Investigating neuroinflammation in the human brain tumor microenvironment using the new RNAscope™ Multiomic assay

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Board Number: B968

Introduction

Glioblastomas are aggressive forms of brain tumors characterized by distinct genetic and molecular signatures. The complex tumor-immune interactions in the microenvironment impact the progression of these tumors and their response to therapy. Compared to other solid tumors, the role of immune cells in progression, invasion, and prognosis is not well studied for Central Nervous System (CNS) tumors. Understanding unique features of the brain tumor microenvironment requires a multiomic strategy to identify unique immune cells infiltrating the tumor and their dynamic interactions with other cells within the tumor.

Using the flagship single-cell spatial RNAscope technology, target gene and protein expression can be visualized to characterize cell types and tissue neighborhoods. Here, we demonstrate a novel method for the simultaneous detection of RNA and protein using a modified TSA-based multiomic assay that eliminates the need for protease for visualizing RNA molecules while preserving the epitopes for sensitive antibody targets. We visualized a few combinations of 3 RNA and three protein marker panels on human FFPE normal brain and brain tumor tissues. Antibodies targeting key immune cell markers such as CD8, IBA1, and CD68 were used. In addition, the protease-sensitive neuronal antibody NeuN was included in the panels. RNA probes targeting chemokines and cytokines such as CXCL10, IFNG, TNFA, and CXCL2. IL-6 was also used in the panels. Immune cells infiltrating the brain tumor tissues were characterized by studying co-expression of essential RNA and protein markers. The tumors demonstrated infiltration of immune cells such as T cells, microglia, and macrophages represented by expression of CD8, IBA1, and CD68 protein markers. In addition, the expression of cytokines was used to assess the activation status of these immune cells, which is an essential indicator for the potential success of specific therapeutic interventions.

Distinct differences in neuroinflammation signatures were also observed between the normal and tumor brain tissues. The assay offers a powerful technique for visualizing target RNA biomarkers in specific cell types identified by cell-marker protein expression. This tool is valuable for multiomic analysis and accurate interrogation of complex tissues such as the brain to obtain insights into novel prognostic and therapeutic biomarkers.

Method

RNAscope RNA-Protein Automated Multiomic Workflow

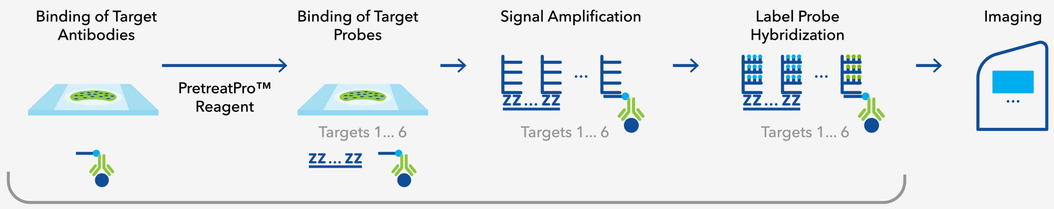


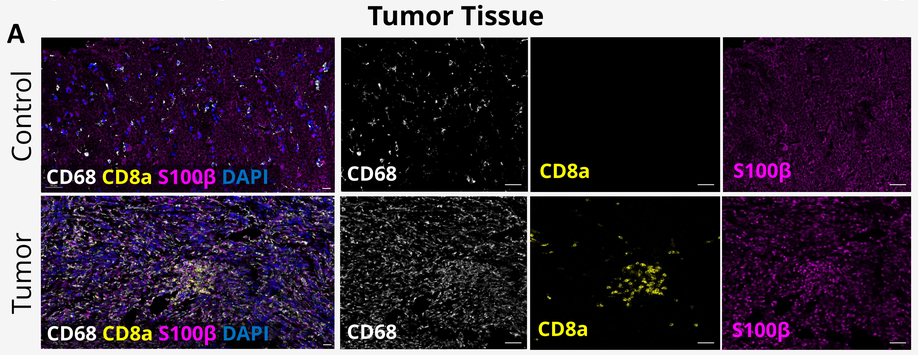
Figure 1: RNA-protein same slide multiomic workflow. This workflow is automated on the Leica BOND RX for the initial steps, which include the addition of target antibodies, RNA probes, building the amplification trees and the first round of label probe hybridization.

Cell types	RNA	Protein
Macrophages	-	CD68
T cells	-	CD8A
Astrocytes	-	GFAP, S100B
Microglia	-	IBA1
Neurons	-	NeuN
Endothelial Cells	*Hs-EMCN-O1, Hs-CLDN5*	-
Cytokines	*Hs-CXCL10, Hs-TNFα, Hs-CXCL2, Hs-IL6, Hs-INFg*	-

- **Samples: FFPE Brain Tumor TMA/Control brain**
- **Assay: RNAscope multiomics Assay**

Results

Targeted Multiplex Antibody Panel Identifies an Increase in Immune Cell Subtypes in Brain Tumor Tissue



Immune Cell Marker Detection of Microglia Shows Activation And Loss of Ramified Morphology in Tumors

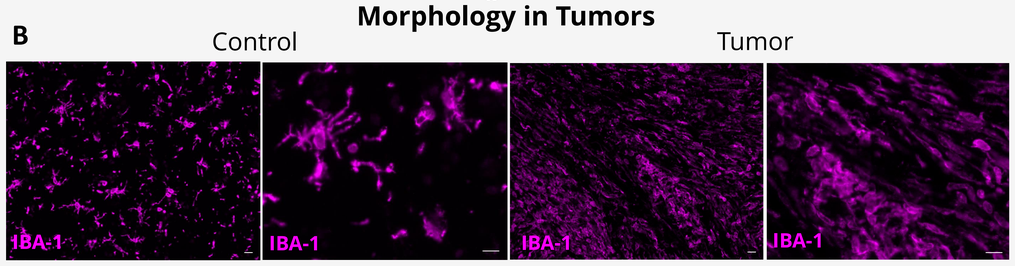


Figure 2: Profiling different cell types within the brain tumor microenvironment. 4 target-specific marker antibodies detect cytotoxic T-cells (CD8a), macrophages (CD68), and astrocytes (S100β) **(A)** and microglia (IBA-1) **(B)** in control and tumor tissues. Scale bar – 50µm.

Novel Non-Enzymatic RNA-Protein Multiomic Workflow Reveals Cytokine Upregulation in CD8⁺ Infiltrated T-Cells in Tumors

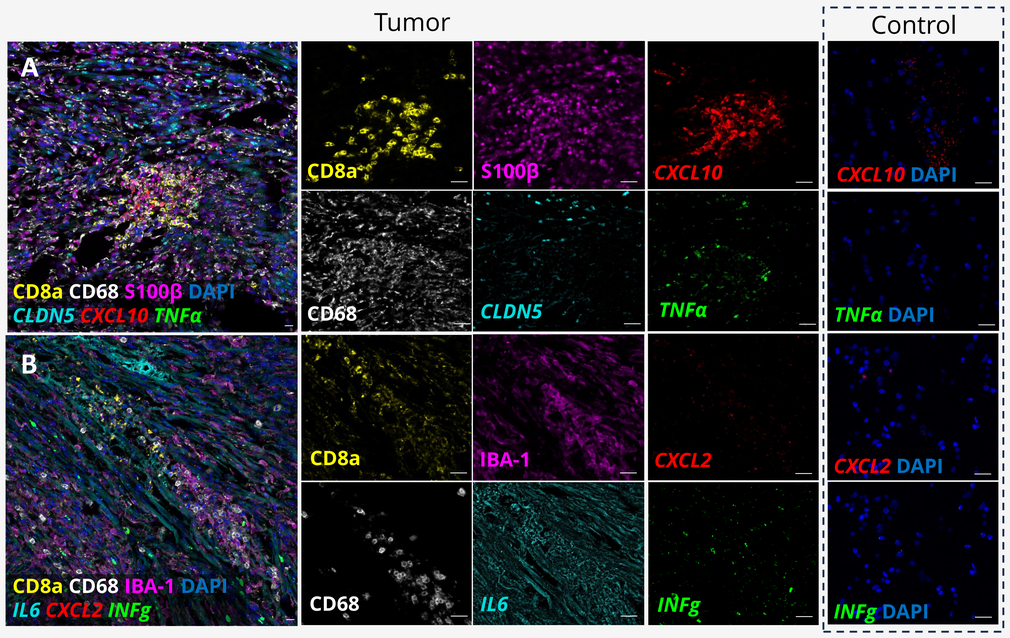


Figure 3: Detection of cytokines, immune and tumor cells using an RNAscope RNA-protein multiomic panel. (A) Simultaneous detection of a panel of 3 marker proteins (CD8a, CD68, S100β) and 3 target RNA probes (*CLDN5, CXCL10, TNFα*) in brain tumor tissue. **(B)** Simultaneous detection of a panel of 3 marker proteins (CD8a, CD68, IBA-1) and 3 target RNA probes (*CXCL2, IL6, and INFg*) in brain tumor microenvironment. Nuclei were stained with DAPI. Scale bar – 50µm.

RNA-Protein Multiomic Assay Enabled The Detection of Increased Gliogenesis And Vascularization in Glioblastoma Tumor Tissue

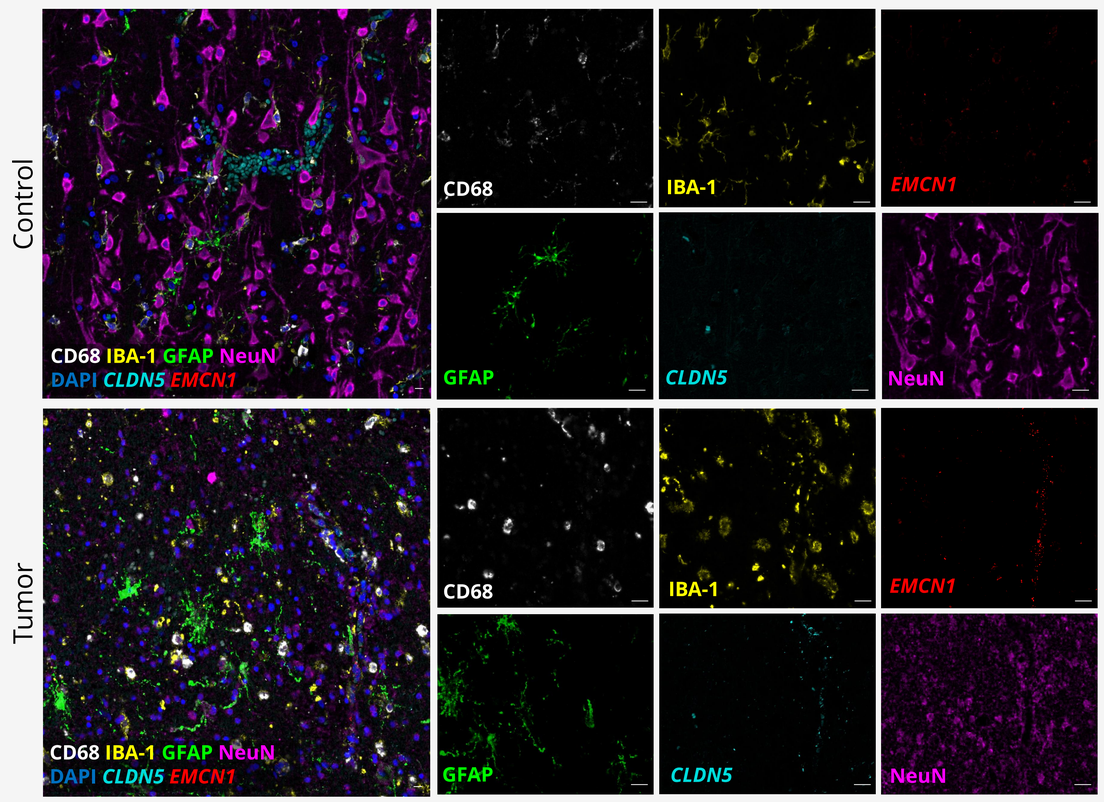


Figure 4: Detection of infiltrating immune in Glioblastoma tumor using an RNAscope RNA-protein multiomic panel. Simultaneous detection of a panel of 4 marker proteins (CD68, GFAP, IBA-1, NeuN) and 2 target RNA probes (*CLDN5, EMCN1*) in glioblastoma tumor and control tissue. Nuclei were stained with DAPI. Scale bar – 50µm.

Summary

- The multiomics workflow allows **simultaneous multiplexing of up to 6 RNA + protein markers** on the same tissue section.
- By combining immune cell marker antibodies with RNA probes for cytokines, this technique can provide **a comprehensive landscape of tumor-immune interactions**.
- Multiomics assay enables the **development of custom panels** based on target proteins and RNAs of interest.
- This automated workflow on the Leica BOND RX ensures **faster turnaround time** and is **less labor-intensive**.

Conclusion

Built on the flagship RNAscope technology, the new protease-free RNAscope multiomic workflow enhances the current capability of the RNAscope assay by allowing a combination of up to 6 target RNAs and proteins to be detected simultaneously.