Spatially Resolved Whole Transcriptome Molecular Investigation of Triple Positive Ductal Carcinoma In Situ using the Visium for FFPE Platform

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1. Introduction
Triple-positive ductal carcinoma in situ (TPDCIS) is a pre-neoplastic tumor positive for human epidermal growth factor receptor 2 (HER2). Such tumors routinely exhibit elevated levels of HER2, along with oncopgenic cell surface estrogen receptors (ER) and progesterone receptors (PR), but the underlying phenotype and survival rates vary. To better understand TPDCIS 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 tumor microenvironments and immune compartments.

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

4. Correlation of FFPE vs Fresh Frozen Tissue
Further, TPDCIS sections from the same block show spatial patterning of gene expression that are highly correlated. FFPE vs Fresh Frozen. (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 TPDCIS FFPE sections and Fresh Frozen.

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.

6. Visium reveals immune infiltration in the microenvironment
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+, TGFM+CD68+, CD163+ macrophage/monocytes, and CD54 (PPLRC) 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 TPDCIS 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.