Small slide, Big world - Fast detection of altered proteins and RNA levels in clinical samples using Nanostring GeoMx® Digital Spatial Profiler

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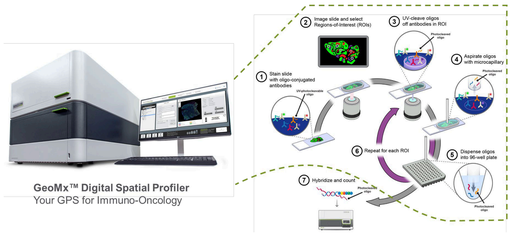
Abstract

Although many new drugs have been approved and more clinical trials are being conducted for various disease therapies in recent decades, efficient identification of reliable therapeutic targets is still a big challenge during drug development. Therefore, tremendous efforts have been put into advancing the technology to quickly determine the altered biomarkers in clinical samples. Nanostring GeoMx Digital Spatial Profiling (GeoMx DSP) has been used in multiple projects and exhibited its outstanding performance on collecting analyzable digital counts for up-to 96 proteins and 18,000 RNAs in a specific area on a single FFPE slide. Tissue regions of interests are identified by fluorescent staining of morphology markers, and either proteins or RNAs in the specific defined regions are collected as the digital nCounter count format. By using this state-of-art technology, spatial distributions of immune- and cancer- relevant proteins were identified in 71 tumor cell rich-, 70 immune cell rich-, and 69 stromal cell rich- area respectively in three Pancreatic cancer patient groups including surgery alone, neoadjuvant FOLFIRINOX (a combination of fluorouracil, folinic acid, irinotecan and oxaliplatin) alone or combined with stereotactic body radiation therapy (F+SBRT). Our results showed that differential regulations among immune checkpoint markers, pan-immune markers and tumor markers in these treatment groups. In tumor-rich regions, CD44 is present at higher levels in FOLFIRINOX treated patients than surgery-alone group. VISTA was significantly down-regulated in F+SBRT group. Also, several key factors from pStat3, PTEN and Bcl2 pathways are also regulated by treatment methods. In immune-rich regions, both T cells markers (CD3 and CD4) and B cell markers (CD19 and CD20) were regulated differently by treatment methods. GeoMx DSP platform provides a novel and more precise view of the spatial regulation of micro-world in the tissue, which enable us to explore more about internal regulation within sub-population of the tissue cells.

GeoMx DSP platform is only for RESEARCH USE ONLY. Not for use in diagnostic procedures.

Methods

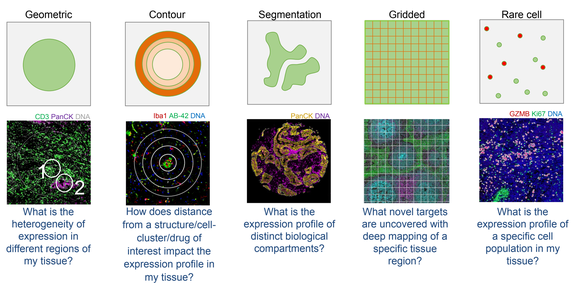
GeoMx DSP sample processing and spatial profiling



GeoMx DSP is a novel high throughput technology, which allow profiling up to 96 proteins, 96 RNA or over 1800 RNA targets from a single FFPE slide at once.

1. **Process:** FFPE tissue or cell pellets slide incubated with a cocktail of oligo-conjugated antibodies.
2. **ROI selection and collection:** Regions of interest (ROIs) are identified with fluorescent images. And oligos from the selected regions are released upon exposure to UV light and collected into 96-well microplate via a microcapillary tube.
3. **Digital counting:** Photocleaved oligos from the spatially-resolved ROIs in the microplate are hybridized to 4-color, 6-spot optical barcodes, enabling up to ~1 million digital counts of the protein targets (distributed across all targets) in a single ROI using standard NanoString nCounter® instruments
4. **Instrument data analysis**: nCounter® counts will be loaded back to DSP to generate exportable digital count of each ROI. Instrument data analysis software helps to generate variance graphs for presentation or publication, including PCA, box plots, heatmap, volcano plot, etc.

GeoMx DSP ROI Strategies



Application of GeoMx DSP Protein Panel in PDAC

Patients information

24 patients who received surgical resection of PDAC tumors at Emory University.

- Six patients had received no treatment for their cancer prior to surgery (surgery alone).
- Six patients received neoadjuvant FOLFIRINOX prior to surgery (FOLFIRINOX).
- Six patients received neoadjuvant FOLFIRINOX followed by stereotactic body radiotherapy (SBRT) (F + SBRT) prior to surgery.

Regions of Interest Strategy

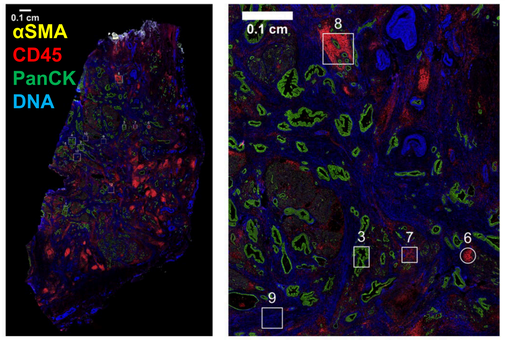


Figure 1. Selection of tumor-rich, immune cell–rich, and stroma-rich regions within PDAC tumors. As part of GeoMx workflow, FFPE slides of PDAC tumors from patients who received upfront surgical resection or surgical resection following neoadjuvant therapy with either FOLFIRINOX alone or FOLFIRINOX + SBRT were stained with fluorescently labeled anti–pan-cytokeratin (green), anti-CD45 (), and anti–α-smooth muscle actin (αSMA, blue). A representative slide is shown. These fluorescently labeled antibodies were used to manually define regions of interest with the intent being to select tumor-rich regions (rich in pan-cytokeratin staining but largely lacking CD45 and αSMA staining), immune cell–rich regions (rich in CD45 staining but largely lacking pan-cytokeratin and αSMA staining), and stroma-rich regions (rich in αSMA but largely lacking the other 2 markers). Scale bar: 0.1 cm.

Results

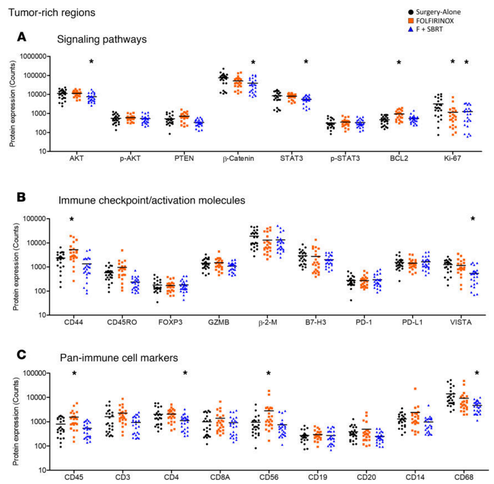


Figure 2. Neoadjuvant therapy alters the expression levels of immunologically relevant proteins in tumor-rich regions. Expression levels of the indicated proteins. Each dot represents a single region of interest. Individual regions of interest were derived from each patient tumor from a total of n = 6 patient tumors. (A) Signaling pathways. Expression level of signal transduction molecules and cell-cycle–related proteins within tumor-rich regions. (B) Immune checkpoint/activating molecules. Expression level of T cell checkpoint molecules, effector molecules, and other proteins that indicate immune cell phenotype within tumor-rich regions. (C) Pan-immune cell markers. Expression level of proteins that indicate immune cell type within tumor-rich regions. *FDR < 0.05 by 1-way ANOVA followed by post test in comparison to surgery-alone patients.

Results (Cont.)

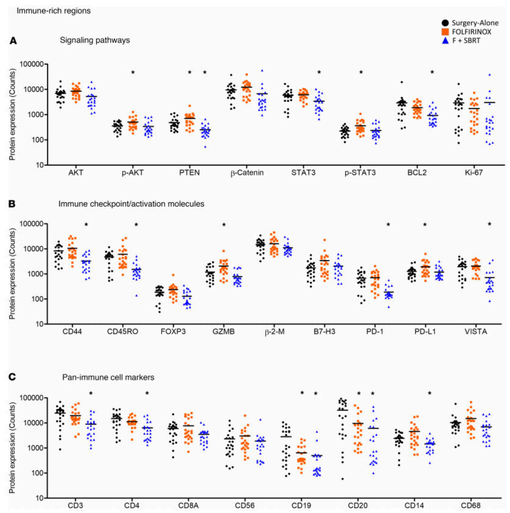


Figure 3. Neoadjuvant therapy alters the expression levels of immunologically relevant proteins in immune cell–rich regions. Expression levels of the indicated proteins. Each dot represents a single region of interest. Individual regions of interest were derived from each patient tumor from a total of n = 6 patient tumors. (A) Signaling pathways. Expression level of signal transduction molecules and cell-cycle–related proteins within immune cell–rich regions. **FDR < 0.05 by 1-way ANOVA followed by post test in comparison to surgery-alone patients. (B) Immune checkpoint/activating molecules. Expression level of T cell checkpoint molecules, effector molecules, and other proteins that indicate immune cell phenotype within immune cell–rich regions. (C) Pan-immune cell markers. Expression level of proteins that indicate immune cell type within immune cell–rich regions

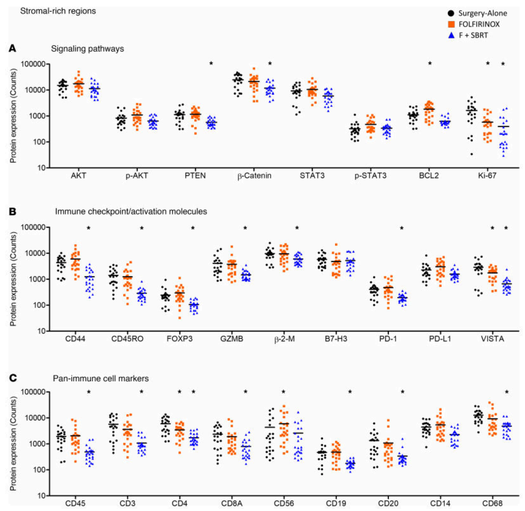
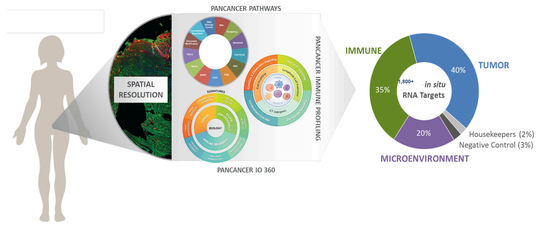


Figure 4. Neoadjuvant therapy alters the expression levels of immunologically relevant proteins in stroma-rich regions. Expression levels of the indicated proteins. Each dot represents a single region of interest. Individual regions of interest were derived from each patient tumor from a total of n = 6 patient tumors. (A) Signaling pathways. Expression level of signal transduction molecules and cell-cycle–related proteins within stroma-rich regions. (B) Immune checkpoint/activating molecules. Expression level of T cell checkpoint molecules, effector molecules, and other proteins that indicate immune cell phenotype within stroma-rich regions. (C) Pan-immune cell markers. Expression level of proteins that indicate immune cell type within stroma-rich regions. *FDR < 0.05 by 1-way ANOVA followed by post test in comparison to surgery-alone patients.

Summary

- GeoMx DSP platform enables researchers to investigate gene expression spatially.
- Segmentation ROI strategies provide effective ways to explore what is happening in specific cell populations.
- RNA expression of up to 1800 genes can be detected on the selected region in one tissue section, which increased research efficiency dramatically.
- The application of GeoMx DSP platform has been extended to more than 100 disease and cell pellet sections.
- Whole transcriptome atlas with 18,000 genes will be launched soon.

GeoMx Cancer Transcriptome Atlas (CTA)



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- **Metabolic signaling**
- **Cell Stress**
- **Tumor Inflammation Signature (TIS)**
- **PAM50**
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Application of GeoMx CTA in Human Tonsil

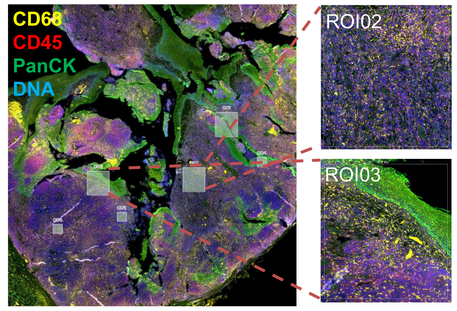

Figure 5. Fluorescent images of human tonsil sample.

Heatmap

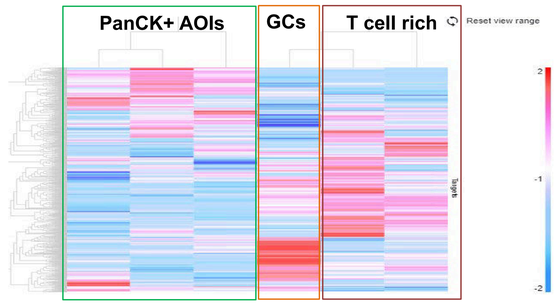

Figure 6. Dendrogram of genes expression of AOIs.

Limit of Quantification

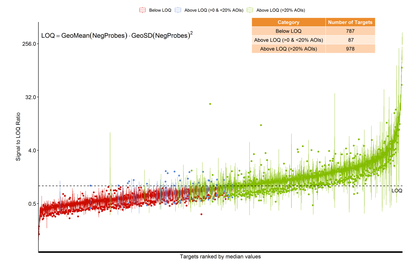

Figure 7. Level of quantification calculation. More than 900 genes were detected with signal to LOQ ratio above 1 in more than 20% AOIs, suggesting the great sensitivity of CTA panel.