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

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INTRODUCTION

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.

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.

METHODS

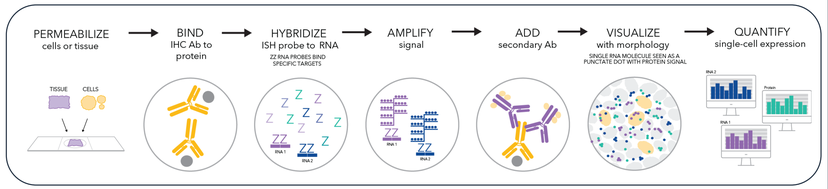

Figure 1: Co-detection of RNA and protein using RNAscope ISH-IHC/IF integrated workflow .

Samples: Tumor TMA (4 slides)
Assay: Codetection assay with RNAscope V2 multiplex LS
Probe Combinations:

Cell types	Probes	Antibody
Macrophages	***CD163 (C1), ITGAM-(C2)***	**CD68**
CTL	***IFNG (C1), GZMB (C2)***	**CD8**
Regulatory T cells	***IL-12 (C1), TGFB1 (C2)***	**CD4**
NK cells	***NCR1(C1), CCL5 (C2)***	**CD56**

RESULTS

1. The new integrated ISH-IHC workflow demonstrates significant improvement in the IHC signal when combined with RNAscope *in situ* hybridization assays

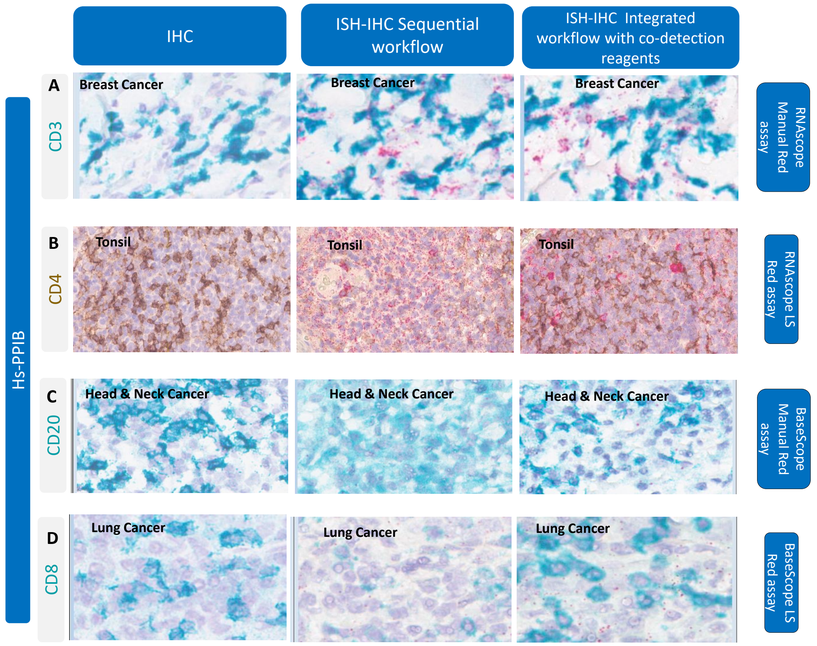

Figure 2: Comparing antibody signals between sequential vs integrated ISH-IHC workflows. A, CD3 IHC signal was robust for both sequential and integrated workflows in breast cancer tissue. **B,** CD4 IHC signal appeared dramatically reduced with the sequential workflow while the integrated workflow rescued CD4 IHC signal comparable to IHC-only assay in tonsil tissue. **C**, Sequential workflow demonstrated diffused CD20 IHC signal when combined with RNAscope ISH while the integrated RNAscope ISH-IHC workflow showed specific staining in head and neck tumor. **D**, CD8 IHC signal was negligible when CD8 Ab was combined with RNAscope ISH but the integrated workflow was able to rescue the CD8 signal in lung cancer. The RNAscope signal for *PPIB* was comparable for the sequential and integrated workflows for all the assays .

2. The new integrated ISH-IHC co-detection workflow can identify tumor infiltrating immune cells

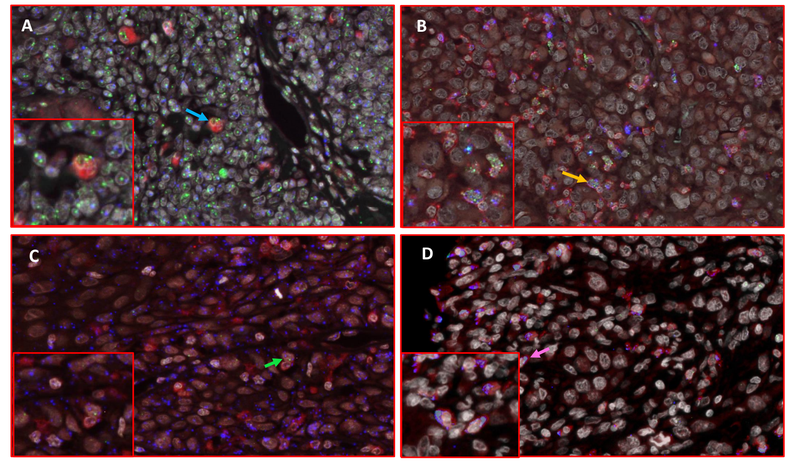

Figure 3: Detection RNA ad protein markers simultaneously enables identification of key immune cell populations in tumors using the co-detection assay. **A**, detection of M1 and M2 macrophages in head and neck cancer tumor with CD68 antibody and *CD163/ITGAM* probe combination. **B**, detection *of* potentially active cytotoxic T lymphocytes in lung cancer tumor with CD8 antibody and *CD8/IFNG* probe combination. **C**, , detection of regulatory T cells in head and neck cancer with CD4 antibody and *TGFB/IL-12* probe combination. **D,** detection of niche NK cells in breast cancer tumor with CD56 antibody and *NCR1/CCL5* probe combination. Arrows indicate either ↑ macrophage, ↑ CD8+ T cell, ↑ CD4+ T cell or ↑ NK cell.

SUMMARY

- The new integrated co-detection assays allows multi-omic profiling in-situ with single cell resolution.
- Combining RNAscope target probes with immune cell marker antibodies enabled detection of key tumor-infiltrating immune cells.
- The new RNA-protein Co-detection Ancillary kit improves IHC antibody compatibility with RNAscope and BaseScope assays creating an extremely powerful technique to answer unique biological questions.

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