Spatial multiomic analysis captures immuno-modulatory programs of RHOA, CEACAM6, and IL-7R in mediating disease heterogeneity of pulmonary fibrosis (PF)

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Background

- The lung histopathology of idiopathic pulmonary fibrosis (IPF) patients undergo extensive tissue remodeling during its asynchronous disease evolution giving rise to the spatial heterogeneity observed in the fibrotic lung.
- Co-occurring pathologic programs within distinct spatial regions are hypothesized to have underlying cellular and molecular interactions that mediate disease pathogenesis.
- Having a multomic understanding of the heterogeneous nature of PF will be critical for improving patient diagnosis and treatment in the future.

Methods

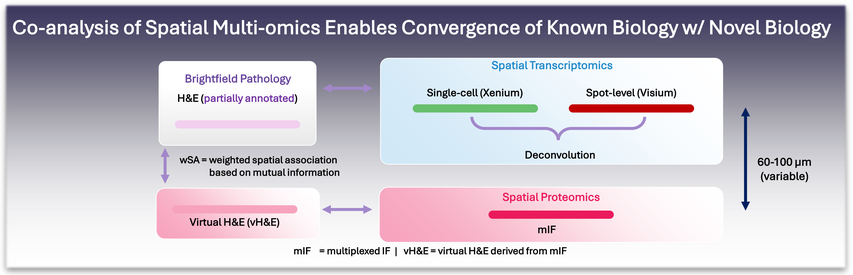
- Using the multiomic SpaceIQ™ platform, we analyzed a publicly available data set presented in Vannan et al. Sci. Adv. 2025 consisting of H&E images and spatial transcriptomics of Xenium from **41 patient lung tissue samples** (GEO GSE250346: 8 healthy controls and 33 peripheral PF patients).
- **34 nuclei morphology and color stain features were derived** from H&E and used to perform unbiased cell typing.
- **PF-specific microdomains were identified** based on differential spatial arrangements of cells between PF and healthy patients.
- 342 cell-level genes were summarized from Xenium data with cell coordinates registered to the H&E images.
- Feature correlation analysis was performed between the tile gene expressions and the spatial microdomains.
- Biological pathway analysis was inferred on the **top correlated genes with discovered microdomains**.

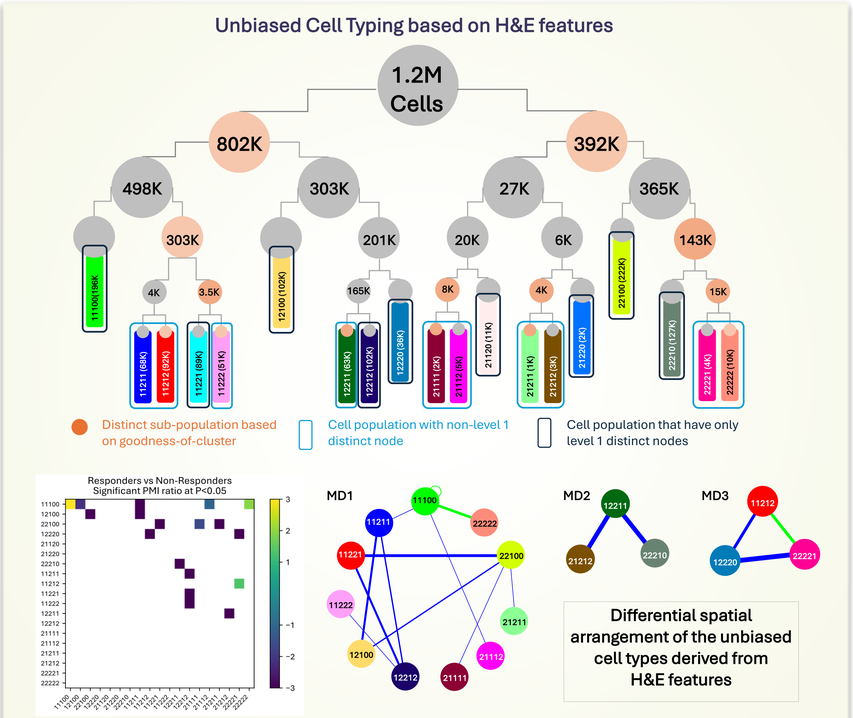
Results

- Unbiased cell typing on the H&E features yielded **19 distinct cell types, from which 4 cell types were identified to be involved in the most significant PF-specific microdomains for downstream feature correlation analysis.**
- Feature correlative analysis identified genes implicated in alveolar epithelial dysregulation (AGR3,SFTPC) and macrophage polarization (RHOA, IL-7R). We found association with these genes to quantitative morphology/color features derived from H&E images
- Biological pathway inference on the correlated genes identified epithelial mesenchymal transition (EMT), TGF-beta regulation of ECM, IL-4 regulation of apoptosis, and CD4 Effector/CD8 memory T cell involvement as significant pathways (p<0.001) implicated in the PF-specific microdomains.

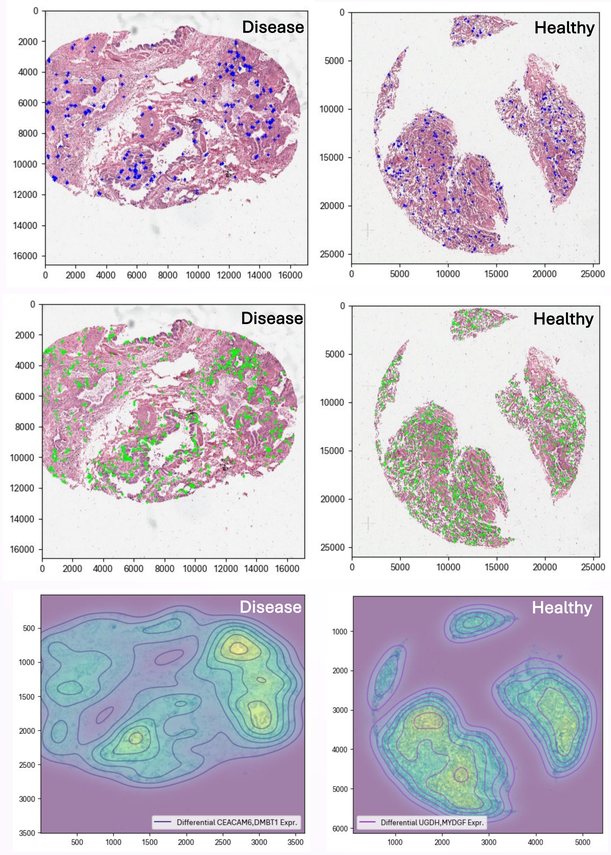
Conclusions

- Our multiomic analysis with SpaceIQ platform reveals microdomains showing consistent known biology associated with PF.
- Interactions between H&E unbiased cell types 11221 and 12212 in healthy patient samples inferred genes involved in cellular stress response (HSPA5) [PMID : 35923704] and cellular growth regulation (UGDH, MYDGF) [PMID : 30787260, 40752010] associated with quantitative H&E features.
- Interactions between H&E unbiased cell types 11100 and 22222 in disease patient samples inferred genes involved in dysregulation of airway epithelial cells (AGR3, SFTPC) [PMID : 34177583, 30102252], inflammatory processes (DMBT1, CEACAM6) [PMID : 35249163, 36341379], epithelial-mesenchymal transition (IL-32) [PMID : 33097029], and profibrotic macrophages (RHOA, IL-7R) [PMID : 38913005, 41092115] associated with quantitative H&E features.
- **This study demonstrates the benefit of using a multiomic spatial approach to generate hypotheses that are both confirmatory and potentially novel to improve our understanding of a complex and heterogeneous disease like PF.**





Differential spatial arrangement of the unbiased cell types derived from H&E features



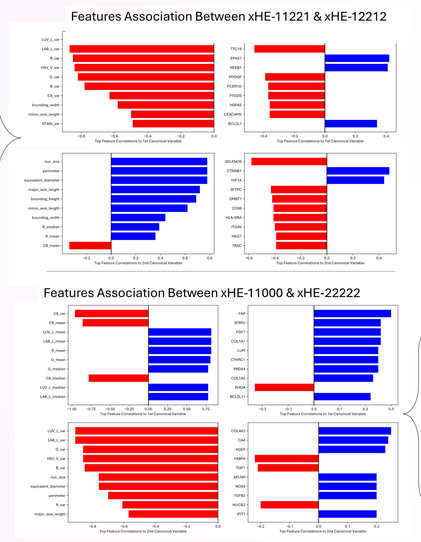
Disease PMI 11100:22222 in Healthy Patient vs Disease Patient

Healthy PMI 11221:12212 in Healthy Patient vs Disease Patient

Differential Pro-inflammtory Genes (Disease) and Cell Growth Regulation Genes (Healthy)

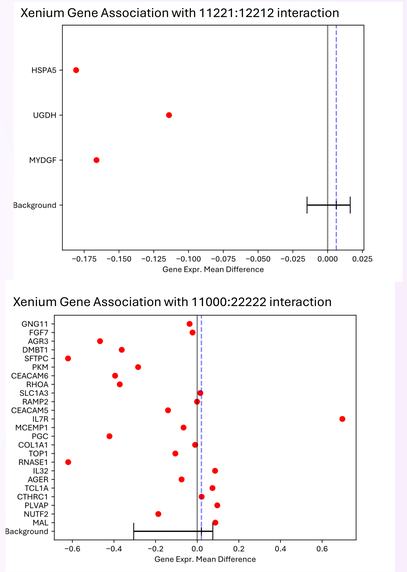
Disease PMI
5 out of 33 disease patient samples
Genes:
IL7R, AGR3, CEACAM6, DMBT1, IL32, RHOA, SFTPC
Pathways :
PBMC-L3-CD4+ Central Memory T (p<0.001)
Bone Marrow-L2-CD4 Effector (p<0.001)
Lung 1-L2-CD8+ Memory/Effector T (p<0.001)
MAL role in Rho-mediated activation of SRF (p<0.001)
KRAS Signaling Up (p=0.006)
IL-4 regulation of apoptosis (p=0.01)
IL-2 signaling pathway (p=0.01)
TGF-beta regulation of extracellular matrix (p=0.05)

Sample patient H&E regions for feature correlation analysis revealing genes associated with H&E features with inferred biological pathways

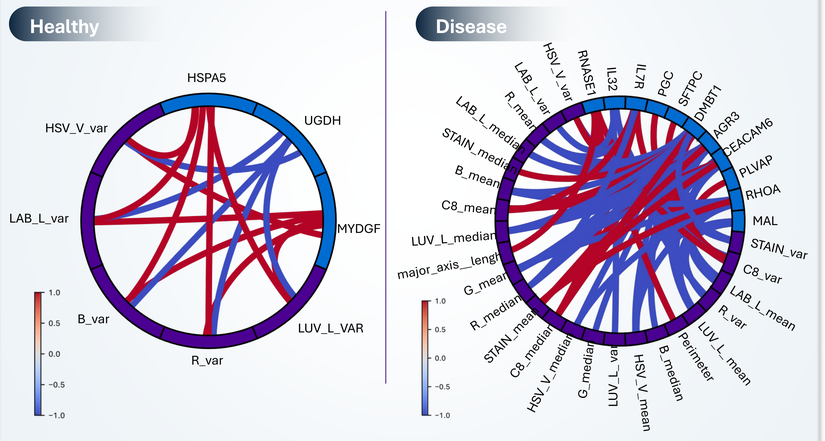


Genes
AGR3 – Regulation of Airway Epithelial junctions
SFTPC – Linked to AT2 epithelial cell dysfunction in interstitial lung disease
DMBT1 – Upregulated in cystic fibrosis; inflammatory process, ciliary motility
RHOA – M2 macrophage polarization
CEACAM6 - pro-inflammatory in classical monocytes
IL32 – EMT and ER stress
IL7R – profibrotic interstitial SPP1-producing macrophages

Healthy PMI
2 out of 8 disease patient samples
Genes:
HSPA5, UGDH, MYDGF
Pathways :
Plasma Cell Nasopharynx (p < 0.001)
BDNF signaling pathway (p<0.001)



Differential Genes correlated with H&E Features



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