Comprehensive characterization of biology of inflammatory bowel disease-associated colorectal cancer using the GeoMx® Cancer Transcriptome Atlas panel

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#3801

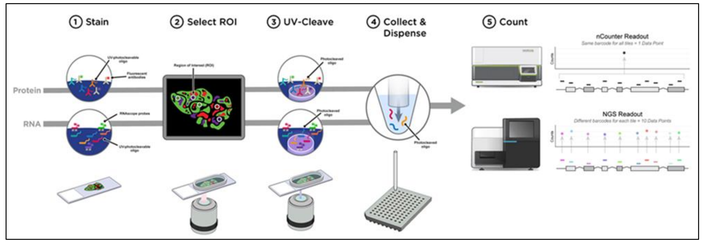
Introduction

"Inflammatory bowel diseases (IBD) ulcerative colitis (UC) and Crohn's disease (CD) significantly increases the risk for development of colorectal cancer (CRC). However, the exact molecular mechanisms that underlie the transition from inflammation to cancer are not fully understood, and a systematic characterization of the immunopathogenesis of IBD-associated CRC is lacking. To address this issue, we performed a comprehensive and spatial tissue analysis using the Cancer Transcriptome Atlas (CTA) panel on the NanoString GeoMx® Digital Spatial Profiler (DSP). The CTA panel enables high-plex data profiling of 1,800 RNA targets simultaneously combined with spatial information from any region of interest from a single tissue section using NGS readout. The CTA panel is designed for comprehensive profiling of the tumor, tumor microenvironment, and tumor immune status including the 18-gene Tumor Inflammation Signature (TIS).

In this study, a total of 20 FFPE samples (5 CRC patients, 4 UC patients, 6 CD patients, and 5 healthy donor samples) were spatially profiled for up to 1,800 genes included on the CTA panel. For all samples, H&E staining and pathology assessment were performed to assess tumor %, and determination of location of tumor and immune-enriched locations. Regions of interest (ROIs) were selected based on fluorescent antibody morphology markers on tissue sections stained for DNA (**Syto13**), epithelial cells (**PanCK**), and immune cells/stroma (**CD45**). The profiling of tumor and stroma regions were achieved through geometric ROI. To thoroughly profile the spatial differences (epithelial vs. stromal/immune), for each sample, 3 groups of ROI were selected, ROIs 1-4: geometric ROIs in epithelial/PanCK+ region, ROIs 5-8: geometric ROIs in CD45+ region proximal to PanCK+ ROIs, ROIs 9-12: geometric ROIs in CD45+ region distal to PanCK+ ROIs. ROI selection was followed by collection of indexed oligonucleotides and sequencing on NextSeq 550 Illumina instrument. Using the GeoMx NGS Pipeline software, Illumina FASTQ sequencing files were automatically processed to GeoMx readable digital counts (DCC) and input back into the Data Analysis Suite for analysis. The digital counts were mapped back to each ROI, generating a map of transcript activity within each ROI. The R BioConductor package DESeq2 was used to test for differential expression by a negative binomial generalized linear model. Models tested for significant differences in expression were between ROIs: PanCK+/Proximal Stroma/Distal Stroma. Transformations from raw digital counts used the Variance Stabilizing Transform function, for Principal Components and heatmap plots.

Overview of Study Workflow

1A. GeoMx DSP Assay Workflow



Signaling Pathways Annotation Summary			
Signaling Pathways	**# Genes**	**Signaling Pathways**	**# Genes**
AMPK Signaling	44	mTOR Signaling	76
Androgen Signaling	32	Myc	26
EGFR Signaling	17	NO Signaling	10
ERBB2 Signaling	21	Notch Signaling	74
Estrogen Signaling	84	p53 Signaling	76
FGFR Signaling	40	PDGF Signaling	30
FoxO Signaling	79	PI3K-Akt Signaling	242
GPCR Signaling	177	PPAR Signaling	15
Hedgehog Signaling	45	Purinergic Signaling	3
HIF1 Signaling	68	Retinoic Acid Signaling	5
Insulin Signaling	81	TGF-beta Signaling	69
JAK-STAT Signaling	118	VEGF Signaling	69
MAPK Signaling	261	Wnt Signaling	124
MET Signaling	34		

1B. GeoMx Cancer Transcriptome Atlas curated content

Biological Category Summary	
Tissue Compartment	**# Genes**
Tumor Biology	1454
Immune Response	1481
Microenvironment	978

Tissue Compartment Summary	
Tissue Compartment	**# Genes**
Tumor	1622
Immune	1396
Stroma	1024

Immune Response Annotation Summary			
Immune Response	**# Genes**	**Immune Response**	**# Genes**
Chemokine Signaling	121	Lymphocyte Trafficking	109
Cytotoxicity	6	NF-kB Signaling	114
IL-1 Signaling	64	Other Interleukin Signaling	173
IL-17 Signaling	49	Prostaglandin Inflammation	4
IL-2 Signaling	37	TNF Signaling	96
IL-6 Signaling	18	Type I Interferon Signaling	47
Immune Exhaustion	20	Type II Interferon Signaling	49
Interferon Response Genes	25	Type III Interferon Signaling	7
Lymphocyte Regulation	93		

Figure 1A. GeoMx DSP Assay Workflow. For NanoString DSP, slides were stained with fluorescently labeled morphology markers and oligo-conjugated RNA probes. ROI selections in two locations, tumor and immune-enriched/stroma locations were guided by staining with fluorescent markers (PanCK,CD45, Syto 13). Per slide, 12 ROIs of 500 µM diameter circle were selected. Photocleaved oligos were collected and counted using Illumina NextSeq 550 per manufacturer protocols. **1B. GeoMx Cancer Transcriptome Atlas panel curated content.** This panel includes over 100 pathways to explore all aspects of cancer. Only key content shown above.

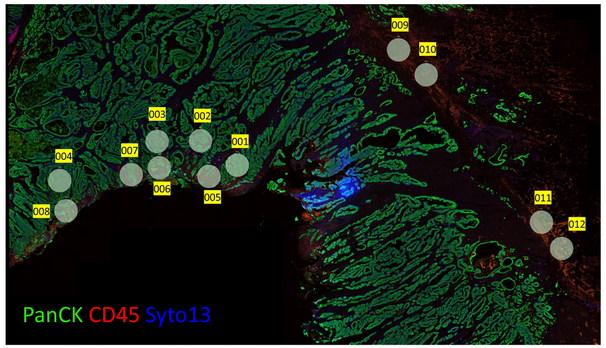
Key Findings

In this study, NanoString GeoMx CTA panel offered by NeoGenomics Laboratories was utilized to perform a large-scale expression profiling of colorectal cancer, ulcerative colitis, Crohn's and healthy donor tissue FFPE samples to understand the genes and pathways involved in the transition from inflammation to cancer.

- Spatially distinct distribution of targets was observed between PanCK+, proximal stroma and distal stroma regions in this study.
- Differential expression analysis helped identify specific genes such as NR4A1 and SOX9 that were altered during intestinal inflammation in IBD and CRC tumorigenesis respectively, in epithelial and stromal/immune fractions.
- Comprehensive spatial profiling by CTA panel allowed us to dissect the spatial target expression alterations in epithelial and stromal regions from colorectal cancer, ulcerative colitis, Crohn's and healthy donor tissue samples.
- The findings from this GeoMx CTA study will be used to further elucidate the underlying mechanisms leading from an IBD disorder to the development of colon cancer.

ROI selection and profiling using NanoString GeoMx DSP

2A.



2B.

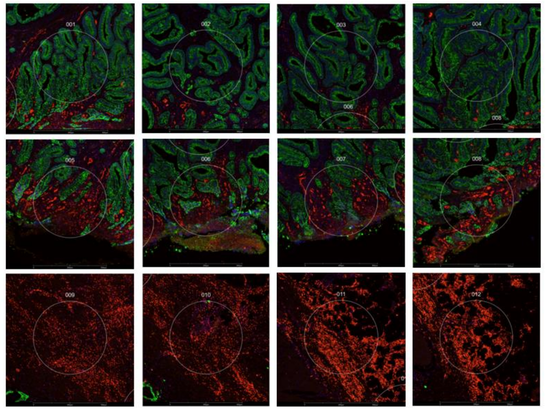


Figure 2A. ROI selection for GeoMx CTA testing. A representative DSP image showing ROI selection PanCK and CD45 in a colorectal cancer FFPE sample. **2B. Distribution of ROIs:** Per slide, 12 ROIs of 500 µM diameter circle were selected based on PanCK and CD45 staining. For each sample, 3 groups of ROI were selected, ROIs 1-4: geometric ROIs in PanCK+ region, ROIs 5-8: geometric ROIs in CD45+ region that are proximal to PanCK+ ROIs, ROIs 9-12: geometric ROIs in CD45+ region that are distal to PanCK+ ROIs.

3.

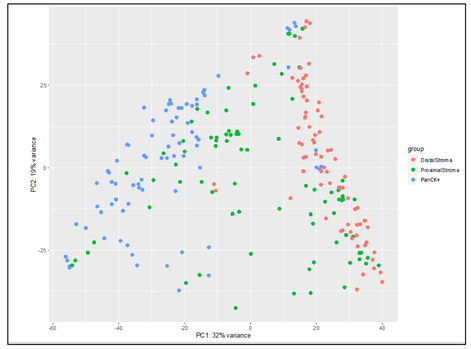


Figure 3. Principal Component Analysis (PCA) plot showing variability between PanCK+, proximal stroma and distal stroma ROIs. For all samples, principal components analysis of PanCK+, proximal stroma and distal stroma ROIs revealed a clear spatial separation of the profiled areas. Targets in the PanCK+ (blue) and distal stroma regions (red) formed distinct clusters with targets in the proximal stroma (green) interspersed in between PanCK+ and distal stroma regions.

Gene expression profiling using GeoMx CTA Panel

4.

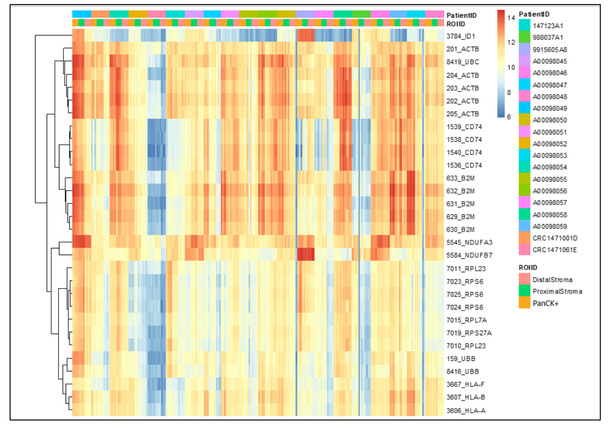
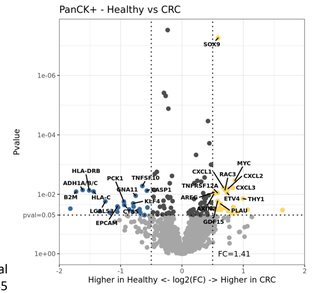
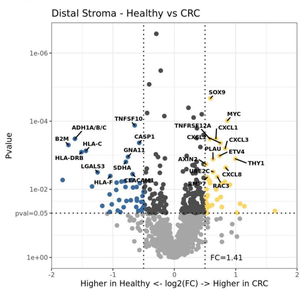


Figure 4. Heatmap plot showing clustering of top 30 maximally differentiated probes across all ROIs for all indications. For each target in the CTA panel, there are 5 separate probes and probes for the same target clustered together. Additionally, targets within the PanCK+ and distal stroma regions were spatially distinct.

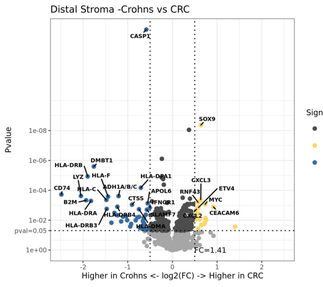
5A.



5B.



5C.



5D.

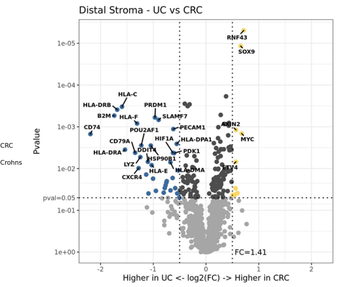


Figure 5A-5C. Differential expression profiling of CTA panel targets. Volcano plot analysis was used to compare target expression between distal stroma ROIs and various indication samples. The dotted horizontal line represents the adjusted P-value cut-off. Differences in key markers for intestinal inflammation in IBD as well as CRC tumorigenesis were observed.

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