Spatial interaction mapping of PD-1/PD-L1 in Head and Neck Cancer reveals the role of Macrophage-Tumour Barriers associated with immunotherapy response

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INTRODUCTION

Head and neck cancer continues to be a global health challenge, with an estimated 50% mortality rate projected for 2024. While immune checkpoint inhibitors (ICIs), like pembrolizumab and nivolumab, have shown promise in extending survival, their effectiveness remains limited, particularly in PD-1/PD-L1 blockade therapies. Traditional measures, such as PD-L1 protein expression, offer only modest predictive value for ICI therapy success. To address this, we employed a novel approach, mapping PD-1/PD-L1 interactions in pre-treatment tissue samples from head and neck squamous cell carcinoma (HNSCC) patients undergoing ICI therapy. Using a combination of high-plex spatial proteomics and low-plex protein spatial profiling (PSP) panels alongside *in situ* proximity ligation assays (isPLA), we visualized these interactions within the tumor microenvironment (TME).

METHODS

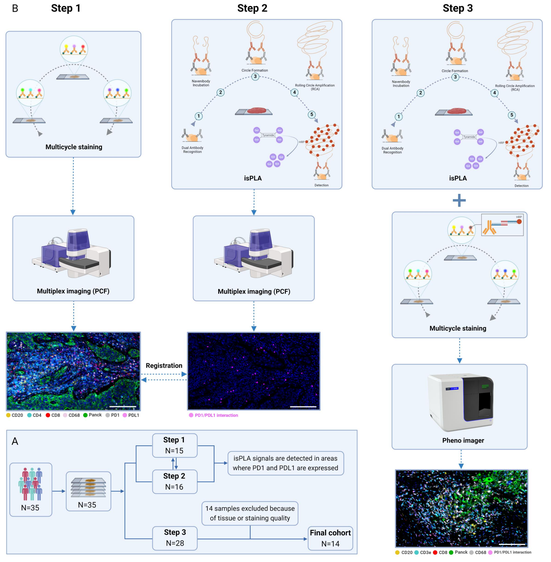


Figure 1. A) A cohort of 35 HNSCC patients was selected for analysis. **B)** Steps 1 and 2 ensure isPLA signals are aligned with PD-1 and PD-L1 expression. In step 3, 28 serial sections were stained with a 6-plex panel.

RESULTS

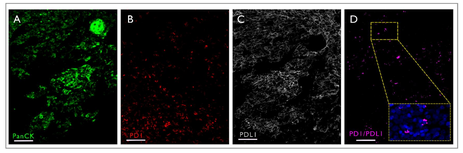


Figure 2. A-C) IF channels for PanCK, PD-1, and PD-L1, compared to panel D, which highlights positive isPLA signals indicating PD-1/PD-L1 interactions. Scale = 50 µm. Green=tumor cells (PanCK), Red=PD-1$^+$ immune cells, White=PD-L1$^+$ tumor cells, Pink=PD-1/PD-L1 ligation.

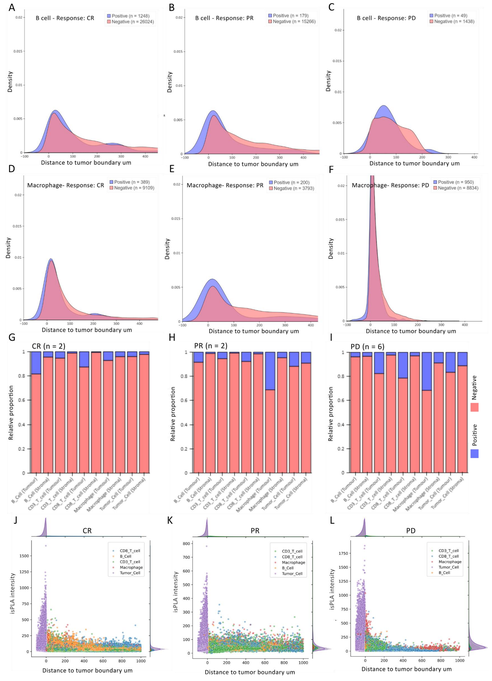


Figure 3. A-F) Display density plots showing the distance to the tumor boundary, cell count, and isPLA status for B cells **(A-C)** and macrophages **(D-F)** across response groups, normalized for comparison. **G-I)** comparison of isPLA-positive cell types per region. **J-L)** Distance to tumor scatter plots against response and isPLA intensity.

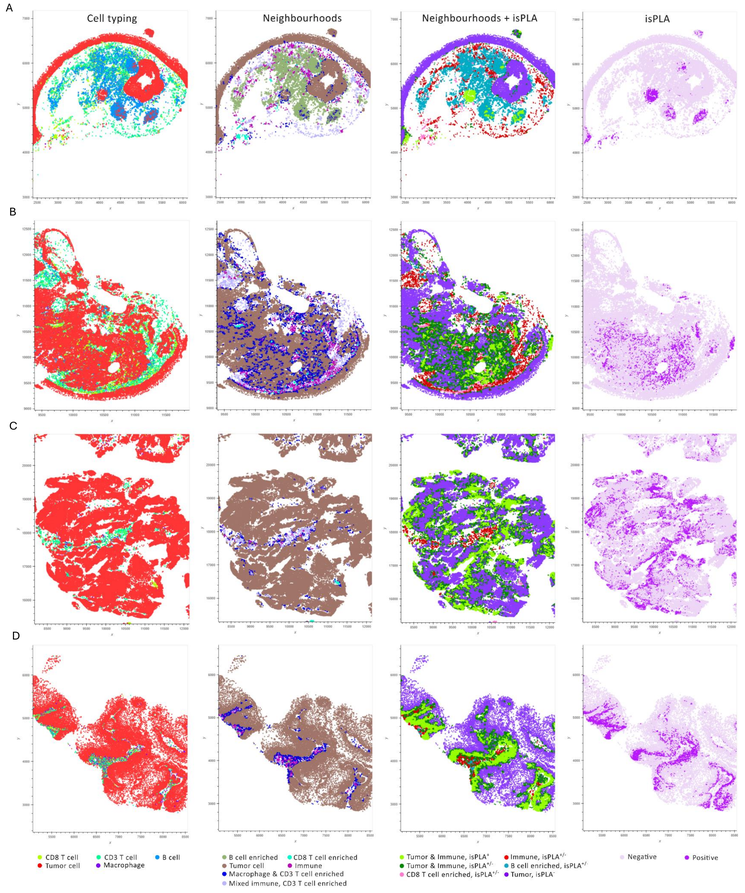


Figure 4. A-D) Cell typing and cellular neighborhoods across response groups (A=CR, B=PR, C=SD, D=PD). Comparison of cellular neighborhoods with and without including isPLA$^+$ clusters showing a different isPLA$^+$ neighborhoods distribution across different response groups.

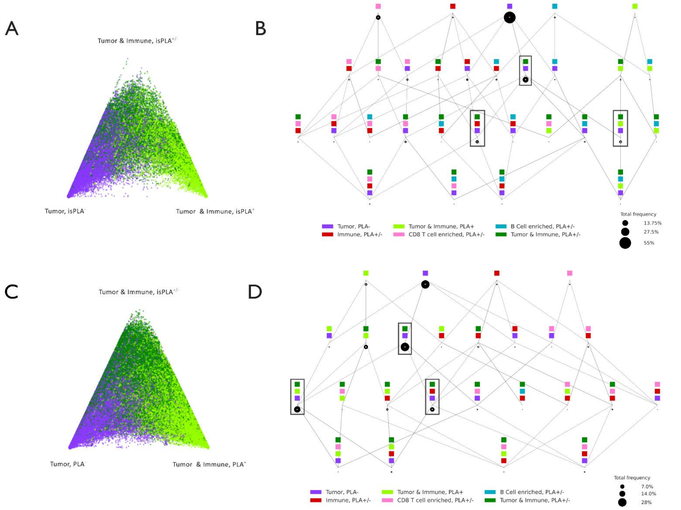


Figure 5. A) Barycentric coordinate projection of windows of cells assigned to Tumor, isPLA$^-$, Tumor & immune, isPLA$^{+/-}$ and Tumor and immune, isPLA$^+$. Outlined regions on edges and vertices indicate abundant CN and interfaces for CR and **C)** for the PD group. **B)** CNM of combinations comprising greater than 0.001% of total cells. Black rectangles indicate SC containing the highest frequency of Tumor, isPLA$^-$, Tumor & immune, isPLA$^{+/-}$ and Tumor and immune, isPLA$^+$ neighborhoods for the CR group and **D)** for the PD group.

CONCLUSION

Our study, which is the first to investigate the function of PD-1/PD-L1 interactions in the immunotherapy response of HNSCC patients, has uncovered a crucial link between these interactions and the spatial arrangement of immune and tumor cells. This complex interplay is essential for understanding the mechanisms behind the varying responses to immune checkpoint inhibitors in HNSCC patients. While more research and different approaches are required for validation, our results provide a novel observation: unique configurations of isPLA$^+$ macrophages, T cells and B cells interacting with immune cells at the tumor periphery correlate with the response to ICI therapy. These methodologies offer a potent platform for enhancing treatment strategies and advancing diagnostic pathologies.

ACKNOWLEDGEMENT

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