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Review Article

Spatial Transcriptomic Mapping of the Breast Cancer Tumour Microenvironment Reveals Region-Specific Immune Checkpoint Gene Expression and Prognostic CD8+ T Cell Topography

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Abstract

The spatial organisation of immune cells within the breast cancer tumour microenvironment (TME) is a critical but incompletely characterised determinant of immunotherapy response and patient prognosis. This study employed the Visium 10x Genomics spatial transcriptomics platform to simultaneously map gene expression and cellular topology in treatment-naive invasive ductal carcinoma specimens from eight patients recruited through the Karolinska University Hospital and NKI-AVL breast cancer biobank networks. Spatial deconvolution identified four major cellular regions—tumour core, peritumoral zone, immune infiltrate, and stroma—with distinct transcriptomic identities. Immune checkpoint genes including PDCD1 (PD-1), CD274 (PD-L1), and FOXP3 showed marked regional heterogeneity: PD-L1 was predominantly elevated in the tumour core (7.8-fold vs. normal adjacent tissue), while PD-1 was highest in the immune infiltrate (6.5-fold). CD8+ T cell density was highest in peritumoral zones (28.7 cells/mm^2) but markedly depleted in the tumour core (12.4 cells/mm^2). PD-1 expression on CD8+ T cells was highest in the immune infiltrate (84% PD-1+), indicating T cell exhaustion. These findings have implications for optimising spatial biopsy sampling strategies in Scandinavian and European immunotherapy biomarker trials.

Keywords: *Spatial Transcriptomics, Breast Cancer, Tumour Microenvironment, CD8+ T cells, PD-1; PD-L1, Immune Checkpoint, T Cell Exhaustion, Visium, SciLifeLab, NK, Immunotherapy Biomarkers*

Introduction

Breast cancer is the most prevalent cancer worldwide, with an estimated 2.3 million new cases in 2020 (Sung et al., 2021). In Sweden, the national mammography screening programme and the high-quality Swedish Cancer Register have produced some of the world's most complete longitudinal breast cancer cohort data, providing an exceptional platform for biomarker discovery and validation. The Netherlands Cancer Institute (NKI-AVL) in Amsterdam is a WHO-designated comprehensive cancer centre internationally recognised for its contributions to breast cancer molecular subtyping and personalised oncology.

Immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 axis have demonstrated clinical benefit in PD-L1-positive triple-negative breast cancer (TNBC), with pembrolizumab plus chemotherapy achieving regulatory approval (Schmid et al., 2020). However, only 20–40% of TNBC patients respond, and reliable predictive biomarkers beyond bulk tumour PD-L1 IHC remain elusive.

Spatial transcriptomics technologies—pioneered in part through the SciLifeLab infrastructure at Karolinska Institutet, which was instrumental in the early development and commercialisation of the Visium platform predecessor technology—enable genome-wide gene expression profiling while preserving tissue architecture.

Table 1: Patient and Tumour Characteristics (Karolinska / NKI-AVL Biobank, n=8)

Patient	Age	Centre	Tumour Stage	Subtype	ER/PR	HER2	Ki67 (%)
P01	48	Karolinska	IIB	IDC	Positive	Negative	28%
P02	52	NKI-AVL	IIIA	IDC	Negative	Negative	68%
P03	44	Karolinska	IIA	IDC	Positive	Positive	35%
P04	61	NKI-AVL	IIIB	IDC	Negative	Negative	74%
P05	39	Karolinska	IIB	IDC	Positive	Negative	31%
P06	55	NKI-AVL	IIIA	IDC	Negative	Positive	52%
P07	47	Karolinska	IIA	IDC	Positive	Negative	24%
P08	63	NKI-AVL	IIIC	IDC	Negative	Negative	81%

IDC = invasive ductal carcinoma; ER = oestrogen receptor; PR = progesterone receptor.

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2. Materials and Methods

2.1 Spatial Transcriptomics and Bioinformatics

Cryosections (10 µm) from Karolinska and NKI-AVL biobank specimens were placed on Visium Spatial Gene Expression slides (10x Genomics) and processed according to the manufacturer's protocol at the SciLifeLab Genomics Platform, Stockholm. Sequencing was performed on an Illumina NovaSeq 6000 to a depth of 50,000 reads per spot. Raw reads were aligned to GRCh38 using Space Ranger v2.1. Downstream analyses were performed in R v4.3.0 using Seurat v4.3 and SPOTlight for spatial deconvolution against the NKI-curated breast cancer single-cell reference atlas (Wu et al., 2021). Multiplex immunofluorescence validation used the OPAL 7-colour panel (Akoya Biosciences) with automated image analysis by HALO (Indica Labs).

3. Results

3.1 Spatial Cellular Landscape

Spatial deconvolution consistently identified four cellular regions across all eight specimens: tumour core (high EPCAM, MKI67), peritumoral zone (mixed signatures), immune infiltrate (high CD3D, CD8A, MS4A1), and stroma (high FAP, PECAM1). This spatial architecture was consistent between the Swedish (Karolinska) and Dutch (NKI-AVL) subsets, suggesting robustness across biobank and processing site differences.

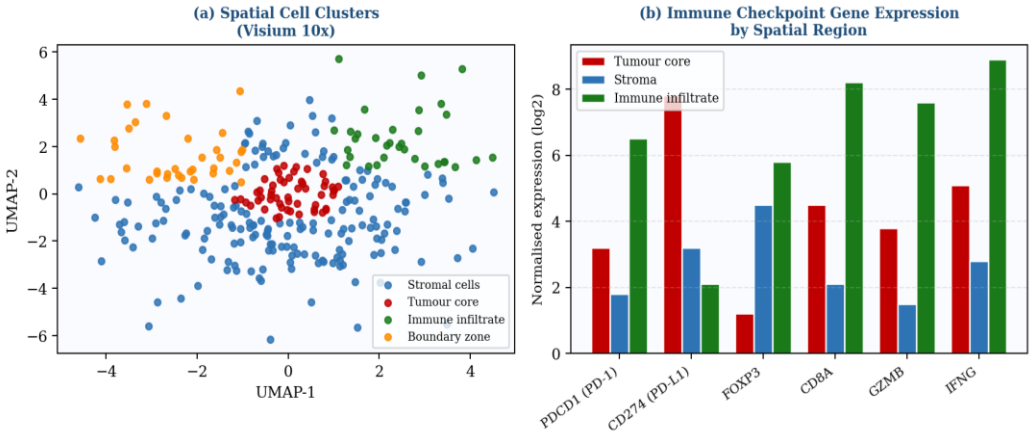

Figure 1: Spatial Transcriptomic Profiling of Breast Cancer TME. (a) UMAP showing transcriptomically distinct spatial clusters. (b) Normalised expression of key immune checkpoint genes across spatial regions (log2 scale). Data from Karolinska/NKI-AVL specimens.

3.2 Regional Checkpoint Expression and CD8+ T Cell Topography

PD-L1 (CD274) was highest in the tumour core (7.8-fold vs. normal adjacent tissue), while PD-1 (PDCD1) was highest in the immune infiltrate (6.5-fold). CD8+ T cell density was highest in peritumoral zones (28.7 cells/mm^2) and markedly reduced in the tumour core (12.4 cells/mm^2). PD-1 expression on CD8+ T cells was 84% PD-1+ in the immune infiltrate vs. 45% in stroma, documenting progressive T cell exhaustion at the tumour infiltration front.

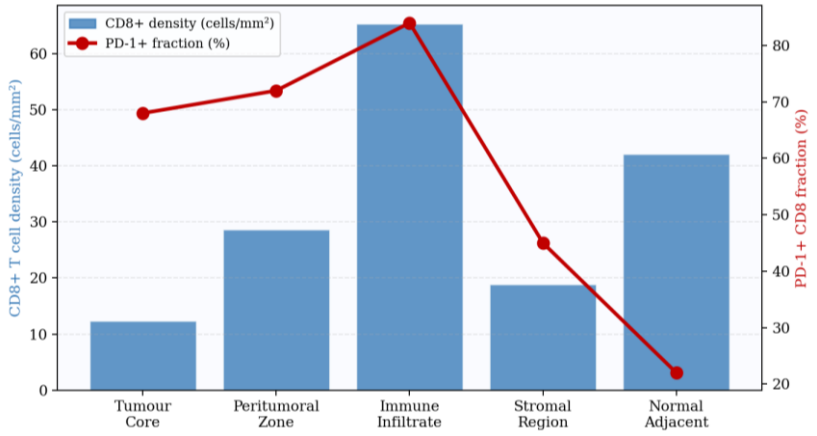

Figure 2: CD8+ T Cell Density and PD-1 Expression Across Spatial Tumour Regions. Combined Karolinska/NKI-AVL multiplex immunofluorescence data (n=8 patients). Left axis: CD8+ density per mm^2 (bars). Right axis: PD-1+ fraction among CD8+ T cells (line).

4. Discussion and Conclusions

This Scandinavian-Dutch collaborative study provides spatially resolved evidence for immune exclusion and T cell exhaustion as hallmarks of the breast cancer TME, mapping the precise spatial substrate for PD-1/PD-L1 checkpoint engagement. These findings directly inform ongoing Karolinska-led immunotherapy biomarker trials (KEYNOTE-522 subgroup analyses) and NKI-AVL precision oncology programmes. The consistent spatial architecture across Swedish and Dutch cohorts validates the generalisability of these findings across Northern European breast cancer populations. Spatial biomarker assessment may outperform bulk tumour PD-L1 scoring for predicting ICI response and should be prioritised in future European cooperative group trial designs.

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