1490 HIGH-PLEX CO-DETECTION OF RNA AND PROTEIN TO EXPLORE TUMOR-IMMUNE INTERACTIONS UTILIZING RNASCOPE WITH IMAGING MASS CYTOMETRY

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Background Future advancements in immuno-oncology will be propelled by the tools capable of deciphering the spatial organization of distinct cell types within the tumor microenvironment (TME). Imaging Mass Cytometry™ (IMC™) has proven its effectiveness in studying complex cellular interactions within the TME. By utilizing CyTOF® technology, IMC allows for the simultaneous assessment of over 40 protein markers with subcellular resolution, eliminating spectral overlap and background autofluorescence. However, the inclusion of certain targets in IMC is impossible if there are no commercially available antibodies that successfully detect these protein targets or if the targets are soluble factors such as cytokines and chemokines. Here we present a new workflow that synergizes the highly sensitive and specific RNAscope™ technology for RNA detection with IMC multiplexing capability to visualize crucial RNA and protein markers simultaneously.

Methods To evaluate the expression of both RNA and protein targets in human FFPE tumor tissue microarrays (TMAs), we combined the RNAscope HiPlex v2 assay with protein detection on the Hyperion XTi™ Imaging System. The RNAscope assay employed 12 target RNA marker probes and their associated metal-labeled detection probes, specifically designed for compatibility with IMC. The recommended workflow for the RNAscope HiPlex v2 assay was followed, with the exception that for RNA detection, metal-conjugated probes were used instead of fluorophores. Metal-conjugated antibodies were used to detect proteins within the same tissue, resulting in a combined 31-marker co-detection panel.

Results The identified target protein markers encompassed a diverse range of extracellular matrix, immune, tumor, stromal, and endothelial cells. Detection of RNA enabled the visualization of various cytokines and chemokines, including *CXCL13*, *CXCL9*, *CXCL10*, *IFNγ*, *IL10*, and *IL8*, thereby facilitating the identification of the cellular sources for these secreted factors. Additionally, the use of marker-specific antibodies allowed for the visualization of immune cell subpopulations and their activation states. Immune cell hubs associated with anti-tumor immune responses were detected in tumor niches throughout the TMA.

Conclusions By integrating RNAscope with the IMC platform, we achieved simultaneous visualization of RNA and protein targets on the same sample to investigate the TME. The superior sensitivity for RNA detection offered by the RNAscope assay unlocks targets previously inaccessible through antibody detection. Thus, this new workflow complements existing multiplexing capabilities of IMC.

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