Spatial characterization of pro-inflammatory pathways in the pathogenesis of IBD-associated colorectal cancer

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#1482

Background

While immunotherapy has transformed the management of metastatic and recurrent solid tumors, survival rates for patients with advanced colorectal cancer (CRC) remain very poor, with treatment still limited to MSI-H and dMMR-expressing tumors in a chemotherapy refractory setting. Ideally, additional insight is needed to investigate the potential role of immunotherapy at all stages of CRC progression, regardless of microsatellite or MMR gene status. To that end, data from a large number of experimental studies have previously demonstrated that chronic inflammation is highly correlated with the occurrence and development of CRC, indeed inflammatory bowel disease (IBD), including, ulcerative colitis (UC) and Crohn's disease (CD) has been proven to be an independent risk factor for CRC. To address the relationship of IBD and CRC pathogenesis, the use of multiplex approaches can be applied to discover common cell types, populations, inflammatory pathways and spatial distribution of infiltrating immune cells that may help in ultimately predicting clinical response. We therefore performed a comprehensive tissue analysis using the Cancer Transcriptome Atlas (CTA) on the GeoMx® Digital Spatial Profiler (DSP, NanoString Technologies Inc. Fig. 1A). The CTA panel is designed to profile the global immune response and all aspects of tumor microenvironment biology, including the various inflammatory cells that participate in the establishment of the chronic inflammatory intestinal microenvironment required for the onset of colorectal cancer (Fig 1B).

ROI Epithelial and Stromal Distribution

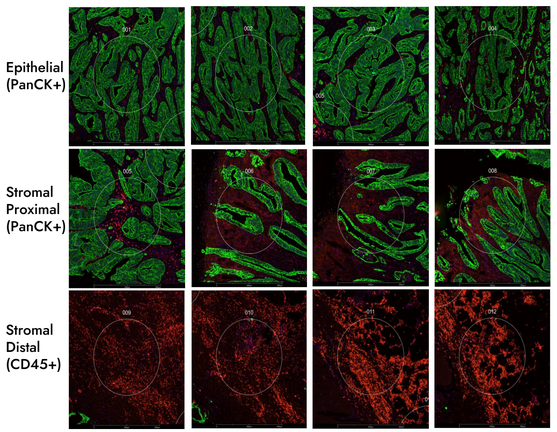

Fig. 2B: Distribution of ROIs. For each slide, 12 ROIs of 500 µM diameter circle were selected based on PanCK and CD45 staining. For each sample, 3 groups of ROI were selected, ROIs 1-4: geometric ROIs in PanCK+ region, ROIs 5-8: geometric ROIs in CD45+ region that are proximal to PanCK+ ROIs, ROIs 9-12: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

Methodology

For DSP analysis, a total of 20 FFPE samples including 5 CRC patients, 5 UC patients, 5 CD patients, and 5 matched normal samples were spatially profiled for up to 1,800 genes. Selection of regions of interest (ROI) was guided by both H&E staining and fluorescent markers (CD45, PanCK, Syto13, Fig 2A), and profiling of tumor and TME regions was achieved through segmenting by PanCK+/PanCK- (Fig 2B), followed by collection of indexed oligonucleotides and sequenced on the NextSeq 550 System (Illumina Inc.) For all samples, crypt and villus regions were selected for a detailed spatial analysis.

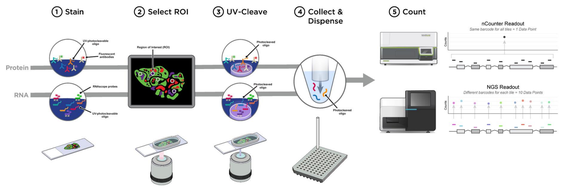

Fig. 1A: GeoMx DSP assay workflow

Gene Expression Profile Analysis

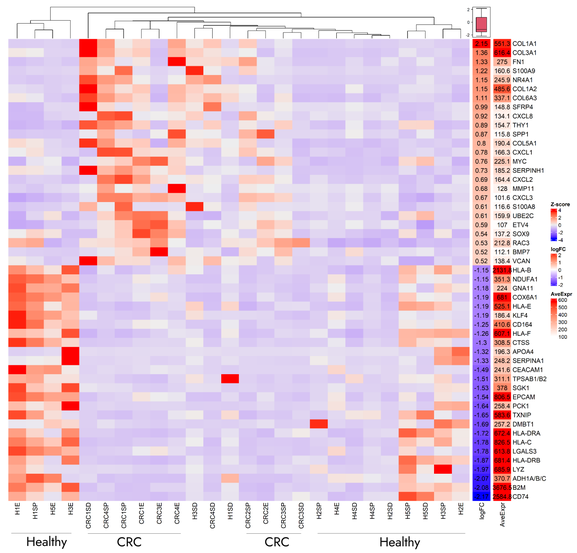

Fig. 3: Heatmap plot Clustering of top 50 genes differentially expressed across all ROIs for CRC and healthy groups. Several genes associated with inflammatory responses (CXCL1, 2,3, Rac3, Sox9) are up-regulated in CRC

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

Increase in Inflammatory mediators in CRC and IBD

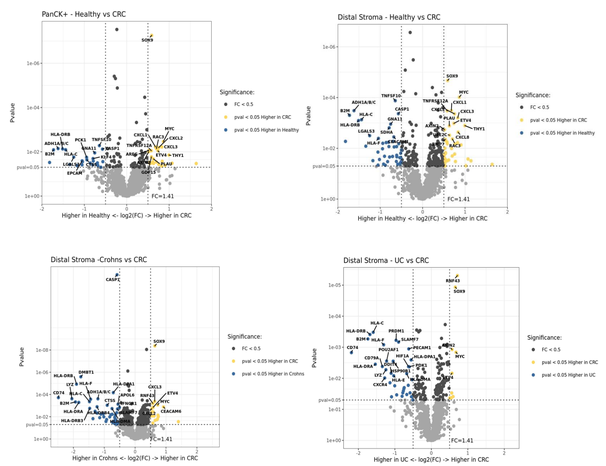

Fig. 4: Differential expression profiling of CTA panel targets. Volcano plot analysis was used to compare target expression between distal stroma ROIs and various indication samples. The dotted horizontal line represents the adjusted P-value cut-off. Differences in key markers for intestinal inflammation in IBD as well as CRC tumorigenesis were observed.

ROI Selection for GeoMx DSP

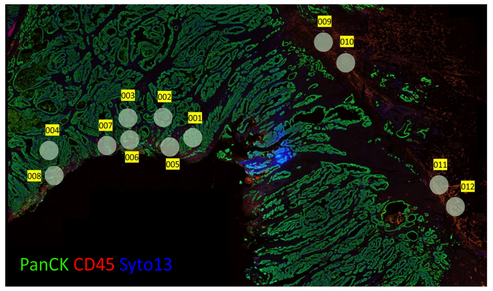

Fig. 2A: ROI selection for GeoMx CTA assay. A representative image showing ROI selection using PanCK and CD45 in a CRC FFPE sample.

Gene Pathway Enrichment Analysis

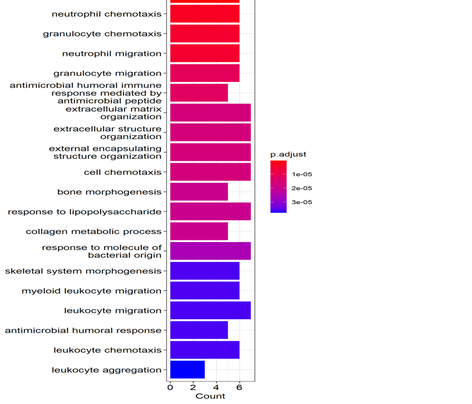

Fig. 5: Gene expression pathway enrichment analysis. Gene Ontology enrichment analysis was performed to identify key signaling pathways in CRC samples based on GeoMx CTA data

Key findings and Conclusions

-Differential expression analysis helped identify specific neutrophil associated inflammatory chemokines such as **CXCL1, CXCL2, CXCL3, Rac3** as well as other important targets such as **ETV4** and **SOX9** (Figure 3 & 4).
-Pathway analysis showed **neutrophil chemotaxis** and **neutrophil migration** targets significantly enriched in CRC samples (Figure 5).
-Spatial resolution was essential in understanding cellular function, and to link biologically relevant interactions to specific cell types, potentially, the highlighted chemokines could participate in the formation, mobilization, and recruitment of neutrophils.
-Future directions should focus on the role of **Tumor-Associated Neutrophils** as an important component of the TME in primary tumors.

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