#1688

High-throughput immune cell phenotyping using GeoMx DSP reveals Non-Small Cell Lung Cancers (NSCLC) are divided into distinct immunological subtypes

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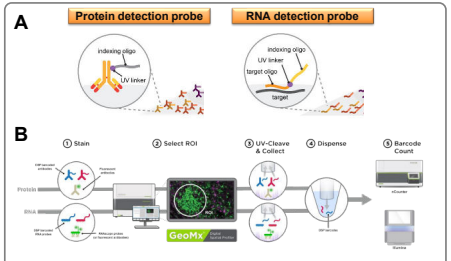
Summary

The GeoMx® Digital Spatial Profiler (GeoMx DSP) is a novel, highly multiplexed-assay platform that digitally and spatially characterizes protein and RNA expression within intact tissue sections. Here, we present a proof-of-concept study on profiling immune infiltrates and classifying immunological subtypes for NSCLCs using spatial RNA and protein expression data from GeoMx DSP.

Quantitative Single Cell Deconvolution (qSCD) development:
- Using scRNA-seq data sets[1], we defined 18 tumor-specific cell types and their gene expression signatures for NSCLCs.
- We calculated cell type scores per tumor subregion using spatial RNA expressions and qSCD and then validated the calculated scores using cell-type specific protein markers.

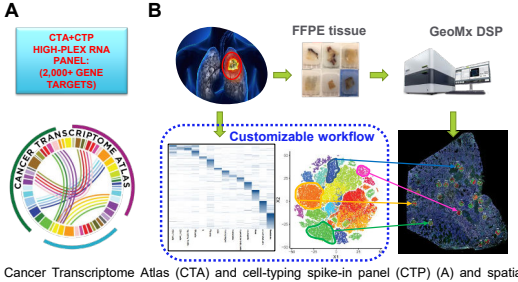
Key results: Using qSCD and RNA expression data from GeoMx DSP, we were able to visualize immunological landscapes across sub-local tumor regions and classify immunological subtypes of tumor tissues.
- Inter-tumoral heterogeneity was visualized better with the spatial segregations of ROIs into Tumor and tumor microenvironment regions.
- We found that grade 2 and 3 tumors contained both lymphoid and myeloid infiltrates, but their spatial distributions were mutually exclusive within a tumor tissue.
- Grade 1 tumor showed little–to-no tumor-infiltrating lymphocytes across the tissue regions.

GeoMx DSP chemistry and workflow



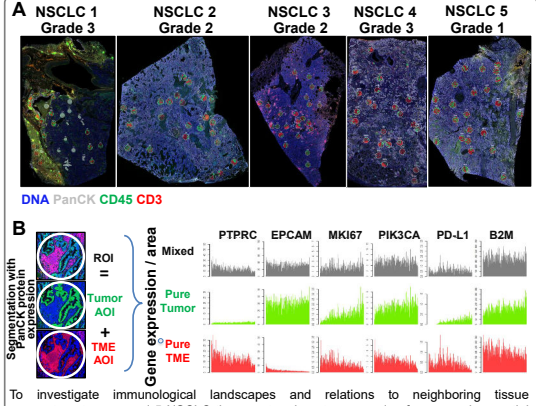
The GeoMx Digital Spatial Profiler (GeoMx DSP) is based on barcoding technology that enables spatially resolved, digital characterization of proteins or RNA in a highly multiplexed (over 2,000-plex) assay. The oligonucleotide tags cleaved from discrete regions are quantitated by NGS or nCounter, and counts are mapped back to a tissue location, yielding a spatially-resolved digital profile of analyte abundance. Using a UV-cleavable linker, epitope-specific antibodies (A) or in-situ-hybridization probes (B) are conjugated with unique DNA-oligo tags. GeoMx DSP shapes and illuminates UV lights over user-defined tissue subregions of interest only to cleave & collect DNA-oligo tags and records x,y coordinates of the subregions (C). Cleaved tags from each ROI are collected and counted using nCounter® or a NGS sequencer.

Quantitative Single Cell Deconvolution (qSCD) algorithm and GeoMx DSP RNA panel



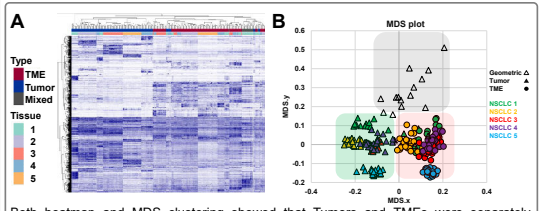
Cancer Transcriptome Atlas (CTA) and cell-typing spike-in panel (CTP) (A) and spatial mapping of cell types of interest using qSCD (B). To gain insights inaccessible to single-cell methods, we demonstrate a harmonized analysis of scRNA-seq and NanoString GeoMx data in tumors. This approach reveals the spatial distribution of cell populations defined via scRNA-seq, enabling detailed descriptions of cells' responses to each other and to their locations within the tumor.

GeoMx DSP's unique feature, "segregating a region of interests (ROI) into multiple sections," allows measuring gene expressions of a pure "Tumor" and "Tumor microenvironment (TME)" region.



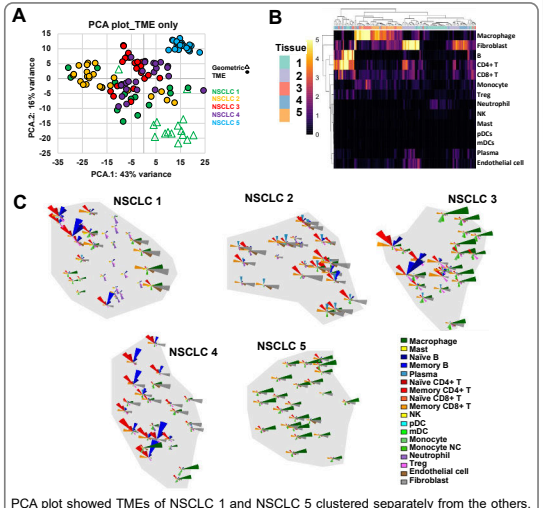
To investigate immunological landscapes and relations to neighboring tissue compartments, we used 5 NSCLC tissues at various tumor grades for measuring spatial gene expressions of Tumors and TMEs (A). To measure gene expression of pure Tumor and TME regions, we segmented each ROI to high and low PanCK protein region, defined as Tumor and TME respectively (B).

GeoMx DSP data suggested that NSCLC 1 and 5 were differentially clustered from the other tumors.



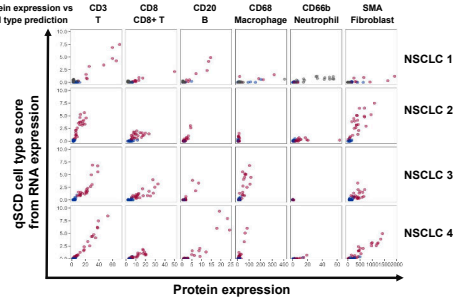
Both heatmap and MDS clustering showed that Tumors and TMEs were separately clustered as expected (A & B). Interestingly, both TMEs and Tumors of NSCLC 5, which is the only grade 1 tumor, were distinct from the other four tumors.

Spatial mapping of immune infiltrates using qSCD suggests that lymphoid and myeloid cells are spatially mutually exclusive, resulting in high intra-tumoral heterogeneity.



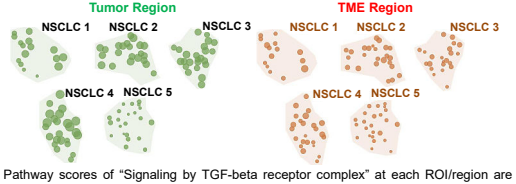
PCA plot showed TMEs of NSCLC 1 and NSCLC 5 clustered separately from the others. (A). We used qSCD to quantitate cell types per TME region and spatially display cell types in TMEs (C). The results suggest that tumor-infiltrating lymphoid and myeloid cells are generally mutually exclusive. Neutrophils are highly enriched in NSCLC 1. B cells are likely co-present with T cells but not other cell types. Macrophages, fibroblast, and neutrophils are dominant and rarely present with the other cell types.

Validation shows that cell type scores from qSCD are quantitative and linearly correlated with cell-type specific protein markers.



To validate qSCD results, serial sections of NSCLC tissues were used for measuring cell-type specific protein expression of same TME regions. **The correlation values of cell type scores and marker-protein expression ranged up to 0.98.**

TGF-beta signaling was depleted at tumor regions of NSCLC 5 (G1) contest to other higher grade tumors while TMEs did not show such strong pattern.



Pathway scores of "Signaling by TGF-beta receptor complex" at each ROI/region are plotted.

Conclusions

- GeoMx DSP generates spatially annotated multidimensional data sets with a single run, such as spatial gene-expression quantitation, pathway analysis, quantitative Single Cell Deconvolution (qSCD) analysis and fluorescent tissue images with visualization markers of user's choice.
- Quantitative Single Cell Deconvolution (qSCD) analysis allows GeoMx DSP to identify spatial abundances of tumor-specific cell types.
- Such multi-dimensional GeoMx DSP capacity provides more spatially enriched information than any traditional method alone, such as tissue imagers, flow cytometry (FACS), scRNA-seq and RNA-seq, can do.

Reference

1. Zilionis R, et al, Single-Cell Transcriptomics of Human and Mouse Lung Cancers Reveals Conserved Myeloid Populations across Individuals and Species, Immunity. 2019 May 21;50(5):1317-1334.e10.