Comparison of immune microenvironment between primary and metastatic breast tumors

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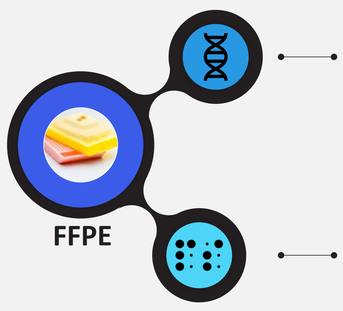
Introduction

- Immune evasion has been described as one of the mechanisms by which cancer cells gain the ability to metastasize from the primary tumor to distant sites in the body.[1]
- In triple-negative breast cancer (TNBC), metastatic tumors are shown to be more immunologically silent than primary tumors. As a result, there are varying degrees of responses to immunotherapy between early-stage and metastatic TNBC.[2]
- Positive clinical responses to immune checkpoint inhibitors (ICIs) are seen in patients with early-stage TNBC regardless of PD-L1 expression, whereas greater benefits to ICIs are seen in metastatic TNBC with higher PD-L1 expression.
- In this study, we investigated the differences in the immune signatures of primary and metastatic breast cancer in a real-world patient population.

Methods

- A retrospective cohort of 529 breast tumors tested in the real-world clinical setting were evaluated by comprehensive genomic and immune profiling (CGIP) of the tumor microenvironment (Figure 1).
- Tumor specimens were classified as primary breast, any lymph nodes (regional and non-regional) or metastatic visceral sites. Lymph node samples were chosen as positive controls due to expected elevated inflammatory signaling.



Genomic Profiling

SNV/INDEL/Fusion/CNV for 523 genes[3]
Tumor mutational burden (TMB)
Microsatellite Instability (MSI)

Immune Profiling

- RNA-seq expression profiling of 395 immune transcripts[4]
- PD-L1 IHC[4]
- Cell Proliferation[5]
- Tumor Inflammation[5]

Figure 1. CGIP methods description.

- Over-representation and proportion analysis using chi-squared test was applied to determine the association of specimen sites to various genomic and immune correlates.

References

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2. Carlino F, Diana A, Piccolo A, et al. Immune-Based Therapy in Triple-Negative Breast Cancer: From Molecular Biology to Clinical Practice. Cancers (Basel). 2022 May; 14(9): 2102.
3. Conroy JM, et al. (2021) A scalable high-throughput targeted next-generation sequencing assay for comprehensive genomic profiling of solid tumors. PLOS ONE 16(12): e0260089.
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5. Pabla S et al. Integration of tumor inflammation, cell proliferation, and traditional biomarkers improves prediction of immunotherapy resistance and response. Biomark Res 9, 56 (2021).

Metastatic breast tumors show immune signatures suggestive of a less active tumor immune microenvironment than primary breast tumors.

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Table 1. Cohort characteristics.

Variable	Group	N (%)
Age	Median: 63.2 years, Range: 25.5-93.5 years	529 (100%)
Gender	Female	519 (98%)
	Male	10 (1.9%)
Sample Source	Lymph node	72 (14%)
	Metastatic	232 (44%)
	Primary breast	224 (42%)
Tumor Histology	Invasive ductal carcinoma, NOS	287 (54%)
	Invasive lobular carcinoma	26 (4.9%)
	Mammary adenocarcinoma, NOS	207 (39%)
	Other	10 (1.9%)
All Samples		529 (100%)

Results

Tumor inflammation landscape

Samples of primary breast lesions harbored a greater degree of immune infiltration, demonstrating a higher TIGS score than metastatic visceral lesions ($p=4.4\times10^{-5}$).

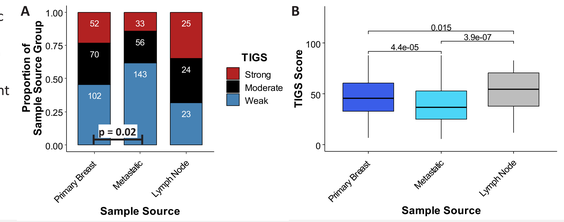

Figure 2. Tumor immunogenic score (TIGS) distributions by sample source: A) TIGS group prevalence in each sample source group with total patient number in each group indicated; B) TIGS score distribution in each sample source group. Wilcoxon Rank Sum p values indicated.

Biomarkers of response to checkpoint inhibitors

Primary lesions also demonstrated a greater proportion of PD-L1 positive tumors than metastatic lesions (44% vs 21%, $p < 0.001$) and higher expressions of other immune checkpoints such as TIGIT ($p<0.001$),LAG3 ($p=0.037$) and TIM3 ($p<0.001$).

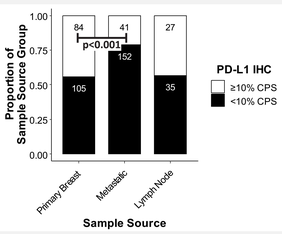

Figure 3. PD-L1 IHC group (Positive: >10% CPS) prevalence in sample source groups with total patient number in each group indicated.

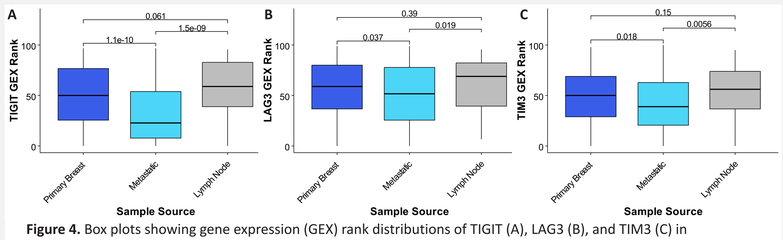

Figure 4. Box plots showing gene expression (GEX) rank distributions of TIGIT (A), LAG3 (B), and TIM3 (C) in each sample source group. Wilcoxon Rank Sum p values shown.

Conclusions

- Non-lymph node breast cancer metastases harbor a less active immune response than primary breast lesions, showing a lower degree of immune cell infiltration and decreased expression of immune checkpoint markers.
- These findings support the notion that the immune microenvironment of breast cancer metastases is immunosuppressive and may exhibit a tempered response to ICIs.

Future Directions for Research:

- Although further clinical validation of these immune biomarkers is required, this study demonstrates the potential for CGIP to provide immunotherapy treatment decision support when selecting an ICI in metastatic breast cancer.
- Combination treatments of ICIs with chemotherapy, targeted therapies or cancer vaccines may be promising therapeutic approaches to enhance the immune responses and potentially overcome resistance to ICIs in metastatic breast cancer.