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Single-cell spatial whole transcriptome reveals tumor heterogeneity and stromal dynamics in invasive ductal carcinoma of the breast

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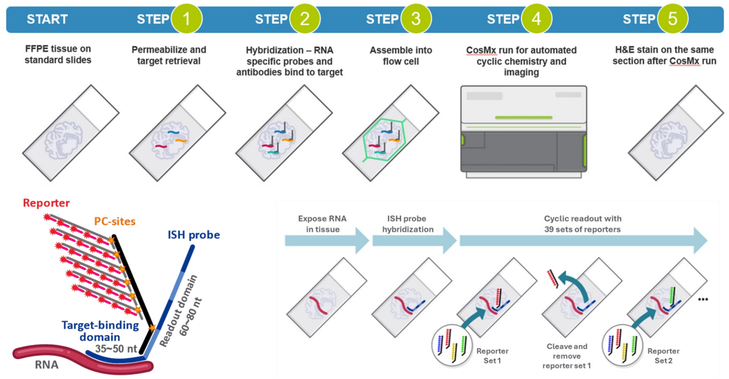
Introduction

Invasive ductal carcinoma (IDC) is the most common and aggressive form of breast cancer, characterized by tissue invasion and metastasis, contributing to significant morbidity and mortality. The tumor microenvironment (TME), composed of stromal and immune cells, plays a critical role in IDC progression and therapy resistance. However, the spatial organization and molecular heterogeneity within the IDC TME remain underexplored. Spatial transcriptomics enables the preservation of tissue architecture while revealing molecular signatures at single-cell and subcellular resolution, offering new insights into cancer biology.



Method

We utilized the CosMx® Spatial Molecular Imager with the Whole Transcriptome (WTX) assay (nearly 19,000 genes) to analyze a formalin-fixed, paraffin-embedded (FFPE) IDC tumor sample. This imager provides high-resolution, multiplexed RNA and protein detection within its native tissue context. Transcriptomic data were analyzed using semi-supervised cell typing and spatial domains to identify cell clusters and spatial patterns. Perturbation methods were used to identify enriched genes and pathways in cellular neighborhoods. We also identified genes that were differentially expressed from the center to the edge of the primary tumor.

Results

Our analysis identified two distinct tumor regions (primary tumor and invasive tumor), each subdivided into subregions with unique and shared gene expression profiles. We observed region-specific stromal compositions, with T cell subpopulations exhibiting distinct spatial patterns. The analysis of spatial domains revealed fine structures in the tumor. For example, two tumor domains with similar expression profiles showed distinct spatial layering. Perturbation analysis identified perturbed marker genes and pathways in each spatial domain. Trajectory analysis revealed gradients of expression: S100A7 was elevated in the tumor core, potentially promoting angiogenesis, whereas ALCAM was enriched at the periphery, possibly supporting invasion. These results highlight the molecular and spatial heterogeneity of IDC and the complex interactions within the TME.

Conclusion

Spatial transcriptomics uncovers complex and finely resolved patterns of tumor behavior and immune adaptation in IDC. By mapping how specific cell types, like activated CD8 T-cells, respond to local microenvironments, we gain insights into tumor-stroma crosstalk, metabolic stress, and immune evasion. These findings provide a valuable foundation for developing spatially informed biomarkers and therapeutic targets, especially in heterogeneous tumors like IDC where context matters. The CosMx imager enables a deeper understanding of both well-characterized and novel genes in their native spatial context, advancing precision oncology approaches.

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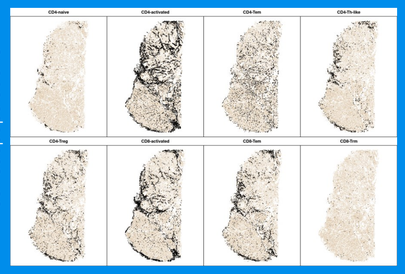
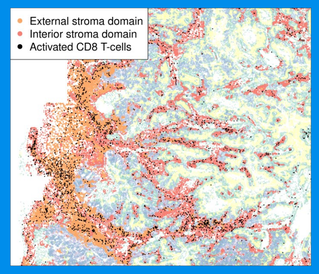
Cell Typing



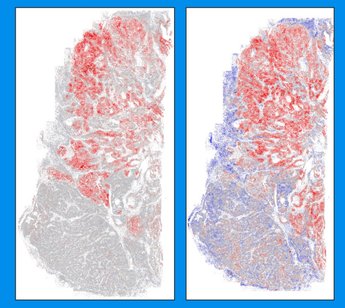


Top genes depleted in tumor interior:
ARL4C, C11orf21, CCND3, CD52, CD69, CDAN1, CTRL, EPHA1, FCMR, *GIMAP2, GNG2, GPRIN3, GZMK, HLA-E, IKZF3, IL2RG, IL32, IL7R, LCP1, MSL2, MYCBP2, POLG2, PRKACB, RGS1, RPS29, RUNX3, THEMIS, TRAT1, TRDC, TTC13

*T-cell survival
Not in any other high-plex spatial Imager assays

Activated CD8 T-cells change based on their spatial location. We analyzed transcriptome-scale readouts of a single cell type's response to its environment. We compare activated CD8 cells in two different stroma domains, one in the tumor and one just outside it. Inside the tumor, we see T-cells up-regulating metabolism, perhaps struggling in a nutrient poor environment, and down-regulating proliferation.

Perturbations

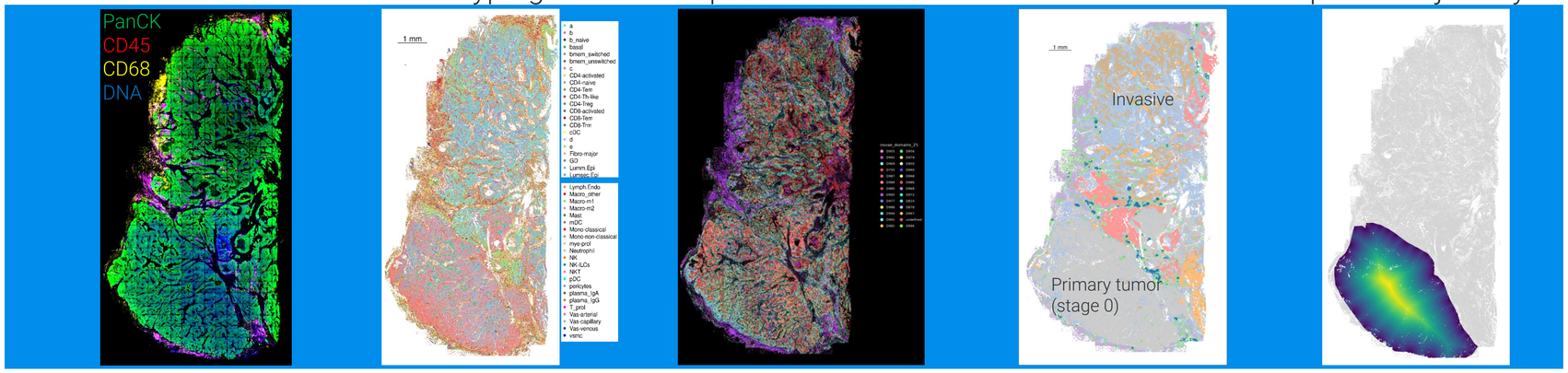


ACSM1:
- Lipid metabolism, apocrine breast cancer subtype
- Not well-studied, only 37 articles in PubMed

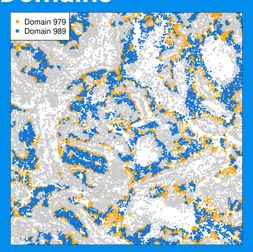
TXN:
- Oxidative stress response
- Up-regulated by breast cancers

Both genes are only available in the WTX assay

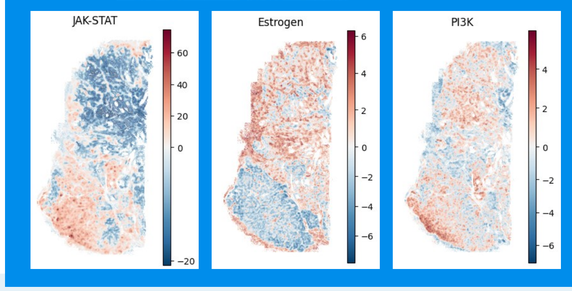
Define spatial domains in the **invasive** tumor based on perturbations to cellular neighborhoods (image below)
- Light blue domain: stress response to nutrient deprivation, protein translation
- Tan domain: proliferation & invasion



Spatial Domains

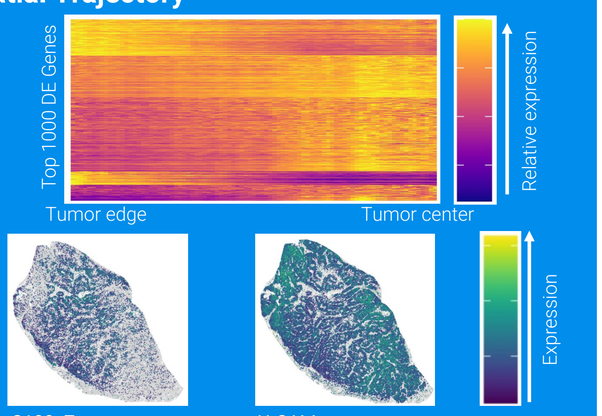


Two tumor domains with similar expression profiles, yet spatially distinct: orange is the border to blue domain. (Both are 99% tumor cells)
Comparing their expression profiles, we find:
- Pathways upregulated in the orange domain involved in remodeling the ECM and activating PD1 signaling.
- The blue domain, isolated from the stroma, is metabolically and transcriptionally active, and we can see the pathways it uses to sustain that state.



- The JAK-STAT pathway plays a crucial role in interferon signaling, which is important for antiviral and antitumor immunity. Its downregulation in the upper region suggests that the tumor is actively evading certain immune attacks.
- Estrogen enrichment in the upper region suggested a more pronounced inflammatory microenvironment or response.
- The enrichment of the PI3K pathway along the margins of the lower tumor suggests increased cell growth and motility.

Spatial Trajectory



S100a7:
Elevated expression of S100A7 in the tumor core may promote angiogenesis, enhancing tumor survival

ALCAM:
High expression of ALCAM at the tumor periphery may facilitate tumor progression and metastasis