3763: Comparative Spatial Analyses of the Tumor Immune Landscape in Different Mouse Models of Glioblastoma

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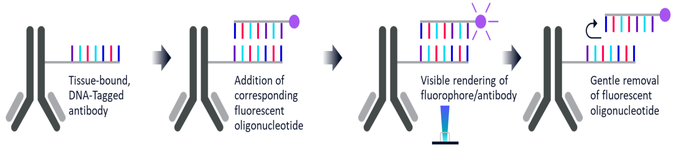
1. Mouse Models of Glioblastoma

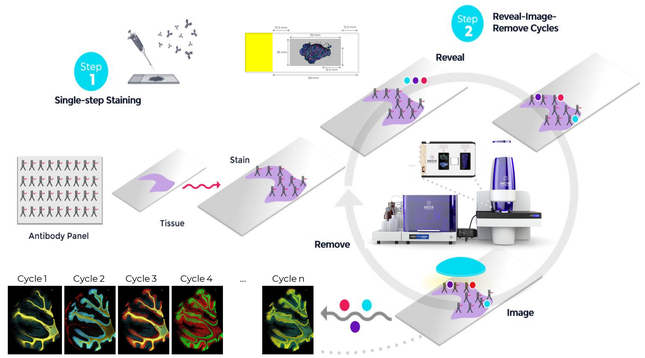
Despite advances in treatment, glioblastoma (GBM) remains one of the most difficult types of cancer to treat, and the prognosis is poor. The current median survival time for patients with GBM is about 12-15 months, and the five-year survival rate is less than 10%. There is an unmet need for better GBM treatment options, leveraged from relevant experimental models. To develop new therapies, preclinical animal models are important for analyzing the biology of GBM and evaluating the efficacy of novel therapeutic strategies. While a variety of experimental models are used to study GBM, most preclinical investigations involve mice. In this study we utilize a spatial phenotyping application that permits comprehensive characterization and comparison of key proteins in the brain tumor immune microenvironment (TiME) of the mouse GL261 GBM model of GBM.

2. PhenoCycler-Fusion Workflow

The **PhenoCycler®-Fusion 2.0** (PCF) technology is the fastest whole-slide spatial biology platform that enables simultaneous detection of 100+ biomarkers by combining automated fluidics and iterative imaging of DNA-tagged antibodies.



The **PhenoCycler-Fusion** workflow consists of iterative cycles of labelling, imaging and removing fluorescent reporters. In each imaging cycle, three fluorescent reporters are attached to their corresponding DNA-tagged antibodies and imaged via standard fluorescent optics. Thereafter, the three reporters are removed, and a new cycle images additional reporters. The process is fully automated, and data are acquired **across whole slides at single-cell resolution**. Barcoded antibody technology enables deep spatial phenotyping, combining antibody specificity with molecular barcodes to simultaneously detect 100+ targets at high spatial resolution, preserving tissue integrity.



3. Design and Validation of the FFPE Mouse Neuro Panel

Mature Neurons
NeuN
MAP2
Synaptophysin
Neurofilament
CTIP2
PSD95

Immature Neurons
Doublecortin

GABAergic Neurons
GAD67
Calbindin (CBDN)

Cholinergic Neurons
Choline Acetyltransferase

Serotonergic Neurons
Serotonin Transporter

Glutamatergic Neurons
GLUT-1

Dopaminergic Neurons
Tyrosine Hydroxylase

Astrocytes
GFAP
S100B

Structural
SMA
LamB1
CD31
Collagen
AQP4
Vimentin

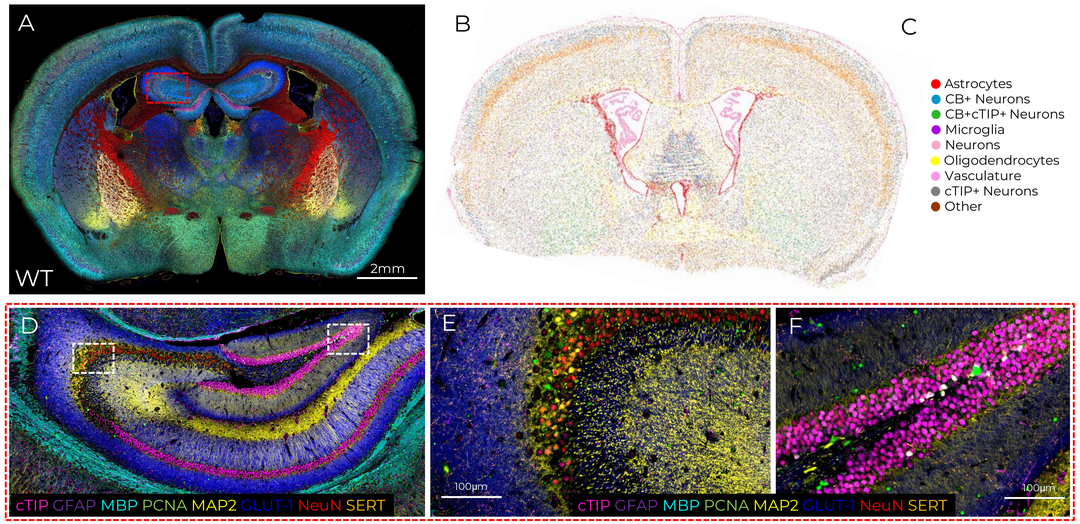
Microglia/Myeloid
Iba-1
TMEM119
F4/80
CD68
CD11b

Oligodendrocytes
Olig-2
MBP

Neural Stem-Progenitor
SOX2

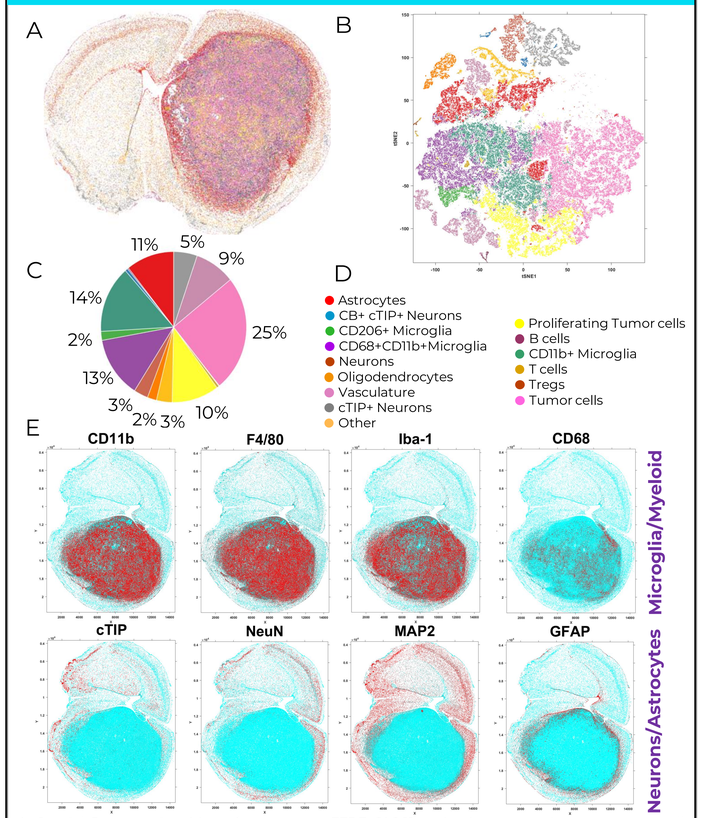
Immune
CD8
CD11c
CD44
Ki67
CD206/MRC1
CD3
PD-L1
CD20
FoxP3
CD56
CD45

Neuropathology
H2AX
pTau (S396)
PCNA
A-Beta



A. Whole-slide image of the healthy mouse brain stained with 43 antibodies and a spatial map (B) showing cell classification results for DAPI-based segmentation masks, obtained using Stardist, colored as per legend (C). D-F Magnified views of the red dotted box region of the mouse hippocampus. G. Multiplexed imaging of the GBM mouse brain and magnified views of the GL261 tumor niches (H-I).

4. Whole-slide Spatial Phenotyping of GL261 Mouse GBM Model



A. Spatial Phenotyping Map of the mouse FFPE GL261 brain tissue recapitulates the overall organization of different cell phenotypes relative to each other. B. The tSNE plot shows distinct phenotype clusters sorted by color as in C and D. C. Pie chart of cell-type fractions for mouse GL261 with infiltrating immune cells colored by cell type (D). E. Expression of individual markers (in red) shows functional specialization across entire GL261 brain tissue.

5. Development of a Mouse FFPE Neuro Panel for Single-Cell Spatial Analysis

Our work encompasses the development of a custom antibody panel, an imaging workflow, as well as a novel bioinformatic analysis method. Deployment of this workflow on mouse GL261 GBM model allowed us to study cell populations, according to biomarker profiles and spatial distribution. This study provides a deeper characterization of the mouse GL261 model of GBM.

We anticipate that further application of this panel will help to determine the optimal model that most accurately recapitulates the complex TiME of human GBM, including key features such as invasive tumor margins, high vascularity, blood-brain barrier, etc. We believe this approach has significant potential for a broad range of applications for which biomolecules' spatial information is important and can deepen our understanding of the GBM TiME.

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