Glioblastoma: Bridging Mouse Model Insights to Human Tumor Microenvironment Using Imaging Mass Cytometry

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Introduction

Human brain neoplasms, such as glioblastoma (GBM), are among the most lethal malignancies, characterized by rapid progression, therapeutic resistance and recurrence. Mouse models are widely utilized in neuro research, as their evolutionary-conserved brain architecture serves as a miniaturized model that permits visualization of whole-tissue spatial relationships. However, translating findings to the human brain requires technologies that can resolve complex high-plex spatial biology at cellular and subcellular levels without compromising on data quality. Imaging Mass Cytometry™ (IMC™) technology enables quantitative spatial proteomic evaluation of the brain without the challenges of autofluorescence, tissue degradation and spectral overlap. This study seeks to demonstrate the value of IMC platforms for bridging translational insights from mouse studies to human disease.

Materials and methods

We used the Hyperion™ XTi Imaging System to simultaneously assess multiple individual protein markers across tissues with high dynamic range. We applied a 40-marker panel composed of the Maxpar™ OnDemand Mouse Immuno-Oncology IMC Panel Kit combined with the Maxpar Neuro Phenotyping IMC Panel Kit to evaluate the spatial biology of whole mouse GBM tissue. For human GBM, the Maxpar Neuro Phenotyping IMC Panel Kit formed the backbone of a 41-marker panel supplemented by the Human Immuno-Oncology IMC Panel, 31 Antibodies. Subsequent pixel clustering using MCD™ SmartViewer and single-cell analyses quantified expression patterns of structural and immune markers in GBM of both species.

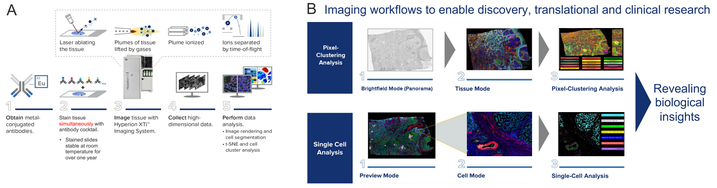

Figure 1. Imaging Mass Cytometry workflows. (A) IMC technology offers a streamlined workflow that simplifies translational and clinical application of multiplexed tissue analysis. The five-step process, which consists of obtaining metal-conjugated antibodies, staining tissues with antibody cocktails, imaging tissues with the Hyperion XTi Imaging System, and the collection and analysis of high-dimensional data, can be accomplished in as little as 72 hours for the whole slide. Additionally, the slide loader can accommodate two cassettes of 20 slides each (40 slides total) to greatly increase throughput. (B) Whole slide imaging (WSI) modes for IMC systems: Preview Mode offers a rapid scan of the sample and generates useful data for guiding region of interest (ROI) placement for Cell Mode acquisition for single-cell analysis application. Tissue Mode can be applied to generate a high-quality scan of entire tissue sections in a matter of hours with higher spot-size ablations enabling entire tissue analysis using pixel-clustering methods. Both workflows offer unique advantages for specific research requirements.

Conclusion

IMC technology establishes a critical bridge between preclinical models and human therapies. Cross-species validation accelerates marker discovery using mouse models as potential predictors of human tumor microenvironment (TME) development, **stratifies immunotherapy candidate selection** by utilizing high-throughput whole tissue visualization with high dynamic range, **and simultaneously explores multiple biological outputs** to advance translational and clinical applications.

Key takeaways

- Conserved spatial features were detected in both human and mouse GBM samples, highlighting striking heterogeneity of human and mouse GBM. Organized necrotic areas were surrounded by replicating Olig2+ cells, indicating elevated tumor growth capabilities.
- A high degree of vascularization was observed in non-necrotic areas. A high concentration of lymphoid and myeloid immune cells was detected in tumor margins and in necrotic cores.
- Analysis identified distinct tumor regions: subsets of differentiated tumor cells, immune hot and cold zones, stromal compartments, *de novo* vascularization and extracellular matrix deposition
- It is possible to improve the outcome of translational studies by utilizing the rapid, high-throughput whole slide visualization with high dynamic range of IMC technology

Results – insights into mouse GBM

Deep functional profiling with IMC technology reveals multiple spatial features of the tumor microenvironment

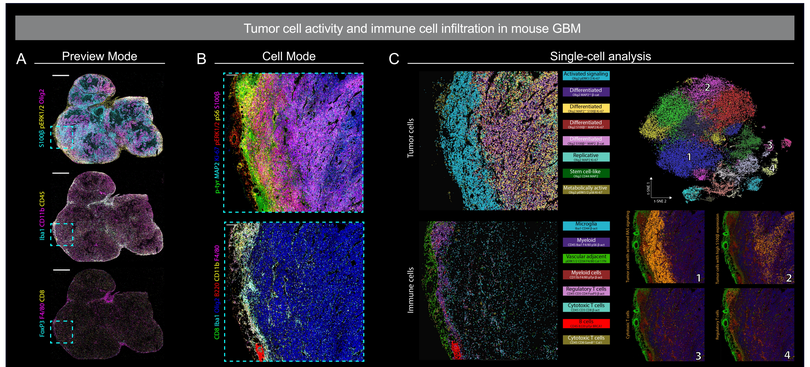

Figure 2. Application of IMC technology on mouse GBM using the Mouse Neuro-Oncology Panel. (A) Preview Mode scan rapidly identified areas with high tumor and immune cell activity, which was used to identify relevant ROIs for detailed Cell Mode investigation. (B) Rendered multiplexed Cell Mode images using tumor- (top) and immune- (bottom) specific markers demonstrate the heterogeneity of the TME. (C) Single-cell analysis through Cellpose and histoCAT enabled detection of tumor and immune cell populations on both tissue samples, as shown in the segmented cell mask. t-SNE and PhenoGraph clustering analysis successfully resolved specific subsets of tumor and immune cell populations, which then were mapped back to the segmented cell mask for each tissue. Scale bar is 2 mm (Preview Mode) and 200 µm (Cell Mode).

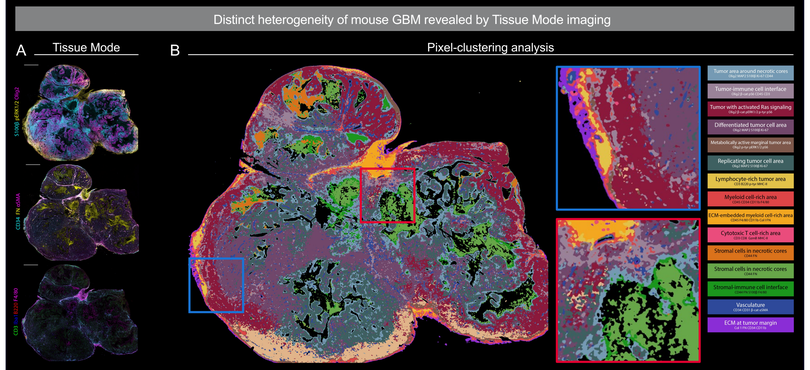

Figure 3. Visualizing the entire mouse GBM immuno-oncological landscape using the Mouse Neuro-Oncology Panel. (A) Tissue Mode imaging demonstrates the tumor and immune cell heterogeneity of mouse GBM tissue and the spatial positioning of tissue compartments. The activation of the Ras signaling pathway can be observed at the tumor margins. Expression of tumor cell activation marker S100β can be observed in specific tumor compartments. The presence of fibroblasts in fibronectin-enriched necrotic cores was detected. Metabolically active tumor cells were detected at the periphery of the tumor and cell replication markers were observed in virtually all tumor cells. (B) Unsupervised pixel-clustering analysis with hierarchical clustering using MCD SmartViewer successfully and quantitatively segregated highly specialized subcompartments in mouse GBM and detected various tumor areas containing subsets of differentiated tumor cells, immune hot and cold areas, stromal compartments, vasculature and the extracellular matrix. Scale bar is 2 mm.

Results – insights into human GBM

IMC analysis of human GBM identifies diverse heterogeneity and similar features with mouse GBM

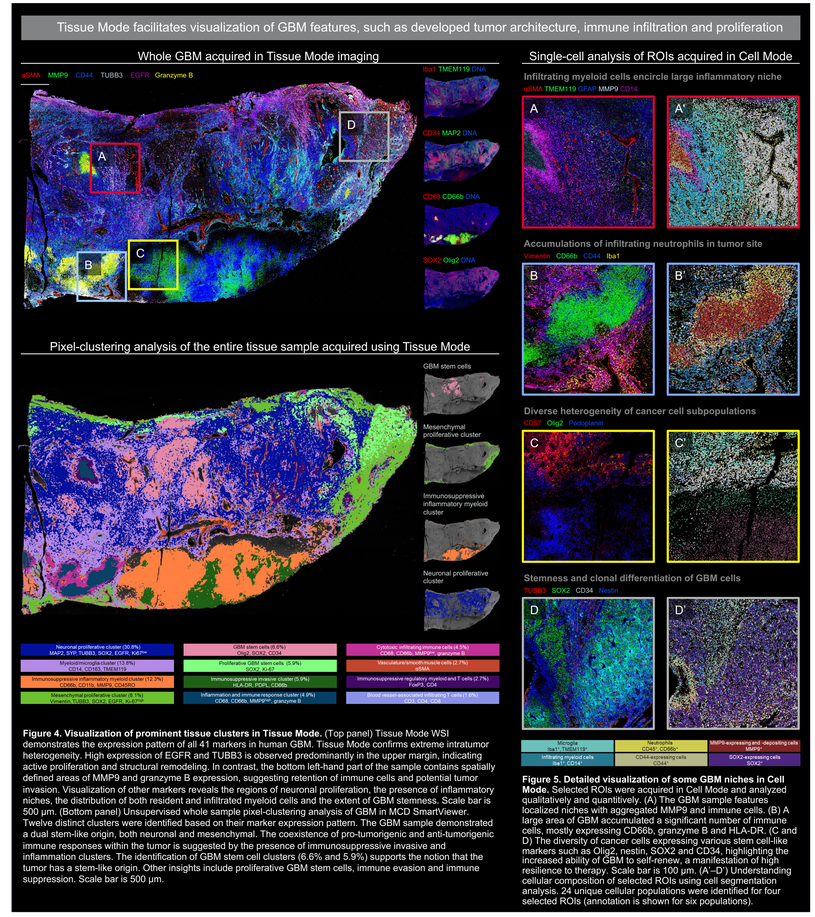

Figure 4. Visualization of prominent tissue clusters in Tissue Mode. (Top panel) Tissue Mode WSI demonstrates the expression pattern of all 41 markers in human GBM. Tissue Mode confirms extreme intratumor heterogeneity. High expression of EGFR and TUBB3 is observed predominantly in the upper margin, indicating active proliferation and structural remodeling. In contrast, the bottom left-hand part of the sample contains spatially defined areas of MMP9 and granzyme B expression, suggesting retention of immune cells and potential tumor invasion. Visualization of other markers reveals the regions of neuronal proliferation, the presence of inflammatory niches, the distribution of both resident and infiltrated myeloid cells and the extent of GBM stemness. Scale bar is 500 µm. (Bottom panel) Unsupervised whole sample pixel-clustering analysis of GBM in MCD SmartViewer. Twelve distinct clusters were identified based on their marker expression pattern. The GBM sample demonstrated a dual stem-like origin, both neuronal and mesenchymal. The coexistence of pro-tumorigenic and anti-tumorigenic immune responses within the tumor is suggested by the presence of immunosuppressive invasive and inflammation clusters. The identification of GBM stem cell clusters (6.6% and 5.9%) supports the notion that the tumor has a stem-like origin. Other insights include proliferative GBM stem cells, immune evasion and immune suppression. Scale bar is 500 µm.

Figure 5. Detailed visualization of some GBM niches in Cell Mode. Selected ROIs were acquired in Cell Mode and analyzed qualitatively and quantitively. (A) The GBM sample features localized niches with aggregated MMP9 and immune cells. (B) A large area of GBM accumulated a significant number of immune cells, mostly expressing CD66b, granzyme B and HLA-DR. (C and D) The diversity of cancer cells expressing various stem cell-like markers such as Olig2, nestin, SOX2 and CD34, highlighting the increased ability of GBM to self-renew, a manifestation of high resilience to therapy. Scale bar is 100 µm. (A'–D') Understanding cellular composition of selected ROIs using cell segmentation analysis. 24 unique cellular populations were identified for four selected ROIs (annotation is shown for six populations).

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