Spatial Multiparametric Investigation of Mouse and Human Brain Tumor Microenvironment Identifies Critical Neuro-Oncological Processes in Brain Neoplasms

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Introduction

Brain neoplasms represent a complex form of cancer that is one of the most challenging to classify and treat. Over 120 tumor subtypes originate from various parts of the central nervous system, which makes identifying the composition of the tumor microenvironment (TME) vital for early assessment of progression, treatment, and prevention. We developed high-parameter proteomic analysis tools to thoroughly characterize the TME of both human and mouse brain tissues using Imaging Mass Cytometry™ (IMC™). IMC offers unprecedented insight into the TME by simultaneously uncovering the spatial distribution of 40-plus distinct molecular markers without autofluorescence data artifacts, facilitating the research of brain neoplasms. Here, we demonstrate the application of high-plex human and mouse neuro-oncology IMC panels on normal and tumor formalin-fixed paraffin-embedded (FFPE) brain tissues.

A basic neuro phenotyping panel was developed and used to customize Maxpar® Human Immuno-Oncology IMC Panel Kits and Maxpar OnDemand™ Mouse Immuno-Oncology IMC Panel Kits. These neuro phenotyping panels consist of cross-reactive clones and enable flexible panel design for brain-specific research goals, such as brain tumor classification and assessment of neuronal inflammation, degeneration, and development. We compiled the high-plex neuro-oncology panels that included **neuro phenotyping panels** and applied them on the tissue microarrays (TMAs) containing a variety of human brain tumors and mouse glioblastoma tissues. We successfully identified major cell populations that make up human and mouse brain matter, such as neurons, astrocytes, microglia, and oligodendrocytes. Various tumor cell phenotypes, resident and infiltrating cells, and resting and activated microglia were detected in brain tumor subtypes. Subsequent single-cell analysis provided a comprehensive and quantitative assessment of the brain TME in mouse and human glioblastoma tissues. Empowered by high-plex neuro-oncology panels, IMC can accelerate brain tumor research and provide insights into the spatial complexity of neuronal neoplasms.

Methods and Materials

A 40-parameter human antibody panel and a 36-parameter mouse antibody panel, both designed to highlight central features of normal and diseased brain tissue, are presented in this poster (middle section). These panels contain antibodies that identify major cell populations in normal brain and the specific states of tumor and immune cell populations in the diseased brain. The respective high-parameter panels were applied on human and mouse normal and tumor tissues. Tissue slides were prepared and stained using optimized antibody dilutions and were ablated using the Hyperion™ Imaging System at 200 Hz with 1 µm pixel size. Qualitative data analysis, multiplexed image rendering, and single-channel image extractions were performed using MCD™ Viewer (see Imaging Mass Cytometry and single-cell analysis workflow below).

Resulting images were rendered in MCD Viewer and exported for single-cell analysis. All antibodies were titrated and tested multiple times on relevant tissues. Segmentation was performed using CellProfiler™ v4.2.1. Nuclei and cell membrane were detected using Cell-ID™ Intercalator-Ir (Cat. No. 201192B) and the Maxpar IMC Cell Segmentation Kit (ICSK; Cat. No. 201500), respectively. Cell masks were subsequently exported for single-cell phenotyping and cellular clustering into histoCAT™ v1.76. t-SNE maps and corresponding PhenoGraph cell clusters were identified to assess relevant and specific tumor and immune cell subpopulations. Cell masks highlighting specific clusters were generated and overlaid on the corresponding IMC image.

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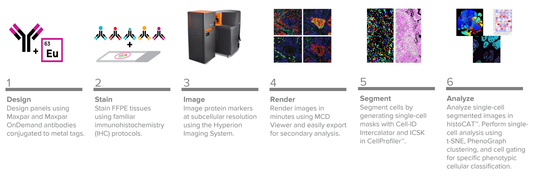
Imaging Mass Cytometry and single-cell analysis workflow



Antibody panels for human and mouse FFPE tissues

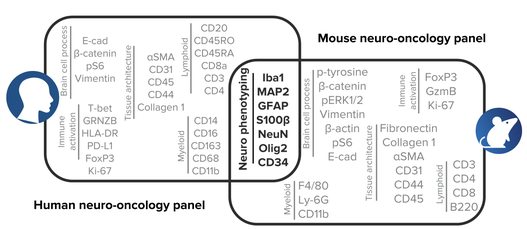


Figure 1. Human and mouse neuro-oncology panels for FFPE tissue application. A 40-parameter human antibody panel and a 36-parameter mouse antibody panel designed to highlight central features of the TME is presented. These panels are subdivided into 6 modules, each revealing critical insights about normal and tumor tissue composition, state, and biology. The **tissue architecture** module identifies the underlying cellular and structural markers of the tumors. The **brain cell process** module identifies activation of signaling pathways, metabolism, and growth in brain cells. The **lymphoid and myeloid** module delineates lymphoid and myeloid cell subtypes of immune cell infiltrates in brain tissues. The **immune activation** module assesses the functional state of immune cells in brain tissues.

Results

Our data demonstrates performance of markers with high specificity and signal intensity and absence of autofluorescence data artifacts in human and mouse normal and diseased brain tissue. High-parameter human and mouse neuro-oncology panels were successful in determining the spatial landscape of normal and neoplastic brain tissues. Presence or absence of primary brain cell constituents such as microglia, astrocytes, neurons, endothelial cells, and oligodendrocytes is apparent in both human and mouse tissue. Both infiltrating and resident myeloid and lymphoid immune cells are distinguished and their spatial locations within the TME are demarcated. Following sections discuss the performance of neuro-oncology panels and the tumor-specific immuno-oncological insights they offer.

1. Neuro phenotyping panel identifies major cell types in normal human and mouse brain tissues

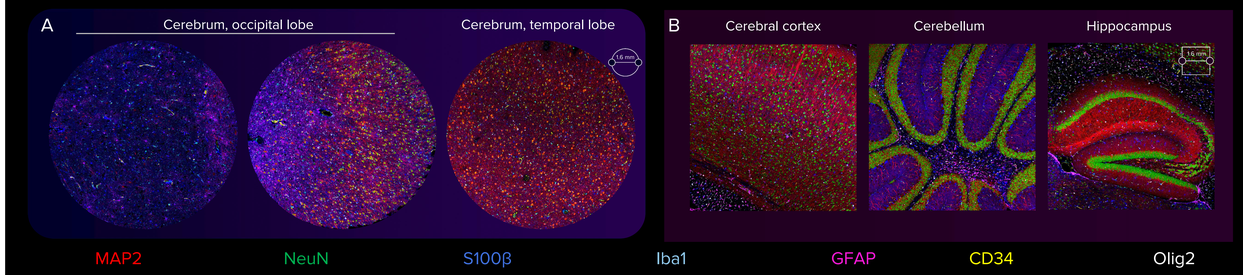


Figure 2. Application of Imaging Mass Cytometry using neuro phenotyping panel demonstrates cellular heterogeneity in normal human and mouse brain tissues. A 7-parameter neuro phenotyping panel consists of human and mouse cross-reactive clones, which enables flexible panel design for brain-specific research goals. The markers in the panel were selected to identify major cell types in brain tissues, such as neurons, resident microglia, astrocytes, endothelial cells, and their activation states. IMC generated images of highly autofluorescent normal FFPE human cerebral cortex tissue (A) and normal FFPE mouse cerebral cortex, cerebellum, and hippocampus tissue (sagittal cut) (B) that were stained with neuro phenotyping panel are shown. Our data demonstrates performance of markers with high specificity, signal, and absence of autofluorescence data artifacts. The following brain tissue constituents can be identified: neuronal cell bodies and axonal extremities (NeuN, MAP2), oligodendrocytes (Olig2), resting and activated astrocytes (GFAP, S100β), resting and activated microglia (Iba1), and brain vasculature (CD34). In human brain sections (A), 3 tissue cores obtained from different locations demonstrate the contrasting cellular composition of the tissue microenvironment. In mouse brain sections (B), images of 3 distinct brain tissues demonstrate unique structural and cellular architecture of tissue compartments. Neuro phenotyping panel can be applied to identify composition of both central and peripheral human nervous system tissue.

2. High-parameter neuro-oncology panels delineate tumor cell states and immune cell infiltration within TME of human and mouse glioblastoma

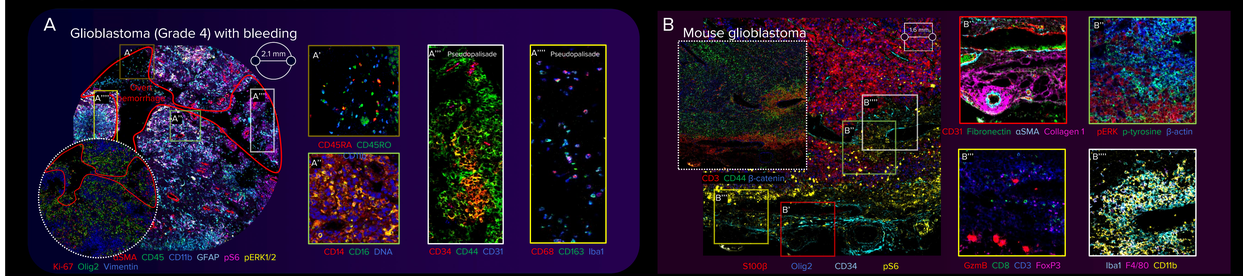


Figure 3. Application of high-parameter neuro-oncology IMC panels on human and mouse glioblastoma tissues. IMC generated images of (A) human glioblastoma (Grade 4) with overt hemorrhage (bleeding) and (B) mouse glioblastoma are shown. In human tissue (A), hemorrhagic tumor areas are demarcated. Smooth muscle cells (αSMA), immune cells (CD45, CD11b), tumor cells (GFAP, Olig2), and activated tumor cells (pERK, vimentin, Ki-67, pS6) can be identified. Inset A' marks infiltrating T cells (CD45RO+, CD45RA+) and monocytes (CD11b) in the hemorrhagic tumor tissue. Inset A'' depicts cells of myeloid origin, both resident and infiltrating (CD14+, CD16+), in the TME. Inset A''' demonstrates one of few pseudopalisade areas with extensive tumor scaffolding expressing CD44 and CD34. Inset A'''' demonstrates presence of immune cells of myeloid lineage within the alternative pseudopalisade area. In mouse tissue (B), tissue area located at the margin of tumor is shown. Tumor cells (Olig2, S100β), endothelial cells (CD34), metabolically active cells (pS6), T cells (CD3), and stem cell-like tumor cells (CD44+) can be identified. Inset B' demonstrates extracellular matrix composition (collagen 1, fibronectin) and smooth muscle covered vasculature (αSMA, CD31). Inset B'' demonstrates activation of receptor tyrosine kinase activity (p-tyrosine), Ras signaling cascade, and cytoskeletal structure (β-actin) in tumor and immune cells. Inset B''' shows presence of T cell subtypes, cytotoxic T cells (CD3, CD8), regulatory T cells (CD3, FoxP3), and areas with cytotoxic immune cell activation (GzmB). Inset B'''' depicts presence of macrophages (F4/80), monocytes (CD11b), and microglia (Iba1).

3. Human and mouse neuro-oncology panels allow deciphering of unique features of various brain neoplasms

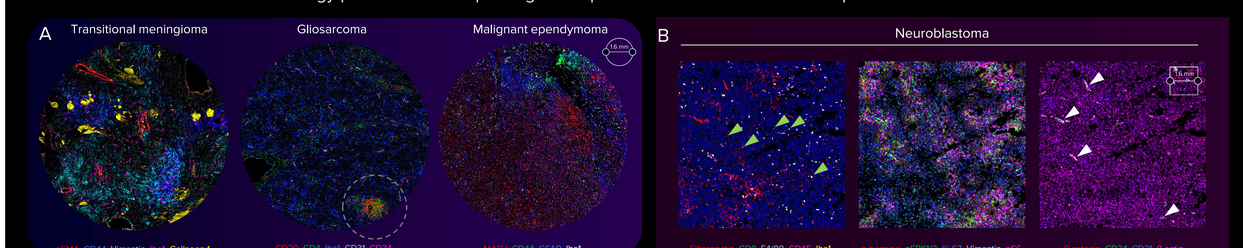


Figure 4. Application of neuro-oncology IMC panel on human and mouse tumors of mixed or non-glial origin. IMC generated images of human transitional meningioma, gliosarcoma, malignant ependymoma (A), and mouse neuroblastoma (B) are shown. Diverse composition of connective tissue (αSMA, collagen 1) can be observed in human transitional meningioma. A cluster of infiltrating B cells (CD20) and T helper cells (CD4) around a blood vessel (CD34, CD31) and microglia (Iba1) scattered around tissue can be observed in human gliosarcoma. Presence of activated amoeboid microglia (Iba1) can be noted in human malignant ependymoma. In mouse neuroblastoma (B) tissues, scattered deposits of fibronectin and presence of myeloid cells (F4/80, Iba1) is noted. Only a few cytotoxic T cells (CD8; green arrowheads) can be observed, indicating a suppressed tissue immune microenvironment. Activation of receptor tyrosine kinase (p-tyrosine). Ras signaling (pERK1/2), mTOR signaling (pS6), replication (Ki-67), activation, and cytoskeletal structure (vimentin, β-actin) across tumor cells can be detected. Relative spatial positioning of vascular network (CD34, CD31, β-catenin; white arrowheads) within the TME (β-actin) can be distinguished.

4. Single-cell analysis

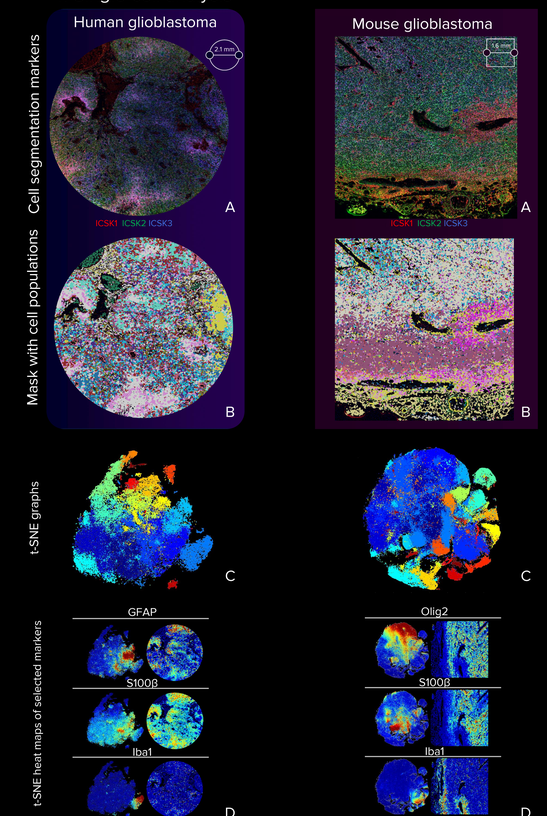


Figure 5. Single-cell analysis identifies specific cell populations in human glioblastoma. ICSK was used to demarcate cellular boundaries in cells to enhance cell segmentation capabilities (A). t-SNE and PhenoGraph analysis identified 18 separate cellular clusters from 2 regions of interest including 58,119 cells defined by the expression of a single or multiple markers from the human neuro-oncology panel. Spatial positioning of cells belonging to individual cellular clusters is shown overlaid on tumor image (B). t-SNE maps further demonstrate the separation of individual cellular clusters by color (C). Heat maps of individual marker expression are displayed as cell masks overlaid on tumor tissues and t-SNE graphs (D). GFAP and S100β expression indicates positioning of tumor cells and activated astrocytes. Iba1 expression highlights microglial cells, which elicit an anti-tumor immune response.

Figure 6. Single-cell analysis identifies specific cell populations in mouse glioblastoma. ICSK was used to demarcate cellular boundaries in cells to enhance cell segmentation capabilities (A). t-SNE and PhenoGraph analysis identified 27 separate cellular clusters from 7 regions of interests including 204,070 cells defined by the expression of a single or multiple markers from the mouse neuro-oncology panel. Spatial positioning of cells belonging to individual cellular clusters is shown overlaid on tumor image (B). t-SNE maps further demonstrate the separation of individual cellular clusters by color (C). Heat maps of individual marker expression are displayed as cell masks overlaid on tumor tissues and t-SNE graphs (D). Olig2 expression indicates positioning of tumor cells, S100β expression highlights astrocytes in activated state. Iba1 expression highlights microglial cells, which can elicit an anti-tumor immune response.

Conclusions

- Neuro phenotyping panel for Imaging Mass Cytometry consists of 7 cross-reactive markers to successfully identify the cellular composition of human and mouse neuronal tissue.
- Spatial positioning of major brain cell types can be recognized without any spectral overlap or tissue autofluorescence.
- Highly customizable and flexible panel design permits addition of custom markers suitable for individual research needs.
- Applications of 40-parameter human neuro-oncology panel and 36-parameter mouse neuro-oncology panel distinguish critical pathophysiological changes in human and mouse glioblastoma TME composition.
- Single-cell analysis of human and mouse glioblastoma identified 18 and 27 cell populations, respectively, offering exceptional opportunities for discovering novel spatial biological insights.

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