Title: Hypoxia shapes Tumor Immune Microenvironment Through Cell-Type Dependent Responses in Diffuse Astrocytomas

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Abstract

Hypoxia is a critical driver of tumor aggressiveness in high-grade gliomas, yet its cell-type-specific effects on immune cell populations within the tumor microenvironment (TME) remain poorly understood. In this study, we investigate how hypoxia shapes the spatial distribution and functional states of monocyte-derived macrophages (MDMs) and brain-resident microglia (MG) in diffuse astrocytomas and glioblastomas (GB). Using cyclic immunohistochemistry (cIHC), single-cell RNA sequencing, spatial transcriptomics, and in vitro cell culture models, we demonstrate that hypoxia induces divergent responses in these myeloid subsets, driving spatial immune patterning. $CD163^-$ MDMs dominate hypoxic niches in GB, while MG populations are excluded from these regions. MG are characterized by hypoxia-induced upregulation of TNF, apoptotic signatures, and dampened interferon responses. Conversely, $CD163^+$ MDMs exhibit hypoxia-associated immunosuppressive traits, whereas $CD163^-$ MDMs are overall immunologically inactive. GBs display heightened hypoxic intensity compared to diffuse astrocytomas, as supported by hypoxia-response gene expression in TCGA datasets. In vitro, MG show heightened sensitivity to hypoxic stress compared to MDMs, suggesting their exclusion from hypoxic zones results from intrinsic vulnerability. Our findings reveal that hypoxia remodels the TME by promoting immunosuppressive MDM accumulation and depletion of MG, creating spatially distinct immune landscapes that may underlie glioma progression. These results highlight hypoxia-driven immune dysregulation as a therapeutic target and underscore the importance of cell-type-specific strategies to counteract TME-driven immunosuppression in malignant gliomas.