Identifying and Analyzing Tumor Subtypes Using Custom Morphology Markers for NanoString® GeoMx® Digital Spatial Profiling

Poster: 6847

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Introduction

Molecular subtyping studies have grouped cancers based on molecular, morphological, and clinical characteristics. This has been beneficial to researchers looking for actionable targets predictive of therapeutic response. Multiomic approaches have been used to analyze morphological characteristics in concert with molecular profiling, assessing tumor subtypes. For non-small-cell lung cancer (NSCLC) tumors, this has been found to be critical for targeted therapies and immunotherapy success (2).

The NanoString GeoMx DSP platform combines morphological context with spatial molecular profiling on a single tissue sample. To evaluate its capability to identify tumor subtypes, NSCLC samples were analyzed with tumor-specific markers developed by Canopy and regions of interest (ROIs) were analyzed using three methods: the Cancer Transcriptome Atlas (CTA), Whole Transcriptome Atlas (WTA) and protein expression modules.

NanoString's standard morphology markers broadly target tumor and immune cells within a sample, but molecular subtyping requires additional stratification at the tissue level enabling meaningful subsequent gene expression analysis. Canopy's ROI markers offer more precise morphological analysis of tissue samples, followed by comparative differential gene expression analysis.

Methods and Materials

Here we present the analysis of NSCLC FFPE samples analyzed using the NanoString GeoMx DSP Platform with parallel approaches of the CTA, WTA, and protein panels (human protein core, IO drug target, immune cell typing and activation status, pan-tumor, and myeloid) for NGS:

- Samples were stained with fluorescent antibodies specific to NSCLC tumor subtypes (TTF1 for adenocarcinoma cells, p40 for squamous cell carcinoma, CD45 for immune cells) and regions of interest (ROIs) were selected based on indication and segmentation goals
- Probes from these ROIs were collected and analyzed using an Illumina Novaseq NGS instrument and the CTA, WTA, and protein panels from NanoString
- Comparisons of specific targets or cell types were generated in the GeoMx DSP Analysis Suite

Segment	Cell Type
TTF-1+	Adenocarcinoma cells
p40+	Squamous carcinoma cells
CD45+	Immune cells
TTF-1-, p40-, CD45-	Tumor microenvironment

Table 1. Segmentation strategy and cell type for molecular profiling.

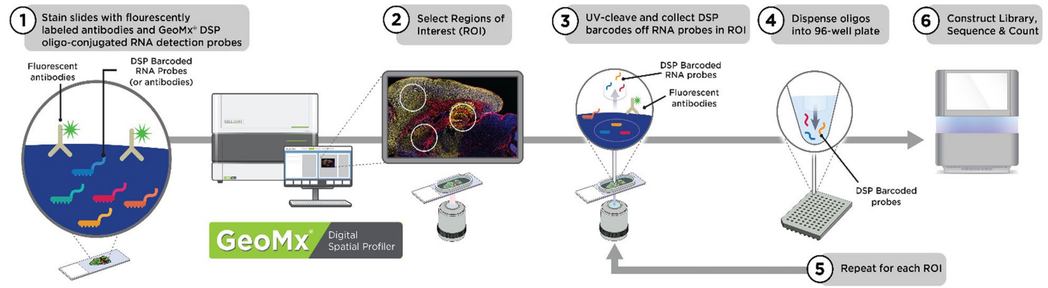

Figure 1. NanoString GeoMx Workflow. Slides are stained with morphology markers to highlight tissue architecture and marker-specific regions. ROIs are selected based on imaging. Probes are collected from ROIs for molecular profiling via NGS.

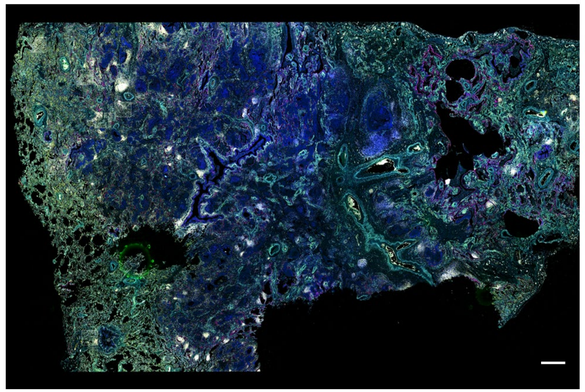

Figure 2. Lung carcinoma whole tissue fluorescence image with biomarkers: DNA (blue), p40 (green), TTF-1 (red), CD45 (yellow). White scale bar is 1 mm.

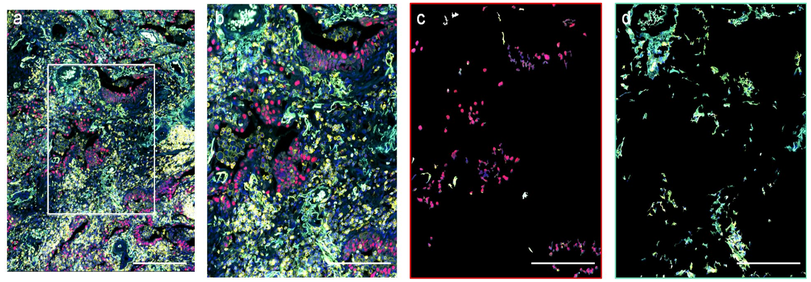

Figure 3. (a) Representative layout of an ROI along the Lung sample outlined within the white box. Fluorescence colors are conserved from Figure 2. White scale bar = 300 µm, **(b)** ROI identified in (a). Scale bar = 200µm, **(c)** TTF-1+ segment of ROI in (a). Scale bar = 200µm, **(d)** p40+ segment of ROI in (a). Scale bar = 200µm.

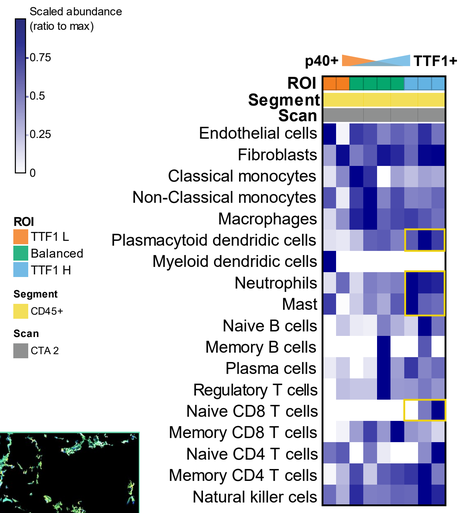

Figure 4. Spatial deconvolution heat map generated based on CTA readout. The heatmap compares relative representations of cell types within the CD45+ segments. Matrix is comprised of separate ROIs for each column, which are ordered and color coded based on the ratio of TTF1/p40 nuclei counts. Cell types enriched in the TTF1 H category are highlighted.

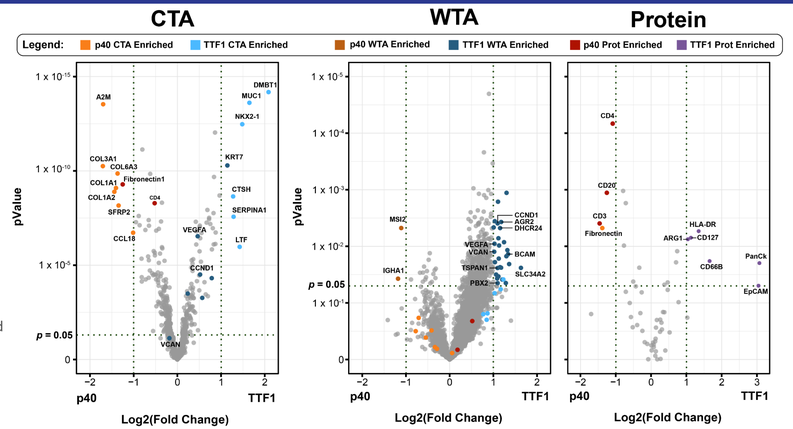

Figure 5. Volcano plots showing differentially expressed proteins between p40+ and TTF-1+ cell compartments. Each panel shows the results from a different GeoMx DSP collection mode (CTA, WTA, or Protein). Colored points represent significantly enriched genes and proteins from each study.

Results & Discussion

- Custom Morphology Markers **(Fig 2-3)** select and enrich for tumor subtypes, even in cell-dense environments.
- Using the CTA expression profile, we determined the most likely representation of cell types for each segment **(Fig 4)**. **Innate immune cell types including Neutrophils, Mast cells, and Plasmacytoid DCs may be enriched in the TTF1 high microenvironment.**
- The combination of CTA, WTA, and Protein panels provide a comprehensive view of RNA and protein expression. **The CTA and WTA readouts are comparable, with most enriched CTA targets showing significantly increased expression in the same compartment for the WTA results.** The CTA panel contains fewer probes, but significance is higher for enriched targets. The protein readout is smaller in scope but helps to present a complete picture of the expression landscape **(Fig 5).**
- Compartments selecting for TTF1+ and p40+ cells in lung ROIs show different expression profiles **(Fig 5). We see significant expression of adenocarcinoma markers: KRT7, ARG2, MUC1, and SLC34A2 in the TTF1 compartment (1,3), as well as oncogenic markers like CCND1 and VEGFA (1).**

Conclusions

These results highlight the utility of combining tumor subtype specific custom morphology markers with GeoMx DSP to gain additional insights into tumor biology. The multiomic analysis of RNA and protein in concert provides a complete picture of the expression landscape.

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References

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