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Multi-omic profiling maps the immune multicellular environment of renal cell carcinoma

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Introduction

Clear cell renal cell carcinoma (ccRCC) is a biologically heterogeneous malignancy shaped by complex tumor–immune–stromal interactions that drive progression and influence therapeutic response. Despite advances in immunotherapy and combination regimens, many patients exhibit intrinsic or acquired resistance. This underscores the need for spatially resolved, multimodal profiling of the tumor microenvironment to elucidate mechanisms of treatment failure and discovery of therapeutic targets.

In this study, we integrated Imaging Mass Cytometry™ (IMC™) technology with Spatial Transcriptomics (ST) to enable simultaneous in situ phenotyping of protein and transcript expression within intact tissue architecture. The multimodal combination of per-cell spatial proteomic and transcriptomic data improves understanding of the tumor-immune microenvironment, and enables the identification of multicellular neighborhoods that are not apparent from single-modality profiling.

Methods

This experimental plan is outlined in figure 1. Serial sections from FFPE ccRCC tumor tissue samples were mounted on Xenium slides (10x Genomics). Slide A underwent Xenium 5K spatial transcriptomics (Xe5K; 10x Genomics) followed by Imaging Mass Cytometry spatial proteomics using a Hyperion™ XTi Imaging System (IMC; Standard BioTools) with a 43-marker immuno-oncology-focused antibody panel on the same tissue section. The second slide (Slide B) underwent hematoxylin and eosin (H&E) staining between the Xe5K and IMC measurements.

Weave® software (Aspect Analytics) was used for data analysis. All images were co-registered at full resolution following the experimental plan outlined in Figure 1. Xenium cell segmentation results (10x Genomics) were imported into Weave for downstream analysis. After the stack fusion, both transcript readout and protein intensity is obtained per cell. Cell type annotation was then performed separately for Xenium and IMC datasets using the cell annotation pipeline implemented in Weave. Cellular neighborhood (CN) analysis was subsequently conducted in Weave using proprietary algorithms.

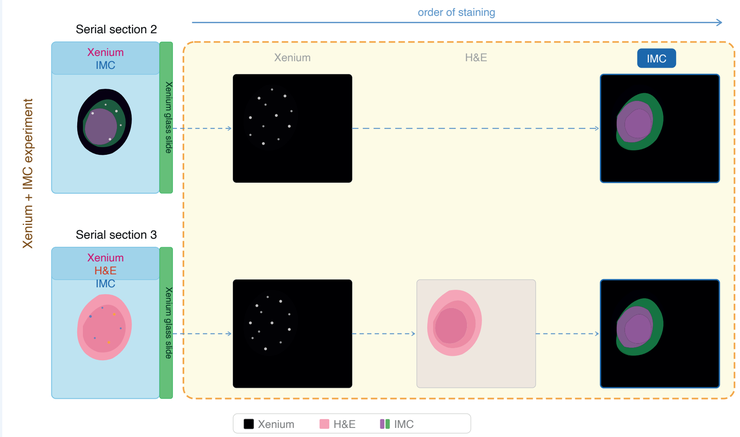

Figure 1: A schematic of the study design.

Results

Reading direction

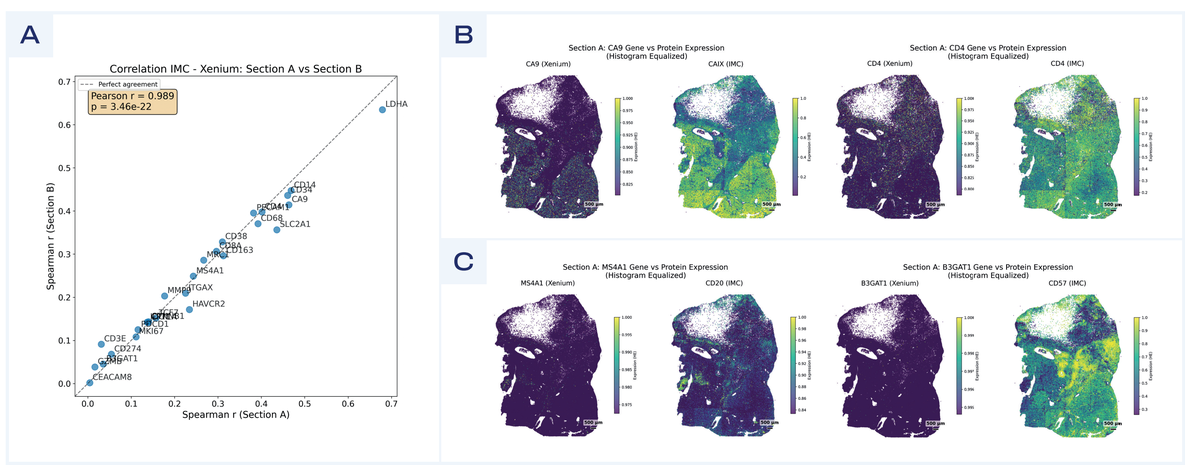

Figure 2: H&E staining does not affect Xenium-IMC correlation integrity
A: Spearman correlations between gene expression (Xe5k) and protein expression (IMC) from Section A (direct workflow) and Section B (with intermediate H&E staining). Each point represents a gene-protein pair. Strong agreement between the sections indicates that H&E staining between Xe5k and IMC acquisition does not adversely affect gene-protein correlation measurements, supporting the feasibility of integrating histological assessment into multimodal spatial omics pipelines.

Examples of gene-protein pairs that show high **(B)** and low **(C)** correlation. CA9/CAIX is highly expressed in corresponding ccRCC tumor areas. CD4 gene and protein are widely expressed across the sample. In comparison, MS4A1/CD20 B cell marker and B3GAT1/CD57 for NK cells have a low spatial correlation.

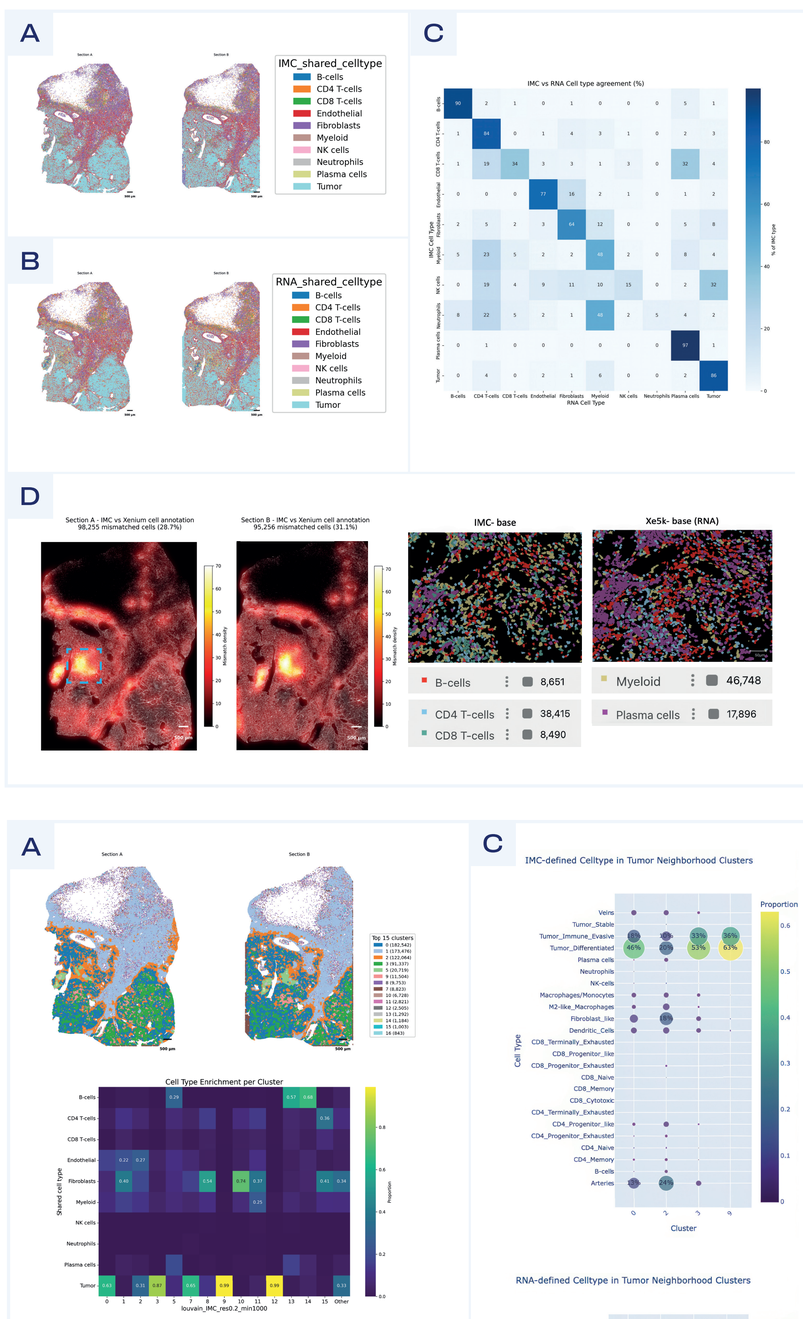

Figure 3: Comparison of cell type annotations between spatial transcriptomics and spatial proteomics
Cell type annotation was performed separately for Xe5k and IMC, resolving cell identities to the deepest lineage level supported by each modality. The resulting annotations were then harmonized by matching corresponding cell types between IMC **(A)** and Xe5k **(B)** to enable fair comparison across platforms.

C: Plot summarising cell type annotation agreement between IMC and Xe5k datasets. While Tumor, Endothelial, Fibroblast are annotated as being the same type when using either protein or transcript data, Immune cell populations e.g. B cells, plasma cells, CD4 T cells have a lower level of agreement. In these datasets, 32% of cells annotated as CD8 T cells using protein expression are annotated as plasma cells when using transcript data. This mismatch is likely due to lineage-specific markers, whereby small differences in marker detection between platforms can result in pronounced mismatches, e.g. MS4A1/CD20 for B cells and B3GAT1/CD57 for terminally differentiated NK cells from figure 2.

D. Hotspot: The bright yellow region (density ~60) indicates a concentrated area where IMC and Xe5k annotations strongly disagree. The pattern in both sections suggests the discrepancies are biologically or technically meaningful, not random noise. Blue dotted box represents the area showing IMC and Xe5K-based cell type result (inset).

Figure 4: Cellular neighborhood (CN) analysis.
CN and further downstream analysis was only performed on cells that had the same IMC and Xe5k annotation.

A: CN analysis of both sections identified recurring regions with distinct cellular composition and spatial organization, e.g. the tumor–stroma interface (cluster 2, orange) and several tumor-associated microenvironments. Heatmap showing cell type enrichment per cluster: Cluster 0 (blue) represents a tumor-stable region surrounding an immune-evasive tumor area (cluster 3, green), whereas cluster 9 (light green) corresponds to an immune-invasive tumor core with near absence of immune cells.

B: Screenshot of CN analysis in Weave for absolute cell count per CN cluster showing again the near absence of immune cells in immune-invasive tumor core

C: Proteomics and transcriptomics provide complementary information for tumour niche characterisation.
1. Tumour cell states: IMC captures functional protein expression, distinguishing Tumour_Differentiated from Tumour_Immune_Evasive states via IDO, and CAIX, while RNA may miss those but provides insight into underlying metabolic programs.
2. T-cell resolution: IMC marker constraints limit T-cells to broad categories, whereas RNA resolves fine-grained functional states: Terminally_Exhausted, Progenitor-like, Cytotoxic, and Memory.
3. Macrophage: agreement between IMC and Xe5k provides stronger confirmation than RNA-based M1/M2 gene signatures alone.

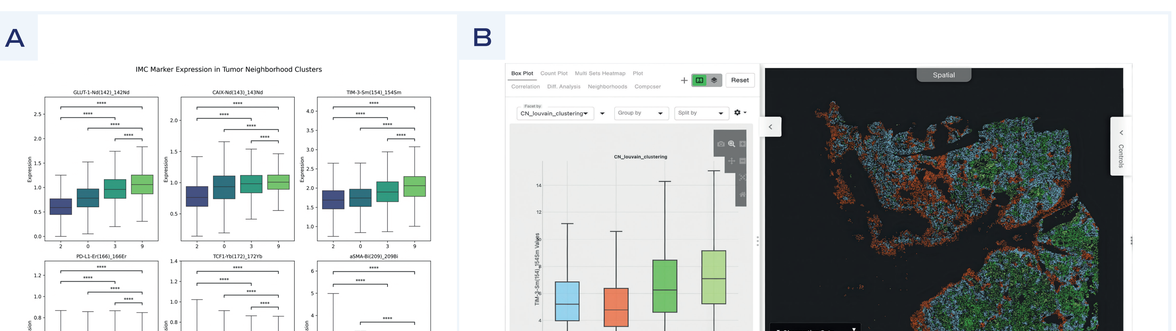

Figure 5: Cellular neighborhood (CN) analysis.
(A) CN analysis revealed distinct tumor microenvironment states characterized by differential IMC marker expression across clusters 0, 2, 3, and 9. Cluster 9 showed the highest expression of GLUT1 and CAIX, consistent with hypoxia, together with increased TIM-3, and PD-L1, indicating a proliferative and immune-suppressed tumor core. This pattern is consistent with previously described immune-evasive and invasive tumor regions in ccRCC. Cluster 3 displayed intermediate expression of hypoxia and immune checkpoint markers, suggesting a transitional microenvironment between tumor core and peripheral regions. In contrast, cluster 0 demonstrated increased alpha-smooth muscle actin (aSMA) consistent with a stromal-rich tumor region. Cluster 2 showed the highest aSMA expression with lowest proliferation and hypoxia markers, indicating a vascularized stromal margin associated with myofibroblasts and cancer-associated fibroblasts (CAFs) in the tumor stroma.

(B) Screenshot of CN analysis in Weave. Elevated TIM-3 and PD-L1 in cluster 9 further support an immune-evasive niche. TIM-3, an inhibitory checkpoint receptor linked to T-cell exhaustion, is associated with suppressed anti-tumor immunity and disease progression, suggesting that checkpoint signaling in this cluster contributes to effector T-cell dysfunction and tumor invasion.

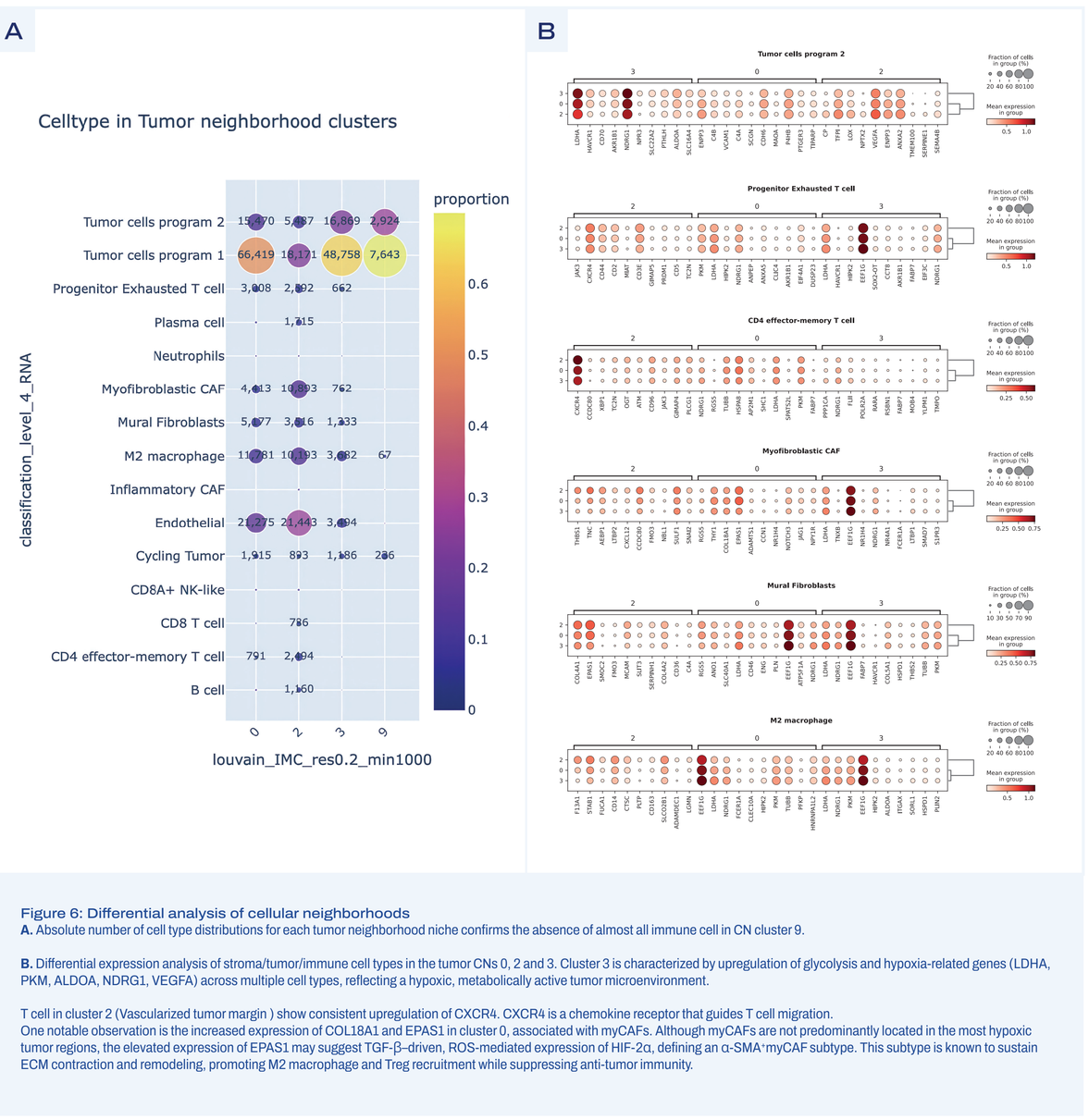

Figure 6: Differential analysis of cellular neighborhoods
A. Absolute number of cell type distributions for each tumor neighborhood niche confirms the absence of almost all immune cell in CN cluster 9.

B. Differential expression analysis of stroma/tumor/immune cell types in the tumor CNs 0, 2 and 3. Cluster 3 is characterized by upregulation of glycolysis and hypoxia-related genes (LDHA, PKM, ALDOA, NDRG1, VEGFA) across multiple cell types, reflecting a hypoxic, metabolically active tumor microenvironment.

T cell in cluster 2 (Vascularized tumor margin) show consistent upregulation of CXCR4. CXCR4 is a chemokine receptor that guides T cell migration.
One notable observation is the increased expression of COL18A1 and EPAS1 in cluster 0, associated with myCAFs. Although myCAFs are not predominantly located in the most hypoxic tumor regions, the elevated expression of EPAS1 may suggest TGF-β–driven, ROS-mediated expression of HIF-2α, defining an α-SMA⁺myCAF subtype. This subtype is known to sustain ECM contraction and remodeling, promoting M2 macrophage and Treg recruitment while suppressing anti-tumor immunity.

Conclusion

We demonstrate a powerful multimodal framework for in situ dissection of tissue architecture and cellular phenotypes, as demonstrated on ccRCC

- **Multimodal spatial profiling feasibility**: Xe5K, H&E, and IMC can be performed on a single FFPE tissue section, enabling profiling of mRNA and protein at single cell resolution and integration of histopathological information. Intermediate H&E staining preserves gene-protein correlation, supporting the integration of histological assessment into multimodal spatial omics workflows of Xe5K - IMC
- **Cell type resolution and modality complementarity:** Cell type annotations can vary based on use of protein or transcript data. However, transcriptomics enables fine-grained T cell and functional state resolution, while proteomics captures tumor cell functional states and confirms macrophage polarization, highlighting complementary strengths of each modality.
- **Tumor niche characterization:** Cellular neighborhood analysis identifies distinct microenvironments in ccRCC: cluster 9 as immune-evasive, hypoxic, and proliferative; cluster 3 as metabolically active with suppressed immune function; cluster 2 as a vascularized tumor margin enriched for CXCR4⁺ T cells; and cluster 0 as a stromal-rich region.
- **Insights into immune regulation and metabolism:** Upregulation of checkpoint markers (TIM-3, PD-L1) and metabolic/hypoxia-associated genes (LDHA, PKM, NDRG1, VEGFA) reveals spatially defined immune suppression, metabolic adaptation, and angiogenesis, reflecting how major cell types adapt to hypoxic stress under tumor-immune interactions in ccRCC.

This approach has the potential to contribute to more accurate and meaningful characterisation of cell populations, their interactions within their spatial locations and their functional potential. Importantly, this provides a more comprehensive view of tissue microenvironments for furthering our understanding of complex biological disease processes.

With the support of

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