

SPATIAL IMMUNE PROFILING OF HUMAN GLIOBLASTOMA TISSUE REVEALS THE PRESENCE OF AGGREGATED LYMPHOID NICHES IN THE TUMOR MICROENVIRONMENT

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Background Glioblastomas (GBM) account for 60% of adult primary brain tumors. With few advances in therapeutics, median overall survival remains 15-months post-diagnosis. Immunotherapies may provide therapeutic benefit in GBM patients; however, no predictive immune features currently inform therapeutic stratification in GBM. We have shown that, independently of known prognosticators, radiographic tumor contact with the lateral ventricle (C-GBM) correlates with 7-months worse survival prognosis compared to patients with ventricle non-contacting GBM (NC-GBM). This study sought to characterize the GBM immune microenvironment and identify targetable mechanisms of immunosuppression correlating with worse outcomes in C-GBM.

Methods Twelve patients presented with pathologically confirmed primary, IDH wildtype C-GBM and thirteen with NC-GBM. Multiplex immunohistochemistry (mxIHC) was performed on formalin-fixed paraffin embedded (FFPE) tissue for each patient interrogating 8 predictive immune markers (CD3, CD4, CD8, FOXP3, CD68, IBA1, PD-1, and PD-L1). Machine learning tools characterized tumor-infiltrating immune populations and identified biomarkers correlating with C-GBM and patient survival. K-means clustering identified immunological neighborhoods within the tissue and a log odds ratio was used to quantify the likelihood of cell-cell interactions in the tissue.

Results C-GBM tumors were enriched in monocyte-derived macrophages (MDM) compared to NC-GBM ($19 \pm 8\%$ vs. $6 \pm 2\%$; $p < 0.001$) and depleted in lymphocytes ($2.9 \pm 1\%$ vs. $7.6 \pm 2\%$; $p < 0.001$) and tissue-resident microglia ($1.8 \pm 0.3\%$ vs. $7 \pm 3\%$; $p < 0.001$). Further, T cells in C-GBM co-expressed the checkpoint receptors PD-1, suggesting T cell exhaustion in the C-GBM tumor microenvironment. K-means clustering identified 10 immunological niches prevalent in GBM tissue. Macrophage-tumor niches were most common niche in the tissue accounting for 17.93% of all niches, followed by T cell-microglia-tumor niches (17.72%). Conversely, tumor-tumor niches were the least prevalent, accounting for only 2.51% of niches. Within niches, T cell-T cell interactions occurred more frequently than expected by random chance (log odds ratio = 0.90) whereas T cell-macrophage interactions occurred less frequently than expected by random chance (log odds ratio = -1.61). Pathological assessment of the tissue confirmed the presence of lymphoid aggregates in regions of myeloid exclusion in the tissue.

Conclusions These findings suggest that factors within the periventricular space may influence antitumor immunity within GBM, and have identified clinically targetable immune biomarkers in glioblastoma. The prevalence of T cell niches in GBM tumors suggests the establishment tertiary lymphoid aggregates may be targetable to improve patient outcomes. Lastly, radiologic assessment of lateral ventricle contact by standard-of-care MRI may guide clinical trial design for immunotherapies in neuro-oncology.

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Ethics Approval Primary glioblastoma tumors obtained in accordance with the Declaration of Helsinki and with institutional IRB approval (#131870) along with patient written informed consent.

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