#2825

High-plex, spatial RNA profiling of tumor infiltrating leukocytes and the tumor microenvironment of microsatellite instable colorectal cancer using GeoMx® Digital Spatial Profiler

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Abstract

Immunotherapy has revolutionized cancer treatment but improved understanding of immunomodulation in tumors is still necessary to expand the reach of these therapies and identify rational combination approaches. An important aspect of this process will be characterizing the molecular differences between tumor-infiltrating leukocytes (TILs) and stromal leukocytes (non-TILs) surrounding the same tissues. Most studies to date have focused on dissociated tissues, which means identifying the origin of the profiled leukocytes is only possible with post-hoc inference. High-plex profiling that retains spatial orientation has proven difficult in fixed tissues, preventing direct understanding of TIL localization beyond a handful of pre-selected targets. To explore the transcriptional profile of TILs in situ, we used the Cancer Transcriptome Atlas (CTA) panel for the GeoMx Digital Spatial Profiler (DSP). The CTA is capable of measuring the expression of 1,400+ genes at once. Here we profiled microsatellite instable (MSI) colorectal cancer (CRC) samples noted to have a high abundance of CD3+ TILs by 4-color immunofluorescence (IF).

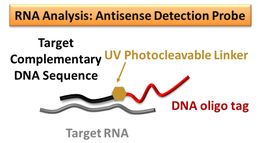
Regions of interest were selected inside (n = 6, per tumor) and outside (n = 6, per tumor) the tumor invasive margin focusing on tumor or stromal regions with high numbers of CD3+ cells. Within each region of interest, we created a custom segmentation strategy to specifically illuminate CD3+ cells, and then sequentially illuminate neighboring cells. These additional segments were defined by extending contours 10 um around the initially selected lymphocytes to determine gene expression differences driven by the local microenvironment of each population.

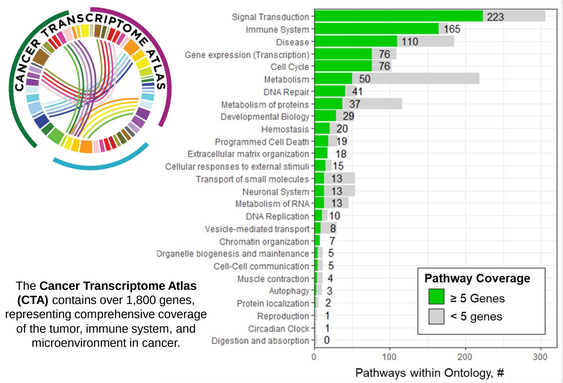
We found that regions neighboring TILs express higher levels of known oncogenic pathways and stromal regions neighboring non-TILs were noted to have higher expression of ECM genes, confirming the specificity of the profiling approach. Furthermore, we found that TILs specifically up-regulate expression of cytolytic pathway genes, as well as several coinhibitory and costimulatory checkpoint genes. We also observe dysregulation of members of the adenosine metabolism pathway within the tumor regions profiled and TILs, but not in regions adjacent to the tumor itself. Together, our results demonstrate the feasibility of profiling specific cell populations with a high plex mRNA panel in situ in FFPE tissue, thus enabling pathway level differential expression analyses and exploration of key interactions between neighboring cell types while retaining their spatial context.

The CTA Enables Rich Readout of Cancer Pathways

Tagged Oligonucleotide Chemistry

GeoMx Digital Spatial Profiler (DSP) uses oligonucleotides which hybridize to target mRNAs to quantitatively read out DNA tags which are selectively released *in situ* by specifically shining UV light into certain regions of the tissue



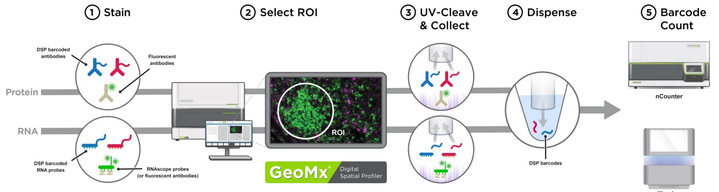


The **Cancer Transcriptome Atlas (CTA)** contains over 1,800 genes, representing comprehensive coverage of the tumor, immune system, and microenvironment in cancer.

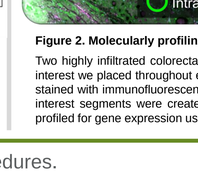
Figure 1. Pathway Coverage of the Cancer Transcriptome Atlas (CTA)

Annotations are sourced from Reactome (reactome.org) and associated with the genes from each panel. Pathways in which more than 5 genes were present on the CTA, the minimum necessary for rich pathway analysis, are colored in green. Pathways in which less than 5 genes from the pathway are present on the panel are colored in gray.

GeoMx Enable Direct *In Situ* Expression Profiling of TILs

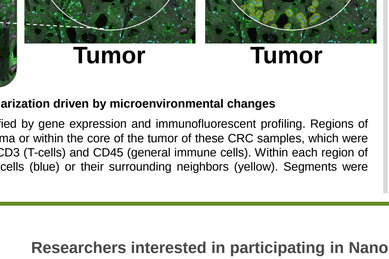


The **GeoMx(R) Digital Spatial Profiler (DSP)** contains a tunable micromirror array which enables profiling of distinct tissue compartments, complex morphological structures, and cell populations dispersed throughout a tissue.



DSP Segments:

- Can be guided by IF staining to specifically profile unique cell populations or structures
- Are dynamically tunable based on intensity
- Can be imported from standard image analysis software such as ImageJ



Selectively Sampling Lymphocytes in Distinct Locations in CRC Samples

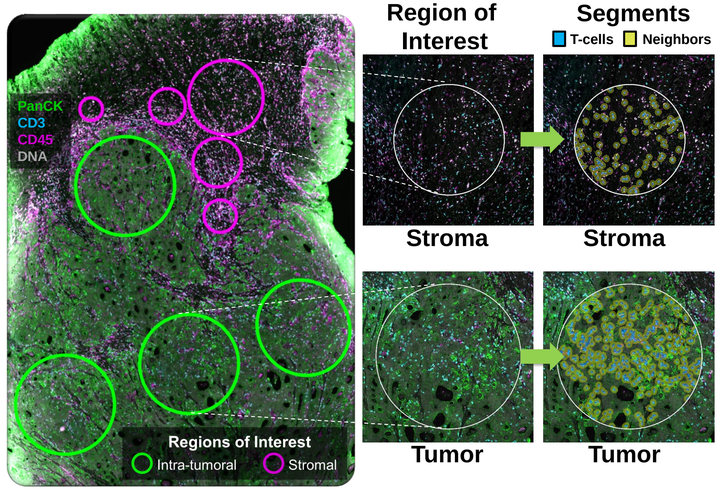


Figure 2. Molecularly profiling TILs *in situ* to identify polarization driven by microenvironmental changes

Two highly infiltrated colorectal cancer patients were identified by gene expression and immunofluorescent profiling. Regions of interest we placed throughout either the tumor-adjacent stroma or within the core of the tumor of these CRC samples, which were stained with immunofluorescent masks for PanCK (tumor), CD3 (T-cells) and CD45 (general immune cells). Within each region of interest segments were created to selectively illuminate T-cells (blue) or their surrounding neighbors (yellow). Segments were profiled for gene expression using the CTA.

T-cells Activation upon Infiltration Captured by DSP

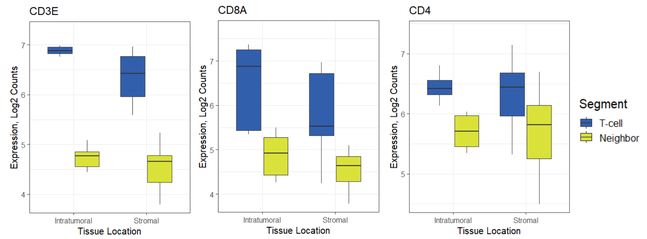
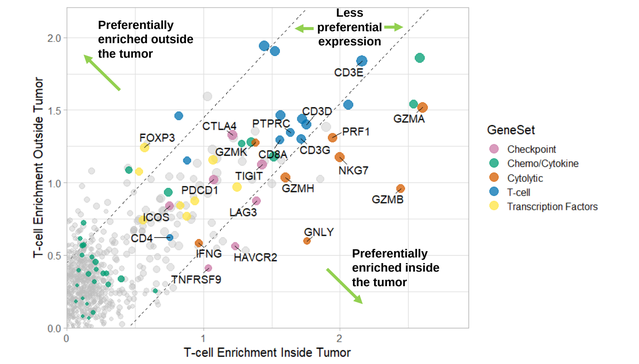


Figure 3. Selective Measurement of T-cell specific Markers

To confirm that DSP specifically profiled CD3 positive cells, we specifically explored the expression of know markers of T-cells which should be expressed at nominal levels by other cell types in CRC samples. We observe that in both intra-tumoral and stromal ROIs that we observe specific expression CD3E, CD8A, and, to a lesser extent, CD4 within the CD3+ segments that were profiled by GeoMx.



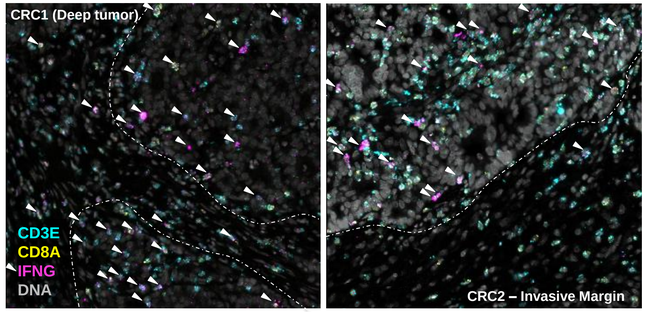


Figure 4: Cytolytic Activity and Checkpoint Expression Preferentially Increased Within Tumor Regions

To measure location-specific T-cell activation, we compared the relative expression of genes within tumor and stromal ROIs relative to their neighboring cells (top). Genes preferentially enriched within the tumor include classic activation checkpoint genes such as LAG3, TIM3 (HAVCR2), and 4-1BB (TNFRSF9). Additionally, cytolytic genes such as granzymes (GZMA/B/H), PRF1, GNLY, and to a lesser extent, IFNG also showed an enrichment within the tumor. Staining by RNA Scope confirmed the tumor-specific increase in IFNG (arrows, bottom panels) in both samples.

Adenosine Pathway Modulation by Tumors

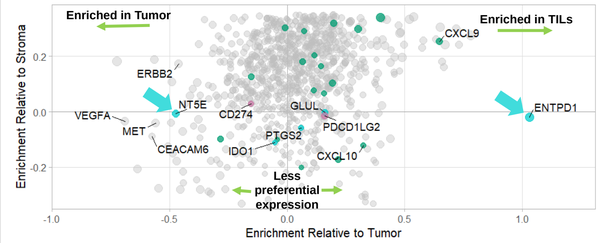


Figure 5: Tumor-Specific Modulation of the Adenosine Pathway

In addition to the modulation of cytolytic and checkpoint molecules, CD73 (NT5E) and CD39 (ENTPD1) were observed to be biased toward tumor-specific or T-cell specific expression within the tumor, respectively. The fact that this finding appeared to be tumor-preferential suggested a mechanism for tumor-specific evolution to modulate T-cell specific response to the tumor while other T-cell metabolism related genes such as IDO1, COX2 (PTGS2), and glutamine synthetase (GLUL), showed little preferential expression.

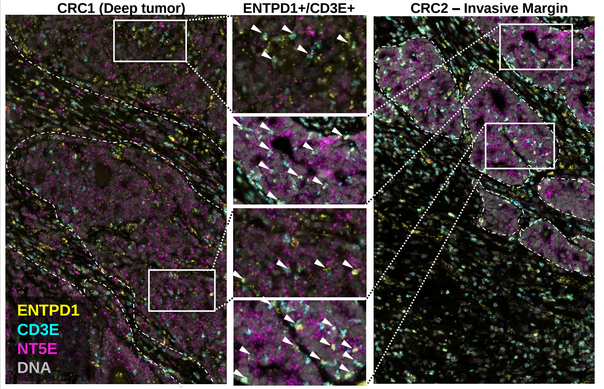


Figure 6: RNA Scope Visualization of Adenosine Pathway

To validate that tumor cells preferentially up-regulate CD73 (NT5E) we explored stained additional sections with 3-color immunofluorescent RNA scope probes for CD39 (ENTPD1), CD73 (NT5E), and CD3 (CD3E). We observed significant, and specific, up-regulation of NT5E within the tumor core. T-cells also appeared to frequently be double-positive for CD3E and ENTPD (middle inserts, arrows), suggesting the that the adenosine pathway may be a compensatory mechanism the tumor is utilizing to modulate T-cell activity.

Conclusions

- The Cancer Transcriptome Atlas and GeoMx DSP enable *in situ* profiling of specific immune cell types and their neighboring cells
- T-cell specific markers were confirmed to be sensitively and specifically captured by GeoMx
- Cytolytic activity and checkpoints are preferentially up-regulated within tumor regions of two highly infiltrated CRC tumors compared to surrounding stromal regions
- CD73 is preferentially up-regulated in these tumors, providing a potential mechanism to modulate T-cell activity within these patients

Researchers interested in participating in NanoString's technology access program for its Digital Spatial Profiling technology should contact us at TAP@nanostring.com.