Spatial Proteomic Profiling of Metabolic and Cell Signaling Heterogeneity in Breast Cancer Tumor Microenvironment Using Imaging Mass Cytometry

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INTRODUCTION

The tumor microenvironment (TME) is a complex cellular ecosystem, which in turn influences tumor development and treatment response. Understanding cellular interactions within the TME is essential for elucidating disease progression and advancing immunotherapy. Imaging Mass Cytometry™ (IMC™) technology is a spatial biology imaging technique that enables deep characterization of the TME. This approach offers scalable and high-throughput acquisition while generating high-quality data without fluorescence-based limitations such as spectral overlap and autofluorescence.

We used IMC technology to map key pathways in metabolic reprogramming and signaling within the TME by utilizing a ready-to-use immuno-oncology IMC panel. This allowed us to investigate processes that regulate energy production, cellular homeostasis and mitogenic signaling pathways. We acquired data using Preview Mode to assess the whole tissue, followed by higher-resolution imaging of selected regions of interest (ROIs) using Cell Mode or whole tissue section imaging using Tissue Mode.

Materials and methods

The 41-marker panels used in this study were created by adding commercially available Metabolism or Cell Signaling expansion panels and single antibodies to the Human Immuno-Oncology IMC Panel, 31 Antibodies. This expands our ability to conduct comprehensive high-plex tumor and immune cell profiling. Whole tumor tissue sections were stained with this comprehensive antibody panel. Tissue Mode imaging of whole slide tumor sections, combined with pixel-clustering analysis, provided a spatially resolved quantitative assessment of specific tumor and immune components within the TME. This approach was further enhanced by a quick tissue scan using Preview Mode, which was used to guide single-cell analysis of selected ROIs in serial tissue sections that were acquired at single-cell resolution using Cell Mode. Together, these methods successfully delivered quantitative spatial biology analyses.

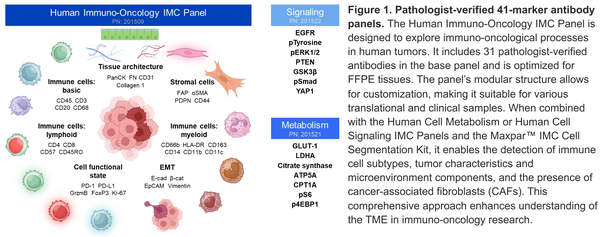


Figure 1. Pathologist-verified 41-marker antibody panels. The Human Immuno-Oncology IMC Panel is designed to explore immuno-oncological processes in human tumors. It includes 31 pathologist-verified antibodies in the base panel and is optimized for FFPE tissues. The panel's modular structure allows for customization, making it suitable for various translational and clinical samples. When combined with the Human Cell Metabolism or Human Cell Signaling IMC Panels and the Maxpar™ IMC Cell Segmentation Kit, it enables the detection of immune cell subtypes, tumor characteristics and microenvironment components, and the presence of cancer-associated fibroblasts (CAFs). This comprehensive approach enhances understanding of the TME in immuno-oncology research.

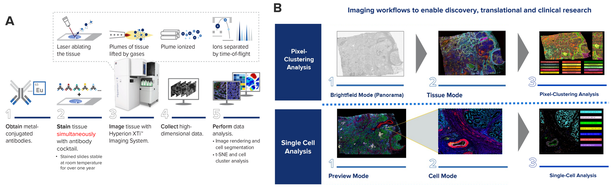


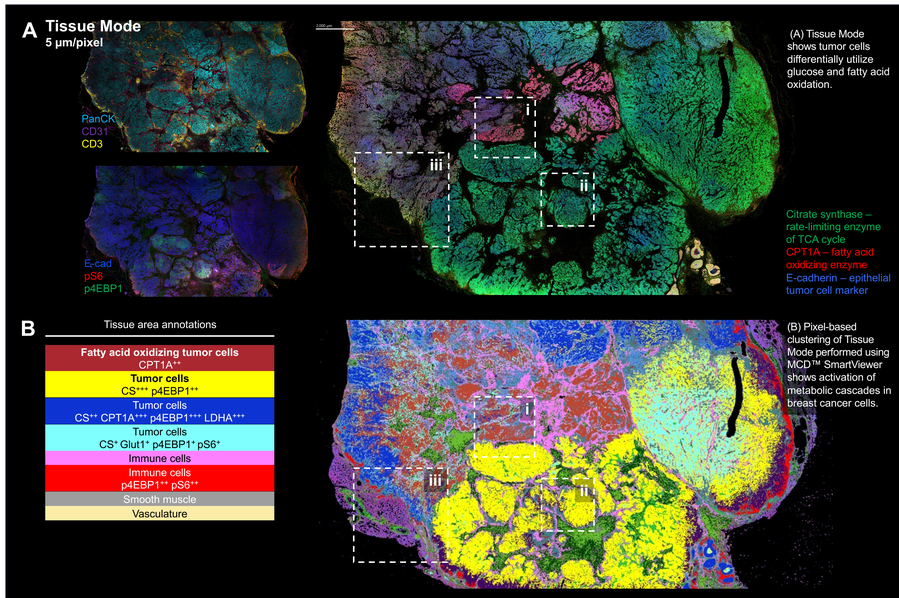
Figure 2. Imaging Mass Cytometry workflows. (A) IMC technology offers a streamlined workflow that simplifies translational and clinical application of multiplexed tissue analysis. The five-step process, which consists of obtaining metal-conjugated antibodies, staining tissues with antibody cocktails, imaging tissues with the Hyperion™ XTi Imaging System, and the collection and analysis of high-dimensional data, can be accomplished in as little as 72 hours (two slides with two 4 mm^2 ROI each). (B) The novel whole slide imaging modes for IMC technology offer a customized workflow for specific imaging applications. Here we highlight two simple ways for a user to get started. For single-cell analysis, start with Preview Mode, which provides a rapid scan of the whole tissue and highlights all your stained markers. This helps guide ROI placement to capture single cell-resolution image data using Cell Mode. For pixel-clustering analysis of an entire tissue section, users can first identify the placement of tissue using the rapid Brightfield Mode, followed by the novel Tissue Mode, which generates a high-quality scan of the entire tissue section in a matter of hours with higher spot-size ablations enabling entire tissue analysis using pixel-clustering analysis. Combining these new workflows with the newly available slide loader for the Hyperion XTi Imaging System streamlines IMC application and makes it a useful resource for high-throughput clinical and translational studies.

CONCLUSION

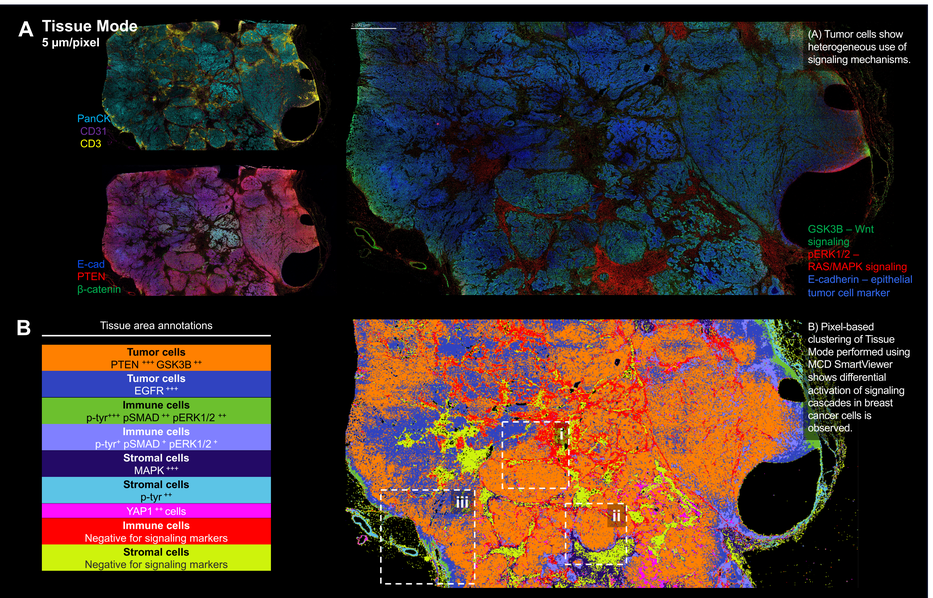
Spatial biology profiling using IMC highlights the interconnected roles metabolism and cell signaling pathways play in promoting tumor survival and resistance to therapies. These findings are crucial for developing future prognostic assessments and have the potential to guide more effective, personalized cancer therapies.

Results

Metabolic activity in breast invasive ductal carcinoma



Cell signaling in breast invasive ductal carcinoma



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