Spatial Whole Transcriptome Analysis of Differential Expression for Biomarker Discovery in Colorectal Cancer

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Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related deaths, often attributed to genetic and epigenetic modification. The investigation of tumor microenvironment (TME) profiling through an in-depth analysis of tissue samples reveals the spatial gene expression patterns of known CRC markers and visualizes cell composition and localization. The aim of this study is to uncover potential therapeutic targets by delving into the mechanisms of tumor formation.

Materials and Methods

The formalin-fixed paraffin-embedded (FFPE) tissue samples from three primary colon adenocarcinoma tissues (PT) and their matched adjacent normal tissues (PN) were selected from BioChain's biorepository. The DV200 of RNA quality was more than 50% (Figure 1). The tissue sections were stained with Hematoxylin and Eosin (H&E), and the stained images were annotated by pathologists. The 10x Genomics Visium Spatial Gene Expression for FFPE assay was performed with more than 18,000 gene probe pairs in human spatial profile, followed by whole transcriptome analysis. This technique used mRNA-binding oligonucleotides to capture gene expression within 4,992 spatially barcoded spots. Integrating imaging and sequencing data enabled the acquisition of a spatially resolved transcriptome of CRC heterogeneity (Figure 2).

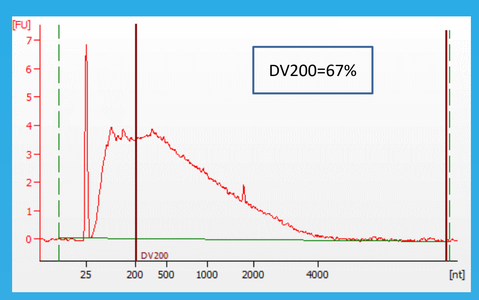


Figure 1. Representative BioAnalyzer electropherogram for calculation of DV200. The FFPE tissue samples were screened by pathologist for selection. The RNA extractions were performed use BioChain's FFPE Tissue RNA Extraction Kit. The DV200 of ≥50% is recommended for the Visium Gene Expression assay.

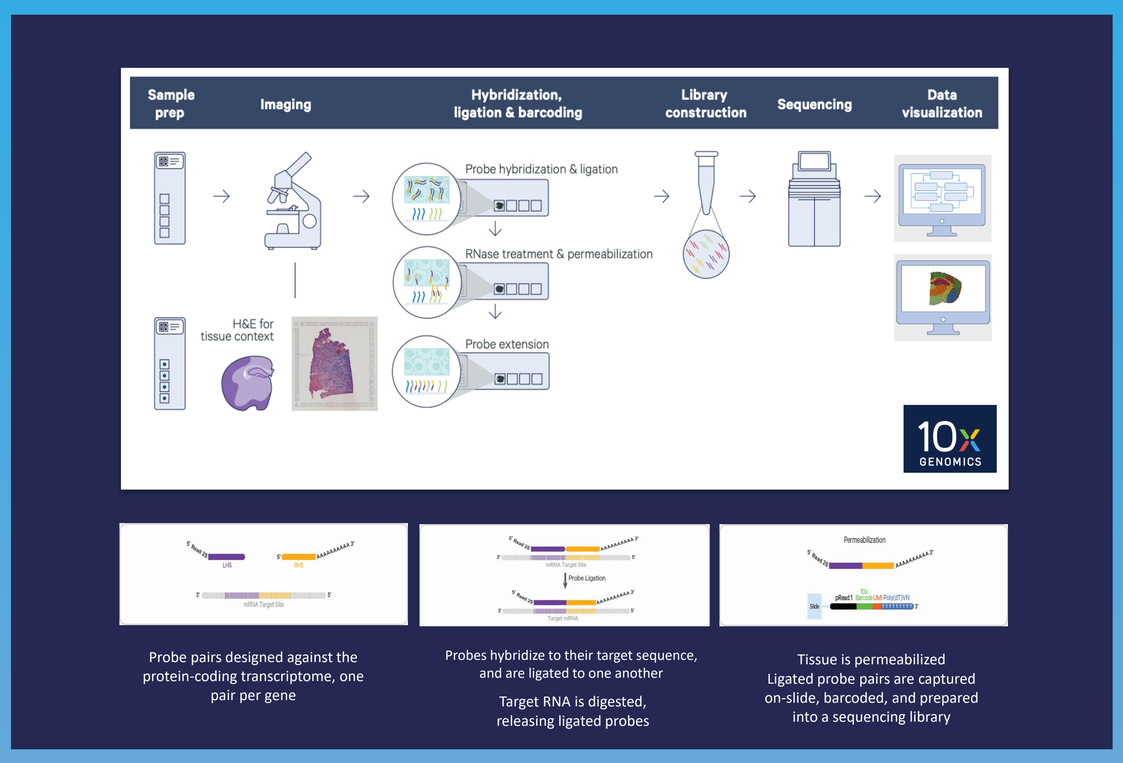


Figure 2. Workflow from tissue preparation to library preparation for the standard Visium Spatial Gene Expression for FFPE tissues.

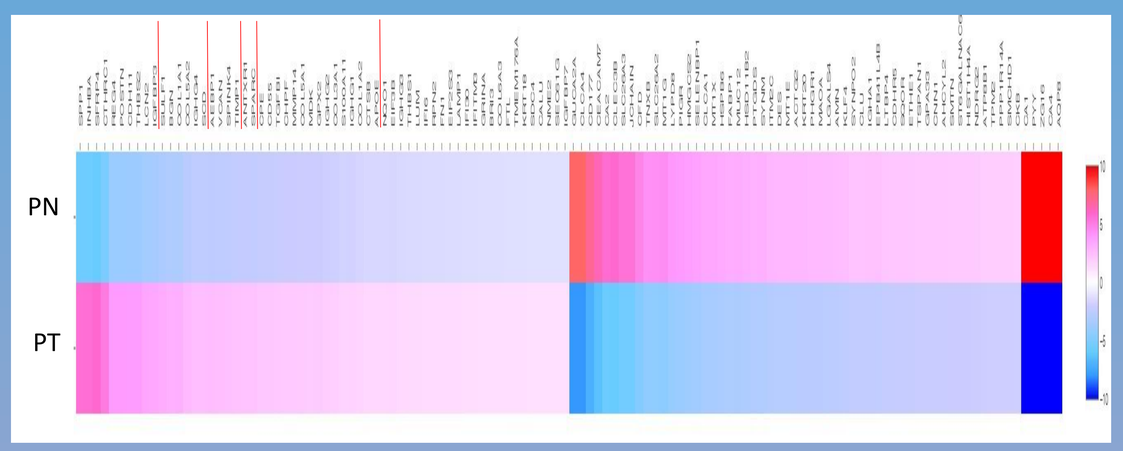
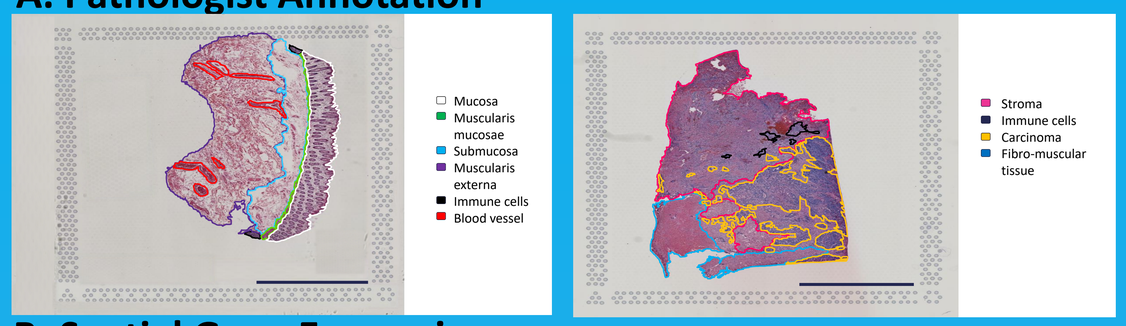
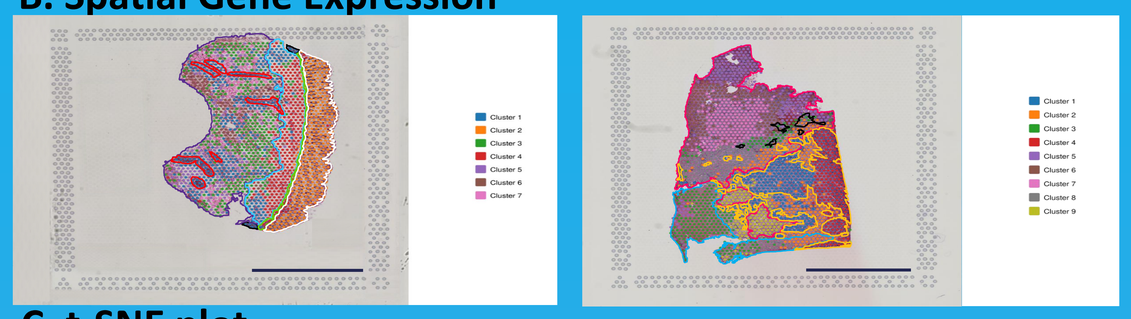


Figure 3. Differential gene expression analysis within the k=2 (PN and PT) clusters. Several known CRC biomarkers (*APOE, SCD, IGFBP3, TIMP1, SPARC*) were detected in Heatmap.

A. Pathologist Annotation



B. Spatial Gene Expression



C. t-SNE plot

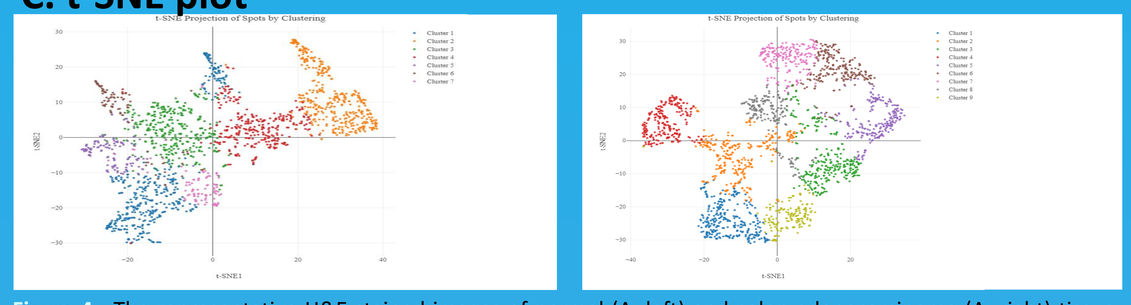


Figure 4. The representative H&E-stained images of normal (A, left) and colon adenocarcinoma (A, right) tissue samples were overlaid with pathologist annotations. The graph-based spot clustering analysis of spatial gene expression and pathologist annotations superimposed on the H&E-stained images of normal (B, left) and colon adenocarcinoma (B, right) tissue samples. The t-SNE plots for each spatial clustering in both normal (C, left) and colon adenocarcinoma (C, right) tissue samples. Scale bars=2.5 mm

Results

The results showed a strong correlation between the annotations of both PT and PN tissue samples and the spatial gene expression clustering (Figure 4). By overlaying the total gene counts on the H&E-stained tissue image, approximately 16,000–17,000 genes were detected in each sample. The PT samples expressed a median of around 5,500 genes per spot, while the PN samples expressed a median of around 1,600 genes per spot. Notably, the study of gene expression in PT samples showed overexpression of well-known CRC markers (*APOE, SCD, IGFBP3, TIMP1, SPARC*), while several markers (*DES, CA1, KLF4*) were down-regulated (Figure 3 and 5A). Furthermore, immune cells are one of the key non-tumor cell population present in TME, affecting both tumor suppression and progression. The gene expression of B-cell associated genes (*IGHA1, IGHG1, JCHAIN, IGKC, IGLC1*) has relatively higher expression levels in PT tissues (Figure 5B).

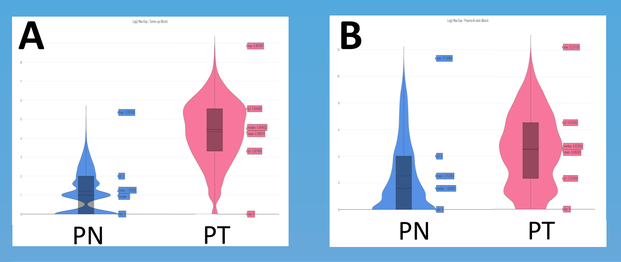


Figure 5. Violin/ Box plots show the distribution of tumor upregulated gene expression (A) and immune cell (B-cell) gene expression (B) in PN and PT samples.

Summary

These findings provide valuable insights into tumor heterogeneity, spatial organization of cells within the TME, and the identification of biomarkers. BioChain Institute, Inc. is uniquely positioned to assist researchers in studying various oncologic and diseased tissue types by offering comprehensive spatial whole transcriptome analysis. This assay enhances understanding of the TME, aids in discovering diagnostic and prognostic targets, and assists in development of personalized therapies for individual patients.

Reference

Ståhl PL, et al. Visualization and analysis of gene expression in tissue sections by Spatial Transcriptomics. Science 353: 78–82 (2016)
Liu HT, et al. Spatially resolved transcriptomics revealed local invasion-related genes in colorectal cancer. Front Oncol. 13:1089090 (2023)

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