In situ detection of PD-1$^+$ CXCL13$^+$ CD8$^+$ T cells in non-small cell lung cancer

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Introduction

Recent reports identified one subset of intratumoral CD8$^+$ cytotoxic T lymphocytes (CTLs) in non-small cell lung cancer (NSCLC) that are PD-1^{high} with distinct molecular and functional properties. Strikingly, these cells produce very high levels of CXCL13 mRNA and protein, which may mediate immune recruitment. Furthermore, the presence of PD-1^{high} CD8$^+$ T lymphocytes are strongly predictive for both response and survival in NSCLC patients treated with PD-1 blockade. Thus, it is of great value to develop a practical biomarker assay to specifically detect these cells in formalin-fixed paraffin-embedded (FFPE) tumor biopsies.

Design

Dual RNAscope® ISH-IF assay:

In this study, we combined the highly sensitive and specific RNAscope multiplex fluorescent RNA *in situ* hybridization (ISH) assay detecting *CXCL13* and *PDCD1* mRNAs with immunofluorescence (IF) detecting CD8 protein in a single tissue section to directly visualize PD-1$^+$ CXCL13$^+$ CD8$^+$ T lymphocytes in NSCLC tissue (figure 1 and 2). Two NSCLC tissue microarrays (TMAs) consisting of 63 independent patient FFPE samples were stained with full automation using the Leica BOND RX instrument. The resulting slides were scanned, and the images were analyzed using the Perkin Elmer Phenochart software.

57 of the 63 TMA cores were available for image analysis. Each tissue core was first examined under 4X magnification, then snapshot images of three independent 40X fields with enriched CD8$^+$ cells (if present) were taken. CD8$^+$ cells, CXCL13$^+$ cells and PD-1$^+$ cells in each snapshot were counted. Every snapshot contained both stromal and tumor regions.

Figure 1. The RNAscope assay and study workflow

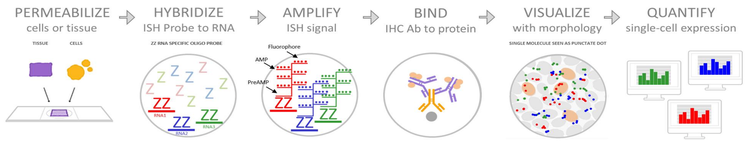
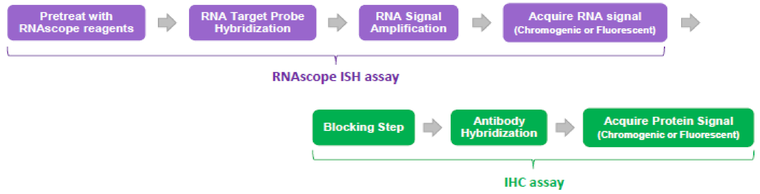


Figure 2. Multiplexed RNAscope dual ISH/IF assay workflow

The RNAscope ISH assay was performed first, followed by the IF assay. Signal was acquired with either chromogenic enzymes or fluorophores. Note: All dual ISH-IF protocols require optimization. It is advisable to perform ISH first followed by IF and also optimize IF separately using the RNAscope pretreatment reagents. Dual ISH-IF works better for highly expressed proteins due to the protease treatment used during the ISH protocol.



Results

Figure 3. Co-detection of *PDCD1* mRNA, *CXCL13* mRNA and CD8 protein in human non-small cell lung carcinoma (NSCLC) FFPE tissue

The RNAscope ISH assay for *PDCD1* (PD1) and *CXCL13* mRNA was performed firstly, followed by the IF assay for CD8 protein. Insets show higher magnification of two regions of interest, demonstrating co-localization of *CXCL13* mRNA (green), *PDCD1* mRNA (red) and CD8 protein (white) in the same cell.

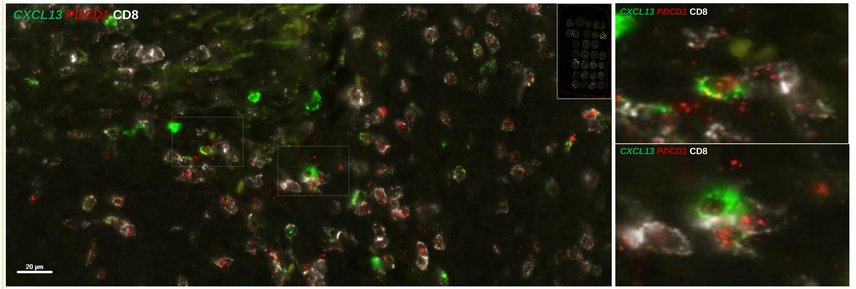
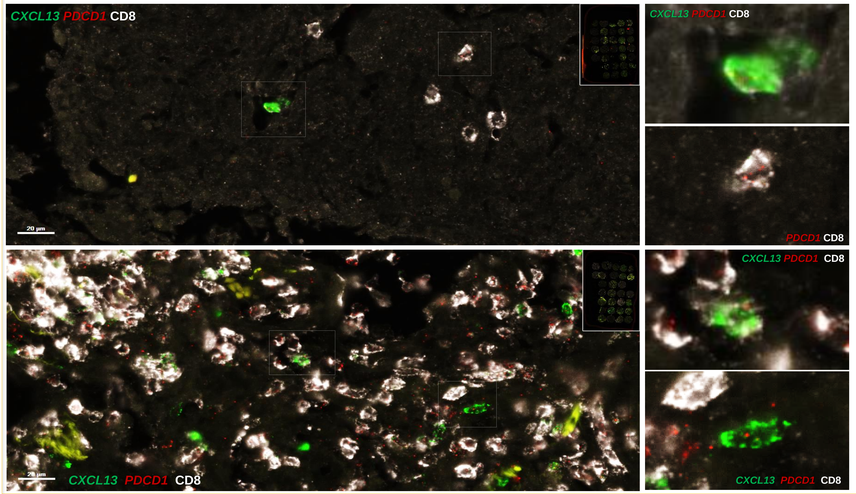


Figure 4. The presence of PD-1$^+$ CXCL13$^+$ CD8$^+$ T cells in both high and low CD8$^+$ infiltrating NSCLC detected by multiplexed RNAscope dual ISH-IF assay

The RNAscope ISH assay for *PDCD1* (PD1) and *CXCL13* mRNA was performed first, followed by the IF assay for CD8 protein. Insets show higher magnification of two regions of interest, demonstrating PD-1$^+$ CXCL13$^+$ CD8$^+$ T cells in both high and low CD8$^+$ CTLs infiltrating NSCLC tissue cores.



Results

Figure 5. The presence of PD-1$^+$ CXCL13$^+$ CD8$^+$ T cells in tumor stromal region and tumor region detected by multiplexed RNAscope dual ISH-IF assay

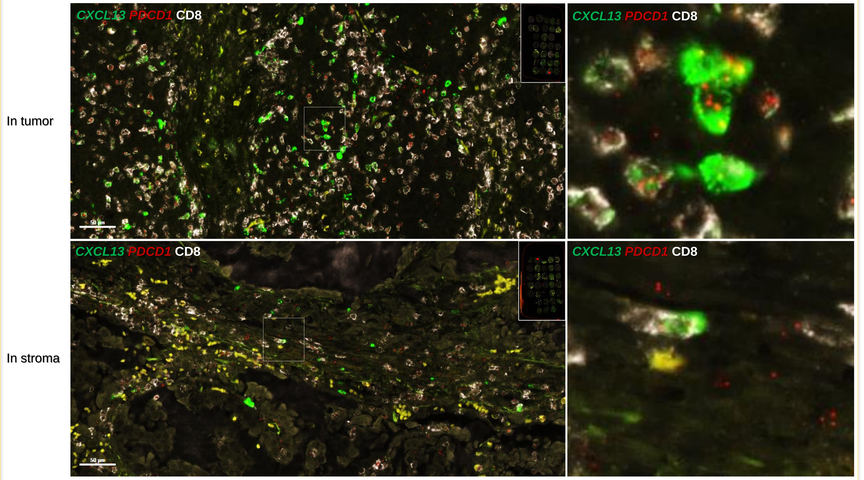


Table 1. The distribution of CD8$^+$ CTLs and PD-1$^+$ CXCL13$^+$ CD8$^+$ T cells in NSCLC TMAs

	>20 cells/sample core	<20 cells/sample core
CD8$^+$ CTLs	43	14
	>10% of CD8$^+$ CTLs/sample core	<10% of CD8$^+$ CTLs/sample core
PD-1$^+$ CXCL13$^+$ CD8$^+$	5	52

Conclusion

These results demonstrate that this fully automated multiplexed RNAscope dual ISH/IF assay allows for co-localization of RNA and protein biomarkers in single cells with morphological context. The ability to detect RNA and protein in a single slide-based assay enables immune profiling applications to include biomarkers such as secreted proteins and non-coding RNAs that are difficult or impossible to detect by IF (or IHC).