AASLD Nov. 4-8, 2022

The Liver Meeting®

WASHINGTON D.C.

The Best of The Liver Meeting®

BASIC AND TRANSLATIONAL RESEARCH

About the program:

Best of The Liver Meeting 2022 was created by the Scientific Program Committee for the benefit of AASLD members, attendees of the annual conference, and other clinicians involved in the treatment of liver diseases. The program is intended to highlight some of the key oral and poster presentations from the meeting and to provide insights from the authors themselves regarding implications for patient care and ongoing research.

Use of these slides:

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Glycolysis promotes extracellular vesicle release in hepatic stellate cells through epigenetic regulation of Rab31 to amplify liver fibrosis

Aim

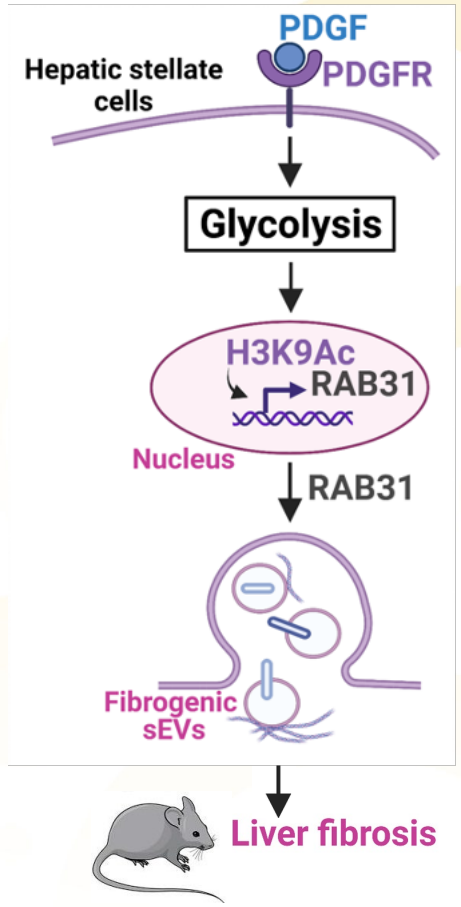
Liver fibrosis is characterized by the activation of hepatic stellate cells (HSCs), which increase their glucose metabolism, termed glycolysis. PDGF-activated HSCs release pro-fibrotic extracellular vesicles (EVs). The aim of this study is to understand the role of glycolysis in PDGF-mediated EV release and liver fibrosis amplification.

Methods

EVs were purified by differential ultracentrifugation and characterized by proteomics. Liver fibrosis in PDGFR$\beta^{CreERT2}$/HK2$^{fl/fl}$ mice and HK2$^{fl/fl}$ littermate controls was induced by CCl_4 administration.

Conclusions

Glycolysis in HSCs promotes fibrogenic EV release by up-regulating RAB31, which might contribute to liver fibrosis amplification.



Khanal S, et al., Abstract 2.

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Transient VEGFA expression using VEGFA mRNA-LNPs induces BEC-driven liver regeneration and reversion of steatosis and fibrosis in a NASH mouse model

Aim

To harness the regenerative potential of biliary epithelial cells (BECs) by promoting their conversion to hepatocytes to treat liver diseases.

Methods

*Krt*19-Cre-ERT2; *R26*-STOP$^{Fl/Fl}$-tdTomato and *Kdr*-2A-Cre^{ERT2}-2A-eYFP, *R26*^{LSL} tdTomato mice were used to fate trace BECs and KDR-expressing cells, respectively. AAV8-*Tbg*-p21 was administered to mimic impaired hepatocyte proliferation observed in many human liver diseases. Chronic injury induced with CDE (0.1% ethionine; 2 weeks) was followed by injections of nucleoside-modified mRNA encoding VEGFA.

Human cirrhotic liver tissues were assessed for BEC-to-hepatocyte differentiation and identification of KDR-expressing BECs and hepatocytes.

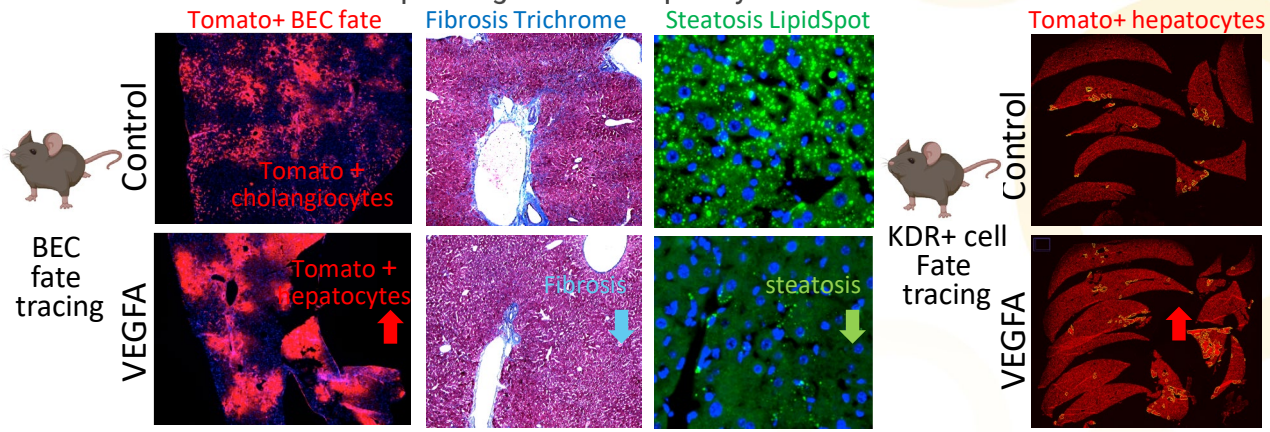
Main Findings

Transient VEGFA expression in chronically injured mice induced robust BEC-to-hepatocyte conversion when fate tracing both BECs or KDR-expressing cells along with reversion of steatosis and fibrosis.
In human and murine-diseased livers, KDR-expressing BECs associated with KDR-expressing cell-derived hepatocytes were identified.

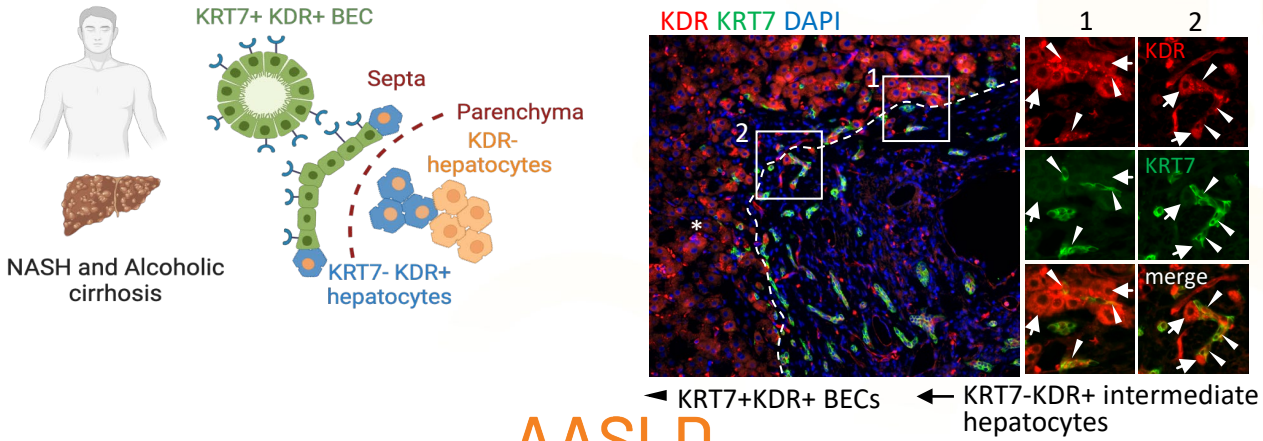
Conclusions

The study reveals novel therapeutic benefits of VEGFA for harnessing BEC-driven repair to potentially treat liver diseases.

MOUSE: VEGFA converts KDR-expressing cells into hepatocytes and reverts steatosis and fibrosis



HUMAN: KDR-expressing BECs are associated with evidence of BEC-to-hepatocyte cell conversion



Rizvi F, et al., Abstract 11.

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Multi-ethnic genome-wide association meta-analysis identifies 17 loci associated with NAFLD that define new disease subtypes, mechanisms, and predict advanced liver disease

Aim

Identify and characterize genetic variants that increase human NAFLD risk.

Methods

- We conducted the largest cross ancestry genome-wide association meta-analysis of imaging- (66,814 individuals) and ICD-based NAFLD (3584 cases vs 621,081 controls) across multiple human cohorts.
- We determined ancestry-specific allele frequency and effect sizes across ancestries and sex.
- We conducted Phenome-Wide Association Studies (PheWAS) on the genome-wide significant variants in UK Biobank, followed by clustering of results to identify 7 subtypes of NAFLD.
- We carried out 2 sample Mendelian Randomization (MR) analyses to define causal phenotypes.
- We generated overall and subgroup Polygenic Risk Scores (PRS) and evaluated their effects on liver-related disease and biomarkers in the Michigan Genomics Initiative and UK Biobank.

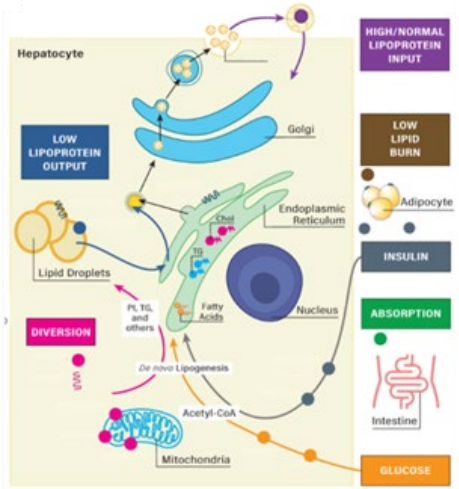
Conclusions

- We identified 17 genome-wide NAFLD promoting genetic variants and 7 subtypes of NAFLD by PheWAS analysis.
- Allele frequency more than effect varies by ancestry at some loci; PNPLA3 and TM6SF2 showed different effects across sex.
- BMI and waist-to-hip ratio are causal for NAFLD. NAFLD is causal to cirrhosis and esophageal varices.
- A PRS can identify 10%, 5%, and 1% of individuals with high risk (odds >2,3, 4, respectively) of NAFLD, cirrhosis, and HCC.
- Subtypes of NAFLD are associated with different outcomes. This opens up new avenues for better matching susceptible individuals to treatments.

Xiaomeng D, et al., Abstract 14.

Main Findings

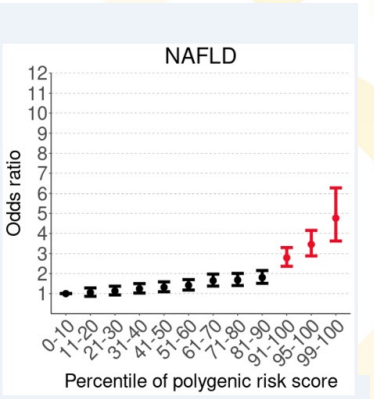
Group	Genes	steatosis	cirrhosis	LDL	MI	TG	HDL	DM	BMI
Low lipoprotein out	*PTPRD PNPLA3 TM6SF2*	↑	↑	↓	↓	↓	↓	↑	↓
High lipoprotein in	*APOE*	↑	↑	↓	↓	↓	↑	↑	↑
Low Lipid Burn	*FTO*	↑	–	↓	–	–	↓	↑	↑
Insulin	*INSR PNPLA2 GRB14 SREBF1*	↑	–	↑	↑	↑	↓	↑	↓
Absorb	*MTTP*	↑	–	↑		↑	↑	↑	–
Glucose	*GCKR TRIB1*	↑	–	↑	↑	↑	↓	↓	↓
Divert	*MBOAT7 MARC1 TOR1B ADH1B GPAM*	↑	↑	↑	↑	↓	↑	↑	–
EPIDEMIOLOGY		↑	↑	↑	↑	↑	↓	↑	↑

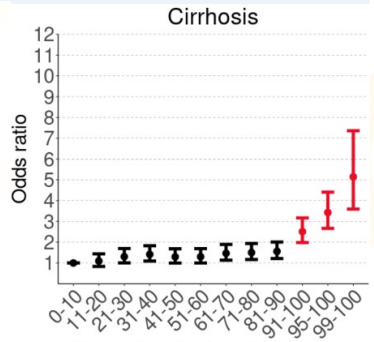


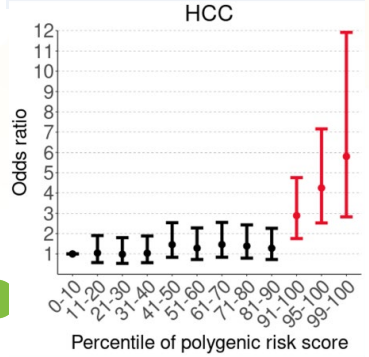
Subgroups of NAFLD identified by biomarkers

PRS of each subgroup affect liver-related traits in the direction noted

17 SNP PRS identifies subgroups with high risks of liver disease







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Targeting YAP-mediated hepatic stellate cell death susceptibility and senescence for treatment of liver fibrosis

Objective

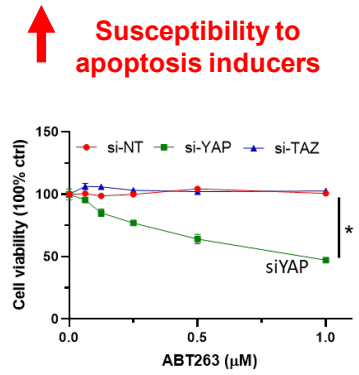
Liver fibrosis is influenced by hepatic stellate cell (HSC) senescence and death. We aimed to study whether YAP (yes-associated protein) regulates these processes and this could be leveraged to treat liver fibrosis.

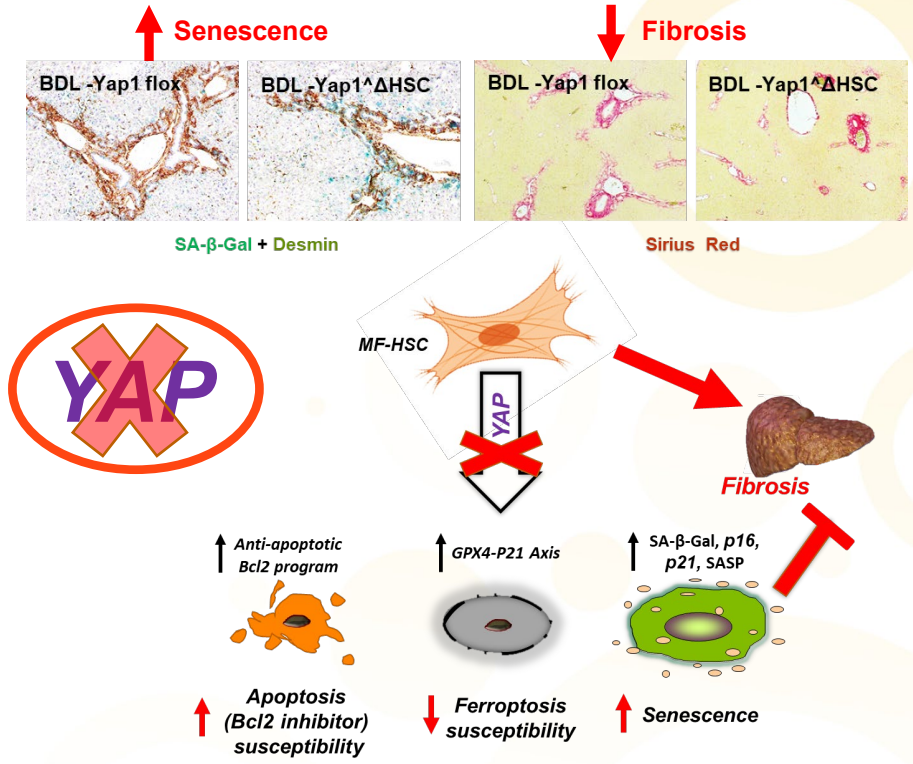
Methods

- YAP activity was manipulated in cultured MF-HSCs to determine how YAP impacts senescence and susceptibility to inducers of different regulated cell death programs (apoptosis, ferroptosis).
- CCl_4-treated mice were treated with inducers of ferroptosis or apoptosis to determine effects on liver fibrosis.
- YAP was selectively depleted in MF-HSCs (aSMA-Cre^{ERT2} x Yap1$^{flox/flox}$ mice →Yap1$^{\Delta HSC}$) to determine effects on HSC senescence and liver fibrosis.

Conclusions

YAP suppression causes MF-HSC vulnerable to pro-apoptotic senolytics but resistant to ferroptosis. However, neither ferroptotic nor senolytic agents improve liver fibrosis when administered systemically. In contrast, MF-HSC specific YAP depletion induces senescence and protects injured livers from fibrosis.





Bcl2: B-cell lymphoma 2
GPX4: glutathione peroxidase 4
SASP: senescence-associated secretory phenotype

Du K, et al., Abstract 53.

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Liver sinusoidal endothelial cell expressed Vascular Cell Adhesion Molecule 1 (VCAM1) promotes liver fibrosis

Hypothesis

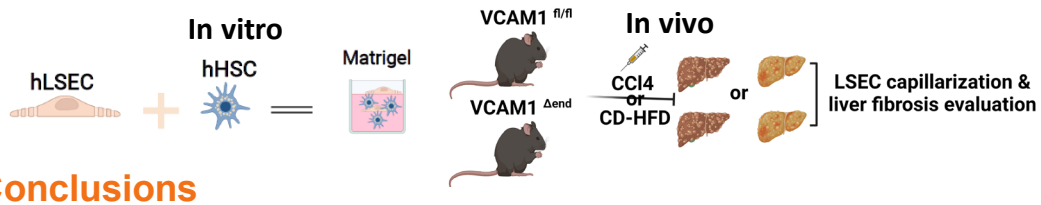
We hypothesize that VCAM1 is implicated in liver sinusoidal endothelial cells (LSECs) capillarization and hepatic stellate cell (HSC) activation in liver injury.

Aims

- To examine the direct role of VCAM1 in LSEC capillarization and HSC activation during liver fibrosis.
- To define the molecular mediators of the profibrogenic angiocrine signaling between LSECs and HSCs.

Methods

Examine the protective effects of loss of VCAM1 in LSECs against activation of HSCs.



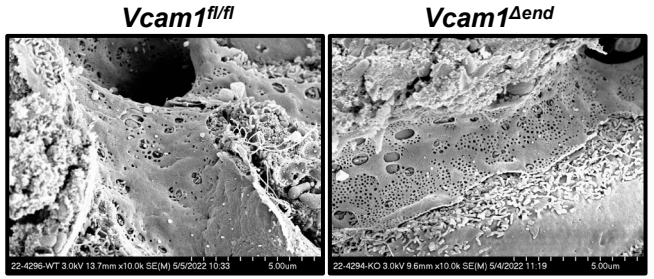
Conclusions

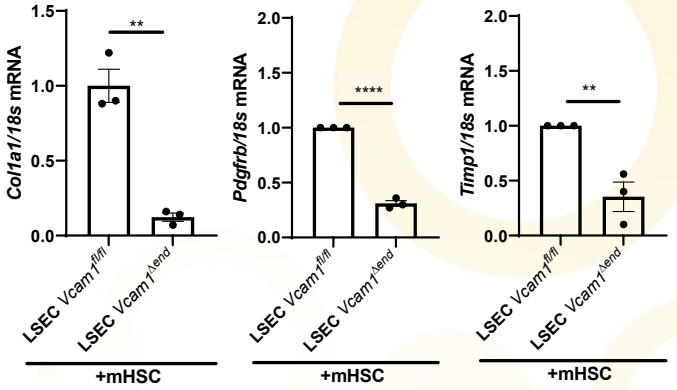
VCAM1 in LSECs is not just a scaffold for leukocyte adhesion, but also a modulator of liver fibrosis.

Guo Q, et al., Abstract 54.

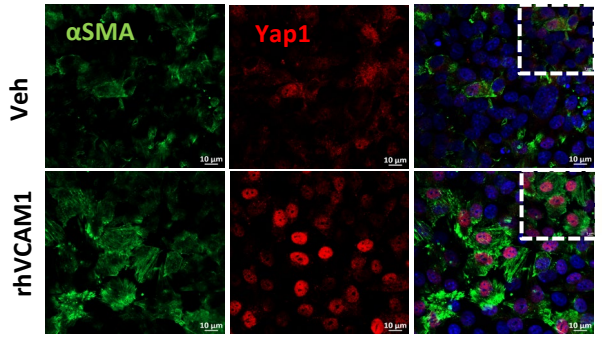
Main Findings

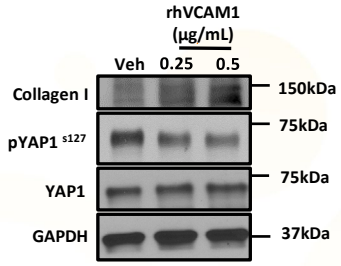
- Exuberant endothelial VCAM1 upregulation in liver injury promotes LSECs capillarization and liver fibrosis.

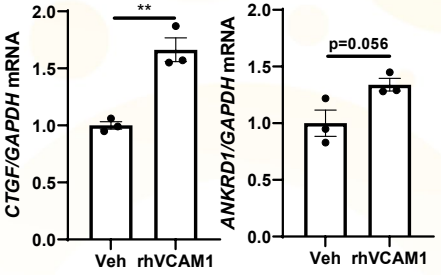


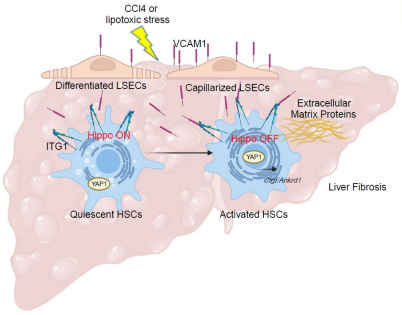


- LSEC VCAM1 is involved in stellate cell activation likely via an ITGB1-YAP1 dependent mechanism.









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Targeting senescent hepatocytes using THBD-PAR1 inhibitor vorapaxar ameliorates NAFLD progression

Objective

We recently discovered that THBD-PAR1 axis is highly upregulated in various senescent cells, and its inhibition suppress senescence. Herein, we examined whether this axis is induced in senescent hepatocytes, and whether this can be leveraged to treat NAFLD.

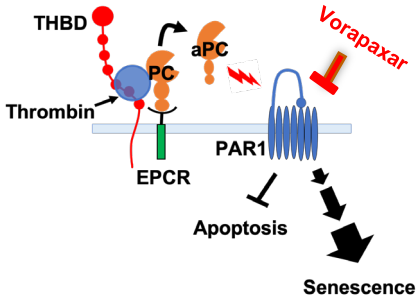
Methods

- Senescence markers and the THBD-PAR1 axis were evaluated in p16-overexpressing mouse hepatocytes and liver biopsies from NAFLD patients.
- THBD-PAR1 axis was inhibited with vorapaxar (specific PAR1 inhibitor) in NAFLD models to determine the effects of senescent hepatocytes on NAFLD progression.

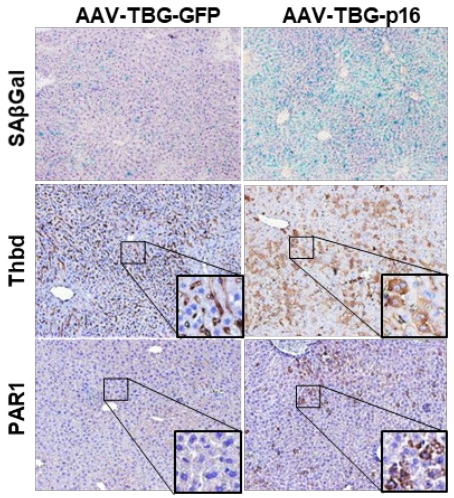
Conclusions

THBD-PAR1 signaling is increased in senescent hepatocytes of NAFLD liver. Its inhibition with vorapaxar suppresses senescence and liver fibrosis in mouse models of NAFLD. The upregulated hepatocyte THBD-PAR1 axis may be a new therapeutic target for NAFLD treatment.

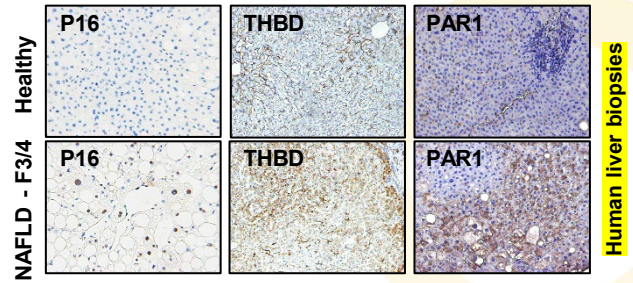
A. THBD-PAR1 axis is a novel senolytic target



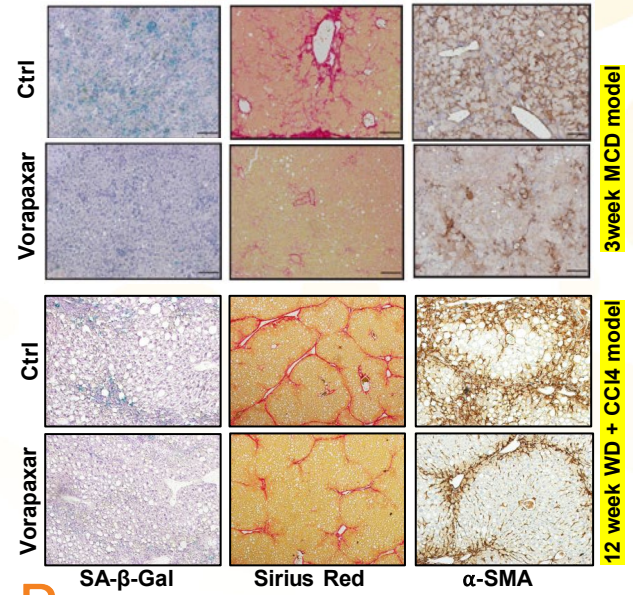
B. THBD-PAR1 axis is increased in senescent hep.



C. THBD-PAR1 axis is increased in human NAFLD liver



D. THBD-PAR1 axis inhibition ameliorates NAFLD



Du K, et al., Abstract 90.

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PKM2-dependent metabolic skewing of hepatic macrophages regulates pathogenesis of nonalcoholic steatohepatitis

Objective

To determine the role of macrophage PKM2 in NASH pathogenesis.

Methods

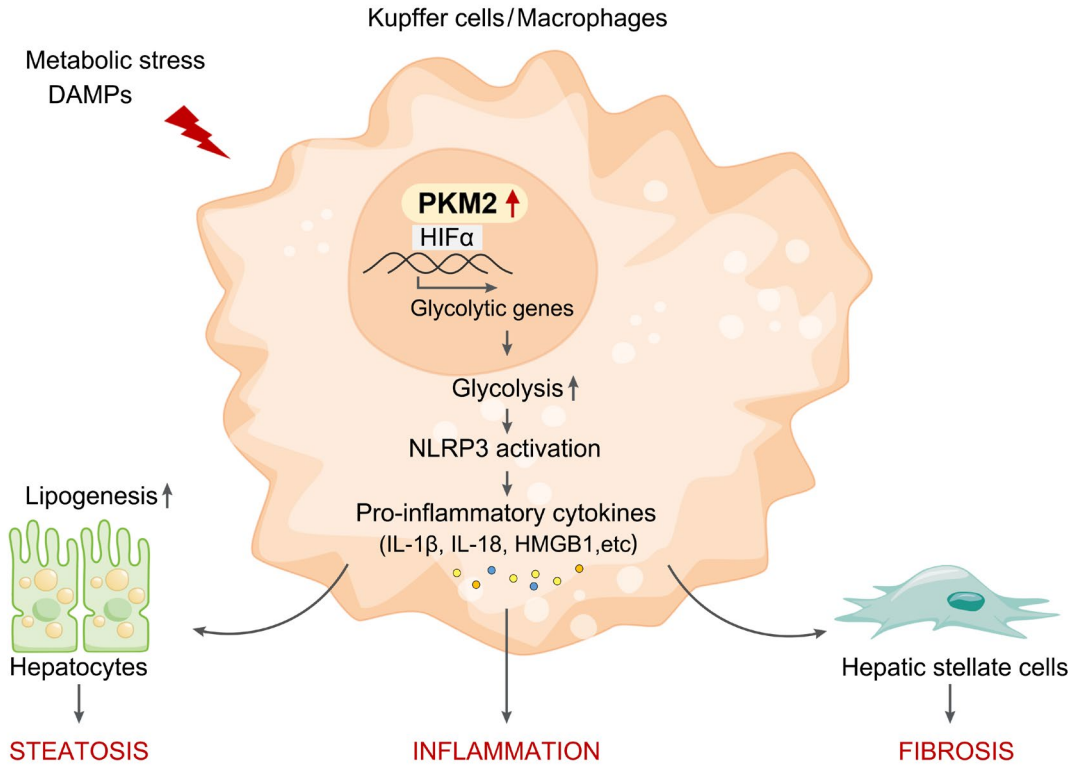
- Macrophage-specific *Pkm2* knockout (*Pkm2*$^{Fl/Fl}$ LysM-Cre) mice were used.
- Multiple NAFLD/NASH models (HFD, MCD, HFHC, WD/CCl_4) were administrated.
- Single-cell transcriptomic analysis of non-parenchymal cells isolated from *Pkm2*$^{Fl/Fl}$ LysM-Cre mice and control mice fed with MCD diet was performed.

Main Findings

- PKM2 is predominantly upregulated in macrophages during NASH progression, and positively correlates with inflammation and fibrosis.
- Macrophage PKM2 knockout ameliorates NASH progression.
- PKM2-dependent glycolysis provokes pro-inflammatory cell traits of hepatic macrophages.
- Limiting PKM2 nuclear translocation with TEPP-46 treatment alleviates NASH pathogenesis in mice.

Conclusions

Steatotic liver microenvironment provokes PKM2^{+}CD86^{+} glycolytic macrophages accrual, and intervention of macrophage PKM2 effectively ameliorates inflammation and NASH pathogenesis.



Qu H, et al., Abstract 92.

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EpCAM+ CD133+ microvesicles: A novel biomarker for steatosis-to-steatohepatitis transition in NAFLD

Hypothesis/Aim/Objective

To evaluate circulating AV+ EpCAM+ CD133+ microvesicles (MVs) as a potential biomarker of the transition from simple steatosis to steatohepatitis in biopsy-proven NAFLD patients.

Methods

- We evaluated the hepatic expression of EpCAM and CD133 proteins and the plasma levels of EpCAM+ CD133+ MVs in 31 C57BL/6J in HFHCC mice diet.
- AlbCrexmT/mG mice were fed with a Western (WD) or with a Dual diet for 23 weeks and was used to confirm hepatic origin.
- We assessed plasma MVs in 130 biopsy-proven NAFLD patients.

Main Findings

- Hepatic EpCAM and CD133 expression increase with the progression of NAFLD (Fig 1).
- The liver produces AV+ EpCAM+ CD133+ circulating MVs (Fig 2).
- Circulating levels of AV+ EPCAM+ CD133 MVs were associated with steatohepatitis in patients with NAFLD (Fig 3).

Conclusions

The circulating levels of EpCAM+ CD133+ MVs in clinical and experimental NAFLD were increased in the presence of steatohepatitis, showing high potential as a non-invasive biomarker for the evaluation and management of these patients.

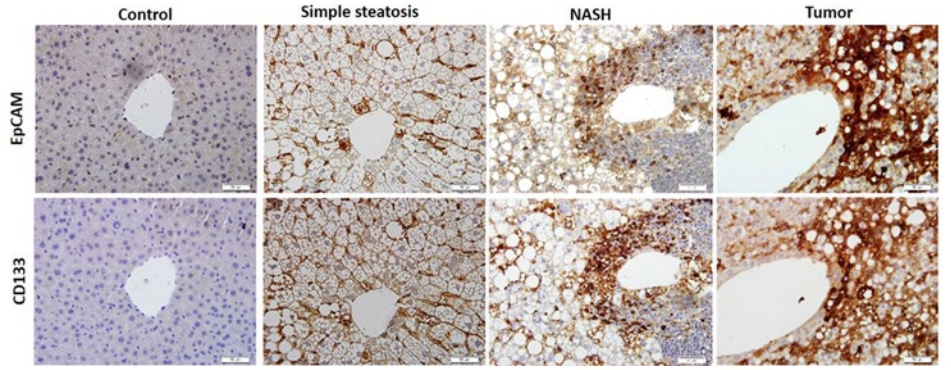

Figure 1: Expression of EpCAM CD133 liver tissue

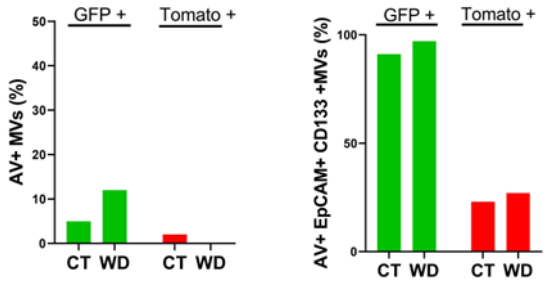

Figure 2 : GFP+/ TOMATO+ EpCAM+ CD133+ MVs levels in AlbCrexmT/mG mice after 23 weeks with WD

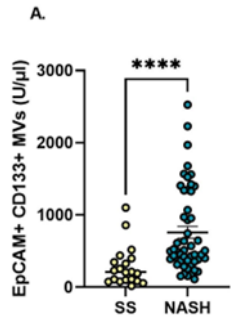

Figure 3 : Levels of EpCAM CD133+ MVs in biopsy-proven NAFLD patients.

Muñoz-Hernández R, et al., Abstract 109.

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Leuconostoc sp. LB-P8 ameliorates liver inflammation and fibrosis in diet-induced non-alcoholic steatohepatitis

Aim

In this study, we investigated the role of *Leuconostoc* sp. LB-P8 in gut bacteria profile, inflammation, and fibrosis in NASH

Methods

- NASH was induced in mice fed with FFC and CDHFD diets
- Hepatic injury, steatosis, inflammation, and fibrosis were measured using histology, IHC, qPCR, picro-sirius red staining, and ELISA
- Intrahepatic leukocytes were analyzed by CyTOF
- Fecal microbiome diversity was analyzed by 16S rRNA gene amplicon sequencing

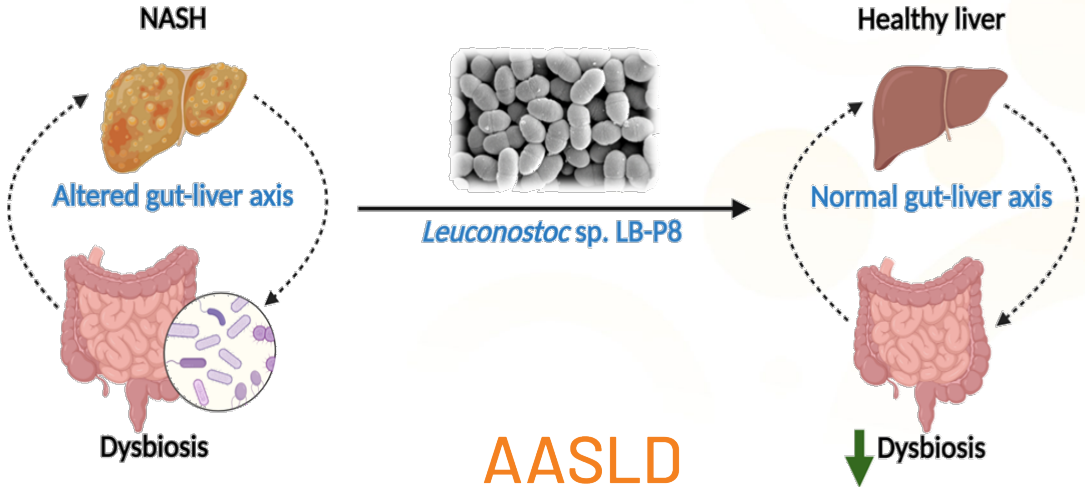
Conclusions

LB-P8 ameliorates NASH progression by modulating the intrahepatic macrophage population, stellate cell activation, and gut bacteria profile. These data suggest that LB-P8 targeting of the gut-liver axis could be a potential novel therapeutic strategy for the treatment of NASH.

Main Findings

LB-P8 administration to mice with diet-induced NASH led to (see figure):

- reduced liver injury as demonstrated by AST, caspase 3 and TUNEL assay
- decreased intrahepatic pro-inflammatory monocyte-derived macrophages
- decreased hepatic expression of TNF-α and IL-1β
- increased intrahepatic anti-inflammatory macrophages
- decreased hepatic fibrosis
- decreased total serum bile acid induced by CD-HFD diet
- improved gut barrier function
- increased the expression of tight junction genes along with lower serum LPS level



Sidhu G, et a;., Abstract 111.

ChREBP and its downstream lipogenic pathways are modulated directly by reductive stress

Objective

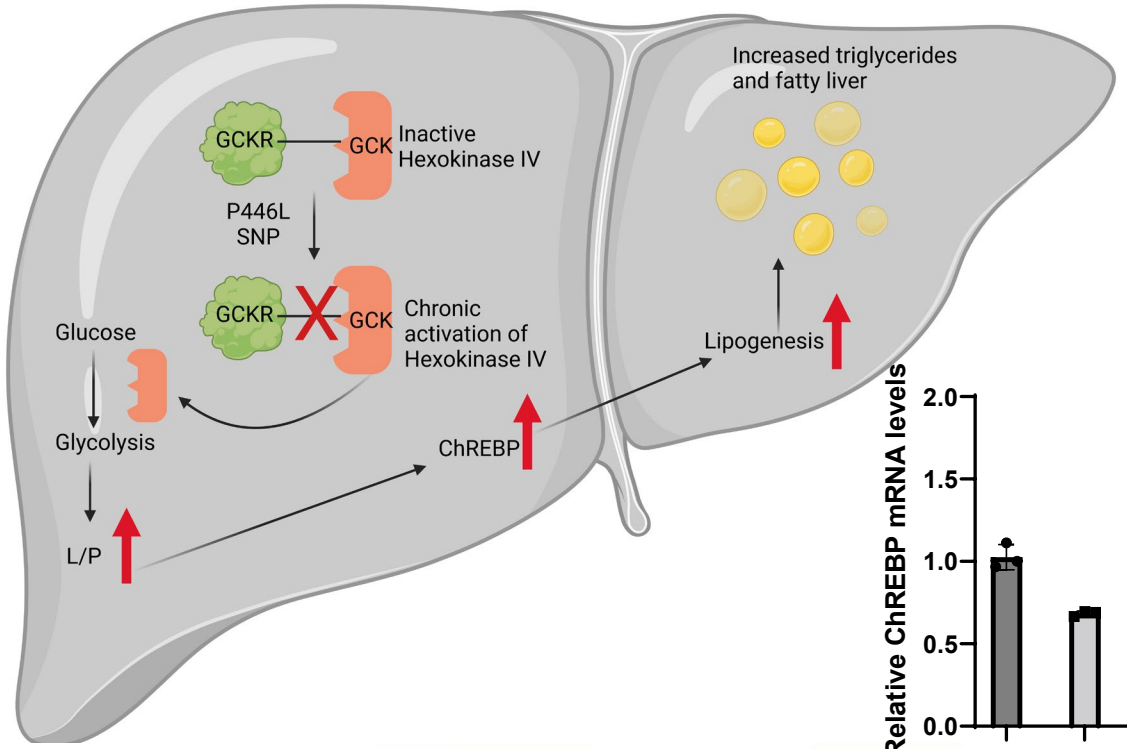
Our aim here was to test if reductive stress, caused by SNPs in the GCKR gene, can regulate the expression of the lipogenic transcription factor ChREBP and its downstream targets.

Methods

- We used three different model systems: mice, mouse primary hepatocytes in culture, and human liver organoids.
- RNA was extracted, converted to cDNA, and qPCRs performed or RNAseq was performed.
- Metabolites were measured on GCMS or LCMS.
- FGF21 protein was assayed with an ELISA kit from Millipore.

Conclusions

We demonstrate the direct regulation of ChREBP and its downstream targets by reductive stress.



Singh C, et al., Abstract 114.

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Defects in primary cilia rewire cholangiocytes glucose metabolism

Objective

The cholangiopathies are a group of diseases affecting the biliary tree including polycystic liver diseases (PLD) and cholangiocarcinoma (CCA), which are associated with defects in primary cilia. Our overall objective was to explore the potential role of primary cilia in the cellular metabolic phenotype.

Methods

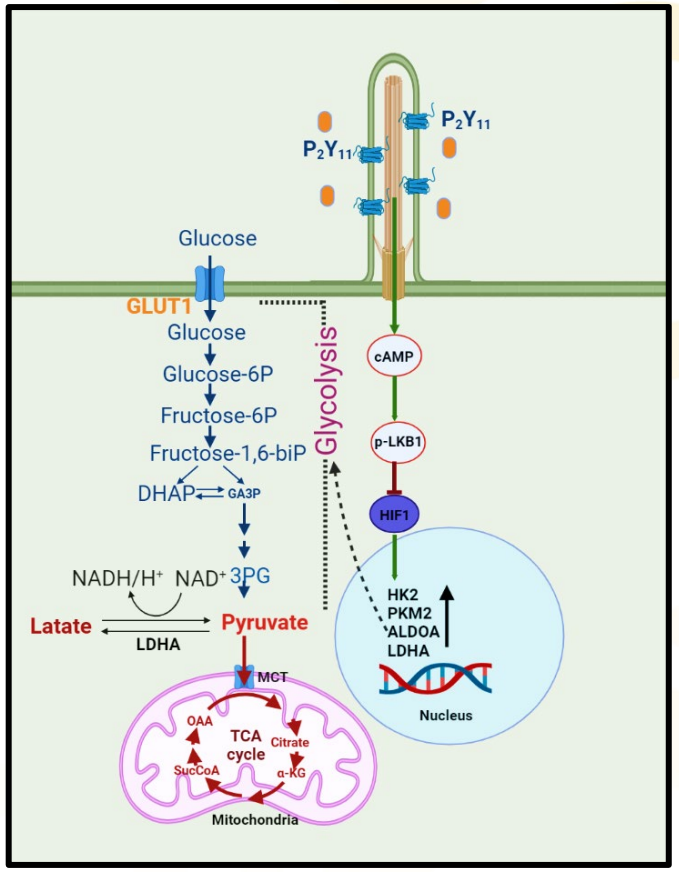
Using normal (NHC, H69, NRC), PLD (PCK, hPLD), and CCA (HuCCT-1, KMCH) cell lines, and PLD (Alb-cre-IFT88, PCK) and CCA (BDEneu) rodent models, and human gene expression data, we characterized the metabolic profiles of ciliary defective cells compared to normal by glucose and lactate assays, Seahorse Real-Time Cell Metabolic analysis, molecular cell biology, Western blots, immunohistochemistry (IHC), and RNA-seq.

Main Findings

- Defects in cholangiocytes primary cilia induce glucose consumption and lactate production *via* the upregulation of glycolytic genes.
- Cilia mediated P_2Y_{11}-LKB1-HIF1a regulatory axis controls glycolysis in cholangiocytes.
- Hesperidin methyl chalcone (HMC) mediated activation of LKB1, inhibited glycolysis and cell proliferation in both *in vivo* and *in vitro* models of PLD and CCA.

Conclusions

Our data propose a relationship between primary cilia defects and glucose metabolism dysregulation, suggesting that cilia negatively regulate glycolysis by repressing HIF1a using the P2Y11-LKB1 pathway as a sensory hub. These experiments uncovered novel mechanisms on the ciliary-dependent regulation of the intermediate metabolism, that may influence survival of PLD/CCA cells.



Pant K, et al., Abstract 137.

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Spatial scRNA expression profiles of hepatocyte subpopulations and non-parenchymal liver cells during fasting/refeeding

Hypothesis/Aim/Objective

To define the metabolic regulatory networks of hepatocytes and non-parenchymal cells (NPCs) in response to fasting/refeeding.

Methods

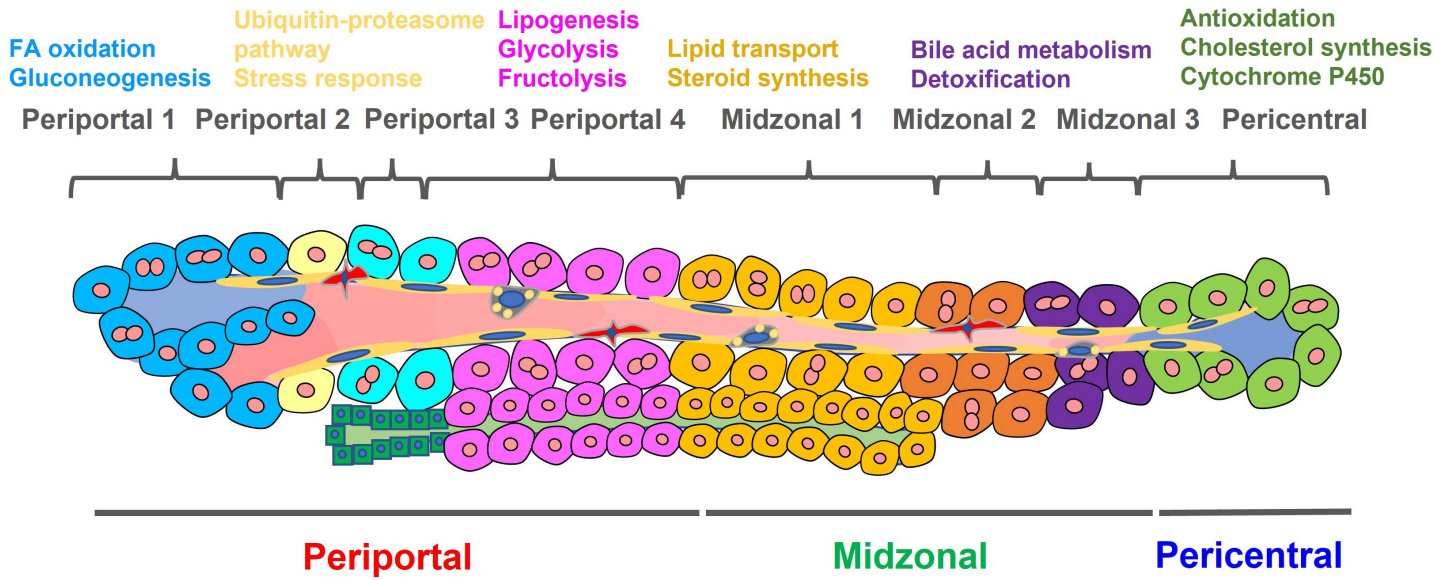
Use of spatial transcriptomics of cryo liver sections and single-cell RNA-seq of liver cells to characterize gene expression levels in hepatocytes and NPCs from mice fasted 24hr and mice refed a fat-free diet for 6hr or 12hr.

Main Findings

Figure summarizes major zonal transcriptional profiles that respond to fasting and refeeding.

Conclusions

This data provides the first transcriptome landscape at a single cell resolution with an in-depth review of the different hepatocyte subsets, their spatial location along with the differentiation trajectory, and transcriptional activity in response to fasting/refeeding.



Wang S, et al., Abstract 187.

AASLD The Liver Meeting®

Spatial organization of hepatocytes in healthy and cirrhotic human liver

Aim

Understanding the cell composition and gene expression patterns of cell types in different zones across the porto-central axis in normal and diseased human liver tissue.

Methods

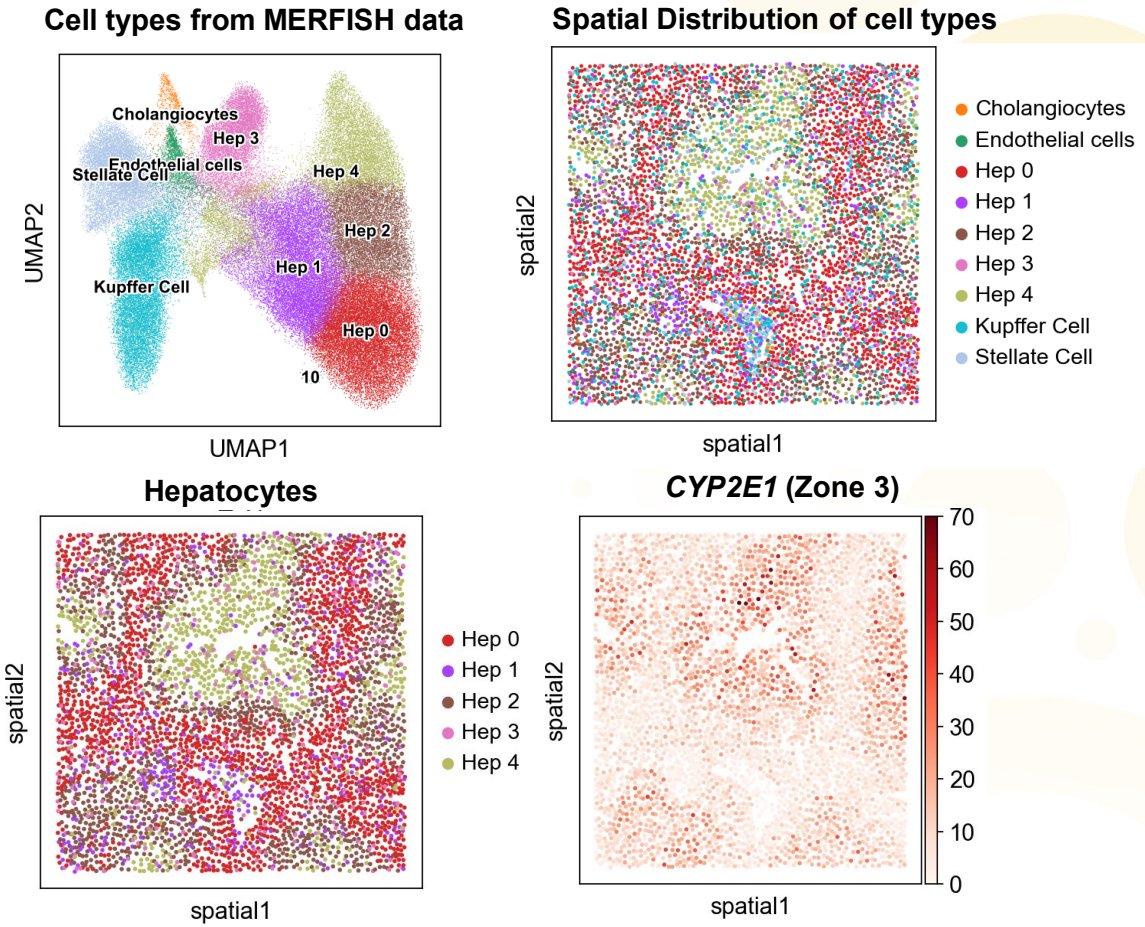
- We performed MERFISH imaging for > 300 genes, which included at least two marker genes for the non-parenchymal and immune cells present in the liver with a focus on genes that define hepatocytes clusters based on single-cell RNA-seq.
- Integrated MERFISH data with snRNA-seq to better understand the genome-wide expression of genes across the porto-central axis.

Main Findings

We identified that multiple clusters of hepatocytes are organized by zones across the normal liver lobule, which are disrupted in cirrhotic liver tissue.

Conclusions

Hepatocyte zonation is disrupted in the cirrhotic liver.



Paul B, et. al., Abstract 189.

AASLD The Liver Meeting®

Mitochondrial dynamics and stasis are critical for hepatocyte function and liver tumorigenesis

Objective

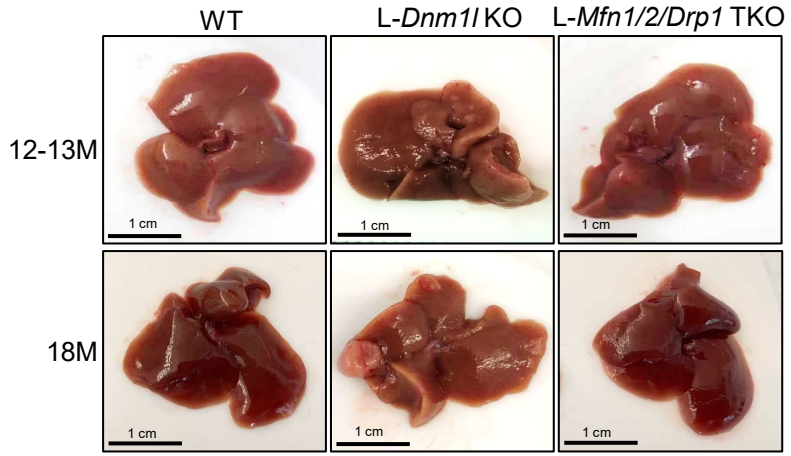
To investigate how mitochondria dynamics would be involved in liver pathogenesis and tumorigenesis.

Methods

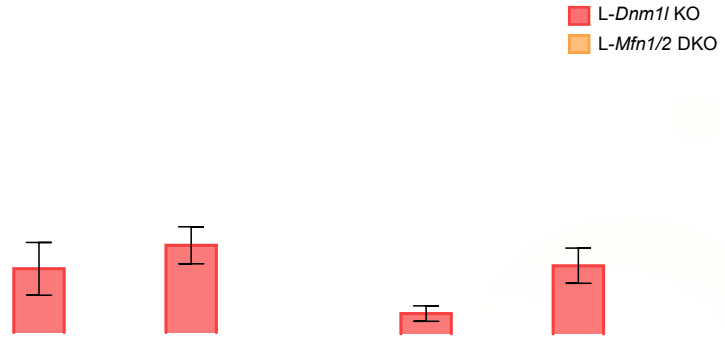
- Liver-specific DRP1 knockout (L-DRP1 KO), L-MFN1 KO, L-MFN2 KO, L-MFN1/MFN2 double KO (DKO), and L-DRP1/MFN1/MFN2 triple KO (TKO) mice were generated and housed for various periods up to 18 months, blood and liver tissues were harvested.
- 5% EtOH diet were applied to 9-month-old L-DRP1 KO mice and lasted for 12 weeks to investigate the role of mitochondrial dynamics in alcohol-associated liver cancer.

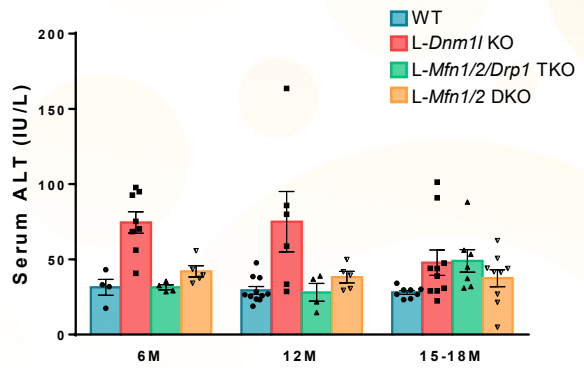
Conclusions

- Mitochondrial dynamics and stasis are critical to maintain hepatic mitochondrial homeostasis and hepatocyte functions.
- Loss of hepatic DRP1 promotes liver tumorigenesis and exacerbates alcohol-associated liver cancer.



Mice Genotype	Sex	Tumorigenic mice/total			
		6M	12M	15M	≥18M
WT	M	0/4	0/16	0/24	0/12
	F		0/16	0/15	0/4
L-*Dnm1l* KO	M	0/8	7/11	2/2	10/10
	F	0/8	2/4	4/8	1/2
L-*Mfn1* KO	M	0/3	0/6	0/5	0/5
	F	0/1		0/1	1/3
L-*Mfn2* KO	M	0/1	0/4	0/5	0/2
	F	0/3	0/5	0/3	0/3
L-*Mfn1/2* DKO	M	0/5	0/6	1/5	3/9
	F		0/3	1/7	0/1
L-*Mfn1/2/Dnm1l* TKO	M	0/4	0/2	0/3	0/5
	F	0/4	0/2	0/4	0/4





Ma X, et al., Abstract 190.

In vivo CRISPRi screen identifies Neurl1b regulates hepatocyte repopulation, ploidy, and tumorigenesis through centrosome and spindle assembly

Aim

To identify regulators of liver repopulation through an *in vivo* CRISPR inhibition (CRISPRi) screen and to study the underling mechanisms

Methods

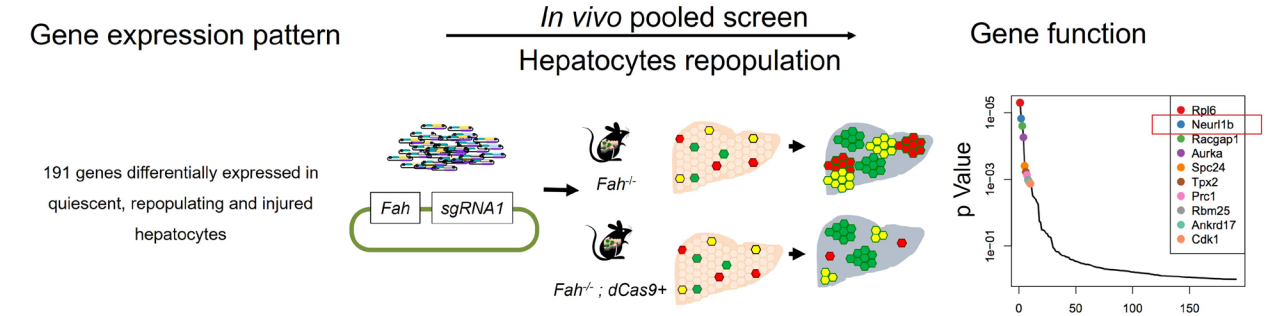
We interrogated the functions of 191 genes differentially expressed during hepatocyte repopulation using an *in vivo* screen in a *Fah*$^{-/-}$;*dCas9*$^{+}$ liver injury model

Main Findings

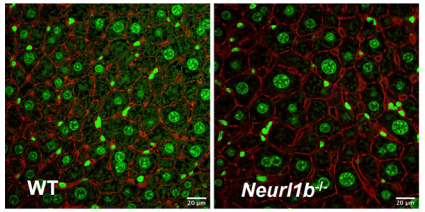
- We found Neurl1b is required for hepatocyte repopulation.
- Neurl1b is an E3 ligase with previously unknown function in mammals.
- *Neurl1b*$^{-/-}$ mice develop normally and accumulate mononuclear, polyploid hepatocytes after weaning.
- Neurl1b localizes to the centrosome and spindle pole and altered expression leads to disorganized mitotic spindles and loss of γ-Tubulin from centrosomes.
- *Neurl1b*$^{-/-}$ mice are resistant to tumorigenesis.

Conclusions

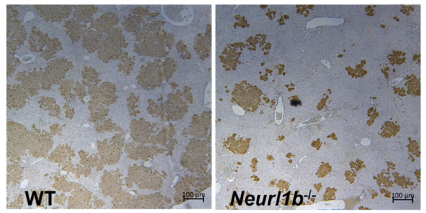
We established a reliable *in vivo* screening system and identified Neurl1b regulates hepatocytes repopulation, polyploidy, and tumorigenesis.



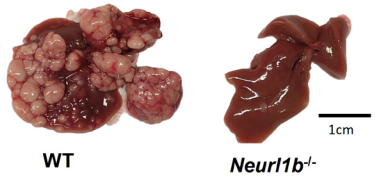
Neurl1b$^{-/-}$ mice have more polyploidy hepatocytes



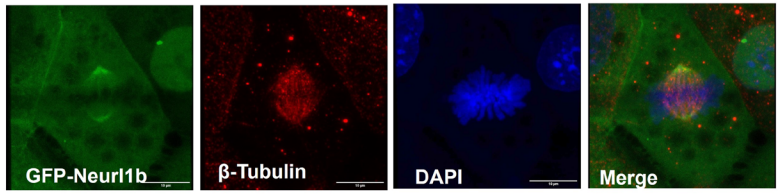
Neurl1b$^{-/-}$ hepatocytes have reduced repopulation capacity when transplanted to Fah$^{-/-}$ mice



Neurl1b$^{-/-}$ knockout mice are resistant to Myc and RasG12V induced tumorigenesis.



Localization of NEURL1B



Yin D, et al., Abstract 191.

AASLD The Liver Meeting®

Establishing long-term functional 3D human liver organoids from multiple-hepatic linage cells

Hypothesis/Aim/Objective

- To establish long-term (**>1 month**) functional 3D Human Liver Organoids (3D-HLOs) model by incorporating **5 major primary liver cells.**
- To mimic the **complete liver microenvironment** that is suitable for modeling drug metabolism toxicity with refined culturing conditions.

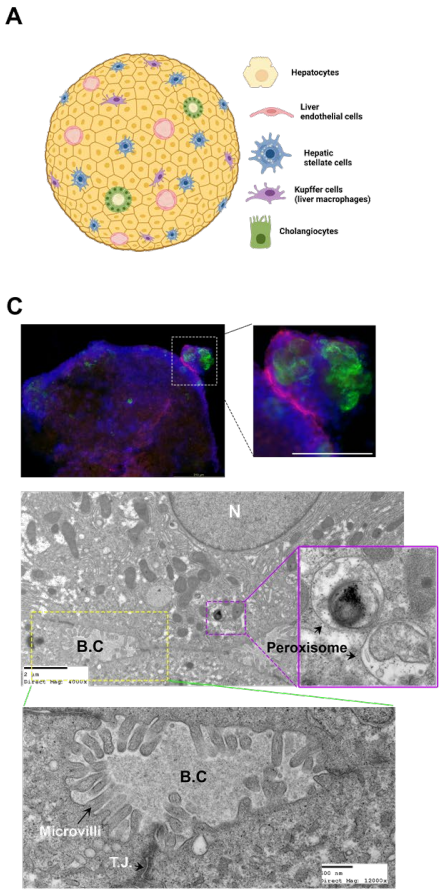
Methods

- Primary human hepatocytes (HCs), hepatic stellate cells (HSCs), liver endothelial cells (LECs), Kupffer cells (KCs), and cholangiocytes (CHO) were isolated and characterized.
- 3D-HLOs were generated by seeding the isolated primary 5 different hepatic cells in low adhesion 96-well plates with HC expansion medium (supplemented with EGF, HGF, Y27632, and A83-01) and followed by culturing in a medium supplied with SB31542, Forskolin, and DAPT for hepatocyte differentiation.

Main Findings (see **Figure1**)

Conclusions

3D-HLOs maintained liver function up to 30 days, demonstrated by the expression of mature HC markers such as ALB, CK18, and several CYP(P450) enzymes and their activities, as well as albumin production and urea synthesis.



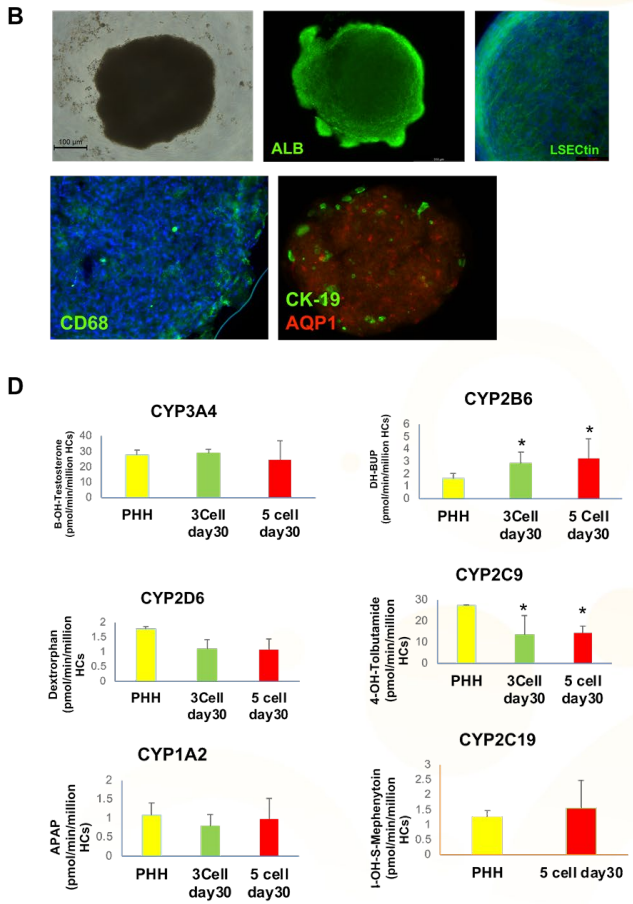


Figure1. **A**. Schematic illustration of 5 cell 3D-HLOs organoids. **B**. brightfield images of 3D-HLOs at day 30 (Upper left), IF staining of Albumin (ALB), LSECtin, CD68 and IF co-staining of Cytokeratin 19 (CK-19) and Aquaporin 1 (AQP-1), which confirms the incorporation of cells in 3D-HLOs. **C**. Transmission electron microscopy (TEM) showing the ultrastructure of 5 cell 3D-HLOs that cultured in for 30 days. The formation of bile canaliculus (B.C) with the microvilli structure inside of lumen was amplified in the lower image. Tight junction (TJ) seal the lumen of bile canaliculi between adjacent hepatocytes. **D**. Drug metabolizing activities of CYP450 enzymes detected by liquid chromatography–mass spectrometry (LC-MS) in 3 cell (HC+HSC+LEC) and 5 cell (HC+HSC+LEC+CHO +KC) 3D-HLOs at day 30 in comparison with fresh Primary Human Hepatocytes (PHH). *, $P<0.05$, V.S. PHH.

Zhang W, et al., Abstract 218.

AASLD The Liver Meeting®

Scaffold-free 3D cholangiocyte organoids to study the progression of primary sclerosing cholangitis (PSC)

Objective

To generate 3D human cholangiocyte organoids (3D-HCOs) using liver cells isolated from normal and PSC patients to better recapitulate the *in vivo* niche driving the progression of PSC.

Methods

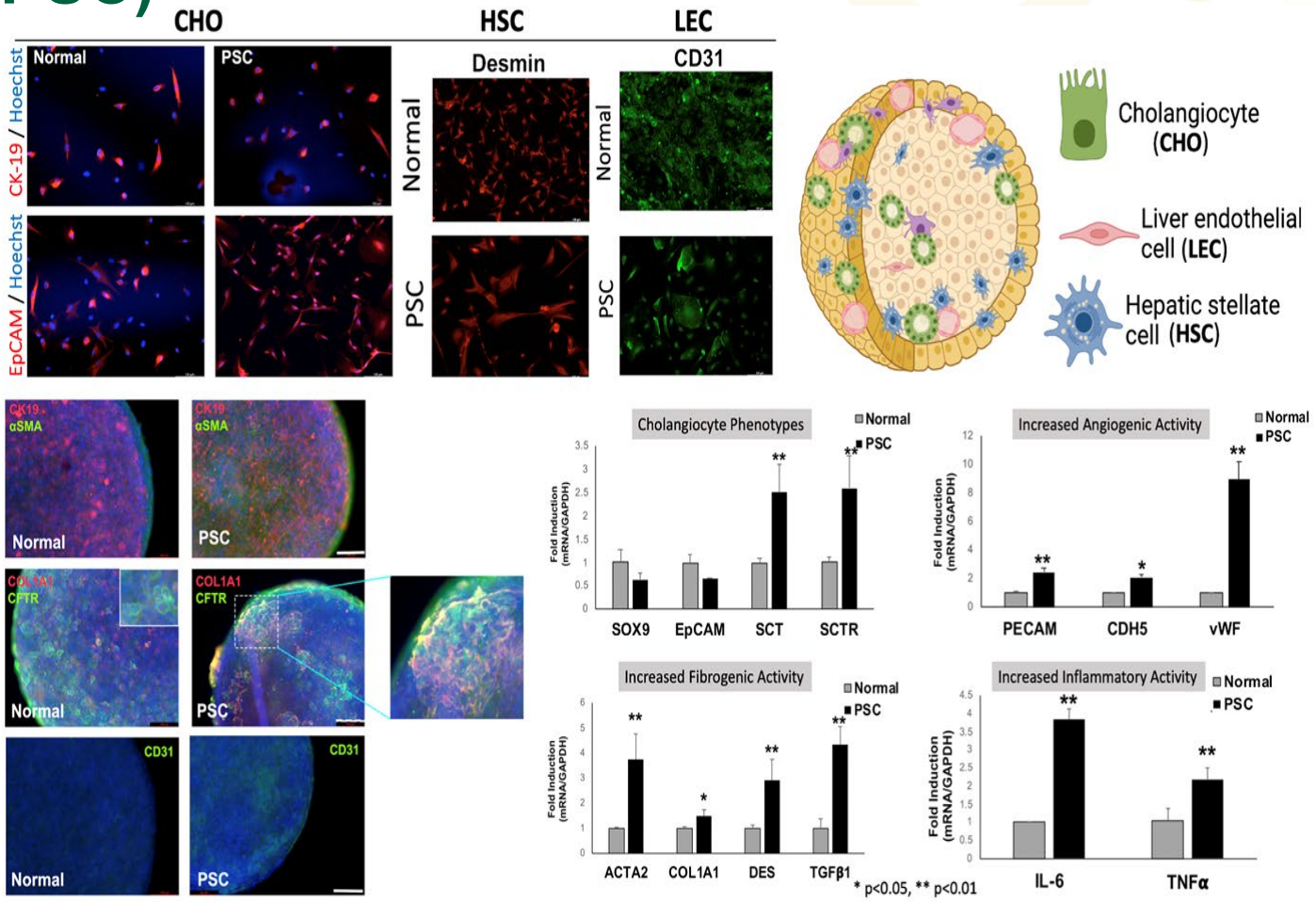
- Primary human cholangiocytes (CHO), hepatic stellate cells (HSC), and liver endothelial cells (LEC) were isolated and purified by flow cytometry and characterized by IF (top right). 3-cell (CHO+HSC+LEC) 3D-HCOs were generated in low-adhesion 96-well plates (top left).
- 3D-HCOs were evaluated for lineage cell integration, viability, ultrastructure formation, and gene expression for CHO (*Sox9, EpCAM, SCT, SCTR)*, fibrosis (*ACTA2, COL1A1, DESMIN, TGFβ1)*, angiogenesis (*PECAM, CDH5, vWF)* and inflammation *(IL-6 and TNFα*).

Main Findings

(Figure1) Above experiments confirmed PSC phenotypes in 3D-HCOs.

Conclusions

We have successfully developed viable scaffold-free 3D-HCOs derived from normal and PSC liver cells to examine the pathophysiological mechanisms driving the biliary inflammation and fibrosis in PSC progression.



Zhang W., et al., Abstract 225.

AASLD The Liver Meeting®

Regulatory T-cells infusion during normothermic *ex vivo* liver perfusion reduces graft immunorecognition after transplantation

Hypothesis

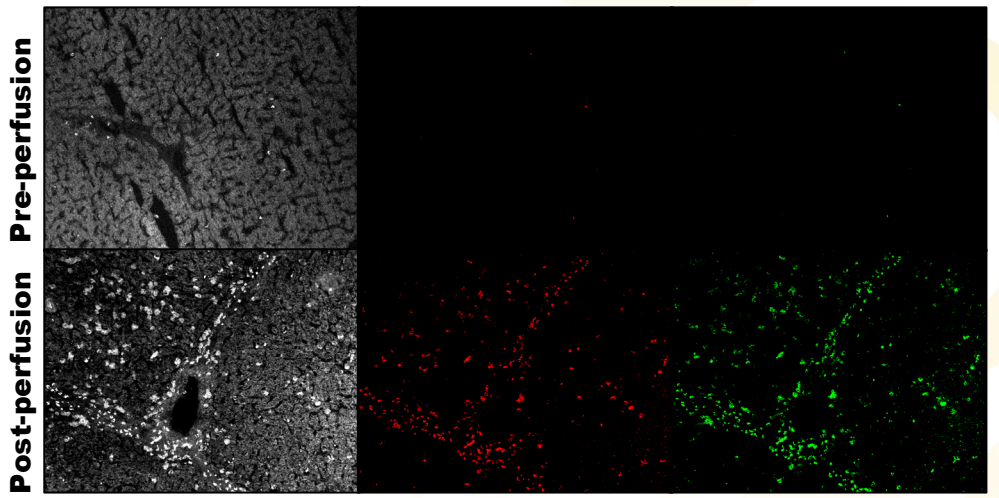
Regulatory T cells (Tregs) can be untaken by the liver during normothermic *ex vivo* machine perfusion (NEVLP), and they can reduce the damage to the liver after liver transplant (LTx) in pig model.

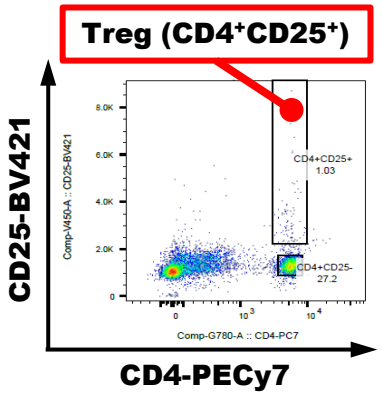
Methods

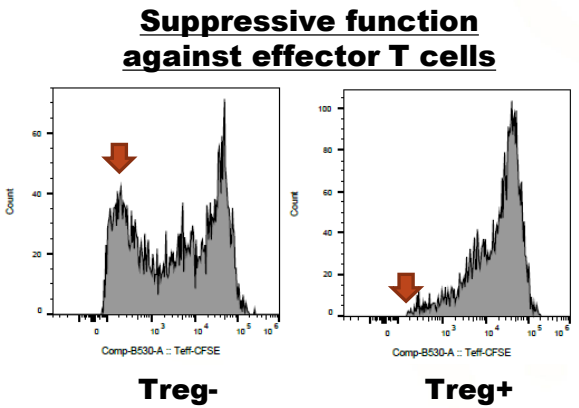
Tregs were isolated from peripheral blood and expanded for 4 weeks, remaining great suppressive function against effector T cells, and then injected into NEVLP with CMTMR (a cell tracker dye), and the perfused liver was transplanted into the recipient.

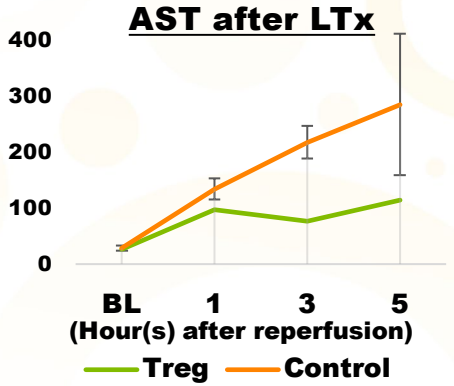
Conclusions

Injection of expanded peripheral-derived Tregs during NELVP allows even distribution and uptake throughout the liver without any negative effects, and those cells may be able to reduce the liver damage after LTx.









Noguchi Y, et al., Abstract 1603.

AASLD The Liver Meeting®

Serum Z polymer is a biomarker for increased liver fibrosis in adults with alpha 1 antitrypsin deficiency

Background

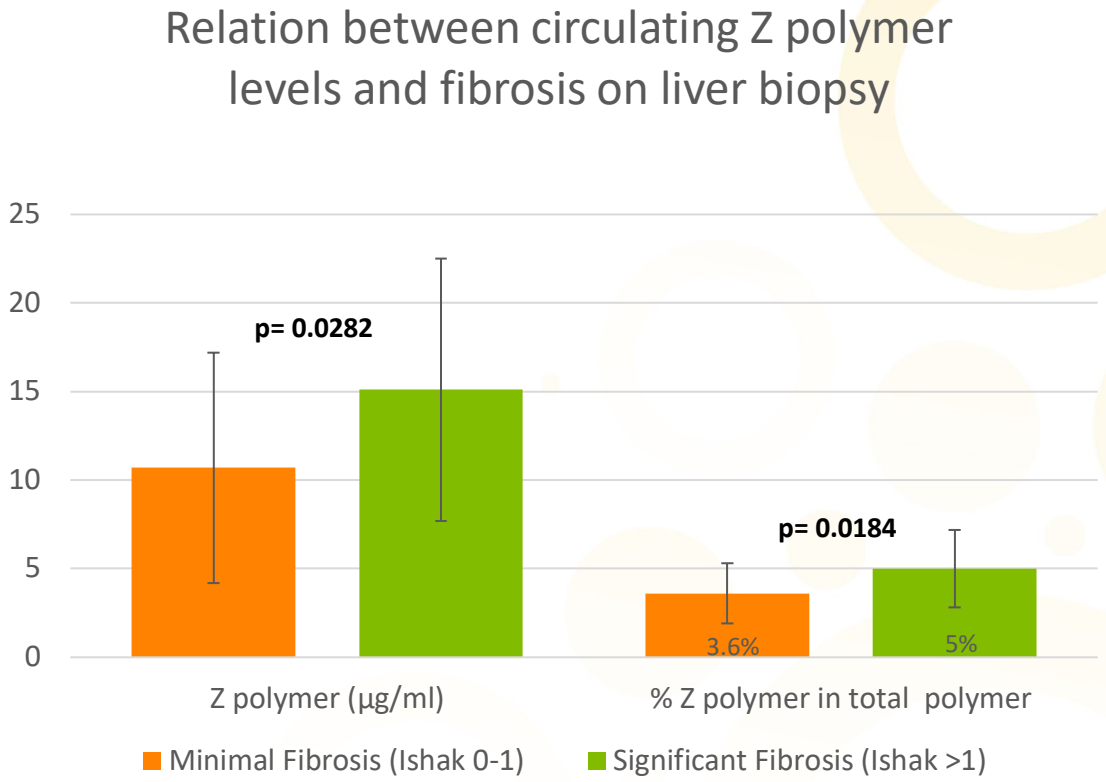
- Predictors and biomarkers of severe disease are being identified. Accumulation of mutant Z protein in polymerized conformation in liver incites injury. Scant levels are seen in serum of unknown significance.
- **Hypothesis**: Increased levels of circulating Z polymer, an unusual, likely toxic but scant conformation variant of Z AAT protein, are associated with more advanced disease in adults with AATD.
- **Objective:** Use data from prospective cohort of AATD ZZ patients enrolled at 3 US sites to examine circulating Z polymer levels as a biomarker for disease severity.

Methods

Enrollment liver evaluations were performed, including liver biopsy, unless the previous biopsy confirmed cirrhosis. Z protein polymer level determined using 2C1 antibody in Meso Scale Discovery (MSD) ELISA-based assay.

Conclusions

High circulating Z polymer levels and higher percentage of the abnormally configured protein in the blood are associated with increased fibrosis, and weakly corelated with reduction in lung function.



Suri A, et al., Abstract LO2.

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