Integration of Whole Transcriptome Spatial Profiling with Single Nucleus Sequencing reveals unique pathways associated with multi-organ phenotypes in COVID-19

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Abstract

In addition to causing viral pneumonia and acute respiratory distress syndrome, COVID-19 impacts multiple organ systems, and manifests in heart and renal failure, liver injury, bone marrow suppression, nerve damage, and thromboembolic events. How the virus manifests in various organs remains poorly understood. To this end, we combined *in situ* tissue transcriptome profiling aided with single nucleus sequencing (sNuc-Seq) to generate integrated atlases on five tissues from five patients who succumbed to COVID-19 at a tertiary medical center. The integrative analyses revealed localized transcriptional programs, capture intercellular communication, and provide novel insights in disease pathogenesis.

Whole transcriptome spatial profiling and sNuc-Seq was performed on lung, heart, liver, kidney, and spleen, from five patients with PCR-confirmed COVID-19 pneumonia. 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 was performed on FFPE tissue sections using the NanoString GeoMx® platform. sNuc-Seq libraries were prepared on 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.

On average, 5,000+ nuclei were sequenced per sample with >1,200 genes expressed per cell, showcasing the robustness of the sNuc-Seq protocol for diverse tissues and frozen samples. 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 and pathways. Importantly, parenchymal cells were extensively represented across tissues, showcasing an additional advantage of the sNuc-Seq protocol. The WTA datasets enabled direct comparisons between microscopic regions with distinct pathological features (e.g. lung alveoli with inflammation vs normal appearing alveoli) and revealed disease-specific pathways. Spatial transcriptomics also supported the generation of atlases capturing the role of tissue architecture on gene expression. For instance, 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 rodent data or a limited set of marker genes. These two modalities exhibited very high complementarity, proving that coupling DSP profiling with single-nucleus assays provides a unique opportunity to understand spatial biological dynamics at a cellular resolution.

COVID-19 exhibits multi-organ phenotypes differentially affecting tissues and cellular programs. The integration of WTA, a novel whole transcriptome spatial profiling assay, with sNuc-Seq maximized the utility of both modalities and mitigated their respective limitations. This integrative approach is broadly applicable and allows mechanistic investigations of complex diseases including 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

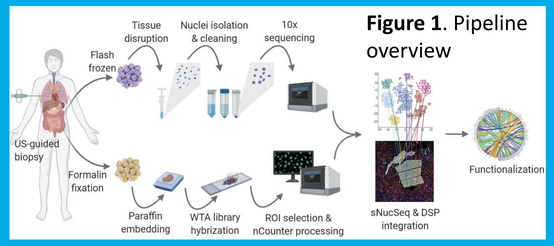


Figure 1. Pipeline overview

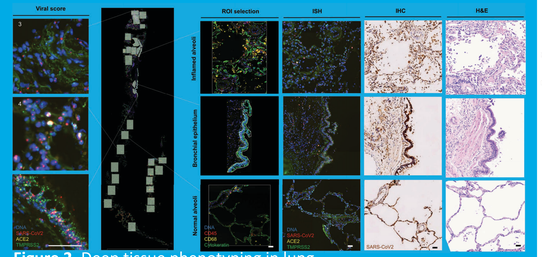


Figure 2. Deep tissue phenotyping in lung

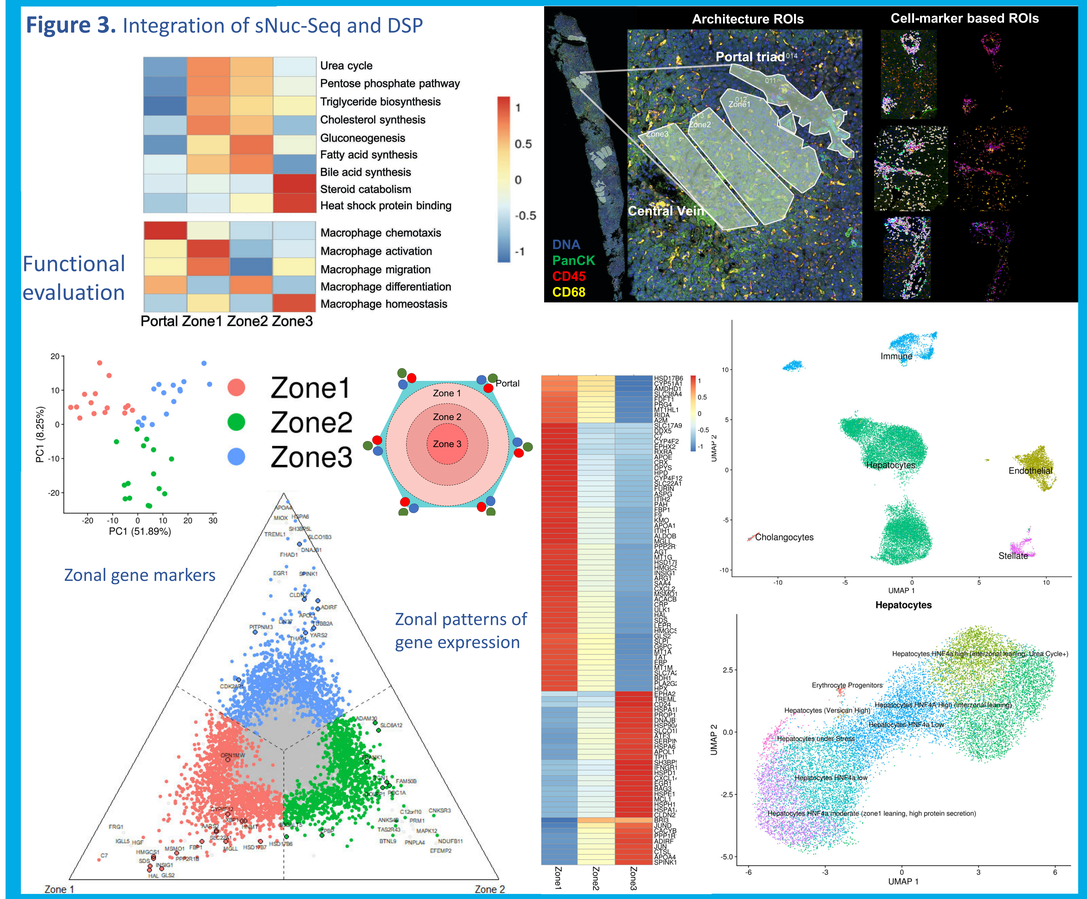


Figure 3. Integration of sNuc-Seq and DSP

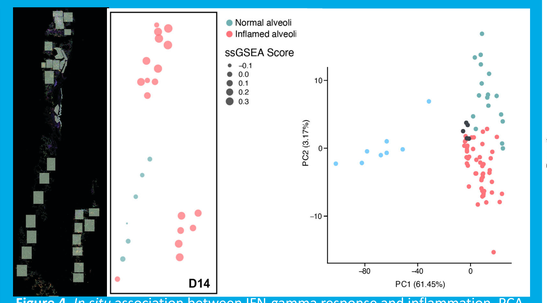


Figure 4. *In situ* association between IFN-gamma response and inflammation. PCA analysis of DSP of lung tissues

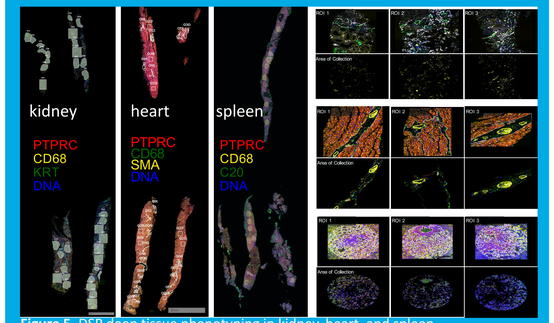


Figure 5. DSP deep tissue phenotyping in kidney, heart, and spleen

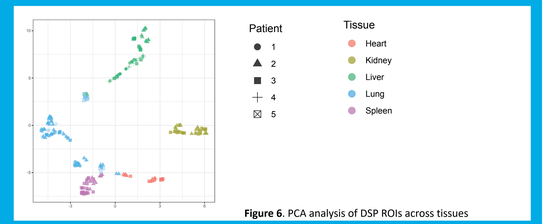
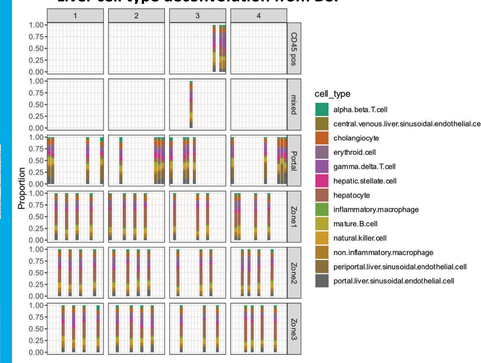


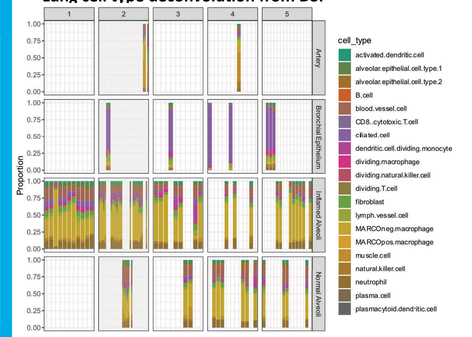
Figure 6. PCA analysis of DSP ROIs across tissues

Supplementary

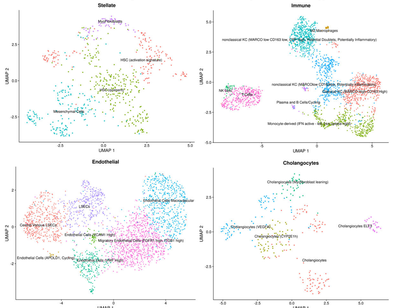
Liver cell type deconvolution from DSP



Lung cell type deconvolution from DSP



Non-Parenchymal Liver Cells (sNuc-Seq)



Acknowledgements

We are deeply grateful to all donors and their families.

We would like to thank Dimitra Pouli who supported the creation of tissue annotation resources.

G.S. acknowledges support from the NIH R01AA0207440 and U01AA026933 research grants

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