



Biomimetic Strategies for the Glioblastoma Microenvironment

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Glioblastoma multiforme (GBM) is a devastating type of tumor with high mortality, caused by extensive infiltration into adjacent tissue and rapid recurrence. Most therapies for GBM have focused on the cytotoxicity and have not targeted GBM spread. However, there have been numerous attempts to improve therapy by addressing GBM invasion, through understanding and mimicking its behavior using three-dimensional (3D) experimental models. Compared with two-dimensional models and *in vivo* animal models, 3D GBM models can capture the invasive motility of glioma cells within a 3D environment comprising many cellular and non-cellular components. Based on tissue engineering techniques, GBM invasion has been investigated within a biologically relevant environment, from biophysical and biochemical perspectives, to clarify the pro-invasive factors of GBM. This review discusses the recent progress in techniques for modeling the microenvironments of GBM tissue and suggests future directions with respect to recreating the GBM microenvironment and preclinical applications.

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INTRODUCTION

Malignant glioma, which is the most common primary brain tumor in adults, arises from star-shaped glial cells or their precursors within the central nervous system (Louis, 2006). Clinically, the World Health Organization grading system classifies gliomas into four stages. Of these, the most aggressive grade IV astrocytoma, glioblastoma multiforme (GBM); this tumor is the most devastating to health and cannot be cured (Louis et al., 2016). One of the main reasons why GBMs are incurable is that they spread widely within intracranial spaces, resulting in an indistinct tumor margin that prevents complete resection (Stummer et al., 2006). Unlike other systemic tumors, GBM rarely metastasizes beyond the central nervous system (Holland, 2000). GBM usually arises from the cerebrum and is prone to micrometastasis, i.e., infiltration at a single-cell level throughout the brain parenchyma, as well as across the corpus callosum from one hemisphere to the other, thus producing a bilateral butterfly like glioma (Dziurzynski et al., 2012). Although GBM invasion is confined within the intracranial spaces, the prognosis of the patients with GBM is still bleak.

The standard therapy for GBM is a surgical resection followed by a combination of radiotherapy and chemotherapy. Typically, postoperative radiotherapy is given along with the alkylating agent temozolomide (Stupp et al., 2005). Unfortunately, GBM cells are very resistant to these conventional therapies, and most patients with GBM end up developing recurrent tumors (Yip et al., 2009). Targeted therapy to overcome the low efficacy and high toxicity of postsurgical adjuvant therapies mostly focuses on treating proliferative cells, not invading cells (Wang et al., 2015). To achieve greater therapeutic efficacy, targeting the infiltration of GBM could be beneficial. Therefore, it is important to understand GBM invasion in brain tissue, to predict and evaluate tumor cell behavior; this is essential for developing new therapeutic inventions. Since GBM cells are predominantly regulated by the complex microenvironment and cause dynamic remodeling of their surroundings that facilitates

invasion (Rao, 2003), it is necessary to investigate the influence of the microenvironment of the brain to understand the unique features of GBM invasion. By reproducing both the composition and structural elements of the complex *in vivo* tumor microenvironment, the pro-invasive factors for GBM cells could be identified in pathophysiologically relevant context, which may eventually lead to novel therapeutic options for clinical trials.

This review focuses on recent research for biomimetic approaches to develop *in vitro* three-dimensional (3D) tumor models of glioblastoma cell invasion. First, the compositional and structural features of brain tumor microenvironment are introduced. Then, state-of-the-art experimental models of GBM invasion are presented and new approaches to mimic the brain microenvironment are discussed. Finally, future directions with respect to constructing *in vivo*-like tumor models for glioblastoma are suggested.

BRAIN TUMOR MICROENVIRONMENT

Brain tumor tissue comprises heterogeneous subpopulations of tumor cells intermingling with normal parenchymal cells (Hambardzumyan and Bergers, 2015). In addition to cellular components, non-cellular components such as brain extracellular matrix (ECM) and brain anatomy play crucial roles in GBM malignancy (Gritsenko et al., 2012). As pro-migratory, pro-invasive factors, these chemical and physical factors facilitate the GBM progression (Figure 1). By understanding the pro-invasive components within GBM microenvironment, we could recreate the *in vivo* behaviors of GBM cells.

Anatomical Features in Glioma Tissue

The unique anatomical structures in the brain include white matter tracts and capillaries, called Scherer's structures (Cuddapah

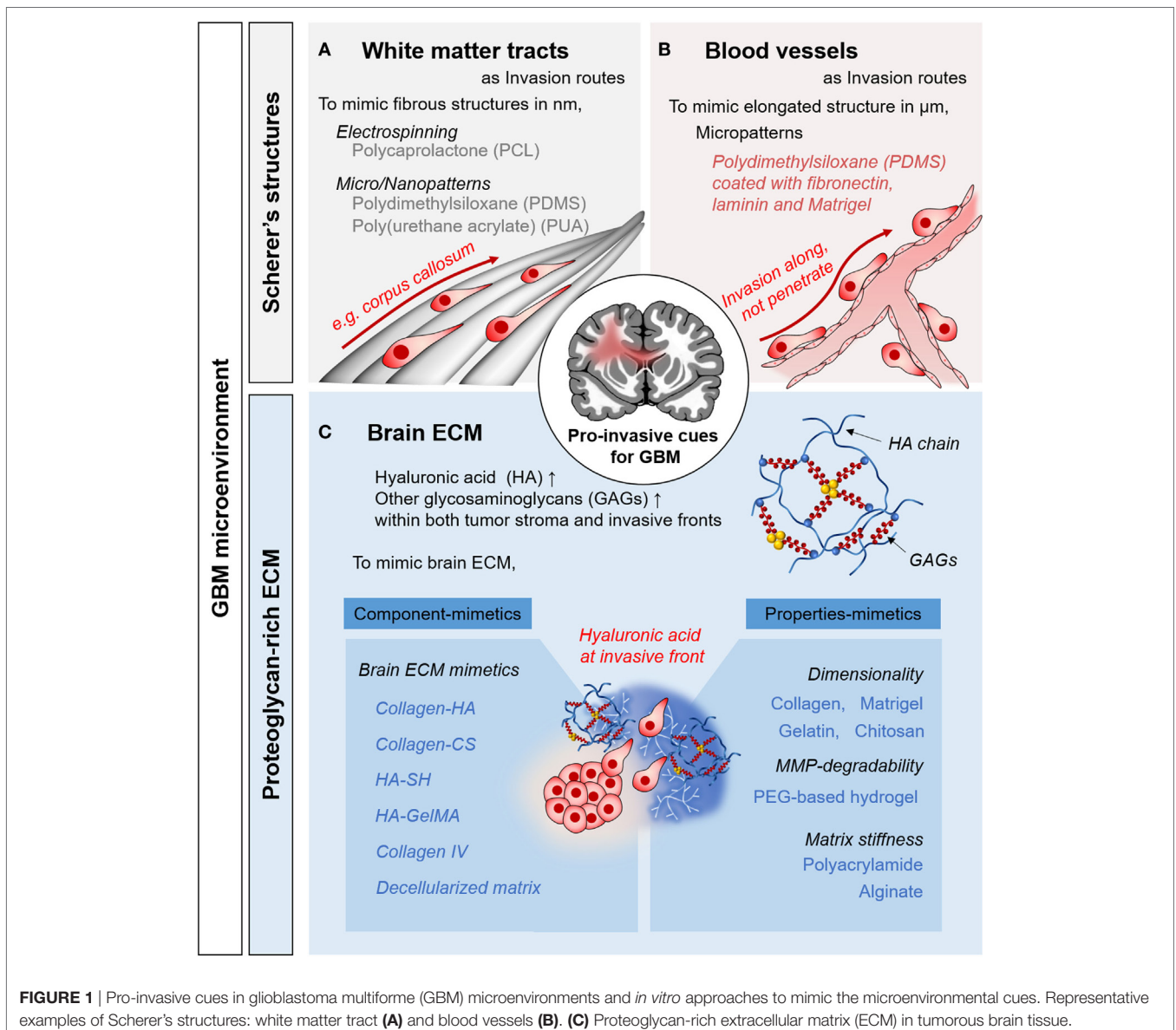


FIGURE 1 | Pro-invasive cues in glioblastoma multiforme (GBM) microenvironments and *in vitro* approaches to mimic the microenvironmental cues. Representative examples of Scherer's structures: white matter tract (A) and blood vessels (B). (C) Proteoglycan-rich extracellular matrix (ECM) in tumorous brain tissue.

et al., 2014). A white matter tract is a bundle of aligned axons. These are bound mainly in corpus callosum, in which fibrous structures consist of submicron-sized fibers. For pro-invasive characteristics, topography is a major mechano-physical cue derived from the ECM structure and the anatomical features or organs, which play key roles in cellular behavior and function (Friedl and Alexander, 2011). Histological observations have revealed that single GBM cells preferentially migrate to these structures (Bellail et al., 2004; Louis, 2006). As biophysical cues for pro-invasive characteristics, tumor cells interact with this existing brain anatomy, which is an important mechanism of invasion by GBM cells.

ECM in Glioma Tissue

The normal brain lacks the stiff fibrillar collagen matrix that is typical component in other organs (Bellail et al., 2004). The brain has a substantially different ECM, composed of glycosaminoglycans (GAGs), proteoglycans (PGs), and other glycoproteins (Cuddapah et al., 2014; Rao et al., 2014; Miyata and Kitagawa, 2017). The interstitial spaces in the brain parenchyma are filled with a PG-based matrix, which interacts with hyaluronic acid (HA)-binding proteins (e.g., CD44 and RHAMM) and tenascins (Gladson, 1999). By contrast, fibrous, adhesive ECM proteins, such as collagen, laminin, and fibronectins, are rarely found in the brain parenchyma, where their expression is restricted to the basement membrane of neural vasculature (Lau et al., 2013). These ECM components function as components of blood-brain barrier, which constitutes both a chemical and physical barrier, inducing the formation of tight junctions.

The composition of the ECM of glioma tissue is distinct from that of normal tissue. During cancer progression, the secretion of neural ECM molecules is increased significantly. The amorphous ECM at the invasive front of a proliferating GBM strongly expresses GAGs and PGs, especially HA (Bellail et al., 2004; Jin et al., 2009). Moreover, fibrous, adhesive ECM proteins such as collagen IV, fibronectin, and laminin are strongly upregulated (Gladson, 1999). Increased production of tumorous ECM leads to a significant increase in its volume, contributing to elevated interstitial pressure in the confined extracellular space (Munson et al., 2013). In addition to acting as a signaling molecule, tumorous ECM activates tumor-associated pathways, promoting cell survival and motility (Rape et al., 2014).

IN VITRO 3D GBM MODELS

There are several advantages of utilizing *in vitro* 3D GBM models to investigate the effects of the microenvironment on GBM invasion. Unlike two-dimensional (2D) models, *in vitro* 3D GBM models can replicate the highly complex microenvironment of *in vivo* GBM niches. Furthermore, well-defined 3D *in vitro* GBM niches are simpler and more reliable than *in vivo* animal models, which involve costly, time-consuming technical procedures (Xiao et al., 2017). Here, we presented an overview of the biomimetic approaches for reconstructing *in vitro* 3D GBM models of anatomical and matrix-related aspects of the GBM microenvironment (see also **Table 1**).

Fibrous Scaffold-Based Culture Models

Histological evidence indicates that GBM cells migrate along pre-existing brain structures and form Scherer's structures by interacting with the neural microenvironment. Brain anatomy, including the brain parenchyma, pre-existing blood vessels, white matter tracts, and the subarachnoid space below the meningeal covering of the brain, plays an important role in GBM invasion (Cuddapah et al., 2014).

To mimic these invasion routes, several synthetic polymer-based micro/nanotechnologies have been used to investigate the behavior of GBM cells. Recently, there have been reports on the effect of 2D topography on GBM invasion, replicating the *in vivo* behavior of GBM cells (Cha et al., 2015; Smith et al., 2016; Sim et al., 2017). For instance, using 2D polydimethylsiloxane (PDMS) substrates, the tapered microtract of the gradient width ranging from 3 to 100 μm was fabricated to identify the effect of topography on GBM motility (Cha et al., 2015). For microtracts smaller than 3 μm , micropatterns can induce *in vivo*-like saltatory migration, even on 2D surfaces. In addition, polyurethane acrylate-based nanotopography (Smith et al., 2016) and nanofabricated polystyrene (Zhu et al., 2004) closely mimic the fibrillar structures of brain.

Techniques to fabricate nanofibers, such as electrospinning, have been used to mimic the delicate fibrous structures of the white matter tracts (Johnson et al., 2009; Agudelo-Garcia et al., 2011; Rao et al., 2013b; Sharma et al., 2013; Beliveau et al., 2016; Cha et al., 2016). The behavior of glioma cells on nanofibers was observed to be a function of substrate topography, as GBM cells migrated much faster on aligned fibers than on random fibers (Johnson et al., 2009; Beliveau et al., 2016). This *in vivo*-like behaviors of glioma cells is associated with STAT3 signaling, a driver of malignancy during GBM progression (Agudelo-Garcia et al., 2011). Modification of both biochemical and biophysical features of nanofibers has a significant effect on GBM migration (Rao et al., 2013b; Sharma et al., 2013), emphasizing the importance of biomimetic approaches to understanding the behaviors of glioma.

Matrix Scaffold-Based Culture Models

To simulate tumorous ECM components in brain, researchers have attempted to reconstruct an HA-rich environment and GAG-based matrices. The biophysical properties of the ECM matrix have also been reported to influence GBM cell invasion; thus, numerous studies have examined the effects of matrix dimensionality, degradability, and stiffness.

Matrices to Mimic the ECM Properties

In addition to the brain anatomy, the brain ECM also has biophysical effects on GBM invasion. To provide the effects of 3D cell-ECM interaction on GBM invasion, naturally derived biomaterials, such as Matrigel (Jin et al., 2009) and collagen (Yang et al., 2010), have been used to develop a 3D ECM microenvironment for GBM. Irrelevant components of the brain ECM, such as chitosan-alginate hydrogels (Kievit et al., 2010) and collagen-agarose hydrogels (Ulrich et al., 2010), were chosen because they have a biologically inert to investigate biophysical effects of the matrices solely, resulting in a mechanosensitive

GBM invasion response. When designing matrices, their physical properties, such as degradability, stiffness, and pore sizes, are usually interconnected and thus influenced each other. Therefore, studies controlling designing parameters, such as

the physical properties of matrix metalloprotease (MMP)-degradable polyethylene glycol gels (Wang et al., 2014, 2017), gelatin methacrylate (GelMA) (Pedron and Harley, 2013), and polyacrylamide (Ulrich et al., 2009; Rape and Kumar, 2014;

TABLE 1 | Representative approaches used to mimic glioblastoma multiforme (GBM) microenvironment.

To mimic Scherer's structures

	Biomaterials	Techniques	Cells	Findings	Reference
Effects of anatomic topography	Polydimethylsiloxane (PDMS)	Soft lithography-based micropatterning	U87, U251	Effective topography to induce saltatory migration	Cha et al. (2015)
	Polyurethane acrylate	UV-assisted capillary force lithography	Patient-derived GBM cells	Mimicry of <i>in vivo</i> three-dimensional (3D) migration, PDGF-sensitive response	Smith et al. (2016)
Fibrous structure of white matter tract	Polycaprolactone (PCL)	Electrospinning	U251, X12, neurosphere glioma from biopsy	Effects of fiber directionality on glioma migration	Johnson et al. (2009)
	PCL	Electrospinning	Dissociated U87, U251, GBM-derived tumor initiating cells (G8, G9)	Involvement of STAT3 signaling in topography-induced glioma migration	Agudelo-Garcia et al. (2011)
	PCL	Electrospinning	Patient-derived GBM cells (GSC11)	Invasion preference along the fibrous structures	Cha et al. (2016)
	Polystyrene	Spinneret-based Tunable Engineered Parameters (STEP)	DBTRG-05MG	Effects of nanofibers on glioma migration and blebbing dynamics	Sharma et al. (2013)
	PCL, gelatin-PCL, PDMS-PCL, PES-PCL, PCL-collagen, PCL-HA, PCL-matrigel	Core-shell electrospinning	Patient-derived GBM cells (OSU-2)	Complex interplay of mechanics, chemistry, and topography on glioma migration	Rao et al. (2013b)

To mimic brain extracellular matrix (ECM)

ECM properties-mimetics	Dimensional effect	Chitosan-alginate (CA) scaffolds	Ionic crosslinking of lyophilized CA mixture	C6 rat glioma, U87, U118	Higher malignancy of 3D cultured GBM than two-dimensional	Kievit et al. (2010)
		Collagen-agarose hydrogels	Physically blended mixture	U373 glioma spheroids	Mechanosensitivity to ECM-based biophysical cues	Ulrich et al. (2010)
Matrix degradability/stiffness/pore size		Multi-arm based polyethylene glycol with matrix metalloprotease (MMP)-cleavable peptide	UV photocrosslinking	U87	Effects of matrix stiffness on ECM deposition and remodeling through modulating HA synthases or MMPs	Wang et al. (2014, 2017)
		Gelatin methacrylate (GelMA)	UV photocrosslinking	U87	Impact of biophysical properties (matrix density, crosslinking density, and degradability) on glioma phenotype	Pedron et al. (2013)
		Polyacrylamide (PA)	Polymerization with APS, TEMED	U373, U87	Impact of microenvironmental stiffness on GBM proliferation	Ulrich et al. (2009) and Umesh et al. (2014)
		Adhesion peptide (RGD)-functionalized HA-methacrylate (Me-HA)	Control of degree of methacrylation at varying ratios of thiols	U373, U87, C6 rat glioma	Matrix stiffness-dependent cell adhesion (spreading), cell speed, cell growth, and cell invasion	Ananthanarayanan et al. (2011)
	Hyaluronic acid-methacrylate (Me-HA)	Functionalization of HA with methacrylic anhydride	U373, U87	CD44-mediated cell adhesion, motility, and invasion in stiffness-dependent manner	Kim and Kumar (2014)	

(Continued)

TABLE 1 | Continued

To mimic Scherer's structures					
	Biomaterials	Techniques	Cells	Findings	Reference
ECM components-mimetics	Adhesion peptide (RGD)-functionalized HA-methacrylate (Me-HA)	Michael-type addition with dithiothreitol	U373, U87, C6 rat glioma	Mechanobiological regulation on brain tumor progression, elevated invasion by CD44-mediated HA	Ananthanarayanan et al. (2011) and Kim and Kumar (2014)
	Collagen I, III, or IV with thiolated HA	Interpenetrating polymer network	Patient-derived GBM cells (OSU-2)	Influence of collagen types on GBM morphology, HA-dependent GBM spreading and migration	Rao et al. (2013a)
	Collagen I-HA	Semi-interpenetrating polymer network	Patient-derived GBM cells (GSC11)	HA-induced GBM invasion and associated mechanisms	Cha et al. (2016)
	HA-MA functionalized GelMA	Photocrosslinking	U87 (+EGFR)	HA-induced GBM malignancy and effects of HA-graded heterotypic tumor microenvironment	Pedron et al. (2013, 2015)
Hybrid scaffolds to mimic the GBM microenvironment					
Combinations					
HA matrices + fibrous structures		3D configuration of collagen-HA semi-interpenetrating polymer network on electrospun fibers	Patient-derived GBM cells (GSC11)	Microenvironmental adaptations in response to drug treatment	Cha et al. (2016)
Hydrogel stiffness + HA contents		Photocrosslinking, of GelMA + HAMA in concentration-dependent manner	U251	Coordinated effect of matrix stiffness, immobilized HA, and compensatory HA production on GBM invasion	Chen et al. (2017)
Vascular basement + HA surfaces (tissue interface)		Interfacial culture between fibronectin-coated PA and HAMA	U373, U87	Mechanochemical feedback at the tissue interfaces	Rape et al. (2014)
GBM-endothelial cell coculture (GBM perivascular niche)		Photocrosslinking, of GelMA + HAMA	U87, HUVECs, NHLF	Contribution of perivascular niche to GBM invasion	Ngo and Harley (2017)

Umesh et al., 2014), have been performed to examine the role of mechanobiological regulation in GBM invasion. For investigating mechanosensing characteristics of GBM cells, the modified forms of HA gels, such as HA-methacrylate (Me-HA) or RGD-functionalized Me-HA (Ananthanarayanan et al., 2011; Kim and Kumar, 2014), were used to control the matrix stiffness, observing the stiffness-dependent GBM features *via* CD44-mediated mechanosensing.

Matrices to Mimic the ECM Components

Hyaluronic acid, which is a major ECM component in tumorous brain, is a linear chain composed of D-glucuronic acid and N-acetyl-D-glucosamine, so it is hard to form cross-linked network itself. Therefore, to investigate the role of HA in GBM invasion, the HA is chemically modified so that it is cross-linked. Chemically modified forms of HA hydrogels include thiolated HA (Rao et al., 2013a), methacrylated HA (Ananthanarayanan et al., 2011; Kim and Kumar, 2014), and mixtures with GelMA (Pedron et al., 2013). GBM cells are strongly influenced by the HA concentration and show increased invasion *via* CD44-mediated HA adhesion (Kim and Kumar, 2014).

Physical blending with other cross-linkable hydrogels, such as collagen, is another means of incorporating HA components

into ECM-mimetic model system. By creating interpenetrating polymer networks with HA solution and collagen gels, HA-rich matrices can be created (Yang et al., 2011; Rao et al., 2013a; Cha et al., 2016). Within this HA-rich matrix, GBM develops highly proliferative, invasive phenotypes (Cha et al., 2016). These results have been confirmed using other HA-rich matrices, such as GelMA-based HA-rich matrices (Pedron et al., 2015, 2017), which have *in vivo*-like characteristics.

Various GAG-based components, such as heparin, chondroitin sulfate, and keratin sulfate, are often incorporated within the ECM of tumorous brain as the scaffolding components. Tenascin-C (Sarkar et al., 2006) and chondroitin sulfate (Yang et al., 2011; Logun et al., 2016) have been incorporated into collagen hydrogels to investigate their influence on the GBM invasion. As expected, the motility of GBM was increased by higher concentrations of both GAGs.

For advanced brain ECM-mimetic model system, decellularized matrix, obtained by isolating the ECM scaffold of an original tissue (Gilbert et al., 2006), shows utility for recreating the ECM components of GBM tissue. Using porcine brain tissue, decellularized ECM was applied to mimic the brain matrices (DeQuach et al., 2011), further indicating the potential of mimicking the tumorous ECM to investigate GBM invasion.

Hybrid Scaffolds for GBM Microenvironment

Recently, researchers have focused on integrating multiple components of GBM microenvironment to reproduce conditions corresponding more closely to *in vivo* conditions. As mentioned earlier, by controlling the various design parameters of ECM matrices, the combined effects of the chemical and mechanical properties of the ECM on GBM invasion were revealed (Chen et al., 2017). Moreover, our group developed 3D GBM models consisting of HA matrices and fibrous structures arranged in a 3D configuration. Using such integrated systems, microenvironmental adaptation was observed during GBM invasion (Cha et al., 2016); changes in the GBM invasion of the HA matrices were mediated by the focal adhesion kinase and MMP, within the fibrous structures, in response to blockade of a HA-mediated pathway of GBM invasion. Recently, other cellular components in the microenvironment were incorporated into 3D GBM models, revealing the roles of glial cells and the perivascular niche in GBM invasion (Grodecki et al., 2015; Iwadata et al., 2016; Gritsenko et al., 2017; Ngo and Harley, 2017).

FURTHER DIRECTION FOR *IN VITRO* GBM MODELS

In recent decades, the focus of cancer treatment has shifted markedly, from targeting cancer itself to understanding its microenvironment; this is because an aberrant microenvironment is pivotal to cancerous tumor progression (Joyce and Pollard, 2009). As suggested in seed-and-soil hypothesis (Fidler, 2003), a favorable environment for the metastatic cancer is crucial for enabling cells to settle down and mature. Indeed, cancers reside in complex tissue environments, including stromal cells, blood vessels, immune cells, and the ECM, which strongly influence the sustained growth, invasion, and metastasis of cancer (Gilkes et al., 2014). During tumor progression, cancer cells remodel dynamically and interact reciprocally with their microenvironment (both the cellular and non-cellular components), leading to the formation of a tumor-favorable environment (Quail and Joyce, 2013). Many recent studies have highlighted the importance of targeting tumor microenvironment to reduce tumor malignancy (Cuddapah et al., 2014; Hambardzumyan and Bergers, 2015; Xiao et al., 2017). For further progress in the development of *in vitro* GBM models, the other components of brain tumor microenvironment should be considered to better understand glioblastoma invasion.

Intratumoral Heterogeneity

Solid tumors have mass transport limitations due to their decreased surface-area-to-volume ratios and longer diffusion lengths. Owing to this limitation in diffusion, the tumor mass develops internal hypoxic areas, causing shortages of oxygen and nutrients. Tumor hypoxia is one of the key factors inducing the development of heterogeneous cell subpopulation within the tumor masses, which leads to an aggressive treatment-resistant phenotype, rapid progression, and poor prognosis (Heddleston et al., 2009; Li and Rich, 2010; Cheng et al., 2011). Recent studies have reported that these subcellular populations within the GBM

mass are cancer stem cells, which exhibit increased tumorigenicity and stem cell-like capacity (Hubert et al., 2016). Histologically, GBM tissue contains a large hypoxic core, called pseudopalisading necrosis (Rong et al., 2006), which in turn contains a stem-like subpopulation (Mamun et al., 2009). Due to its greater therapeutic resistance compared with other tumor population, the roles of cancer stem cells in GBM progression should be considered.

Interaction with Surrounding Cells

Along with cancerous astrocytoma cells, GBM tissues contain other tumor-associated parenchymal cells such as glial cells, vascular cells, microglia, peripheral immune cells, and neural precursor cells. These all play crucial influencing roles in the pathology of GBM (Rape et al., 2014). For example, when activated, astrocytes in GBM tissue promote tumor progression within the GBM microenvironment (Hu et al., 2017). Moreover, the neural vasculature provides a perivascular bed not only for GBM but also for stem-like GBM cells (Bao et al., 2006; Johansson et al., 2017). In addition, microglial cells, which can comprise up to 30% of the brain tumor mass, are heavily involved in GBM invasion (Hambardzumyan et al., 2016). Therefore, by cocultivating these surrounding cells, we can investigate the supportive roles of tumor-associated cells on GBM progression.

Application: *In Vitro* GBM Models As High-Throughput Platforms

By integrating and incorporating the complex components of GBM microenvironment, the model presented herein should be used as a drug-testing tool for GBM patients. For example, by using patient-derived cells and matrices, personalized treatment plans can be constructed. Since the biomimetic approaches used to model the GBM microenvironment contribute to enhancing the similarity and reliability for *in vivo* GBM cells, anti-invasive therapies could be evaluated in a high-throughput manner.

CONCLUSION

This review offers an overview of recent development in *in vitro* GBM models in their microenvironmental context and their further perspectives. Using these biomimetic models, we can investigate and evaluate the invasive features of GBM cells, consequently providing the drug test platform to target their invasion. In the future, we thus expect that the integration of multiple components from complex microenvironment will enhance the understanding of GBM biology and further suggestion of effective therapeutics for the GBM patients.

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JC and PK planned and wrote the manuscript of this mini review.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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