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Investigating the molecular architecture of triple positive breast cancer samples with spatial omics technologies

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Highlights

- Digital Spatial Profiling and ChipCytometry were both used to profile the same breast cancer samples: the first for transcriptomics and the second for single-cell spatial biomarker imaging and analysis.
- Leveraging the strengths of both technology platforms, tumor-associated gene expression patterns were identified and then visualized *in situ* to characterize the TME.

Abstract

Background: Cancers are often heterogeneous in their presentation. These inherent differences affect the severity of the disease, choice of treatment, and treatment effectiveness. To profile these differences, subtyping categorizes cancers based on the expression of specific molecular, morphological, and clinical characteristics. For breast cancer, subtyping is commonly based on expression of ERBB2 (HER2), Estrogen Receptor (ER), and Progesterone Receptor (PR). In triple-positive breast cancer, all three markers are expressed, but the localization of these markers can vary between patients and within the same tumor. Spatial Biology technologies provide novel tools for deciphering the effects of this heterogeneity and allow for a more comprehensive approach to triple-positive breast cancer treatment. The NanoString® GeoMx® Digital Spatial Profiler (DSP) and Canopy Biosciences® CellScape™ are two complementary platforms that can answer questions about heterogeneity through spatial transcriptomic and proteomic analysis.

Methods: To investigate triple-positive breast cancer (BRC), we developed custom morphology markers for use with the GeoMx DSP, including those for PR, ER, and HER2. These markers, in conjunction with a pathologist review of corresponding H&E sections, guided our selection of twelve total ductal carcinoma in situ (DCIS) and infiltrating ductal carcinoma (IDC) regions of interest (ROIs) from each sample with variable PR expression. We transcriptionally profiled the tumor and tumor microenvironment (TME) from these ROIs with the Cancer (CTA) and Whole Transcriptome Atlas (WTA) panels, which monitor expression of 1,800+ and 18,000+ targets, respectively. We use CellScape™ to verify that significant transcriptional changes were correlated with changes in protein expression in individual cells.

Results: Our results showed significant differences in gene expression between the DCIS and IDC regions in both tumor and TME, particularly in the expression of collagen and keratin transcripts. These differences reflect expected changes to tissue structure as tumor cells infiltrate surrounding tissue and are clear in both the CTA and WTA data.

Conclusions: Taken together, these spatial biology solutions will increase our understanding of the molecular architecture of these tumor types by providing a glimpse into the complex cell interactions influencing tumor heterogeneity.

Workflows

Digital Spatial Profiling

Triple-positive breast cancer samples were first stained with H&E and annotated by a pathologist to identify ductal carcinoma in situ (DCIS) and infiltrating ductal carcinoma (IDC) regions. Two FFPE sections were processed for GeoMx whole transcriptome atlas (WTA) and cancer transcriptome atlas (CTA) collections according to NanoString protocols (Figure 1). Samples were scanned on the GeoMx instrument and regions of interest (ROIs) were drawn in DCIS and IDC tumor locations. ROIs were further segmented into PR+/HER2+, HER2+, and tumor microenvironment (TME) compartments. Transcriptional information was collected from these compartments by sequencing barcodes on the Illumina NovaSeq 6000 platform. Data was analyzed with spatial deconvolution and differential expression comparisons in the GeoMx Analysis Suite.

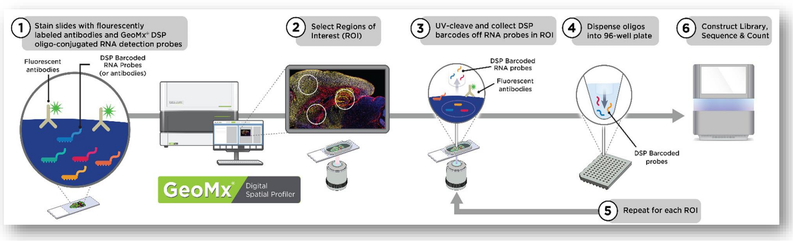

Figure 1. GeoMx Digital Spatial Profiling Workflow. Slides are stained with morphology markers to highlight tissue architecture and marker-specific regions. ROIs are selected based on imaging. Probes are collected from ROIs for molecular profiling via NGS. (Image from nanostring.com)

ChipCytometry

Sections from the same triple-positive breast cancer patient were subsequently analyzed by ChipCytometry according to Canopy Biosciences standard protocols (Figure 2). The samples were stained with a total of 20 markers (PR, ER, VIM, panCK, HER2, CD45, p53, Ki-67, CD27, CD3, EPCAM, CD56, CD45RA, CD20, CD4, CD8, HLADR, COL1, CK17, DNA) across 10 staining and imaging cycles.

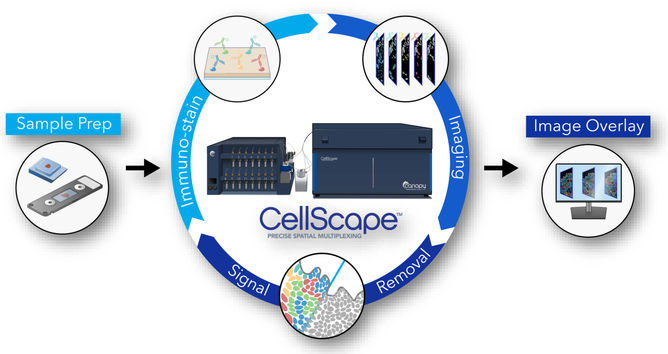

Figure 2. ChipCytometry Workflow. After sample preparation, ChipCytometry consists of successive rounds of immuno-staining, imaging, and signal removal to profile many biomarkers on a single sample. High dynamic range (HDR) imaging uses multiple exposure times to capture a wide range of biomarker expression. An image overlay is created, and cells are then identified based on biomarker expression. The cyclic immunofluorescence staining and imaging process for up to four samples is fully automated with CellScape.

Digital Spatial Profiling identified potential immune response and gene expression differences between DCIS and IDC tumor regions

Spatial transcriptomics allows for the comparison of gene expression in specific tissue regions without the need for microdissection. Leveraging the segmentation capabilities of GeoMx DSP, we explored differences between DCIS and IDC tumor and tumor microenvironment (TME)(Figure 3).

First, immune cell populations in the TMEs were quantified and compared, identifying increased levels of regulatory T cells, natural killer cells, and fibroblasts in the IDC regions, while other immune cell populations like memory B and T cells varied by ROI (Figure 4). However, these findings may be impacted by the sampling methods and differences in segment selection shapes.

Next, transcriptomes in both tumor region types were analyzed and identified several key significant differences in gene expression. IDC regions exhibited increased expression of several collagen proteins, including COL1A1,2, and COL3A1, consistent with other studies showing increases in collagen expression of IDC and the pro-tumorigenic nature of collagen-1[1,2]. Myoepithelial cell markers KRT17, KRT5, and KRT14 were enriched in the DCIS segments (Figure 5).

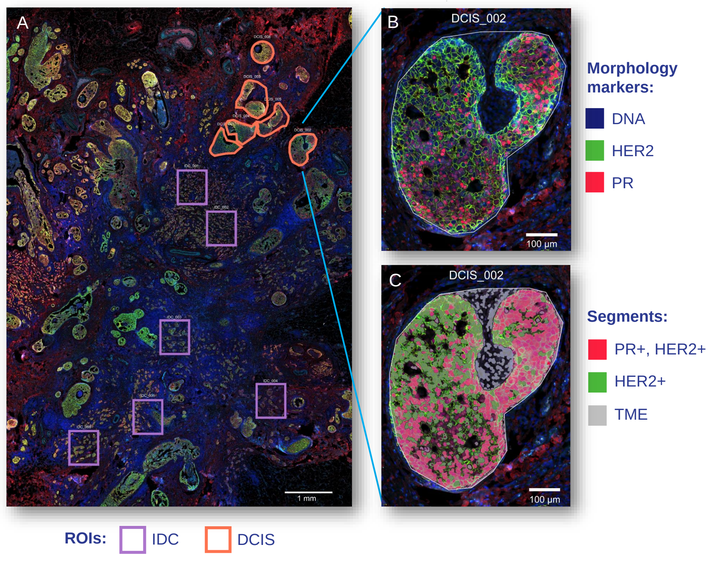

Figure 3. GeoMx ROI selection strategy. Triple positive breast cancer tissue was labeled with DNA, HER2, and PR antibodies.
(A) Six DCIS and IDC ROIs were selected per tissue.
(B) Close-up of DCIS_002 ROI with morphology markers as indicated.
(C) Segmentation strategy implemented for collection of RNA transcripts.

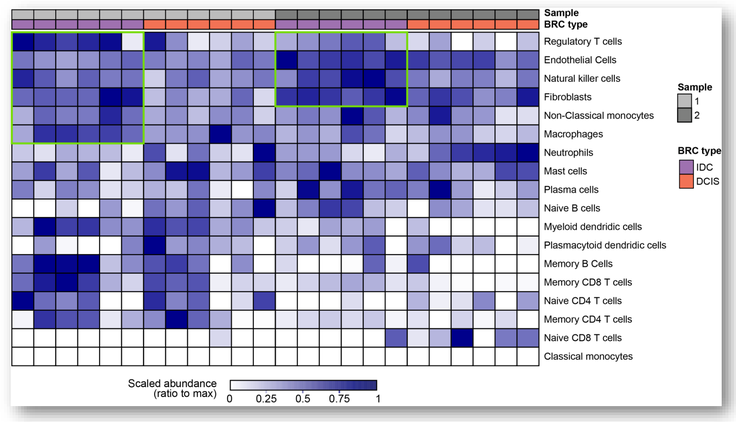

Figure 4 Spatial deconvolution of the tumor microenvironment. Scaled abundances of immune cell types found in each ROI. Values were calculated using the spatial deconvolution algorithm from NanoString.
Green outline: Immune cell types with increased abundance in IDC tumors.

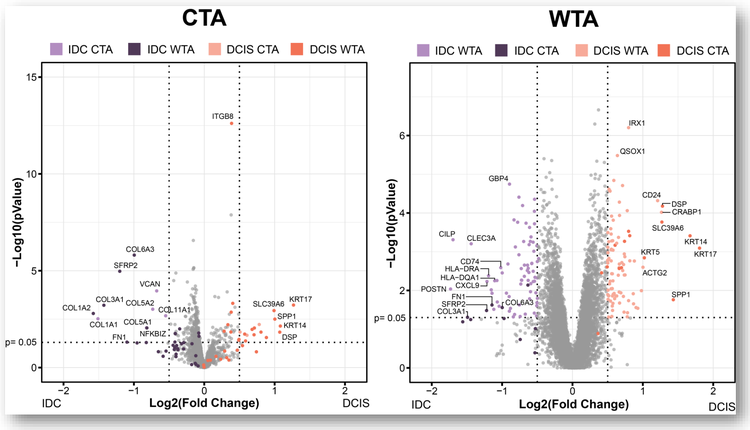

Figure 5 Volcano plots comparing gene expression in IDC and DCIS tumor segments. Points are colored to show overlap between highly expressed targets in the outputs of the CTA and WTA assays.

ChipCytometry visualized TMEs to localize immune infiltration and validate hypotheses generated from transcriptional data

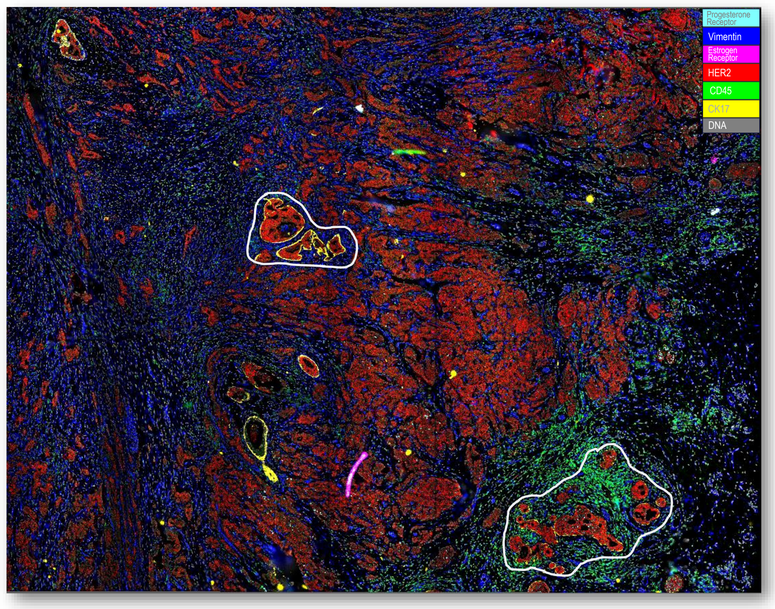

Figure 6. Breast cancer tissue containing DCIS and IDC regions imaged by ChipCytometry. After staining for 20 biomarkers for immune phenotyping and tumor indication, high-resolution images were overlayed to visualize entire tissue landscape and TME. Select markers are shown as indicated in legend. DCIS regions are outlined in white surrounded by IDC.

While GeoMx DSP is a powerful tool for pattern discovery, it only provides spatial information with regional resolution. ChipCytometry is a complementary technique to more deeply query ideas generated from transcriptomic data, providing precise spatial multiplexing for a high number of protein biomarkers. Compatible with any fluorescent antibodies, ChipCytometry is a flexible tool to visualize expression of any biomarker *in situ* with subcellular resolution and HDR imaging.

Immune cell infiltration was assessed by ChipCytometry. CD45+ immune cells were observed outside the boundaries of a major IDC tumor region, closer to DCIS segments (Figure 6), indicating that the DSP findings may be limited by the sampling method.

Looking at the tumor-associated biomarkers, there was a clear difference between IDC and DCIS regions. ChipCytometry visualized the spatial location of the KRT17 (CK17) protein with subcellular resolution and identified specific expression at edges of the DCIS tumors (Figure 7A), consistent with localization of myoepithelial cells[3].

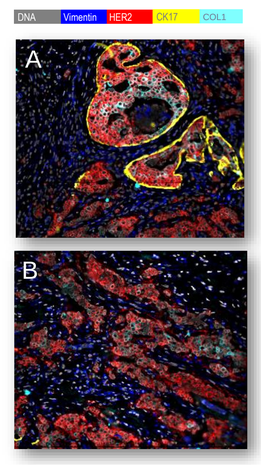

Figure 7. DCIS and IDC regions showing Krt17 (CK17) and COL1 expression. Representative DCIS (A) and IDC (B) regions displaying expression of selected ECM protein markers. Images cropped from the main scan.

Conclusions

- GeoMx DSP identified transcriptional changes associated with IDC and DCIS tumor regions, including immune cell phenotyping and differences in keratin and collagen gene expression.
- ChipCytometry was used to validate GeoMx hypotheses by detecting immune cell infiltration in the TME and localizing subcellular keratin and collagen biomarker expression in the different tumor regions.
- A strength of DSP lies in its broad transcriptional coverage using the WTA probe panel, facilitating discovery of key gene expression patterns. ChipCytometry can precisely validate hypotheses generated using transcriptomics and provide capacity for high-resolution visualization, cell type quantification, and determination of cell-cell interactions. Taken together, the results described here demonstrate how ChipCytometry and DSP can be used in concert to unravel questions about localized biomarker expression in tumor samples.

References

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