Spatially resolve RNA and protein simultaneously in FFPE tumor samples by combining RNAscope in situ hybridization and immunohistochemistry assays

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INTRODUCTION

Spatially resolved gene expression has emerged as a crucial technique to understand complex multicellular interactions within the tumor and its microenvironment. Interrogation of complex cellular interactions within the tumor microenvironment (TME) requires a multi-omics approach where multiple RNA and protein targets can be visualized within the same tumor sample and be feasible in FFPE sample types. Simultaneous detection of RNA and protein can reveal cellular sources of secreted proteins, identify specific cell types, and visualize the spatial organization of cells within the tissue. Examination of RNA by in situ hybridization (ISH) and protein by immunohistochemistry (IHC) or immunofluorescence (IF) are widely used and accepted techniques for the detection of biomarkers in tumor samples. Given the similarities in workflow, co-detection of RNA and protein by combining ISH and IHC/IF in a single assay can be a powerful multi-omics solution for interrogating the complex tumor and its microenvironment. In this report we combined the single cell, single molecule RNA ISH technology known as RNAscope with IHC/IF to simultaneously detect RNA and protein in the same FFPE tumor section using both chromogenic and fluorescence detection methods. We demonstrate co-localization of target mRNA and the corresponding protein in human cancer samples, visualize infiltration of immune cells into the TME, characterize the activation state of immune cells in the TME, identify single cell gene expression within cellular boundaries demarcated by IHC/IF, examine cell type-specific expression of multiple immune checkpoint markers, and distinguish endogenous T cells from activated CAR+ T cells.

Overall, we show that co-detection of RNA by the RNAscope ISH assay and protein by the IHC/IF assay in the same FFPE section is a feasible methodology.

METHODS

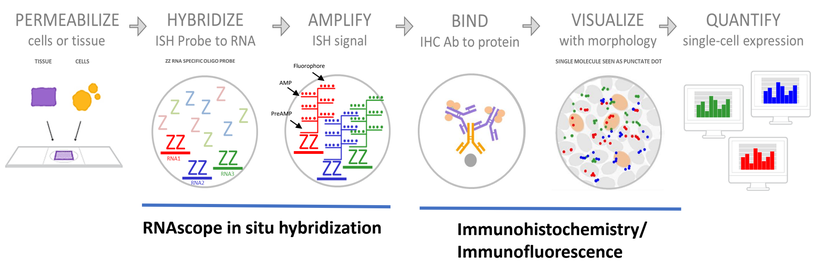

Figure 1: Dual RNAscope ISH-IHC/IF sequential workflow to detect RNA and protein simultaneously.

RESULTS

Visualization of immune cells and soluble factors in cervical cancer tumor microenvironment

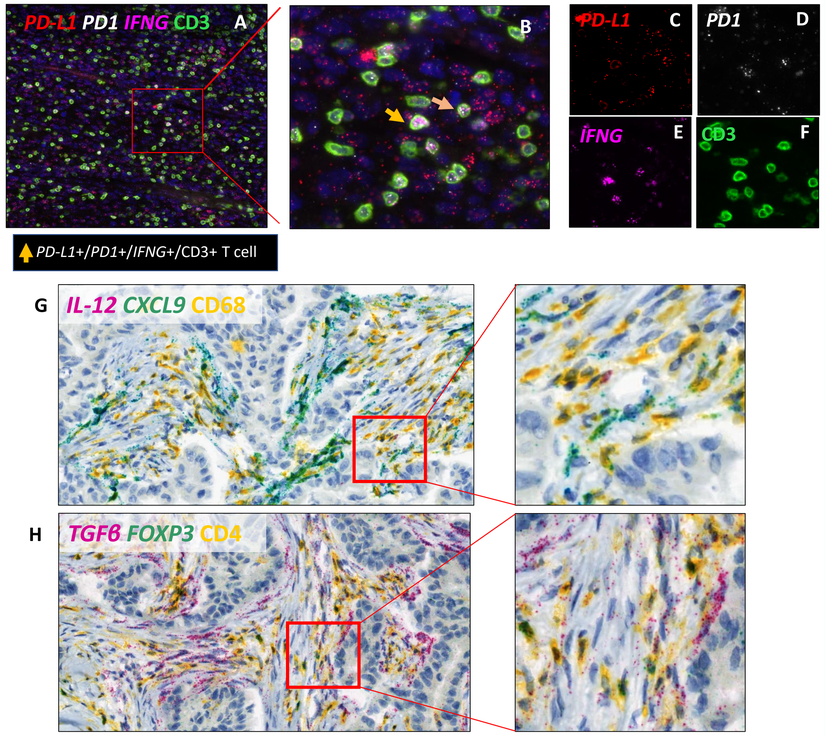

Figure 2: Detection of immune cells, chemokines and cytokines using RNA in tumor tissues. A, Cervical cancer tumor with high T cell infiltration as demonstrated by CD3 protein (green) and *IFNG* (pink), *PD1* (white) and *PD-L1* (red) mRNA expression. **B**, High magnification (40x) image showing CD3+/*PD1+* T cells expressing *IFNG* around *PD-L1* + tumor cells. **C-F**, High magnification (40x) individual images for *PD-L1, PD1, IFNG*, and CD3 in the same cervical cancer tumor section. **G**, RNA transcripts for secreted factors *IL-12* (red) and *CXCL9* (teal) were detected in combination with the macrophage marker protein CD68 (yellow). **H,** RNA transcripts for the secreted factor *TGFB* (red) and the transcription factor *FOXP3* (teal) were detected in combination with the T cell marker protein CD4 (yellow).

Detection of activated CAR-T cell trafficking in xenograft tumors

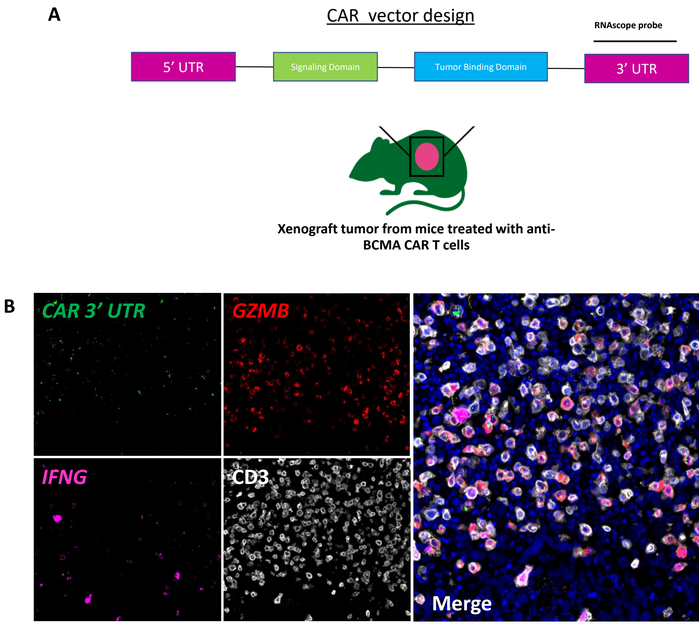

Figure 3: The RNAscope Multiplex Fluorescent V2 assay was combined with IF to visualize tumor infiltration of activated anti-BCMA CAR-T cells. A, RNAscope probe design for the *3' UTR* of the CAR vector of the anti-BCMA CAR-T cell therapy that was delivered to a pre-clinical mouse model with a xenograft tumor. **B,** RNAscope for *CAR 3' UTR* (green), *GZMB* (red), and *IFNG* (pink) was followed by IF for CD3 (white) in xenograft tumors from anti-BCMA CAR-T cell treated RPMI-8226 mice.

CONCLUSION

- This report demonstrates the ability of dual ISH-IF to detect activated T cells and visualize cellular source of secreted cytokines and chemokines.
- Dual ISH-IF/IHC can also be applied to study the trafficking and activation of engineered immune cells such as CAR T cells in tissues.
- The combined RNAscope ISH-IHC/IF workflow is a powerful technique that can be used to study gene expression signatures at the RNA and protein level with spatial and single cell resolution.

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