The Liver is an Inflammatory Mediator of Pulmonary Arterial Hypertension

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Abstract

Rationale
Inflammation is central to pulmonary arterial hypertension (PAH) pathogenesis. The liver regulates systemic immunity, yet its contribution to PAH remains unclear.

Objectives
To characterize hepatic involvement in experimental and human PAH. We hypothesized the liver promotes inflammation and pulmonary endothelial injury through endocrine crosstalk.

Methods
PAH patients without liver disease with pulmonary artery endothelial cell (PAEC) biopsies were included. Liver serologies and imaging were included in unsupervised classification and regression tree (CART) analysis to identify subclinical liver dysfunction clusters. The union of two machine learning models predicted cluster assignment and informed differential expression. PAEC gene expression was compared to liver and lung transcriptomes from monocrotaline (MCT) and Sugen-Hypoxia (SuHx) rats using Ingenuity Pathway Analysis. Hepatic fibrosis was assessed in rat and human PAH livers.

Results
Among 25 PAH patients (76% female, median age 61 [range 30 – 84] years), CART identified clusters distinguished by Model for End-Stage Liver Disease Sodium (MELD-Na) score ≥12, which predicted higher pulmonary vascular resistance (PVR)(ß=0.5 Wood units, 95% CI 0.2-0.8, p=0.005) after adjustment for right atrial pressure. Subjects with MELD-Na ≥12 had decreased 6-minute walk distance (353 m [120 – 576] vs. 411 m [300 – 600] m, p=0.03), with upregulation of apelin, beta-catenin, and immune pathway signaling. Similarly, rat lung ECs demonstrated survival and hepatic growth factor signaling, while rat livers showed immune activation. Both rat (3.5 vs 2.2 % area stained, p=0.01) and human PAH livers revealed fibrosis despite absent right ventricular failure.

Conclusions
Hepatic inflammation and fibrosis accompany pulmonary endothelial activation, supporting a pathogenic lung-liver axis in PAH.

Introduction

Pulmonary arterial hypertension (PAH) is a progressive and fatal disorder that can be associated with systemic conditions including liver disease. Hepatic congestopathy is a known complication of right ventricular (RV) dysfunction, and portal hypertension has been linked to long-term prostanoid treatment in PAH (1). However, recent work suggests that distinct liver injury phenotypes occur in well-controlled PAH independent of RV failure and without primary liver disease (2). While PAH is increasingly recognized as a multisystem disease, the degree to which pulmonary vascular remodeling is perpetuated by inter-organ crosstalk in PAH is unknown (3-9).

The liver has several key functions as an immune organ, including the presence of resident macrophages (Kupffer cells), induction of antigen specific tolerance, and local surveillance of gut bacteria (10). Immunologic and vasoactive factors that first pass through hepatic metabolism (11) and vascular growth factors secreted by the liver (e.g., bone morphogenetic protein 9, a central signaling ligand in PAH pathobiology) may influence distal vascular beds. Hepatic endothelial cells (ECs) secrete cytokines that contribute to left heart failure via *coronary* EC dysfunction (12), suggesting the liver may function as an endocrine organ via EC crosstalk. Despite the liver's role as a regulator of immunity and clinically apparent lung-liver phenotypes in PAH, it's contribution as a driver of pulmonary vascular disease remains unclear.

The current study sought to characterize hepatic involvement in experimental and human PAH without known liver disease. We hypothesized that the liver potentiates a feed-forward cycle of systemic inflammation with distal effects on the pulmonary endothelium in PAH. We tested this hypothesis by first using an unsupervised approach to identify subclinical liver dysfunction in PAH patients as determined by laboratory values and model for end-stage liver disease with sodium (MELD) scores, which have been used to predict outcomes in advanced heart failure (13). We then determined the association of cluster membership with pulmonary vascular resistance (PVR) and differences in differential gene expression in human PAH pulmonary artery endothelial cells (PAECs)(14-16) between the clusters. We compared these results to lung EC gene expression from two experimental pulmonary hypertension (PH) rat models. Finally, we evaluated bulk RNA sequencing of PH rat livers and assessed the degree of hepatic fibrosis both in rats and PAH humans.

Methods

PAH Cohort

The study was approved by the Institutional Review Board at Brown University Health (IRB# 001218 and 2221835). Participants with World Symposium on PH Group 1 PAH as diagnosed by a clinical PH provider PAEC transcriptomic data were included. The clinical diagnosis of PAH was confirmed independently by author N.S. We excluded participants with portopulmonary hypertension. All available clinical data (laboratory, imaging, diagnostic codes) were reviewed to confirm the absence of known liver disease defined by elevated transaminases, evidence of synthetic liver dysfunction (elevated international normalized ratio [INR], hypoalbuminemia, thrombocytopenia), or imaging abnormalities including cirrhotic morphology, hepatosplenomegaly or steatosis. The final study cohort consisted of 25 PAH participants (Supplemental Figure 1).

Human PAEC Biopsies and RNA Sequencing

PAECs were collected at the time of clinically indicated right heart catheterization using our previously published cell biopsy method (14-16). PAECs were cultured from right heart catheter

balloon tips, expanded to passage 3 or 4 and submitted for library preparation and bulk RNA sequencing (Azenta, Cambridge, MA). Libraries were sequenced using a 2 x 50 bp paired-end rapid run on the Illumina HiSeq2500 platform. FastQ files were aligned to the human genome reference sequence GRCh38 using HISAT2 (17) and gene IDs were resolved using annotateMyIDs (18).

Cluster Analysis of Clinical Data and Differential Gene Expression Among Clusters
The medical record was retrospectively reviewed, and data including invasive hemodynamics, all available liver imaging and laboratory data (liver transaminases, albumin, platelets, total bilirubin, sodium, creatinine and INR) were collected. The model for end-stage liver disease (MELD) sodium (MELD-Na) 3.0 (19) and MELD Xi (20) were calculated and included as independent clinical variables. Clinical data and laboratory values were collected as close as possible to the date of PAEC biopsy and within six months of biopsy/RHC catheterization date. An unsupervised classification and regression tree (CART) (21) was used to identify distinguishable clusters within the data. Linear regression was subsequently used to model the relationship between these CART-derived clusters and pulmonary vascular resistance (PVR) at the time of PAEC cell biopsy, adjusting for right atrial pressure. Linear regression was performed to assess if any of the individual components of MELD-Na drove the composite score. Sensitivity analyses were performed to evaluate the influence of connective tissue disease patients and those taking warfarin on the results.

Differentially expressed genes between clusters were generated using linear discriminant analysis (LDA) with principal component analysis (PCA), LDA with weighted gene co-expression analysis (WGCNA), and a random forest model (22-24). All three models were assessed for accuracy to predict group assignment. Gene lists generated by all three models were analyzed using Ingenuity Pathway Analysis (Qiagen), significance for pathway enrichment was calculated using a right-tailed Fisher's Exact Test and gene lists and directional effects predicted using the Ingenuity Knowledge Base.

Bulk RNA Sequencing of Sugen-Hypoxia and Monocrotaline Livers
To validate findings about hepatic dysfunction in human PAH, we next turned to two leading animal models of pulmonary hypertension, the Sugen-hypoxia (SuHx) and monocrotaline (MCT) rat experimental models of PH were performed as described extensively in prior publications by our groups (25, 26). The right lobe of the liver was removed and fixed in formalin, paraffin embedded, sectioned at 5 micrometers and stained with Masson's trichrome per standard protocol. All images were acquired using a confocal microscope (Nikon) with a minimum of three images acquired from each slide. Total RNA was isolated from flash frozen livers from SuHx, MCT, and control rats (four each, all male) using Trizol (Invitrogen). RNA samples were submitted for bulk RNA sequencing to the sequencing core of the University of California, Los Angeles (UCLA); differentially expressed gene lists were generated using *DESeq* in R (27) and then analyzed using Ingenuity Pathway Analysis (Qiagen) where the significance of enrichment for pathways was calculated using a right-tailed Fisher's Exact Test.

Single-cell RNA Sequencing of Sugen-Hypoxia and Monocrotaline Lungs
To further validate our human PAEC findings, we utilized a publicly available data set of single-cell RNA sequencing of SuHX, MCT and control rat lungs (28). Raw expression data were normalized, filtered, and clustered using the *Seurat* R package (29). Cell types were identified using the labels assigned by the study authors. Differential gene expression in endothelial and immune cell populations was determined using the Wilcoxon rank-sum test. Gene expression was subsequently analyzed using Ingenuity Pathway Analysis (Qiagen).

Human Liver Biopsies and Movat Pentachrome Staining
To explore if findings from rat livers were consistent with human disease, we completed complimentary histologic analysis of human tissue from PAH and control patients. Human liver tissue was obtained via warm autopsy at UCLA. In accordance with 45 CFR 46, acquisition of this tissue did not constitute human subjects research and used deidentified human tissues provided by UCLA Pathology departmental honest broker, the UCLA Translational Pathology Core Lab (IRB# 11-002504). Formalin-fixed paraffin-embedded 5 micrometer thick human liver sections (Control n=2, PH n=3) were used for Movat Pentachrome staining following the manufacturer's instructions with slight modifications (Modified Russell-Movat; ScyTek Laboratories). All images were acquired using a confocal microscope (Nikon). A total of four to five images were acquired from each slide.

Results
PAH Patients with High-MELD Scores Have Worse Clinical Outcomes
Characteristics of the PAH participants with PAECs available are included in **Table 1**. CART analysis identified that only MELD-Na was predictive of PVR. Linear regression of the individual variables of MELD-Na revealed that no single variable in the composite score (e.g., creatinine, INR) was responsible for the relationship with PVR. A MELD-Na ≥ 12 was associated with a significantly higher PVR after adjustment for right atrial pressure (i.e., degree of hepatic congestopathy)(ß=0.5 Wood units, 95% CI 0.2-0.8, p=0.005). Nine participants (36%) had a MELD-Na ≥ 12 (High-MELD group) and sixteen (64%) had a MELD-Na < 12 (Low-MELD group). Both groups were similar in age, distribution of race and ethnicity, and renal function. Compared to the High-MELD group, the Low-MELD group had a higher proportion of female patients (94% vs 44%), fewer connective-tissue disease-associated PAH (CTD-APAH) patients (31% vs 44%) and were less likely to be on anticoagulation (0% vs 33%). There was no difference in body mass index (BMI), and there was no evidence of hepatic steatosis on available liver imaging (n=15) indirectly suggesting that hepatic steatosis was not present in this cohort. The six-minute walk distance (6MWD) was higher in the Low-MELD group compared to the High-MELD group (411 m vs 353 m, p=0.03). Because connective tissue diseases are associated with systemic inflammation (30, 31) and warfarin can increase the INR (a component of MELD-Na), we then performed sensitivity analyses excluding patients with connective tissue disease (n=9) and taking warfarin (n=2) and found the relationship between MELD-Na and PVR was unchanged.

PAECs from High-MELD Patients Differentially Upregulate Genes Related to Inflammation
We then used three machine learning models (LDA with PCA, LDA with WGCNA, and RF) to determine gene expression that predicted group assignment (High- vs. Low-MELD); this yielded an accuracy of 0.80, 0.72, and 0.60, respectively. To increase rigor, the union of LDA with PCA and LDA with WGCNA was chosen to build a differentially expressed gene list comparing High- vs. Low-MELD clusters **(Fig 1A).** Pathways analysis demonstrated enrichment for pathways related to apelin signaling, YAP/TAZ, as well as immunity and inflammation **(Fig 1B)**. Network analysis and regulatory networks revealed upregulation of pathways related to apelin, beta-catenin (*CTNNB1*), tumor necrosis factor (*TNF*) and *VCAM-1*, as well as cytokine signaling (e.g., IL-6) in High- vs Low-MELD PAECs **(Fig 2A-B).**

Liver Histology and Gene Expression from Two PH Rat Models Recapitulates Increased Inflammatory Signaling
We next looked to see if a similar pro-inflammatory signature existed in the livers of two small animal models of PH. Trichrome staining revealed increased perivascular and parenchymal fibrosis in SuHx rat livers compared to controls (3.5 vs 2.2 % area stained, p=0.01)(**Fig 3A-B**).

Bulk RNA sequencing of SuHx and MCT rat livers demonstrated similar findings to the High MELD-cluster gene expression from human PAH PAECs. Specifically, we observed increased expression in pathways related to inflammation and TGFβ signaling and decreased expression in pathways related to cellular metabolism. Although the MCT model is known to be characterized by hepatic inflammation and fibrosis (32), these themes were recapitulated in the SuHx model (**Fig 4A and B**). A regulatory network demonstrated activation of pathways related to activation and recruitment of leukocytes in these experimental PH livers (**Fig 4C**).

To understand if our observations in human lungs were consistent with the known biology of these two animal models, differential gene expression in human PAH PAECs from the High-MELD cluster and single cell sequencing data from SuHx and MCT rat lung ECs were then compared. There were 775 genes overlapping in both data sets (**Fig 5A**). Three genes were significantly expressed in both humans and animal regulatory networks: early growth response 1 (*EGR1*), extracellular matrix protein 1 (*ECM1*), and macrophage inhibitory factor (*MIF*). The union of these data sets represented genes with high relevance to cancer biology (**Fig 5B**).

Human PAH Livers Demonstrate Increased Fibrosis

Finally, we examined human liver tissue from five individuals to determine if the hepatic fibrosis seen in SuHx livers was also present in PAH patients without clinical liver disease or RV failure. Livers from a 68-year-old female with CTD-APAH who presented with one-week of dyspnea and a 56-year-old female with idiopathic PAH (IPAH) who presented with dyspnea after a new PAH diagnosis demonstrated increased perivascular and parenchymal fibrosis by pentachrome staining as compared to two male controls aged 84 and 77 years old (**Fig 6**). In the absence of any known clinically detectable underlying liver disease, the IPAH patient's liver had a remarkable increase in parenchymal fibrosis compared to all subjects, including a 47-year-old male with PoPH due to alcoholic cirrhosis.

Discussion

We demonstrate evidence of a lung-liver axis in PAH independent of primary liver disease and chronic RV failure. Specifically, in an unsupervised cluster analysis, the MELD-Na score was predictive of PVR among PAH patients with no clinical liver disease. In PAECs from patients in the High-MELD cluster, i.e., those with subclinical liver dysfunction, there was upregulation in gene expression pathways related to inflammatory activation of the pulmonary endothelium. These findings were recapitulated in two experimental models of pulmonary hypertension, one with known liver injury (MCT) but also in SuHx, a model not known to cause direct hepatic injury. There were concordant findings from human PAH PAECs with High-MELD and the two experimental PH models with upregulation of cell survival genes and those related to hepatic growth factor signaling (*EGR1*). Finally, human liver tissue from well characterized PAH patients demonstrated increased perivascular fibrosis, as seen in SuHx rats, but also had marked hepatic parenchymal fibrosis even in a newly diagnosed IPAH subject without longstanding RV failure.

These findings reinforce prior work demonstrating subclinical liver injury predicts clinical outcomes in PAH patients who have participated in clinical trials (2). In this report, cholestatic liver injury identified with routine laboratory monitoring predicted worse outcomes, suggesting that bile acids and their metabolism play a role in lung-liver communication in PAH. Circulating bile acids may influence pulmonary endothelial and lysosomal activity and cellular metabolism via the nuclear receptor coactivator 7 (NCOA7)(33). In the MCT PH model, ketone metabolism is impaired in the liver and leads to activation of the NLRP3 inflammasome suggesting a link between aberrant hepatic metabolism and activation of a systemic inflammatory process (34), in-line with our observations in both human and animal tissues. Taken together, these data

reinforce our hypothesis that the liver influences the pulmonary circulation as part of a feed-forward loop before (and independent of) underlying liver disease.

While specific mechanisms that link the hepatic and pulmonary vascular beds remain elusive, circulating immunologic and vasoactive factors that pass first through (or escape from) hepatic metabolism (11) and vascular growth factors secreted by the liver are likely important. In children with congenital heart disease, creation of a cavopulmonary shunt causes pulmonary arteriovascular malformations (AVMs)(35, 36). Circulating bone morphogenetic proteins (BMPs), a family of key signaling ligands in PAH (37) produced in the liver, are expressed at lower levels in patients with hepatopulmonary syndrome and PoPH as compared to liver disease controls (38, 39). We noted upregulation of apelin signaling in our PAECs, which we speculate may be a protective compensatory response as apelin is upstream of BMP signaling, including BMPR2 (40).

In human PAH PAECs with High-MELD and PH rat lungs, we noted increased expression of *EGR1*. EGR1 is a transcriptional regulator which plays a role in cell survival, proliferation, and death. Hepatocyte growth factor (HGF) can induce the expression of *EGR1* to regulate these processes (41, 42) and in hepatocellular cancer, EGR1 is implicated in malignant cells' escape from anticancer drugs via stabilization of microtubules and autophagy(43, 44). Increased HGF is associated with worse survival in PAH(45), however HGF supplementation has been shown to be beneficial in experimental PH(46). Hepatokines may modulate endothelial dysfunction in PAH but require additional investigation. We also noted upregulation of gene expression related to cell proliferation (*ECM1*)(47) and escape from cell death via Wnt signaling (*CTNNB1*) (48, 49), reinforcing well established paradigms of PAEC dysregulation in PAH(50).

Perivascular $CD68^+$ macrophages and other inflammatory cells are prominent in plexiform lesions in both animal models and humans(51-54). Macrophages are differentially polarized in PAH(55, 56), and depletion or inactivation of macrophages can prevent disease, including in portopulmonary hypertension(57-61). PAECs are known to recruit leukocytes via increased expression of VCAM-1, ICAM-1 and E-selectin(62). Here, we demonstrated increased expression of *VCAM-1* in the PAECs of High-MELD PAH patients(63, 64). Finally, cytokine signaling (e.g., IL-6, TNF) was upregulated in High-MELD PAECs concordant with established literature that circulating inflammatory cytokines and chemokines are elevated in idiopathic PAH and some, specifically IL-6 and TNF-α, correlate with outcomes(65, 66).

This study has limitations. We cannot definitively exclude hepatic congestion as contributing to our findings, although we adjusted for right atrial pressure in our analysis to minimize confounding. We acknowledge that the MELD-Na was not developed for use in a population without liver disease nor does it comprehensively capture liver function, however given its utility in predicting outcomes in left heart failure (13) we submit that its relevance extends to our patient population. Our results were unchanged when we excluded CTD participants, who may be more prone to autoimmune liver disease and those taking warfarin, which may pharmacologically increase the INR (a component of MELD-Na). We had similar results when we used alternative MELD scores (MELD-Xi). A sensitivity analysis evaluating individual components of MELD-Na demonstrated that no single factor explained the relationship with PVR. Furthermore, we have confirmed the hypotheses generated by our unsupervised analyses in two animal models of PH – one with known liver injury and one without – and human PAH, demonstrating hepatic inflammation and fibrosis is associated with activation of the pulmonary endothelium via expression of *VCAM-1, TNF,* and *MIF.* Whether these observations in SuHx are due to Sugen5416 directly acting on the liver or pulmonary endothelial injury and lung-liver crosstalk is unknown. However, VEGF inhibition in the liver has been shown to be

antiproliferative and antifibrotic (67, 68), whereas we observed increased fibrosis and inflammation in SuHx. Any prior observations of liver injury in SuHx are in chronic RV failure models (69). While BMI is a poor measure of body composition(67), our conclusion that steatohepatitis is not a confounder in this study is supported by the lack of fatty liver infiltration on all available liver imaging. Human liver tissue samples were limited, but similar themes emerged across human and animal tissues. While our observations need to be confirmed beyond the transcript level, we contend that consistent observations in humans, two animal models, and across lung and liver tissue sources are compelling. Future work will continue to explore deeper mechanisms that explain our observations, including the role of circulating growth and vasoactive factors, and expand on our observations in human disease.

In conclusion, we have demonstrated that the liver plays a role in the development of PAH by mediating inflammatory activation of the pulmonary endothelium. This relationship is characterized by hepatic inflammation and fibrosis, even in patients and animal models not known to have detectable liver disease. Taken together, this supports key interorgan modulation and a lung-liver axis early in the PAH disease continuum.

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Table 1. Participant Characteristics.

	Low-MELD-Na (n=16)	High-MELD-Na (n=9)	All PAH Participants (n=25)
Age, years	52 (30 – 75)	68 (43 – 84)	61 (30 – 84)
Female sex, n (%)	15 (94)	4 (44)	19 (76)
Race, n (%)			
Black	1 (6)	1 (11)	2 (8)
White	14 (88)	7 (78)	21 (84)
Asian	1 (6)	0	1 (4)
Other	0	1 (11)	1 (4)
Hispanic Ethnicity, n (%)	2 (13)	1 (11)	3 (12)
Body mass index (kg/m^2)	31 (16 – 40)	35 (23 – 45)	21 (16 – 45)
PAH Etiology, n (%)			
Idiopathic PAH	5 (31)	3 (33)	8 (32)
Hereditary PAH	3 (19)	0	3 (12)
Connective tissue disease-APAH	5 (31)	4 (44)	9 (36)
HIV-APAH	1 (6)	0	1 (4)
Congenital heart disease-APAH	2 (13)	0	2 (8)
PVOD	0	2 (22)	2 (8)
Functional Class III or IV, n (%)	3 (30)	2 (40)	5 (20)
	n=10	*n=5*	*n=15*
6MWD, meters	411 (300 – 600)	353 (120 – 576)	380 (120 – 600)
Hemodynamics			
RAP, mmHg	9 (3 – 19)	10 (4 – 15)	10 (3 – 19)
mPAP, mmHg	41 (22 – 69)	44 (26 – 62)	44 (22 – 69)
PAWP, mmHg	13 (6 – 15)	11 (6 – 17)	11 (6 – 17)
PVR, Wood units	4.6 (1.2 – 16.8)	6.7 (2.2 – 19.6)	5.3 (1.2 – 19.6)
CO, L/min	5.4 (3.3 – 9.9)	5.3 (1.9 – 11)	5.3 (1.9 – 11)
MELD-Na	9 (7 – 11)	15 (12 – 28)	10 (7 – 28)
Creatinine (mg/dL)	0.78 (0.52 – 1.26)	0.96 (0.60 – 1.65)	0.9 (0.52 – 1.65)
Anticoagulation, n (%)	0 (0)	3 (33)	3 (33)

Data are represented as n (%) or median (range). MELD-Na=model for end-stage liver disease with sodium. PAH=pulmonary arterial hypertension. HIV=human immunodeficiency virus. PVOD=pulmonary veno-occlusive disease. 6MWD=six-minute walk distance. RAP=right atrial pressure. mPAP=mean pulmonary artery pressure. PAWP=pulmonary artery wedge pressure. PVR=pulmonary vascular resistance. CO=cardiac output.

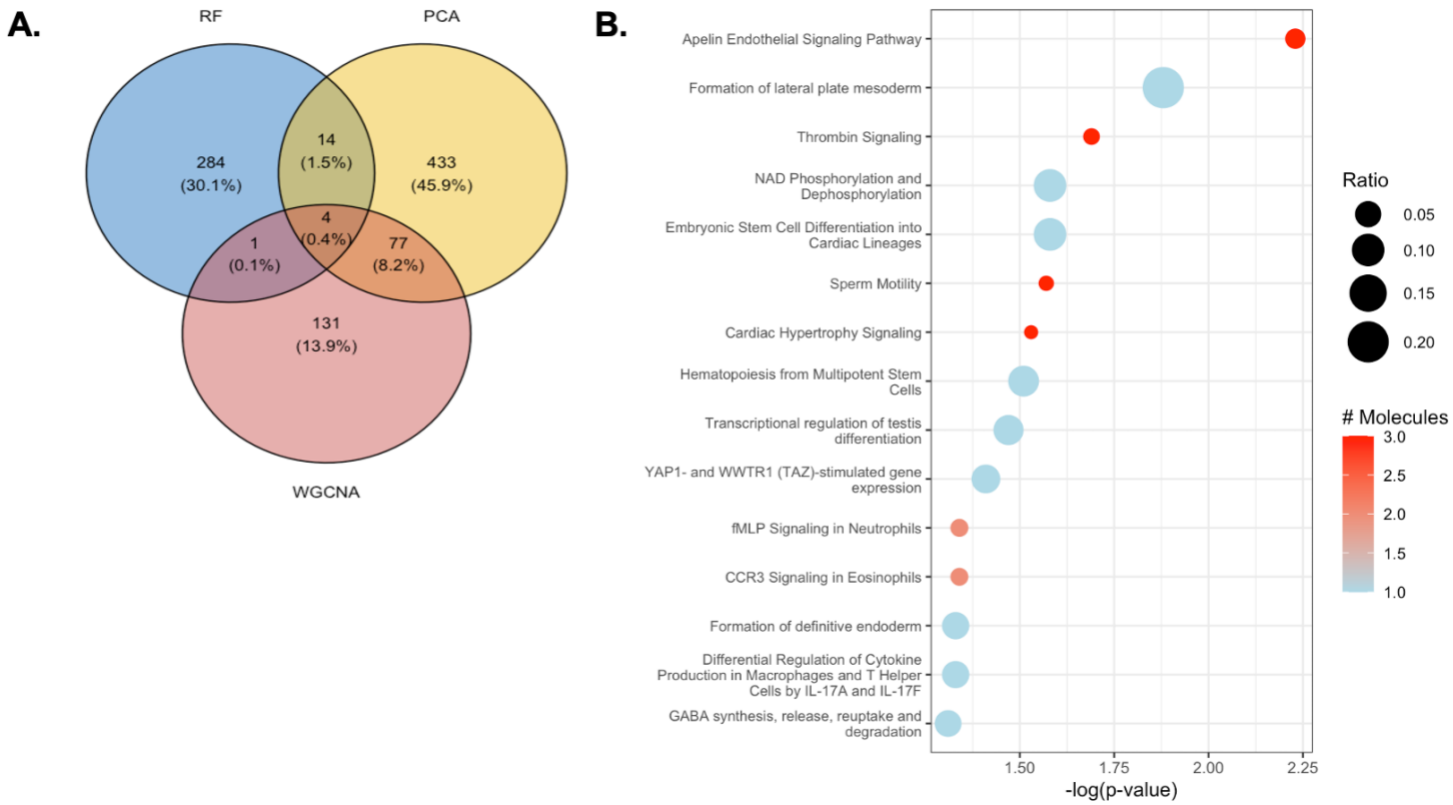


Figure 1. Differences in gene expression between High- and Low-MELD groups were examined using three modeling approaches. **A.** The number and proportion of genes generated by three independent models (linear discriminant analysis (LDA) with principal component analysis (PCA), LDA with weighted gene co-expression analysis (WGCNA), and a random forest (RF) model) and their intersections was reviewed and the intersection of LDA with PCA and WGCNA was used for all subsequent analysis. **B.** Pathways analysis demonstrates enrichment for pathways related to apelin signaling, YAP/TAZ, as well as immunity and inflammation

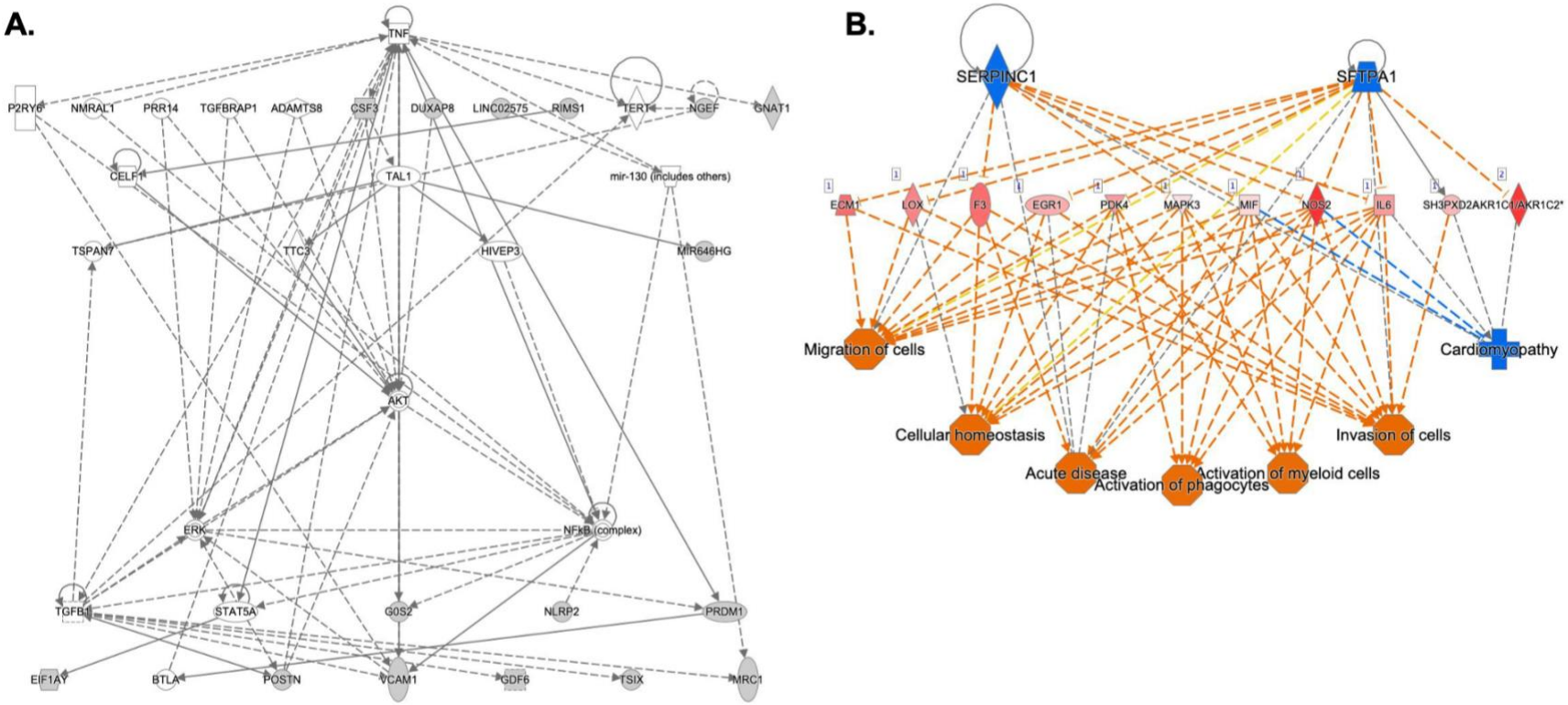


Figure 2. Network analysis of differentially expressed genes in PAECs from High- vs Low-MELD PAH patients reveal High-MELD patients increase expression related to inflammation and immunity. A. Network analysis revealed highly relevant expression related to Wnt signaling (*CTNNB1*), inflammation (*TNF*) and recruitment of leukocytes to the pulmonary vasculature (*VCAM1*). **B.** A regulatory network confirmed activation of pathways related to activation of leukocytes and invasion of cells into the pulmonary vasculature mediated by inflammatory signaling (e.g., IL-6), orange=upregulation, blue=downregulation.

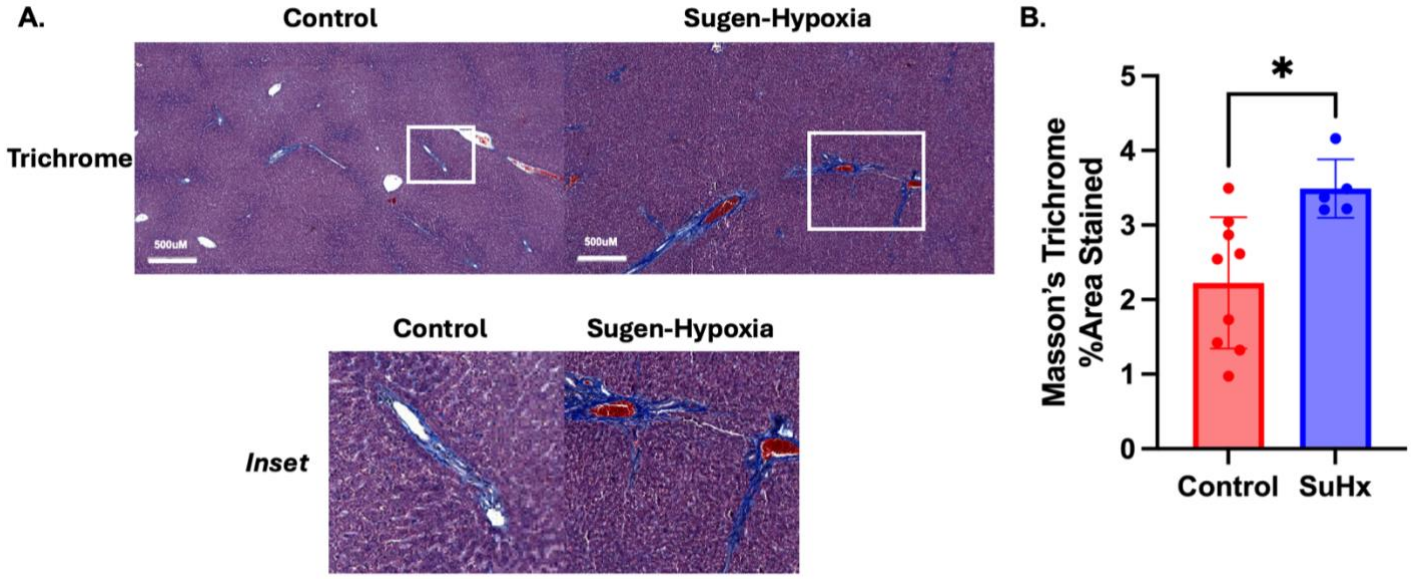


Figure 3. Increased hepatic fibrosis in SuHx rats compared to controls. Control and SuHx rat livers were stained with Masson's Trichrome which resulted in increased perivascular and parenchymal fibrosis (**A**), 3.5 vs 2.2 % area stained, p=0.01 (**B**). n=2-3 rats per group. *=<0.05. SuHx=Sugen-hypoxia. All images taken at 4X. Scale bar = 500 micrometers.

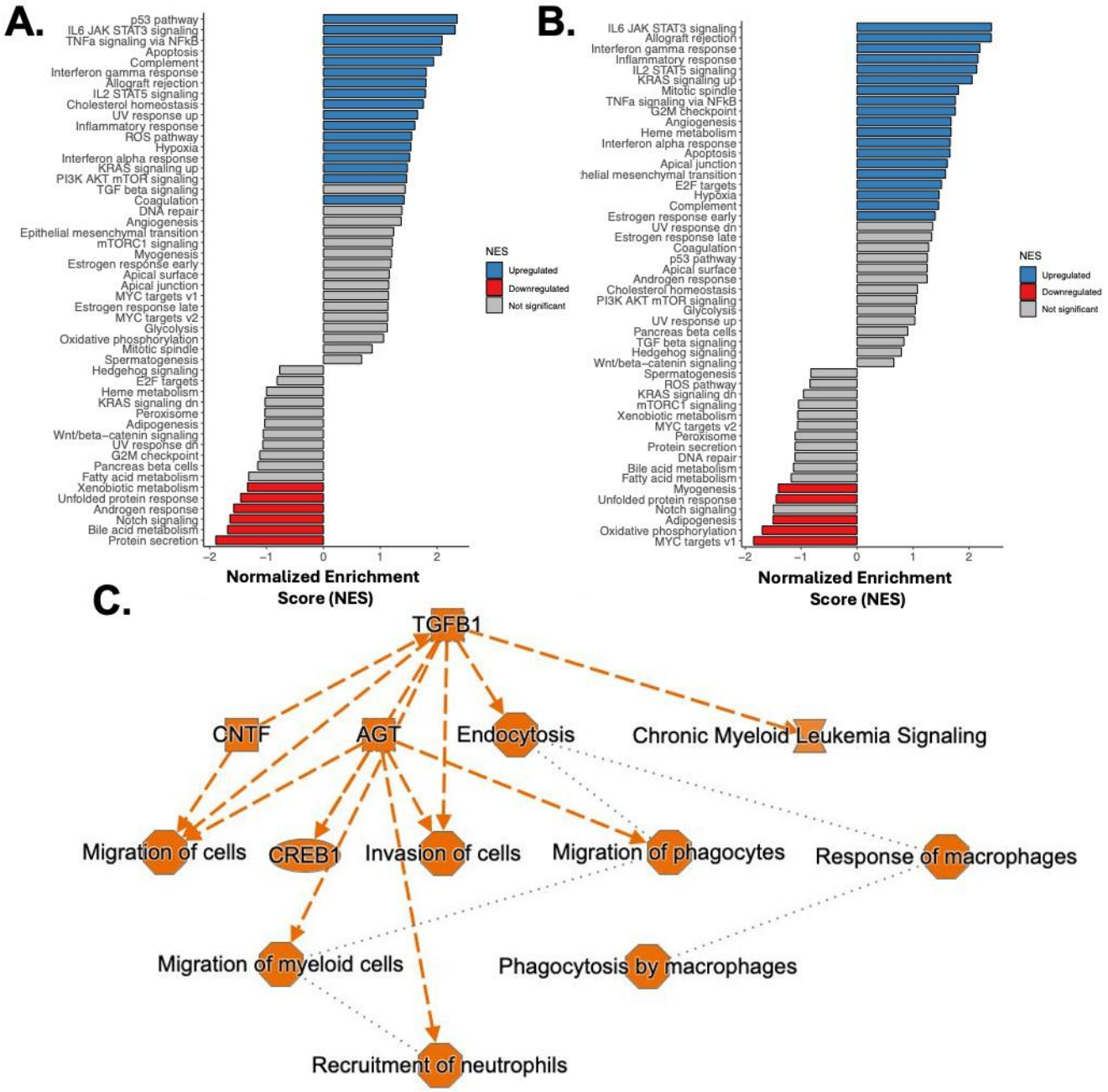


Figure 4. Bulk RNA sequencing of MCT (**A.**) and SuHx (**B.**) male rat livers compared to controls demonstrates increased gene expression in pathways related to inflammation and TGB beta signaling and decreased expression in pathways related to cellular metabolism. **C.** A regulatory network built from differentially expressed genes in both MCT and SuHx rat livers demonstrates activation of pathways related to activation and recruitment of leukocytes.

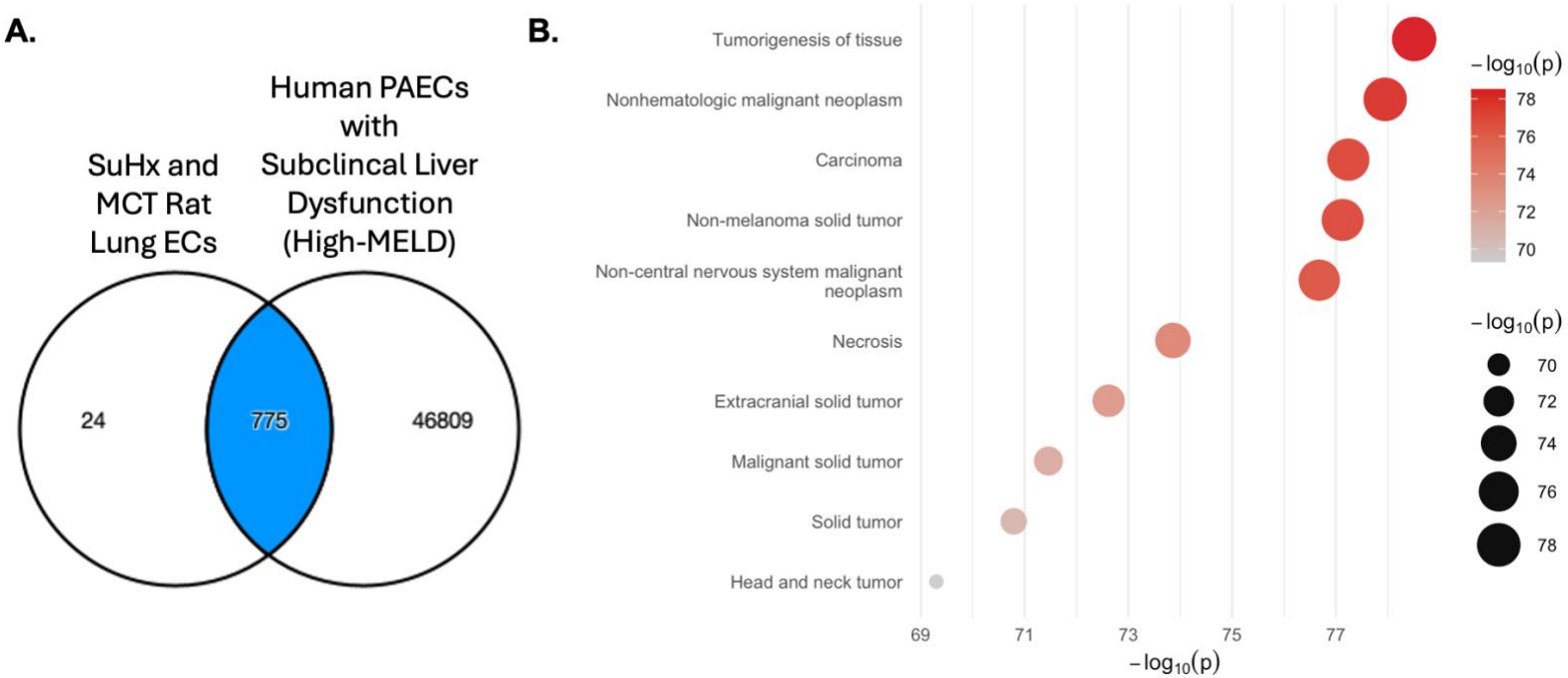
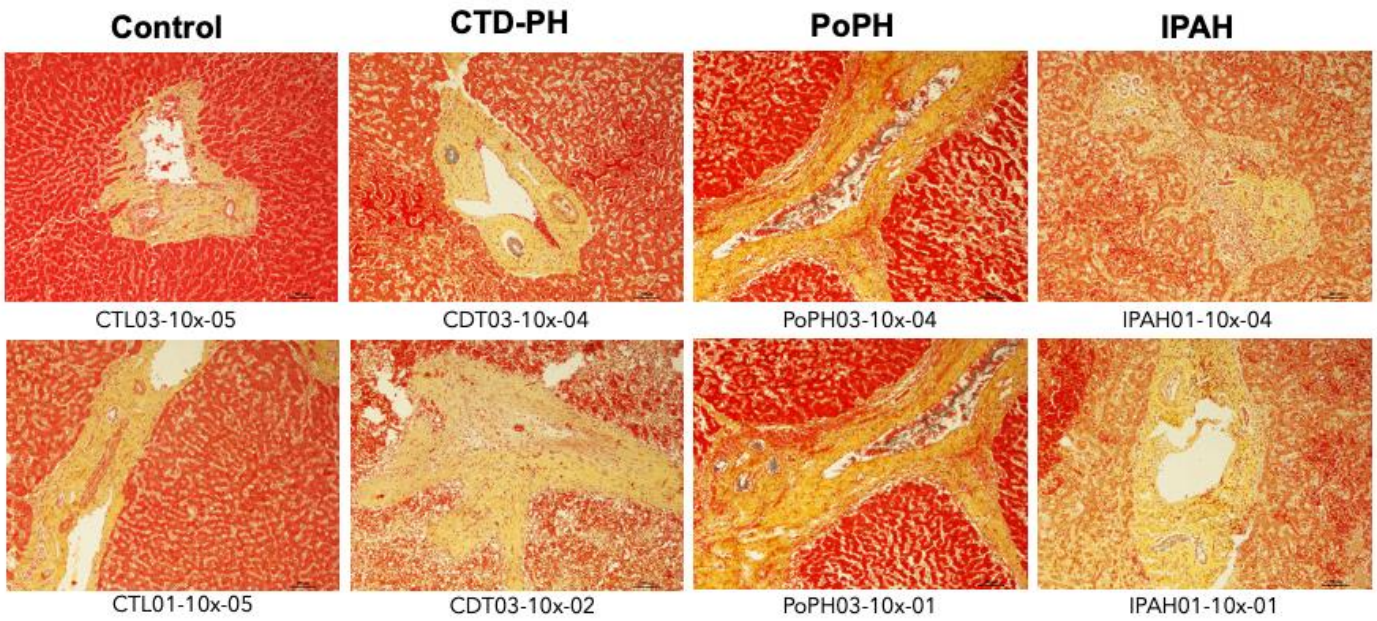


Figure 5. Differential gene expression of SuHx and MCT rat lung ECs demonstrated overlap with PAECs from humans with high-MELD scores (**A**). Genes clustered into highly relevant pathways of cancer biology (**B**). SuHx=Sugen-Hypoxia. MCT=monocrotaline. EC=endothelial cell. PAEC=pulmonary artery endothelial cell.



Subject ID	Age (years)	Sex	PAH Subtype	Clinical Vignette
CTL03	84	Male	Control	--
CTL01	77	Male	Control	--
CDT03	68	Female	CTD-APAH	History of scleroderma, rheumatoid arthritis and lupus; presented with one week of increasing dyspnea.
PoPH03	47	Male	PoPH	History of alcoholic cirrhosis listed for liver transplant complicated by variceal bleed, hepatic encephalopathy who presented with weight gain, hypotension and increasing abdominal distention and lethargy.
IPAH01	56	Female	IPAH	History of chronic kidney disease of unknown etiology presented with a three-week history of progressive dyspnea and newly diagnosed PAH.

Figure 6. Histologic analysis of liver tissue from humans with and without pulmonary arterial hypertension (PAH) demonstrate increased fibrosis in several phenotypes of PAH as compared to controls. All images taken at 10x. Tissue stained with Movat-pentachrome where yellow=collagen. CTD-APH=connective-tissue disease associated pulmonary hypertension. PoPH=portopulmonary hypertension. IPAH=idiopathic pulmonary arterial hypertension.

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