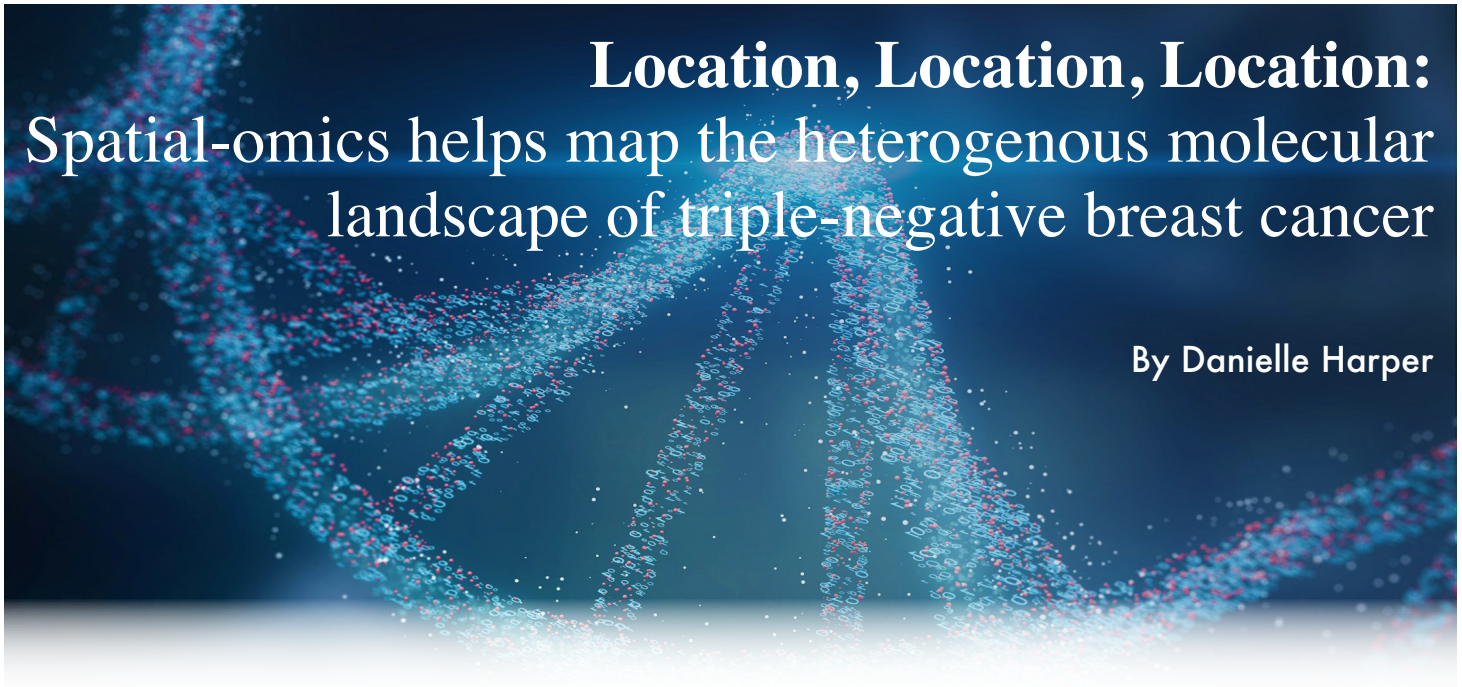

Location, Location, Location: Spatial-omics helps map the heterogenous molecular landscape of triple-negative breast cancer

By Danielle Harper

Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype, characterized by a lack of the clinically actionable targets: ER (estrogen receptor), PR (progesterone receptor), and HER2 (human epidermal growth factor receptor 2).[1] The TNBC subtype represents approximately 15%-25% of all breast cancer diagnoses and compared to other subtypes is more commonly diagnosed in premenopausal women.[1] Until recently, surgery followed by cytotoxic chemotherapy and/or radiation were the only treatment options for TNBC patients. Not only do treatment options remain limited, but drug resistance and disease recurrence are common. Up to 30% of TNBC patients develop metastatic disease, when the cancer spreads to distant organs, for which the average life expectancy is just 8-13 months.[2] This poor prognosis underscores the importance of developing new therapeutic approaches for managing TNBC and preventing disease progression.

Recent advances in multi-omics technologies have revealed TNBC to be a heterogenous group of diseases with distinct gene expression profiles. Lehmann et al used bulk RNA sequencing of 386 TNBC tumours to reveal six distinct subtypes with unique therapeutic vulnerabilities.[3] For example, patients with basal-like tumours exhibit upregulated epidermal growth factor receptor (EGFR) signaling, and may respond to EGFR inhibitors, such as lapatinib.[3] Intratumoural heterogeneity, characterized by subpopulations of cells with varying gene expression profiles and propensities for drug resistance, remains a significant challenge in TNBC management.[4] As we learn more about the molecular features of triple-negative tumours, treatment strategies continue to evolve. Attention has recently shifted toward immunotherapy, which leverages the body's immune system to recognize and destroy cancer cells. Immunotherapy offers a promising new avenue for a subset of TNBC patients, but challenges remain in identifying which patients are likely to respond to treatment.

Transcriptomic studies have demonstrated that ~20% of TNBC tumours express PD-L1 (programmed death ligand-1), making them possible candidates for immune checkpoint inhibitor (ICI) therapies.[5] PD-L1 binds to the programmed cell death protein 1 (PD-1) expressed on the surface of T-cells to prevent their cytotoxic function.[5] Monoclonal antibodies that prevent this interaction between PD-L1 and PD-1 allow T cells to recognize and kill the tumour cells. Susceptibility to immune checkpoint blockade has been shown to correlate with the presence of tumour-infiltrating lymphocytes (TILs) within the tumour.[6] However, increasing evidence suggests that not just the presence, but the location of these TILs within the tumour microenvironment (TME) may provide additional prognostic insight and help guide treatment decisions.[7] These novel insights are made possible by advances in spatial-omics technologies.

Spatial-omics is a field dedicated to profiling the molecular characteristics of a tissue in a way that preserves its positional context.[8] Spatial transcriptomics

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(ST), for example, is a novel technology that measures gene expression from intact tissues samples, such as tumour biopsies.[9] Traditional bulk RNA sequencing provides the average gene expression signatures within a sample, but ST maps gene expression signatures back onto the tumour sample to reveal spatial patterns. These patterns can provide insights into a tumour's molecular features and potential vulnerabilities. While single-cell RNA sequencing approaches can reveal gene expression patterns associated with a particular cell type, ST goes one step further by providing spatial context for the gene expression profiles of individual cells. In other words, researchers can look at cell behaviour relative to neighboring cells and explore how cell-cell interactions shape tumour progression, metastatic spread, immune responses, and treatment resistance.

Briefly, one approach to ST utilizes tissue sections placed on a glass slide containing immobilized reverse-transcription oligo(dT) primers to capture tissue mRNAs.[9] From there, the mRNA is reverse-transcribed and positional barcodes (short DNA sequences that correspond to a particular XY coordinate on the slide) are incorporated to into the resulting cDNA in order to identify where on the tissue section that mRNA molecule originated.[9] The barcoded cDNA library can then be sequenced and mapped back onto the tissue section to reveal spatial patterns of gene expression.[9]

Hammerl et al. defined three spatial immunophenotypes based on the location of TILs, specifically CD8+ cytotoxic T cells, in TNBC tumour samples.10 CD8+ T cells kill tumour cells by releasing a protein called granzyme. Tumours lacking CD8+ T cells were classed as "ignored", while those bordered by CD8+ T cells were considered "excluded", and tumours with infiltrating CD8+ T cells were deemed "inflamed".[10] Gene-expression patterns unique to each spatial immunophenotype were identified and used to predict treatment outcomes. Both ignored and excluded phenotypes were associated with poor response to anti-PD-1 immune checkpoint blockade therapy, while inflamed tumours were associated with more favourable treatment outcomes.[10] While the concept of "hot" and "cold" tumours (indicating immune infiltration or lack thereof) has been around since the early 2000s[11], spatial approaches offer novel insights into the molecular landscapes of TNBC tumours in order to make rapid and accurate treatment decisions.

A more recent study published in Nature Communications used ST to reveal unique patterns in intratumoural organization across TNBC tumour samples.[12] The authors identified a gene expression signature corresponding to the location of tertiary lymphoid structures (TLS), which are aggregates of immune cells within the TME.[12] This thirty-gene signature was able to distinguish infiltrating immune cells from tumour or non-tumour (stromal) cells. As expected, more TLS within a tumour correlated with a higher response to immunotherapy.[13] The presence of TLS within the tumour is believed to correlate with an adaptive immune response and being able to identify these inflammatory structures offers novel predictive insight that traditional bulk transcriptomic methods lacking spatial resolution would miss.[12]

In addition to identifying where specific subtypes of immune cells are located within a tumour, ST has the potential to shed light on host-tumour interactions. Understanding the relationship between a cell's location within a tumour and its gene expression pattern can provide valuable information about how a cell's behaviour is shaped by its neighbours. For example, a 2023 ST study of colorectal cancer (CRC) reported that macrophages with immunosuppressive gene-expression signatures were concentrated at the invasive front of the tumour (the interface between tumour and normal tissue).[14] These macrophages are thought to adopt a pro-tumourigenic phenotype in response to cancer cell secretion of immunosuppressive human leukocyte antigen-G (HLA-G).[14] These findings suggest that targeting HLA-G or anti-inflammatory macrophages at the invasive front may help slow CRC metastasis.[14] In TNBC, ST revealed that crosstalk between tumour-associated macrophages (TAMs) and CD8+ T cells may promote ICI resistance.[15] TNBC patients who did not respond to ICI therapy had a higher proportion of anti-inflammatory Apolipoprotein E (APOE) expressing TAMs and the physical distance between these APOE+ TAMS and exhausted CD8+ T cells was greater.[15] In a mouse model of TNBC, an APOE inhibitor improved ICI efficacy, suggesting that the presence of APOE+ TAMS may be a biomarker for ICI response.[15] As spatial techniques continue to advance, new insights into cellular interactions within the TME and their therapeutic potential in TNBC will emerge.

Spatial-omics incorporates not only transcriptomic approaches, but also proteomics. Spatial proteomics

(SP), named Nature's Method of the Year for 2024, is used to understand the arrangement of proteins within a tissue sample.[16] While fluorescently-conjugated antibodies have long been used to locate particular proteins in cells/tissues, recent advances in multiplex immunofluorescence technologies have allowed researchers to observe localization of dozens of proteins across a single sample. For example, co-detection by indexing (CODEX) is a method that utilizes DNA-conjugated antibodies to visualize up to 60 different proteins at once.[17] The ability to simultaneously visualize such a wide range of targets can reveal novel interactions that will ultimately provide a more comprehensive map of the TME. Mishra et al. recently applied CODEX technology to reveal a novel interaction between S100 calcium-binding protein A7 (S100A7) and phospholipase A2 (cPLA2) within the breast TME.[18] Using this spatial approach, the authors were able to demonstrate that inhibition of S100A7/cPLA2 signaling led to an increased number of proliferating cytotoxic CD8+ T cells within the tumour.[18] These results suggest that of S100A7/cPLA2 inhibition may sensitize breast tumours to ICI therapy. Experts in cancer immunology agree that spatial-omics are transforming the field. In their Nature Methods Comment[19], Daniela Quail and Logan Walsh, researchers at the Rosalind and Morris Goodman Cancer Institute at McGill University, write "for cancer immunology, in which effective innate and adaptive immune responses rely on cellular interactions, understanding these spatial relationships is critical for uncovering mechanisms of antitumour immunity".

As with any emerging technology, spatial-omics faces several key challenges that must be overcome to unlock its full potential. As discussed in a recent review by Alexandrov et al, spatial-omics experiments yield immense amounts of data, posing significant computational challenges.[20] Data analysis requires robust storage infrastructure and bioinformatic expertise.[20] Fortunately, artificial intelligence and machine learning models are rapidly evolving, and are capable of addressing some of these challenges. Other concerns include reproducibility of results, calling for the need to standardize protocols.[20] This has been partially addressed through the commercialization of specific spatial technologies, including the CODEX platform, but we must also acknowledge the potential financial barriers associated with the use of proprietary reagents and equipment.[20]

The ability to map transcriptional, proteomic, or even metabolomic signatures onto a physical tumour landscape provides novel insight into cell behavior and potential therapeutic vulnerabilities. Identifying patients that are most likely to respond to immune checkpoint blockade therapies is key to improving outcomes for TNBC patients. Spatial-omics approaches are paving the way for biomarker discovery and a deeper understanding of TNBC biology.

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