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Glioblastoma Heterogeneity and More Accurate Representation in Research Models

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Angiogenesis; Animal brain tumor model; Glioma; Invasion

Introduction

In their study, Inoue et al. characterized two types of orthotopic brain tumors obtained from cells of a single parental spontaneous glioblastoma (GB) implanted in a murine model. Despite their common origin, these tumors had striking differences in important features, such as angiogenesis dependence and invasive patterns. The finding that a single tumor is able to give rise to two distinct cell types and form different types of brain tumors speaks to the heterogeneity of GBs and shows the possibility of finding different tumor-initiating cells within a tumor.

In adult humans, GB is the most common and lethal primary malignancy of the central nervous system. GB represents approximately 50% of all gliomas. Despite the best treatment modalities presently available, GBs have a median survival of only 14–16 months and a 2-year survival rate of < 10%–15% (26). These tumors are refractory to the combination of surgical resection, radiation, and chemotherapy. Morphologic characteristics of GBs include diffuse infiltration, angiogenesis, and necrosis; molecular characteristics include chromosomal aberrations and genomic instability. Histopathologically, GBs are poorly delineated tumors with abundant undifferentiated mitotic cells and nuclear atypia. Necrosis and microvascular proliferation are also present (17). GBs are characteristically heterogeneous and vary with respect to histologic findings and distinct chromosomal and genetic aberrations (16, 19). A review on the subject by Bonavia et al. (4) deepened the understanding of the origin and maintenance mechanisms of such heterogeneity.

The concept of clonal evolution applies to tumor clonal cell populations, which under selective therapeutic or pathologic pressure acquire properties to resist challenges and survive. A second concept that may explain cancer heterogeneity is associated with the role

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of cancer stem cells (CSCs), a poorly differentiated subpopulation of cells with tumor initiation capabilities. These two concepts are not mutually exclusive because CSCs can undergo a process of clonal evolution as they proliferate (12). The presence of different tumor cells capable of reproducing the pathology in orthotopic models as reported by Inoue et al. may be explained by this phenomenon.

Cancer Stem Cells in Brain Tumors

In the 17th century, Virchow suggested that cancers arise from tissue that resembles an embryonic stage. In modern medicine, the concept of CSCs originated from work on hematologic malignancies such as leukemia (18). Subsequent work showed the presence of similar malignant stem cells in other malignancies such as breast (2), pancreas (8), and brain (10, 25). These findings initiated an important change in the cells used in brain cancer research, and research focused on commercially established lines has shifted to the use of primary-cultured cells. It is now widely recognized that commercial cell lines do not recapitulate the parental tumors (10). Galli et al. (10) showed that primary-cultured glioma neural stem cells not only have in vivo tumorigenic potential but also recapitulate the hallmarks of glioblastoma to a much larger extent than the widely used commercial glioma cell line U87.

The core characteristic of CSCs is their ability to give rise to tumors that recapitulate the parental disease. Other important characteristics of CSCs, and in particular brain CSCs, come from the field of neural stem cells research and include their ability to self-renew and give rise to multiple lineages on differentiation. Although the terms *CSCs* and *tumor initiating cells* have been commonly used interchangeably, it is important to clarify that CSCs refer to a phenotype of tumor cells and not to the cell of origin (27).

Brain Tumor Stem Cell Niches

In the study presented by Inoue et al., the mice injected with the more invasive cell line developed tumors with minimal angiogenesis, whereas less invasive cells formed well-delineated, highly angiogenic tumors. This finding may reflect a varied dependence of the injected cells on vascular niches. GBs are characterized as highly vascular tumors, whose propensity for aberrant angiogenesis is thought to maintain abnormal niches of CSCs. These niches of brain tumor stem cells are highly reminiscent structurally and functionally of developmental neurogenic niches (22). Brain tumor and stem cell niches surround capillaries and are composed of cells, matrix, secreted proteins, and local metabolic conditions that maintain and regulate stem cell fate (11). This intimate association facilitates cell signaling and forms a microenvironment on which developmental and malignant stem cells rely.

Brain tumor cells use the microenvironment perversely to regulate proliferation and self-renewal through signals such as Olig2 and platelet-derived growth factor (15) and promote angiogenesis through vascular endothelial growth factor to recruit tumor vasculature further to the niche (3). Metabolically, the tumor stem cell niche forms a concentric hypoxic gradient, where the stem-like state of cancer cells is maintained at the hypoxic core by endothelial secretion of nitric oxide (6, 20, 23). The connection between brain tumor cells and blood vessels is also involved in cell invasion. Brain tumor cells also often use anatomic

structures such as blood vessels and myelin tracts to invade healthy brain parenchyma, one of the key GB features responsible for tumor recurrence (24). It seems the complex interactions within the tumor perivascular niche function to maintain and promote brain tumor stem cells.

In Vivo and Ex Vivo Models in Brain Cancer Research

Numerous in vivo models have been developed to study gliomas and the possible contribution of neural stem cells to tumors. These broadly consist of murine allografts and human xenografts, in which tumor cells are implanted into mice, and genetically engineered mouse models (GEMMs), in which tumors are created in mice de novo. Xenografts are among the most widely used and allow for primary human glioblastomas to be studied in immunosuppressed mice. Human orthotopic xenografts provide a stromal microenvironment that mimics native stroma, and this model has been shown to be useful in predicting phase II clinical trial performance of cancer drugs (which murine allografts do not) (28). In the context of studying brain tumor stem cells, an advantage lies in the opportunity to test whether specific populations of cells have the capacity to recapitulate the parental tumor. However, the number of cells injected in xenografts does not model spontaneous tumor development, the immunocompromised host does not allow for the role of immune surveillance-escape in tumor development, and the lack of competent immune system limits the host's capacity to tolerate treatments such as radiation (14).

GEMMs provide a unique opportunity to study oncogenes, tumor suppressors, and molecular events involved in tumorigenesis and progression of tumors. From initial murine models, in which viral oncogenes are overexpressed, to Tetregulatable and Cre-recombinase transgenic models, conditional strategies now allow temporal and spatial control of gene expression or inactivation (14). In particular, many GEMMs focus on *Nf1*, *p53*, and *PTEN*, tumor-suppressor genes that have been identified by the Cancer Genome Atlas as the three most frequently mutated in human GB (5). This system has been used more recently as a tool to determine the role of neural stem cells in the development of gliomas. Chen et al. (7) developed a tamoxifen-induced system that allows tumor suppressors to be inactivated specifically in stem/progenitor cells by using a neural-specific Nestin promoter to drive a Cre recombinase-estrogen receptor fusion protein. Using this system, these investigators showed that tumor suppressor inactivation of neural progenitor cells was sufficient to induce malignant glioma formation (1). They also showed that inactivation of tumor suppressors in stem/progenitor cells was not only sufficient but also necessary for tumor formation; this was shown when administration of Cre recombinase to nonprogenitor cells induced loss of tumor suppressors but did not result in the emergence of tumors (1). These conditional murine models support the hypothesis that gliomas may arise from neural stem cells rather than differentiated cells. They provide the means to investigate the natural progression of tumors in a genetic model that resembles human disease and have the potential to be used to test pathway-targeted therapies.

In the search for animal models that better reproduce the features of tumor origin, there is great interest in cell invasion models that represent what occurs in the healthy brain parenchyma as it is invaded. One disadvantage of tumor implanted models is the difficulty

in imaging the invading cells in vivo in real time. An ex vivo alternative to this problem is the use of organotypic cultures of brain slices (21). This method allows the real-time visualization of glioma cells as they invade normal brain parenchyma (9, 29). Combined with the use of intraoperative tissue, this method provides the unique opportunity to use a “human-on-human” model to investigate glioma invasion (13).

In summary, the field of brain cancer research is advancing toward the use of methods that better recapitulate human pathology. From systems aimed at characterizing the origins of tumors to the mechanisms of invasion in the actual brain environment, research is better able to explore the idea of GB heterogeneity and the role of CSCs. Proper use and characterization of primary-cultured cells together with the use of these exciting methods is expected to increase understanding of brain tumor pathology.

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Abbreviations and Acronyms

CSCs	cancer stem cells
GB	Glioblastoma
GEMMs	Genetically engineered mouse models

Biography



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