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REVIEW ARTICLE

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Modeling phenotypes of malignant gliomas

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Abstract

Malignant gliomas are primary tumors of the central nervous system characterized by diffuse infiltration into the brain and a high recurrence rate. Advances in comprehensive genomic studies have provided unprecedented insight into the genetic and molecular heterogeneity of these tumors and refined our understanding of their evolution from low to high grade. However, similar levels of phenotypic characterization are indispensable to understanding the complexity of malignant gliomas. Experimental glioma models have also achieved great progress in recent years. Advances in transgenic technologies and cell culture have allowed the establishment of mouse models that mirror the human disease with increasing fidelity and which support single-cell resolution for phenotypic analyses. Here we review the major types of preclinical glioma models, with an emphasis on how recent developments in experimental modeling have shed new light on two fundamental aspects of glioma phenotype, their cell of origin and their invasive potential.

KEYWORDS
experimental model, genetically engineered mouse model, glioma cell of origin, glioma invasion, malignant glioma

1 | INTRODUCTION

Malignant gliomas are primary tumors that arise in the central nervous system. They are characterized clinically by progression from low to higher malignancy grades and by a response to a limited range of therapies. Biologically, their malignancy is the result in part of a high level of heterogeneity and of diffuse infiltration into normal brain tissue. Our understanding of the genetic makeup and molecular biology of malignant gliomas has seen great advances over the last few years. Progress in genomics, epigenomics, and data-mining is revealing previously unknown characteristics of these tumors. The Cancer Genome Atlas study and several subsequent studies have thus characterized their patterns of somatic mutations, genome-wide DNA copy number alterations, and DNA methylation status.[1,2] As a result of these findings, the World Health Organization classification of gliomas was revised to add a layer of molecular information to the classical pathology-based system.[3] Furthermore, biodata portals now offer access to large international databases of such findings that allows researchers to verify the expression of their molecule of interest, analyze prognosis, and integrate clinical, genomics, and transcriptomics data.[4]

However, high-grade gliomas are heterogeneous, not only at the genetic level but at the cellular level. An equally detailed phenotypic characterization of these tumors and the linking of genomic landscapes to appropriate phenotypes will also be indispensable for the translation of preclinical knowledge into therapeutic strategies. Pertaining largely to cellular biology, questions regarding the cell of origin for gliomas and their diffuse infiltration into the brain parenchyma can be investigated only in animal models.

For more than 50 years, animal models have increased our understanding of human disease and have been instrumental in mechanism discovery and in validation of treatment targets and therapies.[5] Recent advances in the development of such models—in particular, in the establishment of genetically engineered models—have

also opened the door to single-cell resolution for phenotypic analysis.

We here briefly review current experimental models of malignant gliomas and outline the latest findings regarding two defining aspects of glioma biology: the cell of origin and tumor cell invasion.

2 | ANIMAL MODELS OF MALIGNANT GLIOMAS: TYPES AND CHARACTERISTICS

Gliomas are categorized histologically as such on the basis of glial morphology, and graded based on the presence of nuclear atypia (grade II and higher), mitotic activity (grade III and higher), or necrosis or microvascular proliferation (grade IV) on pathological examination. Grade IV gliomas, also known as glioblastomas, are among the solid tumors with the highest recurrence rates, the worst prognosis, and the fewest therapeutic options. These tumors spread insidiously into normal brain tissue and to distant regions beyond the reach of surgery or radiotherapy. They hijack and destabilize the existing microvascular network, and they secrete soluble factors that change the surrounding stroma, lead to self-renewal of malignant cells, and induce aberrant angiogenesis.[6,7]

High grade malignant gliomas, especially glioblastomas, are also known for their large number of genetic aberrations. These include mutations in tumor suppressor genes—such as *TP53*, *CDKN2A*, *RB1*, *PTEN*, and *NF1*—and enhancements in key signaling pathways such as MAPK and PI3K (phosphatidylinositol 3-kinase)–Akt.[1,2]

Ideally, experimental models should reproduce all of these histopathologic characteristics and should closely resemble human gliomas in their genetic and epigenetic profiles. Depending on the focus of the investigation, several additional requirements may arise. To ensure an undisrupted microenvironment with an intact blood-brain barrier (BBB) and immune response, studies on prevention and early detection of glioma would ideally be performed with spontaneous models, or at least with models that avoid physical manipulation of the brain. At the same time, the models should show a penetrance sufficient to allow evaluation of statistical significance. Studies focused on treatment should be performed with models that replicate the heterogeneity of human tumors, given that oversimplification with the use of a single type of cell or genetic aberration might yield results that are misleading with regard to drug efficacy and lead to failure of clinical studies.[8] Furthermore, the requirements regarding the microenvironment also apply to treatment studies. Tumor formation must be highly reproducible and tumor penetrance and latency should also be adequate for assessment of treatment effects.

Even if a single preclinical model of malignant glioma could fulfill all of these conditions necessary to mimic human disease, researchers would still have to adjust for species and strain differences, making validation in several models necessary. To date, available models can be classified into spontaneous, chemically induced, genetically engineered, and transplantation categories, with each type of model being best suited to investigate specific aspects of disease evolution (Figure 1, Table 1).

2.1 | Spontaneous gliomas

Extensive research on brain scrapie with the use of 23 inbred mouse strains led to the discovery that astrocytomas occur with the highest frequency among spontaneous neuroectodermal tumors and that they recapitulate human glioma characteristics such as spread along white matter tracts.[9] However, their development was found to be

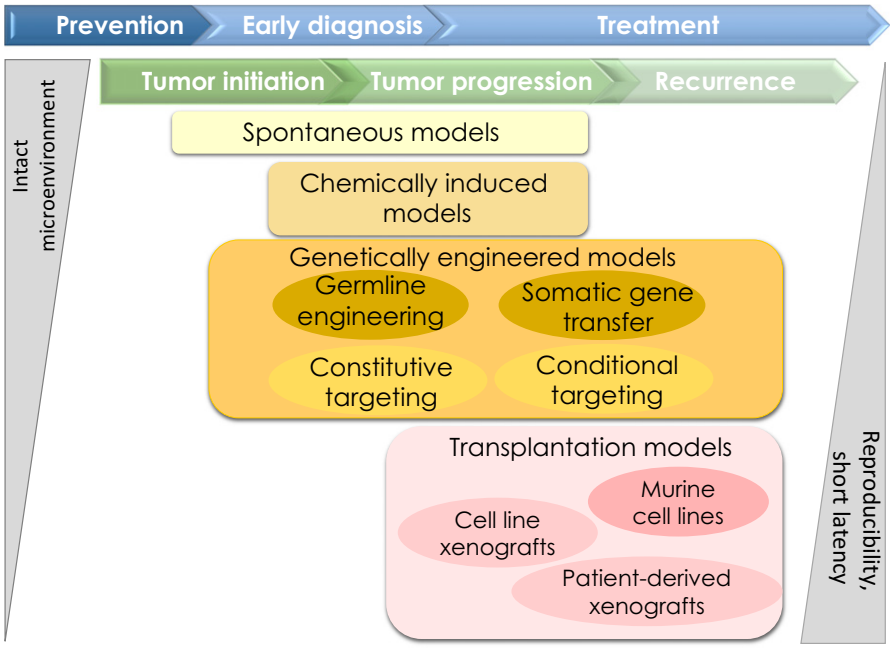

FIGURE 1 Outline of the relations between clinical end points (blue), phases of gliomagenesis (green), and appropriate experimental models

TABLE 1 Overview of glioma model types and of their advantages and disadvantages

Model		Advantages	Disadvantages	Recent advances	Examples
Spontaneous		Intact blood-brain barrier (BBB), microenvironment (ME)/ tumor microenvironment (TME)	Low prevalence		9-11
		Intact immune response	Low reproducibility		
		All stages of tumor development	Strain dependency		
Chemically induced		Intact immune system	Variable predictability		12,13
		No external manipulation of brain	Variable reproducibility		
		All stages of tumor development	Tumors in other organs		
Genetically engineered	Constitutive	Intact BBB, ME/TME	Presence of genetic alteration during development not desirable for adult tumors		14,38
		Mimic gradual tumor development	Do not always recapitulate genomic heterogeneity and instability		
			Reproduce a limited number/type of genetic alterations		
	Conditional	±Intact BBB, ME/TME	Complex genetic engineering	Use in combination with allografts	17-22
		Temporal and spatial control of transgene expression	Do not always recapitulate genomic heterogeneity and instability	Use of genomic editing to improve efficiency and simultaneous induction of multiple alterations	
		Mimic gradual tumor development	Labor-intensive and space-consuming		
		Diagnostic markers/ drug treatment can be tested throughout tumor development			
Transplantation	Xenografts	Relatively short latency	Diminished immune response	Use of serum-free, sphere culture to maintain heterogeneous phenotype	27-30
		Predictable engraftment and proliferation rates	Disrupted BBB and ME due to physical manipulation of the brain	Establishment of "humanized" mouse models	
		High reproducibility	±Limited reproducibility of invasion patterns	Grafting patient-derived tissue that includes stroma	
		Tumors have human expression profiles and heterogeneity			
		±Predict drug response for tumors of patient origin			
	Allografts	Intact immune response	Disrupted BBB and ME due to physical manipulation of the brain		35,36
		Short latency	Do not always recapitulate genomic heterogeneity of human tumors		
		High reproducibility			
		Reproducibility of invasion patterns			
		Evaluation of tumor-host interaction			

limited to a small number of strains, mainly VM and BRVR, and their incidence was still only ~1%.[9,10] On the other hand, dogs show a glioma incidence closer to that in humans and the tumors present with similar mutations such as those in *TP53* and *EGFR*,[11] but ethical considerations and low availability of suitable animal care facilities preclude large-scale studies of these animals.

2.2 | Chemically induced models

The administration of carcinogens to initiate intracranial neoplasms represents one of the earliest types of induced glioma model. Transplacental administration of *N*-methyl-*N*-nitrosourea or *N*-ethyl-*N*-nitrosourea to pregnant rats has mutagenic effects on the intrauterine embryos, resulting in a high incidence of brain tumors.[12] Gliomas established in this manner contain cells that manifest deletions or mutations in $p16^{Ink4a}$ or *Tp53* or aberrant expression of epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor, or Ras,[13] similar to their human counterparts. Their microenvironment and the BBB have not been disrupted by external manipulation, and the immune system remains intact. However, the predictability and reproducibility of tumor formation by chemical induction are variable. Allografts of clones established from such models and propagated in vitro, such as C6 and 9L gliomas, now constitute their most extensive application.[13]

2.3 | Genetically engineered mouse models

The genetic alterations characteristic of human gliomas have been mimicked in genetically engineered mouse models (GEMMs) in a variety of ways depending on the desired outcome. Introduction of an event of interest into embryonic cells or zygotes can result in the engineered change being expressed at the germline level, whereas direct editing of somatic cells has been applied to establish somatic GEMMs. Expression of a transgene can be permanent (constitutive), or it can be induced by several methods (conditional).

2.3.1 | Constitutive engineered models

Murine gliomas can be established by forced gain or loss of one or multiple genes of interest. An example in this category is provided by mice engineered to harbor mutations in the tumor suppressor genes *Nf1* and *Tp53* on the same chromosome (NPcis mice), which develop a range of astrocytomas, from low grade to glioblastoma.[14] In constitutive models, the presence of the genetic alteration (or alterations) during development can have undesired effects, and these models do therefore not necessarily provide a faithful recapitulation of adult gliomas. However, their relative ease of use after establishment is a major advantage. Constitutive engineered mice such as the NPcis model have shown that a combination of alterations in two tumor suppressor genes can be sufficient to elicit glioma formation.

2.3.2 | Conditional engineered models

Generation of mice that harbor a gene of interest flanked by loxP sequences and then crossing them with mice that express Cre recombinase under the control of a cell type–specific gene promoter allows tissue- or cell type–specific transgene expression. In the mouse brain, glial fibrillary acidic protein (GFAP) is expressed in subventricular zone neural stem cells (NSCs) and mature astrocytes, whereas nestin is expressed in NSCs as well as neural and endothelial progenitors.[15,16] Crossing of $p53^{+/-}$;$Nf1^{+/flox}$ cis mice with mice that express Cre recombinase under the control of the human GFAP gene promoter introduces the mutations into developing NSCs and mature astrocytes that express GFAP.[17] Offspring with the targeted genotype form gliomas with 100% penetrance and a survival time of 20-45 weeks.[17]

Expression of transgenes can also be controlled at the temporal level. Using mice that express Cre recombinase under the control of the nestin gene promoter linked to a tamoxifen-responsive sequence (*Nestin-Cre-*ER^{T2} mice) thus allows targeting of the adult NSC population by administration of tamoxifen beginning at 4 weeks of age. This approach, used to achieve conditional inactivation of tumor suppressors *p53*, *Nf1* and *Pten*, also generates grade III or IV gliomas with a high penetrance.[18]

The major advantages of conditional transgenic mice are the high degree of control over the desired genetic outcome and the lack of any direct physical manipulation of the brain before or during tumor formation. Brain homeostasis, the BBB, and the tumor microenvironment therefore remain intact, rendering these models suitable for a large variety of studies, including those focusing on tumor initiation and treatment evaluation.

However, the study of multiple mouse lines is expensive, labor-intensive, and space-consuming. In modeling of brain tumors, these limitations can be circumvented by injection-based gene transfer, albeit with the cost of a degree of interference with the microenvironment. For instance, injection of a lentivirus encoding Cre recombinase into the brain of loxP-based transgenic mice can result in the formation of gliomas. Stereotactic intracranial injection of a self-deleting lentiviral vector that confers Cre expression under the control of the CMV promoter into 8- to 16-week-old mice harboring combinations of floxed alleles of $p16^{Ink4a}$/$p19^{Arf}$, oncogenic $Kras^{V12}$, and *Tp53* results in the formation of high-grade gliomas that are lethal within as short a period as 2 weeks.[19] Another versatile approach based on gene transfer to somatic cells is the replication-competent ALV splice acceptor (RCAS)/Tva system, in which mice are engineered to express the ALV-A retrovirus receptor tv-a under the control of the GFAP or nestin gene promoter (Gtv-a and Ntv-a, respectively), resulting in astrocyte- or neural stem-progenitor cell–specific expression of the receptor.[20,21] The targeted cells are rendered susceptible to infection with avian leucosis virus (ALV)–derived RCAS vectors containing expression cassettes for oncogenes of interest such as *Pdgfb* or *Egfr*. Infection is achieved by injection of chicken fibroblasts producing the RCAS vectors into the desired region of the brain.[20,21] This model has been successfully adopted in

a large variety of studies, ranging from testing the oncogenic potential of various genetic aberrations to elucidation of the molecular characteristics of glioma stem cells.[20-22]

Finally, genome editing technologies have recently started to provide new opportunities in cancer modeling. The clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system can be applied to mouse zygotes to generate animals that harbor the targeted genetic modifications. A detailed protocol for generating mouse models with this system has been described.[23] This versatile technology can produce knockout, mutant, tagged or other targeted alleles in the mouse and might therefore become a preferred choice for cancer modeling in the future.[24] In utero electroporation of the ventricular zone in the cerebrum of wild-type mice at embryonic day 13.5 to achieve the simultaneous delivery of three plasmids encoding Cas9 as well as guide RNAs targeting *Nf1*, *Tp53*, and *Pten* has resulted in the establishment of tumors with glioblastoma-like pathology. With a penetrance of 100% and initiation of tumor formation apparent between 6 and 14 weeks after electroporation, this technique has been proposed as a fast and convenient system for the generation of new animal models.[25]

2.4 | Transplantation models

Stereotactic injection of glioma cells into the forebrain of experimental animals is one of the most reproducible and widely adopted glioma models.

2.4.1 | Xenograft models

Most xenograft models are based on implantation of human glioma-derived cell lines or primary cultures of patient-derived cells or multicellular aggregates into the brain of immunocompromised mice. Cell lines that originate from patient gliomas and have been cultured as a monolayer in the presence of serum have been used extensively for this purpose for many years. They have predictable proliferation and engraftment rates[26] and form tumors that recapitulate expansive growth and manifest a degree of angiogenesis, necrosis, and invasion.[27] However, the formed tumors are more homogeneous than the parental tumors or human gliomas in general, and they usually show relatively limited single-cell invasiveness and invasion along white matter tracts.[26,27] The lack of syngeneic tumor-host interaction—including interaction of the tumor with stromal and immune cells—and the consequent absence of remodeling of the tumor microenvironment may account in part for the differences with the characteristics of the parental tumor.

Culture of glioma cell lines in serum-containing medium has emerged as another factor that limits maintenance of the original tumor phenotype.[28] In contrast, culture of glioma cells in medium conditioned to sustain stem cells—usually by supplementation with epidermal growth factor, basic fibroblast growth factor, as well as a cocktail of antioxidants and insulin—has proved instrumental not only for enrichment of a cell population with stem cell characteristics but also for maintenance of the original properties of primary cultures, including infiltrative potential of individual cells and endothelial cell proliferation.[28,29] Such systems have also been used for the culture of primary oligodendroglioma cells[30] and for derivation of NSCs from human embryonic stem cells for subsequent transformation and establishment of difficult-to-model pediatric gliomas.[31]

Implantation of spheroids from biopsied tumor tissue was one of the earliest approaches introduced for reconstitution of a portion of the original tumor microenvironment. Minced tumor tissue is cultured until multicellular aggregates form. These outgrowths maintain the structure and composition of the biopsied tissue, including endothelial cells, macrophages, and extracellular matrix from the original tumor.[32] In recent years, this approach has been further refined and single-cell suspensions prepared from freshly excised glioma specimens have also been successfully transplanted into rodent brains as orthotopic patient-derived xenografts.

In summary, xenografts are the tool of choice for the modeling and investigation of tumors with human expression profiles, and advances in cell culture and harvesting techniques have expanded the types of analyses that can be performed with this model. Of note, advances in whole-transcriptome analysis, such as the development of RNA sequencing (RNA-seq), now allow improved accuracy in the discrimination of human and mouse transcripts, and such approaches can be used to further dissect the interactions between tumor cells and nonmalignant host cells, as shown for patient-derived xenografts for multiple solid tumors and for cell line–based models of metastatic brain tumors.[33,34]

2.4.2 | Allograft models

Syngeneic transplantation models allow interrogation of both tumor initiation and response to treatment in the context of an intact anti-tumor immune response. The earliest allografts were generated by intracranial injection of mouse cell lines originating from chemically induced tumors. The GL261 mouse cell line was established by the administration of 3-methylcholanthrene to C57BL/6 mice. It harbors *Tp53* and *Kras* mutations, yields tumors with a high penetrance, and has been successfully applied to studies of immune and gene therapies.[35]

Cell lines derived from GEMMs have also been adopted in transplantation experiments. Although this application diminishes the initial advantage of these models for tumor initiation studies, the short and predictable tumor formation time in a syngeneic environment makes the derived cell lines useful for therapeutic studies. Similar results can be achieved by the introduction of specific genetic alterations into isolated mouse NSCs and intracranial implantation of the transformed cells.[36] We have shown that forced expression of oncogenic H-Ras^{V12} in neural stem-progenitor cells isolated from mice with deletion of $p16^{Ink4a}/p19^{Arf}$ gives rise to glioma stemlike cells that form glioblastoma-like tumors. These tumors have a high morphologic heterogeneity and manifest single-cell invasion, pseudopalisading, and pronounced vascular proliferation.[36]

CURRENT RESEARCH TOPICS

Until unequivocal diagnostic procedures and highly effective treatments are established, most research focused on malignant gliomas will continue to be based on both preclinical and clinical studies, making use of all available assays. Certain questions pertaining to the cell biology of gliomas cannot be studied without the help of animal models. Recent advances in modeling techniques and in the resolution of in vivo analyses have improved our understanding of the following topics:

1 Cell of origin

Genetic engineering of mouse cells followed by their implantation into the brain of syngeneic mice has revealed several possibilities for the cell of origin: neural stem cells (NSCs), committed precursor cells, or differentiated astrocytes. Lineage tracing in conditional transgenic mouse models is now used to validate these possibilities for tumors developing in an intact microenvironment.

2 Invasion

In vitro migration or invasion assays have allowed characterization of many of the factors influencing glioma invasion. However, long-term, high-resolution in vivo imaging has revealed new ways in which tumor cells can spread and communicate, such as by the formation of tumor microtubes and the transmission of intercellular calcium waves.

3 | ANIMAL MODELS AT WORK: CURRENT RESEARCH TOPICS

Diversification and fine-tuning of animal models of malignant glioma have helped answer questions unapproachable by other investigative methods. Some of the newly found answers to these questions are summarized below.

3.1 | Cell of origin

Given the histological and molecular heterogeneity of malignant gliomas, it is possible that, by the time of treatment, the cell of origin is no longer present or does not constitute the most therapy-resistant clone in the tumor.[37] However, the ability to unequivocally identify the cells that initiate gliomas not only will deepen our understanding of this malignancy but also might lead to the discovery of molecular markers useful for early diagnosis.

There has been much speculation regarding the differentiation status of the cell of origin, and many animal studies have addressed this issue. The central nervous system of adult mice comprises several types of cell populations that can develop or have developed glial characteristics, including NSCs, astrocytic and oligodendrocyte precursor cells (OPCs), and differentiated astrocytes. Forced expression studies have provided insight into the possible scenarios and combinations of genetic aberrations that can underlie initiation of a mouse glioma.

With the use of mice deficient for $p16^{Ink4a}$, $p19^{Arf}$ or both, Bachoo and colleagues showed that loss of both $p16^{Ink4a}$ and $p19^{Arf}$ is sufficient for dedifferentiation of astrocytes in serum-free culture and also renders these cells susceptible to transformation by a constitutively active mutant of EGFR (VIIEGFR). In contrast, loss of $p16^{Ink4a}$, $p19^{Arf}$, or *Tp53* alone was not sufficient for astrocyte dedifferentiation.[38] Uhrbom et al[39] found that expression of K-Ras, but not of Akt, in Gtv-a–positive astrocytes deficient in $p16^{Ink4a}$ and $p19^{Arf}$ can lead to tumor initiation. Of note, in both studies, the same genetic lesions were also able to induce the formation of tumors from NSCs. Other combinations of genetic alterations have been found to induce the initiation of murine gliomas from neural stem-progenitor cells but not from astrocytes.[40,41] Together, these studies show that, at least in experimental settings and under the right conditions, both astrocytes and NSCs can be primed to initiate murine gliomas.

Advances in refinement of cell lineage markers and GEMM-based lineage tracing have proved instrumental for validation of these possible scenarios. For instance, placing a target gene under control of the nestin gene promoter-enhancer and intron-2 regulatory element restricts its expression to NSCs. Together with the application of tamoxifen-inducible recombination, such a system can lead to highly controllable glioma induction.[18] Mosaic analysis with double markers (MADM) relies on the Cre-loxP system to achieve conditional knockout of target genes during mitotic recombination and, at the same time, to complete the sequence needed to express a green fluorescent reporter in the mutant cells and a red fluorescent reporter in sibling wild-type cells.[42] This model has advanced phenotypic analysis and lineage tracing to the level of single-cell resolution, and it was applied by Liu and colleagues to show that loss of *Tp53* and *Nf1* leads to expansion of OPCs and, ultimately, to glioma formation, without a similar effect on NSCs, astrocytes, or neurons.[43]

Among the many gliomagenic possibilities uncovered by forced expression models, lineage-tracing GEMMs thus allow the selection of combinations of oncogenic events and recipient cells that can coevolve with the microenvironment. However, even such models are not completely free of bias, given that they are also based on predetermined genetic lesions. Refinement of lineage markers, identification of molecular markers for the earliest malignant cells, and the application of these molecular markers to validation studies and to the development of diagnostic markers are the main goals for this area of research.

3.2 | Invasion

Seminal pathology studies showed that gliomas undergo diffuse infiltration into the surrounding brain, manifesting several invasion patterns: perineuronal, perivascular, subpial, and along myelinated fibers.[44] Animal models have been indispensable for validation of in vitro findings concerning molecular regulators of cell motility, chemotactic factors, and tumor cell–matrix interactions. They have

also provided insight into the relation between invasive phenotypes and molecular and epigenetic regulators.[45,46] Furthermore, as with regard to the cell of origin, the combination of new models, visualization at single-cell resolution, and the switch to growth factor–based culture has led to the discovery of novel invasion characteristics.

For glioma cell lines grown in serum-containing medium and then transplanted into the mouse brain, the major pattern of invasion is the collective infiltration of tumor cells from the edge of the mass into the adjacent brain. Cloning of invasive cell subpopulations and microdissection to allow extraction of proteins from invasive cells in paraffin-embedded tissue specimens have been the principal approaches adopted to the establishment and analysis of cells with infiltrative potential.[47] With the advent of stem cell culture, a wide array of cell lines, genetically engineered stemlike cells,[36] as well as glioma cells from fresh surgical specimens[29] has been shown to possess a high infiltrative potential, as demonstrated for murine glioma cells in Figure 2A (right panel). While it remains to be determined to what extent this ability is due to the change in culture conditions (replacement of serum with growth factors) or to an intrinsic characteristic of stemlike cells, the ability to visualize single-cell invasion in real time has helped answer the following questions:

3.2.1 | When does invasion start?

A decrease in oxygen and nutrient availability at the tumor core causes glioma cells to search for more permissive environments. This scenario suggests that invasion occurs relatively late in the tumor formation process, certainly later than mass formation. Using our model of H-Ras^{V12}–expressing glioma-initiating cells, we have shown that this is not necessarily the case: On implantation into the forebrain of wild-type mice, these cells infiltrate the parenchyma before they form a tumor at the injection site.[36] Given that this is an implantation model, the results speak only to the ability of murine glioma-initiating cells to begin invasion at such an early stage. Exploitation of GEMMs with an intact microenvironment should reveal exactly when invasion starts during tumorigenesis, whether transformed NSCs that initiate tumors migrate away from their subventricular zone niches, and how astrocytes behave if they become malignant. Characterization of the trajectory of these cells during tumor initiation might reveal hidden invasion pathways that tumor cells can reuse. For instance, it might explain certain invasion patterns, such as the typical infiltration of tumor cells along the ventricles, infiltration that can lead to a "butterfly" appearance.[29]

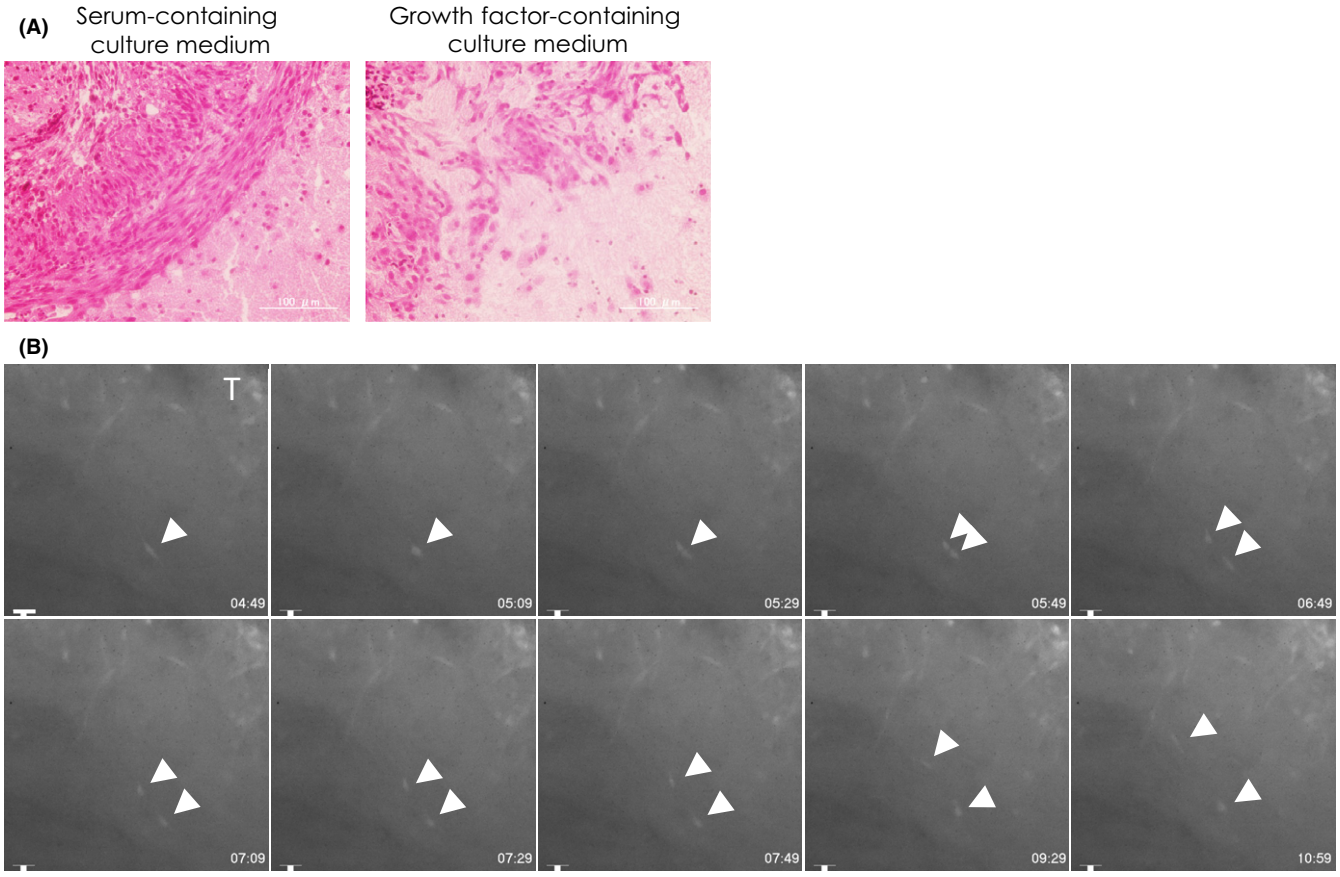

FIGURE 2 Invasion characteristics of a murine glioma model based on *Ink4a/Arf*$^{-/-}$ neural stem-progenitor cells transduced with H-Ras^{V12}. A, Hematoxylin-eosin staining of organotypic brain slices derived from mice 10 days after implantation of 1000 tumor cells and cultured in the presence of serum (left) or growth factors (right). Scale bars, 100 μm. B, Sequential images of a cultured brain slice from the model mice showing a single tumor cell before, during, and after division (arrowheads). Times represent hours:minutes. T, tumor. Scale bar, 30 μm

3.2.2 | Are proliferation and invasion mutually exclusive?

Isolation of invasive subpopulations from glioma cell lines has shown that these cells have a lower proliferation rate than their noninvasive counterparts.[48] The accumulation of similar experimental data led to the hypothesis of an inverse correlation between cell motility and proliferation.[49] Summarized by the expression "go or grow,"[47,49] this dichotomy appears to be partially true. However, real-time imaging of glioma-initiating cells has shown that the period during which proliferating cells cease migration is much shorter than initially thought. Using explants of brain slices harboring H-Ras^{V12}–expressing tumors, we found that tumor cells indeed slow their migration before dividing, but that they can restart migration within hours after mitosis (Figure 2B). Although consistent with results from other transplantation models, in which proliferating cells were shown to cease migration for as little as an hour,[50] this finding will need to be further investigated with GEMMs and intravital imaging. At present, it suggests that invading cells might be more responsive to therapy than previously considered, given that they are not all quiescent. However, it also suggests that anti-invasion therapies need to be continuous and initiated as early as possible.

3.2.3 | How do tumor cells communicate?

Long-term multiphoton imaging of intracranial xenografts formed by patient-derived glioblastoma cell lines maintained in serum-free, stem cell–favorable culture conditions has revealed a previously unknown type of connection between tumor cells known as "tumor microtubes." Tumor microtubes are ultra-long, actin-rich, membrane protrusions that infiltrate the brain at the invasion front and serve as tracks for the travel of nuclei after mitosis.[51] They can achieve a length of up to 500 μm and have been detected even in the hemisphere contralateral to the tumor in the case of astrocytomas. Furthermore, tumor microtubes are long-lived, having been found to persist for months, and they can serve not only as a physical scaffold for invasion but also as a conduit for the propagation of intercellular calcium waves, thus allowing communication between distant tumor cells.[51] They can also convey resistance to surgical lesions and chemotherapy.[52]

4 | FUTURE DIRECTIONS

Although a perfect, all-encompassing experimental system for the study of malignant glioma is not feasible, the quest for better therapies is expected to drive further refinement of animal models. Mice that express fluorescent reporters in all or specific compartments of normal tissue are already available for investigation of the interactions between tumor cells and the microenvironment.[53,54] The success of immune-checkpoint inhibitors in the treatment of other solid tumors has prompted evaluation of their use for brain tumors as well,[55] and the development of humanized mice[56] will likely be adapted to recapitulate populations of immune cells present in the brain. Finally, GEMMs in combination with clinical trials will be instrumental in the fine-tuning of therapy,[57,58] and the models will be further advanced at the same time by feedback from such trials.

DISCLOSURE

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