A Single-Cell Atlas of the Breast Cancer Microenvironment Identifies Subtype-Specific Tumor-Immune Landscapes and Vulnerabilities

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INTRODUCTION

Breast cancer continues to be the most frequently diagnosed cancer among women worldwide and remains a leading cause of cancer-related mortality. In the United States alone, it accounts for approximately 30% of all new cancer diagnoses among women, with an estimated 316,950 new cases and 42,170 deaths in 2025

Recent advances in scRNA-seq have provided unprecedented insights into the tumor microenvironment, revealing the heterogeneity of cellular populations within and across breast cancer subtypes. These findings challenge traditional subtype classifications and underscore the complexity of the TME.

Notably, scRNA-seq has shed light on the classification of tumors as “hot” (immune-inflamed) or “cold” (immune-deserted or excluded), a framework increasingly used to predict response to immunotherapy.

To decipher how molecular and cellular heterogeneity within the breast TME shapes clinical behavior, we performed the most comprehensive to date, integrative analysis of scRNA-seq data. Focusing on subtype-specific TME features and therapeutic vulnerabilities, our results reveal a dynamic shift between “cold” and “hot” microenvironments across disease states.

METHODS

We aggregated 376 samples derived from 263 patients across 31 publicly available datasets.
The cohort included 239 samples from primary breast tumors, 37 originated from adjusted affected lymph nodes and 23 from distant metastatic lesions.
In total, 686,919 cells, all sequenced using 10x droplet-based scRNA-seq platforms, passed our QC process.
Each dataset was processed independently through a standardized pipeline to ensure high data quality and consistency

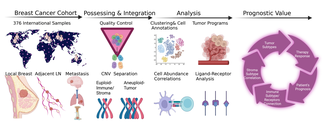


Figure 1: Schematic representation of the study workflow

THE COHORT

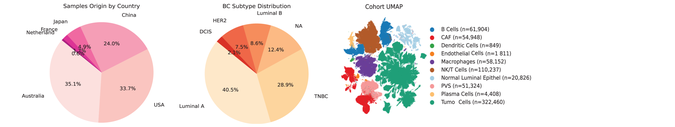


Figure 2: (A) Geographic distribution of cohort samples by country. **(B)** Distribution of breast cancer subtypes across the cohort. **(C)** UMAP visualization of the combined cohort, colored by major cell types

TUMOR MICROENVIRONMENT: HOT VS. COLD PARADIGM

Divergent Immune Infiltration in Primary and Metastatic Breast Cancer

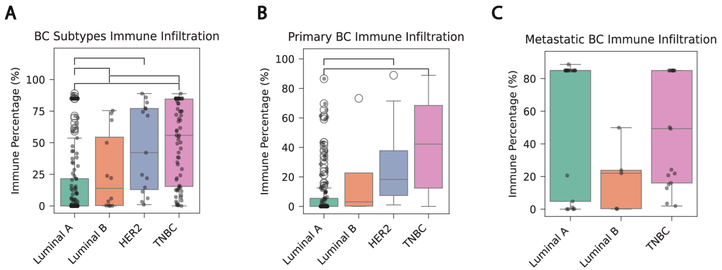


Figure 3: Percentage of immune cell infiltration in breast cancer subtypes across(A) all samples (B) primary tumors and (C) distant metastatic sites.

Tumor Promoting vs. Suppressing Ligand-Receptor Interactions

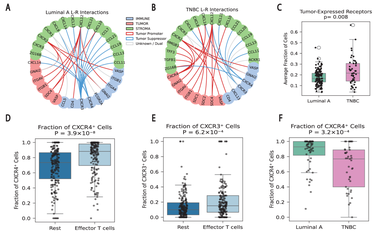


Figure4: (A-B) Ligand–receptor interaction networks across breast cancer subtypes and stages. Panels show interactions in **(A)** Luminal A samples, **(B)** TNBC samples. **(C)** Mean fraction of tumor cells expressing: CXCR4, ITGB1, SDC1, SDC4, VASP in LA vs. TNBC samples **(D-E)** Fraction of (E) CXCR4+ (F) CXCR3+ cells in Effector T cells vs. all other T cells clusters **(F)** Fraction of CXCR4+ cells in effector T cells of LA vs. TNBC samples.

BREAST CANCER SUBTYPE SPECIFIC RESULTS

LUMINAL A

Luminal A ER-Dependent Populations

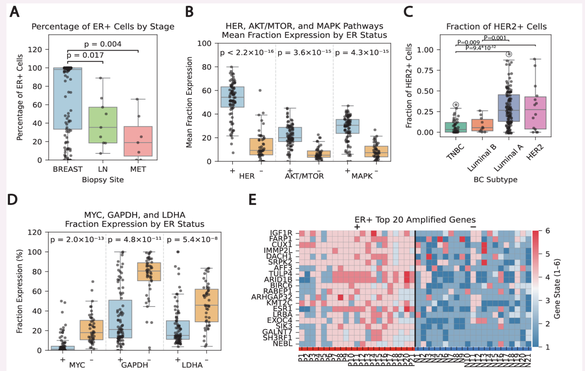


Figure 4: (A) Percentage of ER positive tumor cells by biopsy site. **(B)** Mean fraction of cells expressing HER2-4, AKT/MTOR, and KRAS–BRAF–CERK gene signatures in ER-positive vs. ER-negative tumor cells. **(C)** Fraction of HER2-positive tumor cells across breast cancer subtypes. **(D)** Fraction of cells expressing of MYC, GAPDH, and LDHA in ER (+) versus ER (–) tumor cells. **(E)** Top 20 amplified genes in ER-positive versus ER-negative (CNV clusters.

ER Signature Predicts Survival and Response to ET

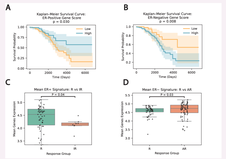


Figure 5: Kaplan–Meier survival analysis for ER+ BC tumors from the TCGA, stratified by high versus low expression of **(A)** ER+ (and **(B)** ER- signatures. Expression of **(C)** ER+ and **(D)** ER- signatures in responders versus resistant patients to endocrine therapy.

ER Signature Correlates With Stroma and Macrophage Populations

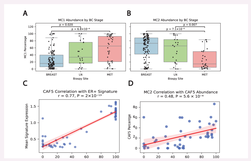


Figure 6: Abundance of macrophage **(A)** MC1 and **(B)** MC2 across different breast cancer biopsy sites. **(C-D)** Correlation between CAF5 abundance and **(C)** ER+ signature and **(D)** macrophage cluster- MC2 abundance.

TNBC

EMT-Associated Tumor Programs in TNBC Reveal TROP2 as a Potential Therapeutic Target and Predict Poor Survival In Metastatic lesions

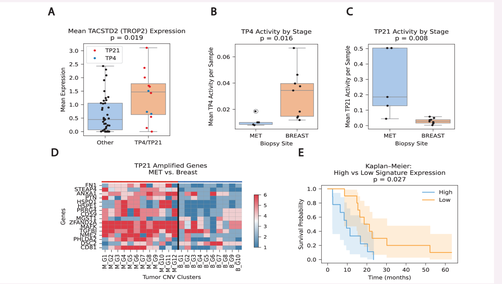


Figure 7: (A) TROP2 mean expression in tumor cells from program 21 (red), program 4 (blue) vs. all other tumor cells. **(B-C)** Mean activity of **(B)** TP4 and **(C)** TP21 across breast cancer biopsy sites.**(D)** Amplified genes in tumor program 21, comparing metastatic versus primary CNV clusters.**(E)** Kaplan–Meier survival probability curves comparing TP21-high vs. TP21-low samples.

TNBC Immune Checkpoint Landscape Correlates With Tumor Programs

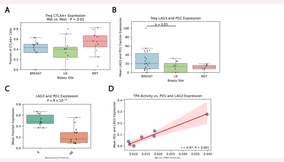


Figure 8: (A-B) Fraction of cells expressing **(A)** CTLA4 and **(B)** LAG3 and PD1 , in regulatory T cells across biopsy sites. **(C)** Fraction of cells expressing LAG3 and PD1 in regulatory T cells from expanded (E) vs. non-expanded (NE) patients. **(D)** Mean immune cells LAG3 and PD1 correlation with TP4 mean activity level.

CONCLUSION

This study presents the first in-depth single-cell analysis of transcriptional heterogeneity within LA and TNBC tumors

In LA we identified two major tumor cell profiles: ER+ and ER-. These were strongly associated with differences in patient survival and response to ET.

MYC was identified as a potential driver of ER- cells and may contribute to therapeutic resistance.

The distinct profiles also corresponded with specific TME characteristics: ER+ tumors were linked to immune exclusion and fibrotic stroma enriched in anti-inflammatory macrophages, whereas ER- tumors showed increased immune infiltration and a pro-inflammatory stromal profile.

In TNBC, we mapped the IC landscape across disease stages—findings that may guide future ICI trial designs.

Finally, EMT-programs with high TROP2 expression nominate a subset of TNBC tumors as potential candidates for targeted therapies such as Sacituzumab-govitecan.

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