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Digital Spatial Profiling Reveals Immune Heterogeneity Across Tumor and Stromal Compartments in Hepatocellular Carcinoma

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Key Takeaway

Digital Spatial Profiling revealed immune heterogeneity in HCC, with stromal regions showing markedly stronger immune activation than tumor areas. CD45 measurements were highly concordant between DSP and IF, confirming the robustness of spatial profiling. These spatially distinct immune patterns highlight the need for compartment-specific analysis to better inform immunotherapy strategies.

Conclusions

Stratification of samples by tissue origin and the distinct expression of immune activation markers in stromal regions underscore the importance of compartment-specific analysis.

The strong concordance between DSP and IF quantification for CD45 expressions supports the robustness of spatial profiling approaches.

These insights highlight the value of compartment-specific immune assessment to guide future immunotherapy strategies and biomarker development.

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Introduction

- Hepatocellular Carcinoma (HCC) is one of the most devastating malignancies around the world, especially in East Asia. Despite significant advances in immune checkpoint inhibitors in the past decade, many patients show poor response, highlighting the need to better understand tumor heterogeneity, especially at the spatial level.
- GeoMx™ Digital Spatial Profiler (DSP) is a spatial biology platform that enables high-plex, in situ profiling of Formalin-Fixed and Paraffin-Embedded (FFPE) samples, allowing molecular characterization within distinct tissue compartments while preserving spatial context. In this study, DSP was exploited for immune profiling utilizing FFPE samples from HCC patients.

Methods

- Patient samples and corresponding clinical data were collected from National Cheng Kung University Hospital, Taiwan. All personal pathological records were de-identified in agreement with ethical commitments, and informed consent was obtained from all patients.
- Thirty HCC FFPE slides were used, and 341 regions of interest (ROI) were selected from tumor and tumor stroma tissue for DSP analysis. Four morphology markers (CD45/Vimentin/CK8/18/SYTO83) were used for Immunofluorescence (IF) staining to guide the ROI selection for the spatial locations.

Figure 1. Schematic of DSP workflow

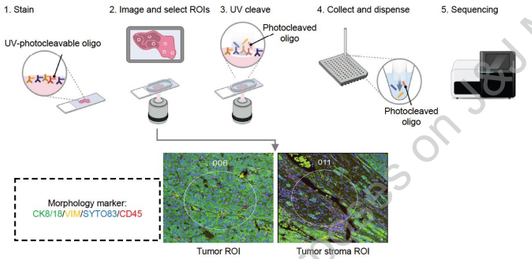


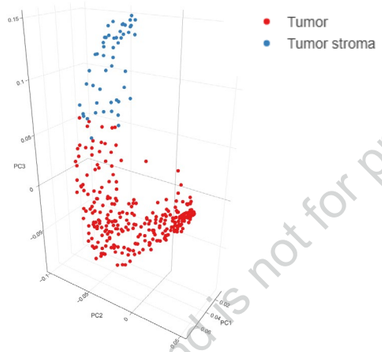
Table 1: DSP panels used for protein profiling

GeoMx DSP Protein Panel (49 markers)	
Human Protein Core (10 targets)	4 specific markers for immune cells (CD45), proliferation (Ki-67), antigen presentation (B2M), and vasculature (CD31), plus 6 positive and negative controls
Immune Cell Typing Panel (10 targets)	CD20, CD3, CD4, CD56, CD8, GZMB, FOXP3, CD34, CD66b, Fibronectin
Immune Activation Status Panel (10 targets)	CD127, CD25, CD80, ICOS, PD-L2, CD44, CD27, PD-1, PD-L1, CD45RO
IO Drug Target Panel (10 targets)	4-1BB, LAG3, OX40L, Tim-3, VISTA, B7-H3, IDO1, STING, GITR, CTLA4
Myeloid Panel (9 targets)	HLA-DR, CD11c, CD40, CD163, CD68, CD11b, CD14, ARG1, CD39

- Raw sequencing data were processed by software GeoMx NGS Pipeline for dcc files. Data normalization was done by R package GeomxTools and negative controls were used to calculate normalization factor. Multivariate linear regression was used to investigate the difference at tissue type, treatment status and individual level.

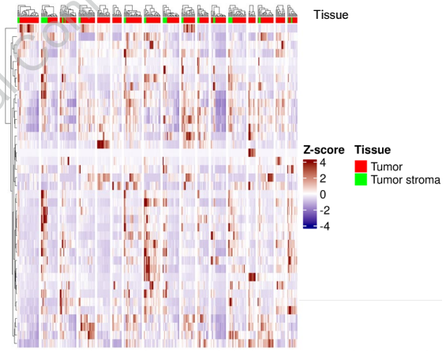
Results

Figure 2. Immune-related markers stratified regions of samples distinctly by tumor and stroma.



Principal component analysis shows clear separation between tumor and stromal ROIs at the protein level, indicating distinct immune profiles across compartments.

Figure 3. DSP analysis revealed distinct immune patterns within tumor and stroma regions in HCC tissues



Note: Protein expression values were standardized into z-scores by subtracting the mean and dividing by the standard deviation for each protein. The resulting scores are represented by a color-coded scale

Heatmap of DSP immune cell-related and immune checkpoint markers across 341 individual ROIs from 17 patients shows heterogeneous expression of immune markers, with stromal regions exhibiting higher immune activation signatures than tumor regions.

Figure 4. Stromal compartments exhibited stronger differential expression of immune related markers

-Stromal compartments exhibited stronger differential expression of immune-activation markers (e.g., CD45).

-T-cell–associated proteins (including CD3, CD4, and CD8) were expressed at higher levels in stromal areas compared with tumor regions.

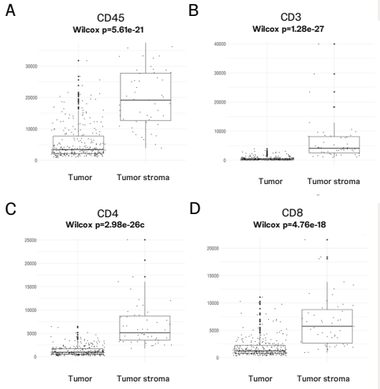
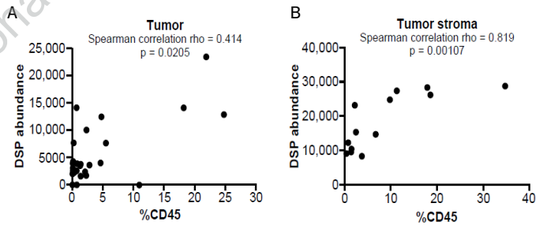
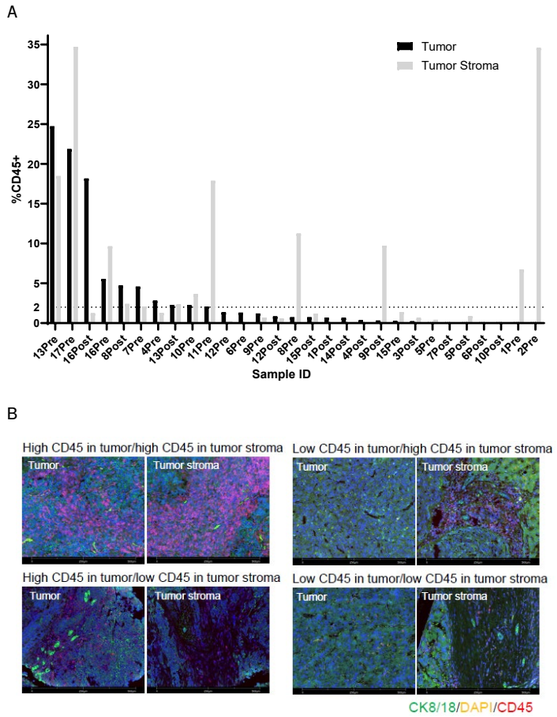


Figure 5. Strong correlation of CD45 expression measured by IF and DSP regardless of tissue compartments



Scatter plots show strong correlation between DSP and IF quantification of CD45 in both tumor (A) and stromal (B) compartments, validating consistency across platforms.

Figure 6. Variable CD45 expression within tumor and tumor stroma regions in HCC tissue



(A) IF staining result shows CD45 levels vary widely across HCC samples, with stromal regions generally showing higher expression than tumor regions. Most tumors show low CD45 (<2%), indicating limited immune infiltration in tumor, yet a subset exhibits elevated CD45 in the stroma, tumor, or both. Together, these patterns indicate heterogeneous immune engagement in HCC.

(B) Representative immunofluorescent staining shows low versus high immune infiltration in the tumor and stroma compartments. Tissue was stained CK8/18 (green), VIM (yellow), SYTO83 (blue), and CD45 (red) for visualization.

Acknowledgement

This study was sponsored by Johnson & Johnson. The authors thank all the patients and technical support from Nanostring.