Integrating single-cell spatial whole transcriptome and histopathology to uncover drivers of tumor heterogeneity in lung adenocarcinoma and squamous cell carcinoma

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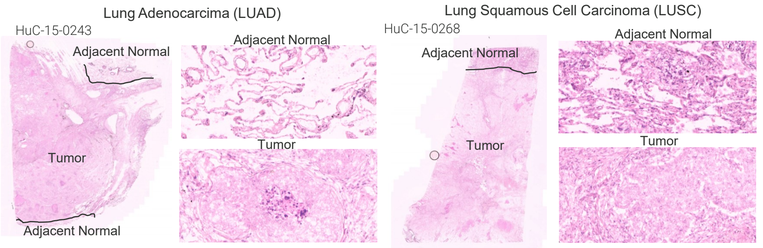
Introduction

Lung cancer, the leading cause of cancer-related deaths worldwide, primarily consists of non-small cell lung cancer (NSCLC), including subtypes like lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). These subtypes exhibit distinct histopathological features but share overlapping molecular characteristics. Understanding the spatial and molecular heterogeneity of these tumors is critical for identifying novel biomarkers and therapeutic targets. This study utilizes the CosMx® Spatial Molecular Imager platform to profile LUAD and LUSC, aiming to uncover shared and unique gene signatures, tumor heterogeneity, and rare tumor cell subtypes, while integrating histopathological insights from H&E staining.

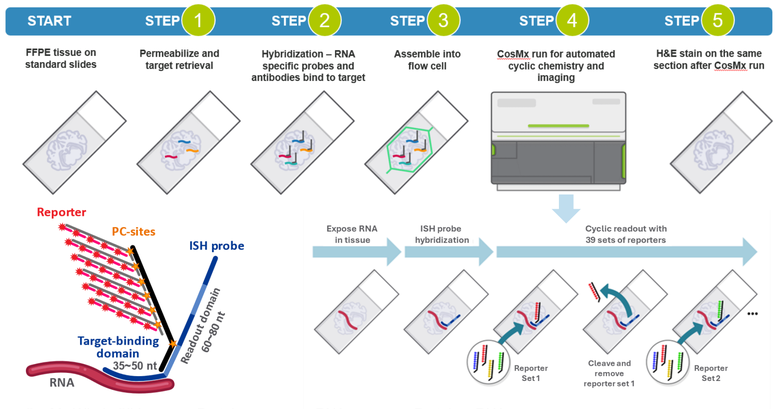
FFPE LUAD and LUSC Tissue

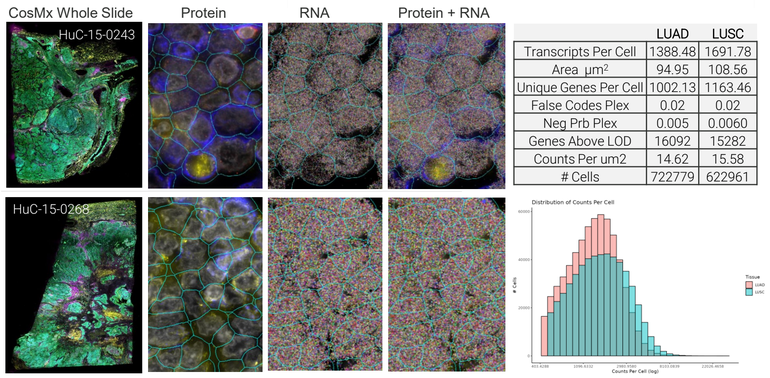


CASE ID	Tissue	SURGICAL DATE	ETHNICITY	GENDER	AGE	ORGAN	DIAGNOSIS	Grade	Tumor(%)	Necrosis(%)
HuC-15-0243	Human Lung	2021	Asian	M	74	lung	adenocarcinoma	III	35%	<20%
HuC-15-0268	Human Lung	2020	Asian	F	51	lung	squamous cell carcinoma	II	40%	10%

Fig. 1 Patient metadata and H&E staining of 2 subtypes of FFPE NSCLC, including LUSC and LUAD.

CosMx WTX Assay Workflow





	LUAD	LUSC
Transcripts Per Cell	1388.48	1691.78
Area μm²	94.95	108.56
Unique Genes Per Cell	1002.13	1163.46
False Codes Plex	0.02	0.02
Neg Prb Plex	0.005	0.0060
Genes Above LOD	16092	15282
Counts Per um2	14.62	15.58
# Cells	722779	622961

Fig. 2 CosMx WTX assay enables co-detection of 4 proteins and 19782 genes on the same slide. Results showed high throughput and high assay sensitivity.

First Step for WTX – Examine Spatial Pathways

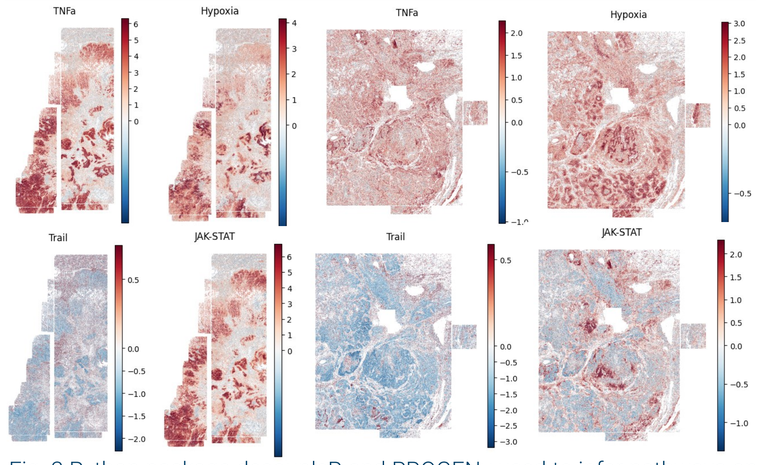
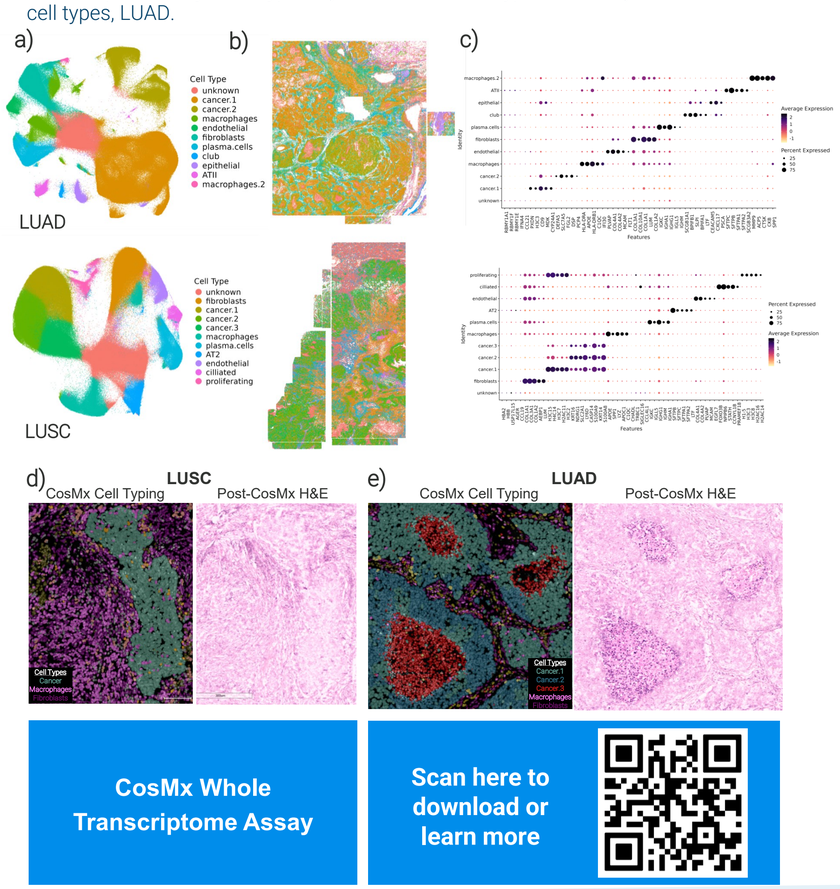


Fig. 3 Python package decoupleR and PROGENy used to infer pathways on single-cell transcriptomics.

Cell Typing

Fig. 4 UMAP (a), spatial (b), and marker gene (c) plots of annotated unsupervised cell types for both lung samples. Clustering algorithm used UMAP coordinates to better separate major cell types. d) Paired H&E / cell types, LUSC. e) Paired H&E / cell types, LUAD.



CosMx Whole Transcriptome Assay

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Insitucor discovers novel spatially correlated pathways

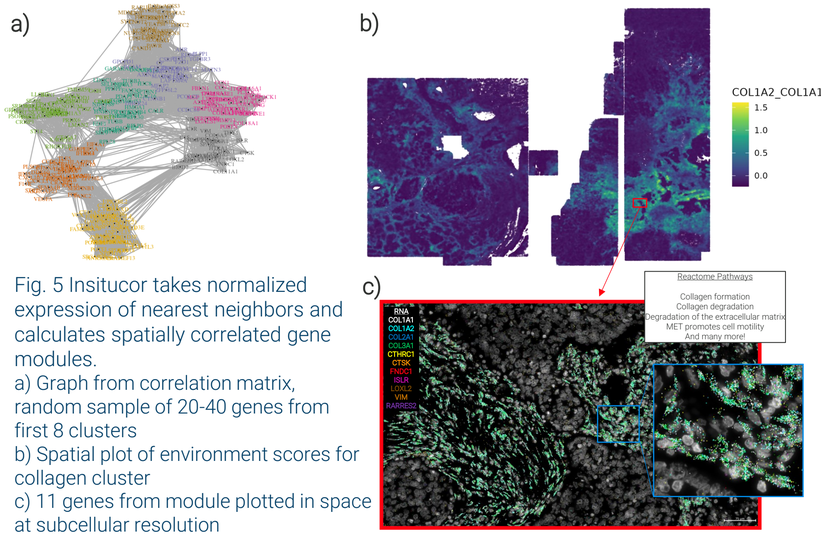

Fig. 5 Insitucor takes normalized expression of nearest neighbors and calculates spatially correlated gene modules.
a) Graph from correlation matrix, random sample of 20-40 genes from first 8 clusters
b) Spatial plot of environment scores for collagen cluster
c) 11 genes from module plotted in space at subcellular resolution

Differential Expression of Cell Types as They Approach Tumors

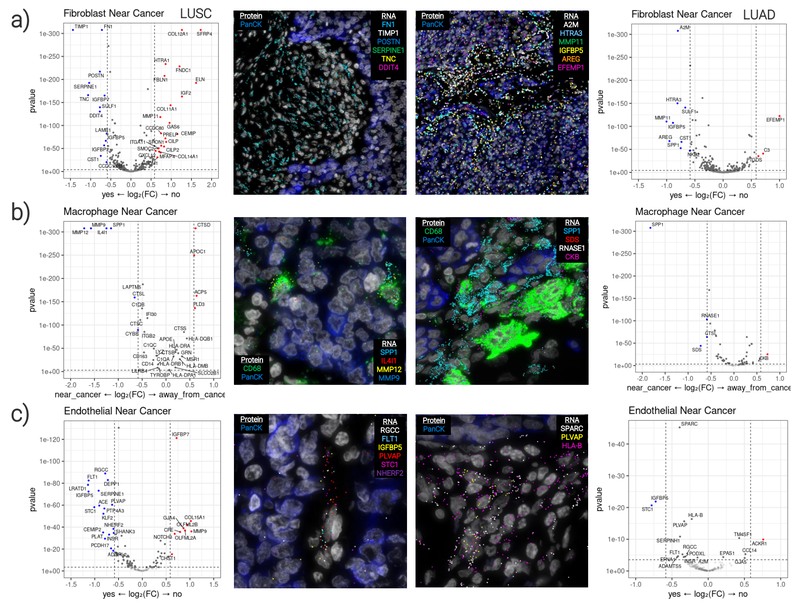

Fig. 6 smiDE applies segmentation error correction to differential expression analysis. These plots show differential expression results for both lung samples, as well as spatial plots of genes of interest, for a) fibroblasts, b) macrophages, and c) endothelial cells that are "near" and "away from" cancer.

Conclusion

Integrating spatial transcriptomics and histopathology using the CosMx imager provided valuable insights into the molecular and spatial complexity of LUAD and LUSC. This study identified rare tumor cell subtypes and mechanisms of tumor progression, underscoring the potential for AI-driven biomarker discovery and therapeutic strategies in NSCLC and beyond.

Badia-i-Mompel P., Vélez Santiago J., Braunger J., Geiss C., Dimitrov D., Müller-Dott S., Taus P., Dugourd A., Holland C.H., Ramirez Flores R.O. and Saez-Rodriguez J. (2022), decoupleR: ensemble of computational methods to infer biological activities from omics data, Bioinformatics Advances.

Schubert M, Klinger B, Klünemann M, Sieber A, Uhlitz F, Sauer S, Garnett MJ, Blüthgen N, Saez-Rodriguez J. 2018. Perturbation-response genes reveal signaling footprints in cancer gene expression. *Nature Communications*: 10.1038/s41467-017-02391-6