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Patterns of Invasive Growth in Malignant Gliomas—The Hippocampus Emerges as an Invasion-Spared Brain Region

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Abstract

BACKGROUND: Widespread infiltration of tumor cells into surrounding brain parenchyma is a hallmark of malignant gliomas, but little data exist on the overall invasion pattern of tumor cells throughout the brain. *METHODS:* We have studied the invasive phenotype of malignant gliomas in two invasive mouse models and patients. Tumor invasion patterns were characterized in a patient-derived xenograft mouse model using brain-wide histological analysis and magnetic resonance (MR) imaging. Findings were histologically validated in a cdkn2a−/− PDGF-β lentivirus-induced mouse glioblastoma model. Clinical verification of the results was obtained by analysis of MR images of malignant gliomas. *RESULTS:* Histological analysis using human-specific cellular markers revealed invasive tumors with a non-radial invasion pattern. Tumors cells accumulated in structures located far from the transplant site, such as the optic white matter and pons, whereas certain adjacent regions were spared. As such, the hippocampus was remarkably free of infiltrating tumor cells despite the extensive invasion of surrounding regions. Similarly, MR images of xenografted mouse brains displayed tumors with bihemispheric pathology, while the hippocampi appeared relatively normal. In patients, most malignant temporal lobe gliomas were located lateral to the collateral sulcus. Despite widespread pathological fluid-attenuated inversion recovery signal in the temporal lobe, 74% of the "lateral tumors" did not show signs of involvement of the amygdalo-hippocampal complex. *CONCLUSIONS:* Our data provide clear evidence for a compartmental pattern of invasive growth in malignant gliomas**.** The observed invasion patterns suggest the presence of preferred migratory paths, as well as intra-parenchymal boundaries that may be difficult for glioma cells to traverse supporting the notion of

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compartmental growth. In both mice and human patients, the hippocampus appears to be a brain region that is less prone to tumor invasion.

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Introduction

Malignant gliomas are the most common intra-axial primary brain tumors and, despite multimodal treatment, survival rates remain poor [1]. Surgical resection is often the primary treatment for these tumors; however, it is not curative due to the widespread infiltration of glioma cells. Such invasive cells are also relatively resistant to radio- and chemotherapy [2], further complicating the management of these tumors. To better understand the mechanisms underlying the invasive behavior of tumor cells and to tailor future therapies targeting invasive glioma cells, more knowledge is needed about tumor cell migratory trajectories and their preferred sites of accumulation in the brain.

Early histopathological studies of brains from glioma patients showed that tumor invasion does not occur in a random manner; glioma cells follow distinct anatomical structures with a propensity to migrate along white matter tracts (WMTs), in perivascular spaces and the subependymal layers while avoiding certain gray matter regions [3,4]. Despite extensive invasion into the brain parenchyma and the perivascular spaces, tumor seeding along cerebrospinal fluid (CSF)-routes is seen in only 2% of the cases [5] and metastasis outside the neuraxis have rarely been reported [6,7]. This supports the notion that glioma cell invasion occurs within certain tissue compartments. The spread of glioma cells within the brain appears to respect some anatomical boarders, giving rise to defined subtypes. As such, optic pathway glioma is a well-known tumor entity residing in the centrobasal midline region, whereas the limbic gliomas are predominantly confined to gray matter structures of the mediobasal temporal lobe, illustrating the diversity of glial tumors [8,9].

Considering that the extracellular space is much smaller than an invasive glioma cell and the astrocytic end-feet cover approximately 99% of the vasculature [10], it is remarkable that a tumor cell is able to move through the brain at all. To this end, studies have shown that glioma cells undergo several geno- and phenotypic changes that enable them to switch to an invasive phenotype. These changes are facilitated by chemo-attractive and repulsive cues that act in a stringent interplay between tumor cells and their microenvironment [11–13].

Orthotopic xenograft studies where human malignant glioma cells are transplanted into rodents have given some indications as to which brain regions are preferred (e.g., corpus callosum and internal capsule) and avoided (e.g., thalamus) by invasive tumor cells [14–16]. These rather old studies were, however, restricted by the methods available for visualization of tumor cells at the time. Many xenograft studies are based on chemically induced glioblastoma (GBM) models and serum-cultured commercial cell lines which either form circumscribed tumors (e.g., U87) or show limited peri-tumoral infiltration of the brain parenchyma (e.g., GL261) without recapitulating the invasive phenotype of gliomas [17]. Although a few studies have used genetically engineered mouse models or xenografts of patient-derived serum-free primary cell cultures that do display invasion of the brain parenchyma [17], a systematic brain-wide characterization of glioma migration patterns has not been performed.

To address this biological and clinically relevant subject, we have investigated the invasive growth pattern of malignant gliomas using two invasive GBM animal models and clinical patient imaging. Analyses were based on histological sections of xenograft tumors formed by transplanting patient-derived GBM-initiating cells (GICs) into severe combined immunocompromised (SCID-) mice and structural magnetic resonance (MR) images. GICs have been shown to reiterate the geno- and phenotype of the parental tumor and to form highly invasive tumors upon orthotopic transplantation [18–20]. Our key findings were validated in a cdkn2a–/– PDGF-β-GFP lentivirus-induced GBM mouse model. In both models, we observed systematic patterns of invasive growth and regional differences in the propensity to harbor or avoid invasive tumor cells.

Material and Methods

Study Populations and Ethical Approvals

Male C.B.-17 SCID mice (Taconic, Ejby, Denmark) were used for intracranial tumor transplantation (n = 22) and serial MR imaging studies (n = 40). Male cdkn2a–/– mice (n = 6) were used to generate PDGF-β-GFP lentivirus models of GBM. All animal experimental procedures were approved by the respective Animal Research Authorities.

Clinical data used for this study included prospectively registered data and MR images from patients who underwent operations for malignant temporal lobe gliomas (TLGs) at Oslo University Hospital, Norway, in a three-year period (July 2013–June 2016; n = 90). GIC cultures were established from GBM biopsies harvested from consenting patients (n = 5). Data collection and the use of human tissue was approved by institutional data protection officials and the Norwegian National Committee for Medical Research Ethics (#07321b).

Tissue Specimens and Cell Cultures

GIC cultures were derived from histologically confirmed GBMs and grown as spheres in serum-free neurosphere medium [21]. We have previously shown that GICs maintain their phenotype and tumorigenicity after *in vitro* culturing in these conditions [20]. The two GIC cultures used the most in this study (T08 and T65) have been characterized in detail with regards to growth, sphere forming ability, and expression of stem cell associated markers [20,22,23]. To identify transplanted human cells and verify human-specific antibody staining, we labeled two GIC cultures with green fluorescent protein (GFP) [23]. GL261, a murine GBM cell line, was obtained from the National Cancer Institute (NCI, Bethesda, MD, USA) and cultured in serum-containing adherent conditions [24].

Intracranial Transplantation, Brain Tissue Processing and Immunolabeling

GICs were stereotactically transplanted into 8–9-week-old C.B.-17 male SCID mice (Taconic, Ejby, Denmark) [20]. Two microliters of