Enhancing histological tissue and cell characterization with simultaneous gene expression and protein measurements

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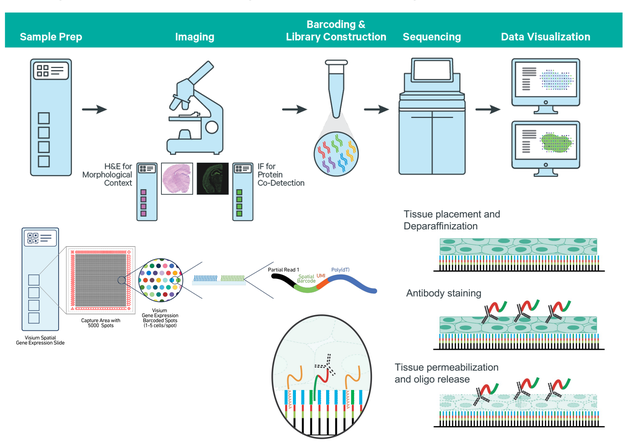
1. Background

Cells establish their function, identity, and state through the careful orchestration of complex molecular mechanisms leading to gene expression. While gene expression can be measured by type and quantity of mRNA transcripts produced, the abundance and isoforms of expressed proteins cannot always be inferred directly from mRNA readout alone. Thus, to characterize cellular identity, condition, and function more accurately, it is important to evaluate gene expression at both transcript and protein levels. Here, we demonstrated a streamlined multiomic tissue analysis by utilizing the highly multiplexed protein capability of 10x Genomics Visium Spatial Gene Expression Solution. The technique combines a human whole transcriptome probe-based panel with an oligo-tagged antibody oncology panel, developed with Abcam conjugated antibodies, to simultaneously assess the transcriptomic and proteomic profiles of sectioned FFPE tissues. This approach allows for the interrogation of the tissue biology in a way that cannot not be captured by traditional image-based techniques or gene expression information alone.

2. Methods

Serial sections of FFPE human tonsils and breast cancer tissues were placed on Visium Gene Expression (GEX) slides. The Visium GEX slides incorporate ~5000 molecularly barcoded, spatially encoded capture spots on which tissue sections were placed, H&E stained, and imaged. Following incubation with the mRNA probes and a panel of oligo-conjugated antibodies, tissues were permeabilized and representative probes were captured. Libraries were generated and then sequenced on an Illumina NovaSeq at a depth of ~50,000 reads per spot. Using the Space Ranger analysis pipeline, the resulting whole transcriptome gene expression and proteomic profiles were mapped onto tissue images where the reads were aligned, clustering performed, and gene and protein expression analyzed. Additional analyses and data visualizations were performed on the Loupe Browser desktop software.



4. Simultaneous transcriptomic and proteomic characterization of invasive ductal carcinoma breast cancer

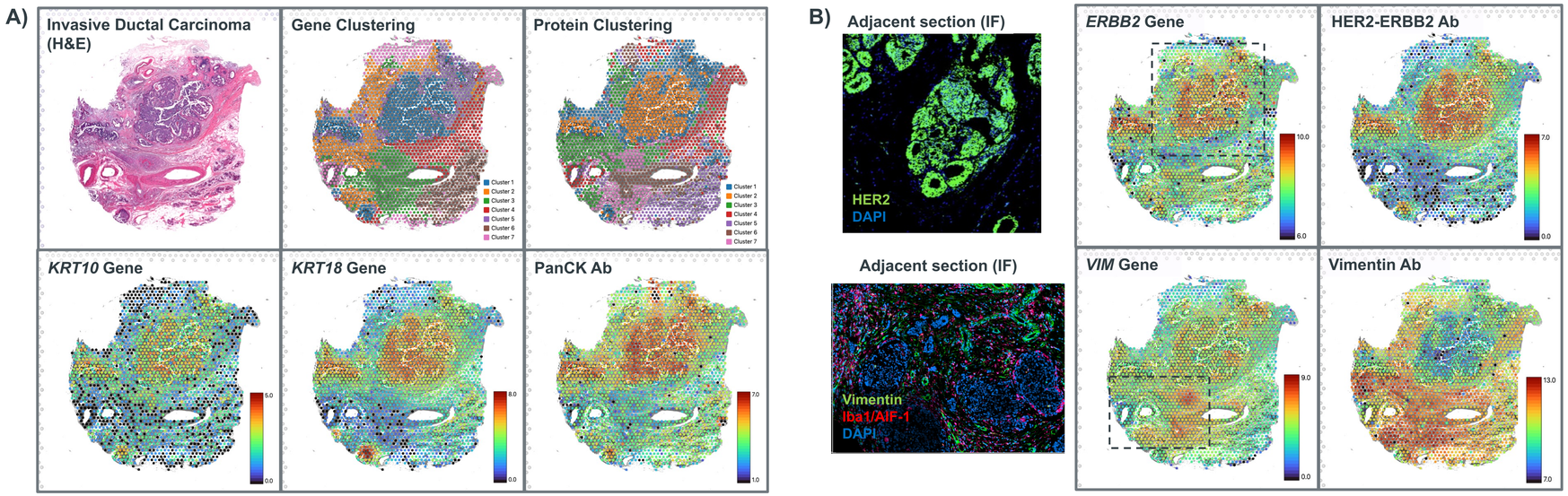


Figure 2. (A) H&E-stained triple positive invasive ductal carcinoma (IDC) tissue section on a Visium slide. Unbiased clustering of mRNA transcripts and protein superimposed on the H&E image demonstrate similar expression patterns especially, the tumor mass at the center of the core. (Bottom) The tumor mass exhibits over expression of tumor markers *KRT10* and *KRT18* relative to the surrounding stroma. Expression patterns of cytokeratin genes spatially correlates with the PanCK immunostaining a pan marker for tumor cells. (B) Adjacent sections immunofluorescent stained for HER2 (left-top) and Vimentin (left-bottom) demonstrate strong signal within the indicated (dashed boxes) regions of the biopsy sample. The positive staining correlates with both the transcriptomic (middle) and proteomic (right) data obtained from the multiomic Visium workflow.

3. Spatial transcriptomic and proteomic characterization of human tonsils

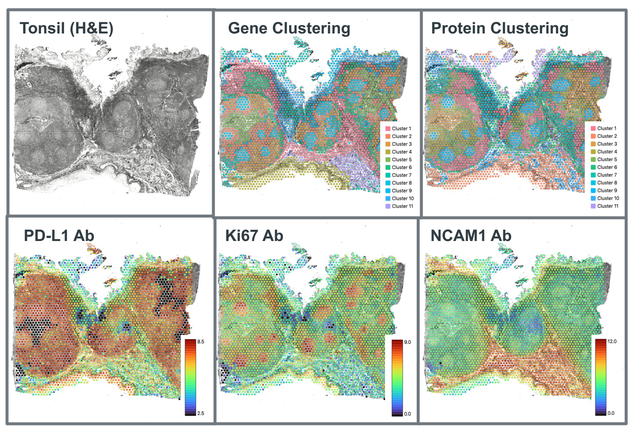


Figure 1. (Top) BW image of H&E-stained human tonsil on Visium slide. Unbiased clustering of mRNA transcripts (middle) and protein (right) superimposed on the H&E image with a correlation of the spatial expression patterns. The expression patterns of both analytes replicates the follicular structure of the FFPE tonsil section. (Bottom) Visualization of the Ki67, PD-L1, and NCAM1 protein markers labeling the whole tonsil, individual follicles, and surrounding epithelial tissue, respectively.

5. Proteomic-directed gene expression profiling

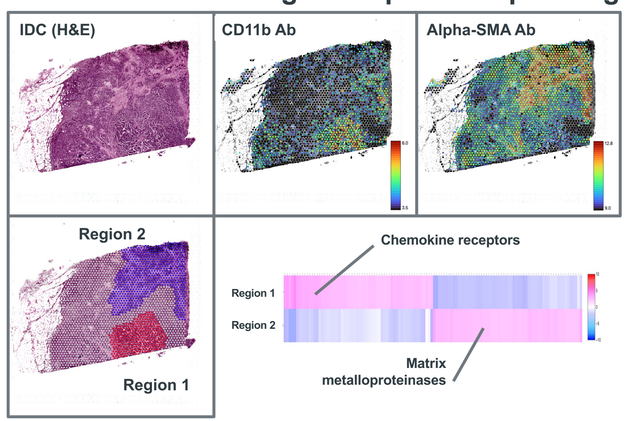


Figure 3. H&E-stained human IDC placed on Visium slide with selected CD11b and alpha-SMA protein markers overlaid on image. Referencing the marker distribution, regions of interest were highlighted on the Loupe Browser. Performing a local differential expression analysis of the both regions displayed the top 50 highly expressed genes presented within each region (gene names redacted), highlighting genes associate with chemokine receptors and matrix metalloproteinases.

6. Conclusion

The Visium Spatial Gene Expression Solution provides a platform that combines traditional histology with the throughput and deep biological insight of next generation sequencing. Here, we demonstrate a novel multiomic solution that offers the ability to combine both mRNA transcript and protein expression data with a histopathology image from the same section. In addition to a simple workflow, this simultaneous high resolution transcriptomic and proteomic view of the tissue biology enables researchers to develop a greater understanding of clinical samples, and provide new insights into the heterogeneity of cellular states across multiple diseases. Together, this spatially resolved multiomic information provides an unprecedented view into the tumor microenvironment, and a powerful new tool for the discovery of new biomarkers and to guide the search for effective therapies.

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