Phenotypic characterization of the tumor microenvironment in patients with colitis-associated cancer using multiplexing immunofluorescence

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Abstract: Inflammatory bowel diseases (IBD) are chronic multifactorial disorders of the intestine, divided into Crohn's disease (CD) and ulcerative colitis (UC). Due to chronic inflammation, one of the most severe complications is the development of colorectal cancer (CRC).

Tumor evolution is strongly influenced by the function of different cell subtypes present in the tumor microenvironment (TME). Among these, the immune infiltrate appears to have a great impact on cancer progression, and its composition is implicated in the clinical outcome of patients. Therefore, in recent years efforts are being made to develop tools to study the composition and organization of the TME to determine which components appear to be most involved in colon cancer progression to identify new molecular targets for cancer prevention and therapy. TME composition studies are commonly performed on fresh or frozen tissue samples, which, however, are not durable and therefore allow only limited analysis. In addition, because most samples stored in biobanks are formalin-fixed and paraffin-embedded (FFPE), new approaches for the analysis of such samples are becoming increasingly popular.

The purpose of this study was to analyze the composition of the TME, with a focus on the immunologic component, of FFPE samples of colitis-associated cancer (CAC) from 19 patients at different stages of the disease and with different tumor localization.

A microarray of tumor tissue was studied by analyzing 27 cellular and molecular markers associated with components of the immune system or the tumor itself, using the multiplex iterative staining by neoantibody deposition (MILAN) protocol. This multiplexing immunofluorescence technique exploits a sequential antibody marking and removal system for visualization and study of different antigens on the same tissue.

Each sample was subjected to several rounds of four-color indirect immunofluorescence staining, image acquisition and antibody removal, thus providing multiplexing analysis. Quantitative image analysis was performed using the HALO® Indica Labs platform.

This technology made it possible to analyze the cohort of CAC patients and characterize the tumor cells and specific immune subpopulations present in the TME. Specifically, it was observed that some patients had a greater degree of immune infiltrate than others, and heterogeneity emerged in the distribution of the different populations, regardless of the stage and location of the tumor and the gender of the patients. In addition, some cellular components, resident in the tissue, characterized by the production of cytokines associated with tumor proliferation, such as interleukin 22 (IL-22), and localized near the tumor area were observed.