A single nucleus and spatial transcriptomic atlas of the liver reveals multicellular changes in response to SARS-CoV2 infection

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Background: The clinical presentations of COVID-19 range from asymptomatic to viral pneumonia, acute respiratory distress syndrome, and multiorgan failure; indicating a highly variable host response. However, the exact cellular mechanisms underscoring these phenotypes across diverse tissues remain unclear. Abnormal liver biochemistry is commonly observed in COVID-19 patients, with reports ranging from 15-65% of infected individuals, and often associated with worse clinical outcomes. To date, studies of COVID-19 using human liver tissue are scarce, hindering in-depth investigation of COVID-19-related liver injury, its main causes, and potential long-term effects. To this end, we combined *in situ* tissue transcriptome profiling aided with single nucleus sequencing (sNuc-Seq) to generate integrated atlases of localized transcriptional programs, capture intercellular communication, and provide novel insights in disease pathogenesis.

Methods: In order to provide an in-depth cellular investigation of the human COVID-19 liver, we performed single nucleus sequencing on 20 liver samples from COVID-19 patients, and whole transcriptome spatial profiling on 6 Regions of interest (ROIs) from 4 concordant patient samples. By comparing to healthy livers, we generated a highly granular characterization of the COVID-19 liver cellular landscape and the viral impact on cell populations, states, and intracellular communications, which potentially helps to recognize and understand liver-associated sequelae. This liver atlas also provides novel insights into the fundamental liver cellular biology as well as how it responds to viral infection and multi-organ failure. Postmortem biopsies were collected within 3h of asystole under ultrasound guidance. Biopsies were formalin fixed and flash frozen for digital spatial profiling (DSP) and sNuc-Seq, respectively. Whole Transcriptome Atlas (WTA) - DSP assay was performed on formalin-fixed parafilm embedded (FFPE) tissue sections using a NanoString GeoMx platform. sNuc-Seq libraries were prepared with a 10x Chromium Controller using isolated nuclei from frozen tissue. SARS-CoV-2 abundance was quantified with qPCR, probes in WTA library, and *In Situ* Hybridization.

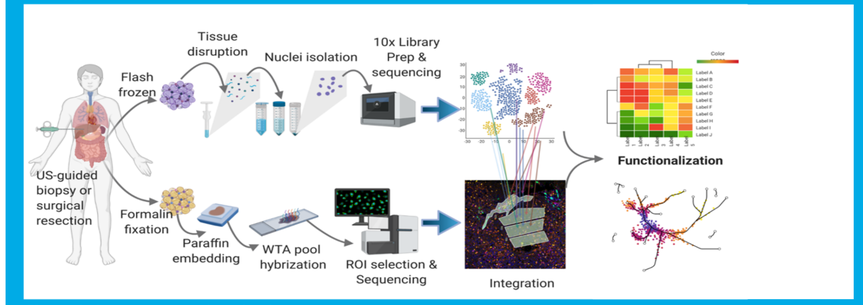

Figure 1. Sample processing pipeline

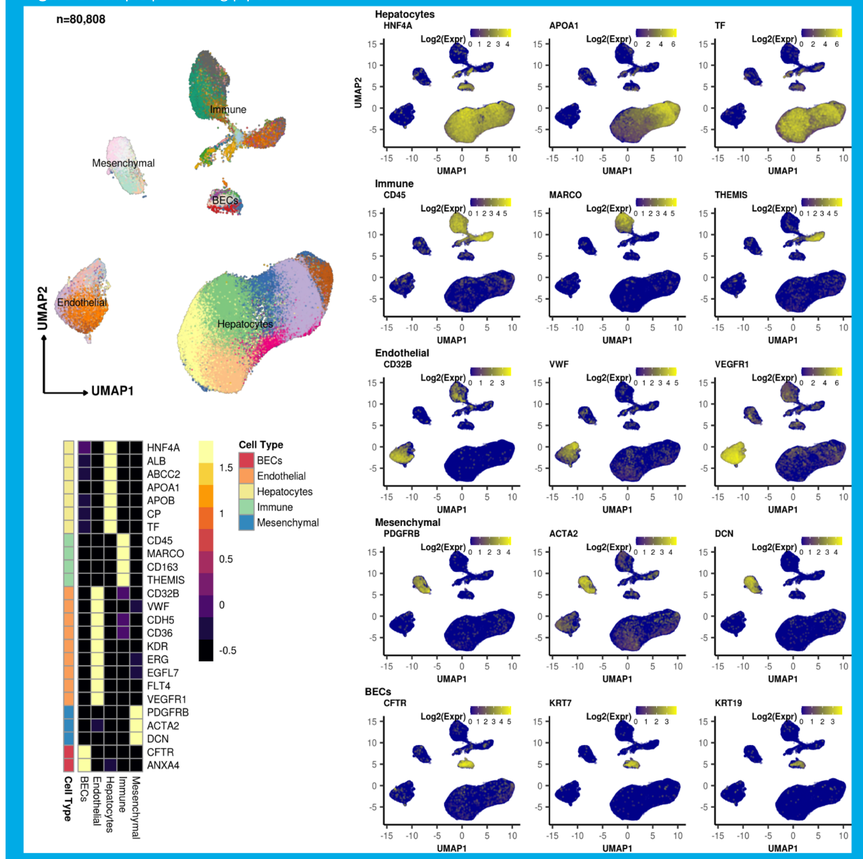

Figure 2. Major cell types present in the sNuc-seq data

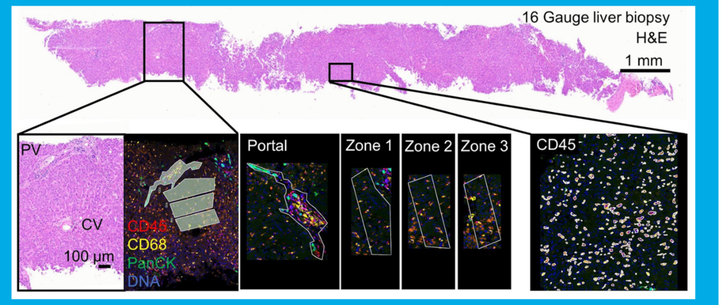

Figure 3. Regions of interest (ROIs) corresponding to the liver lobule, and portal area

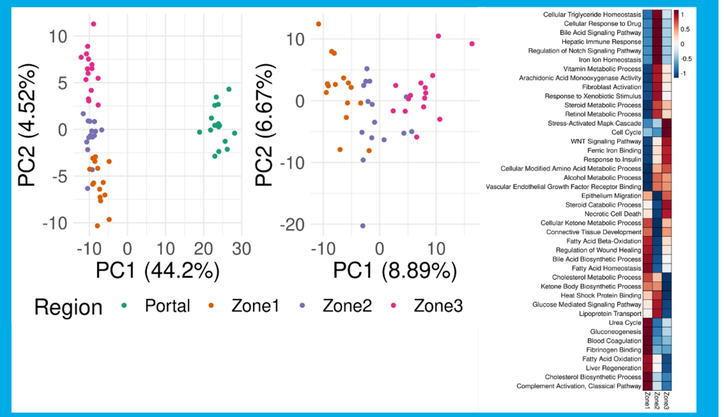

Figure 4. PCA plots and zonation pathways based on WTA-DSP liver data

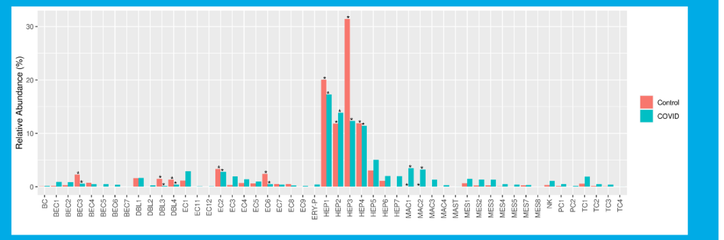

Figure 5. Cell type abundances in control livers and livers from patients affected by COVID-19

ID	Age/Sex	Hospital stay (d)	Comorbidities	SARS-CoV2: lung (Cp/ug RNA)
D13	80+M	10	ESRD on dialysis, HTN	$6.0x\ 10^7$
D14	80+F	16	HTN, DM2, TIA, dementia	$2.1\ x\ 10^4$
D15	50-60F	40	HTN, DM2, obesity, CKD	10
D16	70-80F	15	ESRD on dialysis, HTN	$1.7x10^4$
D17	70-80M	31	Myasthenia gravis, HTN	8,435

Table 1. Human donor characteristics

Results: On average, 5,380 nuclei were sequenced per sample with >1,200 genes expressed per cell. In the spatial analysis, 288 ROIs were quantified across 15 patient samples, providing an average of ~16,000 genes expressed per ROI. sNuc-Seq data permitted the characterization of cellular populations as well as the identification of cell-specific markers, pathways, trajectories, and the expression of ligands/receptors per cell type. The WTA datasets enabled direct comparisons between microscopic regions with distinct pathological features and revealed disease-specific pathways. Spatial transcriptomics also supported the generation of atlases capturing the role of tissue architecture on gene expression. Expression profiles defined by liver zonation were identified by WTA-DSP and directed the annotation of the sNuc-Seq cell clusters, a process which relied in the past mostly on empirical data. These two modalities exhibited very high complementarity, enabling us to assign cell clusters on tissue regions.

Acknowledgements

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