Spatial Analysis of Genomic Signatures on Colorectal Cancer Pathogenesis Using the NanoString GeoMx® Digital Spatial Profiler

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Background

The development of Colorectal cancer (CRC) is a complex process, involving multiple sequential molecular aberrations that contribute to disease progression. Understanding the immunopathogenesis of CRC, particularly within the spatial context of the TME may elucidate genomic and biological changes to improve patient prognosis for adjuvant therapies and identifying potential drug targets. However, this can be challenging, as genomic signatures from sub-cellular populations and varying levels of immune cell infiltration in the TME may be lost during bulk sequencing. In this study, we spatially profiled epithelial, proximal and distal stromal regions of CRC and healthy FFPE for ~1800 genes using the Cancer Transcriptome Atlas (CTA) on the NanoString GeoMx® Digital Spatial Profiler (DSP).

Methods

Regions of interest (ROI) were guided by pathology assessment of H&E images and selected using fluorescent antibody markers (CD45, PanCK, Syto13). Tumor and stroma (proximal and distal) regions were profiled through geometric ROI selection in PanCK+ (epithelial) and CD45+ (stromal/immune) enriched areas respectively, followed by collection of indexed oligonucleotides and sequencing on NextSeq 550 Illumina platform. 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. Differential expression and pathways enrichment analyses was performed using R BioConductor package and DSP GeoMx analysis suite.

Overview of DSP Workflow

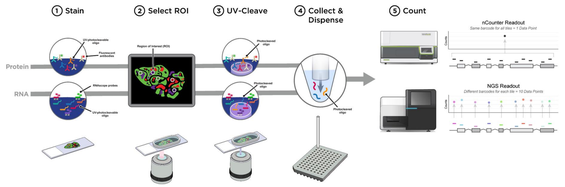

Fig 1A: GeoMx DSP assay workflow

Signaling Pathways Annotation Summary			
Signaling Pathway	**# 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 Signalir	5
Insulin Signaling	81	TGF-beta Signaling	69
JAK-STAT Signaling	118	VEGF Signaling	69
MAPK Signaling	261	Wnt Signaling	124
MET Signaling	34		

Tissue Comparment Summary	
Tissue Compartment	**# Genes**
Tumor	1622
Immune	1396
Stroma	1024
Biological Category Summary	
Tissue Compartment	**# Genes**
Tumor Biology	1454
Immune Response	1481
Microenvironment	978

Fig 1B: GeoMx CTA panel curated content

ROI Selection for GeoMx DSP

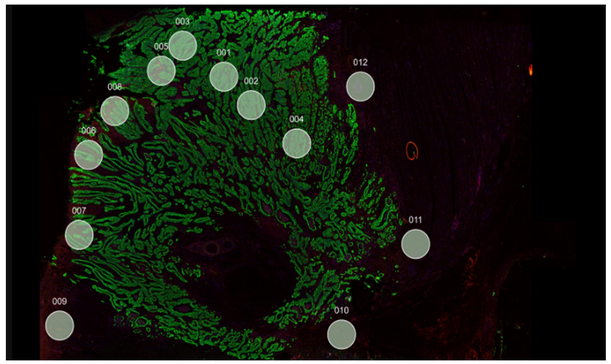

Fig 2A: ROI selection for GeoMx CTA testing. A representative DSP image showing ROI selection using PanCK and CD45 in a CRC FFPE sample.

ROI Epithelial and Stromal Distribution

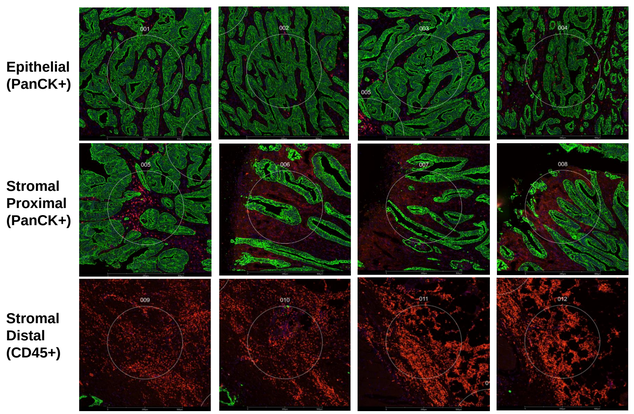

Fig 2B: Distribution of ROIs. Per slide, 12 ROIs of 500 µM diameter circle were selected based on PanCK and CD45 staining. For each sample, 3 groups of ROI were selected, ROIs 1-4: geometric ROIs in PanCK+ region, ROIs 5-8: geometric ROIs in CD45+ region that are proximal to PanCK+ ROIs, ROIs 9-12: geometric ROIs in CD45+ region that are distal to PanCK+ ROIs. This allowed for thorough profiling of the spatial differences (epithelial vs. stromal/immune).

Gene Expression Profile Analysis

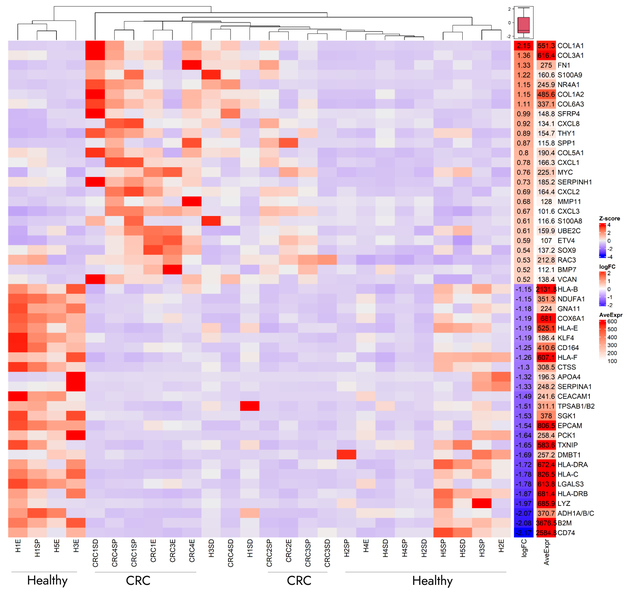

Fig 3: Heatmap plot showing clustering of top 50 genes differentially expressed across all ROIs for CRC and healthy groups. Several genes belonging to the collagen gene family, as well as COL1A1 and COL3A1 are upregulated in CRC samples.

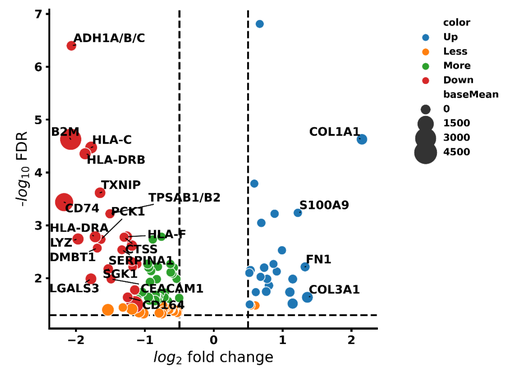

Fig 4: Gene expression profiles analysis. Volcano plot analysis comparing target expression between CRC and healthy samples. The dotted horizontal line represents the adjusted P-value cut-off. Differences in key markers for CRC vs healthy samples were observed.

Gene Pathway Enrichment Analysis

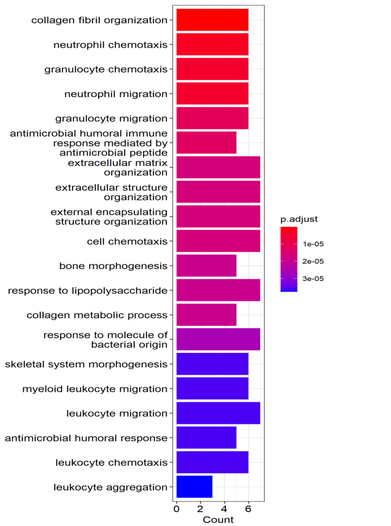

Fig 5: Gene expression pathway enrichment analysis. Gene Ontology enrichment analysis was performed to identify key signaling pathways in CRC samples based on GeoMx CTA data

Key findings

- 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 COL1A1, COL3A1, S100A9 and NR4A1 are significantly upregulated in CRC samples.
- Gene enrichment analysis showed extracellular matrix organization, extracellular structure organization and cell chemotaxis pathway significantly enriched in CRC samples.

Conclusions

With this spatial study using GeoMx high-plexed CTA panel, we have highlighted key genes and pathways that are involved in CRC tumorigenesis in order to provide a much-needed understanding of the underlying mechanisms in the development of colon cancer.

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