Liver cell-type specific molecular signatures marking transition from advanced fibrosis to cirrhosis in human non-alcoholic steatohepatitis

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Background & Aim

Hepatic fibrosis is the strongest predictor of morbidity and mortality in non-alcoholic steatohepatitis (NASH), establishing fibrosis as a critical therapeutic target in NASH. To improve treatment outcomes in NASH, it is pertinent to define hepatic cell phenotype-specific molecular signalling mechanisms involved in the progression of fibrosis towards cirrhosis. Using paired bulk and single-nucleus RNA sequencing, we mapped hepatic transcriptome signatures of parenchymal and non-parenchymal cell types across all stages of fibrosis in human NASH.

Methods

Bulk RNA sequencing (RNAseq) was performed on snap-frozen liver biopsies obtained from a patient cohort of 39 obese individuals with NASH (stages F0-F4) or no/mild NAFLD (macrovesicular steatosis) without fibrosis (control group). Paired single-nucleus (sn) RNAseq analysis was performed on a subset of biopsies (Control, NASH F1, NASH F2-F3, NASH F4; n=4 per group). See study outline in Fig. 1

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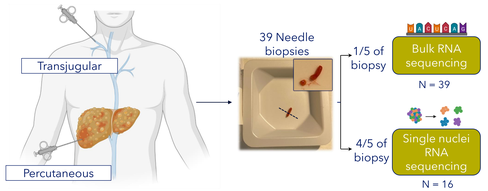
1 Paired bulk + single nuclei RNA sequencing pipeline



										Fibrosis score				NAFLD activity score							
Group	N	M/F	T2DM	HT	DL	Age [yr]	BMI [kg/m²]	ALT [U/l]	AST [U/l]	0	1	2	3	4	0	1	2	3	4	5	6	7	8
Control	12	8/4	5	2	7	55.0±13.0	32.2±8.2	48.0±37.4	30.6±16.2	12	0	0	0	0	4	4	2	2	0	0	0	0	0
F1	10	8/2	6	7	7	50.9±13.5	32.7±4.3	84.3±41.5	53.1±21.4	0	10	0	0	0	0	1	0	2	1	3	2	1	0
F2/F3	11	6/5	8	6	10	53.6±11.8	33.7±5.2	107.0±170.1	70.1±96.3	0	0	7	4	0	1	0	0	1	2	4	1	2	0
F4	6	2/4	4	3	3	65.2±7.5	29.4±4.9	63.3±60.0	73.8±42.9	0	0	0	0	6	1	1	0	2	1	0	1	0	0

2 Transcriptome signatures of cirrhosis

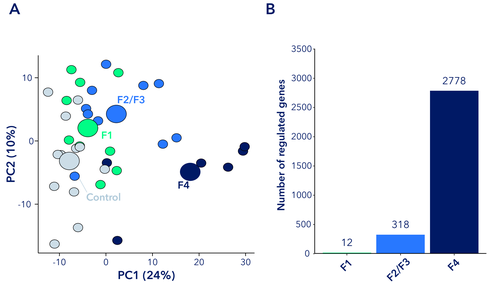


Figure 2. Transcriptome signature of cirrhosis. (A) Principal component analysis (PCA) of top-500 most variable genes. **(B)** Total number of differentially expressed genes relative to Control group (Padj < 0.05).

3 Perturbation of signaling pathways in cirrhosis

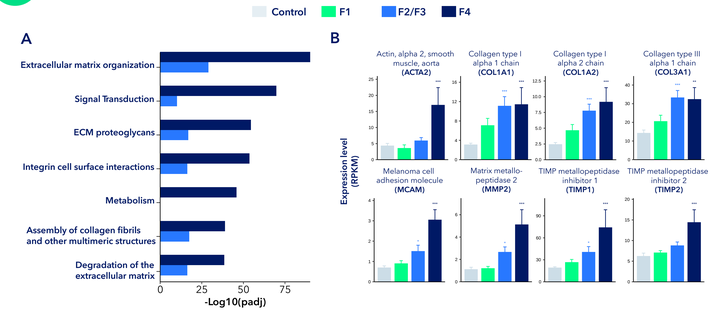


Figure 3. Perturbation of hepatic signaling pathways in NASH-related cirrhosis (A) Unbiased Reactome pathway analysis for comparisons F1, F2/3 and F4 relative to Control. Top-7 most enriched pathways are visualized. **(B)** Expression levels (reads per kilobase million, RPKM) of selected ECM-associated genes. *: padj<0.05, **:padj<0.01, *** padj < 0.001 relative to Control group.

4 snRNAseq recovers all major liver cell types in NASH patients

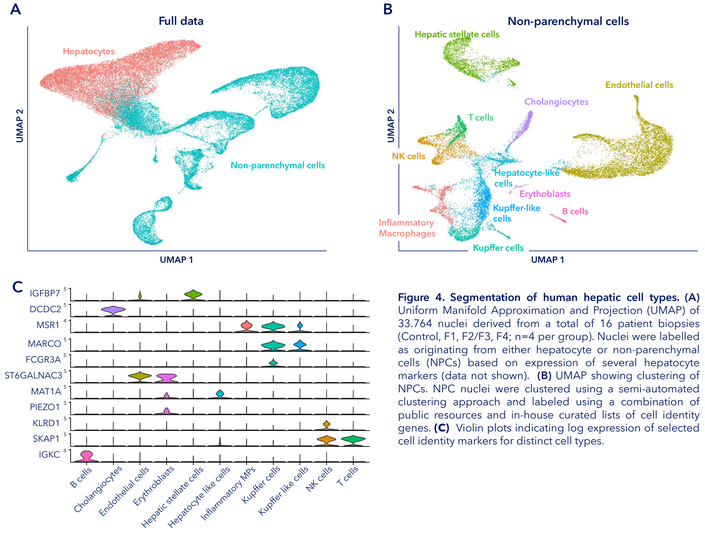


Figure 4. Segmentation of human hepatic cell types. (A) Uniform Manifold Approximation and Projection (UMAP) of 33.764 nuclei derived from a total of 16 patient biopsies (Control, F1, F2/F3, F4; n=4 per group). Nuclei were labelled as originating from either hepatocyte or non-parenchymal cells (NPCs) based on expression of several hepatocyte markers (data not shown). **(B)** UMAP showing clustering of NPCs. NPC nuclei were clustered using a semi-automated clustering approach and labeled using a combination of public resources and in-house curated lists of cell identity genes. **(C)** Violin plots indicating log expression of selected cell identity markers for distinct cell types.

5 snRNAseq identifies several HSC subpopulations

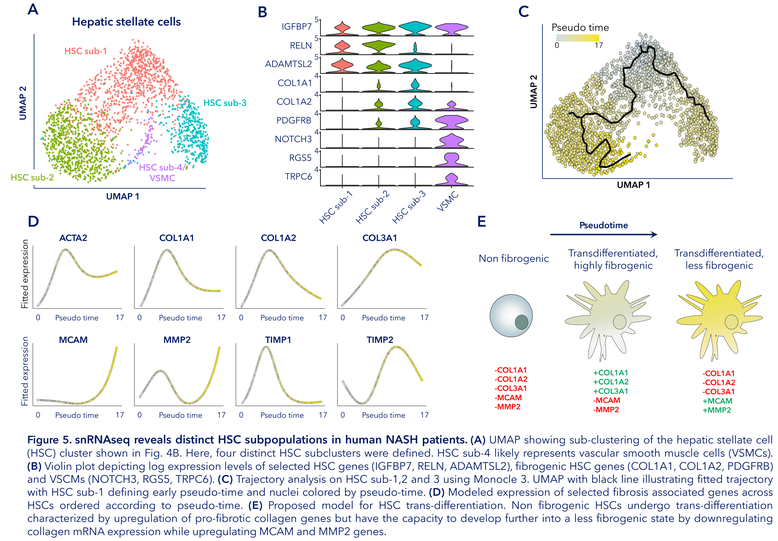


Figure 5. snRNAseq reveals distinct HSC subpopulations in human NASH patients. (A) UMAP showing sub-clustering of the hepatic stellate cell (HSC) cluster shown in Fig. 4B. Here, four distinct HSC subclusters were defined. HSC sub-4 likely represents vascular smooth muscle cells (VSMCs). **(B)** Violin plot depicting log expression levels of selected HSC genes (IGFBP7, RELN, ADAMTSL2), fibrogenic HSC genes (COL1A1, COL1A2, PDGFRB) and VSCMs (NOTCH3, RGS5, TRPC6). **(C)** Trajectory analysis on HSC sub-1,2 and 3 using Monocle 3. UMAP with black line illustrating fitted trajectory with HSC sub-1 defining early pseudo-time and nuclei colored by pseudo-time. **(D)** Modeled expression of selected fibrosis associated genes across HSCs ordered according to pseudo-time. **(E)** Proposed model for HSC trans-differentiation. Non fibrogenic HSCs undergo trans-differentiation characterized by upregulation of pro-fibrotic collagen genes but have the capacity to develop further into a less fibrogenic state by downregulating collagen mRNA expression while upregulating MCAM and MMP2 genes.

Conclusion

- We performed paired high-quality bulk and snRNAseq on small liver biopsies from NASH patients
- NASH patients with cirrhosis demonstrate a unique hepatic transcriptome signature
- snRNAseq recovers all major human liver cell types
- Fibrogenic HSCs may potentially differentiate into a less fibrogenic subtype
- Molecular drivers of HSC trans-differentiation may be targeted to improve outcomes in NASH-related fibrosis