Mapping the cellular architecture of the tumor microenvironment by integrating hyperplex immunofluorescence and automated image analysis.

Imaging

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The tumor microenvironment (TME) is composed of malignant cells and the surrounding healthy counterpart. The precise identification of the TME components is crucial to understanding how this microecosystem remodels during tumorigenesis and responds to treatment in order to identify its vulnerabilities and treatment opportunities. In the past decade, significant efforts have been made to describe the TME using RNA-based technologies. However, RNA-based biomarker expression profiling has limited relevance as it might not always accurately reflect the actual protein levels.

The COMET™ platform is an automated instrument that allows the detection of up to 40 antigens on a single slide using sequential immunofluorescence staining. By integrating multiplex immunofluorescence technology, we profiled the expression of 40 protein biomarkers across a tissue microarray composed of primary lung tumors and their corresponding metastatic lymph nodes. The combination of the hyperplex panel with an automated image and data analysis pipeline based on an unsupervised machine learning clustering algorithm allowed for the identification of several classes of immune cells with preferential accumulation sites. We identified distinct myeloid cells that coexist within the TME but infiltrate to a higher extent either the primary tumor or the metastatic loci. Harnessing the same approach, we also observed a higher frequency of T regulatory cells in the primary tumors. Subsequently, newly identified population frequencies determined by unsupervised clustering was confirmed by a complementary approach of supervised single-cell analysis.

Our data highlights the potential that microfluidics-based multiplex technology brings into the fields of both digital pathology and immuno-oncology, thanks to its single-cell resolution and the simultaneous detection of multiple protein biomarkers. We demonstrate here how the combination of hyperplex images obtained using the COMET™ platform, along with machine learning clustering analysis, results in an easy workflow for analyzing the complex TME and obtaining a single-cell atlas of tissue specimens.