Characterizing tumor-infiltrated immune cells with spatial context using RNAscope ISH-immunohistochemistry co-detection workflow in FFPE tissues

Genomics

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Complex tissues such as tumors are comprised of multiple cells types and extracellular matrix. Characterizing heterogenous populations of tumor-infiltrating immune cells requires a multi-omics approach. Here we demonstrate a newly developed integrated in situ hybridization (ISH) and immunohistochemistry (IHC/IF) workflow that can substantially improve RNA-protein co-detection, enabling the visualization and characterization of tumor immune infiltrates at single-cell and spatial resolution.

To characterize tumor-infiltrating immune cells in a tumor TMA (tumor microarray), we utilized the RNAscope Multiplex Fluorescence assay in combination with the RNA-Protein Co-detection Kit to detect multiple immune cell populations. Immune cells such as macrophages, T cells and NK cells were detected using antibodies against CD68, CD8, CD4 and CD56 in combination with probes targeting CCL5, IFNG, GNZB, IL-12, NCR1 etc.

We identified CD4+ regulatory T cells and CD8+ cytotoxic T lymphocytes. Additionally, we determine the activation states of CD8+ T cells by visualizing IFNG and GZMB expression. Furthermore, infiltrating macrophages were detected by CD68 protein expression while the M1 and M2 subsets were differentiated by using the M2-specific marker, CD163. NK cells were identified by detecting CD56 protein in combination with CCL5 and NCR1 RNA expression. The degree of immune cell infiltration varied based on the tumor type.

In conclusion, the new RNAscope-ISH-IHC co-detection workflow and reagents enable optimized simultaneous visualization of RNA and protein targets by enhancing the compatibility of antibodies, including many previously incompatible antibodies with RNAscope . This new workflow provides a powerful approach to identifying and characterizing tumor infiltrating immune cells.