Spatial temporal map of glioma cancer cells
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Background
- Glioblastoma (GBM) is the most common aggressive, lethal brain tumor in adults.
- GBM is highly heterogeneous and disease progression is attributed to several factors, including the tumor microenvironment, perturbation in signaling pathways, metabolic alterations, and epigenetic modifications.
- The objectives of this study is to determine the cellular phenotype and expression levels of ETV5, an important glial lineage transcription factor that plays a role in tumor growth.
- In addition, we also aimed to determine the signaling pathways affected by MEK inhibition (indirectly blocking ETV5) in mouse xenograft tumors.

**Experimental Methods**

- Brain tissues were fixed in 4% paraformaldehyde for 24 hours, embedded in paraffin, serially cut into 4-μm sections, and stained with H&E (Sigma-Aldrich, St.Louis, MO, USA).
- For immunohistochemical stains, the tumor sections were deparaffinized and subjected to graded rehydration. After blocking the sections in 2.5% serum and performing antigen retrieval (citrate buffer, pH 6.0), we incubated the sections with the primary antibodies overnight at 4 °C.
- Following, we incubated with secondary antibody and a signal stain DAB Substrate Kit, brown stain reveals positively stained human GBM. The cells were counterstained with hematoxylin. The slides were subjected to graded dehydration, and we mounted using DPX.
- Immunofluorescence staining was performed using primary antibody and then we incubated the sections with secondary antibodies. We then incubated the sections with Texas red fluorescein isothiocyanate–conjugated secondary antibodies and green fluorescein isothiocyanate–conjugated secondary antibodies for 120 minutes at room temperature.

**Results**

Conditions for optimal staining were established for most cellular phenotype markers such as SOX9 and ETV5 as EGFR positive cancer cell markers, general astrocyte marker GFAP.

To discriminate between glioma molecular subtypes, we optimized olig2 as proneural and CD44 as mesenchymal. Furthermore, we optimized the staining conditions for proliferative and signaling markers for mouse xenograft tumors. Ongoing studies will establish the use of these antibodies to develop a multiplex antibody panel of cellular phenotype markers to determine the spatial temporal expression of cellular composition of glioma tissues.

**Conclusion**

This study will aid the development and standardization of a panel of multiplex immunofluorescence antibodies to construct a spatial and temporal map of glioma cancer cells and their cellular composition in a single tissue section.

**References**


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