Spatial analysis of tumor-infiltrating immune cells with highly specific RNAscope™ RNA-protein Co-detection assays

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INTRODUCTION

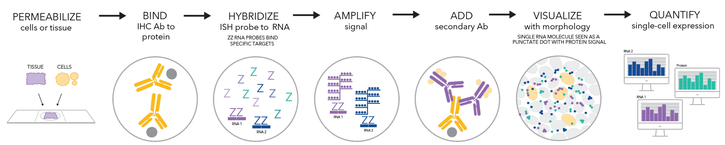
Interrogating complex tumor microenvironment requires a multi-omics approach that can provide high level of sensitivity and specificity. Identifying immune cell subsets within the tumor can be vital for predicting response and determining therapeutic efficacy. Here, we demonstrate a newly developed integrated ISH and IHC/IF (immunohistochemistry/ immunofluorescence) workflow compatible with manual and automated platforms that can substantially improve RNA-protein co-detection.

We demonstrate the use of our RNA-Protein Co-detection assay in combination with the automated RNAscope Multiplex Fluorescent v2 assay, automated RNAscope Chromogenic Duplex assay and manual RNAscope Multiplex Fluorescent v2 assay to detect T cell markers, macrophage markers and checkpoint markers in the tumor microenvironment by using a microarray with different tumor samples. We identified T cells subtypes and their activation states by visualizing *IFNG, GZMB and TNFA* expression. We were also able to identify macrophages detected by CD68 protein expression and the M1 and M2 subsets were differentiated by using the M2-specific marker, *CD163*. We could also delineate tumor-stroma border in the samples by using the Pan-CK probe which distinctly marks the tumor cells and visualize the expression of immunoregulatory receptors PD-L1 and *CTLA4* in the tumor cells.

Overall, 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 and requiring minimal optimization.

METHODS

RNAscope integrated co-detection workflow for simultaneous detection of RNA and protein biomarkers



RNAscope ISH-IHC integrated co-detection workflow for detecting RNA and protein targets on the same section of the tissue

Sample Type: FFPE Tumor TMA (Tissue Microarray)

RNAscope probes + antibody combinations to demonstrate ISH-IHC/IF with manual and automated assays

Combinations	RNA probe	RNA probe	RNA probe	Antibody	Platform
Combination 1	*PanCK*	*PD-1*	*CTLA4*	PD-L1	Multiplex Fluorescent, Manual
Combination 2	*TNFA*	*CCR5*	*IFNG*	CD4	Multiplex Fluorescent, Manual
Combination 3	*GZMB*	*IFNG*	-	CD8	Multiplex Fluorescent, Automated
Combination 4	*CD163*	*ITGAM*	-	CD68	Multiplex Fluorescent, Automated
Combination 5	*TNFA*	-	-	CD3	Chromogenic Duplex, Automated
Combination 6	*CCL5*	*NOS2*	-	CD8	Chromogenic Duplex, Automated

RESULTS

PD-L1 was predominantly expressed in the stromal region of breast cancer tissue while it was co-expressed with Cytokeratin in tumor cells of lung cancer tissue

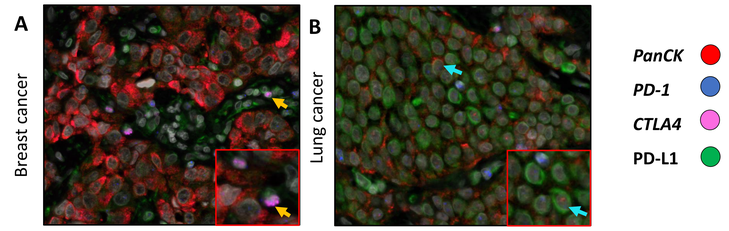


Figure 1: Manual RNAscope ISH-IF co-detection assay was used to visualize immunoregulatory markers in tumor tissues. A and B, expression of *PanCK*, *PD-1* and *CTLA4* was detected by RNAscope ISH while expression of PD-L1 was detected using IF. PD-L1+/*CTLA4*+ cells (▲) , PD-L1+/*PanCK* + cells (▲)

T helper 1 cells were characterized in human breast and lung cancer tissues

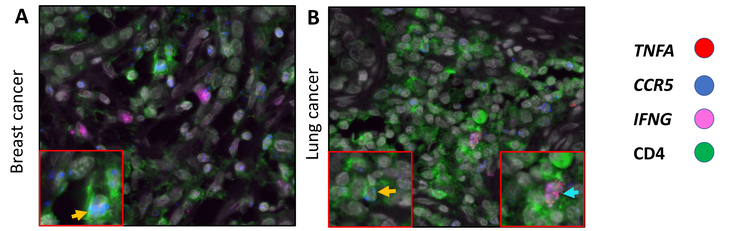


Figure 2: Manual RNAscope ISH-IF co-detection assay was used to visualize Th1 cells in the tumor microenvironment. A and B, expression of *TNFA*, *CCR5* and *IFNG* was detected using RNAscope ISH while CD4 was detected using IF in breast cancer and lung cancer tissues, respectively. CD4+/*TNFA*+/*IFNG*+ Th1 cell (▲), CD4+/*CCR5*+ T cell (▲)

Tumor infiltrating T cells and macrophages were detected in cancer tissues

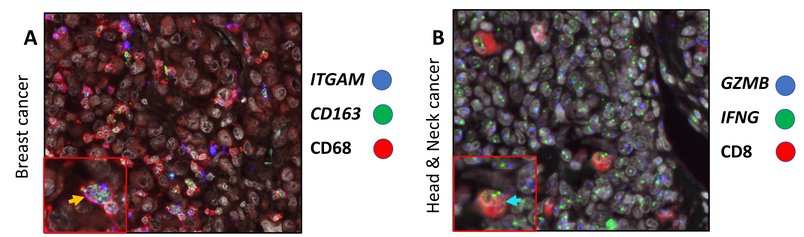


Figure 3: Automated RNAscope ISH-IF co-detection assay was used to detect T cells and macrophages. A, *ITGAM* and *CD163* were detected using RNAscope ISH while CD68 was detected using IF in breast cancer tissue, **B** , *GZMB* and *IFNG* was detected using RNAscope ISH while CD8 was detected using IF in head and neck cancer tissue. *ITGAM*+/*CD163*+/CD68+ (▲) , *GZMB*+/*IFNG*+/CD8+ (▲)

T cells and associated activation markers were visualized in breast cancer tissue

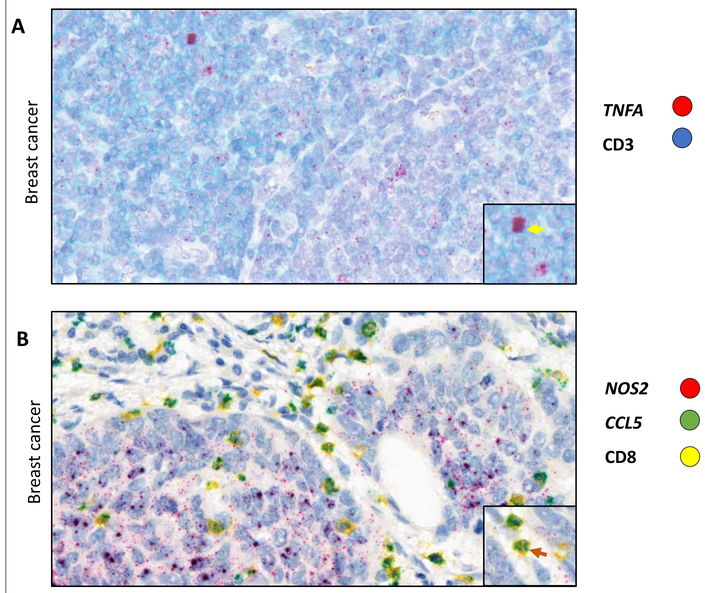


Figure 4: Automated Chromogenic RNAscope ISH-IHC co-detection assay was used to visualize T cells and associated cytokines. A, expression of TNFA was visualized by RNAscope ISH while CD3 was detected using IHC, **B,** expression of *NOS2* and *CCL5* was visualized by RNAscope ISH while CD8 was detected using IHC.CD3+/*TNFA*+ T cells (▲), CD8+/*CCL5*+ T cells (▲)

SUMMARY

- Using the RNAscope RNA protein co-detection assays we assessed the tumor microenvironment and characterized tumor infiltrated immune cells.
- We identified immune cell sub-populations using cell marker-specific antibodies and detected expression of activating chemokines and cytokines using RNAscope ISH.
- The co-detection reagents and workflow are compatible with **manual and automated platforms** and can be combined with most RNAscope, BaseScope and miRNAscope assays.

CONCLUSION

The new **RNA-protein Co-detection workflow** improves IHC antibody compatibility with the RNAscope ISH technology creating an extremely powerful multiomic solution to elucidate cellular heterogeneity, identify novel cell populations while retaining spatial information with morphological context.

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