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Review Article

Organoid models of glioblastoma: advances, applications and challenges

Chaocai Zhang[1], Mingzhu Jin[2], Jiannong Zhao[1], Juxiang Chen[3], Weilin Jin[4]

[1]Department of Neurosurgery, Hainan General Hospital/Hainan Affiliated Hospital of Hainan Medical University, Haikou, PR China; [2]Shanghai Jiao Tong University School of Medicine, Shanghai, PR China; [3]Department of Neurosurgery, Shanghai Institute of Neurosurgery, Changzheng Hospital, Second Military Medical University, Shanghai, PR China; [4]Key Laboratory for Thin Film and Microfabrication Technology of Ministry of Education, Department of Instrument Science and Engineering, Institute of Nano Biomedicine and Engineering, School of Electronic Information and Electronic Engineering, Shanghai Jiao Tong University, Shanghai, PR China

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Abstract: The high mortality and poor clinical prognosis of glioblastoma multiforme (GBM) are concerns for many GBM patients as well as clinicians and researchers. The lack of a preclinical model that can easily be established and accurately recapitulate tumour biology and the tumour microenvironment further complicates GBM research and its clinical translation. GBM organoids (GBOs) are promising high-fidelity models that can be applied to model the disease, develop drugs, establish a living biobank, mimic therapeutic responses and explore personalized therapy. However, GBO models face some challenges, including deficient immune responses, absent vascular system and controversial reliability. In recent years, considerable progress has been achieved in the improvement of brain tumour organoid models and research based on such models. In addition to the traditional cultivation method, these models can be cultivated via genetic engineering and co-culture of cerebral organoids and GBM. In this review, we summarize the applications of GBM organoids and related advances and provide our opinions on associated limitations and challenges.

Keywords: Glioblastoma, organoids, precision oncology, invasion, immunotherapy

Introduction

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumour in adults [1]. Because of its aggressive growth and high heterogeneity, this kind of tumour is often fatal [2]. The main treatment methods for GBM include surgery combined with postoperative radiotherapy and chemotherapy, as well as targeted treatment, immunotherapy and tumour treating fields [3-5]. However, these treatments have not made satisfactory progress [6]. The molecular heterogeneity observed between and within tumours is the main reason for the poor effect of many clinical trials [7-9]. For basic trials and clinical applications, developing a model including tumour atypia in time to verify the effectiveness of individualized treatments for GBM is a major challenge. At present, several research models are helping researchers understand the biological mechanisms of GBM, but they have limitations. First, animal models cannot fully reflect the genetic characteristics of human cancer or capture the heterogeneity of tumours. Second, in vitro culture models, whether two-dimensional (2D) cell line cultures or tumour spheroid cultures, acquire additional mutations during the process of cloning and amplification, which is not conducive to maintaining the expression of key driving genes in multiple cell subtypes and parent tumours. Patient-derived xenotransplantation (PDX) models can retain the important features of the primary tumour. However, these PDX models have the disadvantages of a large workload, high cost, and variable tumour implantation efficiency, so they are difficult to use in high-throughput drug screening. Therefore, a better model system to study GBM is urgently needed. Organoids are three-dimensional structures

that are derived from stem cells, have self-organization ability and can simulate the structure and function of human organs [10]. Compared with traditional 2D culture models, tumour organoids can more closely represent the cell composition and physiological behaviour of the human body and replicate the three-dimensional (3D) tumour microenvironment (TME) [11], especially with respect to hypoxia, which is the key element in cancer biology [12]. Many kinds of tumour organoids retain the heterogeneity of primary tumours and can be widely amplified in culture while maintaining the stability of the genome [13-15]. Therefore, organoid-based models of malignancy are emerging as an indispensable platform for cancer research [16].

Common research models for glioma

Cell line models

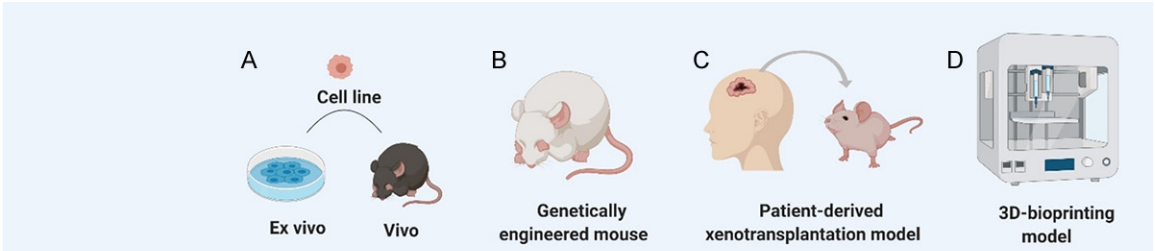
At present, brain glioma cell lines, including U87, U251 and GL261, are the most common two-dimensional cell research models due to their easy access and high cost-effectiveness [17, 18]. However, due to the lack of tumour structure and heterogeneity and the absence of single-cell infiltration, tumour necrosis and microvascular proliferation, 2D cell models are quite different from tumour cells growing in vivo in terms of cell-cell/matrix interactions, proliferation, morphology and signal transduction; thus, these models cannot accurately simulate the clinical response [19]. Moreover, in 2016, Allen compared the original U87MG-GBM cell line with the same cell line from the American Type Culture Collection (ATCC) and Cell Line Service (CLS). The results showed that the original U87MG gene did not match the U87MG gene present in the ATCC and CLS lines, even though these U87MG cell lines came from the same institution [20]. Therefore, cell lines do not usually recapitulate cancer heterogeneity or reveal a credible mechanism for therapeutic resistance.

Genetically engineered mouse models

Huge amounts of animal models have been developed to study the occurrence and development of glioma and serve as a platform for preclinical potential therapies screening [21, 22]. One of the most commonly used models are genetically engineered mouse (GEM) models, which are helpful for exploring the effect of specific mutations/oncogenes on the characteristics of glioma stem cells, as well as their roles in tumour formation, invasion and metastasis. For example, to evaluate the role of the p21ras pathway in GBM, a transgenic mouse model expressing the oncogene v-Ha-ras was developed [23]. This study found that the level of v-Ha-ras was in direct proportion to the development of astrocytoma. At the same time, inoculating these tumour cells into different hosts can induce tumour growth. However, even if these GEM models have combined with powerful new technologies, they cannot fully describe the genetic complexity of tumours. Additionally, due to the lack of non-neoplastic normal host cells, these models often fail to recapitulate many human-specific features of the TME [24], and there may be great obstacles when the treatment results are translated from animals to humans. Therefore, the therapy effect must be examined in human tumours rather than in mouse tumours when preparing human personal therapy regimens.

Patient-derived xenotransplantation models

PDX mouse models are increasingly used as personalized preclinical platforms because they are maintaining the characteristics of parental tumours. They can be used to analyse the genome map and clinical data of a specific tumour tissue as a whole, as well as allowing for the generation of direct correlative associations between the cancer genome and the outcome of drug treatment [25]. By predicting the clinical success rate of a new therapy, GBM-PDX models have been further proven to overcome the limitations of traditional cell lines. For example, bevacizumab has anticancer properties in a mouse model established by U87 cell line xenotransplantation [26]. However, Joo et al. [27] showed that bevacizumab did not change the overall survival (OS) rate using a GBM-PDX model. The survival rate of GBM patients was also not significantly prolonged by these drugs in phase 2 clinical trials [28]. The main challenges of GBM-PDX are its high cost, time-consuming approach, immunodeficient and varying transplant rates [29-31]. Although the use of “humanized” mice (expressing components of the human immune system), the coinjection of human stromal/immune cells and human cytokines and injection of



-Easily accessible	Yes	Yes	No	No	No
-Cost-effective	Yes	Yes	No	No	No
-Time- and labour-consuming	No	No	Yes	Obvious	Yes
-Gene editing	Yes	Yes	Yes	No	Yes
-Maintained parental tumour characteristics	No	No	No	Yes	Yes
-Human tumour microenvironment modelling	No	Good	Good	Good	Excellent
-Immune system	No	Yes	Yes	No	Yes
-High-throughput drug screening	Yes	No	No	No	Yes

Figure 1. Comparison of common research models of glioma: A. Cell line models are divided into 2D ex vivo models and vivo models. Although they are easily accessible and cost-effective, 2D ex vivo models lack a 3D TME, and neither model type can recapitulate the characteristics of the parental tumour; B. Genetically engineered mouse models are conducive to studying specific gene mutations, but the characteristics of the parental tumour are not maintained; C. Patient-derived xenotransplantation models can recapitulate the characteristics of the parental tumour, but challenges include high cost, a time-consuming approach and varying transplant rates; D. 3D-bioprinting models can recapitulate the characteristics of the parental tumour and tumour structures and have the potential advantage of biophysical manipulation.

human GBM into mouse embryos might address the problem of immunodeficient [32, 33], some obstacles remain when using these models for high-throughput drug screening. What's more, there has demonstrated that Patient-derived xenografts may follow mouse-specific evolutionary trajectories which potentially jeopardize cancer modeling researches.

3D-bioprinting models

3D-bioprinting of multiple cell types within optimised extracellular matrices has the potential to more closely mimic the 3D environments involved in human physiology and disease [34]. Using 3D-printing technology [35], Yi et al. [36] reported a bioprinted human glioblastoma-on-a-chip that could identify patient-specific responses to chemoradiotherapy. This ex vivo patient-derived tumour model can not only recapitulate the characteristics of the parental tumour but also has the potential advantage of biophysical manipulation [37, 38]. More importantly, the model can replicate complex tumour structures [39]. As only 1-2 weeks is required to generate the human glioblastoma-on-a-chip, ex vivo GBM models may be good models for high-throughput drug screening. We summarize the characteristics of the four kinds of research models in **Figure 1**.

Generation of brain tumour organoids

Cerebral organoids, also called 'mini-brains', which are a more recent approach to modelling brain in vitro, can be obtained from two types of stem cells: (I) pluripotent stem cells (PSCs), such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), and (II) organ-specific adult stem cells (ASCs). Organoids can grow and differentiate after the addition of an intricate sequence of media and additives.

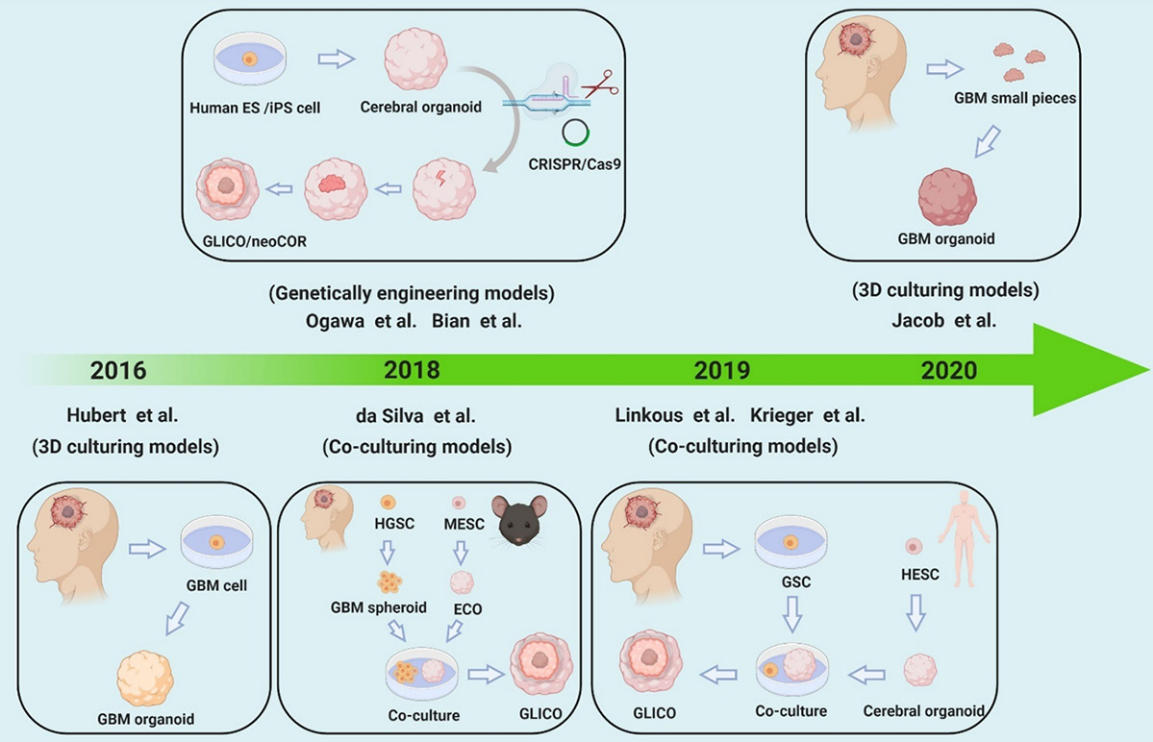


Figure 2. Classification of glioma organogenesis. We summarize three common methods used to generate brain tumour organoids, including traditional 3D culturing, genetic engineering and co-culturing.

Cerebral organoids are eventually cultured in rotating bioreactors, where they grow to contain functionally mature populations of various brain cell types and further mirror the regional differences observed in the human brain [40]. By replacing PSCs with patient-derived glioblastoma stem cells (GSCs), tumour organoids replicating the tumour cytoarchitecture rather than the brain architecture have been generated [41]. Several methods can be used to generate brain tumour organoids (**Figure 2**) (**Table 1**).

Traditional 3D culture models

Traditional 3D culturing usually involves cutting human glioblastoma into small pieces and separating glioma stem cells for in vitro culture under the effects of matrix and exogenous factors. For example, in 2016, with the help of 3D culture methods, Hubert et al. [41] described a novel glioblastoma organoid (GBO) culture system using patient-derived glioblastoma CSCs that recapitulates the hypoxia gradients and stem cell heterogeneity found in tumours in vivo. Similar to Hubert et al.'s [41] research, most GBO studies have used Matrigel to obtain a 3D structure. However, this traditional material has some drawbacks, such as the difficulty of fine-tuning its properties to optimize cell growth. Gjorevski et al. [42] applied polyethylene glycol (PEG) matrix, a synthetic hydrogel system, to generate intestinal cancer organoids, which can serve as a substitute for Matrigel but does not affect biochemical signalling. Additionally, in a recent study, Jacob et al. [43] established a GBO model with the addition of Matrigel. This model directly cut the specimen into small pieces for culture without dissociating them into single cells or adding extracellular matrix or epidermal growth factor (EGF)/basic fibroblast growth factor (bEGF). This model well preserved the diversity, gene expression and mutations of the primary tumour cells. Moreover, only 1-2 weeks are needed to establish the model, which is beneficial for supporting the timeliness of preclinical studies of glioblastoma patients [43]. Continuous improvement of methods to construct organoids will lead to the development of a more realistic preclinical model for cancer research.

Table 1. A summary of the formation of brain tumour organoids

Year/Author	Material	Method	Generation time	Tumour	Vessels and immune cells	Model
Traditional 3D culture						
Hubert et al. 2016 [41]	patient-derived GBM CSCs	3D culture	>2 m	GBM	No	
Jacob et al. 2020 [43]	GBM pieces	3D culture; No extracellular matrix/EGF/bEGF	1-2 w	GBM	Yes	GBO
Gene engineered						
Ogawa et al. 2018 [45]	hESCs	CRISPR/Cas9	3-4 m	GBM	No	
Bian et al. 2018 [44]	hESCs/iPSCs	Transposon- and CRISPR-Cas9-mediated mutagenesis	1-2 m	GBM/ PNET	No	neoCOR
Ballabio et al. 2020 [47]	hESCs/iPSCs	CRISPR/Cas9	35-60 d	G-3MB	No	
Co-culture						
Da Silva et al. 2018 [48]	GBM cells/mESCs	Co-culture	14 d	GBM	No	
Linkous et al. 2019 [46]	GSCs/hESCs/iPSCs	Co-culture	1 m	GBM	No	GLICO
Krieger 2019 [49]	iPSCs/GBM cells	Co-culture	<4 w	GBM	No	

Genetically engineered models

Generating a genetically engineered model requires induction of glioma organogenesis by CRISPR/Cas9 gene editing technology or the addition of oncogenes [44]. In 2018, Ogawa et al. [45] combined technological advances in human organoid technology and CRISPR genome engineering to generate a genetically defined model of human glioblastoma, which was also called the glioma cerebral organoid (GLICO) model, in the study of Linkous et al. [46]. In this model, tumours can be induced in human cerebral organoids by introducing CRISPR/Cas9 and sgRNAs in combination with the activated oncogene $HRas^{G12V}$ and simultaneous disruption of the tumour suppressor TP53. The modified cells proliferate, show invasive phenotypes within organoids, and can be transplanted into immunodeficient mice. These tumours, when transplanted into mice, possess proliferative hallmarks of tumorigenesis, are invasive, and exhibit disease pathology. Additionally, Bian et al. [44] established a 3D in vitro model called a neoplastic cerebral organoid (neoCOR) in which brain tumourigenesis was replicated by introducing oncogenic mutations into cerebral organoids via transposon and CRISPR/Cas9 mediated mutagenesis. They defined mutation combinations that result in glioblastoma-like and central nervous system primitive neuroectodermal tumour (CNS-PNET)-like neoplasms. Therefore, neoCORs are suitable for use in investigations of aspects of tumour biology, such as invasiveness, and for evaluating drug effects in the context of specific DNA aberrations [44]. Moreover, using CRISPR/Cas9 technology, Ballabio et al. [47] recently validated these findings in human cerebellar organoids, where Otx2/c-MYC engendered MB-like organoids harbouring a DNA methylation signature that clusters with human Group 3 medulloblastoma.

Co-culturing models

Another approach is to form brain tumour organoids through cocultivation, such as coculture of glioblastoma and cerebral organoids to generate a GLICO model. For example, in 2018, da Silva et al. [48] established a glioma organoid invasion model by coculturing human GBM spheroids with mouse embryonic stem cell (mESC)-derived early-stage cerebral organoids (eCO). This approach may be used for the identification of anti-GBM invasion strategies. In 2019, Krieger et al. [49] presented an experimental model using human cerebral organoids as a scaffold for patient-derived glioblastoma cell invasion. This GLICO model has proven useful for studying GBM invasion and transcriptional heterogeneity in vitro, with applications for both pharmacological screens and patient-specific treatment selection at a time scale amenable to clinical practice. Additionally, in 2019, Linkous et al. [46] established a GLICO model to retro-engineer patient-specific GBMs using patient-derived glioma stem cells (GSCs) and human embryonic stem cell (hESC)-derived cerebral organoids. The model results showed that the tumours closely phenocopied GBMs from patients and were supported by tumour microtubes that promoted invasion into host tissue.

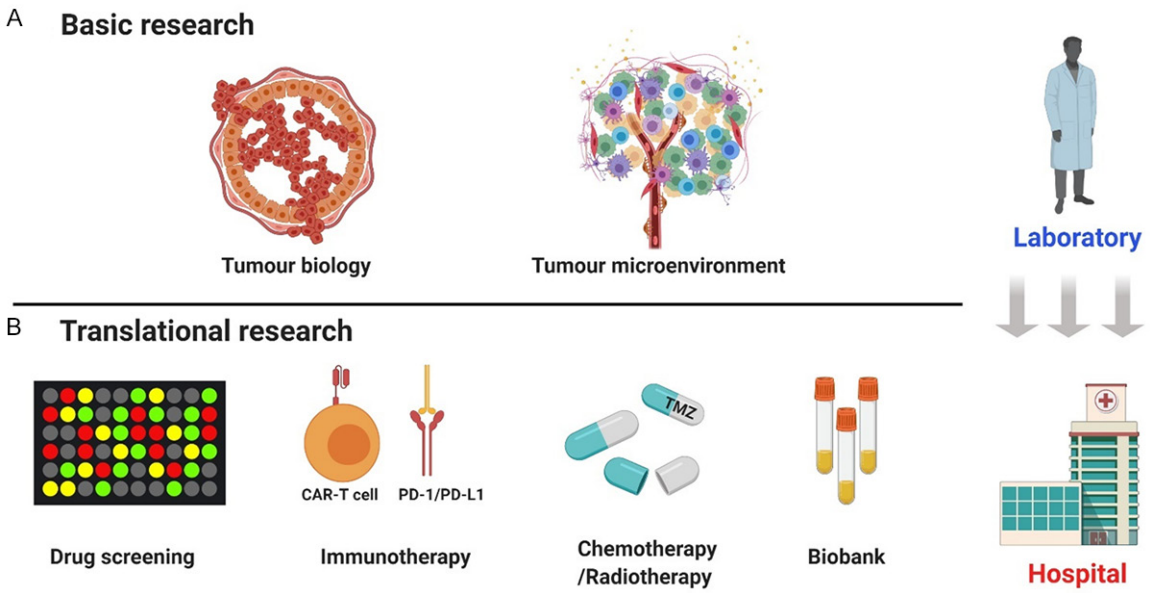


Figure 3. Applications of brain tumour organoids. Glioma organoid models have been widely implemented in basic and translational research: A. In basic research, we focus on tumour biology and the TME; B. In translational research, we summarize these models' application in drug screening, chemotherapy, radiotherapy, and biobanks and focus more on immunotherapy, especially CAR-T cell therapy.

The various tumour organoid models have been widely implemented in glioma research, all of which have their unique advantages. For example, due to its fast production and inclusion of immune cells, the GBO model reported by Jacob et al. [43] is especially suitable for constructing a biobank and mimicking immunotherapy responses. Genetically engineered models are conducive to studying specific gene mutations and developing personalized therapy. Containing both tumour cells and normal brain tissue, GLICO models are suitable for exploring tumour biology and the TME and drug screening.

Applications of glioma organoid models

Glioma organoids have opened new avenues for the development of novel, more physiological human brain tumour models. Such preclinical models are essential for more efficient translation of basic cancer research into novel treatment regimens for patients with glioma (**Figure 3**) (**Table 2**). Wild-type GBOs can be grown from GSCs and display self-organizing capacity, phenocopying essential aspects of human-derived glioblastoma. Genetic modification of organoids allows disease modelling in a setting that approaches the physiological environment. Additionally, organoids can be grown with high efficiency from patient-derived normal and tumour tissues, potentially enabling patient-specific drug testing and the development of individualized treatment regimens [50].

Organoids for basic research

Organoids to explore tumour biology

Organoid fusion is a new strategy to study the mechanism of GBM diffusion in the brain [34]. GSCs can fuse with cerebral organoids to evaluate the infiltration of tumour cells. Linkous et al. [46] observed the network of tumour microtubes in 923 and 827 GSC-formed GBMs growing in cerebral organoids, and these tumour microtubes promote the invasion of GLICO tumours into normal host tissue. These microtubules are also present in human glioblastomas, where they contribute to multicellular communication between cells and provide a pathway for invasion [51]. The detection of these microtubules in the GLICO model provides evidence that the behaviour and phenotype of cancer cells in this system are very similar to those of primary tumours. Thus, it is a good tool to study GBM biology in the human brain environment.

Table 2. A summary of the application of brain tumour organoids

Year/Author	Basic research	Drug screening	Immune-therapy	Chemotherapy/ Radiotherapy	Biobank
Hubert et al. 2016 [41]	Exploration of microenvironmental influences and CSC biology	No reported	No	Yes	No reported
Ogawa et al. 2018 [45]	Model for tumour formation; Platform for tumour cell transplantation	No reported	No	No reported	No reported
Bian et al. 2018 [44]	Studying tumour biology	Afatinib, erlotinib, gefitinib, canertibib, pelitinib	No	No reported	No reported
Da Silva et al. 2018 [48]	Modelling the process of GBM invasion	No reported	No	No reported	No reported
Linkous et al. 2019 [46]	GBM biology, tumour microtubes	TMZ, BCNU	No	Yes	No reported
Krieger 2019 [49]	Studying GBM invasion and transcriptional heterogeneity	Yes	No	Yes	No reported
Jacob et al. 2020 [43]	Exploration of cellular heterogeneity, GBO engraftment and infiltration	TMZ, gefitinib, trametinib, everolimus	CAR-T	Yes	Yes
Ballabio et al. 2020 [47]	Studying medulloblastoma biology	Tazemetostat	No	No reported	No reported

Additionally, da Silva et al. [48] demonstrated that human GBM spheroids possess the ability to spontaneously infiltrate early-stage cerebral organoids (eCOs). The resulting formation of a hybrid organoid demonstrated the existence of an invasive tumour phenotype. These findings provide a basis for modelling and quantification of the GBM infiltration process using a stem-cell-based organoid approach and may be used for the identification of anti-GBM invasion strategies [48]. What's more, Bian et al. [44] established a neoCOR model in which they recapitulated brain tumorigenesis by introducing oncogenic mutations into cerebral organoids via transposon- and CRISPR/Cas9 mediated mutagenesis. They demonstrated that neoCORs are suitable for use in investigations of aspects of tumour biology, such as invasiveness [44].

Modelling the tumour microenvironment

The TME is a self-sufficient ecosystem [52] involving interactions and networking between cells and between cells and non-cells. The main components of the TME include cancer cells, immune cells, stromal cells, and blood vessels as well as physical structures [53-55]. The focus of targeted therapy and immunotherapy for glioma has shifted from tumour cells to the TME [5]. Furthermore, tumour immune microenvironment normalization has the potential to transform a cold tumour into a hot tumour [56-58]. The TME is also critical for the maintenance of cellular states found in primary glioblastomas. By comparing single-cell RNA sequencing results for tumour cells from different GSC-derived model types, Allison R. Pine found that GSCs within the GLICO model were enriched for a neural progenitor-like cell (NPC) subpopulation and recapitulated the cellular states and plasticity found in the corresponding primary parental tumours [59]. They also found that the GLICO differentially expressed not only stemness marker SOX4 and glioma invasiveness marker BCAN but also prominent Notch pathway members (DLL3, HES1, and HES6) that are differentially expressed in other GLICO model cells. Thus, the GLICO model not only contained patient-derived GSCs but also closely resembled the TME physically, which is critical for studying cancer biology and drug screening.

Organoids for translational research

Drug development

Targeting tumour stem cells is the key to preventing tumour recurrence [60, 61]. The therapeutic response to anticancer drugs, especially targeted drugs, strongly depends on the genetic and epigenetic background of the patient [62]. Patient-derived organoids can capture tumour-specific gene mutations, gene expression, and individual histopathological changes, thus representing an ideal preclinical model for individual drug screening [63, 64]. For example, GLICO tumours can maintain the key genetic features, molecular signalling networks, and EGFR amplification found in the parental

tumours, whereas EGFR amplification is lost in 2D cultures. Phosphorylation of EGFR, which is related to RTK, and ephrin type-B receptor 3 (EphB3) was observed in both parental tumours and the corresponding GLICO tumours from GSC lines [46]. This model can provide a basis for screening potential drug targets or therapies by measuring the effects of a drug or gene intervention on tumour cell proliferation, death and invasiveness. More importantly, organoids containing both normal and tumour tissues can be generated, enabling screening for drugs that specifically target tumour cells while leaving healthy cells unharmed. For example, Dijkstra et al. [65] established a modified patient-derived tumour organoid system that allows the expansion of tumour-specific T cells by coculture of peripheral blood lymphocytes (PBMCs) and tumour organoids derived from mismatch repair-deficient colorectal cancer and non-small-cell lung cancer. The induced T cell populations do not recognise healthy organoids or tissue and can be used to assess the efficiency of T cell-mediated tumour killing, thus establishing a foundation for exploration of individual antitumour drugs with functions similar to those of tumour-specific T cells.

Immunotherapy

Immune checkpoint inhibitors: Cancer immunotherapy, which has resulted in exciting and significant treatment outcomes for multiple cancer types, has attracted unprecedented research interest as a treatment for gliomas [66]. Retaining the genetic and phenotypic characteristics of the parent tumour, tumour organoids are a promising model to test immunotherapy effects ex vivo [67, 68]. The newly developed coculture organoid and air-liquid interface (ALI) systems have provided new insights for studying epithelia-immune cell interactions based on their endogenous distributions. In 2018, Neal et al. [69] generated a tumour organoid model including the tumour immune microenvironment using ALI cultures. The model preserved CD8+ T cells, CD4+ T cells, B cells, natural killer cells, natural killer T cells, and macrophages. The conserved T cell receptor repertoire of the parental tumour was retained within the ALI PDOs. More importantly, PD-1/PD-L1 immune checkpoint blockade expanded and activated antigen-specific tumour-infiltrating lymphocytes (TILs) within ALI PDOs derived from melanoma, lung cancer, and renal cell carcinoma, and the results showed a clinical response rate similar to that observed in ALI PDOs [70]. ALI systems also enable oxygen to be transported in a more efficient manner, resulting in a more rational oxygen gradient [71], which is particularly significant for glioma research. Thus, ALI PDOs show potential as a tool to predict clinical responses to immune checkpoint therapies.

Chimeric antigen receptor-T cell therapy: Chimeric antigen receptor (CAR)-T cells represent a potent new approach to treat GBM. The novelty of CAR-T cells partly lies in the genetically engineered chimeric receptor [72, 73]. In contrast to native T-cell receptors (TCRs), CAR-T cells can recognise any cell surface structure independent of MHC presentation [74]. An infusion of CAR-T cells targeting EGFRvIII, IL-13Rα2, or HER-2 has frequently been reported in glioblastomas [75-78]. However, experiments testing the efficacy of CAR-T cells have often relied on overexpression of target antigens in tumour cell lines, which may not recapitulate the endogenous condition [79]. Tumour organoids have recently been used as a model to test CAR-T cell killing due to their 3D architecture [80]. For example, CAR-T cell therapies comprise genetically engineered T cells that are redirected towards tumour cell-surface antigens [81]. The ability to label these lymphocytes will enable potential quantitative intra-GLICO tracking studies of CAR-T cells [82] as well as studies of interactions between CAR-T cells and tumour targets at the invasive front, which is the origin of recurrent disease. Additionally, by coculturing GBOs with 2173BBz CAR-T cells designed to react specifically with cells expressing EGFRvIII [78], Jacob et al. [43] demonstrated the utility of GBOs for rapid testing of antigen-specific CAR-T cell treatment responses with the endogenous target in culture. Although some limitations exist, such as immune components in organoid cultures that can be maintained only for a short period [83], with the development of several advanced technologies, such as microfluidics, organ-on-a-chip technology, 4D imaging technology, next-generation sequencing and transcriptomics [84-87], more potential advantages of brain tumour organoid use in immunotherapy research will be identified.

Radiotherapy and chemotherapy

In addition to applications in tumour immunotherapy, brain tumour organoids can also be

used in the study of radiotherapy and chemotherapy [46, 88]. For example, Linkous et al. [46] demonstrated that isogenic GSC lines are substantially more resistant to chemotherapeutic agents and radiation-induced genotoxic stress when grown within the microenvironment of a cerebral organoid (GLICO) than when grown under traditional 2D conditions. Hubert et al. [41] reported that CSCs in organoids are radioresistant, whereas adjacent non-stem tumour cells are radiosensitive. Furthermore, by using human brain organoids comprised of mature neural cell types as a 3D tissue substrate to model the invasive growth of glioma, Liu et al. [88] found that antisense oligonucleotides targeting lncRNA Glioma Radiation Sensitizer (lncGRS-1) selectively decreased tumour growth and sensitized glioma cells to radiation therapy. Therefore, glioma organoids can be used as a screening tool to identify tumour sensitivity to radiotherapy and chemotherapy.

Living biobank

In view of the urgent need for individualized cancer treatments, many studies are developing a "living organism bank" composed of patient-derived organoids [89]. Studies have shown that tumour organoid cultures can be established from prostate, colon, breast, pancreas, stomach, liver and endometrium cancer tissues [90-96]. Importantly, the genetic and phenotypic characteristics of tumour-derived organoids resemble those of the tumour epithelia from which they were derived. In a recent study, Jacob et al. [43] provided a resource of 70 biobank GBOs from different patients that captures major genomic alterations associated with glioblastoma pathogenesis. They provided detailed characterizations of many of these biobank GBOs, including the histology, RNA-seq, whole-exome sequencing, and responses to different drugs and CAR-T cell therapies. Therefore, these biobanks will be a useful resource for future biological studies and for testing therapeutic agents for glioblastomas.

Challenges and prospects for brain tumour organoids

Although glioma organoids have been widely used in glioma research, some limitations still exist. However, technological innovation and development may increase the potential of glioma organoids as preclinical models.

Deficiency in immune responses

As immunotherapy investigations require an intact immune microenvironment, the absence of blood vessels and immune cell types that act directly on antitumour immune responses will limit the utility of brain tumour organoid models for immunotherapy investigations. However, the improvement of the brain tumour organoid culture method also makes it possible to study immunotherapy ex vivo. For example, Jacob et al. [43] generated the GBO model, which provides a platform to test and optimize CAR-T cell therapies for solid tumours in vitro. Importantly, these GBOs recapitulate the endogenous expression of antigens, allowing for more accurate assessment of CAR-T cell target reactivity, response thresholds, and specificity. Furthermore, due to preservation of the primary tumour epithelium en bloc with native endogenous TILs, ALI PDOs can be used to predict the clinical response of the PD-1/PD-L1 immune checkpoint [69]. In addition, tumour organoids can be co-cultured with peripheral blood lymphocytes [65], and such models can be used to evaluate the sensitivity of T cells to tumour cell-mediated killing, which can also be extended to evaluate the effectiveness of immunotherapy at different time points.

Lack of a vascular system

The lack of endothelial cells and tumour vasculature is also a major problem that limits the utility of brain tumour organoids, as the microvascular system is a major component of the tumour environment [5], which can affect the growth of tumour cells and the antitumour immune effect [53, 54, 97, 98].

Several methods to induce vascularization in cerebral organoids in vitro have been developed. First, coculture with endothelial cells may facilitate vascularization in cerebral organoids [99-101]; for example, human endothelial cells (hECs) can be embedded in Matrigel for coculture with early-stage organoids, and the hECs will assemble into capillaries around the organoids and generate a vascular network over time. Moreover, a recent study showed that treatment with VEGF from the beginning of hESC differentiation could successfully generate blood vessels to facilitate further maturation of the vasculature in cultured cerebral organoids [102]. Additionally, by engineering

hESCs to ectopically express human ETS variant 2 (ETV2), B. Cakir found that ETV2-expressing cells in human cortical organoids (hCOs) contributed to forming a complex vascular-like network in hCOs. These vhCOs form vasculature-like structures that resemble the vasculature in the early prenatal brain and represent a robust model to study brain disease in vitro [103]. Moreover, these researchers reported that vhCOs acquired several blood-brain barrier (BBB) characteristics, including increases in the expression of tight junctions, nutrient transporters and trans-endothelial electrical resistance; these characteristics allow vhCOs to more closely and realistically replicate brain function.

Controversy in structural or genetic fidelity

In addition, some studies showed that the structural and genetic fidelity of brain organoids is still unclear [104-106]. Recently, Aparna and other researchers found that brain organoids do not produce unique cell subtypes or regional circuit structures unique to normal human brain circuits and that brain organoids express high levels of cell "stress" genes (including the glycolysis gene pgk132 and the endoplasmic reticulum stress genes arcn133 and gorasp2) [107]. Moreover, for some gene knock-in mouse strain-derived organoids, there is still a lack of robust knock-in methods for the precise integration of exogenous DNA sequences into human-derived organoids [108, 109]. However, recently, the Hans Cleaver research group established gene editing technology independent of the homologous arm of DNA repair. Based on NHEJ, a novel and robust gene typing technology, CRISPR-hot, has been established, which provides a reliable gene editing method for adult stem cell-derived organoid visualization research [110]. With the improvement of culture methods and techniques, more realistic brain organoids and brain tumour organoids will be produced in the future.

Conclusion

The glioma organoid model is one of the most promising models for glioma research and can not only retain the genetic phenotype of the primary tumour but also provide a 3D TME similar to that of the parental tumour. Several methods can be used to generate glioma organoids, including traditional 3D culturing, genetic engineering and coculturing. These methods may be used to generate other brain tumour organoids, such as medulloblastoma and brain metastasis organoids. To date, brain glioma organoids have been widely used in basic research and clinical transformation research and have considerable potential particularly in the study of immunotherapy. By combining innovative technologies such as 3D bioprinting and 4D real-time imaging, a more realistic brain tumour organoid model will contribute to anti-cancer research in the near future.

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Disclosure of conflict of interest

None.

Address correspondence to: Jiannong Zhao, Department of Neurosurgery, Hainan General Hospital/Hainan Affiliated Hospital of Hainan Medical University, Haikou, PR China. E-mail: zjn@vip.163.com; Juxiang Chen, Department of Neurosurgery, Shanghai Institute of Neurosurgery, Changzheng Hospital, Second Military Medical University, Shanghai, PR China. E-mail: juxiangchen@smmu.edu.cn; Weilin Jin, Key Laboratory for Thin Film and Microfabrication Technology of Ministry of Education, Department of Instrument Science and Engineering, Institute of Nano Biomedicine and Engineering, School of Electronic Information and Electronic Engineering, Shanghai Jiao Tong University, Shanghai, PR China. E-mail: weilinjin@sjtu.edu.cn

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