(RE-) SOLVING THE BIOLOGY OF COLORECTAL CANCER ONSET AND PROGRESSION TO IMPROVE TREATMENT AND PREVENTION

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Background Colorectal cancer (CRC) development is accompanied by the gradual accumulation of genetic alterations in epithelial cells of the colon and rectum.^{1,2} The paradigm of the adenoma-carcinoma sequence was originally centered around cancer cells; however, it is now clear that the tumor microenvironment plays a substantial role in cancer progression and patient outcome.³ In recent years, technologies have evolved rapidly, allowing the multiplexed quantification of gene expression while preserving spatial context.⁴ Furthermore, some spatial transcriptomic technologies also allow the parallel interrogation of different cell populations in the tumor microenvironment. Here, we performed digital spatial profiling on early-stage CRC samples to elucidate the biological processes that are at the basis of malignant transformation and to identify novel therapeutic targets and (immune) biomarkers.

Methods Endoscopically resected early-stage CRC samples were obtained at Leiden University Medical Center. In total, 144 areas of illumination were interrogated with GeoMx digital spatial profiling using the Cancer Transcriptome Atlas (>1,800 genes). In each of eight samples, nine regions of interest with different levels of cancer progression were selected, including normal epithelium, transition areas, low-, and high-grade dysplasia, and invasive carcinoma (figure 1A). We segmented each region based on cytokeratin and vimentin protein expression (figure 1B). Immunohistochemical detection was performed on these samples and 26 additional samples to validate targets associated with disease progression.

Results Digital spatial profiling allowed us to dissect transcriptional alterations in epithelial and stromal fractions between different regions from healthy tissue, different degrees of dysplasia, and cancer. Gene expression data revealed a clear separation of profiled areas by histologic category. Interestingly, gene expression features in the stromal compartment provided a better data-driven separation of histologic categories than the epithelial fraction (figure 1C). Substantial changes in immune-related pathways were identified, including differential expression of specific immunomodulators. We validated the expression of several candidate biomarkers/targets that demonstrated consistent alterations from normal tissue to cancer by immunohistochemistry. Several proteins were identified that could clearly discriminate benign from malignant tissue.

Conclusions We here demonstrated the unique biological insights that are provided by spatial examination of early-stage CRC by digital spatial profiling. We identified specific genes that were altered during CRC tumorigenesis, in epithelial and stromal/immune fractions. Furthermore, our results indicate an essential role for innate immunity in colorectal cancer onset and progression. The genes identified by this approach could potentially serve as novel biomarkers and targets for early interception or prevention of CRC development.

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Trial Registration N/A

Abstract 673 Figure 1 Transcriptional alterations in early-stage colorectal cancer. Digital spatial profiling defines transcriptional alterations in early-stage colorectal cancer. (A) Schematic representation of an early-stage CRC sample containing regions with different levels of cancer progression, including normal epithelium, transition areas, low-, and high-grade dysplasia, and invasive carcinoma. (B) Segmentation based immunofluorescent labelling with antibodies directed against PanCK and Vimentin in one of the early-stage CRC samples. Artificial overlay of implemented segmentation is indicated for each ROI, visualizing Vimentin+ (pink) and PanCK+ (orange) segments. Inset: higher magnification of an individual ROI. (C) Dimension reduction of expression of all quantified genes by t-Distributed Stochastic Neighbor Embedding (tSNE). tSNE plots are annotated by segment (left), and histological region (right).

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Ethics Approval This study was approved by the METC Leiden-Den Haag-Delft (protocol B20.039). Patient samples were anonymised and handled according to the medical ethical guidelines described in the Code of Conduct for Proper Secondary Use of Human Tissue of the Dutch Federation of Biomedical Scientific Societies.

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