Spatially resolving the host immune-epithelial landscape in lung tissue from patients infected with SARS-CoV-2 with proteomic and whole transcriptomic profiling

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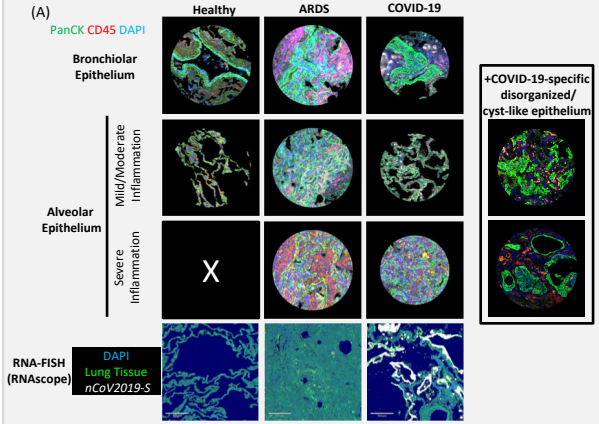
Background and Study Design

- The novel SARS-CoV-2 coronavirus can induce a wide variety of symptoms and pathologies
- Studying the impact of SARS-CoV-2 infection on lung tissue and the host response may elucidate mechanisms underlying this pathobiology and the molecular and histological consequences of infection
- Understanding the complex spatial interplay of the viral load and the host immune response may explain the heterogeneity observed in pulmonary disease manifestation and progression of COVID-19

PROJECT AIM: Assess the immune and epithelial cell gene and protein expression patterns between healthy, ARDS, and COVID-19 lung tissue with GeoMx® Digital Spatial Profiling (DSP) technology using the Whole Transcriptome Atlas (WTA) and Human Immuno-Oncology Protein assay panels

Lung tissue samples and ROI selection strategy



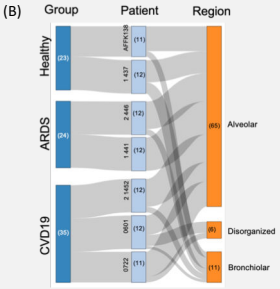


Figure 1:
(A) Bronchiolar and alveolar regions of interest (ROIs) selected across FFPE distal lung tissue sections from patients who died from non-respiratory-related reasons (Healthy; n=2), Acute Respiratory Distress Syndrome (ARDS; n=2), or from coronavirus disease 2019 (COVID-19; n=3). Sections were labeled for either epithelial tissue (PanCK) and immune cells (CD45), or for *nCoV2019-S* by RNA-FISH (RNAscope™), nuclei are labeled with DAPI.

(B) Sankey plot illustration of the distribution of selected ROIs for DSP analysis across health status and anatomical region of the lung tissue

Tissue labeling and GeoMx® DSP workflow

- RNA-FISH (RNAscope™): Used *nCoV2019-S* probe to identify regions with SARS-CoV-2 virus
- Whole Transcriptome Atlas (WTA): >18,000 human gene targets
- Human Immuno-Oncology Protein Assay: >70 protein targets
- Included COVID-19 specific gene and protein spike-in panels

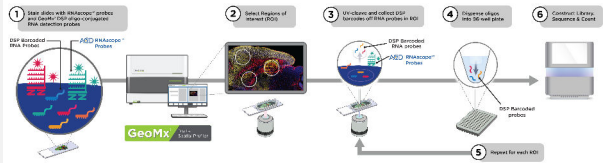
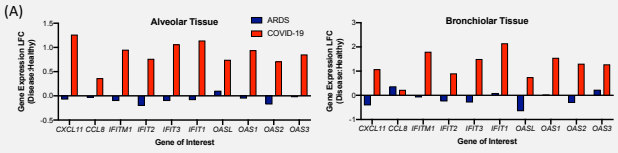


Figure 2: NanoString GeoMx® labeling, ROI selection, and data acquisition workflow

SARS-CoV-2-infection drives anti-viral immune response and inflammatory responses unique from inflammation seen in ARDS

- Alveolar and bronchiolar COVID-19 lung tissue expresses increased levels of viral-induced cytokines (*CXCL11/CCL8*), interferon response genes (*IFIT* genes) and anti-viral innate immune responses (*OAS* genes) compared to both healthy and ARDS lung tissue



- COVID-19 inflammatory response drives increased levels of inflammatory pathway mediators and cytokines
- Alveolar tissue gene expression profiles are distinctly unique between COVID-19 and ARDS subjects

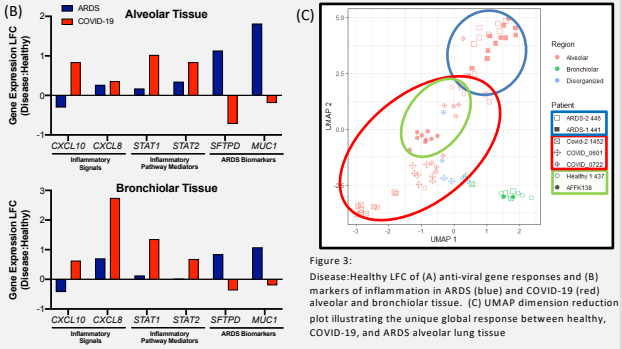
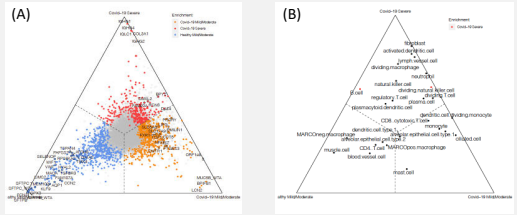


Figure 3:
Disease:Healthy LFC of (A) anti-viral gene responses and (B) markers of inflammation in ARDS (blue) and COVID-19 (red) alveolar and bronchiolar tissue. (C) UMAP dimension reduction plot illustrating the unique global response between healthy, COVID-19, and ARDS alveolar lung tissue

Inflammation- and disease-specific immune cell infiltration in COVID-19

- A distinct inflammatory, immune cell, and epithelial cell response can be seen between mild/moderate and severe inflammatory states of COVID-19 (gene expression; panel A)
- COVID-19 drives a unique tissue-associated cell type profile in ROIs with mild/moderate or severe inflammation compared to ROIs from healthy subjects (enriched cell types; panel B)



- Proteomic profiling demonstrates T-cell, B-cell, and fibroblast protein signals that are higher in expression in COVID-19 alveolar tissue compared to healthy or ARDS alveolar lung tissue

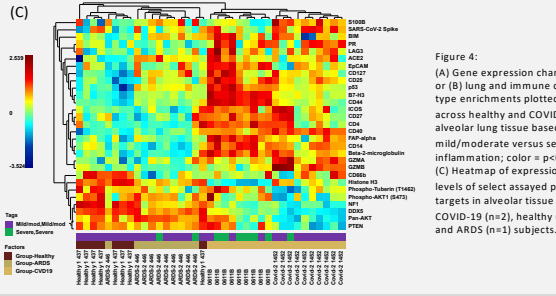


Figure 4:
(A) Gene expression changes or (B) lung and immune cell type enrichments plotted across healthy and COVID-19 alveolar lung tissue based on mild/moderate versus severe inflammation; color = p<0.01; (C) Heatmap of expression levels of select assayed protein targets in alveolar tissue from COVID-19 (n=2), healthy (n=1), and ARDS (n=1) subjects.

Conclusions

- COVID-19 drives a robust anti-viral gene expression response in both alveolar and bronchiolar lung tissue
- Lung tissue from COVID-19 patients demonstrates a unique inflammatory expression profile, in both cytokine signals and inflammatory pathways, compared to lung tissue from ARDS patients
- Differing states of COVID-19-driven inflammation alters immune cell type presence in COVID-19 tissue
- Inter-donor and intra-donor heterogeneity in COVID-19 subjects is significant and drives differing levels of inflammation and anti-viral responses, immune cell infiltration, and gene and protein expression patterns in both alveolar and bronchiolar airways and lung tissue

Acknowledgements/Disclaimers

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