Introduction

Glioblastoma multiforme (GBM) is the most prevalent type of primary brain tumor among adults, with a rate of about 3.2 cases per 100,000 individuals \([1,2]\). Surgical resection, and radio- and chemotherapy are the standard GBM treatments \([1]\). However, the prognosis remains poor, with a median overall survival of 12 to 15 months upon diagnosis. Despite significant improvements in GBM research, therapeutic options are still limited and survival rates have not significantly improved. Accordingly, clinical and translational studies are hampered due to the lack of suitable preclinical models that accurately reflect the brain tumor architecture and its microenvironment. Scientists have recently developed cerebral organoids, which are artificial 3-dimensional brain-like tissue. Organoid technology provides new cancer modeling options, which could help us better understand GBM pathogenesis and design personalized treatments. In this review, we summarize recent developments in organoid GBM models, highlighting their advantages in cancer modeling, as well as their challenges and limitations and potential future directions in GBM therapy.

Keywords: Glioblastoma; Glioma; Stem cell; Organoids; Therapeutics
that resist targeted therapy and promote relapse within the tumor [4].

Several preclinical models have been developed to understand the fundamental pathobiology and discover therapeutic approaches in GBM. However, these have demonstrated minimal efficacy or have failed in clinical settings [12]. Most GBM studies have relied on 2-dimensional (2D) cell culture methods, in which cells are maintained in serum-containing media. This method, however, does not account for the actual variety of tumors, which are subject to clonal selection and genetic drift [13]. Meanwhile, glioma stem cell (GSC) cultures cultivated in 3-dimensional (3D) spheres in serum-free environments can preserve the tumor’s genetic background, degree of phenotypic heterogeneity, and molecular gradients, as well as maintain invasive behavior when intracranially implanted in immunodeficient mice [14]. However, GSCs do not sustain a complex tissue architecture, such as the ECM and tumor microenvironment (TME), which can play an important role in proliferation. Moreover, late cell passages are susceptible to clonal selection and genetic drift [13]. Furthermore, the commonly used animal models in GBM are xenografts, genetically engineered, and syngeneic mouse models [15]. However, these in vivo models do not fully reflect the genetic characteristics of tumors or capture their heterogeneity [16]. Immuno compromised mice models also have the disadvantages of being time-consuming, costly, and limited in sample number [17]. Thus, current in vitro and in vivo models must be reconsidered, and new approaches must be developed to fill the gap between preclinical and clinical outcomes.

Recent advances in cerebral organoids are starting to address the drawbacks of traditional GBM models. This method can capture GBM complexity, heterogeneity, and responsiveness to treatment. In this review, we examine the current state of GBM organoid models and their importance, limitations, and possibilities as preclinical models for studying tumor architecture and microenvironment, invasion, and drug screening for precision and personalized medicine.

Established GBM models

1. In vitro GBM models

2D cultures, which are maintained in a monolayer in a culture flask or petri dish, are commonly used as models to better understand cell biology, disease mechanism, drug action, production of proteins, and development of tissue engineering [18]. In the 1950s, the first brain tumors were cultivated in vitro. In 1968, Pontén and Macintyre established the Uppsala (U) series of malignant glioma cell lines obtained from human gliomas [19]. Glioma cell lines like U87MG, U251MG, U373MG [20], LN229 [21], and T98G [22] have been extensively used in 2D models [16]. Although the 2D culture method is simple, low-cost, and capable of being used to conduct functional tests [18], it has several limitations, including (1) lack of interactions between cancer stem cells (CSCs) and the TME causes genetic and epigenetic alterations; (2) lack of oxygen, nutrient, and pH microenvironment gradients; (3) absence of physiological input from other metabolically active organs; and (4) genetic alterations after long-term culture [23]. Likewise, when glioma cell lines are implanted in immunodeficient mice, the tumors generated fail to replicate several GBM features, including microvascular proliferation, diffuse infiltration, and necrosis [24].

Meanwhile, the presence of a subgroup of GSCs adds to the overall treatment resistance machinery in GBM [24]. GSCs share some characteristics with healthy neural stem cells, including the ability to self-renewal capacity and differentiate into various cellular lineages [25], and the expression of stem cell-related biomarkers (e.g., CD15, CD133, CD44, CD70, ALDH1A3, S100A4, OCT-4, SOX2, Nanog, and Nestin) [26]. Also, the proliferative and quiescent characteristics of GSCs, as well as their treatment resistance, are defined by their gene signatures (proneural [PN] and mesenchymal [MES]), metabolic pattern, and biological activities [27].

GSCs are grown in spheroid cultures in serum-free media with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), as well as a nutrient, mix such as N2 or B27, to preserve their self-renewal ability and generate cellular lineages seen in the parental tumor [28,29]. Gliomabase (also known as neurosphere) cultures can preserve key mutations and generate tumors that reflect human GBM after xenotransplantation, which has proven their efficacy as a model [28]. Meanwhile, GSCs can also be grown in a monolayer over a laminin-coated plate, ensuring that all stem cells have equal access to growth stimuli, and allowing them to maintain a highly pure stem cell population while suppressing differentiation [30]. However, late passage cell cultures are susceptible to clonal selection and genetic drift [13].

2. In vivo GBM models

Mice are by far the most accessible in vivo model for GBM research due to their biological similarities to humans, as well as their flexibility for genetic manipulation and short breeding durations [4]. Each GBM mouse model has unique advantages and limitations that can affect the validity and translatability of experimental data [31]. The usual Cre-LoxP approach targets
GBM-related genes selectively, however it takes a long time to analyze. Although the CRISPR-Cas9 approaches take less time to develop animal models, off-target effects result in a wide range of GBM phenotypes. While transposon-based insertional mutagenesis systems can prevent and enhance transcription, the amount of insertional transgenes is strictly limited [32]. Also, injecting allograft cell lines into immunocompetent mice reduces immunological rejection, it fails to replicate certain aspects of human GBM [32].

Tumor cells are implanted into immunocompromised mice such as nude, NOD/SCID, and NOD/SCID gamma (NSG) in xenografts in an attempt to imitate human GBM. Patient-derived xenografts (PDXs) are seen as more promising due to their ability to accurately imitate human GBM in a mouse model [31]. Early passages may allow PDXs to retain the key histological and genetic characteristics of their source tumor [33]. PDX models also accurately simulate the heterogeneity of varied individual tumors as compared to genetically engineered human cancer models [34]. However, xenograft models also have limitations. Cultured tumor cell xenograft GBM models are frequently unable to mimic GBM interactions with components of the TME, such as vasculature [35]. Moreover, xenograft tumors also develop quickly after being injected subcutaneously and are confined to the subcutaneous spaces, which is fundamentally different from the brain microenvironment. On the contrary, tumors that are intracranially injected interact with the host brain microenvironment, and tumorigenesis and therapeutic effect often take time [36]. Likewise, variation occurs based on the type of brain tumor and the cell line being studied. For a multitude of factors, including the host’s immune system, the mouse species used has a major effect on how the outcomes must be interpreted. Graft-versus-host disease will occur in a mouse with an intact immune system, obscuring the significance of the immune system in cancer. Consequently, when immunodeficient mice are used, a key component of the immune TME is absent [37].

3D Organoid models

Scientists have recently developed an organoid model that allows cells to grow in a 3D environment in vitro and captures the phenotypic and molecular heterogeneity present in different organs [24]. Organoids are created by embedding patient-derived stem cells in a Matrigel matrix and cultivating them with various growth factors. Within a few days, these cells multiply and differentiate, self-organizing into an organic structure that mimics the function and structure of an organ in vivo (“mini-organs”) [38,39]. Several organoid models have already been developed for the colon, kidney, liver, brain, pancreas, ovary, stomach, and lung, among other organs [40]. The organoid model is used in various applications including basic research, disease modeling, biobanking, precision medicine, drug screening, host-microbe interactions, and regenerative therapy [40–42].

Organoids are commonly derived from embryonic or induced pluripotent stem cells (iPSCs) or adult stem cells (ASCs) [41]. ASC-derived organoids are created directly from postnatal or adult tissues, either from a single ASC or an ASC-containing tissue unit. The culture medium is aided by several growth factors that stimulate signaling control in normal tissue homeostasis [39]. Equally, iPSCs are obtained by reprogramming somatic progenitor cells [43]. Organoids derived from PSCs are generated through directed differentiation, which comprises germ-layer specification, induction, and maturation by culturing with precise growth and signaling factors to achieve the appropriate cell types. To more precisely resemble their in vivo counterparts, PSC-derived organoids may include cells from distinct germ layers [41]. Organoids derived from iPSCs are an effective model for studying developmental processes and genetic disorders since they must be generated by replicating physiological differentiation processes [44].

Organoids have recently been created from various cancers including colorectal, esophageal, stomach, brain, prostate, pancreas, liver, brain, bladder, endometrial, and lung cancers. These models are very useful for mimicking human tumor biology and the interactions of neoplastic cells with the ECM, immune cells, and tumor vasculature [38], making it possible to gain a better understanding of the pathogenesis and characteristics of these cancers [45].

The most common 3D model for maintaining GBM in vitro is the neurosphere assay. This approach, however, has the drawback of enabling cells to create their niche, resulting in more differentiated cells in the core than on the surface, and a mixed population of cells as well as a limited number of true stem cells [46]. In 2013, Lancaster and Knoblich constructed the first human cerebral organoids (known as “mini-brains”) from PSC-derived embryonic bodies [47]. The neuroectodermal tissue samples were cultured in 3D and embedded in a Matrigel droplet to form a scaffold for the initiation of more complex tissues. The Matrigel droplet was kept in a spinning bioreactor to aid nutrient uptake. This method resulted in rapid brain tissue development, with neuronal identity appearing in 8–10 days and defined brain areas forming in 20 to 30 days [48]. The cerebral organoid model offers an in vitro platform for recreating the 3D architecture of the human brain to better understand the molecular dynamics that occur during brain development [49,50].
Furthermore, brain organoids provide a unique advantage for modeling neurological disorders and developing treatments for these conditions [50–52].

3D organoids have been emerging as an *ex vivo* experimental system for GBM research. Fig. 1 shows the various 3D organoid GBM models, including a GBM organoid in Matrigel (Fig. 1A) [53], a glioblastoma organoid (GBO) in a matrix- and serum-free conditions, on an orbital shaker (Fig. 1B) [54], the

---

**Fig. 1.** Three-dimensional glioblastoma multiforme (GBM) organoid models. (A) GBM organoid in Matrigel. (B) Glioblastoma organoid (GBO) in a matrix- and serum-free condition. (C) Genetically engineered neoplastic cerebral organoid (neoCOR) model. (D) Glioma cerebral organoid (GLICO) model. CSC, cancer stem cell; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; GSC, glioma stem cell.
genetically engineered neoplastic cerebral organoid (neoCOR) model (Fig. 1C) [55], and the glioma cerebral organoid (GLICO) model (Fig. 1D) [56]. Table 1 also presents an overview and comparison of the different 3D GBM organoid models [53–59].

1. Patient-derived GBM organoids

In 1989, Rolf Bjerkvig and his team demonstrated the use of patient-derived stereotactic biopsies and tumor resections to generate 3D multicellular organotypic spheroids. The spheroids had connective tissue, preserved vessels, and macrophages, which matched those of the original tumor in vivo [13,60,61]. In 2016, Hubert and colleagues developed a novel approach to maintaining a 3D culture system of GBM obtained from patient primary cultures, genetically engineered glioma models, and xenografts [53]. GBM organoids were created using a modified cerebral organoid technique. Standard tumorspheres maintained in a neurobasal serum-free media can grow up to 300 μm in two weeks. Conversely, organoids can grow up to 3 to 4 mm after two months when embedded in the Matrigel matrix. GBM organoids can be stable and viable for more than a year in continuous culture, although the growth rates decrease noticeably during several months of culture. Organoids contain sphere-forming CSCs at rates comparable to tumorspheres. Patient-derived organoids that were dissociated after 5 to 6 months of culture and orthotopically transplanted into the frontal lobes of mice permitted tumor development with a 2-month latency. This shows that patient-derived organoids can proliferate and retain their tumorigenic potential [53].

Jacob et al. [54] later improved on this model, coining the term “GBOs” to represent these GBM organoids [62]. GBOs were generated without mechanical or enzymatic dissociation of patient-derived samples into single cells. This was done to preserve the original tumor’s local cytoarchitecture and native cell-cell interaction while avoiding the clonal selection of specific cell population culture. GBOs were grown for 1 to 2 weeks and kept in a fully defined, serum-free environment without EGF/bFGF or ECM that could contribute to clonal selection. These GBOs mimicked the heterogeneity of their parental tumors as evidenced by similarities in tissue architecture and cellular morphology, the presence and continuous generation of diverse cell types, the maintenance of transcriptomic signatures, the preservation of somatic variants and copy number variations (CNVs) at similar frequencies, and the maintenance of different cell populations with distinct gene expression profiles [54].

2. Genetically engineered GBM organoids

GBM organoids can be created by genetically modifying healthy tissue stem cells or cerebral organoids to induce tumor growth. In 2018, Bian et al. [55] created a 3D in vitro model called a neoCOR, in which oncogenic mutations were initiated in cerebral organoids to mimic gliomagenesis. Combining Sleeping Beauty (SB) transposon-mediated gene insertion for oncogene activation with CRISPR-Cas9-based mutation of tumor suppressor genes resulted in tumorigenic events in cerebral organoids. Three mutation combinations were found to cause glial-oriented differentiation and abnormal overgrowth. The neoCOR model was produced by overexpressing the oncogene MYC, and it displays histopathological characteristics, cellular signatures, and transcriptome profiles that are comparable to those seen in central nervous system primitive neuroectodermal tumor-like neoplasms. Bian et al. [55] were able to use this method to test the tumorigenic potential of gain- and loss-of-function mutations, either alone or in combination.

Similarly, Ogawa et al. [57] used CRISPR/Cas9 technology to insert an HRasG12V-IRES-tdTomato construct into the TP53 gene via homologous recombination. Tumorigenesis in cerebral organoids was triggered by CRISPR/Cas9 modification of oncogenes or tumor suppressors. The modified cells were highly proliferative and showed an invasive phenotype within an organoid. During a few weeks, human cerebral organoid tumors became invasive, eliminating all normal organoid tissue and replacing it with malignant tissue. Organoid-derived tumor cells exhibited uncontrolled invasive growth in immunodeficient NOD/SCID IL2RG−/− mice, eventually killing the mice in vivo [57]. Ballabio et al. [63] recently confirmed that Otx2/c-MYC gives rise to medulloblastoma-like organoids with a DNA methylation feature that corresponds to group 3 medulloblastoma in human cerebral organoids by using a CRISPR/Cas9-mediated loss-of-function strategy to eliminate the identified potential oncosuppressors.

3. Co-cultivation of iPSC/human ESC organoids and GSC

In 2018, da Silva and colleagues cocultured human GBM spheroids with mouse ESC (mESC)-derived early-stage cerebral organoids (eCOS) [58]. GBM spheroids were established from a patient-derived GBM that expressed stem-cell-like features such as Nestin/SOX2, clonal growth and tumorigenicity, and invasion potential in vivo. The aggregation of 500 GBM cells that stably expressed green fluorescent protein produced homogeneous GBM spheroids. To boost experimental efficiency, a 12-day mESC differentiation phase was used, which was sufficient
<table>
<thead>
<tr>
<th>Model</th>
<th>Source</th>
<th>Culture method</th>
<th>Generation time</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D traditional organoid models</td>
<td>GBM organoid</td>
<td>Patient-derived single-cell GBM CSCs</td>
<td>2 mo</td>
<td>Rapid and long term growth and expansion</td>
<td>Does not mimic invasive behavior due to a lack of host tissue</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td>GBM organoid (GBO)</td>
<td>Micro-dissected GBM tumors</td>
<td>1-2 wk</td>
<td>Modeling parental tumor gene expression, cellular heterogeneity, and mutations</td>
<td>Tumor resection and tissue processing are critical for maximum reliability of GBO generation</td>
<td>[54]</td>
</tr>
<tr>
<td>Genetically engineered GBM organoid models</td>
<td>NeoCOR</td>
<td>hESC/iPSCs</td>
<td>&lt;1-2 mo</td>
<td>Modeling GBM progression in genetically engineered brain-like organoid tissue</td>
<td>Lack of vasculature</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td>Cerebral organoid gene manipulation</td>
<td>hESCs</td>
<td>3-4 mo</td>
<td>Time-lapse microscopic imaging of GBM progression</td>
<td>Lack of endothelial cells, which limits the ability to reconstruct the natural history of GBM</td>
<td>[57]</td>
</tr>
<tr>
<td>Co-culture GBM organoid models</td>
<td>Glioblastoma spheroid infiltration of early-stage cerebral organoids</td>
<td>Patient-derived GBM/ mESCs Co-culture</td>
<td>14 d</td>
<td>Modeling and quantification of GBM invasion</td>
<td>Observing GBM cell invasion along vasculatures and into the corpus callosum is impossible</td>
<td>[58]</td>
</tr>
<tr>
<td></td>
<td>GLICOs</td>
<td>GSC/hESCs/iPSCs Co-culture</td>
<td>1 mo</td>
<td>Modeling GBM biology and TME</td>
<td>Does not replicate the invasive nature of GSCs</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td>Human brain organoids</td>
<td>iPSCs/patient-derived GBM Co-culture</td>
<td>1 mo</td>
<td>Modeling interactions of invasive GBM cells, and the interaction mechanism is not observed</td>
<td>Other cell types, such as mature astrocytes, are not represented, limiting the results' accuracy.</td>
<td>[59]</td>
</tr>
</tbody>
</table>

GBM, glioblastoma multiforme; 3D, 3-dimensional; GBO, glioblastoma organoid; CSC, cancer stem cell; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; neoCOR, neoplastic cerebral organoid; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; mESC, mouse embryonic stem cell; GSC, glioma stem cell; GLICOs, glioma cerebral organoids; TME, tumor microenvironment.
to stimulate brain tissue identity in eCOs before the assay. Adult NP spheroid integration into eCOs resulted in hybrid organoids with aggressive tumor characteristics that were unique from non-malignant adult NP spheroids [58].

In 2019, Linkous et al. [56] employed a GLICO model system to reverse-engineer patient-specific GBMs using patient-derived GSCs and human ESC (hESC)-derived brain organoids. The GLICO model can sustain phospho-RTK signaling, patient-specific EGFR amplification, and the in situ GLICO microtube formation. Since the model was developed in vivo, it may be modified experimentally, chemically, and controlled accurately in terms of physiological and environmental aspects [56]. In 2020, Krieger et al. [59] demonstrated a method of observing the interaction between GBM and normal neuronal lineage brain cells using iPSC-derived cerebral organoids as a 3D platform for the infiltration of patient-derived GBM cells. They were able to observe tumor microtubule formation via tissue clearance, confocal imaging, and semi-automated quantification. The GBM co-culture model showed transcriptional alterations involved in invasion and potential ligand-receptor interactions in GBM and organoid cells, pointing to new GBM therapeutic targets [59].

Lu et al. [64] recently demonstrated that cell-to-cell interactions between hematopoietic stem and progenitor cells (HSPCs) and GBM cells boost cell proliferation and PD-L1 expression. Interestingly, enhanced tumor cell proliferation and PD-L1 expression require cell-cell interaction between HSPCs and GBM. GBM cells can also sustain HSPCs in vitro for lengthy periods, resulting in the generation of chemokines and cytokines related to tumor proliferation. These results corroborate in vivo evidence of a connection between immunosuppressive features and clinical outcomes. Azzarelli et al. [65] likewise cocultured GSCs and iPSCs to construct a model to examine GSC features and the impact of cell-cell and cell-TME interactions on cancer progression and metastasis. The findings show that GSCs can fully integrate into brain organoids while maintaining cell heterogeneity.

Applications of GBM organoid models

Organoids have been established successfully in various types of cancers, including liver, breast pancreas, prostate, and colorectal cancer. Fig. 2 presents GMB organoid technology as an emerging preclinical model to study the tumor biology and microenvironment, as well as the proliferation, invasive and self-renewal capacity of tumors. Organoids are also used as models in the development of anti-tumor drugs and personalized medicine for GBM patients.

1. Modeling the TME

Cancer cell heterogeneity, clonal development, and multi-drug resistance all require dynamic interactions between cancer cells with the TME, resulting in progression and metastasis. Understanding the fundamental mechanisms that regulate these connections is important to developing an effective and safer treatment by indirectly disrupting cancer cell crosstalk [66]. Tumor organoids can mimic the TME, which includes both cancer cells and non-cancerous host components [67]. Hubert et al. [53] have shown that organoid culture can be used to investigate interactions between CSCs in proliferative and hypoxic niches. Similarly, Jacob et al. [54] demonstrated that GBOs could mimic particular aspects of the TME, such as hypoxia gradients, microvascularity, and immune cell populations. The GLICO model of Linkous et al. [56] used hESC-derived cerebral organoids and patient-derived GSCs to explore GBM in a primitive human brain microenvironment. Pine et al. [68] underscored the importance of tumor-host cell interactions and the TME is essential for recreating GSCs’ biological state. By comparing the cellular heterogeneity of four organoid models (GSC spheroid, GBO, GLICO, and PDX) using single-cell transcriptome analysis, GLICO had a higher number of neural progenitor-like cells (NPC) and oligodendrocyte progenitor-like cells (OPC) than the PDX model. GBM invasiveness biomarkers such as KPNA2, SOX4, and BCAN were also significantly elevated in GLICO...
tumors. Also, the cellular state composition of the GLICO tumors was comparable to that of the original patient samples, with a predominance of OPC-like and NPC-like states. However, without the organoid microenvironment, GLICO cells lose a significant fraction of their NPC- and AC-like compartments when cultivated in 2D culture conditions.

2. Modeling tumor invasion
Uncontrolled cancer proliferation and metastasis have been the focus of cancer research for many years. Studies show that individual and collective cell invasion patterns in tumor cells overcome ECM barriers and migrate into nearby tissues [69]. These invasion patterns can be demonstrated using cancer organoid models [70]. The GLICO model has demonstrated that brain organoid tumors can arise rapidly and are maintained by a network of interconnected microtubes that supports the invasion of healthy host tissue [56]. Goranci-Buzhala et al. [71] have shown that GSC invasiveness in host organoids is inversely related to the organoid's age, based on tissue clearance and quantitative 3D imaging. In addition, invasion experiments can distinguish between primary and recurring GSC invasive patterns. Chemical and pharmacological interventions that alter the invasive properties of GSCs in brain organoids can also be adapted using the model. Ogawa et al. [57] showed that normal organoids had a smooth sphere structure, whereas tumor-dominated organoids exhibited tumor mass projections beyond the organoid limits, indicating that the invasive phenotype had intensified. With the neoCOR model of Bian et al. [55], the expression levels of invasion-related genes such as migration-related receptor (CXR4), transcription factors related to the epithelial-mesenchymal transition (TGFBI1, TGFBI, SNAI2, STAT3, ZEB1, ZEB2), ECM molecules (ITGA5), and proteases (PLAU, ADAM10, ADAM17, CTSD, MMP14, MMP2), were upregulated in GBM organoids compared with normal cell organoids.

3. Modeling tumor biology
Organoids are important as hybrids that combine the experimental tractability of standard 2D cell lines with the biological features of in vivo model systems because of their 3D structure and ability to imitate differentiation, self-renewal, and pathogenesis [72]. Recent works of Jacob et al. [54] and Bhaduri et al. [73] used the GBM organoid model and single-cell RNA sequencing to address GBM heterogeneity, providing a useful tool and insights into GBM biology [74]. Jacob et al. [54] demonstrated that the GBO model could preserve the features of the parental tumors evident by similarities in tissue architecture and morphology, the presence and continuous generation of diverse cell types, the maintenance of transcriptomic signatures, the maintenance of various cell populations and their gene expression profile, and the preservation of somatic variants and CNVs at similar frequencies. Similarly, Bhaduri et al. [73] found that individual GBM tumors had a heterogeneous cellular composition, with a subpopulation of cells partly recapitulating glial and neuronal lineage at varying stages of development. Stemness-related transcriptional programs were widely expressed and could be activated in practically every cell type within the tumor. Radial glia-like and other progenitor cell populations also play a role in GBM propagation. Hubert et al. [53] proved that most of the cells located at the peripheral rim of the organoid model had higher proliferative activity since this region contains higher levels of oxygen, nutrients, and growth factors.

4. Drug screening and personalized medicine
Cancer organoids have become extensively utilized models for preclinical drug evaluation, biomarker discovery, and personalized medicine [75]. In GBM, Bian et al. [55] demonstrated that the neoCOR organoid model is suitable for drug screening. neoCORs were treated with afatinib, an EGFR inhibitor and after 40 days of treatment, neoCORs were sorted with fluorescence-activated cell sorting. The results showed that neoCORs with EGFR overactivation had significantly lower numbers of GBM cells due to the strong effect of afatinib. Hence, neoCORs can be used to evaluate how chemical compounds affect cancers caused by certain driver mutations. The GLICO model demonstrated that patient-derived GBMs responded differently to treatment with various compounds [56]. Specifically, some samples responded well to bis-chloroethyl nitrosourea (BCNU), while other samples responded better to TMZ. Patient samples grown in 2D, on the other hand, are more responsive to TMZ and BCNU. As a result, the model is more scalable, allowing several patient-specific GLICOs to be generated for high-throughput drug screening [56]. Goranci-Buzhala et al. [71] investigated GSC organoids with pharmacological therapy, making use of the powerful imaging and manipulation options offered by GSC invasion assays, and used an ADAM10 inhibitor (GI254023X) to treat recurrent GSC organoids. ADAM10 cleaves NLGN3 and releases functional NLGN3 into the TME, which is found in postsynaptic neurons and oligodendrocyte progenitors. When compared to the control treatment, GI254023X greatly inhibited the integration of GSC spheres into organoids. As a result, once established, such systems could act as effective preclinical in vitro tools for drug screening and GBM individualized therapy [71].

Meanwhile, Jacob et al. [54] have also shown that GBO
responses to various treatment therapies are varied and that the success of targeted treatments is generally consistent with mutational status and pathway enrichment in tumors. Krieger et al. [59] demonstrated that an organoid model could be used to perform high-throughput drug screening to study patient-specific therapeutic activity in tumor and healthy brain cells, assisting in the identification of the most effective therapy over clinically relevant timeframes.

5. Patient-derived orthotopic xenograph model
Organoids have recently been generated from diverse patients to give rise to patient-derived orthotopic xenografts (PDOXs) and serial transplantation in vivo enables the consistent expression of tumor cells [76]. Golebiewska et al. [77] generated around 160 organoids including 40 PDOX models, both from treatment-naïve GBM patients and those who had standard-of-care treatment. The PDOX model retained the core histopathological features of grade III and IV gliomas replicated tumor-intrinsic molecular and genetic properties in individual patients and retained intra-tumoral transcriptome signatures and stem-cell-associated heterogeneity. Jacob et al. [54] demonstrated that GBOs could be successfully xenografted into an adult mouse brain, exhibiting fast and aggressive invasion, as well as the expression of essential driving mutations. Organoid culture may help to sustain a patient tumor cell population that can replicate this infiltrative nature. Infiltrative mouse xenografts formed from organoids and solid xenograft tumors derived from tumourspheres may respond to potential treatments [53]. Similarly, Ogawa et al. [57] demonstrated that organoid-derived tumor cell xenograft models showed uncontrolled aggressive proliferation, resulting in mortality in the implanted mice, suggesting that organoid-generated tumors possess fundamental oncogenic traits.

Limitations of the current GBM organoid models
Brain organoids are highly promising technologies that have the potential to change how we understand and treat brain disorders. These 3D neural tissues are produced from self-organizing PSCs that stimulate the maturation of the human brain, including progenitor regions and basic cortical layers [50]. In GBM, the slow pace of translational research is due in part to a lack of preclinical models that accurately reflect tumor biology and the TME [25]. The development of organoid models perfectly compensates for the drawbacks of earlier models, and organoids have become the most acceptable platform for GBM research [38]. Indeed, current GBM organoid models hold considerable promise for studying tumor biology [55], with applications in biobanking [54], genetic repair, and drug screening studies, both for drug development and personalized treatment, as well as immunotherapy [53–56,78]. Furthermore, the co-culture of GBM organoids with PSCs has become a powerful model to study GSC behavior and the impact of cell-to-cell and cell-to-TME interaction on tumor growth and invasion [65]. Also, GBM organoid models are useful to understand tumor biology and heterogeneity, which support their invasive and proliferative nature [55–58].

However, established GBM organoid models also have some limitations. It is crucial to work closely with a neuropathologist during tumor tissue acquisition to verify the diagnosis and reduce the time during resection and tissue processing, which is crucial for GBO generation dependability. Furthermore, more work needs to be done to generate and maintain tumors with aggressive growth phenotypes [56]. Meanwhile, organoids require longer to establish than tumourspheres and are difficult for high-throughput screening approaches when compared to other GBM models [53].

The invasiveness of patient-derived samples varies; therefore, future studies should establish differences in invasive features among glialoma cell lines within the same organoids [59]. Highly aggressive cells express increased levels of TNFRSF12A, CTGF, and genes associated with cell cycle pathways, survival, and migration. Also, the increased invasion was linked to the MES gene signature. The expression of GFAP was higher in non-invasive cells from the tumor core, as was a feature of genes containing hypoxia, VEGFA, and chemo-repulsive signals [79]. Further, GSCs maintain their stemness signature in co-culture with organoids, which is an unusual characteristic. Hence, it is also crucial to clarify how GSCs develop along their lineage at later stages of culture and whether settings can enable GSC differentiation into glial cells [65]. Although promising, the cerebral organoid model lacks major cell types that are necessary for tumor development [57]. Due to the paucity of vasculature in neoCORs, certain GBM characteristics such as perivascular palisading necrosis and glomeruloid microvascular growth are not visible [55]. Other cell types, including immune cells, and the formation of blood vessel-like structures within organoids could help to mimic the complex TME, making the GSC lineage progression and treatment response even more comparable to those seen in vivo [65].

Orthotopically injected primary patient-derived cells were unable to kill mice and infiltrate the organoid parenchyma in a PDOX-organoid model, whereas established cell lines easily
killed mice and infiltrated the organoid interior. Therefore, more research with a larger number of primary patient-derived isolates and cell lines is needed to confirm the invasive properties and mortality of these models in vivo. Furthermore, whole-genome sequencing analysis is needed to determine whether the organoid model's niche contains features that preserve genomic stability [57]. Meanwhile, the GLICO model can model a variety of drug and radiation responses and has the potential to improve the predictive efficacy of in vitro and ex vivo therapeutic screening. These features will facilitate more research comparing drug sensitivity in vivo, allowing us to compare tumor heterogeneity in GLICO with orthotopic xenografts using the same GSCs [56].

Conclusions and future directions

3D human brain organoids have unexpectedly opened up new possibilities for studying biology and visualizing numerous facets of GBM pathogenesis. The various cell types in organoid models may potentially be used to recreate the complex TME associated with GBM in a dish. These models also provide more information on the mechanism of tumor invasion, and the heterogeneity seen at various levels of differentiation makes them a good tool for investigating GSC biology. GBM organoids have been employed extensively in fundamental research and clinical translational investigations. These models have the potential to enhance drug screening techniques, the identification of new therapeutic targets, and efforts to tailor personalized treatments. Thus, a more realistic brain tumor organoid model will soon contribute to further anti-cancer research by merging revolutionary technologies such as 3D bioprinting and 4D real-time imaging.

Notes

Conflict of interest
No potential conflict of interest relevant to this article was reported.

Funding
This research was supported by the National Research Foundation of Korea (NRF) grants funded by the Korean government (No. 2019R1I1A3A01059211 and 2021R1A2C1003561).

Author contributions
Conceptualization: SHK; Writing-original draft preparation: DCRB, SHK; Writing-review and editing: DCRB, SZ, MCC; Visualization: DCRB; Approval of final manuscript: all authors.

ORGID
Don Carlo Ramos Batara, https://orcid.org/0000-0003-0805-4428
Shuchang Zhou, https://orcid.org/0000-0002-5700-3830
Moon-Chang Choi, https://orcid.org/0000-0002-3148-7718
Sung-Hak Kim, https://orcid.org/0000-0003-4882-8600

References

13. Klein E, Hau AC, Oudin A, Golebiewska A, Niclou SP. Glio-
26. Hassan Mersati M, Behrooz AB, Y Abuhmad A, Syahir A. Understanding glioblastoma biomarkers: knocking a moun-
40. Dutta D, Heo I, Clevers H. Disease modeling in stem cell-de-


