Abstract number: #72

A spatial interactomics approach reveals interplay between the tumor microenvironment and T cell activation

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Introduction

T cells play a fundamental role in immune response to tumors. However, the tumor microenvironment (TME) – a complex network of cells, signaling molecules, and extracellular matrix components – employs various strategies to evade immune surveillance and suppress T cell activity. Among the many molecular interactions taking place in the TME, the role of CD8/MHC-I, LAG3/MHC-II and PD1/PDL1 is pivotal in modulating anti-cancer response and the escape routes that cancer in turn uses to withstand immune defense. LAG3/MHC-II and PD1/PDL1 interactions are inhibitory, causing T cell dysfunction and exhaustion, while the CD8/MHC-I interaction is essential for initiating an effective anti-tumor response. Here, we used a cutting-edge *in situ* proximity ligation strategy combined with immunofluorescence (IF) to reveal these key interactions and TME biomarkers in hepatocellular carcinoma and Hodgkin lymphoma. Since the method does not compromise the structural integrity of tissues, communication between proteins can be studied in their native milieu.

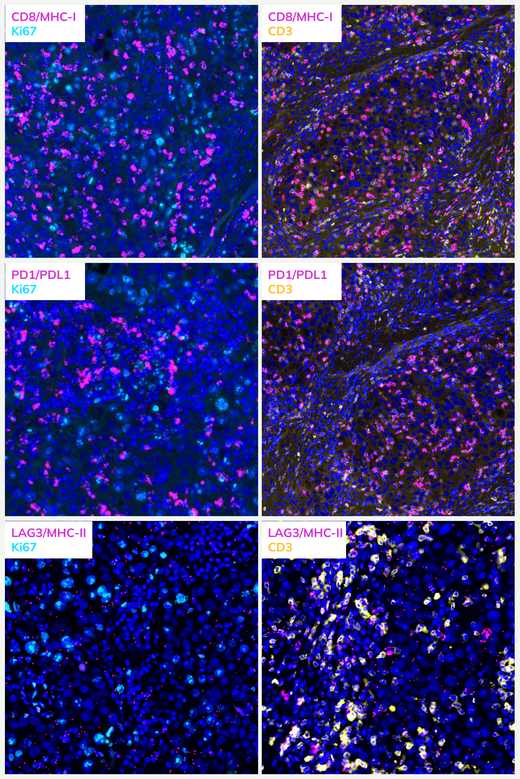


Fig. 2. FFPE sections of hepatocellular carcinoma show T cell priming and activation of inhibitory pathways in the TME.
Liver cancer samples were highly positive for primed CD8+ T cells (as evidenced by the abundant CD8/MHC-I interaction) and for the PD1/PDL1 interaction, whereas the interaction between LAG3 and MHC-II was sparser. High proliferation was demonstrated with Ki67 IF co-staining. T cells were visualized with CD3 IF co-staining.

Results

All hepatocellular carcinoma stainings (Fig. 2) originated from the same tissue block. The tumor microenvironment was visualized by either Ki67 (a proliferation marker indicative of the aggressiveness of the tumor) or CD3 (a general T cell marker) as shown by IF. The studied samples were of “hot” tumors with high proliferation activity. The presence of active and functional CD8+ cells was evident by the high number of CD8/MHC-I interactions detected with NaveniFlex™ Tissue. At the same time, inhibitory pathways like PD1/PD-L1 and LAG3/MHC-II were also activated. The proximity between the Ki67+ cells, CD3+ cells and the inhibitory interactions suggests crosstalk between proliferating tumor cells and the immune system.

In the case of Hodgkin lymphoma (Fig. 3), cytotoxic T cells (positive for CD8/MHC-I interaction) and cells undergoing immune checkpoint activation (positive for the PD1/PDL1 interaction) were detected in similar areas, whereas in healthy tissue (tonsil, data not shown) the opposite pattern is observed. CD3 co-staining partially overlapped with interaction signals, indicating the presence of immune cells in the TME and the possible crosstalk between them and cancer cells.

Technology and methods

Protein-protein interactions (PPIs) were detected with the NaveniFlex™ Tissue Atto647N kit in human FFPE tissues according to the manufacturer’s protocol. This technique can detect proteins located within interaction range (<40 nm) via oligonucleotide-antibody conjugates that create amplified fluorescent signal. Parallel IF co-staining for Ki67 and CD3 was performed during the Naveni® detection step 5 in Fig. 1A. Slides were mounted and imaged on the Olympus VS200 automated scanner.

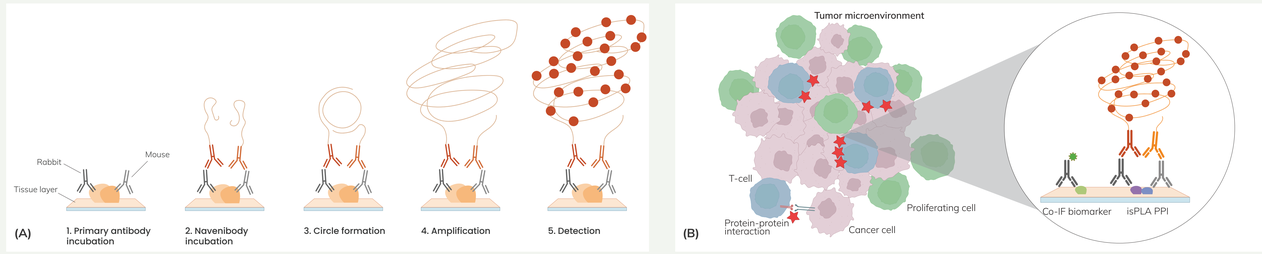


Fig. 1. Schematic depiction of the Naveni® technology for PPI detection and the integration of IF for co-staining of single biomarkers.
(A) Outline of the NaveniFlex™ Tissue workflow, demonstrating the binding of target-specific primary antibodies, Navenibodies, circle formation when the Navenibodies are bound within interaction range, amplification and detection of the fluorescent signal in Atto647N. (B) Illustration of the TME, showing interactions detected by the Naveni® *in situ* proximity ligation assay (isPLA) and individual markers detected by co-IF.

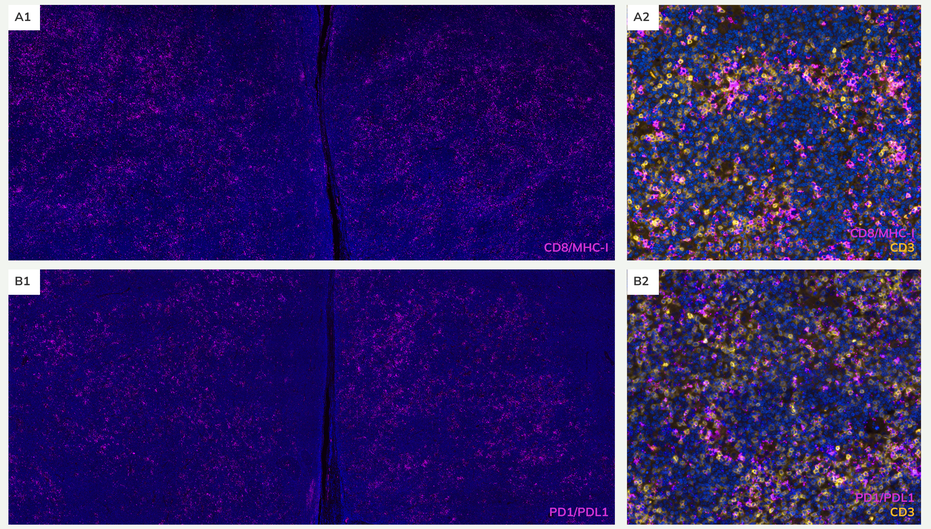


Fig. 3. FFPE sections of Hodgkin lymphoma show activation of the PD1/PD-L1 axis along with potent cytotoxic T cell activation.
Images A1 and B1 show overview (20x magnification) of the tissue staining for the CD8/MHC-I and the PD1/PD-L1 interactions, respectively (magenta). A2 is a zoom-in of A1, including anti-CD3 IF co-staining (yellow). T cell activation is demonstrated by the abundant interaction staining. As expected, CD8/MHC-I partially coincides with CD3 staining as it represents a sub-population of T cells. B2 is a zoom-in of B1, including anti-CD3 IF co-staining (yellow). The powerful activation of the PD1/PD-L1 axis is evident by the strong interaction stain (magenta) and is adjacent to T cells, indicating a “hot” tumor with strong immune system involvement.

Conclusion

The interactomics approach, employing *in situ* proximity ligation for observing protein function as opposed to mere expression, allows the detection of key PPIs in the TME. Understanding the intricate crosstalk between them is crucial in developing effective immunotherapies. Targeting these pathways simultaneously or in combination, such as using immune checkpoint inhibitors against PD1/PDL1 while exploring strategies to modulate LAG3/MHC-II and CD8/MHC-I interactions, holds promise in reinvigorating T cell responses against cancer and improving the efficacy of immunotherapies within the complex landscape of the TME.

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