Spatial Transcriptomics Reveals Heterogeneity and Microenvironmental Shifts in Breast Cancer Subtypes and Metastatic Progression

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Introduction

Breast cancer is a clinically heterogeneous disease, where the diverse tumour and microenvironmental landscape determines cancer progression and prognosis. Despite advances in and optimisation of cancer treatment, over 3,000 Australians die of breast cancer each year[1], highlighting the need to further define the biological mechanisms that drive tumour growth.

Cancers are supported by a complex network of surrounding cells (stroma). Stromal cells, including cancer-associated fibroblasts (CAFs), communicate with cancer cells to activate signalling pathways that promote cancer growth, metastasis and protect cancer against drug treatments[2-3]. A better understanding of this dynamic interaction between cancer and stromal cells is thus critical for identifying novel drug targets against this disease.

Traditional bulk tissue analyses fail to capture the complex cellular and molecular interplay within tumours due to the loss of spatial information during tumour dissociation. In this study, we utilised the ground-breaking spatial transcriptomics profiling to reveal previously hidden biological interactions that will support unbiased discovery of clinically relevant signatures.

Aims

We aim to investigate the spatial and cellular diversity across primary and regional metastasis of breast cancer using spatial transcriptomics.

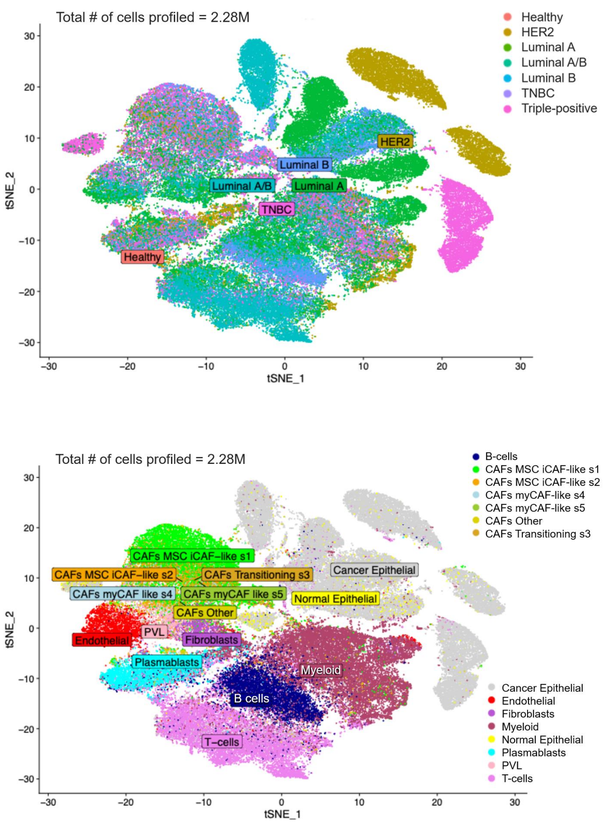
Methods

Sample cohort: We applied the Human 6K Discovery Panel on the CosMx Spatial Molecular Imager to our cohort of 28 breast cancer patients diagnosed with major molecular subtypes, including luminal A (n=8), luminal B (n=5), luminal A/B (n=4), triple-negative (n=8), HER2-enriched (n=2) and triple-positive (n=1), along with 4 healthy women.
Construction of tissue microarrays (TMA): 8 TMAs were generated from FFPE tumour blocks of these patients capturing the invasive front and core of the tumour, as well as patient-matched adjacent normal breast and lymph node tissues and lymph node metastases.

Niche analysis: Niches were identified using the R package, Scider. BuildNicheAssay function in R was utilised to visualise the spatial distribution of niches across each tissue core. Density contours were generated within each core, centering on a niche of interest, to capture its spatial context within the surrounding neighbourhood.

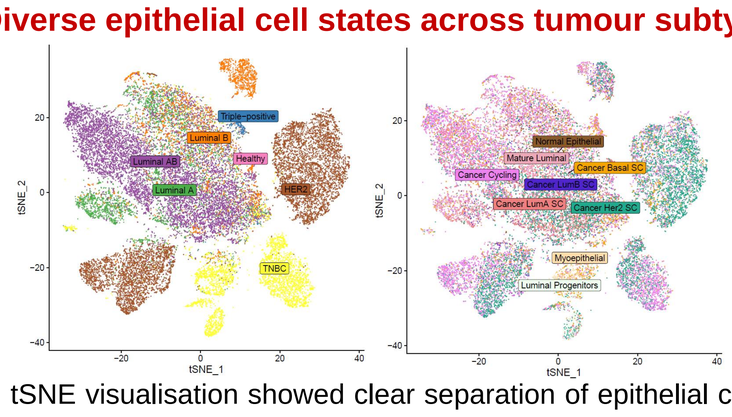
Results

Global clustering distinguishes molecular subtypes and cell types



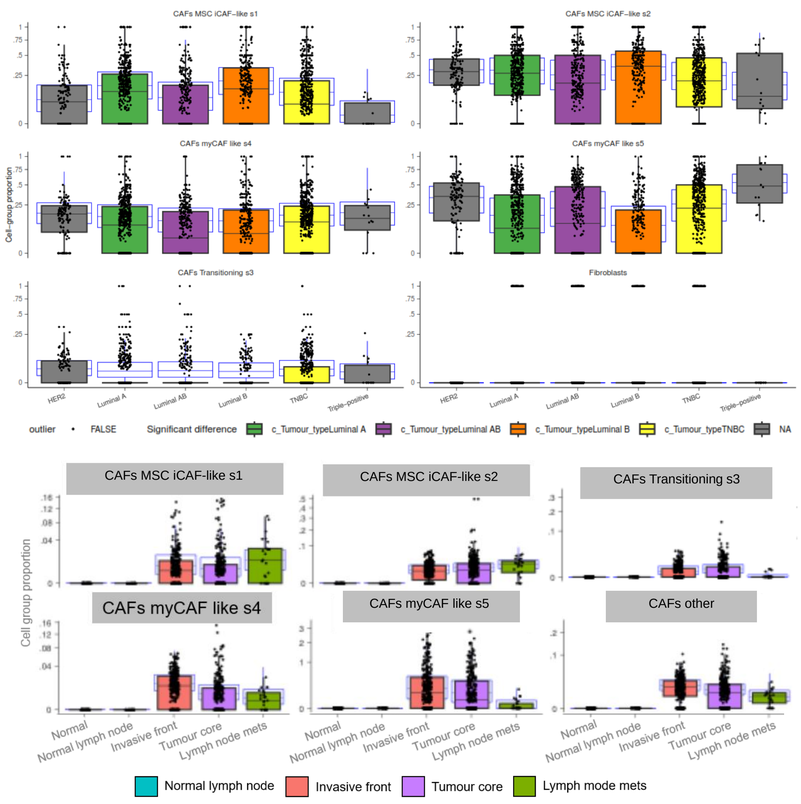
Using published scRNA-seq dataset[4] as reference, tSNE analysis revealed common and subtype-specific transcriptomic patterns across tumour subtypes and healthy controls (top), and clustered cells into 15 broad populations (bottom).

Diverse epithelial cell states across tumour subtypes



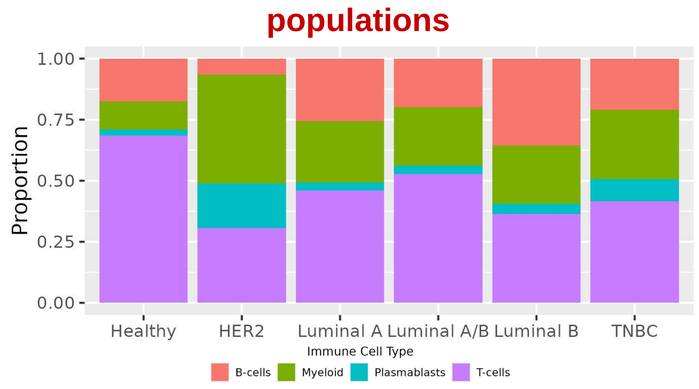
tSNE visualisation showed clear separation of epithelial cells by tumour subtypes (left), and clustering of major epithelial subpopulations (right).

CAF heterogeneity across molecular subtypes and tissue locations



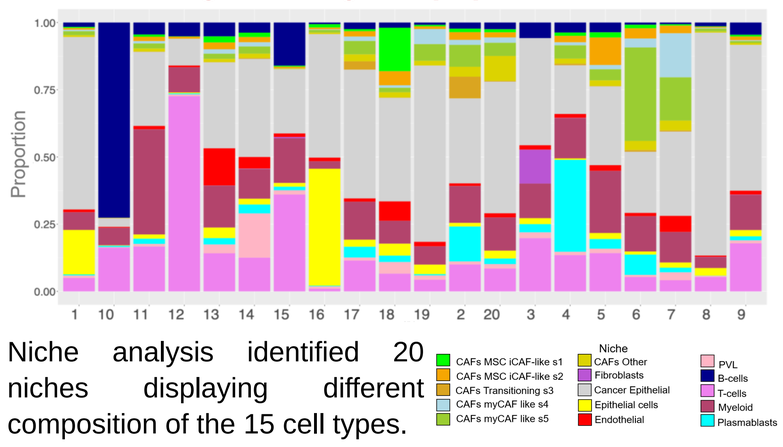
Cell type proportion analysis revealed variation in phenotypes and spatial distribution of CAF subpopulations across molecular subtypes of breast cancer (top). Significantly higher abundance of CAFs in the tumour regions of the breast and the lymph node was also observed (bottom), indicating their potential role in disease progression.

Subtype-specific variations in immune cell populations



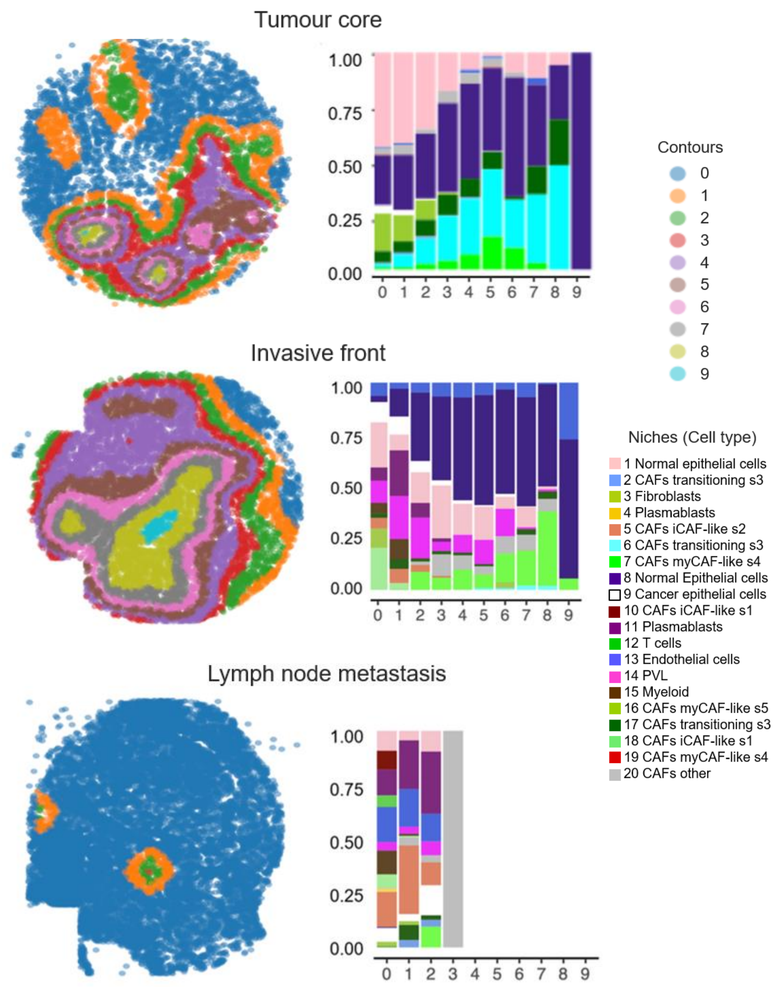
Immune cell compositions differed between healthy and tumour samples, with distinct shifts observed across cancer subtypes, highlighting subtype-specific immune landscapes.

Niche analysis reveals varying composition of neighbouring cell populations



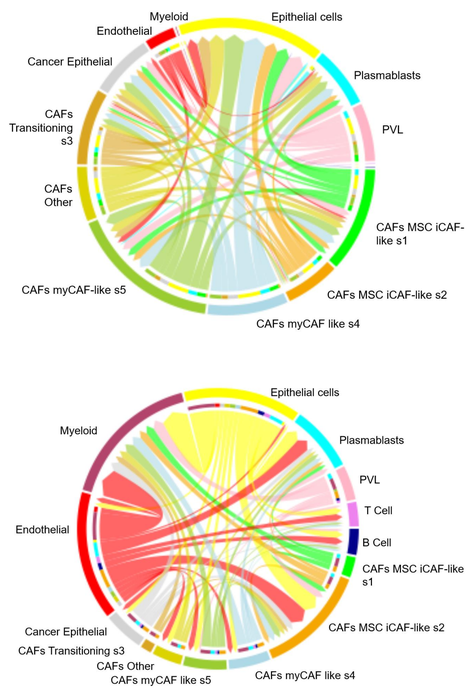
Niche analysis identified 20 niches displaying different composition of the 15 cell types.

Distinct niche patterns observed across tissue locations in triple-negative breast cancer (TNBC)



Density contours encompassing gradient distribution of CAF s4-high niche (niche 19) identified unique neighbouring niches and cellular interactions. Niche 19 is most abundant in contour 9 and least represented in contour 0. Differences in relative co-occurrence of other niches in these contours (up to 10) across the tumour core, invasive front and lymph node metastasis of TNBC were observed (left). Niche composition plot of the 10 density contours revealed enrichment of various cell types (right), suggesting location-specific cellular compositions and interactions that may be responsible for disease progression.

Spatial architecture reveals unique cellular communications between cell types



Based on their physical proximity and gene expression profiles, we identified communication between CAFs and epithelial cells via the collagen pathway in the tumour core. Additionally, endothelial cells and epithelial cells appear to interact with CAFs at the invasive front via the amyloid precursor protein (APP) pathway which produces amyloid-beta peptides.

Conclusion

By analysing the diverse molecular signatures and cellular architecture, our in-depth spatial atlas will help gain deeper insights into the intercellular communication that characterises major breast cancer subtypes and contributes to disease progression.

References

1. Australian Institute of Health and Welfare 2018.
2. Wu et al. *EMBO J.* 2020.
3. Danenberg et al. *Nat Genet.* 2022.
4. Wu et al. *Nat Genet.* 2021