Journal of Cancer Research and Practice

journal homepage: https://journals.lww.com/jcrp

Review Article

Spatial Profiles in Triple-negative Breast Cancer: Unraveling the Tumor Microenvironment and Biomarkers for Immune Checkpoint Inhibitors

Cheng-Han Wu[1,2,3], Yu-Chiao Chiu[3]*

[1]Division of Hematology/Medical Oncology, Department of Medicine, Taichung Veterans General Hospital, Taichung, Taiwan
[2]Institute of Biomedical Informatics, School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan
[3]Division of Hematology/Oncology, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Abstract

Objective: Immune checkpoint inhibitors (ICIs) have become an important treatment option for cancer. However, the predictive power of current biomarkers is limited for treatment response, especially in triple-negative breast cancer (TNBC). Investigation of the tumor microenvironment (TME) may provide biological insights into the response to ICIs by uncovering the interactions among tumor and immune cells. Emerging technologies of spatial transcriptomics (ST) and proteomics allow clinical researchers to better understand the TME. **Data Sources and Study Selection:** We reviewed the results of articles published in the past 10 years worldwide. **Results:** Emerging spatial profiling technologies can be classified into image-based and sequencing-based methods, both of which preserve information on tissue architecture with gene expression and/or protein abundance profiles. Here, we reviewed articles studying TNBC using spatial profiling techniques. By integrating spatial profiles, recent studies showed the relevance of gene and protein expression profiles in the TME of different subgroups. These ST and proteomic characteristics were shown to be associated with patients' survival. **Conclusion:** The application of spatial profiling techniques to cancer research has significantly advanced our understanding of breast cancer biology, particularly in the context of TNBC. We are confident that the technology has the potential to revolutionize the prediction of treatment outcomes in the near future. By elucidating the nuances within the TME, spatial profiling opens up new possibilities for personalized strategies for immunotherapy.

Keywords: Breast cancer, immune therapy, spatial transcriptomics

Introduction

Immune checkpoint inhibitors (ICIs) have emerged as a pivotal approach to treat cancer, offering sustained responses and significant survival benefits in a subgroup of patients across multiple cancer types. In clinical practice, several predictive biomarkers have been developed for ICIs based on tumor mutation burden, microsatellite instability, DNA mismatch

Submitted: 30-Jul-2023 **Revised:** 09-Oct-2023
Accepted: 15-Nov-2023 **Published:** 26-Jun-2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jcrp

DOI: 10.4103/ejcrp.eJCRP-D-23-00030

Address for correspondence: Prof. Yu-Chiao Chiu, Division of Hematology/Oncology, Department of Medicine, University of Pittsburgh School of Medicine, 5051 Centre Ave, Pittsburgh 15213, PA, USA.
E-mail: yuc250@pitt.edu

How to cite this article: Wu CH, Chiu YC. Spatial profiles in triple-negative breast cancer: Unraveling the tumor microenvironment and biomarkers for immune checkpoint inhibitors. J Cancer Res Pract 2024;11:62-6.

repair deficiency status, programmed cell death-ligand 1 (PD-L1) expression, and neutrophil-to-lymphocyte ratio in peripheral blood.[1]

Breast cancer is the most prevalent tumor type in women, and it poses significant biomedical challenges. Patients with triple-negative breast cancer (TNBC) lack the expression of common therapeutic targets of breast cancer, such as hormone receptors or human epidermal growth factor receptor 2. Thus, identifying effective biomarkers for ICIs in this vulnerable patient population is critical. In fact, not all TNBC patients respond well to ICIs. A recent clinical study demonstrated that the combination of ICIs with chemotherapy did not improve overall survival (OS) or progression-free survival (PFS) in TNBC patients with a "combined positive score" ≥10 or ≥1.[2] Thus, there is an urgent need for accurate and robust biomarkers to better understand and leverage the tumor microenvironment (TME) in this specific breast cancer subtype.

Single-cell RNA sequencing has revolutionized cancer biology and immunology by offering valuable insights into the gene expression profiles at a single-cell resolution. However, a limitation of conventional single-cell RNA sequencing is the inability to capture spatial information, which is crucial for understanding the spatial context of cell identity and biological functions in the TME. This limitation has recently been overcome by spatial transcriptomics (ST), a cutting-edge technique that provides spatially resolved, high-dimensional assessments of gene transcripts with the flexibility to create accompanying protein profiles. By integrating ST with advanced bioinformatics methods, studies have provided a deeper understanding of cell–cell interactions within the complex TME.[3]

In this review article, we summarize the emerging technologies of spatial profiling and discuss the latest discoveries in unraveling intricate interactions within the TME. We focus on TNBC as it remains a significant clinical challenge where the efficacy and biological investigation of ICIs is an active area of research.

Spatial Profiling Techniques

In 2009, the single-cell digital gene expression profiling assay was introduced, marking a significant milestone in single-cell transcriptomics.[4] This technology allowed transcriptome-wide analysis of individual or several cells, but it necessitated the disassociation of viable cells from tissue, posing challenges for studying intact tissue, and preserving spatial information among cells. To address this, two major approaches were developed.[5] The first approach involves imaging RNA *in situ* through microscopy, known as image-based ST. The second approach is based on next-generation sequencing. It involves direct capture of mRNAs within a tissue block using barcoded beads to annotate the spatial information, which is then subjected to sequencing.

Image-based technologies

In situ hybridization (ISH) enables direct imaging of transcriptional heterogeneity in intact tissue architecture. Fluorophore-labeled probes are specifically bound to target transcripts, which are then quantified through fluorescence spot counting under high-resolution microscopy.[3] One representative technique, multiplexed error-robust fluorescence ISH, utilizes binary codes for each probe, with an algorithm of extended Hamming code to process the binary codes of each transcript and correct detection errors during reading.[6]

Sequencing-based technologies

Sequencing-based techniques involve capturing mRNAs directly from the tissue and sequencing the captured mRNAs to determine their identity. The chosen tissue is placed on a slide, and the mRNAs from tissue sections are then transferred onto the surface covered in DNA-barcoded beads with known positions.[7] To maintain spatial information while transferring mRNAs, microdissection or ligation of the mRNAs to spatially-barcoded probes in a microarray is required.[5] Once the mRNAs are captured together with the positional information, cDNAs are synthesized and subject to next-generation sequencing.

Unlike image-based technologies, which offer spatial resolution at the molecular level, the resolution of sequencing-based technologies is determined by the diameter of the barcoded beads being used. An early example of this technique is ST, which was introduced in 2016 and has a resolution of about 100 μm.[8] By 2022, the technique had been further improved to a 55 μm resolution and was commercialized by 10X Genomics under the name *Visium*. Another technique called *slide-seq* utilizes barcoded beads with a diameter of 10 μm.[7]

Comparison of the techniques

Generally speaking, image-based techniques feature subcellular resolution but are significantly limited in gene coverage. On the other hand, sequencing-based techniques can detect the entire transcriptome simultaneously, but the spatial resolution is constrained by the density and size of barcoded beads. In addition, the capture areas may not conform to the intricate contours of cellular morphology. As a result, cells frequently span multiple capture areas, leading to the contribution of mRNAs to more than one pixel. ISH-based techniques have not been widely used so far, possibly owing to the availability of this emerging technology, extended imaging duration, and the cost associated with specialized probes. The most popular commercialized platforms include 10X Genomics Xenium and NanoString CosMx. A comparison of these two main techniques is shown in Table 1.

For clinical research, the availability of fresh frozen tissues and the readiness of commercial profiling techniques are the two major concerns. Most techniques were initially developed for frozen sections, and only a few techniques are compatible with formalin-fixed paraffin-embedded (FFPE) samples. Visium is FFPE compatible. However, the genes detected in FFPE samples per spot are 5–10 times less than the frozen

Table 1: Comparison of image-based and sequencing-based techniques for transcriptomic profiling

	Image based	Sequencing based
Gene detection methodology	Fluorescence signal from ISH	mRNA capturing by barcoded beads
Resolution	Single molecular resolution (<1 μm)	A few cells, depending on the bead size (around 10–50 μm)
Techniques	smFISH; MERFISH	LCM; array-based capture of mRNA
Number of measured genes	500–6000	Whole transcriptome
Commercial platforms	MERSCOPE, Xenium, CosMx	Visium, GeoMx

ISH: *In situ* hybridization, smFISH: Single-molecule fluorescence ISH, MERFISH: Multiplexed error-robust fluorescence ISH, LCM: Laser capture microdissection

counterparts due to RNA fragmentation.[9] GeoMx is also FFPE compatible and predominantly used on pathological FFPE tissues.[5] There is a trade-off between the number of targeted genes and transcript errors.[5] Choosing an ST method from the commercially available options necessitates careful consideration of factors such as spatial resolution, tissue coverage, mRNA detection sensitivity, and the range of genes.[3]

In this review article, we discuss several published studies to showcase how these spatial techniques have been utilized in clinical research on breast cancer, with a primary focus on TNBC. By employing these techniques, we can obtain gene expression and/or protein abundance profiles together with the positional information of the cells. Of note, the groundbreaking technology of spatially resolved transcriptomics was selected as the Method of the Year 2020 by *Nature Methods*.[10]

Tumor Microenvironment in Breast Cancer

The TME plays critical role in tumor progression and resistance to chemotherapy. Dynamic cell–cell interactions taking place within the TME govern cancer initiation, progression, and invasion.[11] This complex microenvironment comprises various components, including stromal cells, immune cells, and nonstromal factors.[12,13] Both innate immune cells and adaptive immune cells present in the TME are involved in tumorigenesis.[14] Notably, immune infiltration in breast cancer consists of intratumoral and stromal tumor-infiltrating lymphocytes (TILs). Traditional methods for analyzing TILs involve primary morphological assessments of immune cells by hematoxylin and eosin or immunohistochemistry (IHC) staining.[15] Previous studies using these conventional techniques have shown that the quantity of TILs is positively associated with disease-free survival and OS in early breast cancer.[16]

ST represents significant advantages over traditional IHC staining. It offers detailed gene expression profiling that is more sensitive than conventional histological methods, allowing for a deeper understanding of the underlying molecular mechanisms within the TME. Moreover, ST can reveal intricate effects that may not be evident in immunofluorescence images, offering a more comprehensive view of the cellular interactions and signaling pathways.[17] This technological advancement has been widely adopted to investigate a broad range of disease contexts, with cancer being a particularly promising area of application.[18] By employing ST, researchers can gain valuable biological insights into the complex cellular interactions within the TME, paving the way for more targeted and effective therapeutic strategies. Numerous studies have already leveraged ST and reported preliminary results with exciting implications. In the following sections, we explore some of these studies and showcase how ST has contributed to advancing our understanding of breast cancer, the TME, and treatment responses.

Applications of Spatial Profiling Technologies in Breast Cancer Research

By utilizing the commercialized spatial transcriptome profiler Visium, researchers have evaluated the gene expressions of cancer cells with unprecedented precision. Tashireva *et al*. conducted a study comparing gene and protein expressions between breast cancer cells with positive and negative presence of PD-L1.[19] The gene expression profiles were analyzed using CIBERSORT, a method designed for cell decomposition and assessment of immunocyte constituents. The investigation revealed that PD-L1-positive cases had higher percentages of CD4+ naive T-cells and M2 macrophages within the tumor and immune stromal cell adjacent regions compared with PD-L1-negative cases. Furthermore, pathway analysis revealed an enrichment in immune-related pathways in PD-L1-positive tumors. Interestingly, PD-L1-negative tumors exhibited only the antigen presentation pathway without any effector phase of the immune response. This finding suggests a potential absence of specific immune reactions against the tumor in the TME, possibly due to limited contact between PD-L1 and PD1-positive cells, which might negatively impact the efficacy of ICIs.

Carter *et al.* used the NanoString GeoMx Digital Spatial Platform (DSP) to quantify the protein abundance of immune markers in two cohorts with systemic therapy-naïve TNBC.[20] The immune protein profiles within CD45-rich and CD68-rich stromal microenvironments exhibited significant differences, indicating that the stroma microenvironment might impact immune proteins in the tumor and adjacent area. After incorporating clinical outcomes in the analysis, the results showed that intraepithelial CD40 or HLA-DR enrichment was associated with better outcome, independently of other prognostic factors. This study also used eigenprotein scores to evaluate antigen-presenting and T-cell activation state.

Only cases with high scores for both types of cells showed significantly lower risks of recurrence. This observation might support the hypothesis that the proximity of the tumor and immune cells would lead to a better outcome.

In another study using NanoString GeoMx, Kulasinghe *et al.* investigated the prognostic features of adjuvant therapy in TNBC.[21] The study focused on proteomic analysis using the GeoMx DSP to examine the TME of TNBC and its association with the outcome of adjuvant chemotherapy. The study specifically targeted proteomics of two groups of TNBC samples: chemosensitive and chemoresistant cases. Chemosensitive cases were defined as those exhibiting complete remission after receiving adjuvant chemotherapy of 5-fluorouracil, epirubicin, and cyclophosphamide, whereas chemoresistant cases were defined by the occurrence of progressive disease during the follow-up period. The analysis revealed significant associations between protein profiles within the TME and the response to chemotherapy. Specifically, a higher expression of estrogen receptor-α (ER-α) protein and lower expression of MART1 protein within the stromal region were found to be associated with a stronger sensitivity to chemotherapy. Moreover, an increased expression of ER-α and decreased expression of MART1 were also correlated with better OS in TNBC patients.

Patwa *et al.* used the image-based technique multiplexed ion beam imaging (MIBI) to measure protein expressions at the subcellular level.[22] MIBI leverages secondary ion mass spectrometry to visualize antibodies labeled with isotopically pure elemental reporters. It captures spatial information and enables the measurement of 40 proteins simultaneously. The study enrolled 38 TNBC patients, incorporated their clinical outcomes and tissues, and measured immunoregulatory proteins. The results showed that single-cell expression levels of functional proteins were not associated with survival, and, instead that the co-expressions of proteins and interactions involving immunoregulatory proteins might have prognostic value. For example, co-expressions of immunoregulatory proteins, including PD-1, PD-L1, IDO, and Lag3, were associated with a lower risk of recurrence.

Taken together, the findings from these recent studies shed light on the importance of understanding the intricacies of the TME, where various immune cell populations interact with heterogeneous TNBC tumor cells, and the critical roles in governing the response to ICIs. Spatial profiles and cell compositions identified by the studies provide valuable insights that may lead to improved strategies for enhancing the effectiveness of treatment in breast cancer.

Future Perspectives

The studies reviewed here investigated the complex interplay among gene and protein expressions in cells, protein co-expressions in the TME, activation of immune pathways, and differences in survival between different TMEs. The proximity of immune cells and antigens showed a favorable association with patient outcomes. Another important finding is that the co-expressions of paired functional proteins can potentially trigger immune responses and lead to better survival. This may be a promising research topic in the context of various immune-desert cell types.

To meaningfully utilize the enormous data generated by spatial profiling, the role of computational biologists is becoming important. The results of data-driven analysis also need to be biologically interpretable.[23] We believe that cross-disciplinary collaboration will become critical in this endeavor.

Conclusion

ST profiling is a promising technology with the ability to provide information on gene and protein expressions with a spatial context. By leveraging ST, researchers have gained a deeper understanding of the complex interactions among various cells and the intricate molecular landscape within the TME, leading to significant implications for predicting treatment response. A single parameter, such as PD-L1 expression, cannot precisely interpret the complex TME or predict tumor immunogenicity. As precision medicine continues to evolve, the insights gained from ST could pave the way for improved patient outcomes and more effective immunotherapy approaches in the battle against breast cancer. We believe that future clinical practice will integrate spatial techniques to enable more accurate predictions of treatment responses.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bai R, Lv Z, Xu D, Cui J. Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors. Biomark Res 2020;8:34.
2. Winer EP, Lipatov O, Im SA, Goncalves A, Muñoz-Couselo E, Lee KS, *et al.* Pembrolizumab versus investigator-choice chemotherapy for metastatic triple-negative breast cancer (KEYNOTE-119): A randomised, open-label, phase 3 trial. Lancet Oncol 2021;22:499-511.
3. Williams CG, Lee HJ, Asatsuma T, Vento-Tormo R, Haque A. An introduction to spatial transcriptomics for biomedical research. Genome Med 2022;14:68.
4. Tang F, Barbacioru C, Wang Y, Nordman E, Lee C, Xu N, *et al.* mRNA-Seq whole-transcriptome analysis of a single cell. Nat Methods 2009;6:377-82.
5. Moses L, Pachter L. Museum of spatial transcriptomics. Nat Methods 2022;19:534-46.
6. Chen KH, Boettiger AN, Moffitt JR, Wang S, Zhuang X. RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells. Science 2015;348:aaa6090.
7. Rodriques SG, Stickels RR, Goeva A, Martin CA, Murray E, Vanderburg CR,

et al. Slide-seq: A scalable technology for measuring genome-wide expression at high spatial resolution. Science 2019;363:1463-7.
8. Ståhl PL, Salmén F, Vickovic S, Lundmark A, Navarro JF, Magnusson J, *et al.* Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science 2016;353:78-82.
9. Gracia Villacampa E, Larsson L, Mirzazadeh R, Kvastad L, Andersson A, Mollbrink A, *et al.* Genome-wide spatial expression profiling in formalin-fixed tissues. Cell Genom 2021;1:100065.
10. Marx V. Method of the year: Spatially resolved transcriptomics. Nat Methods 2021;18:9-14.
11. Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y, *et al.* Role of tumor microenvironment in tumorigenesis. J Cancer 2017;8:761-73.
12. Mehraj U, Dar AH, Wani NA, Mir MA. Tumor microenvironment promotes breast cancer chemoresistance. Cancer Chemother Pharmacol 2021;87:147-58.
13. Mittal S, Brown NJ, Holen I. The breast tumor microenvironment: Role in cancer development, progression and response to therapy. Expert Rev Mol Diagn 2018;18:227-43.
14. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. Cancer Res 2019;79:4557-66.
15. Dieci MV, Miglietta F, Guarneri V. Immune infiltrates in breast cancer: recent updates and clinical implications. Cells 2021;10:223.
16. Loi S, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, *et al.* Tumor-infiltrating lymphocytes and prognosis: A pooled individual patient analysis of early-stage triple-negative breast cancers. J Clin Oncol 2019;37:559-69.
17. Whitsitt QA, Koo B, Celik ME, Evans BM, Weiland JD, Purcell EK. Spatial transcriptomics as a novel approach to redefine electrical stimulation safety. Front Neurosci 2022;16:937923.
18. Rao A, Barkley D, França GS, Yanai I. Exploring tissue architecture using spatial transcriptomics. Nature 2021;596:211-20.
19. Tashireva LA, Kalinchuk AY, Gerashchenko TS, Menyailo M, Khozyainova A, Denisov EV, *et al.* Spatial profile of tumor microenvironment in PD-L1-negative and PD-L1-positive triple-negative breast cancer. Int J Mol Sci 2023;24:1433.
20. Carter JM, Chumsri S, Hinerfeld DA, Ma Y, Wang X, Zahrieh D, *et al.* Distinct spatial immune microlandscapes are independently associated with outcomes in triple-negative breast cancer. Nat Commun 2023;14:2215.
21. Kulasinghe A, Monkman J, Shah ET, Matigian N, Adams MN, O'Byrne K. Spatial profiling identifies prognostic features of response to adjuvant therapy in Triple Negative Breast Cancer (TNBC). Front Oncol 2021;11:798296.
22. Patwa A, Yamashita R, Long J, Risom T, Angelo M, Keren L, *et al.* Multiplexed imaging analysis of the tumor-immune microenvironment reveals predictors of outcome in triple-negative breast cancer. Commun Biol 2021;4:852.
23. Ohnmacht AJ, Stahler A, Stintzing S, Modest DP, Holch JW, Westphalen CB, *et al.* The oncology biomarker discovery framework reveals cetuximab and bevacizumab response patterns in metastatic colorectal cancer. Nat Commun 2023;14:5391.