

Poster 22 Spatial Multiomics Reveals T-cell Activation and Exhaustion States in the Tumor Microenvironment

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Spatial Multiomics allows for context-based study of the tumor microenvironment (TME). TME consists of diverse cells that collectively influence tumor progression, therapeutic response, and immune activation. CD8 T-cells, essential for antitumor immunity, often become exhausted in the TME. Recent studies show that PD1+ TCF1+ stem-like CD8 T-cells retain differentiation potential and can be rejuvenated through cytokine signaling and immune checkpoint modulation. This detailed spatial profiling of RNA and protein expression in the TME is crucial to understanding immune cell function, activation, and differentiation. Here we introduce a protease-free workflow for RNAscope™ Multiplex Fluorescent v2 assay combining RNA *in-situ* hybridization (ISH) and protein immunofluorescence to study cell marker proteins (CD8, PD1) with RNA targets (*TNFA*, *TCF7*, *IFNG*) in a tumor microarray (TMA). The Manual Multiplex Protease-Free workflow enables simultaneous RNA and protein visualization without enzymatic disruption, preserving antigen integrity and tissue morphology.

Using this Multiomics assay we can study CD8 T-cell differentiation and activation. Co-detection of *TNFA*, *TCF7*, and *IFNG* transcripts with CD8 and PD1 proteins provides insights into specific T cell phenotypes and their activation/exhaustion status. Data reveal robust co-expression patterns of these targets, particularly in the tumor-cell neighboring stromal and immune cells in breast, cervical and stomach cancers, reflecting immune activation and exhaustion across distinct TME niches. This methodology lays a foundation for refining immunotherapeutic strategies targeting PD1+ TCF7+ CD8 T-cells, enhancing effector functions, and advancing precision oncology interventions.