49 IMMUNO-ONCOLOGY IMAGING MASS CYTOMETRY STUDY OF THE STRUCTURAL AND CELLULAR COMPOSITION OF THE TUMOR MICROENVIRONMENT IN HUMAN CANCERS

Thomas Pfister*, Christina Loh, Sam Lim, Qanber Raza, Shaida Ouladan, Nick Zabinyakov.
Standard BiTools, Toronto, Canada

Background Multiplex imaging is becoming an increasingly valuable tool to study the tumor microenvironment (TME) in immuno-oncology. Imaging Mass Cytometry™ (IMC™) provides a means for visualization and analysis of the complex spatial biology of the TME. IMC permits detailed assessment of cell phenotype and function using 40-plus markers simultaneously at subcellular resolution on a single slide without spectral overlap or background autofluorescence. The ability to analyze multiple markers can facilitate accurate prediction of disease progression and clinical outcome measures from precious tissue samples and microarrays. Here, we demonstrate how our reagents support these applications.

Methods Antibodies targeting T cells, natural killer cells, myeloid cells, immune checkpoint markers, epithelial-to-mesenchymal transition (EMT) and extracellular matrix (ECM) proteins were used to study the immune microenvironment in multiple cancer histologies. Antibodies were selected from the Maxpar® and Maxpar OnDemand™ catalog. Data acquisition was performed using a Hyperion™ Imaging System (Standard BioTools™). To facilitate cell segmentation, an IMC Cell Segmentation Kit (Standard BioTools) was applied to enhance nucleus and cell membrane boundaries. Single-cell analysis was completed using custom MATLAB® script for pixel classification, CellProfiler™ for cell segmentation and histoCAT™ for phenograph clustering.

Results IMC analysis of human cancer tumor microarrays (TMAs) stained with panels of Maxpar and Maxpar OnDemand antibodies is presented here. We identified major cell populations and phenotypes via clustering (PhenoGraph). Immune cell phenotype and ECM staining data are overlaid on IMC images and complemented with t-SNE embeddings and signal intensity heat map. Additionally, we classified the activation state of immune cell populations, EMT progression and molecular composition of the TME ECM.

Conclusions Highly multiplexed IMC has enabled us to compare the TME across cancer histologies. Spatial analysis of the tumor architecture revealed details of tumor immune cell interaction at single-cell resolution. This work demonstrates the capability of IMC for quantitative and spatial identification of multiple immune parameters and detailed analysis of the TME from a single slide from cancer patients.

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