Using Additional Morphology Markers in NanoString® GeoMx® Whole Transcriptome Atlas Assay to Assess NSCLC Tumor Subtypes

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Background

- Molecular subtyping of NSCLC tumors is critical for targeted therapies and immunotherapy success
- The NanoString GeoMx DSP platform provides spatial context about cells and their interactions with a tumor along with high-plex gene expression data.
- To evaluate the capability of GeoMx DSP to selectively identify tumor subtypes using a histological approach paired with molecular profiling, NSCLC samples were analyzed.
- NanoString's standard morphology markers broadly target tumor and immune cells within tissue.
- Molecular subtyping within a tumor type requires additional stratification at the tissue level enabling meaningful subsequent gene expression analysis.
- Canopy Biosciences' ROI Selection markers enable more precise morphological analysis of tissue samples, allowing for tumor subtyping, followed by comparative gene expression differences in these two tumor cell types.

Methods

- Here we present the analysis of non-small-cell lung cancer (NSCLC) FFPE samples analyzed using the NanoString® GeoMx® DSP platform using the Whole Transcriptome Atlas (WTA) panel for molecular profiling, and NGS protein quantification.
- Samples were stained with fluorescent antibodies specific to NSCLC tumor subtypes 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.
- Data was generated in the Software Analysis Suite using pre-determined comparisons for specific targets or cell types.

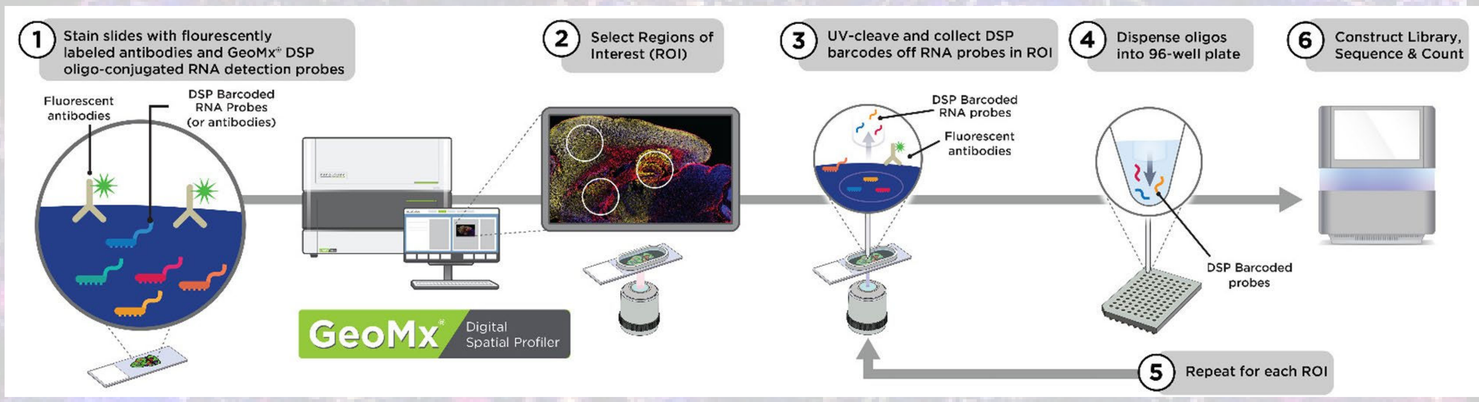

Figure 1. NanoString GeoMx workflow. Slides are stained with morphology markers to highlight tissue architecture and marker-specific regions. ROIs are selected based on imaging. Probes are collected from ROIs for molecular profiling via NGS.

Table 1. Sample list with segmentation strategy and comparison list for molecular profiling analysis

Sample	Lung Carcinoma – NGS protein assay	Repeat Lung Carcinoma – NGS RNA assay
Segmentation Strategy	TTF-1+ (Adenocarcinoma cells) p40+ (Squamous cell carcinoma) CD45+ (Immune cells) DNA+ (Remaining cell types)	Repeat ROIs of protein assay: TTF-1+ (Adenocarcinoma cells) p40+ (Squamous cell carcinoma) CD45+ (Immune cells) DNA+ (Remaining cell types)
Analyte Profiling Comparisons	Adenocarcinoma vs Squamous cell carcinoma differential expression of proteins involved in drug targeting, tumor presence, and immunity (59 proteins total).	Adenocarcinoma vs Squamous cell carcinoma differential expression of RNAs from the NanoString WTA (18818 RNAs total).

Results

- Fluorescence imaging and segmentation on the GeoMx system (Figure 1 and Figure 2) can select and enrich for tumor subtypes, even in cell-dense environments.
- Compartments selecting for TTF1+ and p40+ cells in lung ROIs show differences in expression of numerous proteins (Figure 3). This includes key immune maturation factors, noted to have increased presence in squamous cell carcinomas, EpCam and ribosomal protein S6, known to be upregulated in adenocarcinomas (1).
- Using the WTA expression profile, a spatial deconvolution in the GeoMx analysis suite determined the most likely representation of cell types for each segment (Figure 4). This showed enrichment for the expected cells types based on protein differential expression in Figure 3.
- Differential RNA expression of gene pathways identified in p40+ and TTF-1+ tumor subtypes show key significant differences (Figure 5). Pathways previously identified as differentially expressed between the two subtypes are identified (see citations) and select pathways contributing to tumor growth and response are marked.

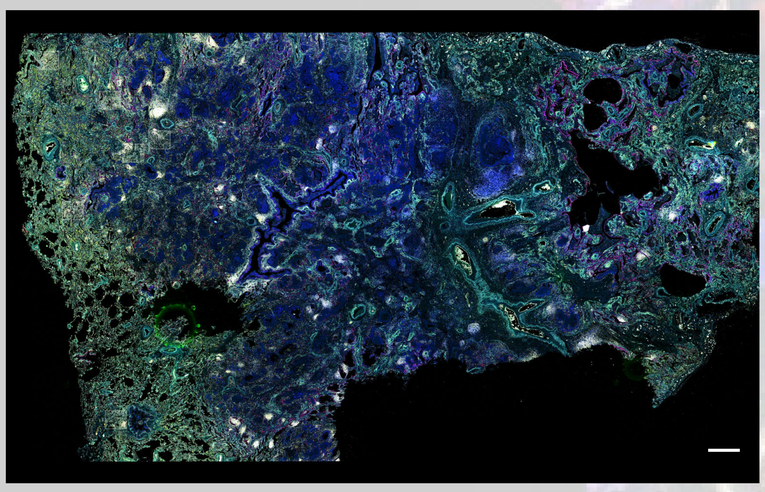

Figure 1. Lung carcinoma whole tissue fluorescence image with biomarkers: DNA (blue), p40 (green), TTF-1 (red), CD645 (yellow). White scale bar is 1 mm.

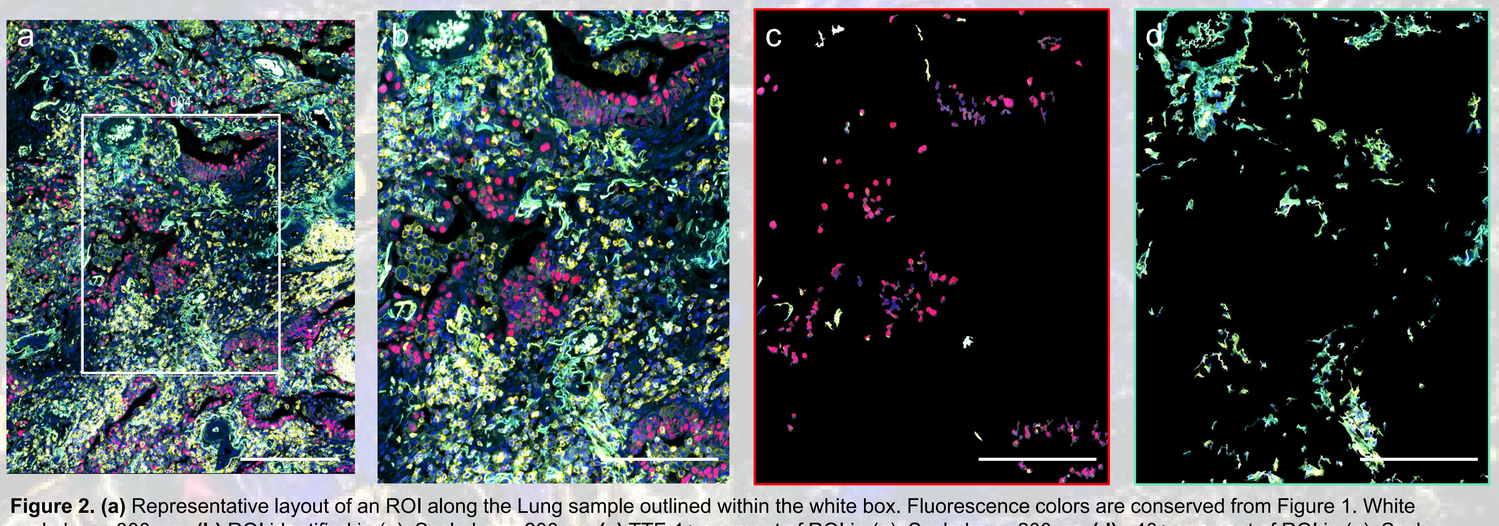

Figure 2. (a) Representative layout of an ROI along the Lung sample outlined within the white box. Fluorescence colors are conserved from Figure 1. White scale bar = 300 µm, **(b)** ROI identified in (a). Scale bar = 200µm, **(c)** TTF-1+ segment of ROI in (a). Scale bar = 200µm, **(d)** p40+ segment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

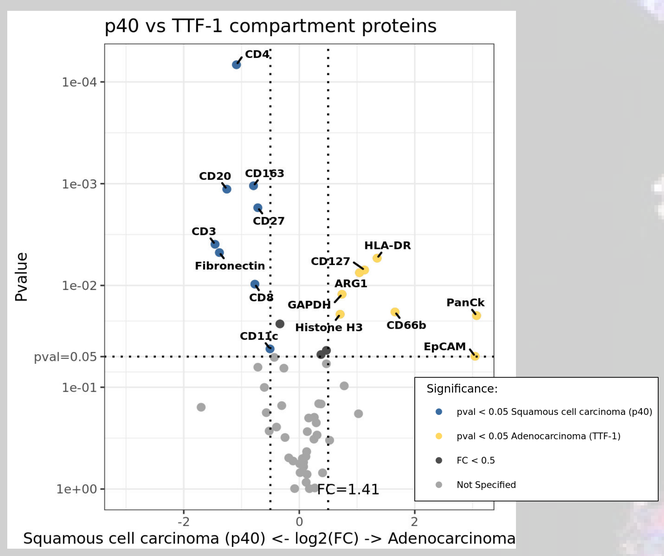

Figure 3. Volcano plot showing differentially expressed proteins between p40+ and TTF-1+ cell compartments. Labeled proteins are those with significant change.

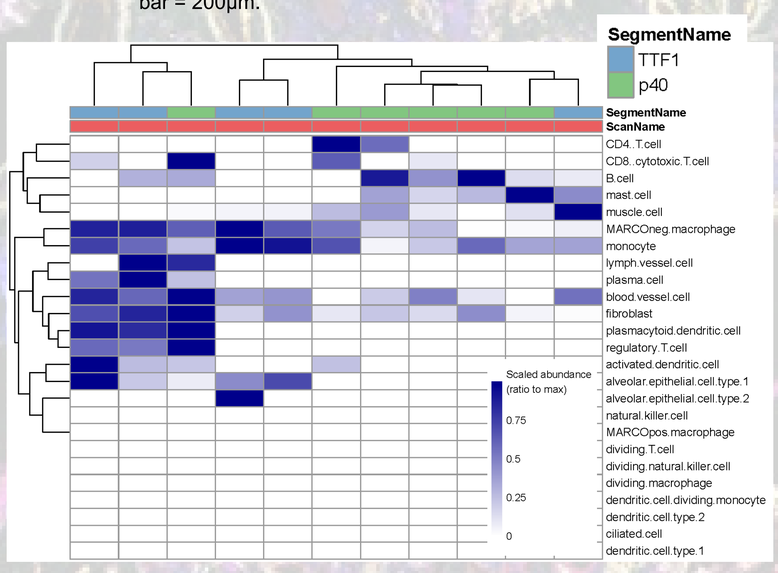

Figure 4. Spatial deconvolution heat map generated based on RNA readout. The heatmap compares relative representations of cell types within TTF-1+, and p40+ compartments. p40+ shows expected increase in immune cells based on Figure 3. Matrix is comprised of separate ROIs for each segment, and cell type.

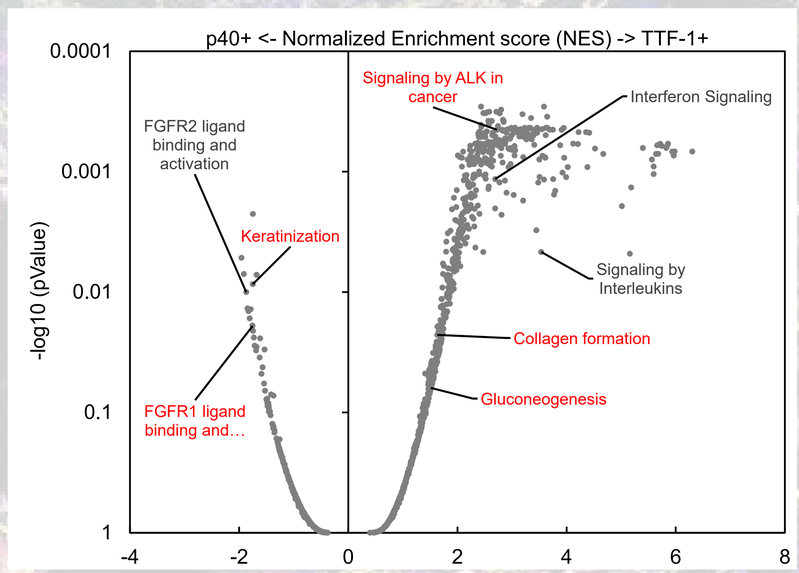

Figure 5. Volcano plot showing differentially expressed pathways identified by differences in RNAs between adenocarcinoma (TTF-1 positive) and squamous cell carcinoma (p40 positive) compartments of Lung sample. Notable pathways are marked. Red pathways have been previously identified to be relatively upregulated in the given tumor subtype (see citations).

Conclusions

- Identification of p40+ and TTF-1+ compartment protein expression reveals differential levels for several included proteins. Recognition of genes known to be relatively upregulated in squamous cell carcinoma (immune maturation factors), and in adenocarcinoma (EpCam, S6), and as-of-yet unimplicated genes in tumor subtype growth and immune response, all show differential expression levels (1). The identification of these unimplicated proteins highlights the potential of GeoMx DSP to guide research into tumor subtype specific treatments.
- Use of the Spatial Deconvolution within the GeoMx DSP analysis suite confirmed enrichment of expected cell types based on the protein assay. The assay suggested increased interaction and infiltration of immune cells into the squamous cell carcinoma. This relative increase between tumor subtypes has been previously identified (2). This demonstrates the utility of GeoMx DSP in characterizing the cellular makeup of tissue and tumors using the WTA in the RNA assay.
- The GeoMx DSP and analysis suite identified previously known differential pathways specific to tumor subtypes (3). However, pathways which could advance tumor subtype specific treatment, including those in tumor growth (FGFR2 activation), and immune response (interleukin and interferon signaling) were also identified.

Presenter Bio

Jessica Runyon is the Director of Product Management for Canopy Biosciences, specializing in CRO Services including NanoString GeoMx and nCounter, single-cell RNA Seq, IHC, Histopathology and Gene Expression Analysis. She received her Masters in Biochemistry from Boston University School of Medicine focusing on epigenetics.

Citations:
1. Song Q, Yang Y, Jiang D, et al. Proteomic analysis reveals key differences between squamous cell carcinomas and adenocarcinomas across multiple tissues. *Nat Commun*. 2022;13(1):4167. Published 2022 Jul 18. doi:10.1038/s41467-022-31719-0
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3. Heist RS, Mino-Kenudson M, Sequist LV, et al. FGFR1 amplification in squamous cell carcinoma of the lung. *J Thorac Oncol*. 2012;7(12):1775-1780. doi:10.1097/JTO.0b013e31826aed28