IMAGING MASS CYTOMETRY IDENTIFIES IMMUNO-ONCOLOGY-BASED PATHOPHYSIOLOGICAL FEATURES OF THE MOUSE TUMOR TISSUE MICROENVIRONMENT

Thomas Pfister*, Christina Loh, Michael Cohen, Sam Lim, Qanber Raza. Standard BiTools, Toronto, Canada

Background Mouse tumor models are widely utilized as the preferred model organism for cancer studies and pre-clinical drug development. An obstacle in predicting therapeutic drug efficacy is the ability to quantitatively evaluate the multi-parametric post-treatment response in the tumor microenvironment (TME). Particularly, identification of immunological and oncological processes that dictate tumor growth, metastasis and immune response are essential for selecting promising drug candidates for further clinical evaluation. Imaging Mass Cytometry™ (IMC™) enables analysis of 40-plus distinct tissue and cellular markers simultaneously on tumor samples, providing a thorough assessment of the spatial landscape of the TME on a single slide without spectral overlap or background autofluorescence. Here, we showcase application of the Mouse Immuno-Oncology (IO) IMC Panel Kit for identification of pathophysiological features of the mouse TME.

Methods We compiled the Maxpar® OnDemand™ Mouse Immuno-Oncology IMC Panel Kit to quantitatively assess IO-related processes and applied it to a tissue microarray containing multiple mouse tumors. Antibodies in panel kits were selected from the Maxpar and Maxpar OnDemand catalog. Data acquisition was performed using a Hyperion™ Imaging System (Standard BioTools™). To facilitate cell segmentation, the IMC Cell Segmentation Kit (Standard BioTools) was applied to enhance nuclear and cell membrane demarcation. Single-cell analysis was completed using a custom MATLAB® script for pixel classification, CellProfiler™ for cell segmentation and histoCAT™ for PhenoGraph clustering.

Results The Maxpar OnDemand Mouse Immuno-Oncology IMC Panel Kit successfully identified pathophysiological processes such as immune cell infiltration and activation, signaling pathway activation, biomarkers of epithelial to mesenchymal transition, metabolic activity, growth and the tissue architecture of the TME. Single-cell analysis of non-small-cell lung carcinoma and B cell lymphoma separated distinct cellular clusters representing tumor, immune, stromal and vascular cells. Based on expression of activation markers in the panel kits, cellular processes associated with signaling, growth and metastasis were identified in tumor cells. In addition, cytotoxic and inflammatory activation in lymphoid and myeloid immune cell subtypes was detected.

Conclusions Application of IMC based multiparametric analysis successfully identified the spatial landscape of the TME at single-cell resolution. Quantitative analysis of tumor composition revealed critical insights regarding prognostic parameters such as metastatic and growth potential of tumors, and identification and activation of immune cell infiltrates. Overall, this work demonstrates the power of IMC technology and provides evidence of its successful application in mouse tumor models.