Rapid identification of comprehensive multi-omic protein and RNA biomarkers on a single FFPE tissue section using a novel integrated nCounter® workflow

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Abstract #3764

Introduction

Simultaneous transcriptomic and proteomic profiling from a single tissue section is critical for elucidating tumor biology. However, multi-omic analysis of FFPE samples remains challenging due to formalin-induced crosslinking and degradation, leading to variable analyte quality and limited yield. These limitations, compounded by tumor heterogeneity, hinder data integration and interpretation. To address this, NeoGenomics, a leading clinical research organization, developed and validated a streamlined workflow using the nCounter® Analysis System for concurrent mRNA and protein quantification. Enabled by direct hybridization chemistry and Bruker Spatial Biology's platform, this approach enhances data robustness.

Methodology

The core innovation of this approach is the simultaneous measurement of mRNA and protein from a single FFPE slide using a streamlined three-step workflow. This assay integrates nCounter mRNA panels, including the PanCancer IO 360™ Panel (up to 800 gene targets), with newly optimized protein panels enabling analysis of up to 800 protein targets within a unified protocol (Figure 1A–B). To validate this multi-omic application, performance specification of accuracy, precision and analytical specificity was assessed in this study using diverse FFPE tumor types, including breast (including TNBC), lung (NSCLC), colorectal, bladder, and urothelial cancers (Figure 1C).

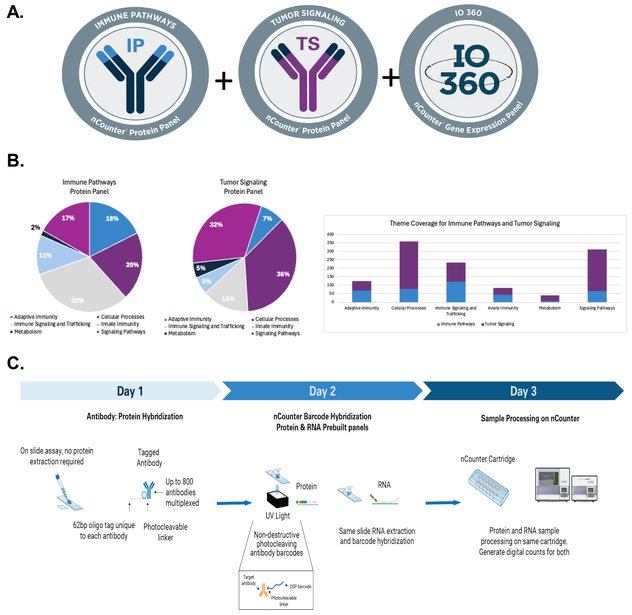

Figure 1: *Whole slide combined RNA and Protein assay.* ***A & B****. nCounter immune pathways protein panel (204 targets), nCounter tumor signaling protein panel (325 targets) and nCounter IO360 (770 targets). Panels encompass adaptive immunity, innate immunity, immune signaling & trafficking, metabolism, cellular processes and signaling pathways.* ***C****. Multiomics workflow*

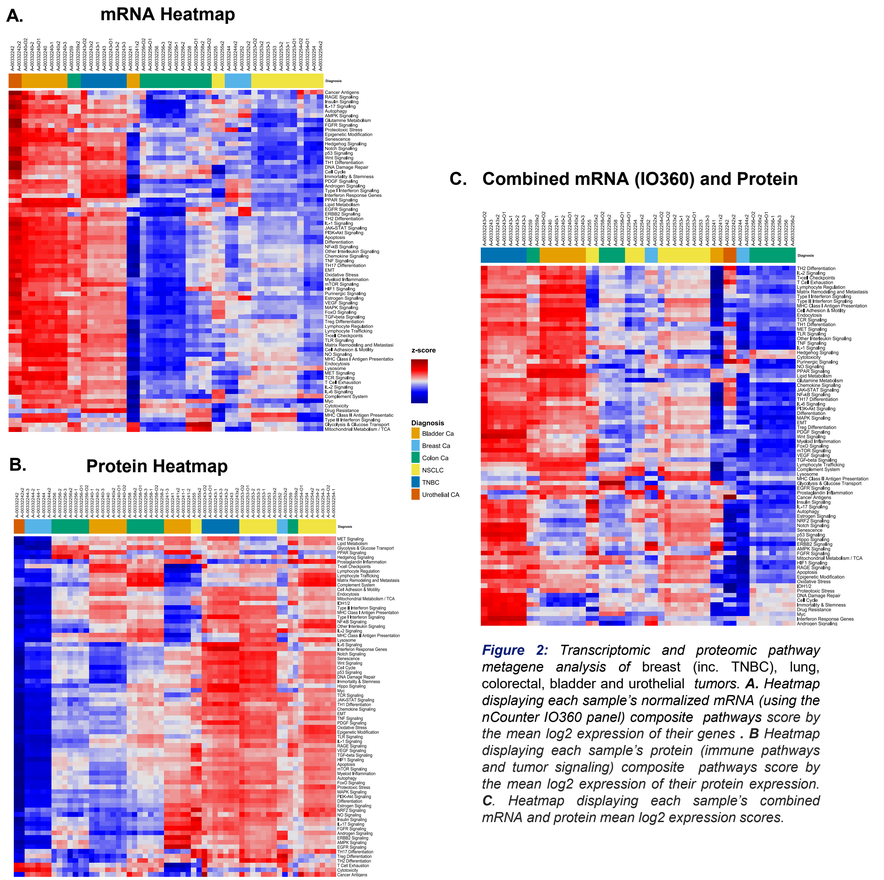

Figure 2: *Transcriptomic and proteomic pathway metagene analysis of* breast (inc. TNBC), lung, colorectal, bladder and urothelial *tumors.* ***A****. Heatmap displaying each sample's normalized mRNA (using the nCounter IO360 panel) composite pathways score by the mean log2 expression of their genes .* ***B*** *Heatmap displaying each sample's protein (immune pathways and tumor signaling) composite pathways score by the mean log2 expression of their protein expression.* ***C****. Heatmap displaying each sample's combined mRNA and protein mean log2 expression scores.*

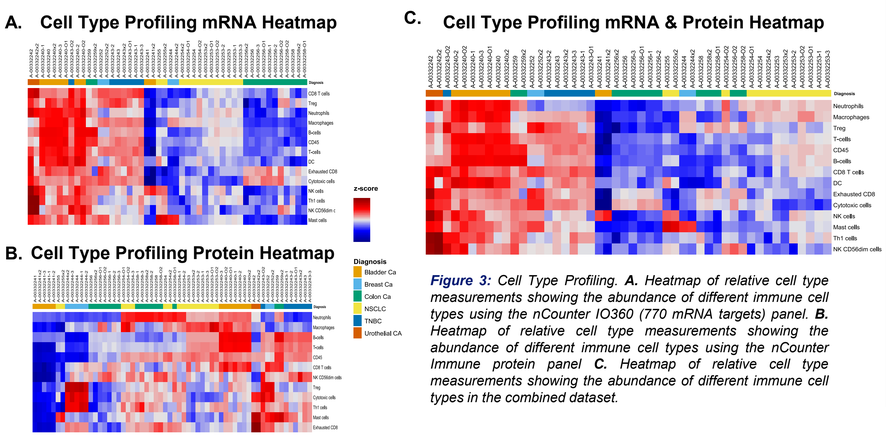

Figure 3: *Cell Type Profiling.* ***A.*** *Heatmap of relative cell type measurements showing the abundance of different immune cell types using the nCounter IO360 (770 mRNA targets) panel.* ***B.*** *Heatmap of relative cell type measurements showing the abundance of different immune cell types using the nCounter Immune protein panel* ***C.*** *Heatmap of relative cell type measurements showing the abundance of different immune cell types in the combined dataset.*

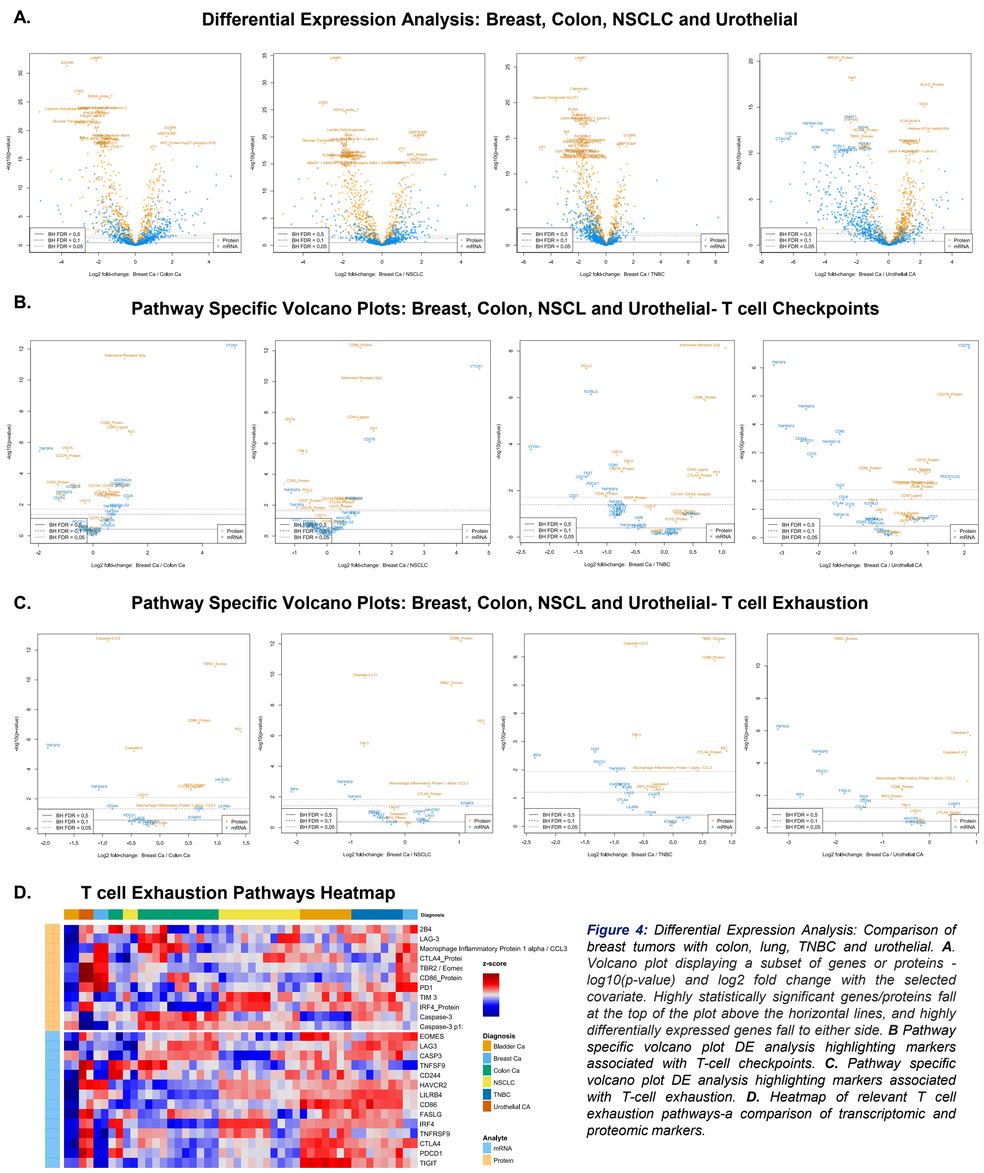

Figure 4: *Differential Expression Analysis: Comparison of breast tumors with colon, lung, TNBC and urothelial.* ***A****. Volcano plot displaying a subset of genes or proteins - log10(p-value) and log2 fold change with the selected covariate. Highly statistically significant genes/proteins fall at the top of the plot above the horizontal lines, and highly differentially expressed genes fall to either side.* ***B*** *Pathway specific volcano plot DE analysis highlighting markers associated with T-cell checkpoints.* ***C.*** *Pathway specific volcano plot DE analysis highlighting markers associated with T-cell exhaustion.* ***D.*** *Heatmap of relevant T cell exhaustion pathways-a comparison of transcriptomic and proteomic markers.*

- Across all tissue types, the streamlined assay demonstrated high specificity, strong correlation between replicate runs, and excellent dynamic range for both mRNA and protein analytes.
- By enabling integrated mRNA+Protein analysis on a single tissue section, the nCounter platform allows researchers to identify disease-related signatures to accelerate translational workflows with greater confidence in biological insights and reduced technical complexity.

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