suggests that endothelial PGC1α may be a key novel regulator to maintain liver vascular health which is critical to whole-body energy metabolism.

#27. A LIVER-ON-A-CHIP WITH PERFUSABLE VASCULATURE FOR COMPOUND SCREENING.

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The liver sinusoid acts as a microcirculatory system, responsible for blood flow regulation along the liver, and facilitates the key functions of the liver. The sinusoid is lined with liver sinusoidal endothelial cells (LSECs) that, through their fenestrae, act as mediators for hepatic homeostasis and inflammatory responses to hepatic injury. Previously, we have shown primary human hepatocytes (PHHs) maintain phenotypic functions in 3D collagen microtissues when co-cultured with non-parenchymal cells, providing an in vivo-like microarchitecture. However, vascularization and ¬in vivo¬-like diffusion remains a

challenge for engineered liver disease models and drug screening. Here, we sought to test the novel hypothesis that combining liver microtissues with a perfusable vasculature system formed de novo in a microfluidic device would recapitulate the sinusoidal environment for compound screening. A microfluidic chip was fabricated containing a main channel coated with human umbilical endothelial cells (HUVECs). HUVEC monolayers formed confluent lumen after 24 hours and maintained circulatory for over one week. LSEC-coated microtissues were then seeded in a fibrin gel with HUVECs and human lung fibroblasts. Lumen-like structures formed de novo from the main HUVEC channel after two days, providing a conduit to the LSEC-coated microtissues. Fluorophores were perfused through the main HUVEC channel showing direct perfusion to the hepatic microtissues. Future efforts are focused the diffusion of compounds through endothelial to elucidate hepatocyte-endothelia crosstalk for ¬in vitro disease modeling and drug screening.

SYMPOSIUM 3: CELL-FATE TRACING IN LIVER DISEASE

#28. INSIGHT PROFILING OF NON-PARENCHYMAL CELLS IN LIVER FIBROSIS PROGRESSION AND REGRESSION BY SINGLE-CELL RNA SEQUENCING ANALYSIS.

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Background: Liver fibrosis regression has been described in both human and animal models of liver diseases. However, the mechanism and driven factors are still widely unknown. Single-cell RNA sequencing is transforming our understanding of novel subpopulations, cell-cell interaction, and potential factors that contribute to liver fibrosis regression, resulting in the discovery of therapeutic targets. **Methods:** ScRNA-seq was performed on primary liver non-parenchymal cells (NPCs) isolated from the age-matched control, CCl4-induced liver fibrosis, and recovery group 2 weeks after CCl4 injection using the 10X Genomics Chromium platform. Computational analysis was performed in R. **Results:** Integration and annotation of NPCs from control, fibrosis, and recovery revealed nine cell types. Five cell types that contribute to liver fibrosis progression have been characterized including capillarized LSECs, M2 macrophages with three subpopulations: scar-associated macrophages, monocytes-derived macrophages, and proliferated macrophages, activated B cell, profibrogenic and pro-inflammatory BECs, and activated HSCs. Furthermore, we found four cell types that contribute to liver fibrosis regression: periportal angiogenic LSECs, pro-resolution macrophages (Cd45+F4/80+Tim4+Folr2+), NKT cell activation, and inactivated HSCs. Cell-cell interaction analysis revealed that autocrine was the hallmark signal that promoted the activation of HSCs involved in fibrosis formation. 5 secreted proteins and one transcription factor HANDS were found and confirmed as the specific markers of qHSCs. Trajectory analysis using Monocle 3 showed that Emilin1 was novel gene related to activation of HSCs. **Conclusion:** Both liver fibrosis

progression and regression exposed cellular heterogeneity. Target of HSCs autocrine signals and regulate its activation state could be promising antifibrosis therapies.

#29. UNRAVELING THE ROLE OF WNT SIGNALING IN LIVER ORGANOGENESIS USING HUMAN HEPATOBLAST ORGANOIDS.

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Hepatoblasts play a key role in liver organogenesis. These fetal liver stem cells have the capacity to differentiate into hepatocytes and cholangiocytes which represent the main functional cell types of the liver. Lineage tracing and genetic studies in the mouse have shown the association of Wnt signaling with proliferation and differentiation of hepatoblasts, as well as progression of different liver diseases. However, the exact function of this pathway in hepatic development and cell fate choice is not fully uncovered, especially in human where access to primary tissues is challenging. Here, we took advantage of human hepatoblast organoids (HBOs) to investigate the importance of Wnt in self-renewal and cell fate decisions. Notably, HBOs display a transcriptomic profile characteristic of hepatoblasts and they maintain the capacity to differentiate into hepatocytes and biliary cells. We first showed that Wnt plays a key role in hepatoblast self-renewal in vitro by maintaining their proliferative state through regulation of cell cycle-related genes. However, Wnt was not sufficient to block differentiation of HBOs into hepatocytes or cholangiocytes. Finally, single-cell transcriptomic analyses showed that Wnt signaling activity correlates with proliferation of hepatoblasts in the human fetal liver, thereby suggesting that the role for Wnt could be conserved in vivo. Taken together, our results support a model where Wnt signaling acts to preserve the proliferative capacity of hepatoblasts without being sufficient to maintain their bipotent state. These results could help to further understand the role of Wnt in the adult liver homeostasis and in disease.

#30. DETERMINING THE ROLE OF PLASTICITY FACTORS IN HUMAN LIVER REGENERATION.

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Although liver regeneration is well-established in the context of acute liver injury, its relevance for chronic diseases remains to be demonstrated. Studies performed on animal models have suggested three different mechanisms: i) hepatocytes and cholangiocytes can dedifferentiate into a liver cell progenitor state, restoring the corresponding cell compartment; ii) cholangiocytes can transdifferentiate into hepatocytes and vice versa; iii) hepatic stem cells can be activated giving rise to new liver cells. However, these

processes have not been validated in humans due to technical and ethical obstacles. To address these limitations, we mapped at the single cell level the progression of metabolic dysfunction-associated steatotic liver disease (MASLD) and then investigated potential regenerative events. These analyses revealed that hepatocyte and cholangiocyte can transdifferentiate into each other during chronic liver injury. This process involves a set of transcription factors that might play a role in the acquisition of plasticity necessary for transdifferentiation. To test this hypothesis, we first confirmed the induction of these plasticity factors in transdifferentiating cells by immunostaining on human tissue slides. We also performed gain of function experiments in vitro by overexpressing candidate factors in intrahepatic cholangiocyte organoids derived from patients with end stage liver disease and hepatocyte like cells generated from induced pluripotent stem cells. Phenotypic analyses suggest a role of these factors in ICOs and hiPSC-generated hepatocyte plasticity. Together our data uncover in part the molecular mechanisms controlling regeneration during chronic liver disease and thus path the way to develop new therapies promoting tissue repair during chronic injury.

#31. TEMPORALLY CONTROLLED EXPRESSION OF A SPLICING FACTOR IN SINGLE CELLS COORDINATES THE METABOLIC AND PROLIFERATIVE ACTIVITIES OF REGENERATING LIVERS.

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The exact mechanics of liver regeneration, such as how quiescent hepatocytes transition into a proliferative state and how regenerating livers sustain normal metabolic activities while the tissue recovers from injury, remain largely unknown. The role of alternative splicing and RNA binding proteins during liver regeneration has remained completely uninvestigated. Epithelial splicing regulator protein 2 (ESRP2) is an RNA splicing factor that acts as the developmental switch for splicing targets during postnatal liver maturation, including the Hippo signaling pathway, which is critical for organ development and regeneration. ESRP2 promotes the production of adult Hippo pathway splice variants, thereby limiting hepatocyte proliferation in a quiescent mature liver. We have previously demonstrated that ESRP2 and its splicing targets are transiently reprogrammed within regenerating hepatocytes to promote a proliferative state. We used scRNA sequencing to determine altered cell states and gene expression changes in ESRP2 KO cells compared to WT during the initiation and termination stages of liver regeneration. Here we show that regenerating hepatocytes bifurcate into proliferating or metabolically-hyperactive cell states, such that ESRP2 is highly expressed in the metabolically active hepatocytes and nearly absent in the proliferating hepatocytes. Remarkably, the adult quiescent ESRP2 KO hepatocytes exhibited an altered starting cell state, one more closely related to the fetal hepatocytes. We further show that forced expression of ESRP2 inhibits the proliferation of hepatocytes, whereas ESRP2 deletion increases their proliferative index during liver regeneration. Taken together, these data imply that tightly controlled expression of ESRP2 coordinates the metabolic and proliferative activities of regenerating livers.

SYMPOSIUM 4: INTERCELLULAR COMMUNICATION IN LIVER DISEASE

#32. GLYCOLYSIS-DEPENDENT EXTRACELLULAR VESICLES FROM HEPATIC STELLATE CELLS PROMOTE IN VIVO LIVER FIBROSIS.

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Liver fibrosis is characterized by the activation of hepatic stellate cells (HSCs) and the release of fibrogenic nano-sized extracellular vesicles (EVs). Activated HSCs increase their glucose metabolism via glycolysis to satisfy high energy demands. Nevertheless, the mechanism of how glycolysis in HSCs coordinates fibrosis amplification in the fibrogenic zones in the liver is elusive and is the scope of this study. Single cell RNA sequencing (scRNAseq) and bulk RNAseq demonstrated that several glycolysis enzymes, including hexokinase 2 (HK2), were upregulated in activated HSCs. HSC-selective glycolysis-deficient mice (HK2ΔHSC) showed abrogated CCl4-mediated fibrosis as compared to littermate controls (HK2fl/fl). Spatial transcriptomics revealed an upregulation of several EV-related pathways in the fibrotic pericentral zone during liver fibrosis in control HK2fl/fl mice. However, glycolysis-deficient HK2ΔHSC mice showed downregulation of these EV-related pathways in the pericentral zone. Consistently, induction of glycolysis in HSCs in vitro, either by glucose or platelet-derived growth factor B (PDGF), upregulated the expression of several extracellular vesicle (EV)-related genes, including RAB31. Glycolysis in HSCs epigenetically enhanced RAB31 expression through histone-3 lysine-9 acetylation (H3K9ac) on the promoter region, leading to increased EV release. Functionally, glycolysis-dependent EVs were enriched with fibrogenic molecules and increased the expression of fibrotic markers in recipient HSCs. Finally, EVs derived from glycolysis-deficient HK2ΔHSC mice abrogated liver fibrosis amplification as compared to EVs derived from littermate control HK2fl/fl mice. In summary, glycolysis in HSCs amplifies liver fibrosis by promoting fibrogenic EV release in the pericentral fibrotic regions in the liver.

#33. A PROTEIN COMPLEX OF LIVER ORIGIN ACTIVATES A PRO-INFLAMMATORY PROGRAM THAT DRIVES HEPATIC AND INTESTINAL INJURY IN ALCOHOL-ASSOCIATED LIVER DISEASE.

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Objective: there is limited information on how the liver-to-gut axis contributes to alcohol-associated liver disease (ALD). We previously identified that high-mobility group box-1 (HMGB1) undergoes oxidation in hepatocytes and showed that serum levels of oxidized HMGB1 ([O] HMGB1) are elevated in alcoholic patients. Since interleukin-1 beta (IL1B) increases in ALD, we hypothesized that hepatocyte-derived [O] HMGB1 could interact with IL1B to activate a pro-inflammatory program that, besides being detrimental to the

liver, drives intestinal barrier dysfunction. **Design & Results:** alcohol-fed *Rage*$^{\Delta Mye}$ mice showed a decrease in NFκB signaling, a pro-inflammatory signature, and total intestinal permeability, which resulted in protection from ALD. Furthermore, [O] HMGB1 bound and signaled through the receptor for advanced glycosylation end-products (RAGE) in myeloid cells to drive hepatic inflammation, intestinal permeability, and increased portal blood lipopolysaccharide in ALD. We identified that [O] HMGB1 formed a complex with IL1B, which was found in the livers of patients with acute alcoholic hepatitis and mice with ALD. This complex was of liver origin because it was absent in the intestine when hepatocytes did not produce [O] HMGB1. Mechanistically, the complex bound RAGE in Kupffer cells and macrophages to stimulate a pro-inflammatory program. Moreover, it bound RAGE in intestinal macrophages and epithelial cells, caused intestinal inflammation, altered intestinal epithelial cell tight junction protein expression, increased intestinal permeability and portal blood lipopolysaccharide, enhancing ALD pathogenesis. **Conclusion:** We identified a protein complex of liver origin that amplifies the pro-inflammatory feedback loop in ALD; therefore, targeting this complex could have significant therapeutic potential.

#34. POST-TRANSLATIONAL MODIFICATIONS DRIVE THE EFFECTS OF HMGB1 IN ALCOHOL-ASSOCIATED LIVER DISEASE.

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Background and Aims: high-mobility group box-1 (HMGB1) is a non-histone chromatin-associated protein involved in the pathogenesis of chronic liver disease. We identified that HMGB1 is increased and undergoes post-translational modifications (PTMs) in response to alcohol consumption. Thus, we hypothesized that specific PTMs could drive the pathogenic effects of HMGB1 in alcoholic liver disease (ALD). **Methods:** mice with cell-specific ablation of *Hmgb1* or the Receptor for advanced glycation end-products (*Rage*) were generated. Mice were injected with an HMGB1-neutralizing antibody or with the HMGB1 isoforms. The Lieber-DeCarli model of ALD was used. **Results:** *Hmgb1* ablation in hepatocytes (*Hmgb1*$^{\Delta Hep}$) or myeloid cells (*Hmgb1*$^{\Delta Mye}$) partially protected while ablation in both (*Hmgb1*$^{\Delta Hep\Delta Mye}$) prevented ALD. Neutralization of HMGB1 prevented while injection of [H] HMGB1 promoted ALD, which was worsened by injection of [O] HMGB1. Ablation of [O] HMGB1 protected whereas ablation of [Ac] HMGB1 exacerbated ALD due to inflammatory cell infiltration, which was blocked by ablation of both. Ethanol-fed *Rage*$^{\Delta Mye}$ mice were significantly protected, indicating a crucial role of RAGE in myeloid cells for ALD. [O] HMGB1 signaled through RAGE in myeloid cells. It was critical for driving steatosis, inflammation, IL1B production, and alcohol-induced liver injury, whereas [Ac] HMGB1 was protective by blocking the noxious effects of [O] HMGB1. **Conclusion:** [O] HMGB1 signals through RAGE in myeloid cells to drive the pathogenesis of ALD while [Ac] HMGB1 offsets the effects of [O] HMGB1.

#35. HEPATOCYTE EXTRACELLULAR VESICLES DELIVER MIR-122 TO HEPATIC STELLATE CELLS TO PROMOTE ERASTIN-INDUCED FERROPTOSIS.

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Background: Extracellular vesicles (EVs) participate in intercellular communication through delivery of their cargo molecules to target cells. miR-122 is mainly produced by hepatocytes and constitutes 70% of the total miRNA pool in the liver. miR-122 regulates cholesterol and iron metabolism but here we identify novel functions of mir-122 after its EV-mediated delivery to hepatic stellate cells (HSC). **Methods and Results:** Hepatocyte EVs promoted erastin-induced ferroptosis in HSC. Small RNA sequencing identified more than 70 miRNAs in hepatocyte EVs with miR-122 being the most abundant. Transient transfection or stable expression of miR-122 in LX2 cells led to drastic ferroptosis induced by erastin, RSL-3, FIN56, or cystine depletion but no apoptosis. miR-122-induced cell death was reversed by ferroptosis inhibitors but not apoptosis inhibitors. Wildtype or 3' GU-rich motif mutant but not 5' seed sequence mutant miR-122 mimic maintained the ability to promote ferroptosis. GPX4 (glutathione peroxidase 4), a master gene controlling ferroptosis, was downregulated at both transcript and protein levels in miR-122-expressing cells. Glucose-regulated protein 78 (GRP78, also called Bip or HSPA5), which reportedly interacts with and protects GPX4 from degradation, is a predicted target of miR-122 and its protein level was reduced by miR-122. EGCG, a GRP78 antagonist, caused more cell death in LX2 cells when co-treated with erastin. Stable knockdown of GPX4 or GRP78 but not of scramble controls led to enhanced ferroptosis in LX2 cells. **Conclusion:** Hepatocyte EVs potentiate HSC ferroptotic cell death by delivering miR-122, which may directly repress GRP78 expression, thereby reducing GPX4 expression and driving ferroptosis.

#36. A NOVEL ROLE OF EXOSOMAL MAT2A IN THE DEVELOPMENT OF COLORECTAL LIVER METASTASES.

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Background and Aims: The liver is the most common site of metastatic disease for patients with colorectal cancer (CRLM). Methionine adenosyltransferase 1A (MAT1a) catalyzes the formation of S-adenosylmethionine (SAMe), the principal methyl donor and precursor of glutathione in the liver. We reported that SAMe treatment inhibits colon cancer growth in a metastatic liver expansion model. In contrast, MAT2A, the extrahepatic MAT enzyme, is highly expressed in colon cancer and promotes cell proliferation and malignancy. In this study, we wanted to further elucidate the interplay between these MAT isozymes to understand a potential novel pathway for CRLM proliferation. **Methods:** In vitro studies of human hepatocytes and RKO cells. Exosome isolation, ChIP-seq, immunoprecipitation, western blot. Immunohistochemistry and immunofluorescence.

Results: Human hepatocytes cultured in conditioned media from RKO cells showed decreased expression of MAT1A and increased level of MAT2A. In addition, MAT2A was predicted to bind MAT1A promoter and confirmed using ChIP sequencing. Although MAT2A was discovered to be secreted by RKO cells and internalized by human hepatocytes localizing inside the nucleus. Also, we found that treating RKO cells with overexpressed MAT2A derived-exosome promoted cell proliferation and invasion. Conclusions: Our results suggest CRLM cells potentially proliferate in the liver via secretion of exosomal MAT2A, may acting as a transcription factor when internalized. Liver with decreased expression of MAT1A may be more susceptible to not only dysfunction but metastases as well. This potential novel pathway could be used to develop novel targets for metastatic inhibition.

#37. DISCOVERY AND VALIDATION OF NOVEL SINUSOIDAL BIOMARKERS FOR THE DIAGNOSIS AND PROGNOSIS OF CHRONIC LIVER DISEASE.

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Background and aims: Single-cell RNA sequencing has described new hepatic cell subpopulations, although their relevance and applicability in chronic liver disease (CLD) is still unknown. This study aimed to describe a panel of specific sinusoidal biomarkers which correlate with CLD diagnosis and prognosis. **Methods**: We performed gene deconvolution in an internal cohort of patients (n=24) to define which sinusoidal cells subpopulations are the most relevant for CLD. We generated the final sinusoidal scores (endothelial, mesenchymal and macrophage) using logistic regression with the most specific genes for each relevant sinusoidal subpopulation. The predictive power of these scores for clinical parameters (fibrosis, HVPG, MELD, Child-Pugh) was evaluated in a second internal cohort (n=110 patients including compensated and decompensated cirrhosis) and validated in three external cohorts (n = 1,316). **Results:** The generated sinusoidal scores efficiently predicted clinical parameters for diagnosis: compensated vs decompensated (AUROC = 0.90), hepatic fiber > 10%, 20% and 30% (AUROC = 0.72, 0.86 and 0.82, respectively), HVPG > 12mmHg and 16mmHg (AUROC = 0.75 and 0.79), CHILD-PUGH >A and >B (AUROC = 0.92 and 0.87) and MELD > 20 (AUROC = 0.70). The results obtained in the internal cohort were also validated in the extensive external cohort. **Conclusions:** We describe a panel of hepatic markers specifically reflecting the phenotype of sinusoidal cells, which correlates with relevant clinical parameters of CLD. Current studies analyzing extensive cohorts of patients with follow-up data will reveal the potential of these scores to predict patients' progression in a precision medicine manner.

#38. EXPRESSION OF LSEC MARKERS IN PATIENTS WITH DIFFERENT NAS SCORES AND FIBROSIS STAGES USING MULTISPECTRAL IMAGING MICROSCOPY AND AI APPLICATIONS.

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Introduction: The complex hepatic microenvironment is essential for liver homeostasis and is crucial in the development of steatotic liver disease (SLD). In situ methods that study the relationships between hepatocytes and non-parenchymal cells, including Kupffer cells (KC), hepatic stellate cells (HSC), and liver sinusoidal endothelial cells (LSEC) are needed. The role of LSECs in the progression of SLD is poorly understood. We describe a workflow for non-parenchymal cell characterization and spatial distribution while maintaining tissue architecture. **Methods:** Liver biopsies from controls and SLD patients in different fibrosis stages (F1-F4) were stained simultaneously with a panel of markers for LSECs (CD31, CD34, CD36), KCs (CD163), and HSCs (cytoglobin) using multiplex immunofluorescence. Imaging data was collected and analyzed with AI applications and the expression area for LSEC markers was calculated. Guided phenotyping quantified the numbers of CD163+ and cytoglobin+ cells. The expression of LSEC markers was correlated with cytoglobin expression and fibrosis stages. Results: CD31, a marker of LSEC capillarization, was the only marker with increased expression from F1-F3. CD31 and cytoglobin positively correlated, consistent with reported associations between capillarized LSECs, HSC activation, and fibrosis progression. Cirrhotic patients (F4) showed reduction in expression of LSEC markers (CD31, CD34, CD36) and HSC (cytoglobin), likely due to replacement of sinusoids with fibrosis and parenchymal extinction. **Conclusions:** CD31 shows potential as an indicator of capillarization in SLD fibrosis progression. Approaches such as multispectral imaging, which can capture liver cell heterogeneity and spatial relationship between them, could lead to a greater understanding of SLD progression.

SYMPOSIUM 5: INTERORGAN COMMUNICATION IN LIVER DISEASE

#39. INTESTINAL EPITHELIAL CELL OSTEOPONTIN PROTECTS FROM MASH BY CHANGING THE COMPOSITION OF THE GUT MICROBIOME AND BILE ACIDS.

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Previous studies reported that intestinal Osteopontin (OPN) regulates the gut microbiota. However, the role of intestinal epithelial cell (IEC)-derived OPN in MASH is unknown. We generated Spp1 knock-in (*Spp1*$^{KI\ IEC}$) and knock-out (*Spp1*$^{\Delta IEC}$) mice in IECs and fed both genders with high fat, fructose, and cholesterol diet to induce MASH or with an isocaloric control diet for 6 months. Immunohistochemistry revealed decreased OPN expression in IECs in WT mice with MASH compared to the control diet. *Spp1*$^{\Delta IEC}$ showed worse hepatic inflammation than WT mice regardless of diet but developed more liver fibrosis and increased serum ALT than WT mice when fed a MASH-inducing diet. *Spp1*$^{\Delta IEC}$ mice displayed clutched IECs with condensed cytoplasm and pyknotic nuclei, increased TUNEL+ IECs, downregulation of tight junction proteins, increased serum LPS and

hepatic bacterial load, compared to WT mice, regardless of diet. Metagenomic analysis of gut microbiota revealed that *Spp1*$^{\Delta IEC}$ reshaped gut microbiota. Functional analysis with a publicly available microbiome genome database suggested a down-regulation of bile acid (BA) deconjugating bacteria regardless of diet. Total BAs increased in portal serum and decreased in feces from *Spp1*$^{\Delta IEC}$ compared to WT mice with MASH, indicating less BA excretion. Liver BA was increased independent of liver cholesterol. The MASH diet and the *Spp1*$^{\Delta IEC}$ genotype independently increased conjugated primary BAs, particularly taurocholic acid (TCA) and taurodeoxycholic acid (TDCA), in both genders. Treatment of BAs with a concentration equivalent to serum from *Spp1*$^{\Delta IEC}$ with MASH showed increased hepatocyte damage. *Spp1*$^{KI\ IEC}$ mice were protected from MASH, which was shown by reduced liver inflammation and fibrosis.

#40. OVEREXPRESSION OF OSTEOPONTIN (*SPP1*) IN BILIARY EPITHELIAL CELLS CAUSES DUCTULAR REACTION.

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Background: Osteopontin (OPN, encoded by *SPP1*) expression increases in chronic liver diseases such as metabolic dysfunction-associated steatotic liver disease (MASLD), metabolic dysfunction-associated steatohepatitis (MASH), alcohol-associated liver disease (AALD), fibrosis, and hepatocellular carcinoma (HCC). Biliary epithelial cells (BECs) maintain the structural integrity of bile ducts (BDs), form a continuous barrier between hepatocytes and bile, and play a major role in bile acid (BA) composition and secretion. The proliferation of BECs occurs in cholangiopathies, including primary sclerosing cholangitis (PSC) and biliary atresia. While OPN is highly expressed in BECs, the role of BEC-derived OPN is still unknown. **Methods:** Publicly available datasets from patients with MASLD and MASH were analyzed for *SPP1* expression. Mice with overexpression (*Spp1*$^{KI\ BEC}$) or ablation (*Spp1*$^{\Delta BEC}$) of OPN in BECs were generated by breeding *Spp1.Stopfl/fl* and *Spp1fl/fl* with *Sox9-CreERT* mice. Tamoxifen (75 mg/kg) was i.p. injected into 8-week-old mice (two cycles of 5 consecutive days, every 15 days) until sacrificed 4 weeks later. Histopathological analysis of livers was performed using H&E staining. Fibrosis was observed by Sirius red/Fast green staining. Ductular reaction was detected by immunostaining for CK7 and SOX9. mRNA levels of *Krt7*, *Krt19*, and *Col1a1* were analyzed by qPCR. **Results:** scRNAseq data analysis showed BECs express increased *SPP1* in MASLD and MASH. Histological analysis of *Spp1*$^{KI\ BEC}$ mice shows increased inflammation and ductular reaction compared to *Sox9-CreERT* (control) mice. Sirius red/Fast green staining shows fibrosis increased in *Spp1*$^{KI\ BEC}$ and decreased in *Spp1*$^{\Delta BEC}$ mice. Moreover, CK7 and SOX9 immunostaining of the liver tissue revealed increased bile ducts per portal triad in *Spp1*$^{KI\ BEC}$ mice compared to control mice. **Conclusion:** BECs increase SPP1 expression in chronic liver disease. *Spp1*$^{KI\ BEC}$ mice show increased inflammation, ductular reaction, portal fibrosis, and more bile ducts per portal triad.

#41. GUT-LIVER AXIS REVISITED: PROTECTIVE ROLE OF LYMPHANGIOGENESIS IN PORTAL HYPERTENSION AND CIRRHOSIS.

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Background: VEGFC-VEGFR3 lymphangiogenic pathway maintain structural and functional integrity of lymphatic vascular system, which regulates immunity and fluid distribution in our body. We modulated the lymphagiogenenic pathway in mesenteric lymphatic vessels (MLVs) and explored its effects on several physiological and pathological parameters in cirrhosis. **Methods:** Rat models of cirrhosis were prepared. Lymphangiogenic pathway was enhanced by recombinant pro-lymphangiogenic factor, vascular endothelial growth factor C (rVEGF-C, Cys156Ser) and was inhibited by an inhibitor, Saroglitazar (SAR). Molecular and histological characterization of MLVs was performed. Lymph drainage of MLVs was analysed by tracer dyes. Portal and systemic physiological assessments and computed tomography was performed. Immune cells were quantified in mesenteric lymph nodels (MLNs) by flow cytometry. Bacterial load was quantified in MLNs and other organs. **Results:** In cirrhotic rats, MLVs were dilated and leaky with impaired fluid drainage. SAR treatment in cirrhotic animals led to decreased drainage and enhancement of portal pressure while using rVEGF-C attenuated both abdominal ascites and portal pressures by improving the lymphatic drainage. As compared to vehicle, rVEGF-C-treated cirrhotic rats showed decrease in bacterial counts in MLNs and other organs. After exogenous bacterial challenge, we found significant increase in activated TH cells in MLNs of rVEGF-C-treated rats compared with vehicle. Serum IL-6 was also significantly reduced in rVEGF-C compared to untreated and SAR-treated animals. rVEGF-C upregulated the expression of vascular endothelial-cadherin in lymphatic endothelial cells and functionally improved their permeability. **Conclusion:** Restoration of new MLVs by rVEGF-C in cirrhosis ameliorates mesenteric lymph drainage, portal hypertension and gut immune-surveillance.

#42. NEUTROPHIL EXTRACELLULAR TRAPS INDUCE HEPATOCYTE PYROPTOTIC AND NECROPTOTIC DEATH IN A NOVEL MULTI-ORGAN DAMAGE MODEL OF ALCOHOL-INDUCED ACLF.

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Background: Mechanisms underlying alcohol-induced ACLF pathophysiology are poorly understood. In our recently developed an acute-on-chronic liver failure (ACLF) murine model triggered by a single alcohol binge we tested mechanisms leading to acute liver damage, hepatocyte dysfunction, systemic inflammation encephalopathy-like phenotype, and kidney dysfunction. **Methods:** Liver fibrosis was induced in 12-week old male C57BL/6 or gasderminD knockout (GSDMD KO) mice by BDL for 28days (n≥4 per group). Alcohol binge (5g/Kg) was given to induce ACLF. Sham surgeries were used as controls. Tissue analysis was performed 9h after binge. LTCDC provided human liver specimens

from patients with ACLF. **Results:** Neutrophil extracellular trap (NET) formation (citH3, ELANE and lipocalin2) was increased in the serum, liver, brain and kidney of ACLF mice compared to BDL. In vitro, we demonstrate that bile acids or alcohol promote NET formation in primary neutrophils. Co-culture of cell-free NETs with HepG2 cells induced LDH, RIPK3 and IL-1β release by hepatocytes. Livers from ACLF mice also exhibited increased necroptosis (RIPK3 expression) and pyroptosis (increased cl-GSDMD staining and circulating IL-1β and IL-18) compared to BDL alone. NETosis, pyroptosis and RIPK3 activation were validated in human livers with ACLF. GSDMD KO mice were partially protected from developing ACLF compared to WT as exhibited by reduced neutrophil infiltration in the liver, NET formation, fibrosis and reduced necroptotic and pyroptotic cell death. **Conclusions:** Our data highlight NETs as a key mechanism inducing pyroptotic and necroptotic hepatocyte death during ACLF. GSDMD-deficient mice undergoing ACLF showed attenuated neutrophil infiltration, NET formation, fibrosis and cell death.

#43. TGFB2 UPREGULATION ON REACTIVE CHOLANGIOCYTES LIMITS THE THERAPEUTIC EFFICACY OF TGFB TRAP RAP-1332 IN MOUSE MODELS OF CHRONIC BILIARY INJURY AND FIBROSIS.

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Background: We report therapeutic effect of RAP-1332 (a ligand trap for TGFβ1 and TGFβ3) in comparison with TGFβ signaling inhibitor of ALK5, in mouse models of primary sclerosing cholangitis (PSC)-like biliary injury. **Methods:** RAP-1332 (1, 3 and 10 mg/kg twice a week) or ALK5 inhibitor (30 mg/kg/day) was tested in a BALBc.*Mdr2*$^{-/-}$ mice starting at 4 weeks (early therapy) or 6 weeks (delayed therapy) of age for the following 4-6 weeks, respectively. Portal venous pressure (PVP), serum liver function tests and histologic analyses were assessed. 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) feeding model of PSC was used as a validation model. **Results:** Alk5 inhibitor led to detectable improvement of liver injury and fibrosis parameters in both *Mdr2*$^{-/-}$ and DDC models. In contrast, both early and delayed administration of RAP-1332 into *Mdr2*$^{-/-}$ mice did not ameliorate liver injury and liver fibrosis, and led to paradoxical worsening of disease at high dose. In delayed therapy arm, ALT and AST were increased by 53.50% and 44.14%, respectively at 10 mg/kg dose RAP-1332. Exacerbated ductular reaction and worsened periductular fibrosis was observed in mice receiving high dose of RAP-1332, with 2-fold increase of collagen compared to placebo. Likewise, high dose of RAP-1332 in DDC model led to similar exacerbation of biliary injury and fibrosis. Realtime PCR and in situ hybridization showed remarkable upregulation of TGFβ2 expression on actively proliferating reactive cholangiocytes. **Conclusion:** Our results suggest that selective blocking TGFβ1 and TGFβ3 in chronic biliary fibrosing disease is inefficient due to compensatory upregulation of TGFβ2 on reactive cholangiocytes.

#44. GUT-DERIVED AMMONIA CONTRIBUTES TO ALCOHOL-RELATED FATTY LIVER DEVELOPMENT.

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Dysbiosis contributes to alcohol-associated liver disease (ALD); however, the precise mechanisms remain elusive. Given the critical role of the gut microbiota in ammonia production, we aimed to investigate whether and how gut-derived ammonia contributes to ALD. Blood samples were collected from human subjects with/without alcohol drinking. Mice were exposed to the Lieber-DeCarli isocaloric control or ethanol-containing diets with and without rifaximin (a nonabsorbable antibiotic clinically used for lowering gut ammonia production) supplementation for 5 weeks. Both in vitro (NH4Cl exposure of AML12 hepatocytes) and in vivo (urease administration for 5 days in mice) hyperammonemia models were employed. RNA sequencing and fecal amplicon sequencing were performed. Ammonia and triglyceride concentrations were measured. The gene and protein expression of enzymes involved in multiple pathways were measured. Our results showed that chronic alcohol consumption causes hyperammonemia in both mice and human subjects. In healthy livers and hepatocytes, ammonia exposure upregulates the expression of urea cycle genes, elevates hepatic de novo lipogenesis (DNL), and increases fat accumulation. Intriguingly, ammonia promotes ethanol catabolism and acetyl-CoA formation, which, together with ammonia, synergistically facilitates intracellular fat accumulation in hepatocytes. Mechanistic investigations uncovered that ATF4 activation, due to ER stress induction and general control nonderepressible 2 activation, plays a central role in ammonia-provoked DNL elevation. Rifaximin ameliorates ALD pathologies in mice, concomitant with blunted hepatic ER stress induction, ATF4 activation, and DNL activation. In conclusion, an overproduction of ammonia by gut microbiota, synergistically interacting with ethanol, significantly contributes to ALD pathologies.

#45. SEX DIFFERENCES IN SMALL INTESTINAL MICROBIOTA DISTINCT PORTAL BILE ACID COMPOSITION.

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Studies focused on microbial communities in the small intestine are few and lacking. Here, we investigated mucosal microbiota across the intestine (duodenum, jejunum, distal ileum, proximal colon) in addition to cecum and feces under normal conditions in female and male WT mice. We measured portal and systemic bile acids. We found robust sex-specific variations in the gut microbiome across the different regions, but the overall bacterial richness was higher in males than females. Despite variations in populations, the abundances of microbial genes involved in bile acid metabolism were present

throughout the intestine and displayed region-specificity. We uncovered in males had abundant BA transformations which correlated with changes in secondary unconjugated BAs. Taken together, our data reveals sexual dimorphism in microbes and bile acids compositions within the enterohepatic loop, which may contribute to the inherent sex-differences in GI physiology, and diseases incidences.

#46. SEX DIFFERENCES IN BILE ACID COMPOSITION AND DYSBIOSIS IN A MURINE MODEL OF HEPATOCELLULAR CARCINOMA.

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Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths, and men have a higher incidence than women. If caught early, HCC has a high curative rate. However, the current surveillance methods fall short. Bile acids (BAs) have been indicated as potential prognostic markers for HCC. Primary BAs are made in the liver and conjugated to amino acids prior to being secreted into the intestines for fat digestion. In the intestines, resident microbes modify BAs to make secondary BAs. We aimed to understand how BA composition relates to HCC and influences microbial composition. Further we assessed if there were associations between tumor marker genes, BA composition, and the microbiome. The double knockout (DKO) mouse model for BA-related nuclear receptors, farnesoid X receptor (FXR) and small heterodimer partner (SHP) mimics human HCC incidence. Intriguingly, we observed that the male DKO mice have higher primary BA unconjugated cholic acid and reduced proportion of secondary BA deoxycholic acid in the serum. We found that microbial genera known for BA deconjugation, Bifidobacterium and Faecalibaculum, have altered abundance in the DKO model. These findings demonstrate that dysregulated BA metabolism and subsequent dysbiosis contributes to the sex differences in HCC. There are changes in the serum BA composition that correlate to HCC tumor marker genes and changes in the microbial composition. Overall, these observations may lead toward coupling BAs and microbiota as additional HCC prognostic markers.

#47. AN ENGINEERED GUT-LIVER PLATFORM UTILIZING PRIMARY HUMAN HEPATOCYTES AND INTESTINAL EPITHELIAL CELLS.

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Orally administered drugs undergo intestinal metabolism and selective absorption before transport to the liver for further metabolism. Such intestinal-liver crosstalk can significantly impact drug bioavailability and efficacy by the time it reaches a patient's systemic circulation. Unfortunately, most in vitro gut-liver models fail to recapitulate in vivo metabolic functionality, such as cytochrome P450 (CYP) activity, as they rely on immortalized tumor cell lines and/or de-differentiated primary cell monolayers. Despite considerable progress in developing human intestine and liver models using primary cells,

little has been done in engineering integrated primary human gut-liver models. We have previously developed a micropatterned co-culture (MPCC) model of the human liver containing primary human hepatocytes (PHHs) and supportive fibroblasts that displays stable hepatic functions for several weeks in vitro. Here, we utilize MPCCs with primary intestinal epithelial cells to develop a modular and human gut-liver model for drug screening, disease modeling, and to investigate organ-organ crosstalk in chronic liver.

#48. EXPLORING THE ROLE OF HEPATIC STELLATE CELL DERIVED CXCL12 IN LIVER FIBROSIS.

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Background: Chemokines are secreted proteins that play a crucial role in stimulating immune cell migration. CXCL12 is an important chemokine with 5 isoforms in human and 3 in mouse. The role of CXCL12 has been unexplored in the hepatic stellate cells (HSCs). **Methods:** Gene expression was assessed using RNA-sequencing and single cell RNA-sequencing (scRNA-seq) in primary human HSCs (hHSCs) and CCl4-murine models. hHSCS were treated with TGFβ and evaluated by qPCR, agarose gel electrophoresis and Western blotting. RNA-fluorescence in situ hybridization (RNA-ISH) on mouse livers was used to investigate the spatial distribution of CXCL12. **Results:** Data mining of public datasets demonstrated that CXCL12 is mainly expressed in the liver by HSCs. RNA-seq analysis of hHSCs and CCl4-murine models treated with TGFβ (vs vehicle) exhibited decreased CXCL12. scRNA-seq from HSCs isolated from vehicle and CCl4-injected mice displayed a CXCL12 downregulation in a collagen-enriched HSCs subset ([Log2(FC)=-0.4,P<0.01). These findings were further confirmed in vitro by qPCR, where TGFβ decreased by 60% the transcripts of total CXCL12 and isoforms 1-3 in hHSCs (p<0.0001). Next, RNA-ISH of CXCL12 in murine models showed that most CXCL12 mRNA transcripts were redistributed along the fibrotic septae compared to the homogenous distribution in the vehicle. **Conclusion:** The TGFβ-mediated downregulation of HSCs-derived CXCL12 along with its striking spatial redistribution can underline a paramount importance in the directional trafficking of cells to the fibrotic septae. The rationale of the redistribution of the CXCL12 and isoforms in the diseased model and the TGFβ-mediated transcriptional regulation of CXCL12 are under current investigation.

#49. ALCOHOL REDUCES CIRCULATING LEVELS OF LIVER-EXPRESSED ANTI-MICROBIAL PEPTIDE, LEAP2: PUTATIVE ROLE IN THE DEVELOPMENT OF ALCOHOL-ASSOCIATED LIVER DISEASE.

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Introduction: Excessive alcohol consumption is a global healthcare problem. The liver sustains the greatest degree of tissue injury by chronic heavy drinking which causes a wide spectrum of pathology, the most characteristic of which are steatosis, hepatitis, and fibrosis/cirrhosis. Pathophysiological mechanisms involved in development and progression of alcohol-associated liver disease (ALD) are complex and multifactorial, including gut, pancreas and adipose tissue dysfunction and altered organ crosstalk. Recent research from my laboratory has established that alcohol-induced alterations in circulating gut peptide hormone level perturb organ crosstalk and promote liver injury. Specifically we have shown that alcohol-induced increases in serum levels of stomach-derived ghrelin impairs insulin secretion from pancreatic β-cells. The consequent reduction in the circulating insulin levels promotes adipose lipolysis and mobilization of fatty acids to the liver, ultimately contributing to the development of hepatic steatosis. We have further shown that inhibiting ghrelin-ghrelin receptor interaction prevents ALD development. A recently discovered predominantly liver and intestine-expressed peptide named liver-expressed antimicrobial peptide 2 (LEAP2) is an allosteric inhibitor of the ghrelin receptor and noncompetitively reduces the magnitude of the detrimental effects of ghrelin. The goals of this study were (i) to explore the status of LEAP2 expression and levels in clinical samples and in animal models of ALD and (ii) to examine the function and regulators of LEAP2 in the context of ALD. **Methods:** We measured LEAP2 levels in serum obtained from a previous study of rats fed control or ethanol Lieber-DeCarli diet for 5-6 weeks using a commercial ELISA kit (MyBiosource . We also determined LEAP2 levels in serum obtained from Nebraska Biobank and Liver Tissue Cell Distribution System (LTCDS) Nebraska biobank of alcoholic cirrhotic patients and controls. To identify the regulators of LEAP2 synthesis and secretion, hepatocytes in culture were treated with molecules (ghrelin, insulin, free fatty acid), which are altered with alcohol administration. In addition, we examined the antimicrobial effects of LEAP2 by using an in vitro assay to assess bacterial burden. **Results:** Serum LEAP2 levels were significantly lower in chronic ethanol-fed rats and alcoholic cirrhotic patients compared with their respective controls. We observed that insulin promoted while ghrelin and oleic acid reduced LEAP2 expression and secretion from primary hepatocytes. In addition, purified LEAP2 significantly attenuated bacterial overgrowth in vitro. **Conclusion:** Our studies indicate that the alcohol-induced increased free fatty acid & ghrelin circulating levels and decreases in serum insulin levels may be responsible for the reduction in LEAP2 levels seen in patients and animal models of ALD. This decrease in the circulating levels of the protective LEAP2 could have several detrimental functional ramifications via the reduction in ghrelin receptor antagonism and antimicrobial effect to promote the initiation and progression of ALD.

#50. ABERRANT SPLICING FUNCTIONALLY ALTERS THE LIVER IN ALCOHOL-ASSOCIATED LIVER DISEASE, EXPLAINS COAGULOPATHY.

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Alcohol-associated fatty liver disease (ALD) is an emerging global health issue of the 21st century. Largely unknown is how hepatic alternative splicing program changes as ALD progresses from benign hepatic steatosis to symptomatic steatohepatitis, or, severe alcohol-associated hepatitis (SAH/AH), cirrhosis, and, ultimately, hepatocellular carcinoma. The liver, besides being the master regulator of metabolic processes, is a major hub for making secretory proteins, especially blood clotting factors. Therefore, coagulopathy associated with a diseased liver is commonly observed in patients. Currently, the field reasons abnormal expression of coagulation factors for coagulopathy in liver disease. However, tests often show normal coagulation factor levels in liver disease despite clotting issues – a paradox that lacks explanation. We used a multipronged approach to overcome an intrinsic challenge for genome-wide studies, i.e., transitioning from a descriptive analysis of alternative splicing changes to addressing their functional impact on corresponding proteins. Therefore, our latest research revealed unique hepatic alternative splicing programs in human ALD and aberrant splicing in key members of the secretome. Exemplifying our approach, we predict that fibrinogen Bß chain (FGB, ΔPSI>35%) missplicing and increased secreted isoform of plasminogen activator inhibitor 1 (PAI1, ΔPSI>20%) alters the kinetics of blood clot formation in human ALD. Our findings potentially explain coagulopathy and the physiological response in advanced liver disease. Based on these results, we will redirect the splicing of a select set of disease-linked exons with antisense oligonucleotides in mouse and probe if they contain coherent information with respect to related cellular processes in ALD-associated pathophysiology.

#51. POTENTIAL MECHANISM FOR HEPATIC HEXOKINASE DOMAIN CONTAINING 1 (HKDC1) REGULATION OF GESTATIONAL GLUCOSE LEVELS AND LIVER SIZE.

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Pregnancy demands pronounced maternal physiological adaptations for the sustainable viability of offspring, including increased insulin resistance (IR) and liver size. Unchecked IR can result in gestational diabetes mellitus (GDM) which significantly increases the risk of pregnancy complications and future morbidity; yet the cause of GDM is poorly understood, with the role of the liver being even less characterized. Genome wide association studies have identified genetic polymorphisms in the promoter sequence of hexokinase domain containing 1 (HKDC1) to be associated with gestational glucose levels and GDM. This recently identified protein is expressed under certain conditions in adult liver, including pregnancy. Our previous studies over expressing HKDC1 in liver have shown protective effects against gestational IR and a role in the adaptive increased liver size during pregnancy through unknown mechanisms. Our current work shows, with increasing gestational HKDC1 expression, there is increased Forkhead box protein O1 (FOXO1)—a well categorized transcription factor for glucose homeostasis—activity

based on downstream expression, yet increased FOXO1 phosphorylation. There is also increased products of yes associated protein 1 (YAP) activation and mTOR1 phosphorylation, suggesting HKDC1 may be playing a role in gestational hepatic proliferation via these pathways. Classically, activation of YAP and mTOR1 is associated with inhibition of FOXO1 through phosphorylation, but with increased HKDC1, there is increased O-GlcNAcylation of proteins—a post-translation modification, which has been shown to bypass classical regulation. Collectively, these have begun to help us decipher a mechanism for HKDC1 to increase hepatic glucose utilization and liver size during pregnancy, allowing for protection from impaired gestational glucose levels.

SYMPOSIUM 6: CELLULAR STRESS IN LIVER DISEASE

#52. ROLE OF SQSTM1/P62 IN REGULATING HEPATIC STRESS GRANULES AND MALLORY-DENK BODY ALCOHOL-INDUCED LIVER INJURY.

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Alcohol-associated liver disease (ALD) is a global health problem without an effective treatment. Mallory-Denk Body (MDB) is a protein aggregate commonly found in alcohol-associated hepatitis (AH). It primarily contains ubiquitinated proteins, cytokeratin 8/18, and SQSTM1/p62. However, the role and mechanisms of alcohol-induced MDBs in the pathogenesis of ALD remain largely unknown. Previous studies have shown that chronic plus binge alcohol (Gao-binge alcohol) impairs the proteasome and autophagy-lysosome pathways, which are crucial for removing protein aggregates. In this study, our aim was to investigate the role of autophagy receptor protein SQSTM1/p62 in alcohol-induced protein aggregates, specifically stress granules (SGs) and MDBs, in mouse livers. Our research found that livers of AH patients had higher levels of p62, CK8 (MDB marker), and G3PB1 (SGs marker) when compared to healthy donors using IHC staining. We further discovered that Gao-binge alcohol feeding increased insoluble SG markers, such as Hu antigen R protein (HuR), and phosphorylated Eukaryotic Initiation Factor 2 (p-eIF2α) in mouse livers. Mice fed a DDC diet with Gao-binge alcohol had greater hepatic MDB formation (increased insoluble CK8) and liver injury (increased serum ALT and AST levels) than those fed either diet alone. Loss of p62 led to reduced protein aggregation involved in SGs and MDBs but increased liver injury in DDC plus Gao-binge alcohol-fed mice, indicating that SQSTM1/p62 is required for MDB formation. In conclusion, our data suggests that chronic plus binge alcohol increases hepatic SGs and MDBs, which are mediated by SQSTM1/p62 as an adaptive protective mechanism against ALD.

#53. S-ADENOSYLMETHIONINE PROTECTS AGAINST FOLFOX-INDUCED LIVER INJURY BY INHIBITING PLASMINOGEN-ACTIVATING INHIBITOR-1 IN MICE.

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Background and Aims: FOLFOX leads to poor clinical outcomes in patients with colorectal liver metastases, with sinusoidal obstruction syndrome (SOS) playing a critical role. Consequently, we hypothesized that this could be improved by a hepatoprotective agent such as S-adenosylmethionine (SAMe). SAMe is the principal methyl donor, synthesized in all mammalian cells but most abundantly in the liver. In this study, we explored the protective effect of SAMe against FOLFOX-induced hepatotoxicity along with its underlying mechanism. **Methods:** Murine model of FOLFOX-induced SOS. Hepatocyte specific PAI-1 knockout mice. Primary mouse and human hepatocytes, Kupffer cells (KCs), hepatic stellate cells, and liver sinusoidal endothelial cells (LSECs). Co-immunoprecipitation, chromatin immunoprecipitation and western blot. ELISA, TUNEL assay and RT-PCR. Immunohistochemistry fluorescence. **Results:** SAMe co-treatment completely blocked the induction of markers increased in FOLFOX-induced SOS and protected against liver injury. The most upregulated gene was Serpine1, which encodes for PAI-1. SAMe blocked FOLFOX induced expression and activation of NF-κB, which in turn activated SERPINE1/Serpine1 promoters. Interestingly, FOLFOX failed to activate hepatic NF-κB or cause liver injury in Serpine1 knockout mice. Treatment of mouse hepatocytes, KCs, and LSECs with recombinant PAI-1 (rPAI-1) induced NF-κB activation, expression of proinflammatory cytokines, and CD31, respectively. Conditioned media from rPAI-1 treated hepatocytes also induced activation of KCs and LSECs. FOLFOX and IL-1β induced interaction between PAI-1 with uPAR and vitronectin in mouse liver and hepatocytes, respectively, which was blocked by SAMe. Lastly, rPAI-1 requires interaction with uPAR and vitronectin for full activation of NF-κB in hepatocytes. **Conclusions:** Our results suggest PAI-1 is required for FOLFOX-mediated NF-κB activation, which then further induce PAI-1 expression in a feedforward manner to cause liver injury. SAMe protects against FOLFOX-mediated liver injury in part by inhibiting NF-κB activation and PAI-1 induction.

#54. PHOSPHORYLATION OF UBIQUITIN CONJUGATING ENZYME 9 REGULATES DE-NOVO LIPOGENESIS IN ALCOHOL-ASSOCIATED LIVER DISEASE.

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Background and Aims: Chronic alcohol consumption leads to hepatocellular injury and liver inflammation by increasing translocation of gut-derived endotoxins to the portal circulation and activating Kupffer cells. Activated Kupffer cells (KCs) lead to SREBP-1c and CEBP-δ induction, the key players of de novo lipogenesis in hepatocytes, through the NF-κB mediated mechanism. We recently found that alcohol-induced phosphorylation of ubiquitin conjugating enzyme 9 (UBC9), the sole E2 conjugating enzyme for SUMOylation, leads to NF-κB activation in KCs. In this study, we revisited this crosstalk between KCs and hepatocytes to identify novel therapeutic strategies for ALD. **Methods:** NIAAA animal model and primary mouse hepatocytes and KCs, human steatosis livers.

Phospho-peptide mapping, CRISPR/Cas9 UBC9 Y68 editing in vitro and vivo, proximity ligation assay (PLA). Co-immunoprecipitation and western blot. ELISA and RT-PCR. Oil red O and H&E stain. **Results:** We found that Tyr68 (Y68) of UBC9 is phosphorylated specifically in KCs upon alcohol exposure and it is mediated by SRC kinase. Alcohol-mediated liver injury and lipogenesis in NIAAA model were prevented by inhibition of SRC (PP1 inhibitor) and by CRISPR/Cas9 editing of the UBC9 Y68 to F68 residue that prevented phosphorylation and suppressed inflammatory markers in KCs. Further, blocking UBC9 Y68 phosphorylation attenuated the SREBP1-c and CEBPd levels in hepatocytes and NIAAA mice livers. **Conclusion:** Phosphorylation of UBC9 at Y68 by SRC kinase promotes de novo lipogenesis via SREBP1/CEBPδ induction, suggesting Y68 of UBC9 as a potential therapeutic target for ALD.

#55. HEPATIC STELLATE CELL-INTRINSIC ROLE FOR CYTOPLASMIC STRESS GRANULES IN TGFB-INDUCED FIBROGENESIS.

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A growing body of literature has implicated anti-viral defense pathways, such as nucleic acid-induced activation of the transcription factor interferon regulatory factor 3 (IRF3), in sterile metabolic liver diseases. Furthermore, cell-intrinsic IRF3 activity promotes hepatic stellate cell trans-differentiation and transforming growth factor-beta (TGFβ)-induced fibrogenesis. In order to clarify the underlying molecular mechanisms, we performed a mass spectrometry-based proteomic analysis in the human hepatic stellate cell line LX2 to identify novel IRF3-interacting proteins. Gene ontology analysis of IRF3-binding proteins revealed striking enrichment in RNA metabolism and cellular responses to stress. Unexpectedly, several component proteins of cytoplasmic stress granules were identified, most notably G3BP stress granule assembly factor 1 (G3BP1). Stress granules are large, organized conglomerates of untranslated mRNAs, stalled ribosomes, and RNA-binding proteins that form under various conditions of cellular stress and serve as a form of liquid-liquid phase separation. Immunofluorescence revealed co-localization of G3BP1 and IRF3 within punctate cytoplasmic regions after stimulation with the double-stranded RNA analogue poly(I:C). Finally, pharmacologic inhibition of G3BP1 activity with (−)-epigallocatechin gallate (EGCG) decreased TGFβ-induced expression of collagen 1A1 (COL1A1), α-smooth muscle actin (α-SMA), and monocyte chemoattractant protein 1 (MCP1).Collectively, these results identify that cytoplasmic stress granules likely contribute to the pro-fibrogenic response of hepatic stellate cells to TGFβ, which may occur through altered activation of IRF3.

#56. ROLE OF RECEPTOR-INTERACTING PROTEIN 3 (RIP3) KINASE IN REGULATING HEPATOCYTE ENDOPLASMIC RETICULUM STRESS.

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Background and Aims: Endoplasmic reticulum (ER) stress plays a critical role in the pathogenesis of liver diseases, where hepatocyte death contributes significantly to disease progression. Receptor-interacting protein 3 (RIP3), known for its involvement in programmed necrosis, is implicated in cellular stress responses. In this study, we investigated the impact of RIP3 kinase and its downstream effector MLKL in regulating ER stress and viability of hepatocytes. **Methods:** Rip3-/- and wild-type littermate mice were injected intraperitoneally with either vehicle or tunicamycin at the dose of 0.5 µg/g body mass to induce ER stress. Primary hepatocytes isolated from wild-type and Rip3-/- mice were first pretreated with either Rip3 kinase or MLKL inhibitors for 2h and then challenged with or without thapsigargin and tunicamycin for up to 6 h. ER stress markers were assessed in liver and hepatocytes using western blot and qPCR. Hepatocyte viability was evaluated using MTS assay. **Results:** Administration of tunicamycin to wild-type, but not Rip3-/-, mice increased expression of hepatic RIP3 and MLKL Similarly, tunicamycin increased expression of ER stress markers and cleaved PARP in the liver of wild-type, but not Rip3-/-, mice. In primary hepatocyte cultures, Rip3 deficiency or pharmacological inhibition of RIP3 kinase activity or MLKL attenuated ER stress markers at both mRNA and protein level, including p-IRE1α, CHOP and GRP78. Furthermore, hepatocytes isolated from RIP3-deficient mice had enhanced cell viability under ER stress conditions compared to hepatocytes from wild-type mice. **Summary:** Combining both in-vivo and in-vitro experiments, our study underscores the significant contribution of RIP3 and its downstream effector MLKL to hepatic ER stress. This reveals a potentially novel role for RIP3 and MLKL in ER stress responses.

#57. CHARACTERIZATION OF THE ROLE OF EPITHELIAL SPLICING REGULATORY PROTEIN 2 IN REGENERATION INDUCED BY ACUTE LIVER INJURY.

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The liver maintains an extensive ability to regenerate in response to various physical and toxic injuries. Despite this, acute liver failure may occur due to severe drug induced liver injury, wherein a dose threshold is surpassed, and regeneration is prevented. This failure to regenerate typically occurs after initial drug clearance; and is associated with the inhibition of cell proliferation or overproduction of immature hepatocytes incapable of carrying out desired metabolic functions. However, the underlying mechanisms driving such maladaptive regenerative responses are unclear. Recently, we demonstrated that regulated changes in alternative splicing are required for proper liver regeneration, and we identified Epithelial Splicing Regulatory Protein 2 (ESRP2) as the key splicing factor that coordinates the liver's regenerative response. Here, I studied the role of ESRP2 in drug-induced acute liver injury and its effects on alternative splicing that support liver regeneration. Using an acetaminophen (APAP) overdose model and ESRP2 knockout mice, I identified ESRP2-dependent differences in the injury, progression, and regeneration phases after APAP-induced acute liver injury. Importantly, I found a temporally linked suppression of ESRP2 within regenerating hepatocytes after acute

injury with a corresponding shift in splicing of its direct RNA targets. Together, these data highlight the importance of ESRP2 and its splicing activities in liver repair and regeneration following drug-induced acute liver injury.

#58. MONOCARBOXYLATE TRANSPORTER-1 IS DISPENSABLE FOR HEPATOCELLULAR CARCINOMA DEVELOPMENT IN DEN/CCL4 MOUSE MODEL.

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Background: Hepatocellular carcinoma (HCC) is the most prevalent type of liver cancer and the deadliest liver disease. It is imperative to understand the underlying molecular mechanisms involved in the development of HCC. Monocarboxylate transporter-1 (MCT1) is a proton-coupled protein that facilitates the bidirectional transport of monocarboxylates, such as lactate and pyruvate, across the plasma membrane to maintain the cellular metabolism and energy supply. MCT1 was found to be upregulated in human specimens, and its inhibition reduced xenograft tumor growth. However, the role of MCT1 in HCC remains to be further investigated using immune-competent in vivo models. **Methods and Results:** To better understand the role of MCT1 in HCC, we established liver-specific MCT1 knockout mice. We found that deletion of MCT1in hepatocytes did not affect morphology, proliferation, or apoptosis. DEN/CCl4 model, where a single injection of DEN is followed by repeated injections of CCl4, was used to induce HCC in mice. Intriguingly, we found that liver-specific knockout of MCT1 was not sufficient to reduce the size or count of DEN/CCl4-induced liver tumors. In addition, we used immunohistochemical staining to evaluate the expression of Ki67, collagen A1, and myeloperoxidase (MPO), and we found that MCT1 knockout was not able to hinder the proliferation, fibrosis, and inflammation in the DEN/CCl4-induced HCC tumors. **Conclusions:** MCT1 is dispensable for HCC development and its inhibition was insufficient to alleviate the phenotypic repercussions of HCC tumors in DEN/CCl4-induced HCC model.

#59. AGING EXACERBATES ALCOHOL-INDUCED LIVER INJURY VIA IMPAIRED AUTOPHAGY AND INTEGRATED STRESS RESPONSE.

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Background: Alcohol-associated liver disease (ALD) is a major chronic liver disease with no successful treatment. Aging is a well-known risk factor associated with ALD. However, the mechanisms of how alcohol and aging cooperatively contribute to ALD in older adults remain incompletely understood. Alcohol and cellular aging are known inducers of endoplasmic reticulum (ER) stress leading to the activation of the integrated stress response (ISR). ISR activation is not well studied in aging and alcohol, thus the goal of the study was to investigate changes in autophagy and ISR in alcohol-induced liver injury in aged mice. **Methods:** Young (3-month-old) and aged (22-month-old) C57BL/6N mice

were subjected to Gao-binge alcohol feeding. Unbiased liver tissue RNAseq was performed. Tail-vein injection of adenovirus-ATF3 was performed to overexpress hepatic ATF3 in mouse livers with Gao-binge alcohol. Liver injury, steatosis and autophagy were determined. **Results:** Analysis of RNAseq showed that aging and alcohol have distinct effects on hepatic gene expression. The levels of hepatic triglycerides increased while lysosomal protein expression decreased in ethanol-fed aged mice compared to young control diet-fed mice. Genes associated with UPR were upregulated due to alcohol consumption and increased with age. Hepatic expression of proteins associated with ISR were significantly induced by Gao-binge alcohol and increased further with age. Overexpression of ATF3 in livers protected against alcohol-induced liver injury in aged mice. **Conclusions:** Aging exacerbates alcohol-induced hepatic steatosis and ISR, which may function as an adaptive response in protecting against alcohol-induced liver injury.

SYMPOSIUM 7: METABOLIC STRESS IN LIVER DISEASE

#60. HEPATOCYTE-DERIVED ARG2 PROTECTS FROM METABOLIC DYSFUNCTION ASSOCIATED STEATOHEPATITIS.

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Arginase 2 (ARG2) is a mitochondrial protein that regulates cell respiration. We have previously reported that induction of ARG2 in hepatocytes mediated the protective effect of *Spp1*^{high} macrophages in the context of metabolic-associated steatohepatitis (MASH). However, the mechanism of ARG2 protected against MASH is unknown. *Arg2* knock-out mice in hepatocytes (*Arg2*$^{\Delta Hep}$) were generated. Mice were fed a MASH-inducing or an isocaloric control diet for 6 months. Control littermates fed with the MASH-inducing diet developed key features of NASH, including steatosis, hepatocyte ballooning degeneration, inflammation, and chicken-wire fibrosis. These events were more prominent in male than in female mice. However, *Arg2*$^{\Delta Hep}$ worsened the MASH phenotype, as shown by a significant increase in liver inflammation, ductular reaction, and fibrosis. In both genders, *Arg2*$^{\Delta Hep}$ increased the level of metabolic syndrome characterized by increased body weight, content of epididymal adipose tissue, baseline glucose, and insulin resistance. WT hepatocytes treated with siRNA targeting ARG2 show decreased basal respiration, maximal respiratory capacity, spare respiratory capacity, and ATP production when using palmitic acid as an energy source. Therefore, ARG2 protects from MASH by regulating fatty acid oxidation in mitochondria.

#61. MECHANISMS OF RETINOIC ACID SIGNALING RESPONSE TO ETHANOL IN ALCOHOL-ASSOCIATED LIVER DISEASE.

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Vitamin A (VA, retinol) possesses hepato-protective properties and reductions to hepatic VA correlate with the progression of alcohol-associated liver disease (ALD). However, hepatic mRNA levels of the key signaling effectors of VA, the retinoic acid receptors (RARa, b, g), do not always reflect the hepatic VA or its bio-active metabolite retinoic acid (RA) levels. Using short (10 days) and long-term (3, 4 and 8 weeks) durations of a murine Lieber De Carli liquid alcohol-diet model, the aim of this study is to examine the temporal effect of alcohol on hepatic mRNA expression of RARa, b, g and other markers of hepatic RA-signaling. After 10-days of alcohol treatment, hepatic retinol (ROL) and retinyl palmitate (RP) levels decreased by 2.5-fold and 5-fold, respectively, compared to control diet-fed mice. Conversely, hepatic mRNA levels of the RA-receptor, RARb, and Cyp26A1, a key RA-catabolism enzyme, increased over 7-fold. After 3-weeks of alcohol treatment, hepatic ROL and RP remained 1-fold and 3-fold lower compared to control-fed mice, however RARβ and Cyp26A1 mRNAs markedly declined until their levels showed no increases at 8-weeks of ethanol treatment. RARα and γ mRNA levels exhibited no significant changes in expression throughout the treatments. Taken together, these data show that short-term alcohol-induced increases in the RARb-signaling pathway were not sustained but rather declined with long-term alcohol exposure, possibly due to alcohol-induction of RA-catabolism enzyme Cyp26A1. In summary, this work defines how ethanol shapes the VA signaling in early stages of ALD.

#62. EXPLORING THE EFFECT OF HEPATOCYTE-SPECIFIC BHMT IN THE REVERSION OF METABOLIC DYSFUNCTION-ASSOCIATED STEATOHEPATITIS (MASH).

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Metabolic dysfunction-associated liver disease (MASLD) prevalence is exceptionally high, and there is no FDA-approved pharmacological treatment yet. We showed that the knockout of hepatocyte peroxisome proliferator-activated receptor gamma reduces the progression of diet-induced MASH, partly through the upregulation of genes involved in methionine and homocysteine (Hcy) metabolism. Of note, elevated levels of Hcy in MASH are associated with reduced expression of betaine-homocysteine S-methyltransferase (Bhmt), and correlate with hepatic inflammation and fibrosis. Here, we explored the effect of AAV8-mediated restored activity of BHMT, a hepatocyte-specific enzyme that transfers methyl groups from betaine to homocysteine, in mice with MASH. Briefly, male mice were fed a high-fat, high-cholesterol, and high-fructose (HFCF) diet for 20 weeks. Then, a subset of mice was injected with AAV8-TBG-Bhmt, and livers were collected 8 weeks later. HFCF-fed mice developed MASH with fibrosis, and the AAV-mediated expression of BHMT reduces liver steatosis and MASH progression. In addition, we measured a panel of 307 metabolites in the liver with LC/MS. HFCF diet regulated 66 metabolites, including Hcy (increased). Our enrichment analysis revealed that the HFCF diet impacted sets of metabolites related to carbohydrate metabolism and downregulated components in the Pentose Phosphate Pathway, Starch/Sucrose, and Fructose/Mannose Metabolism.

Conversely, AAV-mediated restoration of BHMT activity regulated 47 metabolites, including Hcy (reduced). The restored BHMT altered sets of metabolites related to Hcy, polyamines, and methionine metabolism. Our findings extend the knowledge of the effects of diet-induced NASH in hepatic metabolism and the positive effects of BHMT on liver physiology and amino acid metabolism.

#63. EXPRESSION OF GLYCOLYTIC GENES IN NAFLD CORRELATES WITH THE RISK OF HEPATOCYTES DYSFUNCTION.

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NAFLD is the most prevalent liver disease, of which currently has no approved therapies for treatment. The liver is a hub for metabolism, specifically glycolysis. In unhealthy livers, there is a shift from oxidizing glucose via glycolysis to converting pyruvate to lactate. The specific mechanistic metabolic changes in the progression of liver disease remain unclear, but the hypothesis suggests that alterations in glucose metabolism contribute to exacerbating the progression of liver disease. To address the gap in knowledge, the study conducted a differential gene expression analysis using two datasets from the Gene Expression Omnibus (GEO) database, GSE126848 and GSE89632. Differential expression of glycolytic genes was analyzed using an ordinary one-way ANOVA test ($p<0.0001$), and significant differences among means were determined with $p<0.05$. Prism GraphPad was used for the analysis. Genes related to glycolysis, oxidative phosphorylation, and the oxidative pentose phosphate pathway were downregulated in unhealthy/diseased patients. Conversely, those involved in the tricarboxylic acid (TCA) cycle, such as IDH2 and SDHB, were highly expressed. The heightened activity of the hepatic TCA cycle suggests an increase in mitochondrial respiration correlating with liver disease progression. The conclusion supports the findings of decreased expression of glycolytic genes, explaining that citrate, an intermediate of the TCA cycle, inhibits multiple glycolytic enzymes, suppressing glycolysis. The differentially expressed genes offer insights into potentially modifying the metabolic changes occurring in the progression of liver disease.

#64. LOSS OF CDKN1A PROTECTS AGAINST METABOLIC STRESS IN MASLD/METALD.

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Background: Cyclin-dependent kinase inhibitor 1 (CDKN1A) is involved in cell cycle arrest in response to a variety of stimuli. However, its role in MASLD/MetALD remains elusive. **Methods:** CDKN1A analysis was performed in 5 patient cohorts encompassing a whole spectrum of CLD. The preclinical models consisted of 8-13-week-old B6j mice fed with a WD for 14 weeks, DuAL diet consisting of WD and 10% vol/vol absolute ethanol in drinking sweetened water for 18 weeks, and a Lieber-deCarli diet plus multiple EtOH binges for 8 weeks. Additionally, the efficacy of Palbociclib, a CDK4/6 inhibitor that activates p21 in p53-independent manner, was tested in a DuAL diet for 10 weeks. **Results:** CDKN1A expression was found to correlate with the severity of liver damage. Significant increase in CDKN1A expression was observed in patients with cirrhosis or fibrosis stages >3 compared to lower stages. Mutant mice lacking CDKN1A exhibited reductions in various aspects compared to CDKN1A-intact counterparts when subjected to a WD and DuAL. (i) Decrease liver damage markers, (ii) hepatic steatosis was diminished, (iii) oxidative stress was lowered, (iv) cell death decreased, (v) reduced inflammation and fibrosis. Remarkably, treatment with Palbociclib led to a decrease in liver damage and fibrosis in mice subjected to DuAL for 10 weeks. However, no significant differences were observed between mutant and WT mice in preclinical MetALD, except for decreased lipid accumulation. **Conclusions:** CDKN1A-/- deletion protected against preclinical MASLD by promoting cellular senescence, inflammation, and metabolic reprogramming. Our novel results indicate that CDKN1A could be potential theragnostic target for the treatment of MASLD.

#65. A NOVEL ROLE OF STARD10 IN ERBB2-MEDIATED LIPOGENESIS IN ALCOHOL-ASSOCIATED LIVER DISEASE.

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Background and aims: In developed countries, one of the most important risk factors for liver cirrhosis and HCC is alcoholic steatohepatitis (ASH). Recently, ErbB2 (transmembrane receptors with tyrosine kinase activity) has been described in ASH and correlated with severity of inflammation and signs of longstanding liver injury like liver fibrosis. ErBb2 has the ability to translocate into the nucleus and mediates survival effects in injured hepatocytes. StAR-related lipid transfer domain containing 10 (StarD10) is a phosphorylated protein that positively regulates ErBb2 signalling pathway. We recently reported that ethanol administration lowers the phospho status of StarD10 leading to increased plasma membrane fluidity and induced ErbB2 activity in breast cancer. This study aims to investigate whether phosphorylation of StarD10 influences ErbB2 nuclear trafficking and activity in alcohol associated liver disease (ALD). **Methods:** NIAAA mice livers and primary mouse hepatocytes. Western blot and immunoprecipitation. Duolink PLA and immunofluorescence. **Results:** We found increased protein levels of StarD10 and nuclear ErbB2 in NIAAA livers as well as in ethanol-treated hepatocytes. Also, co-

immunoprecitated and co-localized StarD10 and ErbB2 were observed in vitro and in vivo. Overexpression of StarD10 increased membrane fluidity in hepatocytes that presumably caused ErbB2 nuclear translocation from plasma membrane. In addition, overexpressed ErbB2 or ethanol treatment induced cyclin D1 expression and its downstream target genes such as FAS and ACC, the de novo lipogenesis related gene. **Conclusion:** This study suggests a potential role for StarD10-induced nuclear ErbB2 to cause liver steatosis upon alcohol treatment. It also opens a wide field of research about functional implications of StarD10 and nuclear ErbB2 cross-talk deducing possible therapeutic strategies in ALD.

#66. ABSENCE OF LIVER PEX16 LEADS TO HEPATOCYTE PROLIFERATION: POSSIBLE ROLE OF BILE ACIDS.

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Peroxisomes are classic membranous organelles present in all eukaryotic organisms. Peroxisomes grow by employing PEX16 along with PEX19 and PEX3 to import newly synthesized proteins and lipids into the peroxisomal membrane to form a complete peroxisomal structure. PPARα agonist WY-14,643 induced peroxisome proliferation via inducing PEX16, which was not observed in pparα knockout mice. In liver-specific PEX16 knockout mice (Pex16-Alb-Cre), structural peroxisomes were absent as indicated by the absence of peroxisome marker PMP70, but peroxisomal matrix enzymes ACOX1 and catalase are upregulated. Due to lack of peroxisomes, the upregulated ACOX1 and catalase were in cytoplasm. Interestingly, hepatomegaly and hepatocyte proliferation were observed in the Pex16-Alb-Cre mice as indicated by elevated staining of proliferation markers PCNA and Ki67 as well as elevated expression of cell division genes, which was not further induced by WY-14,643. RNA-Seq analysis showed that sterol and bile acid metabolism pathways were upregulated in the Pex16-Alb-Cre mice, and consistently, expression of cholesterol and bile acid synthesis enzymes and transcriptional regulators were upregulated in the Pex16-Alb-Cre mice. While serum cholesterol was not changed, serum bile acids were upregulated in the Pex16-Alb-Cre mice. Bile acids have recently been identified as liver-specific metabolic signals and promote liver regeneration. The last a few steps of bile acid synthesis are in peroxisomes. It is probably that peroxisome association with hepatocyte proliferation is mediated by bile acid.

#67. LOSS OF HEPATOCYTE GROWTH HORMONE RECEPTOR PROMOTES DUCTULAR REACTION AND FIBROSIS THAT IS REDUCED BY STAT5B.

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Growth hormone (GH) deficiency is associated with MASH, which is reduced by GH therapy. Some of the protective actions of GH may be direct since we previously reported chow-fed male mice with adult-onset, hepatocyte-specific, GH receptor knockdown (aHepGHRkd) develop liver steatosis, and with age a mild MASH-like phenotype. To determine if aHepGHRkd accelerates diet-induced MASH, GHR-intact control and aHepGHRkd male mice were fed a high-fat diet with glucose/fructose in the drinking water (HFS) for 3 months. Since GHR signals through the transcription factor STAT5b, and STAT5b is required to sustain hepatocyte insulin-like growth factor 1 (IGF1) production, a subset of aHepGHRkd mice were treated with AAVs that express IGF1 or a constitutively active STAT5b (STAT5bCA) in hepatocytes. In the context of HFS diet, aHepGHRkd did not alter liver weight, hepatic triglyceride content or lipid composition compared to GHR-intact mice. However, aHepGHRkd dramatically advanced liver injury, increasing plasma ALT levels, F4/80-stained macrophages forming crown-like structures (hCLS; marking dying hepatocytes) and periportal and invasive ductular reaction (DR, Krt19+ cells) associated with fibrosis, all features not observed in GHR-intact controls. STAT5bCA, but not IGF1, reduced DR and fibrosis in aHepGHRkd livers, without impacting hCLS. RNA-seq analysis revealed genes related to hepatocyte regeneration (Lifr, E2f8, Onecut1/HNF6) were decreased by aHepGHRkd and increased by STAT5bCA. We hypothesize that hepatocyte GHR/STAT5 acts independent of steatosis to support hepatocyte regeneration in the context of chronic diet-induced liver injury, while loss of GHR/STAT5 leads to maladaptive regenerative programs (DR) and fibrosis.

#68. ASSESSING THE ROLE OF ABL2 IN THE PROGRESSION OF ALCOHOL-ASSOCIATED LIVER DISEASE.

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Background: Alcohol-associated liver disease (ALD) is a primary cause of chronic liver disease worldwide. Outside of alcohol abstinence, there are no FDA-approved treatments for ALD, necessitating a greater understanding of ALD pathogenesis and molecular targets for intervention. ABL2 is a non-receptor tyrosine kinase that participates in diverse cellular functions and is highly activated in ALD patient liver samples and liver tissues from mice subjected to alcohol feeding. **Methods and Results:** To better understand the role of ABL2 in ALD, we established a strain of liver-specific ABL2 knockout mice. Following alcohol feeding, the knockout of ABL2 attenuated alcohol-induced steatosis, liver injury, and inflammation. Subsequent RNA-seq and GSEA of mouse liver tissues revealed that ABL2 knockout alcohol-fed mice exhibited significantly decreased focal adhesions and PPAR signaling. First, we confirmed that focal adhesion kinase (FAK) is activated in the presence of alcohol, however a liver-specific knockout of FAK was not able to attenuate alcohol-induced liver damage. Subsequent investigation into PPAR signaling revealed that PPARg was induced upon alcohol feeding in wild-type mice, but

not in ABL2 knockout mice, suggesting a novel role for ABL2 in the regulation of PPARg. Furthermore, we assessed the ability of FAK to mediate this novel regulation of PPARg by ABL2 but instead found that HIF1a is required for the ABL2-mediated induction of PPARg expression. **Conclusions:** We propose that alcohol-induced ABL2 activation promotes ALD through increasing HIF1a and subsequent PPARg expression, and ABL2 inhibition may serve as a promising target for the treatment of ALD.

#69. ELUCIDATING THE ROLE OF CONSTITUTIVE ANDROSTANE RECEPTORS IN MAINTAINING HEPATOCYTE PLOIDY.

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The nuclear receptor Constitutive Androstane Receptor (CAR/NR1i3) is primarily known for its detoxification function in the liver. Apart from detoxification, it also has a role in metabolism and proliferation. Hepatocytes make up to 80% of the liver population and they exhibit polyploidy. Here, we are investigating the role of CAR in maintaining hepatocyte ploidy. Preliminary studies with FACS sorting have shown that the CARKO hepatocytes have enriched 2c hepatocytes compared to the WT (wild type), whereas CAR activation increased polyploidy. On further examination, we have discovered that CAR on activation binds and promotes Ribonucleotide reductase- M2 (RRM2) transcription. RRM2 is a subunit of Ribonucleotide reductase enzyme (RNR) complex, and it's the rate-limiting step in the de novo dNTP synthesis. Consistently, we find that CAR activation led to an increase in Deoxynucleotide triphosphates (dNTPs) levels in the liver. We also found that we were able to rescue some of the lost ploidy in CARKO by overexpression of RRM2. These results suggest that the CAR helps in regulating the liver ploidy via RRM2-mediated DNA synthesis.

#70. MYOTONIC DYSTROPHY TYPE 1 CAUSES HEPATIC STEATOSIS AND REDUCES HEPATIC DRUG METABOLISM.

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Myotonic Dystrophy type 1 (DM1) is multi-systemic muscular dystrophy, affecting 1 in 2800 people. While DM1 affects multiple systems, recent studies highlight its link to liver pathology, glucose intolerance, and drug sensitivity, suggesting the disease may interfere with hepatic function. To understand the effects of DM1 in the liver, we generated a DM1 mouse model that expresses CUG repeat-containing RNA within hepatocytes. Through these mice, we show that the expression of CUG RNA in hepatocytes sequesters MBNL RBPs, resulting in an altered transcriptome and causing a reduction in mature hepatocellular activity. Characterization of the misregulated hepatic events led to the discovery that DM1 drives morphological changes, increased inflammation, and lipid accumulation in the liver. Additionally, DM1 reduces the liver's capacity to respond to

xenobiotics and sensitizes the liver to diet-induced NAFLD, as genes involved in drug metabolism and lipid biosynthesis, transport, and metabolism-related functions are misregulated. The 28th exon of acetyl-CoA carboxylase 1 (ACC1), the rate-limiting enzyme in fatty acid synthesis, is of interest. This exon shows increased inclusion in the livers of the DM1 mice and may affect ACC1 phosphorylation and activity. We have shown that ACC1 levels in the DM1-afflicted mice increase in addition to the exon switching, and that inhibition of ACC1 reverses the lipid accumulation brought on by DM1. These results reveal that expression of CUG repeat-containing RNA disrupts normal hepatic functions and predisposes the liver to injury and fatty liver disease, which jeopardizes the health of DM1 patients and complicates the treatment of DM1.

#71. GROWTH ARREST AND DNA DAMAGE-INDUCIBLE 45A (GADD45A) DIFFERENTIALLY REGULATES FIBROGENESIS IN HUMANS AND MICE.

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Cirrhosis involves the accumulation of extracellular matrix driven by activated hepatic stellate cells (HSCs). Endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) are critical in promoting HSC activation and fibrogenesis. The UPR-responsive gene GADD45A regulates the cell cycle and directly regulate protein kinase activity in skeletal muscle, but its contribution to liver fibrosis is unclear. We hypothesize that GADD45A regulates HSC proteostasis to facilitate prolonged activation, survival, and fibrogenesis. GADD45A increased in cirrhotic patients (GSE14323), fibrotic mice (GSE120281/GSE149508), and primary human HSCs (hHSC) treated with TGFβ. Inhibition of UPR pathways revealed that ATF6α or PERK regulated TGFβ induction of GADD45A in HSCs. Additionally, autophagy inhibition increased intracellular GADD45A, revealing post-translational regulation of GADD45A. siRNA-mediated reduction of GADD45A in immortalized HSCs (LX-2 cells) limited TGFβ-induction of collagen-Iα1, αSMA, and phosphorylation of p38, while increasing expression of senescence marker p18, implicating GADD45A in HSC activation, fibrogenesis, and senescence, potentially through p38. We isolated HSCs from Gadd45af/f mice, deleted Gadd45a using an adenoviral-delivered Cre or GFP control, and harvested HSCs after 3 or 7 days. Surprisingly Gadd45a loss increased Col1α1 expression compared to control HSCs, and HSC specific ablation of GADD45A mice using PDGFRBCreERT2, showed no change in fibrosis after 6 weeks of CCl4 injection compared to wild-type mice. In conclusion, GADD45A expression increased in activated HSCs, but this expression may be temporal- and stimuli-dependent, which could differentially impact fibrosis. Thus, inhibition of GADD45A signaling may be an additional important approach for the management of fibrosis phenotypes by limiting HSC activation.

#72. HEPATOCYTE-SPECIFIC DELETION OF THE NOVEL HEXOKINASE, HKDC1 PROTECTS AGAINST METABOLIC ASSOCIATED STEATOHEPATITIS (MASH).

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Metabolic-associated steatohepatitis (MASH), the inflammatory stage of MASLD will soon be one of the primary causes of liver-related complications including hepatocellular carcinoma. Our published data shows a clear role of novel hexokinase HKDC1 in the progression of liver disease, and targeting HKDC1, which is highly expressed in pathological hepatocytes (MASH) but negligible in normal hepatocytes, represents a highly selective approach. Therefore, we hypothesized that hepatocyte-specific deletion of HKDC1 will protect against diet-induced obesity and progression to MASH. To test our hypothesis, we used two different mouse models of hepatocyte-specific knockout (HKO) of HKDC1: (a) early-onset (eHKDC1HKO) and (b) adult-onset (aHKDC1HKO). We fed the Western Diet to these two groups of female mice along with HKDC1 floxed mice (HKDC1fl/fl; as Controls) for 28 weeks. Our data shows that HKDC1 deletion (both early and adult-onset) significantly decreases body weight and fat mass compared to HKDC1fl/fl mice with no changes in food intake and energy expenditure. Both knockout groups have greater glucose tolerance and lower fasting glucose levels than HKDC1fl/fl mice. Our data also shows that both knockout groups have smaller livers, healthier liver parameters, less steatosis and lower NAS and fibrosis scores. Furthermore, both knockout groups had significantly low proinflammatory and profibrogenic gene expression. Although we did not see any changes in triglyceride levels, there was a significant reduction of serum cholesterol concomitant with enhanced expression of cholesterol metabolism genes in both knockout groups. Taken together, our data suggest a promising protective role of HKDC1 deletion against diet-induced obesity and MASH mice.

#73. LIPIDOMICS APPROACH TO ANALYZE THE MECHANISMS OF PHARMACO-NUTRITIONAL PREVENTION FOR METABOLIC DYSFUNCTION AND STEATOHEPATITIS.

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Dietary glycine has been shown to prevent a variety of liver injuries including metabolic dysfunction-associated steatohepatitis (MASH) in rodent models. Here, we evaluated the effect of dietary glycine on hepatic lipid profile and glucose metabolism in advance to develop overt steatohepatitis in KK-Ay mice. Mice were fed a semisynthetic diet containing 5% glycine or the equivalent amount of casein as controls for 1 week. Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were then performed. Hepatic expression of Male, 8-week-old KK-Ay mice fed a

diet containing 5% glycine for 1week showed marked improvement of IPGTT, as well as IPITT, indicating that dietary glycine prevents insulin resistance. Increases in hepatic mRNA levels for sterol regulatory element-binding protein (SREBP) 1c and fatty acid synthase (FAS) in KK-Ay mice were blunted nearly 1/3 in mice fed a glycine-containing diet. Further, a widely targeted lipidomics by ultra-high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS) analysis revealed that dietary glycine changes lipid composition in the liver dramatically. Dietary glycine dramatically alters hepatic lipid profiles in KK-Ay mice prior to develop overt pathological features of steatohepatitis, with decreased hepatic contents of saturated fatty acids (SFAs) and mono-unsaturated fatty acids (MUFAs) but increased levels in poly-unsaturated fatty acids (PUFAs), especially omega-3 PUFAs. Glycine most likely prevents hepatic lipotoxicity, leading to suppression of SREBP1c and downstream lipid metabolizing enzymes, thereby ameliorating steatohepatitis in these mice. It is therefore concluded that pharmaco-nutritional approach utilizing glycine is promising for prevention and treatment of MASH.

#74. EFFECT OF FASTING AND HIGH CARBOHYDRATE DIET ON HISTONE ACETYLATION TURNOVER IN MICE LIVER.

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Histone acetylation connects substrate metabolism with transcription, relying on glucose as the primary source of acetyl-CoA. However, the contribution of other substrates to histone acetylation in response to nutritional changes remains unknown. Utilizing 2H2O-labeling, we investigated acetyl-CoA sources and acetylation turnover during a high-carbohydrate diet (HC) and fasting in mice. Wild-type mice, with free access to chow or subjected to a 48-hour fast, and a third group on an 8-week HC diet, were euthanized at various time points following 2H2O exposure. Liver histones were analyzed using a proteomics approach. Fasting depleted adipose fat, associated with a decreased respiratory exchange ratio and elevated hepatic free fatty acids. 2H rapidly incorporated into acetylated peptides, selectively labeling the acetyl moiety but not the peptide backbone. Kinetic analysis revealed swift acetylation turnover with K23 site of H3.2 acetylating three times faster than K18 in control mouse livers. Fasting, not a HC diet, significantly reduced acetyl plateau labeling by 34%, suggesting dilution by fatty acid oxidation and fasting increased acetylation turnover at K9 and K14 sites of H3.2 without altering stoichiometry. However, fasting decreased the acetylation stoichiometry of H4k12ac and H4k16ac ($P<0.05$) compared to controls. HC decreased acetylation turnover of histone H4k16ac compared to controls ($P<0.001$) without significant changes in acetylation stoichiometry. Irrespective of the diet, acetylation levels and turnover rates exhibited a reverse correlation in both histones H3 and H4. In conclusion, glucose is the primary source of acetyl-CoA in all conditions, with a partial contribution from fatty acids in the fasting state.

#75. ETHANOL-INDUCED POST-TRANSLATIONAL ACETYLATION ALTERS HEPATIC METABOLISM IN MICE.

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Post-translational acetylation of proteins at lysine side chains, driven by the central metabolite acetyl-CoA, is a crucial regulator of proteostasis. Ethanol metabolism in the liver induces protein acetylation, leading to disruptions in hepatic substrate metabolism. While acetylation can influence gene transcription, enzyme activity, and stability of proteins, the role of ethanol-induced acetylation in hepatic metabolism is still unclear. We used a 2H2O-based metabolic labeling approach to investigate the impact of ethanol-induced acetylation on liver metabolism in a murine model of chronic ethanol-induced liver injury. Mice were fed an ethanol containing diet for 25 days; liver proteins and acetylation patterns were monitored during the final 21 days of 2H2O labeling. The proteome, acetylome, and targeted metabolic profiling were conducted to evaluate ethanol-induced alterations in hepatic metabolism. Ethanol induced hepatic steatosis, inflammation, and oxidative stress. It led to reduced turnover of mitochondrial proteins and increased turnover of cytosolic stress response proteins and metabolic enzymes. Ethanol elevated acetylation levels of mitochondrial metabolic enzymes and nuclear histones, with no significant changes in the cytosol. Acetylation stabilized mitochondrial proteins but destabilized histones. The ethanol-induced reduced mitochondrial protein turnover was associated with increased acetylation. Ethanol-induced mitochondrial acetylation was associated with altered levels of acyl-CoAs and acyl-carnitines, amino acids, and citric acid cycle intermediates, reflecting impaired fatty acid oxidation, urea cycle and citric acid cycle activities. In conclusion, ethanol-induced alterations in acetylome dynamics could modify hepatic substrate metabolism and contribute to liver injury in alcohol-associated liver disease through acetylation-dependent epigenetic changes and the regulation of metabolic enzymes.

#76. KK-AY MOUSE: THE ANIMAL MODEL REFLECTING THE IMPACT OF METABOLIC DYSFUNCTION ON SUSCEPTIBILITY TO ACUTE LIVER INJURY.

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Clinical studies have indicated that metabolic dysfunction-associated steatotic liver disease (MASLD) causes poor prognosis, by its own progression or by exacerbation of a wide spectrum of liver diseases. KK-Ay mice are a strain derived from crossing the diabetic KK mouse with the agouti gene-mutated Ay mouse, and spontaneously develop obesity and steatohepatitis. We used KK-Ay mice to elucidate the impact of MASLD on acute liver injury. KK-Ay mice developed severe liver injury with hepatic necrosis and apoptosis compared to wild-type C57Bl6J mice after administration of acetaminophen. We demonstrated that hepatic glutathione content was originally low in KK-Ay mice, so

glutathione was easily depleted after acetaminophen, leading to phosphorylation of JNK and oxidative stress. Oxidative stress in hepatocytes isolated from KK-Ay mice was significantly more enhanced than in hepatocytes isolated from C57Bl6J mice after acetaminophen. Additionally, KK-Ay mice fed a high-fat diet (HFD) died in significantly greater numbers after administration of sublethal doses of lipopolysaccharides (LPS). Expression of inflammatory cytokines was significantly increased in the livers of KK-Ay mice fed an HFD compared to KK-Ay mice fed a control diet after LPS administration. Chemokine expression was increased in the livers of HFD-fed KK-Ay mice, leading to macrophage accumulation and increased expression of LPS pathways-related molecules such as TLR4 and CD14. Our research results using KK-Ay mice indicate that MASLD is a risk of exacerbation of acute liver injury through by enhanced oxidative stress in hepatocytes and accumulation of macrophages with the enhancement of LPS-related signals.

#77. MASH-ASSOCIATED REPROGRAMMING OF HEPATOCYTE CELL STATE AND SIGNALING REVEALED BY SINGLE NUCLEUS RNA SEQUENCING.

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Metabolic dysfunction-associated steatohepatitis (MASH) pathophysiology is complex due to the involvements of multiple cell types within the liver. Hepatocyte stress and injury serve as the primary driver of hepatic inflammation and fibrosis and disease progression. However, the nature of MASH-associated reprogramming of hepatic signaling and its pathophysiological significance remain incompletely defined. Here we employed single nucleus RNA sequencing (snRNA-seq) to delineate the mechanisms underlying transcriptomic reprogramming and changes in cell states during diet-induced MASH. Analysis of 36,690 mouse hepatic nuclei revealed zone-specific transcriptomic reprogramming within hepatocytes, characterized by de novo activation of disease-associated signaling pathways. Our analysis uncovered ectopic activation of Themis signaling, most notable within pericentral hepatocytes, that contributes to the development of MASH pathologies. Hepatic Themis mRNA and protein expression are markedly induced in mouse and human MASH. Themis ablation exacerbates hepatic steatosis, liver inflammation and fibrosis in mice upon MASH diet feeding. Accordingly, hepatic overexpression of Themis resulted in decreased liver triglyceride content and improved liver fibrosis. Our findings illustrate a novel pathophysiological signaling pathway that promotes key aspects of MASH pathogenesis in mice.

#78. LIPOTOXICITY-INFLUENCED EPIGENETIC ACTIVATION UPREGULATES S100A11 ALARMIN EXPRESSION IN MASH.

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Lipotoxic endoplasmic reticulum (ER) stress is a hallmark characteristic of metabolic dysfunction associated steatohepatitis (MASH) and causes release of proinflammatory signals via extracellular vesicles (EVs). Epigenetic and transcriptomic perturbations mediate individual susceptibility and risk of disease progression in MASH. The epigenetic regulation of lipotoxic ER stress mediated inflammatory signaling remains understudied. We hypothesized that lipotoxic ER stress mediated epigenetic mechanisms regulate enrichment of inflammatory signals within EVs secreted by lipotoxic hepatocytes. We identified S100A11 as a novel stress signal, from an unbiased proteomic screening of EVs secreted from palmitate (PA) treated hepatocytes. We noticed a significant enrichment of both S100A11 mRNA and protein levels in PA-treated hepatocytes, correlating with increased S100A11 in PA-stimulated EVs. Lipotoxic ER stress can activate all three unfolded protein response receptors spanning ER transmembrane on hepatocytes. Hence, we pharmacologically inhibited each of the ER stress sensors and demonstrated that PA-induced upregulation of S100A11 transcripts was IRE1α dependent, and did not involve PERK or ATF6α sensors. To interrogate an epigenetic mechanism by which IRE1α may induce the expression of S100A11, we inspected the S100A11 promoter for active enhancer mark, denoted by H3K27acetylation. We identified an H3K27acetylated, putative cis-regulatory distal genomic DNA region upstream to the transcription start site of S100A11 gene. *In vitro* ChIP-qPCR studies confirmed PA dependent acetylation of this genomic DNA region, which we termed as a *Lipid Influenced Enhancer* (LIE). We observed that PA induced lipotoxic ER stress significantly enriched recruitment of spliced XBP1 onto the promoter of S100A11, and also enhanced H3K27acetylation on the LIE domain which in coherence upregulated the transcriptional burst of S100A11. We verified the functionality of the LIE domain within PA induced transcriptional program of S100A11 using a CRISPRi based approach. Transient introduction of LIE-sgRNAs into dCas9-KRAB expressing Huh7 cells significantly alleviated PA induced H3K27acetylation marks on the LIE domain, with expected repression of S100A11 transcripts. Taken together, our studies have identified a functional lipotoxic ER stress-dependent enhancer which upregulates the expression of the pro-inflammatory alarmin S100A11. As the livers of human MASH patients and murine MASH models have upregulated S100A11 levels, we anticipate targeting S100A11 via an epigenetic axis to be a novel and plausible therapeutic avenue to repress MASH phenotype.

SYMPOSIUM 8: THE FIBROTIC ECM ENVIRONMENT IN LIVER DISEASE

#79. EXPLORATION OF THE MATRISOME COMPOSITION OF HEPATOCELLULAR CARCINOMA AND CIRRHOSIS UNRAVELS A CANCER-SPECIFIC EXTRACELLULAR MATRIX.

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Background and Aims: HCC is the third leading cause of cancer-related death and arises in the context of liver fibrosis. Although HCC is generally poorly fibrogenic, some tumors harbor focal intratumor extracellular matrix (ECM) deposits associated with stem-like phenotype; called “fibrous nests.” Our goal was to unravel the protein composition of the ECM of these fibrous nests in comparison with non-cancer associated liver fibrosis, i.e. cirrhosis, and to assess the clinical relevance of this histological phenotype. **Approach and Results:** We performed quantitative matrisome analysis by tandem mass tags mass spectrometry in 20 decellularized human HCCs with high or low-grade intratumor fibrosis and matched non-tumor tissues, as well as in 12 livers from mice treated with vehicle, carbon tetrachloride, or diethylnitrosamine. We found 94 ECM proteins differentially abundant between high and low-grade fibrous nests, including interstitial and basement membrane components, such as several collagens, glycoproteins, proteoglycans, enzymes involved in ECM stabilization and degradation. Pathway analysis revealed a metabolic switch in high-grade fibrosis, with enhanced glycolysis and decreased oxidative phosphorylation. Integrating the quantitative proteomics with transcriptomics from HCCs and non-tumor livers (n = 2,285 samples), we identified a subgroup of fibrous nest HCCs, characterized by cancer-specific ECM remodeling, expression of the WNT/TGFB (S1) subclass signature, poor patient outcome and T-cell exhaustion. We identified a short fibrous nest signature associated with poor outcome by multivariate Cox analysis and measurable by multiplex immunohistochemistry. Overall, we showed that histological analysis of focal intratumor ECM deposition in HCC is of clinical relevance.

#80. MATRISOME GENE-BASED SUBCLASSIFICATION OF PATIENTS WITH LIVER FIBROSIS IDENTIFIES CLINICAL AND MOLECULAR HETEROGENEITIES.

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Background & Aims: The excessive deposition and crosslinking of extracellular matrix (ECM) increases liver density and stiffness, promoting fibrogenesis and enhancing resistance to fibrolysis. Targeting the composition of the ECM or interrupting pathogenic communication with surrounding cells presents a promising therapeutic approach for liver fibrosis. However, the nature and extent of extracellular changes triggering liver fibrosis vary depending on the underlying etiology. This study aimed to unveil matrisome genes clinically relevant to liver fibrosis, independent of etiology. **Approach & Results:** Transcriptomic profiles from liver fibrosis cases of various etiologies were utilized to identify and validate liver fibrosis-specific matrisome genes (LFMGs) and determine their clinical and biological relevance. Dysregulation patterns and cellular landscapes of LFMGs were further explored in mouse models of liver fibrosis progression and regression using bulk and single-cell RNA sequencing (scRNA-seq). A total of 35 LFMGs, independent of etiology, were identified, collectively forming a signature defining liver

fibrosis. Expression of the LFMG signature depended on histological severity and was reduced in regressive livers. Patients with liver fibrosis, even with identical pathological scores, could be subclassified into $LFMG^{Low}$ and $LFMG^{High}$ subgroups, exhibiting distinguishable clinical, cellular, and molecular features. Furthermore, scRNA-seq revealed that microfibrillar-associated protein 4+ (MFAP4+) activated hepatic stellate cells (aHSCs) increased in $LFMG^{High}$ patients and were primarily responsible for the expression and dysregulation of the LFMG signature. Conclusions: the MFAP4+ aHSC-derived LFMG signature classifies liver fibrosis patients with distinct clinical and biological characteristics. Our findings unveil hidden information from liver biopsies that is undetectable using traditional histologic assessments.

#81. MODELING ANTI-FIBROTIC ACTIONS OF EXTRACELLULAR VESICLES USING HUMAN LIVER ORGANOIDS.

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Background: Extracellular vesicles (EVs) are nano-sized particles containing a complex molecular cargo that may have beneficial effects in liver disease. Here, we investigate the therapeutic effects of EVs from mouse AML-12 hepatocytes or bone marrow mesenchymal stem cells (BM-MSC) in human liver organoids (HLOs) or mouse livers and find that HLOs are a novel platform for anti-fibrotic EV therapy. **Methods and Results:** C57Bl6J mice received a normal chow diet or a choline-deficient L-amino-defined diet containing high (60%) fat (HF-CDAA) for 8 weeks. During the last 6 weeks, EVs from AML-12 cells or BM-MSC were administered 3 times a week (1e+9 particles i.p. per injection). HLOs were induced from iPSC over a 20-day period in Matrigel cultures. They were then treated with 0-500mM palmitate and each type of EV (0-25µg/ml) for the next 7 days. Cell damage, hepatocyte functional deficits, and fibrosis-related gene expression (Col1α1, αSMA, CCN2) in CDAA-HF-exposed livers or palmitate-treated HLOs were significantly attenuated by EVs of both types. **Conclusions:** EV anti-fibrotic actions were manifested both in mice in vivo and HLOs in vitro. Since the HLO system is fully human, multicellular, and highly reproducible, it has strong potential for advancing studies of EV therapy in the liver.

#82. ELUCIDATING THE INTERACTIONS BETWEEN IQGAP1-YAP-ECM STIFFNESS IN LIVER CANCER.

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Hepatocellular Carcinoma (HCC) is the second leading cause of cancer-associated deaths worldwide. Complex signaling mechanisms and several cellular processes in cancer are coordinated using scaffolding proteins. One such scaffold, IQ motif-containing GTPase Activating Protein 1 (IQGAP1) is associated with metastasis, inflammation and tumor growth. Previous work has shown that overexpression of IQGAP1 activates Yes-

associated Protein (YAP) in mice and both proteins are induced in clinical HCC samples. Conversely, we found YAP inhibition, also reduces IQGAP1 levels. Extracellular matrix (ECM) stiffness, due to liver fibrosis in HCC, can activate YAP to promote tumor progression. Our preliminary data show that IQGAP1 protein stains in a punctate manner. The characteristics of this puncta are being investigated currently. Remarkably, we find IQGAP1 protein induction and predominant localization at the plasma membrane when HepG2 cells (an HCC cell line) are grown onto a stiff ECM (25 kPa). But the stiffness did not change the IQGAP1 transcript levels. ECM stiffness led to IQGAP1 induction, and high YAP staining in the nucleus. Intriguingly, we find that IQGAP1 colocalizes with actin, a mediator of biomechanical signals in HepG2 cells. This colocalization with actin is maintained irrespective of the stiffness. When we inhibit the actin polymerization with LatA, we noted increased IQGAP1 protein expression. Based on this data, we speculate that IQGAP1 levels may be regulated by actin dynamics and that IQGAP1-Actin-YAP-Stiffness impact hepatic carcinogenesis.

#83. A NOVEL CHEMICALLY DEFINED ORGANOID CULTURE METHOD TO IMPROVE CHOLANGIOCYTE DIFFERENTIATION.

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Cholangiocyte organoids provide a powerful platform for various applications, from *in vitro* modelling of biliary diseases to tissue engineering for regenerative medicine. However, cholangiocyte organoids are commonly expanded and differentiated in animal-derived hydrogels (*e.g.,* Matrigel and equivalent), which hampers the maturation of the organoids into fully mature cholangiocytes. This is mainly due to the batch-to-batch variability of hydrogels and the abundant presence of laminins that keep the cells immature. Moreover, the clinical application of cholangiocyte organoids is greatly limited by such culture system as the animal derived hydrogels are derived from tumors and and poorly defined. Recently, it has been shown that liver organoids can be expanded in a chemically defined hydrogel. However, the wider applicability of these defined hydrogels, *e.g.,* for cholangiocyte maturation, remains unclear. Here, we investigated a synthetic hydrogel for differentiation of intrahepatic cholangiocyte organoids (ICOs) into functional cholangiocytes. Cultured in the synthetic polyisocyanopeptide (PIC) hydrogels, the ICOs showed a more mature phenotype as indicated by gene expression profiling and protein expression of key cholangiocyte markers. The mature cholangiocyte exhibited farnesoid X receptor (FXR) functionality and bile acid homeostasis. Interestingly, the mature cholangiocyte organoids in the synthetic PIC displayed apical-to-basal polarity, opposite to organoids in collagen I supplemented Matrigel, as shown by stainings for zonula occludens 1 (ZO1), multidrug resistance transporter 1 (MDR1) and laminin receptor (subunit CD49f/integrin α6) . Moreover, we successfully used the mature cholangiocyte

organoids to mimic biliary fibrosis, a phenotype induced by transforming growth factor beta (TGFβ). Taken together, we established a chemically defined culture system that can advance the maturation of ICOs into more mature cholangiocytes with reversed polarity as compared to organoids cultured in Matrigel/collagen I. These results indicate that the chemically defined culture system supports cholangiocyte organoids differentiation and clinical applications in the future.

SYMPOSIUM 9: MECHANISMS DRIVING LIVER REPAIR

#84. USE OF A NANO CARRIER FOR EFFECTIVE SIRNA DELIVERY TO LIVER SINUSOID ENDOTHELIAL CELLS.

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Small interfering RNA (siRNA) is a repressive molecule that can cause gene inhibition leading to mRNA degradation. It is presently under investigation for use as a therapeutic intervention, however RNA cannot freely diffuse across the cell membrane and requires an efficient delivery system to facilitate uptake that also overcomes any potential for nonspecific off-target effects or immune stimulation. Previously, we utilized silver sulfide quantum dots (QDs) for targeted delivery of medications and peptides to liver sinusoid endothelial cells (LSECs) with no immune recognition. Here we will demonstrate the effectiveness of the QDs for delivery of bioactive siRNA for their potential use in mRNA targeting therapy. In this study, the delivery of siRNAs using a nano carrier of siRNA conjugated to an organic quantum dot and coated in various polymers that guide endocytosis, lysosomal determination and cytosolic release has demonstrated a reduction in GAPDH gene expression following oral administration. In vitro studies found a reduction in both LSEC and hepatocyte expression of GAPDH following 4, 24 and 48hr siRNA treatment compared to healthy controls, with no toxicity indicated by cell death and cell proliferation assays. In vivo studies demonstrated a dramatic reduction in LSEC GAPDH expression following orally delivered nano siRNA comparable to healthy controls. Gene knockdown of the nano carrier was comparable to galNAc tail vein injection treated mice. Hepatocytes demonstrated minimal GAPDH downregulation, indicating LSEC specific targeting and delivery. This preliminary work suggests that our nano carrier is a viable delivery agent for gene therapeutics and warrants further investigations.

#85. ORAL NANOTHERAPEUTIC FORMULATION OF INSULIN WITH REDUCED EPISODES OF HYPOGLYCAEMIA.

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Injectable insulin is an extensively used medication with potential life-threatening hypoglycaemic events. Here we report on insulin-conjugated silver sulfide quantum dots coated with a chitosan/glucose polymer to produce a responsive oral insulin nanoformulation. This formulation is pH responsive, is insoluble in acidic environments and shows increased absorption in human duodenum explants and Caenorhabditis elegans at neutral pH. The formulation is sensitive to glucosidase enzymes to trigger insulin release. It is found that the formulation distributes to the liver in mice and rats after oral administration and promotes a dose-dependent reduction in blood glucose without promoting hypoglycaemia or weight gain in diabetic rodents. Non-diabetic baboons also show a dose-dependent reduction in blood glucose. No biochemical or haematological toxicity or adverse events were observed in mice, rats and non-human primates. The formulation demonstrates the potential to orally control blood glucose without hypoglycaemic episodes.

#86. TRANSPLANTATION OF HUMAN IPSC-DERIVED LIVER SINUSOIDAL ENDOTHELIAL CELLS IN AN ACUTE LIVER INJURY MOUSE MODEL AS A CELL THERAPY FOR IMPROVING LIVER REGENERATION.

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Liver sinusoidal endothelial cells (LSECs) are essential for liver homeostasis and function, playing pivotal roles in metabolism, immune response, and regeneration post-injury. Specifically, activation of the VEGFA/KDR axis in LSECs has been reported to support hepatocyte proliferation following hepatectomy. Building on this, we propose that LSEC transplantation therapy will enhance hepatocyte-mediated liver regeneration post-injury. We developed a modified protocol from previous studies to differentiate human induced pluripotent stem cells (hiPSC) into induced LSECs (iLSECs), generating a pure iLSEC population expressing key markers (CD31, CD144, KDR, LYVE1) by day 11 in culture. To evaluate iLSEC engraftment and ability to reconstruct the sinusoid vasculature, luciferase-tagged iLSECs were transplanted into an acute acetaminophen (APAP)-induced liver injury model via intrasplenic injection 24 hours post-APAP administration, and mice were sacrificed 7 days later. In an attempt to investigate the potential role of KDR activation in transplanted iLSECs to improve cell engraftment, a low dose of VEGFA was delivered to the liver via mRNA complexed to lipid nanoparticles. Bioluminescence imaging showed sustained iLSEC engraftment in both VEGFA mRNA-LNP and control mRNA-LNP injected groups for 7 days, indicating successful iLSEC engraftment. We are currently testing increasing doses of VEGFA mRNA-LNP in further improving iLSEC engraftment and function. Immunofluorescence staining for human LYVE1 and CD31 confirmed the integration of iLSECs within host sinusoids. These findings highlight the potential of iLSEC therapy to alleviate liver diseases. This study opens avenues for advanced therapeutic strategies in liver regeneration, leveraging the critical interplay between endothelial cells and hepatocytes.