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Multiomic characterization of colorectal cancer using MICS technology reveals interaction of antigen presenting cancer associated fibroblasts and T cells

RNAsky Detection Probes

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Introduction

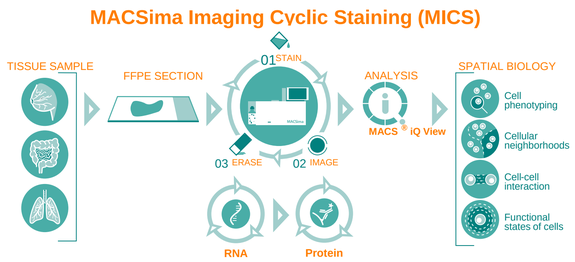

Fig 1. Same-section multiomics with MACSima Cyclic Staining (MICS) technology on the MACSima platform.

In solid tumors, the tumor microenvironment (TME) is composed of diverse cell types including cancer cells, immune cells, stromal cells, and other tissue specific cell types. The complex intercellular interactions that occur between these various cell populations determine cancer development and progression. Cancer-associated fibroblasts (CAFs) have been identified as key players in the TME, capable of promoting tumor cell growth and invasion, as well as manipulating immune responses. To better resolve potential subpopulations, spatial relationships, and signaling occurring between cell types within the TME, we performed same-section multiomic profiling of colorectal cancer (CRC) using the MACSima™ Imaging Cyclic Staining (MICS) technology.

We combined a custom 48plex RNA panel with a 40plex antibody panel to identify subsets of CAFs and characterize the activation state of immune cells. FFPE CRC specimens were reviewed by a pathologist for clinical assessment and region of interest selection. Then, gene expression profiles were generated using RNAsky™ technology, with each gene being detected by a single detection probe during cyclic rounds of detection probe hybridization, image acquisition, and signal erasure. Subsequently, fluorescently labeled antibodies were applied to the same section following an equivalent acquisition process. Finally, multiomic cell population analyses were performed using MACS® iQ View software. The analyses revealed four, spatially separated subpopulations of CAFs. Functional characterization of cellular neighborhoods and the cell-to-cell interactions occurring within the TME showed that one of the CAF populations potentially promoted cancer cell growth while another subpopulation, resembling antigen-presenting CAFs (apCAFs), closely interacted with T cells in the TME. These findings will deepen our understanding of tumor progression in colorectal cancer and potentially other solid tumors.

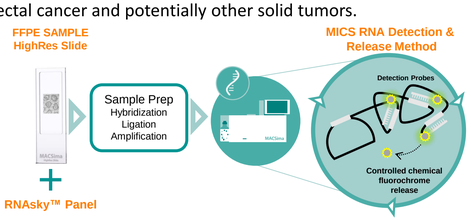

Fig 2. RNAsky molecular workflow.

Results

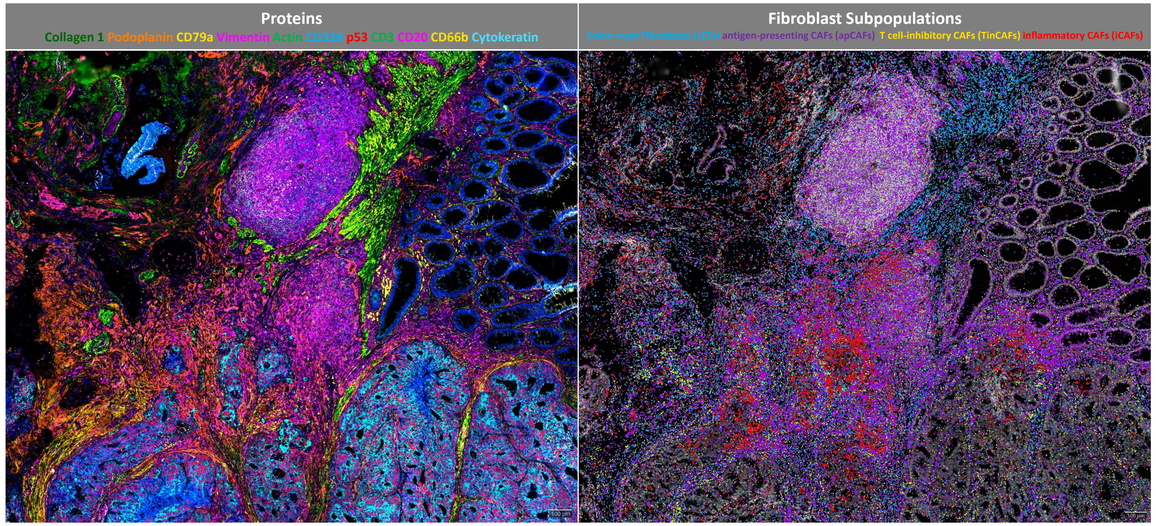

Fig 3. Investigating TME dynamics with MICS technology. High resolution imaging shows the interaction between immune, stromal, and cancer cells in CRC. We identified four fibroblast subpopulations with spatially distinct expression patterns in the tissue section, including a previously undescribed population of CAFs that we termed T cell-inhibitory CAFs. The fibroblast subpopulations were identified based on the expression of four unique hallmark genes per population. Colon crypt fibroblasts (cCFs, blue) were distributed throughout the sample and showed enrichment in areas with positive Actin staining. Antigen-presenting CAFs (apCAFs, purple) also had a broad distribution and showed high expression in regions with dense immune cell presence. Conversely, inflammatory CAFs (iCAFs, red) and novel T cell-inhibitory CAFs (TinCAFs, yellow) had more sparse and localized expression. Scale bar = 100 µm.

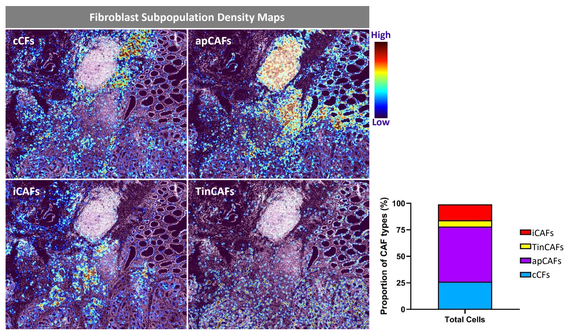

Fig 4. Density maps of fibroblast cell subpopulations show distinct patterns. cCFs were found throughout stromal regions of the sample and represented 26% of identified fibroblast cells. apCAFS were the most abundant subpopulation (52%) and exhibited the highest density in immune cell rich regions including the tumor edge and a tertiary lymphoid structure (TLS). iCAFs represented 15% of identified CAFs and were localized to tumor edges. TinCAFs were the sparsest population (6% of cells), with the highest density of cells located in close proximity to tumor cells. Scale bar = 100 µm.

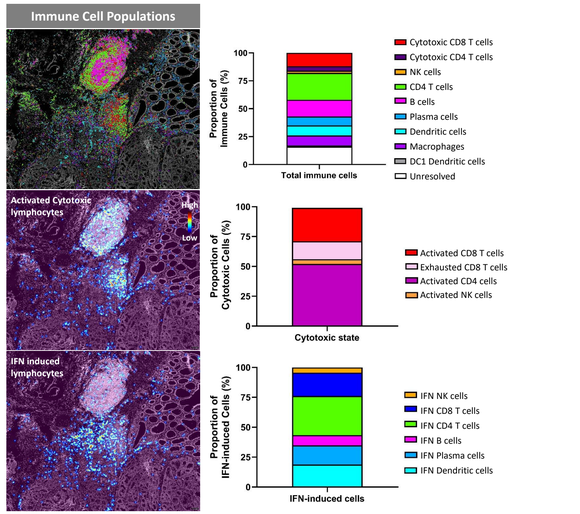

Fig 5. Immune cell population analysis. Immune cells were identified based on protein and gene expression. There is a prominent lymphocyte presence at the tumor edge, as well as a TLS distal to the tumor. To further resolve immune dynamics, we evaluated immune cell activation states. Cytotoxic lymphocytes represented 19% of immune cells, and of these 36% were activated, as defined by the presence of two hallmark genes. The highest density of activated cytotoxic lymphocytes was at the tumor edge and in the TLS. Interferon (IFN) induced lymphocytes were identified by the presence of two additional hallmark genes, represented 5% of immune cells, and were also densest at the tumor edge. Scale bar = 100 µm.

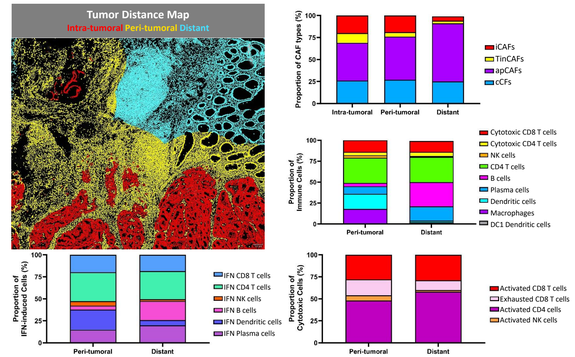

Fig 6. Distance map analysis. We analyzed the spatial distribution of CAFs and immune cells by defining intra-tumoral, peri-tumoral, and distant areas relative to the tumor. iCAFs and TinCAFs showed the highest concentrations intra and peri-tumoral regions. cCFs were evenly distributed, regardless of proximity to the tumor, and apCAFs were more concentrated in the peri-tumoral and distant regions. For our analysis, we established exclusion gates between immune and cancer cells, so immune analyses focused on the peri-tumoral and distant regions. Both cytotoxic lymphocytes and CD4 T cells were present in similar quantities in the peri-tumoral and distant regions, whereas macrophages and dendritic cells showed highest concentrations in the peri-tumoral region. B cells were concentrated in the distant region due to the presence of a TLS. IFN-induced and activated cytotoxic NK cells were most concentrated in the peri-tumoral area. The highest amount of exhausted CD8 T cells was also in the peri-tumoral region. Scale bar = 100 µm.

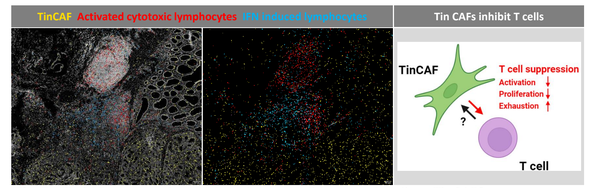

Fig 7. TinCAFs and activated lymphocytes did not colocalize. TinCAFS were present in intra- and peri-tumoral regions, but were absent in areas with high density of activated lymphocytes. We hypothesize that TinCAFs are immuno-suppressive and act to inhibit T cells via the immune-interacting function of the hallmark genes for this population. Scale bar = 100 µm.

Conclusions and Outlook

Miltenyi's MICS technology generates highly specific and quantitative protein signatures and gene expression profiles for investigating TME dynamics. We assessed the heterogeneity of fibroblasts and immune cells in colorectal cancer, and identified a novel immunosuppressive CAF subpopulation. Due to the immune-interacting genes that define this novel CAF population, TinCAFs show promise as a possible target for future immunotherapies. Same-section, spatial tumor profiling has the potential to improve biomarker identification, tumor cell phenotyping, and clinical prognoses for patients.