Inhibition of integrin $\alpha_V\beta_1$ attenuates profibrogenic gene expression by myofibroblasts in fibrotic human liver explants

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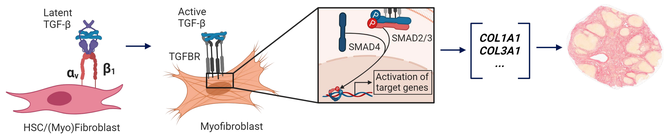
BACKGROUND

Integrin $\alpha_V\beta_1$ is a (myo)fibroblast-specific integrin that activates transforming growth factor (TGF)-β, promoting fibrogenesis. Inhibition of $\alpha_V\beta_1$ is antifibrotic in mouse models of liver fibrosis; however, data in human tissue are limited. Precision-cut liver slices (PCLivS) bridge the gap between cell-based models and *in vivo* models of liver fibrosis, providing a translational assay platform for investigating fibrogenesis in small sections of intact fibrotic human tissue cultured *ex vivo*. Here we use human PCLivS and single nuclei RNA-sequencing (snRNA-seq) to evaluate the effects of an $\alpha_V\beta_1$-selective inhibitor on individual cell populations present in fibrotic human liver tissue.

Figure 1. Role of $\alpha_V\beta_1$ in Liver Fibrosis

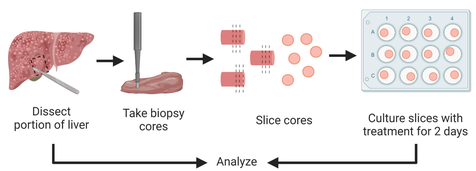


METHODS

Precision-cut Liver Slices

Human liver tissue with or without evidence of fibrosis (fibrotic and normal, respectively) was obtained from rejected organ donors. Integrin $\alpha_V\beta_1$ protein levels in donor tissues were quantified by custom Meso Scale Discovery electrochemiluminescence assay. PCLivS were generated from fibrotic liver tissue and cultured for 2 days in the presence of a selective $\alpha_V\beta_1$ inhibitor ($\alpha_V\beta_1$ inh) or vehicle (DMSO). While systemic TGF-β inhibitors have limited clinical utility, a TGF-β receptor I kinase inhibitor (ALK5 inh, R-268712), which blocks TGF-β signaling downstream of integrin activation, was also evaluated as a positive control, to demonstrate a similar mechanism of action with $\alpha_V\beta_1$ inhibition in the fibrotic human liver.

Figure 2. Generation and Culture of PCLivS



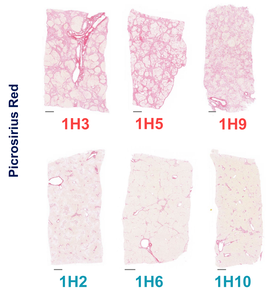
snRNA-seq and Analysis

Nuclei were isolated from three pooled liver slices per treatment and processed for single nuclear barcoding using 10x Chromium Next GEM 3' HT kits. Resulting libraries were sequenced, processed using CellRanger, and analyzed using Seurat. Custom annotation of cell types was performed using gene markers established from published data sets[1,2]. Differential gene expression was determined using a non-parametric Wilcoxon rank sum test. Pathway enrichment analysis was performed with EnrichrGO[3].

RESULTS

Characterization of Livers for PCLivS

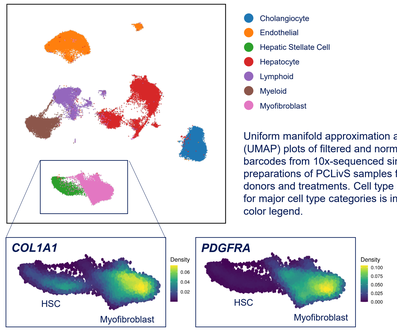
Figure 3. Fibrosis in Donor Livers



Picrosirius red staining for collagen in liver explant tissue from 6 donor livers with fibrosis
Scale bars = 1 mm.

Identification of Cells Present in PCLivS

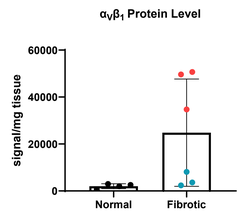
Figure 5. snRNA-seq Analysis Identified Major Hepatic Cell Populations



Uniform manifold approximation and projection (UMAP) plots of filtered and normalized unique barcodes from 10x-sequenced single nuclei preparations of PCLivS samples from all donors and treatments. Cell type annotation for major cell type categories is indicated by color legend.

UMAPs indicating *PDGFRA and COL1A1* expression by density plot in myofibroblast and HSC clusters

Figure 4. $\alpha_V\beta_1$ Levels in Fibrotic Livers



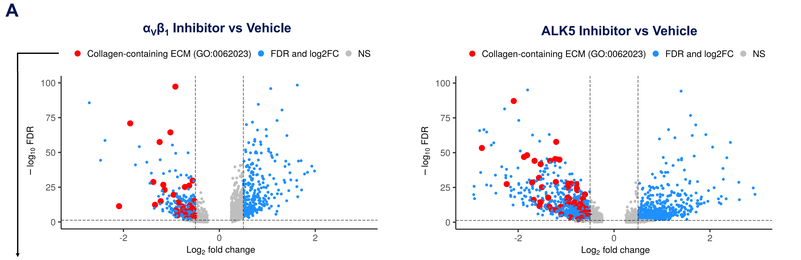
- Picrosirius red staining shows fibrosis in donor explants
- $\alpha_V\beta_1$ protein levels were most elevated in the livers with more advanced fibrosis (red labels and dots)
- Major hepatic cell populations were identified in PCLivS post-culture and treatment

Relative $\alpha_V\beta_1$ protein levels in human liver tissue with (Fibrotic) or without (Normal) evidence of fibrosis measured by custom Meso Scale Discovery electrochemiluminescence assay

Evaluation of Differentially Expressed Genes in Myofibroblasts

Figure 6. $\alpha_V\beta_1$ Inhibitor Significantly Decreased Profibrogenic Pathways in Myofibroblasts

A



TIMP1, FN1, COL1A1, COL8A1, GPC6, MGP, BGN, S100A6, TGFBI, LTBP2, TGFB2, SRPX2, SULF1, AEBP1, LAMC2, COL16A1, TIMP3, HSPG2, CTSC, GREM1, TGFB1, COL18A1, POSTN, SERPINE2, WNT5A, ANGPT2, MFGE8, CTHRC1, CALR, WNT5B, CD151, THSD4, TGM2, COL10A1, COL15A1, EDIL3, PCOLCE, ACTA2

B

$\alpha_V\beta_1$ Inhibitor vs Vehicle

GO: Biological Process	Adj. P-Value
extracellular matrix organization	6.65E-08
extracellular structure organization	6.65E-08
external encapsulating structure organization	6.65E-08
cell-substrate adhesion	0.000419
collagen fibril organization	0.000579
epithelial to mesenchymal transition	0.001029
collagen metabolic process	0.002352
regulation of collagen metabolic process	0.003501
collagen biosynthetic process	0.004777

ALK5 Inhibitor vs Vehicle

GO: Biological Process	Adj. P-Value
extracellular matrix organization	3.87E-13
extracellular structure organization	3.87E-13
external encapsulating structure organization	3.87E-13
collagen fibril organization	6.98E-08
collagen metabolic process	1.22E-05
collagen biosynthetic process	6.32E-05
cell-substrate adhesion	0.000127
regulation of collagen metabolic process	0.000151
epithelial to mesenchymal transition	0.000232

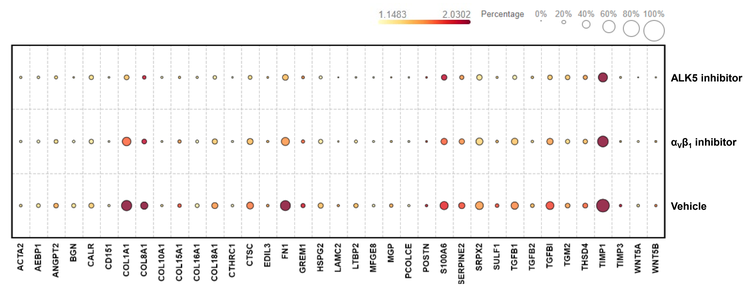
(A) Volcano plots of differentially expressed genes in $\alpha_V\beta_1$ inhibitor-treated or ALK5 inhibitor-treated vs vehicle-treated comparison in myofibroblasts. Genes indicated in blue are $|\log2FC| > 0.5$ and FDR < 0.05. Enriched gene lists for the significantly downregulated collagen-containing extracellular matrix term (adj.p=2.55E-17) are shown and highlighted in each volcano plot (red), and specifically listed below for $\alpha_V\beta_1$ inhibitor. (B) Select GO:Biological Process terms from pathway enrichment analysis of downregulated genes ($|\log2FC| > 0.5$, FDR < 0.05).

- Differential gene expression analysis of $\alpha_V\beta_1$ inhibitor-treated or ALK5 inhibitor-treated versus vehicle-treated PCLivS showed downregulation of genes related to extracellular matrix and collagen fibril organization

Comparison of $\alpha_V\beta_1$ Inhibition and ALK5 Inhibition

Figure 7. $\alpha_V\beta_1$ and ALK5 Inhibitors had Similar Effects on Fibrogenic Gene Expression in Myofibroblasts

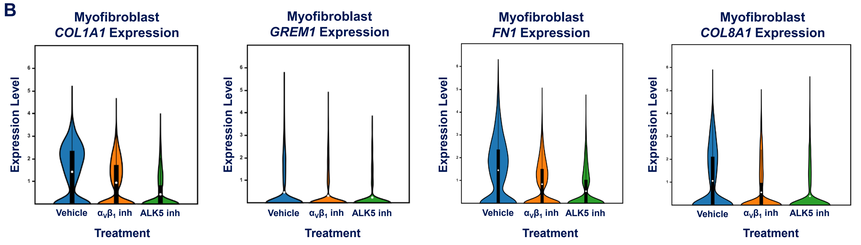
A



- Differential gene expression analysis of $\alpha_V\beta_1$ inhibitor-treated or ALK5 inhibitor-treated versus vehicle-treated PCLivS showed downregulation of similar genes and processes

(A) Bubble heat map of expression of genes from the enriched GO:Cellular Component term collagen-containing ECM (GO:0062023) by treatment in myofibroblasts. (B) Violin plots for *COL1A1, GREM1, FN1, and COL8A1* expression by treatment in myofibroblasts.

B



CONCLUSIONS

- **Treatment of fibrotic human PCLivS with an $\alpha_V\beta_1$ inhibitor resulted in clear reductions in profibrogenic gene expression by myofibroblasts.**
- **The similar degree of effect of $\alpha_V\beta_1$ and ALK5 inhibition on myofibroblasts demonstrates the importance of the $\alpha_V\beta_1$ integrin-TGF-β activation pathway in fibrotic liver disease.**
- **These data support $\alpha_V\beta_1$ integrin inhibition as a promising approach for inhibition of TGF-β signaling in fibrotic liver disease.**

References: 1. Ramachandran P, et al. Nature 2019; 575(7783), 2. Andrews TS, et al. Hepatol Commun 2022; 6(4). 3. Yu G, et al. OMICS 2012; 16(5).

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Disclosures: SH, MA, RA, VR, ST, MD, and JS are employees and shareholders of Pliant Therapeutics