Spatially Resolved Whole Transcriptome Molecular Investigation of Triple Positive Breast Cancer FFPE Tumors using the Visium Platform

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1. Introduction

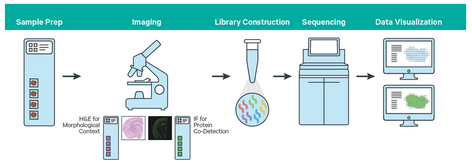
Triple-positive breast cancer (TPBC) is a rare but aggressive form of human epidermal growth factor receptor 2 (HER2) positive cancer accounting for fewer than 10% of all new breast cancer diagnoses in the United States annually. Such tumors routinely exhibit elevated levels of HER2, along with oncogenic cell surface estrogen receptors (ER) and progesterone receptors (PR), but the underlying phenotype and survival rates vary. To better understand TPBC heterogeneity, we examined the tumor microenvironment by characterizing the spatial distribution of cellular transcriptomes.

2. Methods

Spatial transcriptomics technology combines the benefits of histological techniques with the massive throughput and discovery power of RNA sequencing, addressing the limitations of traditional pathological examination. However, standard clinical workflows collect formalin-fixed paraffin-embedded (FFPE) tissue, which can significantly damage molecules such as RNA, making investigation of the underlying biology at the transcriptomic level intractable.

Our team utilized the 10x Genomics Visium Spatial Gene Expression for FFPE tissue to analyze and resolve tumorigenic profiles of serial sections of TPBC samples. This assay incorporates ~5,000 molecularly barcoded, spatially encoded capture probes in spots over which the tissue is placed, imaged, and permeabilized. Native RNA is detected using an FFPE compatible whole transcriptome probeset. Imaging and sequencing data are processed, resulting in a spatially resolved transcriptional readout.

3. Visium for FFPE Workflow



4. Correlation of FFPE vs Fresh Frozen Tissue

Expression profiles and spatially mapped clustering data from FFPE sections processed using the whole transcriptome probeset highly correlated with data from fresh-frozen tissue. Further, TPBC sections from the same block show spatial patterning of gene expression that are highly correlated.

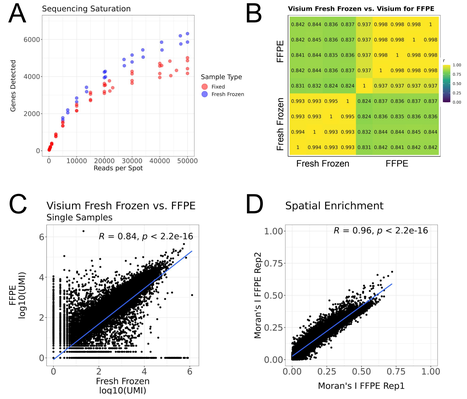


Figure 2. (A) Saturation Curves. (B) Correlation of Visium for FFPE and Fresh Frozen. (C) Single sample comparison between Visium for FFPE and Fresh Frozen. (D) Correlation of Moran's I (Spatial Autocorrelation) between serial TPBC FFPE sections

4. Visium Gene Expression aligns with pathologist annotations

The expression signatures of HER2 (*ERBB2*), ER (*ESR1*), and PR (*PGR*) align with pathologist annotations of TPBC tissues where IHC staining was used to demarcate the tumor, stromal, and immune compartments.

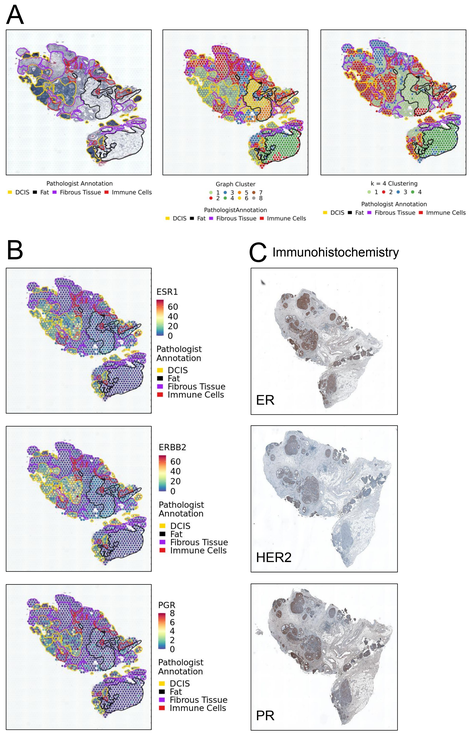


Figure 3. Visium For FFPE. (A) Pathologist annotation of TPBC, Graph based spot clustering, and K = 4 spot clustering. DCIS = Ductal Carcinoma *in situ*. (B) Expression of TPBC markers. UMI counts shown. (C) Immunohistochemistry staining of TPBC.

5. Single Nucleus Integration Reveals Spatially Resolved B-Cell to Plasma Cell Progression

Spatial data were paired with single nucleus RNA-seq (Chromium Single Cell 3' assay), generating cell type expression profiles for estimating spot level cell type proportions and giving insight into cell-type co-localization. Shown below is the developmental process of memory B-cell → plasmablast → mature plasma cell transition.

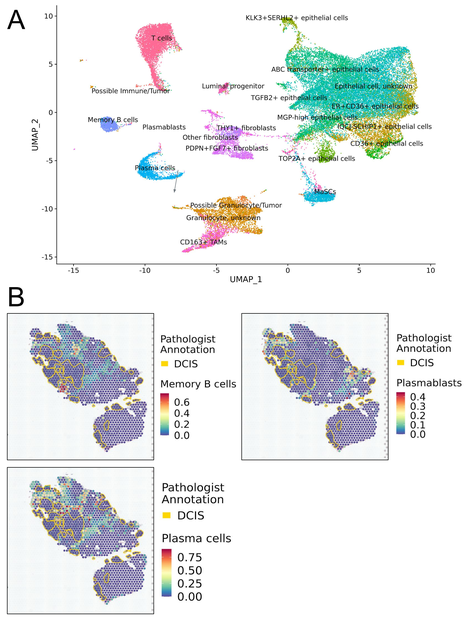
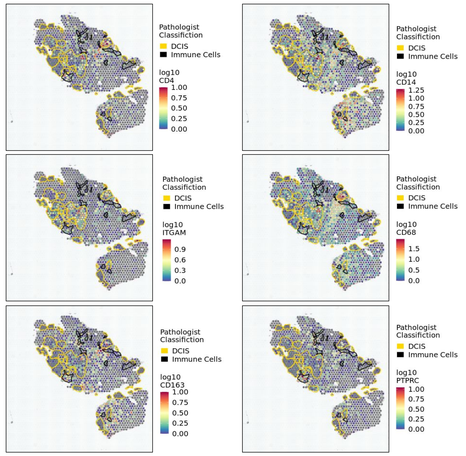


Figure 4. snRNA + Visium Investigation of B cell lineage. (A) UMAP Projection of single-nuclei taken from the same TPBC block as Visium sections. (B) Memory B-cell → plasma blast → mature plasma cell lineage transition estimations overlaid H&E image with annotation of DCIS. Prediction score shown

6. Visium reveals immune infiltration in the TME

Tumor Infiltrating Lymphocytes (TILs) are a hallmark of the immune response in cancer and has prognostic utility in the clinic. Here we show log10 expression of infiltrating CD4+ T-cells, CD14+, ITGAM+, CD68+, CD163+ macrophage/ monocytes, and CD45 (*PTPRC*) activated B cells



7. Conclusion

These results demonstrate that whole transcriptome profiling of FFPE tissues using the Visium platform provides a powerful complement to traditional histopathological methods. By pairing analysis of the whole transcriptome profiles across TPBC FFPE tissue sections with high sensitivity and specificity, with morphological context and protein co-detection, Visium for FFPE provides a comprehensive understanding of the tumor architecture. This in-depth knowledge can provide new insights into tumor biology, disease progression, predictive biomarkers, drug response and resistance, and development of therapeutic targets.

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