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

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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 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 tumour 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).

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 sequencing on the NextSeq 550 System (Illumina Inc.). For all samples, crypt and villus regions were selected for a detailed spatial analysis.

ROI Selection for GeoMx DSP

Increase in Inflammatory mediators in CRC and IBD

ROI Epithelial and Stromal Distribution

Fig. 1B: GeoMx CTA panel curated content

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Fig. 1A: GeoMx DSP assay workflow

Gene Pathway Enrichment Analysis

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 cutoff. Differences in key markers for intestinal inflammation in IBD as well as CRC tumorigenesis were observed.

Gene expression 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.