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REVIEW

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Applications of organoid technology to brain tumors

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Abstract

Lacking appropriate model impedes basic and preclinical researches of brain tumors. Organoids technology applying on brain tumors enables great recapitulation of the original tumors. Here, we compared brain tumor organoids (BTOs) with common models including cell lines, tumor spheroids, and patient-derived xenografts. Different BTOs can be customized to research objectives and particular brain tumor features. We systematically introduce the establishments and strengths of four different BTOs. BTOs derived from patient somatic cells are suitable for mimicking brain tumors caused by germline mutations and abnormal neurodevelopment, such as the tuberous sclerosis complex. BTOs derived from human pluripotent stem cells with genetic manipulations endow for identifying and understanding the roles of oncogenes and processes of oncogenesis. Brain tumoroids are the most clinically applicable BTOs, which could be generated within clinically relevant timescale and applied for drug screening, immunotherapy testing, biobanking, and investigating brain tumor mechanisms, such as cancer stem cells and therapy resistance. Brain organoids co-cultured with brain tumors (BO-BTs) own the greatest recapitulation of brain tumors. Tumor invasion and interactions between tumor cells and brain components could be greatly explored in this model. BO-BTs also offer a humanized platform for testing the therapeutic efficacy and side effects on neurons in preclinical trials. We also introduce the BTOs establishment fused with other advanced techniques, such as 3D bioprinting. So far, over 11 brain tumor types of BTOs have been established, especially for glioblastoma. We conclude BTOs could be a reliable model to understand brain tumors and develop targeted therapies.

KEYWORDS
brain metastases, brain tumor, glioblastoma, medulloblastoma, meningioma, organoid, precision medicine, tumor microenvironment

1 | BACKGROUND

Brain tumors cause high morbidity and mortality globally and are challenging to treat due to the complexity of the anatomical location and biological characteristics; predictably, the incidence and 5-year survival rate of malignant brain tumors have not changed considerably in the past decades.[1] Unfortunately, patients with brain tumors receive minimal benefits from current treatments.[2] For instance,

the median survival period of glioblastoma (GBM) patients has only been extended by 3.7 months on average compared to the 1980s, despite advances in neurosurgical resection, chemotherapy, and radiotherapy.[3–5] Moreover, multiple novel therapies for brain tumors have recently been developed, including targeted therapy, immunotherapy, tumor vaccines, and oncolytic viruses. However, the limited success of these therapeutics has restricted their clinical application prospect.[6]

The key obstacle is the lack of an appropriate model to comprehensively mimic the characteristics of brain tumors, which hampers the investigation of tumor biology and the development of novel therapies and drug screening for precision treatment.[4] Two-dimensional cell culture is convenient and represents accurate molecular signatures in the early generations.[7] However, subsequent generations may show genetic and transcriptional changes owing to spontaneous variations and selection of cells with rapid proliferation.[8,9] It also loses three-dimensional functional cell–cell interactions, which further reduces its applicability under in vivo conditions. Tumor spheroids retain the three-dimensional architecture and physical cell interactions[10] but consist of tumor cells with limited intra-tumor heterogeneity and lack a tumor microenvironment (TME).[11,12] The TME, where stromal interactions, immune responses, and extracellular matrix (ECM) generation occur, plays an important role in tumorigenesis and therapeutic resistance. Brain tumor TMEs include specific cell types, such as neurons, astrocytes, microglia, macrophages, tumor-infiltrating lymphocytes, vascular cells, and fibroblasts.

Compared with two-dimensional cultures and spheroids, patient-derived xenografts (PDXs) can maintain TME. PDXs are generated from surgical tissues transplanted into immunosuppressed rodents and consistently maintain primary tumor phenotypes and heterogeneity.[13,14] However, species differences at the gross neuroanatomical, cellular, and molecular levels have led to varied results[15]; additionally, low success rate, prolonged latency, and high cost impede the broad application of PDXs. Tumor organotypic explant cultures are established from patient tumors mechanically and preserve the cellular composition and TME as present in situ.[16] While this model has been applied to investigate tumor invasion and drug responses, it showed short-term survival and poor expandability.[16–18] The manipulative complexity and the subsequent cellular reaction after mechanical slicing also impeded the application.[19] Altogether, none of the current models for brain tumors are optimal and technical innovations are required (Table 1).

Organoids are three-dimensional cellular self-aggregates that precisely mimic the source tissue and are commonly derived from human pluripotent stem cells (hPSCs) or cancer stem cells (CSCs). The first brain organoids and brain tumor organoids (BTOs) were reported in 2013 and 2016, respectively.[20,21] Organoids can maintain multiple cellular lineages and preserve complex cell–cell communications.[22,23] Importantly, this model recapitulates the genotype and phenotype, including the heterogeneity of the parental tumor.[20,24] Furthermore, organoids provide a humanized TME to investigate brain tumors. Currently, BTOs can be generated within 1–2 weeks with success rates far higher than those of PDX. They can be cultured for long-term biobank application.[24] Different forms of BTOs can be customized to research objectives and brain tumor features (Table 2). These advantages of BTOs have attracted attention in preclinical research. In this comprehensive review, we introduce the different forms of established BTOs and their characteristics, including their strengths and applications in the study of CSCs, therapy resistance, drug testing, and preclinical research.

TABLE 1 Characteristics of the three mainstream preclinical cancer models.

Features	2D cell lines	PDXs	Tumor organotypic explant	BTOs
Basic criteria for preclinical model				
Time demand	+++	+	+	++
Success rate	++	+	+	+++
Cost	+++	+	+	++
Technical difficulty	+	+++	+	++
Long-term stability	+	++	–	+++
Real-time imaging	+++	+	++	+++
Manipulability	+++	+	+	++
Representation of primary tumor				
Molecular preservation	+	++	+++	+++
Phenotype preservation	+	+++	+++	++
TME preservation	–	+++	+++	++
Application in basic research				
Tumorigenesis	++	+	+	+++
Interactions with TME	–	+++	+++	++
Cancer stem cell	+	++	+	+++
Therapy resistance	+	+	++	+++
Application in precision therapy				
Drug testing	++	+	+	++
Biobank	++	+	–	+++
Preclinical research	+	++	+	+++
Reducing side effects	+	++	+	+++

Abbreviations: +++, best; ++, suitable; +, possible; –, unsuitable.

2 | NORMAL BRAIN ORGANOIDS

In. 2013, Lancaster et al[21] established brain organoids derived from embryonic stem cells (ESCs), recapitulating the three-dimensional structural organization with neural identity and differentiation. The procedure entailed: (1) inducing hPSCs to generate embryoid bodies (EBs); (2) feeding EBs and initiation of germ cells; (3) induction of the neural ectoderm; (4) transfer of neuroepithelial tissues to Matrigel droplets and neuroepithelial bud expansion; (5) brain tissue growth and expansion.[21,25,26] Using this technique, normal brain organoids

TABLE 2 Characteristics of the different forms of BTOs.

Form of BTOs	Advantages	Shortcomings	Suitable application	Current establishment
BTOs derived from patient somatic cells	1. Preserving the intrinsic germline mutations 2. Providing a precious platform for human-specific, systematic genetic diseases with pathology of brain tumors	1. Long-time establishment 2. No immune and vascular cells	1. Discovering the biological mechanism and interventable targets of the diseases 2. Monitoring the natural trajectories of tumorigenesis and development	TSC, NF1
BTOs derived from hPSCs with genetic manipulations	1. Modeling tumorigenesis and development Flexibility of the timing to introduce driver mutations 2. Capability to artificially introduce mutations 3. Containing both the tumor and normal cells	1. Long-time establishment 2. No immune and vascular cells 3. Ignorance of cellular heterogeneity 3. The complexity of genetic manipulation techniques 4. Unknown effects of the artificially introduced genes on their own gene expression	1. Identification of the oncogene and understanding the genetic function 2. Discovering therapeutic targets 3. Studying invasion and cell–cell interaction 4. Investigating susceptibility of brain tumors	GBM, MB, ATRT, CNS-PNET, NF1
BTOs derived from tumor cells	1. Maintenance of molecular features (genetics, epigenetics, transcriptomics, metabonomics) 2. Maintenance of phenotypes when orthotopically transplant into animals 3. Diversity of niche and cell subtypes 4. Maintenance of intra- and inter- heterogeneity 5. Maintenance of functional cell–cell interaction among tumor cells 6. Maintenance of partial stromal cells (e.g., immune and vascular cells) 7. Capability to co-cultured with immune cells directly 8. Similar responses to therapies as original tumors 9. Fast and scalable establishment 10. High success rate for establishment 11. Providing a precious platform for benign or slow-proliferated brain tumors 12. Biobankability	1. Lacking TME, gradual reduction of stromal cells 2. Limited interactions with non-tumor cells 3. Gene drift after high passage	1. Drug testing and high-throughput screening 2. Preclinical studies (targeted drugs, immunotherapy, oncolytic virus) 3. Establishing biobank 4. Discovering the biological mechanism (therapy resistance, cancer stem cells, tumorigenesis)	GBM, LGG, BM, MB, Schwannoma
Brain tumor cells/spheres co-cultured with brain organoids	1. Containing both the tumor and normal cells 2. Maintenance of the interactions between tumor cells and TME (neurons, astrocytes) 3. Maintenance of phenotypes 4. Closest model to the original tumor (molecular features) 5. Real-time imaging of invasion	1. Long-time establishment 2. No immune and vascular cells	1. Studying the mechanism of interactions between tumor cells and TME, such as invasion and tumor-promoting effect of TME 2. Evaluation of the therapeutic effects on invasiveness 3. Evaluation of the dosage and side effect of therapy	GBM, LGG, Meningioma, BM, MB, ATRT

Abbreviations: ATRT, atypical teratoid rhabdoid tumor; BM, brain metastasis; GBM, glioblastoma; LGG, lower grade glioma; MB, medulloblastoma; NF1, neurofibromatosis type 1; CNS-PNET, primitive neuroectodermal tumor; TSC, tuberous sclerosis complex.

can be generated within a month and maintained for more than a year, exhibiting the spatial topography identified by region-specific markers (Figure 1A).[21,25,26] To date, different region-specific brain organoids have been established, including the forebrain, midbrain, hindbrain, choroid plexus, cerebellum, hypothalamus, and pituitary.[27–33] Normal brain organoids, which are differentiated and self-aggregated from hPSCs, also preserve multiple cell types including neuronal and astrocytic sublineages. Oligodendrocytes,[34] vascular endothelium,[35] and microglial cells[36] can be derived using a modified protocol. These features enable normal brain organoids to mimic the human brain to a high degree, and extensively model neural diseases.

3 | BRAIN TUMOR ORGANOIDS DERIVED FROM PATIENT SOMATIC CELLS

Some types of brain tumors resulting from specific germline mutations emerge and grow during neurodevelopment, but lack an appropriate in vivo or in vitro model.[37,38] Several studies have collected somatic cells (blood mononuclear cells and fibroblasts) from patients with these tumors, reprogrammed these cells to induced pluripotent stem cells (iPSCs), and generated brain organoids from reprogrammed iPSCs with intrinsic genetic defects. During the growth of this form of brain organoids, brain tumors initiate at a specific developmental point and proliferate within the brain organoids, exhibiting morphological progression, biological behavior, and signaling mimicking human disease (Figure 1B). Such models have been demonstrated in studies of neurofibromatosis (NF) and tuberous sclerosis complex (TSC).[39,40] However, the ability to simulate the developmental trajectories of these brain tumors driven by germline mutations cannot be supported by other models. Moreover, animal models cannot recapitulate human-specific neurodevelopmental diseases, and cell lines lack the three-dimensional cell–cell interactions that are essential for neurodevelopment. Eichmüller et al. generated brain organoids for TSC derived from patients with *TSC2* mutations. The organoid

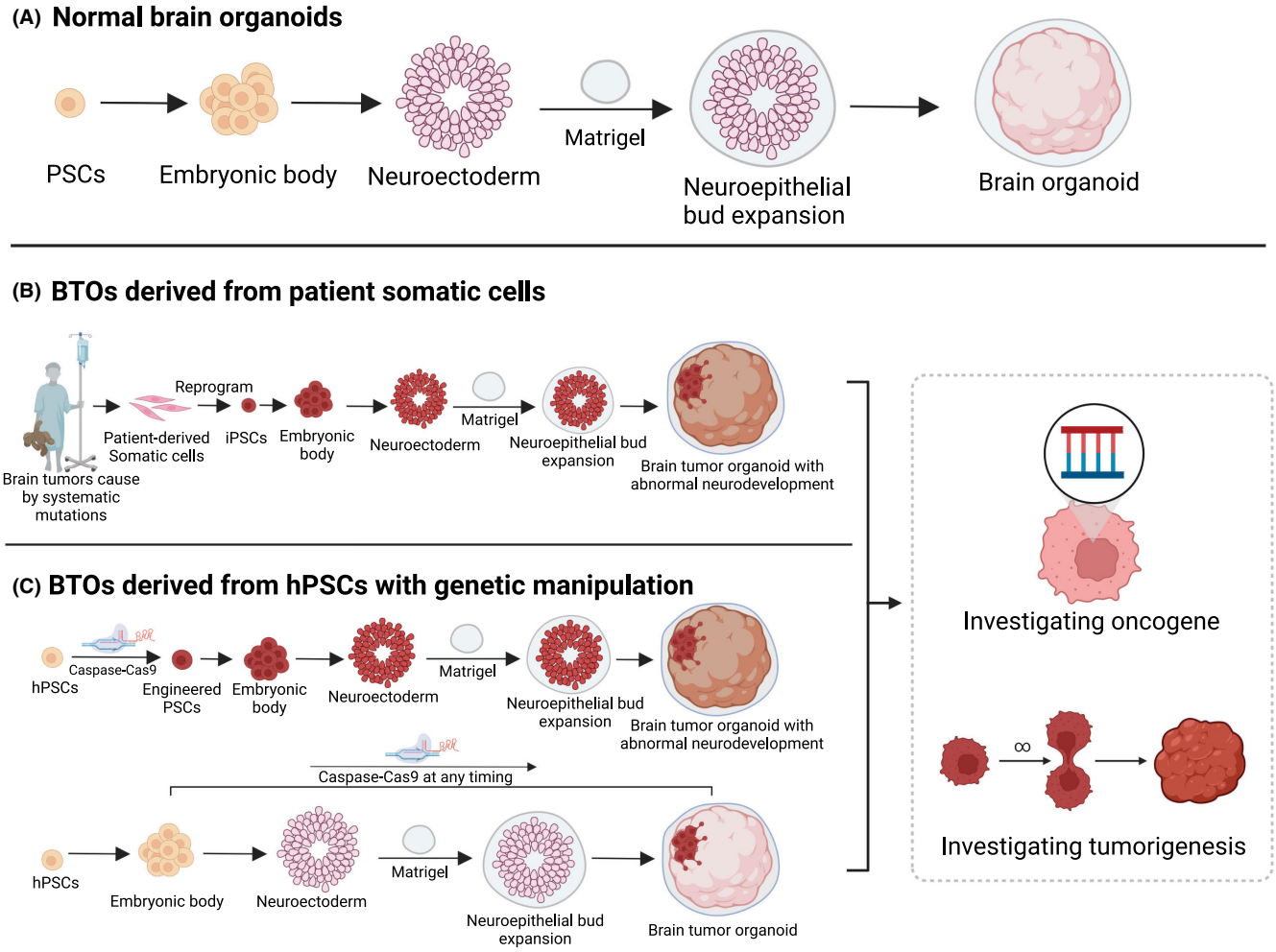

FIGURE 1 Establishment of BOs and BTOs derived from hPSCs. (A) Protocol of generating normal BOs. (B) Protocol of generating BTOs derived from patient somatic cells. (C) Protocol of BTOs derived from hPSCs with genetic manipulation. Genetic manipulation could be carried on at multiple timepoints during the generation of BTOs to model the role of genetic mutations during neurodevelopment. These two types of BTOs could be applied for investigation of oncogene function and tumorigenesis process. The diagram was created with BioRender.com.

model recapitulated the pathological features of TSC, exhibiting both brain tumors and dysplastic cortical regions. Using scRNA-seq and extensive histological validation, a specific interneuron progenitor population called the caudal late interneuron progenitor (CLIP) cells was identified, which are dispensable and responsible for the initiation of both tumor and cortical tuber lesions. These cells originated from the caudal ganglionic eminence during mid-gestation in the fetal brain and were previously unidentified. Excessive CLIP cell proliferation initiates both tumor and brain abnormalities during neurodevelopment, depending on EGFR signaling, indicating a possible therapeutic target.[39] This organoid technology provides a valuable humanized platform to model these rare genetic diseases.

4 | BRAIN TUMOR ORGANOIDS DERIVED FROM hPSCs WITH GENETIC MANIPULATION

In addition to BTOs established from patient-derived iPSCs with inherent genetic defects, BTOs can also be established from PSCs (including iPSCs and ESCs) by introducing oncogene overexpression or loss of tumor-suppressor gene function at different developmental stages (Figure 1C). This form of BTOs can be used to investigate the role of genetic mutations in tumorigenesis and tumor development (Table 3). EGFRvIII is a common mutation in GBMs. EGFRvIII was introduced into ESCs to generate EGFRvIII^{OE} organoids, which exhibit excessive cell proliferation and astrogenesis at the expense of neurogenesis, similar to that observed during GBM pathogenesis. At the EBs stage, neural stem and progenitor cells, which are considered the origin of many different brain tumors, expand on the surface of the EBs.[41–43] Bian et al[44] introduced plasmids containing oncogene-amplifying and tumor-suppressing mutations into EBs via electroporation at the end of neural induction culture, prior to Matrigel embedding. They modeled the formation of GBM-like and primitive neuroectodermal tumor (CNS-PNET)-like tumors, which induce tumor overgrowth and showed similar transcriptomic signatures. CNS-PNET is a rare and malignant brain tumor that lacks in vivo and in vitro models, necessitating the use of organoid technology to further investigate this rare tumor type. At the brain organoid expansion stage, brain organoids do not achieve complete postmitotic maturity and never completely lose their neural stem cell population.[25] GBM[45] and medulloblastoma[46] organoids were established using oncogene electroporation of brain organoids in matrigel. Because only a small proportion of the cells in brain organoids are genetically engineered, these BTOs partly mimic human tumorigenesis because they contain both tumor and normal tissues. This allows for the study of interactions between tumors and normal cells, and their invasiveness. Another potential advantage of this model is that organoids established from iPSCs derived from patients or susceptible populations can be further used to test the susceptibility of individuals to different combinations of driver mutations, thereby meeting the needs of precision oncology.[44]

Brain tumors have been suggested to arise from or be driven by neural stem-like cells.[47–51] Recurrent mutations in brain tumors also affect neurodevelopment.[52,53] Similarly, some perturbed signaling pathways in neurodevelopment lead to the initiation and proliferation of brain tumors.[54,55] Therefore, tumorigenesis may be closely associated with neurodevelopment. Brain organoid growth mimics neurodevelopment and contains multiple cellular lineages in the human brain. By performing genetic manipulation at different stages of organoid establishment, brain organoids can be developed as an optimal model to study tumorigenesis, especially for pediatric brain tumors that appear during active neurodevelopment. For example, atypical teratoid rhabdoid tumors (ATRTs) are challenging pediatric brain cancers caused by the inactivation of *SMARCB1* during neurodevelopment. During neuronal differentiation in brain organoids, *SMARCB1* was knocked down using CRISPR/Cas9. The *SMARCB1*$^{-/-}$organoids exhibited a transcriptomic profile similar to that of ATRTs and demonstrated instability among neural progenitors and failure in neural maturation, contributing to tumorigenesis.[56]

5 | BRAIN TUMOROIDS

Based on the tumoral property of infinite proliferation, the models of brain tumors can be generated from brain tumor specimens, such as immortalized tumor cell lines, patient samples, xenografts, and genetically engineered glioma models. In 2016, Hubert et al. established brain tumoroids directly from GBM specimens. They dissociated samples derived from patient tumors into single cells and embedded approximately 1000 suspended cells per organoid into Matrigel (Figure 2). GBM tumoroids (GBOs) expanded prolifically to sizes of 3–4 mm in 2 months, demonstrating reduced growth, stability, and viability for more than a year without passaging.[20] When dissociated into single cells and implanted into mouse brains, these GBOs could maintain invasiveness, while GBM tumor spheres lost their invasive phenotype, indicating that cell growth conditions may help maintain the phenotype. The greatest strength of Hubert GBOs is their ability to recapitulate cellular diversity and the TME. Because GBOs do not have a vascular system, a gradient was observed resulting from exposure to growth-supporting materials (oxygen, exogenous growth factors, nutrients) from the outer zone to the core of the GBOs, resulting in microenvironmental variation. The outer zone of Hubert GBOs modeled the perivascular niche with sufficient growth-supporting materials and exhibited rapid proliferation. The inner zone modeled the perinecrotic niche far from the vasculature and exhibited hypoxic, quiescent, and even necrotic properties. The TME exerts considerable effects on tumor cells, including GSCs. In Hubert GBOs, GSCs were distributed more densely and proliferated faster in the outer zone, but were sparse and tended toward quiescence in the inner zone. Importantly, this model spontaneously contained different states of GSCs, providing an experimental platform for studying their biological characteristics. For example, GSCs could transform into quiescent state under chemotherapy to develop resistance and keep living with a possibility to recur.[57]

TABLE 3 Summary of tumorigenesis studies using BTOs.

Brain tumor type	Driver gene	Stage of mutations occurring	Findings	References
Glioma	*EGFRvIII*^{OE}	hESCs	Excessive gliogenesis at the expense of neurogenesis	138
	1.*CDKN2A*$^{-/-}$/*CDKN2B*$^{-/-}$/*EGFR*^{OE}/*EGFRvIII*^{OE} 2.*NF1*$^{-/-}$/*PTEN*$^{-/-}$/*TP53*$^{-/-}$ (*p53*$^{-/-}$) 3.*EGFRvIII*OE/*CDKN2A*$^{-/-}$/*PTEN*$^{-/-}$	End of neural induction culture	1. Inducing tumor over-proliferation and invasion in organoids 2. Similar transcriptome as GBM 3. Exhibition of distinct cellular identity 4. Viability and invasion when transplant in vivo; 5. Identification of interactions between tumor and normal cells; suitability for targeted drug testing	44
	TP53$^{-/-}$/*PTEN*$^{-/-}$/*MEOX2*^{OE}	Cerebral organoids expanding	MEOX2 cooperated with p53 and PTEN loss to induce excessive proliferation	139
	TP53$^{-/-}$/*HRas*$^{G12V\ OE}$	Cerebral organoids expanding	1. Inducing tumor over-proliferation and invasion in organoids 2. Similar transcriptome as GBM 3. Tumorigenesis and invasion when transplant in vivo 4. Serial transplantability	45
Medulloblastoma	*Otx2*^{OE}/*c-MYC*^{OE}	Cerebellar organoids expanding	1. Inducing over-proliferation of cerebellar progenitor cells and impairing their differentiation 2. Similar cellular identity and methylation profile as medulloblastoma 3. Identification of SMARCA4 and EZH2 as therapeutic targets	46
CNS-PNET	*MYC*^{OE}	End of neural induction culture	1. Inducing tumor proliferation 2. Similar transcriptome as CNS-PNET 3. Exhibition of distinct cellular identity 4. Proliferation and exhibition of characteristic pathological features when transplant in vivo	44
ATRT	*SMARCB1*$^{-/-}$	hiPSCs During neuronal differentiation	Inducing defects in neuron formation 1. Inducing instability among neural progenitors and failure in neural maturation which contribute to tumorigenesis 2. Similar transcriptome as ATRT	56
NF1	*NF1*	hiPSCs	Differential effects of NF1 mutations on cerebral organoid neural progenitor cells proliferation, apoptosis, and differentiation	40
TSC	TSC$^{+/-}$	hiPSCs	1. Recapitulating the emergence of both brain tumors and dysplastic cortical regions during organoids development 2. Identification of a specific interneuron progenitor population (CLIP cells) which result in both tumor and cortical tuber lesions 3. Over-proliferation of CLIP cells depending on EGFR signaling, suggesting a therapeutic target	39

However, currently the models for quiescent GSCs are lacking owing to rapidly proliferating populations. Hubert GBOs may help to solve this dilemma and allow the identification of quiescent GSC markers and targets.

Metabolism is a key facet of glioma growth and metastasis, and cannot be accurately represented outside the influence of the TME. The diverse microenvironment in Hubert GBOs also triggers metabolic alterations that can serve as therapeutic targets. For example, hypoxia can induce lipid droplet biogenesis to protect cells from oxidative damage and provide energy.[58] Lipid enrichment has been identified in hypoxic GBOs. Because GSCs are mainly distributed in the outer zone, they have low lipid droplet accumulation compared to non-GSCs. Deeper lipidomic analysis showed that GSCs contained reduced levels of major classes of neutral lipids, but displayed higher polyunsaturated fatty acid production compared to non-CSCs, due to high expression of fatty acid desaturase (FADS1/2). Upon knocking down FADS1/2, the viability and self-renewal ability of GSCs are damaged, indicating a therapeutic target.[59] Therefore, Hubert GBOs provide a platform to identify the abnormal metabolisms and target them.

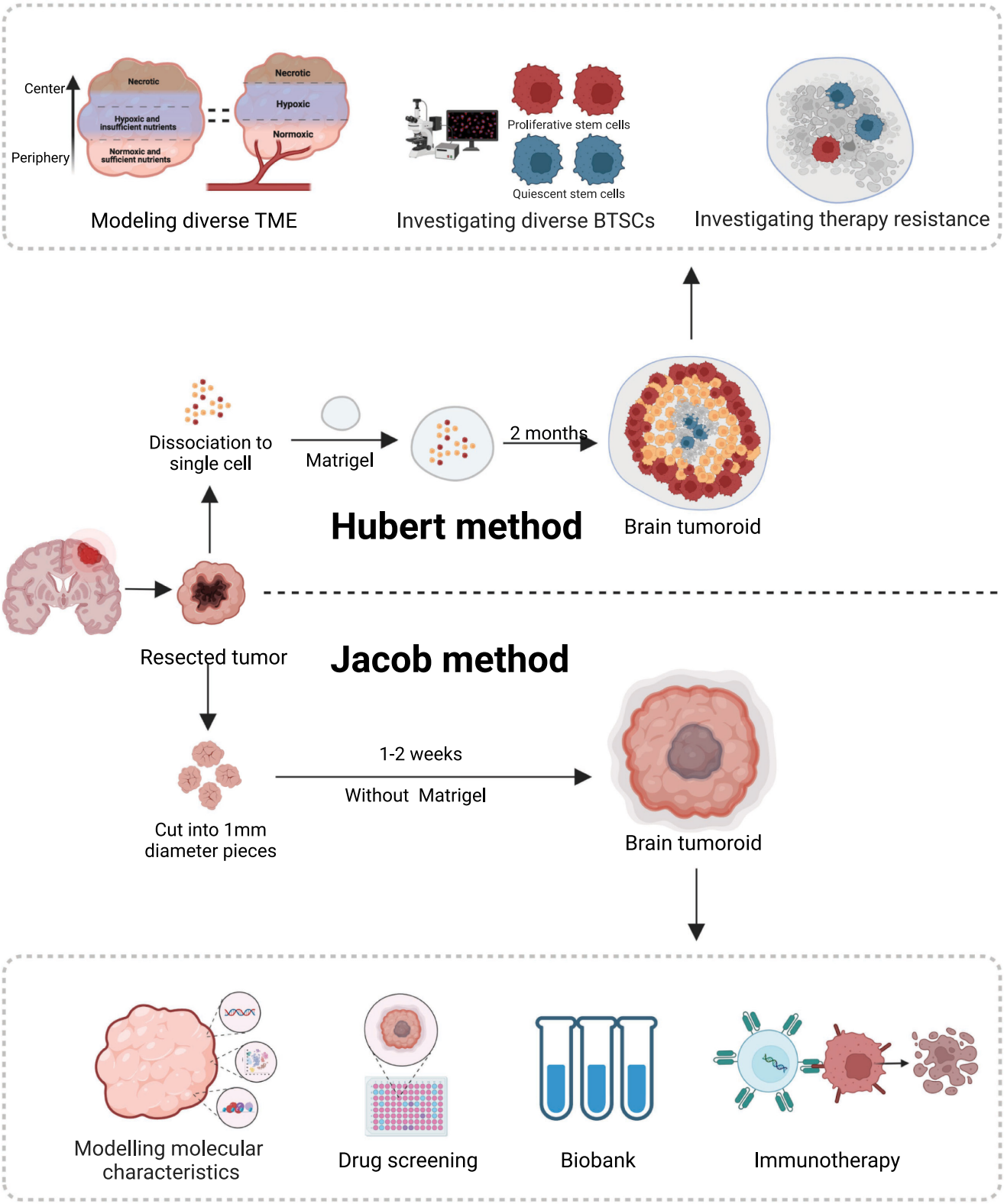

FIGURE 2 Establishment of brain tumoroids and their applications. Hubert brain tumoroids are generated within matrigel as BOs, composed of multiple TME due to gradient exposure to nutrients and oxygen. Jacob brain tumoroids could be generated within 2 weeks without cell–cell dissociation and matrigel, which are the most clinically applicable BTOs. The diagram was created with BioRender.com.

In Hubert GBOs, different cells exhibited different responses to therapies and more resistance compared to cell lines and tumorspheres (Table 4).[20] By using the techniques of cellular tracking and sorting, specific subpopulation of GBM cells could be identified from GBOs to investigate.[60,61] For example, by isolating quiescent GBM cells, bioinformatics analyses and functional assays showed

TABLE 4 Different therapy responses of preclinical in vitro models.

Therapy	2D	Tumorsphere	GBOs	References
TMZ	+	+	–	61,94
Radiation	+	+	–	61,94
TMZ + Radiation (Stupp)	+	+	–	61,140
Vismodegib	+		–	80
Vismodegib+Stupp	+		–	80
Disulfiram	+		–	80
Disulfiram+Stupp	+		–	80
Omipalisib	+		–	80
Omipalisib+Stupp	+		–	80
Parthenolide+Stupp	+		–	80
Compound JVM-3-55		+	–	141
Compound PNR-5-88		+	–	141
Compound PNR-7-84		+	–	141
Ruxolitinib		+	–	61
Ibrutinib		+	–	61
ruxolitinib		+	–	61

Abbreviations: +, sensitive; –, resistant.

that hypoxia and TGFβ signaling may drive the identity of quiescent GBM cells, providing a potential mechanism to ameliorate therapy resistance.[60] While targeting populations of resistant cells is promising, complicated cell–cell crosstalk within GBOs could also be a potential mechanism supporting therapy resistance. Tunneling nanotubes (TNTs) and tumor microtubes (TMs) have both been found in Hubert GBOs, of which TMs have not been previously identified in vitro. TMs are membranous extensions that allow ion flux through GAP-junctional proteins, providing rapid neurite-like communication between cells. TNTs are membranous structures that are open at both extremities, allowing cytoplasmic continuity and transportation of organelles such as mitochondria between connected cells.[62] Due to this transfer, tumor cells acquire new abilities such as metabolic plasticity and treatment resistence.[62–64] In GBOs, mitochondrial transfer through a functional TNT connection was observed among tumor cells. TMs cooperate with TNTs to participate in therapy resistance.[65] Mitochondrial transfer between tumor cells can provide metabolic support and rescue aerobic respiration for recipient tumor cells in response to treatment-related stress, revealing a partial mechanism of therapy resistance in patients.[64] Therefore, glioblastoma cells in GBOs may overcome therapy through cooperation in the TME, aided by complicated cellular connections, which may be closer to the responses observed in parental tumors and an appropriate model to study therapy resistance.

In 2020, Jacob et al reported a revolutionary method that could generate GBM tumors directly from resected GBM samples rather than via dissociation, retaining native cell–cell interactions (Figure 2). By optimizing a chemically defined medium, they cultured tumoroids with few exogenous growth factors and no Matrigel to minimize clonal selection and decrease potential treatment confounders. Besides, growing without matrigel, which is a kind of undefined and complex ECM, could also avoid the unstability and matrigel-specific effects.[66] Tumoroids derived from patients without dissociation also retained a heterogeneous cellular composition, including immune and endothelial cells. In this model, immune and endothelial cells can persist for more than 8 months and gradually decrease over time. Moreover, this method generated GBM organoids approximately 1–2 weeks after initial surgical resection with high fidelity and an overall success rate of 91.4%.[24,67] Jacob GBOs depend on the gradient exposure of growth-supporting materials and precisely recapitulate the intra- and inter-tumoral heterogeneity from genotype to phenotype. Profiling of somatic variants and copy number variants (CNV) in GBOs is largely similar to tumors derived from different patients, indicating inter-tumoral heterogeneity. The GBOs derived using this method from different subregions in the same patient also showed subregion-specific mutations.[24] Specifically, Jacob GBOs preserved EGFR mutation, a driver in GBM, which was rapidly lost in two-dimensional culture.[67]

GBOs also showed high similarity to parental GBM samples at the transcriptome level for over 12 weeks. Even for the macrophage/microglial-related genes, the expression was comparable between GBOs and parental tumors for 2 weeks. Due to the disability of replication and immortality for non-tumor cells, the most differentially downregulated genes between GBOs and parental tumors were immune- and blood-related genes, indicating incomplete retention of immune cells and blood cells over a long period of time relative to in vivo conditions. Furthermore, scRNA-seq analysis showed that cellular and molecular signatures in GBOs were highly similar to those of the parental tumor, maintaining cell-type heterogeneity and molecular properties.[24] Finally, the GBOs preserved similar morphology compared to parental tumors and could be transplanted into the mouse brain intact, displaying not only invasiveness, but also angiogenesis.

The omics revolution has led to the identification of various targets and a more comprehensive view of the molecular signaling underlying brain tumors through the integration of genomic, epigenomic, transcriptomic, metabolomic, and proteomics data.[68–70] The classification of patients based on multi-omics profiling enhances precise diagnosis and therapy. However, intratumoral heterogeneity and limited amounts of tumor material available for omics analysis may hamper these advances.[71–73] The extensibility and precision modeling offered by GBOs provide a platform for solving this dilemma. To establish a living biobank for storing omics information, Jacob et al optimized the procedures to cryopreserve GBOs long-term by: (1) cutting GBM tumoroids into small pieces, (2) pre-incubating them in freezing medium to allow complete perfusion before freezing, and (3) incubating GBM tumoroids with the ROCK inhibitor before freezing and during thawing to inhibit cell death.[67,74] After recovering these GBOs from the cryopreserved state, they were capable of maintaining their characteristics and growth. GBOs expanding exponentially on serial passage are generally deemed biobankable, which means that the current tumoroids for all brain tumor types are potentially useful for this purpose.[75] Importantly, the establishment of biobanks guarantees reproducibility.[76]

Because of the rapid establishment and precise recapitulation of parental tumors, tumoroids have been applied in drug testing and could potentially be used to select efficacious therapies for individual patients. At the whole cancer level, patient-derived tumor organoids accurately predicted patient responses to therapy with 81% sensitivity and 74% specificity.[77] For GBM, in an observational study, Jacob et al. reported that the responses of GBOs were consistent with those of patients, with 83% sensitivity and 88% specificity.[24] Loong et al[78] used GBOs to prospectively screen drugs for patients to identify targetable mutations using genetic sequencing, which finally selected everolimus and achieved a real effect in the patient. Currently, many studies have used GBOs to test drugs (Table 5). Because treatment for brain tumors, especially malignant brain tumors such as GBM is time-constrained, GBOs are the most frequently used BTOs for drug testing. Owing to the manipulability of GBOs, several techniques have been incorporated to quicken and scale up drug testing, such as 3D bioprinting and microarray establishment. Importantly, the process of generating GBOs for drug testing can be completed within a month, possibly enabling clinical management and high-throughput drug screening. Similar to the results of Loong et al,[24,79] many studies have reported that the responses of GBOs to targeted drugs were not consistent with targeted mutations, confirming that mutation analysis alone without functional testing is insufficient to predict response to treatment. GBOs could also provide a platform to study the biological mechanisms of novel effective drugs that have not been reported in GBM. For example, the proteasome inhibitor carfilzomib was identified as a targeted drug from high-throughput screening of 320 drugs combined with proteomic and bioinformatic analyses and a series of functional assays in GBOs.[80] GBOs can also be used to predict the effects of combination therapy, which can improve outcomes in patients with malignancies.[81] The responses of GBOs to combination therapy showed greater effects than those of monotherapy.[82]

While immunotherapy has achieved great success in several types of cancer, its efficacy on brain tumors is limited.[83] Current in vitro models for brain tumors often lack cellular heterogeneity and do not maintain mutations that lead to altered surface antigens, which hampers preclinical studies of immunotherapies. Because Jacob GBO retains immune cells, they can serve as in vitro model to develop immunotherapies. For example. inhibiting HSPA7, an immune-related pseudogene, increased the efficiency of anti-PD1 therapy in GBOs by reducing macrophage infiltration and shifting the TME from an immunosuppressive state to an immune-activated state.[84] Chimeric Antigen Receptor T (CAR-T) cells could also be co-cultured with GBOs to test the efficacy and specificity. Using the co-culturing system, 2173 CAR-T cells targeting EGFRvIII^{+} cells were found to specifically kill the targeted cells, but retain EGFRvIII^{-} cells in GBOs.[24,67] In the future, more syngeneic immune cells and tumor-infiltrated lymphocytes can be sorted and added to BTO co-culture systems to promote the development of immunotherapy technologies against brain tumors.

Tumor-treating fields (TTFields) have achieved great efforts for GBM patients in clinical trials. However, patients are also possible to resist to TTFields and it is still unknown which patients are likely to maximally benefit from TTFields. Besides, the mechanisms of TTFields resistance are undiscovered. Using the patient-derived GBOs, Nickl et al[85] observed different responses to TTFields and found a TTFields-resistant GBO. The fast establishment and the close representation make GBOs suitable for screening patients sensitive to TTFields. Meanwhile, GBOs were also capable of further investigating the mechanism of TTFields resistance and find the target to sensitize the efficacy. However, the accuracy for the response to TTFields between GBOs and patients is needed to be confirmed in future studies.

Although GBM is the most common malignant tumor source of brain tumors, many other brain tumoroid models have been established. Organoid technology can be used to establish models of benign or slowly proliferating brain tumors, which have limited experimental models or are difficult to culture in vitro. For example, in vitro models for lower grade gliomas (LGGs) are hard to generate; interestingly, they have been established as tumoroids in 4 weeks with an 87% success rate and could be maintained for months by modifying the Jacob method and using lower oxygen conditions (5%) during culture, probably due to the activated HIF2α under 5% oxygen. The established LGG tumors not only presented the same histology, stem cell markers (SOX2), proliferation (Ki67), vascular composition (CD31), macrophages/microglia (Iba1), and genetic alterations as parental tumors, but also showed similar metabolomics. Oncoprotein IDH enzymes were observed pervasively using immunohistochemistry. More importantly, 2HG accumulation was observed in LGG tumors, comparable to parental tumors, using liquid chromatography-mass spectrometry analysis.[86]

Meningioma is the most common primary tumor of the brain and is derived from the neural crest.[1] Meningiomas have a high

proportion of interstitial matrix; therefore, dissociating the original tumor samples using enzymatic methods is hard to perform without disrupting cell viability. Most meningiomas are benign and proliferate slowly. This has led to a lack of models for this disease.[87] Organoid techniques can be used to establish meningioma tumoroids by embedding the dissociated meningioma cells into matrigel with the supplementation of growth factors similar to the generation of cerebral organoids.[88] The establishment was within 2 weeks with 100% success rates, recapitulating multiple characteristics of the parental tumors.[88] Molecular features, genetic mutations, chromosome structure, DNA methylation, and RNA expression were all maintained in meningioma tumoroids from parental tumor tissues.[88] These meningioma tumoroids showed histological and morphological features similar to those of the parental meningiomas of different grades. Meningioma markers and low proliferative features were also observed in meningioma tumoroids.[87,88] FOXM1 expression is correlated with increased proliferation in meningiomas, and inhibiting FOXM1 using thiostrepton combined with radiotherapy could efficiently kill tumor cells, indicating a novel targeted therapy.[88]

Other brain tumors, including medulloblastoma (MB),[89] brain metastases,[20] and schwannoma[90] tumoroids, have also been established and used for scientific exploration. For example, in MB tumors, the proteasome inhibitor NPI-0052 combined with γ-radiation showed synergistic apoptotic effects on MB cells.[89] However, these tumoroids were still established based on the matrigel and multiple supplements supporting growth, more optimized approaches were encouraged.

6 | BRAIN ORGANOIDS CO-CULTURED WITH BRAIN TUMORS (BO-BT)

To study tumor invasion and cell–cell interactions between tumors and normal cells, brain organoids can be co-cultured with brain tumor cells/spheres (Figure 3). CSCs are a group of cells that most recapitulate tumors molecularly and phenotypically and are most commonly co-cultured with organoids.[45,91,92] Depending on the tissue-clearing method and microscopy technique used, invasive protrusions, and microtube networks formed in brain tumor cells can be observed and measured as surrogates of invasive ability. Reporter genes, such as luciferase, can also be ectopically expressed in brain tumor cells for real-time live imaging. Three types of co-culture patterns were established. First, co-cultured brain tumor cells with iPSCs and then induced brain tumor organoids. In this pattern, recurrent GBM stem cells (GSCs) exhibited enhanced invasiveness compared to primary GSCs at an early stage. However, both recurrent and primary GSCs stopped growing after day 10 and survived for up to day 20. Second, brain tumor cells were implanted into the established brain organoids. Distinguishing invasiveness between recurrent and primary GSCs was also significant. The invasive protrusions and microtube-like structures of surgical GBM tumor specimens resembled GSCs formed in this pattern. Third, brain tumorspheres were co-cultured with established brain organoids. In this system, individual GSCs invade brain organoids, showing a profound tropism of GSCs to the brain tissue. While GSCs were compact in spheres, invasive protrusions, and microtubes could not be quantified.

The generation of GBM cells co-cultured with cerebral organoids (GLICOs) often takes over a month, which is longer than that for GBOs. However, GLICOs are reported to be the most accurate models when compared with the two-dimensional, GBOs, and PDX models. Among these models, GLICOs exhibited the highest correlation with parental tumors at many levels, such as similar transcriptomes, diversity of cellular states, and strong stemness and invasiveness signatures.[91] GSCs in GLICOs also preserved key genetic and signaling components of the parental tumors,[93] in addition to pathological features and progression capacity. In one study, GLICO models identified aggressive infiltration of tumor cells into cerebral organoids, a pathological feature of grade IV GBM, in a patient with grade II astrocytoma. After 18 months, the second diagnosis of recurrent tumors in the same patient revealed a pathological advancement to grade IV GBM.[79] These advantages may result from the existence of a suitable TME from brain organoids that contain neurons, axons, myelinated axons, and dendritic synapses. GSCs in GLICOs exhibited reduced apoptosis and markedly enhanced proliferation and tumor microtubes compared to GSCs in GBO, which may also be due to the TME. The interactions of brain tumor cells with TME components and how they affect tumor growth and behavior are now gradually being revealed, although the specific mechanisms remain unknown.[94]

Brain organoids provide a platform for studying the mechanism of interaction between the brain and brain tumors. Compared with PDX, which also contains a TME, brain organoids are humanized, manipulable, and fast for the establishment with a higher success rate, allowing real-time imaging. scRNA-seq analysis of GBM cells before and after co-culture with brain organoids showed that GBM cells could sense the neuron once co-cultured and upregulate the gene expression related to dispersion and ligand-receptor interaction between GBM and organoid cells. Therefore, targeting and breaking the cell–cell connections could be a novel therapeutic strategy.[95] TMs and TNTs have been observed in GLICOs. Their connections with normal cells in brain organoids may be the reason for the enhancement because tumor growth in the brain has been shown to require neighboring cellular activity.[96] TMs can form synapses with neurons and astrocytes and drive tumor progression in primary brain tumors[97,9] or brain metastases.[99] In GLICOs, TMs are found in an interconnected network that can effectively propagate calcium signals for cellular communication. They deeply penetrate the brain organoids and provide potential routes for invasion, proliferation, and interconnection over long distances.[100] In brain metastases co-cultured with brain organoids, astrocytes also form GAP junctions with metastatic lung cancer cells, which promotes tumor growth.[101,102] TNTs were observed in GLICOs under electron microscopy, and cytoplasmic fusions were formed not only between neighboring tumor cells, but also between neurons and tumor cells. Multiple organelle transfers, including mitochondria, were observed between the cell nuclei. Using organoid techniques, tumor cells can

TABLE 5 Summary of drug testing studies using GBOs.

Treatment	Target	Cases	Consistency with mutations	Generation time	Treating time	Combined methods	Indicator	References
TMZ Dianhydrogalactitol Geftinib Erlotinib AZD3759 AG490 Daphtenin Abemaciclib Palbociclib 42 FDA-approved drugs	Alkylating agent Bi-functional alkylating agent EGFR/ErbB inhibitor EGFR/ErbB inhibitor EGFR/ErbB inhibitor EGFR/ErbB inhibitor EGFR/ErbB inhibitor EGFR/ErbB inhibitor CDK4/6 inhibitor CDK4/6 inhibitor Various	18 18 16 16 16 16 16 16 16 1	Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes	3 days	1 week	1.3D bioprinting 2.GBOs derived from mice transplanted with GBOs	High content imaging system recognizing viable cells	142
TMZ+Radiation (Stupp) Gefitinib Trametinib Everolimus	Standard therapy EGFR inhibitors MEK inhibitor mTOR inhibitor	8 10 4 3	No Yes Yes No	1–2 weeks	1 week	None	Immunohistology of Ki67	24
TMZ BEZ235 Niraparib TMZ+BEZ235 Niraparib + BEZ235	Alkylating agent PI3K/mTOR inhibitor PARP inhibitor Combined therapy Combined therapy	2	– – Yes – Yes	1–2 weeks	3 days	4D bioprinting	1. Intracellular ATP cell viability assays (no detailed description) 2. Live cell imaging and Realtime apoptosis by measuring activated caspase 3 3. Immunofluorescence of GFAP, BMI1, pS6	82
Dacomitinib NSC59984	EGFR inhibitors p53 pathway activator	2	–	1 week	3 days	3D bioprinting	Intracellular ATP cell viability assays by Celltiter Glo	126
Compound JVM-3-55 Compound PNR-5-88 Compound PNR-7-84	NFk-B inhibitor COX-2 inhibitor Tubulin inhibitor	3	–	15 days	1, 2, 3, 6, 8, 15 days	Select drugs from 22 drugs by cytotoxicity and invasion effects on tumorspheres	Automatic microscopic measurement of the area of cells invading into the matrix	142
Stupp Vismodegib w/wo Stupp Disulfiram w/wo Stupp Omipalisib w/wo Stupp Costunolide w/wo Stupp Parthenolide w/wo Stupp	Standard therapy Hh/GLI inhibitor ALDH inhibitor PI3K/mTOR inhibitor hTERT inhibitor HDAC/IKK-β/NF-κB inhibitor	1	–	10 weeks	10 days	Select drugs from 65 drugs by cytotoxicity effects on 2D cells	Intracellular ATP cell viability assays (no detailed description)	140

(Continues)

TABLE 5 (Continued)

Treatment	Target	Cases	Consistency with mutations	Generation time	Treating time	Combined methods	Indicator	References
Everolimus	mTOR inhibitor	1	Yes	–	–	Select drugs based on target capture sequencing	Cytotoxic sensitivity (no detailed description)	78
Cobimetinib	MEK inhibitor		Yes					
Erlotinib	EGFR/ErbB inhibitor		Yes					
Vemurafenib	Raf inhibitor		Yes					
TMZ	Alkylating agent		No					
EPZ015666	PRMT5 inhibitor		No					
Carfilzomib	Proteasome inhibitor	2	Yes	–	2 or 3 days	Select carfilzomib from 320 drugs by cytotoxicity effects on tumorspheres and analyses of drug dose–response curve	Immunofluorescence of cleaved caspase 3 and CD133	80
Temozolomide	Alkylating agent	6	–	–	6 days	Creation of organoid microarrays	1. Intracellular ATP cell viability assays by Celltiter Glo 2. DNA content analysis 3. Immunofluorescence of cleaved caspase 3 and SOX2 4. Immunohistochemistry staining	61
Ibrutinib	JAK/STAT3 inhibitor							
Lomustine	JAK/STAT3 inhibitor							
Ruxolitinib	JAK1/2 inhibitor							

form functional TMs and TNTs, thus cooperating with neighboring tumor cells, neurons, and astrocytes in the brain organoids. Tumor cells acted as a synergistic community in organoids, comparable to in situ conditions. Once GLICOs were transferred into two-dimensional culture conditions, breaking the three-dimensional cell–cell interaction, the GBM cells exhibited transcriptomes similar to the tumor cells in two-dimensional culture conditions, downregulation of several genes related to tumor stemness such as SOX4, NFIA, and BCAN, and lost diversity in cellular subtypes. This evidence suggests that the ability of BO-BT to model parental tumors can be attributed to cell–cell interactions in the TME.

Invasiveness is linked to cancer-TME crosstalk. By changing the characteristics of brain organoids, the consequent invasive capacity can be altered, thus offering an opportunity for a deeper understanding of the invasive process of brain tumors and discovery of potential therapeutic targets. For example, GSCs exhibited faster and deeper invasion in mature brain organoids compared to younger ones; this was linked with the synaptic protein Neuroligin-3, which is generated by mature neurons only. By blocking Neuroligin-3 function, the invasiveness of GSCs was markedly reduced, indicating a potential target. More variants can be changed in brain organoids to further study the interactions between the brain and brain tumors, such as different brain regions or brain organoids derived from syngeneic and non-syngeneic iPSCs.

Patient-derived meningioma cells also exhibited phenocopy invasiveness when co-cultured with brain organoids. In brain organoids, higher grades of meningioma cells exhibited an invasive phenotype, and lower grades of meningioma cells only formed tumorspheres at the surface of the brain organoids. Meningioma cells co-cultured with brain organoids showed the greatest number of overlapping genes with parental tumors when compared to two- and three-dimensional monocultures. CDH2 and PTPRZ1 have been identified as oncogenes driving the tumorigenesis of meningiomas in brain organoids, indicating potential targets.[103]

With the incorporation of brain components in organoids, GSCs exhibit resistance to chemotherapy and radiotherapy compared with the cells in two-dimensional culture.[93] This means that the BO-BT model can also serve as a model for studying therapy resistance and is even more suitable than brain tumors because of the preservation of TME. The tumor cells in BO-BT are more sensitive to therapy than cell lines, including GBM treated with TMZ[79] and NSCLC brain metastases treated with Gefitinib.[103] However, the reason remains unknown, and though tumor cells in two-dimensional culture are speculated to be restricted in terms of growth and malignant behavior and thus protected from chemotherapy or radiation therapy, further investigation is required.[79]

GLICOs can be used to evaluate the efficacy of novel therapies in preclinical trials, and have been widely used for this purpose, including the evaluation of cytotoxicity, invasion inhibition, and radiotherapy sensitization. For example, doxycycline is a Nek2-KD inducer that can activate Nek2 to induce ciliogenesis, thereby causing GSCs to differentiate. Among GLICOs, GSCs exhibit significantly decreased invasiveness caused by doxycycline-induced differentiation,

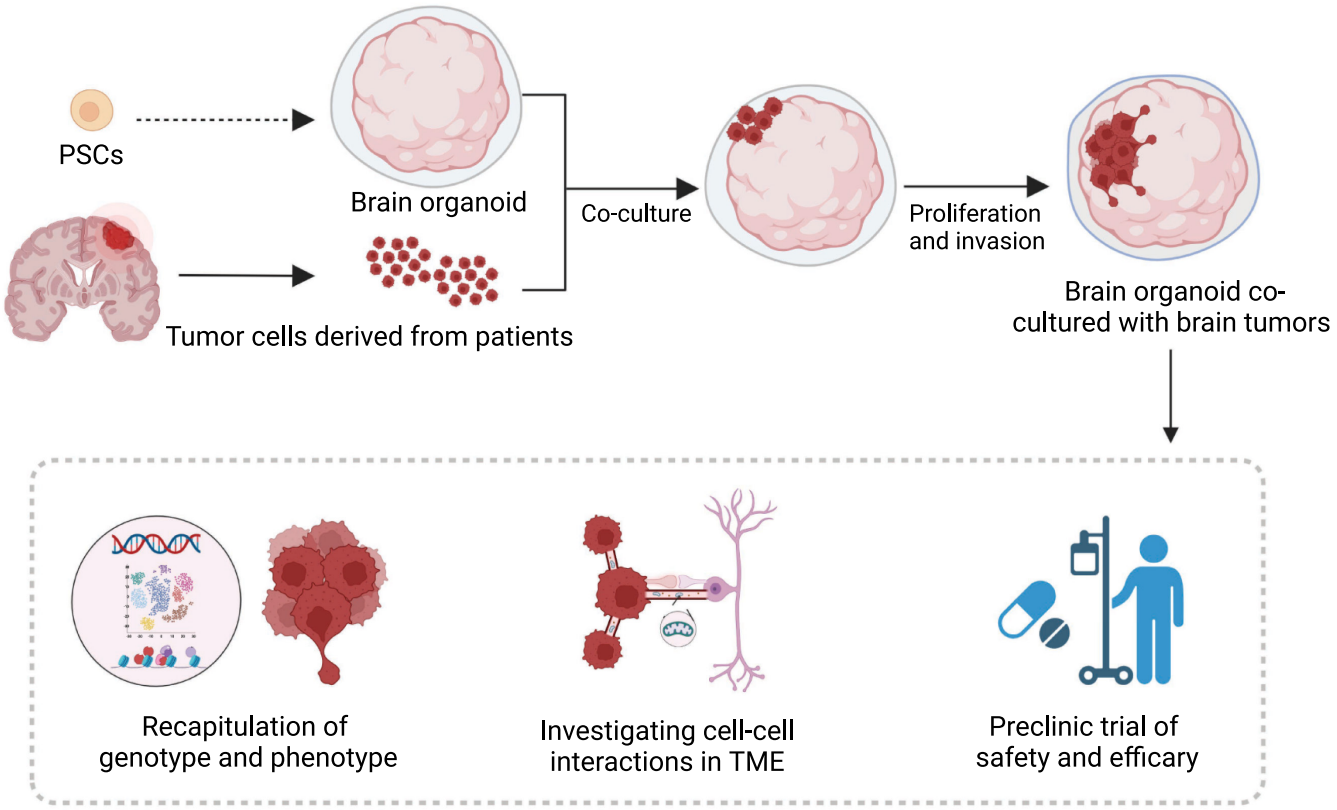

FIGURE 3 Establishment of brain organoids co-cultured with brain tumors and their applications. BO-BTs are the most similar BTOs as original tumors, which could model humanized interactions between tumor cells and brain components in vitro. The diagram was created with BioRender.com.

paving the way for GSC-targeted therapy. Moreover, GLICOs offer a humanized platform close to the parental tumors in situ because certain therapeutic targets do not exist in animal hosts, such as some long non-coding RNAs (lncRNAs). Inhibiting a certain primate-conserved lncRNA screened by the CRISPR interference technique in GLICOs showed decreased tumor growth and stronger radiotherapy effects, which could not be modeled in PDX.[104]

Oncolytic viruses are emerging antitumor therapies that selectively target, internalize, and kill tumor cells while sparing normal cells.[105] The Zika virus can enter the brain, and this viral infection can lead to neonatal microcephaly and other neurodevelopmental defects; infected adults are often asymptomatic.[106] Recently, Zika virus was engineered as an oncolytic virus for patients with brain tumors by targeting SOX2 cells.[107–109] Normally, SOX2 is a transcription factor that is expressed at high levels during human neurodevelopment and contributes to the induction of pluripotency.[110] SOX2 is also highly expressed in many brain tumor stem cells (BTSCs) such as GBM and medulloblastoma.[111] Using humanized BT-BO, the effects of oncolytic viruses on both tumor cells and normal cells could be evaluated. In BT-BO, Zika virus preferentially and effectively infected and killed BTSCs, including GBM, ATRTs, and medulloblastoma, but had limited effects on mature brain organoid size.[107–109] In the future, more oncoloytic viruses that can enter the brain may be engineered to target brain tumor cells, and BTOs represent a major opportunity for preclinical studies of this emerging treatment modality.

The BO-BT models can also be used to evaluate the safety and tolerance of novel therapies. The neurological impairment caused by tumors and associated therapies can have serious lifelong consequences on daily function and deeply influence the quality of life, including fatigue, memory loss, emotional distress, and sleep disorders. Minimizing the side effects of therapies is as equally important as inhibiting the tumor. The identification of the effective and tolerable range of dosage and therapeutic intensity is important in preclinical trials.[112,113] Brain organoids as a "mini brain" can be used as a surrogate to evaluate the side effects of antitumor therapy and provide valuable information for clinical decisions. For example, TTFields showed inhibitory effects on GBM cell proliferation at both 75% and 100% duty cycles; the neurotoxicity of brain organoids at 75% was less prominent than at 100%, indicating that 75% may be a better choice.[114] The targeted drug UM-002 employed in GLICO showed that higher concentrations (>500 nM) reduced GBM cell proliferation but also induced toxicity in normal brain organoids. In a dose–response study, 100 nM was found to not only be cytotoxic for GBM cells, but also safe for brain organoids.[115] The neural side effects of radiation[116,117] and Zika virus[62,63] have also been evaluated in brain organoids. In addition, normal organoids of other organs that are frequently impaired in systemic therapy, such as the heart, liver, and stomach, can be used to test toxicity.[118,119] Notably, multi-organ organoids with tumoroids have been constructed into a connected system with circulation using

microfluidic techniques to synchronously test toxicity and treatment efficacy.[119]

7 | BTO ESTABLISHMENT USING OTHER ADVANCED TECHNIQUES

Internal hypoxia and cell death due to insufficient diffusion of culture media and oxygen are prominent causes for current brain organoid culture methods generating insufficient numbers of mature neurons. However, organotypic slices can bypass diffusion limitations to prevent cell death and enhance neuronal maturation and viability.[120,121] Sliced brain organoids co-cultured with brain tumor cells/spheres have been identified as a feasible method for assessing how mature neurons interact with brain tumors. For example, network structures comparable to those of synapses between neurons and GSCs have been observed in this system.[92] Other types of cells, including microglia, astrocytes, immune cells, and molecules/drugs, can be added to the culture system of organotypic slices to enrich the microenvironment and evaluate the tumor response. The limitations of this method include its relatively short-term maintenance ability in culture, which lasts for only several weeks, and lack of scalability.[121]

Another deficiency of BTOs is the lack of stromal cells such as immune and vascular cells. Although the Jacob method can retain some of these populations, their presence varies across GBOs because of the heterogeneous occurrence of stromal cells among different regions in tumors and different patients.[24] Before Jacob's work, BTOs were embedded into an ECM, such as Matrigel. There are three methods to coculture stromal cells with organoids: (1) submerged Matrigel culture, (2) air-liquid interface (ALI) culture, and (3) microfluidic 3D culture.[122] Using the submerged Matrigel culture method, astrocytes, and microglia were co-cultured with brain tumor cells and organoids underneath the culture medium.[103,123] ALI systems enable more efficient oxygen transportation to sustain the growth of hybrids comprising multiple types of cells.[124] Using the ALI method, immune cells, and fibroblasts were successfully co-cultured with meningioma and schwannoma tumors, while oligodendroglioma and GBM failed, perhaps due to relatively smaller samples. Endogenous and syngeneic tumor-infiltrated lymphocytes were preserved in this system for 60 days.[90] Both methods required customized culture media with varying additives depending on the cell type. The microfluidic method has been applied to establish BTO as a technical part of the 3D bioprinting technique. GBOs generated following Jacob's protocol can be directly co-cultured with immune cells more conveniently and faster within 1–5 days, providing a platform to develop immunotherapies. This co-culture method can also be extended to other stromal cells and types of brain tumors.[67]

To efficiently scale up the generation of BTOs and reduce variability within and between batches, a 3D bioprinting technique was applied. This bioprinting technique involves computer-controlled additive biofabrication, with the potential to build or pattern viable organ-like structures in 3D using cells and biomaterials. By refining bioinks with key ECM components that propagate cellular viability and bioprinting BTOs in 96-well plates, BTOs can be used for high-throughput drug screening after only 7 days of culturing.[125] In 4D bioprinting, 3D bioprinting is combined with smart materials that respond to stimuli, and this has been used to form 4D organoid arrays. This technique not only allows high-throughput drug screening, but also reduces manual operation, thus simplifying the process and increasing reproducibility.[82] However, the ability of these bioprinted organoids to recapitulate cellular heterogeneity and organization comparable to that of parental tumors remains uncertain. In other words, presently, it is more appropriate to regard them as "biofabricated spheroids" until further characterization studies prove their ability to recapitulate their source tissue.[125,126]

8 | CHALLENGES AND FUTURE PROSPECTS

BTOs are an important new platform for understanding tumor development and developing precision oncology for brain tumors. Below, we detail the current limitations and future prospects of this technology:

1. Accurate recapitulation of brain cellular architecture

Brain tumors mostly occur in adults.[1] Although the current brain organoids are remarkably similar to the fetal brain, the mature components in brain organoids are insufficient, and neural functions in brain organoids differ from those in the adult brain.[127,128] Some define "mature" brain organoids as at least 6 months old—a time period in which most NPCs differentiate into neurons and astrocytes and express mature markers.[128,129] A more extended culture period (>9 months) was proposed to facilitate greater functional maturity, including the formation of dendritic spines and active neuronal networks.[130] The main reasons for immaturity in BOs include (1) diffusion limitation of culture media, (2) non-physiological ECM, (3) and missing cell types, such as microglia.[131] Organoid maturity was positively correlated with BT invasiveness, suggesting that establishing BOs that recapitulate adult brain was critical.[92] Several optimizations have been made to promote BO maturity, such as the application of human brain ECM to model real ECM,[132] air-liquid interface,[133] microfluidic devices,[132] and sliced culture to alleviate diffusion.[120] Moreover, the lack of a functional vascular system is the primary reason for diffusion limitation in BOs. Although the GBOs established by Jacob et al[24] could maintain some vascular endothelial cells, no fully formed blood vessels were present. Because of this limitation, the use of BOs for testing drugs or CAR-T cells, which depends on vascular diffusion, is limited. Recently, vasculature in BOs was shown to be created using engineering hESCs to ectopically express hETV2, leading to the acquisition of several blood–brain barrier characteristics and enhanced functional maturation of BOs.[35] Intracerebral implantation of BOs into immunodeficient mice also generated blood vessels in the BOs.[134] However, to date, these BOs

have not been used in conjunction with BTs. The lack of persistent immune cells is also a defect in BTOs. Co-culturing could be a solution, as was discussed in this review.

2. Expanding clinical relevance for BOs and BTOs

BTOs could potentially be used to guide personalized therapy in patients with BTs. However, perhaps because of ethical considerations, only two studies have correlated patient drug responses with drug testing in BTOs and responses in patients. Both these studies showed consistency, giving confidence in the wider clinical use of BTOs for patients.[24,78] Currently, 5 clinical trials on BTOs (NCT04865315, NCT03971812, NCT04868396, NCT03896958, and NCT04478877) are ongoing. BTOs remain a promising tool for precision medicine, but further clinical correlation analysis is required. Thus, more observational research should be performed to reduce ethical risks while expanding the use of this technology.

3. Standardization and automatization of BO and BTO techniques

As emerging state-of-the-art models, techniques for BOs and BTOs are constantly being optimized. No acknowledged standard protocol exists for all BOs or BTOs. Inter- and intra-batch variability are common across studies because BO and BTO generation largely depends on self-patterning and self-organization of PSCs/BTSCs without guided differentiation.[21,25,130,135] The complexity of manual processes in culture is also a major source of variability and error that hampers large-scale production. Developing standardized protocols and automatized devices will be helpful in ensuring authenticity and expanding the application of precision oncology. Furthermore, organoid factories can be used for high-throughput drug screening and target investigations. Three-dimensional bioprinting, computational automatic techniques, and microfluidic techniques can help achieve this goal.

4. Identification of pathogenic factor driving brain tumors

Although the pathogenesis of brain tumors is mainly related to genetic mutations, microenvironmental factors and their relationship with susceptibility are also important factors leading to brain tumors. Ionizing radiation (hazardous factor) and history of allergies (protective factors) are well-documented risk factors for brain tumors. Other possible risk factors have also been reported by analyzing large clinical databases that require further validation.[136] Brain organoids can be used to confirm the relationship between tumorigenesis and exposure to risk factors in future studies. For example, exposing brain organoids to hormonal contraception could help determine if associations exist between maternal hormonal contraception use and central nervous system tumors.[137] Through the genetic manipulation of brain organoids, the relationship between risk factors and genetic susceptibility may also be revealed.

9 | CONCLUSIONS

Since the BOs and BTOs emerged in 2013 and 2016, the worldwide application of organoid technology has resulted in remarkable advances in the study of precision oncology for brain tumors. In this review, we described the current literature on the establishment of several forms of BTOs and how precisely they modeled different types of brain tumors. Additionally, the promoting effects of BTOs for deeper biological understanding and personalizing therapy for brain tumors are also described. In summary, even though current BTOs are facing some challenges and required optimizations for complete cancer modeling and precision medicine, BTOs are on the way to be indispensable tools for preclinical and clinical research.

AUTHOR CONTRIBUTIONS

Jie Wen and Xisong liang drafted the manuscript and prepared the figures. Fangkun Liu, Quan Cheng, and Fan Fan collected the related references. Nathaniel Weygant revised the manuscript. Chuntao Li, Liyang Zhang, and Zhixiong Liu generated the organization and designed this review and also revised the manuscript. All authors consented the final manuscripts.

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CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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REFERENCES

1. Miller K, Ostrom QT, Kruchko C, et al. Brain and other central nervous system tumor statistics, 2021. *CA Cancer J Clin*. 2021;71:381-406. doi:10.3322/caac.21693
2. GBD 2016 Brain and Other CNS Cancer Collaborators. Global, regional, and national burden of brain and other CNS cancer, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. 2019;18:376-393. doi:10.1016/s1474-4422(18)30468-x
3. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987-996. doi:10.1056/NEJMoa043330

4. Aldape K, Brindle KM, Chesler L, et al. Challenges to curing primary brain tumours. *Nat Rev Clin Oncol*. 2019;16:509-520. doi:10.1038/s41571-019-0177-5
5. Nowogrodzki A. How cerebral organoids are guiding brain-cancer research and therapies. *Nature*. 2018;561:S48-S49. doi:10.1038/d41586-018-06708-3
6. Xu S, Tang L, Li X, Fan F, Liu Z. Immunotherapy for glioma: current management and future application. *Cancer Lett*. 2020;476:1-12. doi:10.1016/j.canlet.2020.02.002
7. Zanders E, Svensson F, Bailey D. Therapy for glioblastoma: is it working? *Drug Discov Today*. 2019;24:1193-1201. doi:10.1016/j.drudis.2019.03.008
8. Gillet J, Calcagno AM, Varma S, et al. Redefining the relevance of established cancer cell lines to the study of mechanisms of clinical anti-cancer drug resistance. *Proc Natl Acad Sci USA*. 2011;108:18708-18713. doi:10.1073/pnas.1111840108
9. Neve R, Chin K, Fridlyand J, et al. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell*. 2006;10:515-527. doi:10.1016/j.ccr.2006.10.008
10. Nunes A, Barros A, Costa E, Moreira A, Correia I. 3D tumor spheroids as in vitro models to mimic in vivo human solid tumors resistance to therapeutic drugs. *Biotechnol Bioeng*. 2019;116:206-226. doi:10.1002/bit.26845
11. Tannock I, Lee C, Tunggal J, Cowan D, Egorin M. Limited penetration of anticancer drugs through tumor tissue: a potential cause of resistance of solid tumors to chemotherapy. *Clin Cancer Res*. 2002;8:878-884.
12. Trédan O, Galmarini C, Patel K, Tannock I. Drug resistance and the solid tumor microenvironment. *J Natl Cancer Inst*. 2007;99:1441-1454. doi:10.1093/jnci/djm135
13. Joo K, Kim J, Jin J, et al. Patient-specific orthotopic glioblastoma xenograft models recapitulate the histopathology and biology of human glioblastomas in situ. *Cell Rep*. 2013;3:260-273. doi:10.1016/j.celrep.2012.12.013
14. Hermans E, Hulleman E. Patient-derived orthotopic xenograft models of pediatric brain tumors: in a mature phase or still in its infancy? *Front Oncol*. 2019;9:1418. doi:10.3389/fonc.2019.01418
15. Oberheim N, Takano T, Han X, et al. Uniquely hominid features of adult human astrocytes. *J Neurosci*. 2009;29:3276-3287. doi:10.1523/jneurosci.4707-08.2009
16. Hovinga K, Shimizu F, Wang R, et al. Inhibition of notch signaling in glioblastoma targets cancer stem cells via an endothelial cell intermediate. *Stem Cells*. 2010;28:1019-1029. doi:10.1002/stem.429
17. Merz F, Gaunitz F, Dehghani F, et al. Organotypic slice cultures of human glioblastoma reveal different susceptibilities to treatments. *Neuro Oncol*. 2013;15:670-681. doi:10.1093/neuonc/not003
18. Parker J, Lizarraga M, Waziri A, Foshay K. A human glioblastoma organotypic slice culture model for study of tumor cell migration and patient-specific effects of anti-invasive drugs. *J Vis Exp*. 2017;125:53557.
19. Soubéran A, Tchoghandjian A. Practical review on preclinical human 3D glioblastoma models: advances and challenges for clinical translation. *Cancer*. 2020;12:2347. doi:10.3390/cancers12092347
20. Hubert C, Rivera M, Spangler LC, et al. A three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found in vivo. *Cancer Res*. 2016;76:2465-2477. doi:10.1158/0008-5472.Can-15-2402
21. Lancaster M, Renner M, Martin CA, et al. Cerebral organoids model human brain development and microcephaly. *Nature*. 2013;501:373-379. doi:10.1038/nature12517
22. Sasai Y. Next-generation regenerative medicine: organogenesis from stem cells in 3D culture. *Cell Stem Cell*. 2013;12:520-530. doi:10.1016/j.stem.2013.04.009
23. Xu R, Zhou X, Wang S, Trinkle C. Tumor organoid models in precision medicine and investigating cancer-stromal interactions. *Pharmacol Ther*. 2021;218:107668. doi:10.1016/j.pharmthera.2020.107668
24. Jacob F, Salinas RD, Zhang DY, et al. A patient-derived glioblastoma organoid model and biobank recapitulates inter- and intra-tumoral heterogeneity. *Cell*. 2020;180:188-204.e122. doi:10.1016/j.cell.2019.11.036
25. Lancaster M, Knoblich J. Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc*. 2014;9:2329-2340. doi:10.1038/nprot.2014.158
26. Lancaster M, Knoblich J. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science*. 2014;345:1247125. doi:10.1126/science.1247125
27. Pellegrini L, Bonfio C, Chadwick J, Begum F, Skehel M, Lancaster MA. Human CNS barrier-forming organoids with cerebrospinal fluid production. *Science*. 2020;369:eaaz5626. doi:10.1126/science.aaz5626
28. Muguruma K, Nishiyama A, Kawakami H, Hashimoto K, Sasai Y. Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. *Cell Rep*. 2015;10:537-550. doi:10.1016/j.celrep.2014.12.051
29. Cederquist G, Asciolla JJ, Tchieu J, et al. Specification of positional identity in forebrain organoids. *Nat Biotechnol*. 2019;37:436-444. doi:10.1038/s41587-019-0085-3
30. Zagare A, Barmpa K, Smajic S, et al. Midbrain organoids mimic early embryonic neurodevelopment and recapitulate LRRK2-p.Gly2019Ser-associated gene expression. *Am J Hum Genet*. 2022;109:311-327. doi:10.1016/j.ajhg.2021.12.009
31. Qian X, Jacob F, Song MM, Nguyen HN, Song H, Ming GL. Generation of human brain region-specific organoids using a miniaturized spinning bioreactor. *Nat Protoc*. 2018;13:565-580. doi:10.1038/nprot.2017.152
32. Valiulahi P, Vidyawan V, Puspita L, et al. Generation of caudal-type serotonin neurons and hindbrain-fate organoids from hPSCs. *Stem Cell Rep*. 2021;16:1938-1952. doi:10.1016/j.stemcr.2021.06.006
33. Kasai T, Suga H, Sakakibara M, et al. Hypothalamic contribution to pituitary functions is recapitulated in vitro using 3D-cultured human iPS cells. *Cell Rep*. 2020;30:18-24.e15. doi:10.1016/j.celrep.2019.12.009
34. Madhavan M, Nevin ZS, Shick HE, et al. Induction of myelinating oligodendrocytes in human cortical spheroids. *Nat Methods*. 2018;15:700-706. doi:10.1038/s41592-018-0081-4
35. Cakir B, Xiang Y, Tanaka Y, et al. Engineering of human brain organoids with a functional vascular-like system. *Nat Methods*. 2019;16:1169-1175. doi:10.1038/s41592-019-0586-5
36. Cakir B, Tanaka Y, Kiral FR, et al. Expression of the transcription factor PU.1 induces the generation of microglia-like cells in human cortical organoids. *Nat Commun*. 2022;13:430. doi:10.1038/s41467-022-28043-y
37. Henske E, Jóźwiak S, Kingswood J, Sampson J, Thiele E. Tuberous sclerosis complex. *Nat Rev Dis Primers*. 2016;2:16035. doi:10.1038/nrdp.2016.35
38. Fisher M, Belzberg AJ, de Blank P, et al. 2016 Children's Tumor Foundation conference on neurofibromatosis type 1, neurofibromatosis type 2, and schwannomatosis. *Am J Med Genet A*. 2018;176:1258-1269. doi:10.1002/ajmg.a.38675
39. Eichmüller O, Corsini NS, Vértesy Á, et al. Amplification of human interneuron progenitors promotes brain tumors and neurological defects. *Science*. 2022;375:eabf5546. doi:10.1126/science.abf5546
40. Anastasaki C, Wegscheid ML, Hartigan K, et al. Human iPSC-derived neurons and cerebral organoids establish differential effects of germline NF1 gene mutations. *Stem Cell Rep*. 2020;14:541-550. doi:10.1016/j.stemcr.2020.03.007
41. Marino S, Vooijs M, van Der Gulden H, Jonkers J, Berns A. Induction of medulloblastomas in p53-null mutant mice by somatic

inactivation of Rb in the external granular layer cells of the cerebellum. *Genes Dev*. 2000;14:994-1004.
42. Zheng H, Ying H, Yan H, et al. p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. *Nature*. 2008;455:1129-1133. doi:10.1038/nature07443
43. Momota H, Shih A, Edgar M, Holland E. c-Myc and beta-catenin cooperate with loss of p53 to generate multiple members of the primitive neuroectodermal tumor family in mice. *Oncogene*. 2008;27:4392-4401. doi:10.1038/onc.2008.81
44. Bian S, Repic M, Guo Z, et al. Genetically engineered cerebral organoids model brain tumor formation. *Nat Methods*. 2018;15:631-639. doi:10.1038/s41592-018-0070-7
45. Ogawa J, Pao G, Shokhirev M, Verma I. Glioblastoma model using human cerebral organoids. *Cell Rep*. 2018;23:1220-1229. doi:10.1016/j.celrep.2018.03.105
46. Ballabio C, Anderle M, Gianesello M, et al. Modeling medulloblastoma in vivo and with human cerebellar organoids. *Nat Commun*. 2020;11:583. doi:10.1038/s41467-019-13989-3
47. Taylor M, Poppleton H, Fuller C, et al. Radial glia cells are candidate stem cells of ependymoma. *Cancer Cell*. 2005;8:323-335. doi:10.1016/j.ccr.2005.09.001
48. Lathia J, Mack S, Mulkearns-Hubert E, Valentim C, Rich J. Cancer stem cells in glioblastoma. *Genes Dev*. 2015;29:1203-1217. doi:10.1101/gad.261982.115
49. Goodrich L, Milenković L, Higgins K, Scott M. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science*. 1997;277:1109-1113. doi:10.1126/science.277.5329.1109
50. Hakes A, Brand A. Neural stem cell dynamics: the development of brain tumours. *Curr Opin Cell Biol*. 2019;60:131-138. doi:10.1016/j.ceb.2019.06.001
51. Sanai N, Alvarez-Buylla A, Berger M. Neural stem cells and the origin of gliomas. *N Engl J Med*. 2005;353:811-822. doi:10.1056/NEJMra043666
52. Pei Y, Brun SN, Markant SL, et al. WNT signaling increases proliferation and impairs differentiation of stem cells in the developing cerebellum. *Development*. 2012;139:1724-1733. doi:10.1242/dev.050104
53. Northcott P, Jones DT, Kool M, et al. Medulloblastomics: the end of the beginning. *Nat Rev Cancer*. 2012;12:818-834. doi:10.1038/nrc3410
54. Gonçalves C, Le Boiteux E, Arnaud P, Costa B. HOX gene cluster (de)regulation in brain: from neurodevelopment to malignant glial tumours. *Cell Mol Life Sci*. 2020;77:3797-3821. doi:10.1007/s00018-020-03508-9
55. Curry R, Glasgow S. The role of neurodevelopmental pathways in brain tumors. *Front Cell Dev Biol*. 2021;9:659055. doi:10.3389/fcell.2021.659055
56. Parisian A, Koga T, Miki S, et al. SMARCB1 loss interacts with neuronal differentiation state to block maturation and impact cell stability. *Genes Dev*. 2020;34:1316-1329. doi:10.1101/gad.339978.120
57. Xie X, Laks DR, Sun D, et al. Quiescent human glioblastoma cancer stem cells drive tumor initiation, expansion, and recurrence following chemotherapy. *Dev Cell*. 2022;57:32-46.e38. doi:10.1016/j.devcel.2021.12.007
58. VandeKopple M, Wu J, Auer EN, Giaccia AJ, Denko NC, Papandreou I. HILPDA regulates lipid metabolism, lipid droplet abundance, and response to microenvironmental stress in solid tumors. *Mol Cancer Res*. 2019;17:2089-2101. doi:10.1158/1541-7786.Mcr-18-1343
59. Shakya S, Gromovsky AD, Hale JS, et al. Altered lipid metabolism marks glioblastoma stem and non-stem cells in separate tumor niches. *Acta Neuropathol Commun*. 2021;9:101. doi:10.1186/s40478-021-01205-7
60. Tejero R, Huang Y, Katsyv I, et al. Gene signatures of quiescent glioblastoma cells reveal mesenchymal shift and interactions with niche microenvironment. *EBioMedicine*. 2019;42:252-269. doi:10.1016/j.ebiom.2019.03.064
61. Sundar S, Shakya S, Barnett A, et al. Three-dimensional organoid culture unveils resistance to clinical therapies in adult and pediatric glioblastoma. *Transl Oncol*. 2021;15:101251. doi:10.1016/j.tranon.2021.101251
62. Pinto G, Brou C, Zurzolo C. Tunneling nanotubes: the fuel of tumor progression? *Trends Cancer*. 2020;6:874-888. doi:10.1016/j.trecan.2020.04.012
63. Hekmatshoar Y, Nakhle J, Galloni M, Vignais M. The role of metabolism and tunneling nanotube-mediated intercellular mitochondria exchange in cancer drug resistance. *Biochem J*. 2018;475:2305-2328. doi:10.1042/bcj20170712
64. Vignais M, Caicedo A, Brondello J, Jorgensen C. Cell connections by tunneling nanotubes: effects of mitochondrial trafficking on target cell metabolism, homeostasis, and response to therapy. *Stem Cells Int*. 2017;2017:6917941. doi:10.1155/2017/6917941
65. Pinto G, Saenz-de-Santa-Maria I, Chastagner P, et al. Patient-derived glioblastoma stem cells transfer mitochondria through tunneling nanotubes in tumor organoids. *Biochem J*. 2021;478:21-39. doi:10.1042/bcj20200710
66. Kozlowski MT, Crook CJ, Ku HT. Towards organoid culture without Matrigel. *Commun Biol*. 2021;4:1387. doi:10.1038/s42003-021-02910-8
67. Jacob F, Ming G, Song H. Generation and biobanking of patient-derived glioblastoma organoids and their application in CAR T cell testing. *Nat Protoc*. 2020;15:4000-4033. doi:10.1038/s41596-020-0402-9
68. Wang L, Karpova A, Gritsenko MA, et al. Proteogenomic and metabolomic characterization of human glioblastoma. *Cancer Cell*. 2021;39:509-528.e520. doi:10.1016/j.ccell.2021.01.006
69. Archer T, Ehrenberger T, Mundt F, et al. Proteomics, post-translational modifications, and integrative analyses reveal molecular heterogeneity within medulloblastoma subgroups. *Cancer Cell*. 2018;34:396-410.e398. doi:10.1016/j.ccell.2018.08.004
70. Northcott P, Pfister S, Jones D. Next-generation (epi)genetic drivers of childhood brain tumours and the outlook for targeted therapies. *Lancet Oncol*. 2015;16:e293-e302. doi:10.1016/s1470-2045(14)71206-9
71. Patel A, Tirosh I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*. 2014;344:1396-1401. doi:10.1126/science.1254257
72. Reinartz R, Wang S, Kebir S, et al. Functional subclone profiling for prediction of treatment-induced intratumor population shifts and discovery of rational drug combinations in human glioblastoma. *Clin Cancer Res*. 2017;23:562-574. doi:10.1158/1078-0432.Ccr-15-2089
73. Kelly R. Single-cell proteomics: progress and prospects. *Mol Cellular Proteomics*. 2020;19:1739-1748. doi:10.1074/mcp.R120.002234
74. Lisanti M, Tanowitz H. Translational discoveries, personalized medicine, and living biobanks of the future. *Am J Pathol*. 2012;180:1334-1336. doi:10.1016/j.ajpath.2012.02.003
75. Larsen B, Kannan M, Langer LF, et al. A pan-cancer organoid platform for precision medicine. *Cell Rep*. 2021;36:109429. doi:10.1016/j.celrep.2021.109429
76. Li S, Wang M, Zhou J. Brain organoids: a promising living biobank resource for neuroscience research. *Biopreserv Biobank*. 2020;18:136-143. doi:10.1089/bio.2019.0111
77. Wensink G, Elias SG, Mullenders J, et al. Patient-derived organoids as a predictive biomarker for treatment response in cancer patients. *NPJ Precis Oncol*. 2021;5:30. doi:10.1038/s41698-021-00168-1
78. Loong H, Wong AM, Chan DT, et al. Patient-derived tumor organoid predicts drugs response in glioblastoma: a step forward in personalized cancer therapy? *J Clin Neurosci*. 2020;78:400-402. doi:10.1016/j.jocn.2020.04.107

79. Zhang L, Liu F, Weygant N, et al. A novel integrated system using patient-derived glioma cerebral organoids and xenografts for disease modeling and drug screening. *Cancer Lett*. 2021;500:87-97. doi:10.1016/j.canlet.2020.12.013
80. Benitez J, Finlay D, Castanza A, et al. PTEN deficiency leads to proteasome addiction: a novel vulnerability in glioblastoma. *Neuro Oncol*. 2021;23:1072-1086. doi:10.1093/neuonc/noab001
81. Sicklick J, Kato S, Okamura R, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. *Nat Med*. 2019;25:744-750. doi:10.1038/s41591-019-0407-5
82. Chadwick M, Yang C, Liu L, et al. Rapid processing and drug evaluation in glioblastoma patient-derived organoid models with 4D bioprinted arrays. *iScience*. 2020;23:101365. doi:10.1016/j.isci.2020.101365
83. Lyon J, Mokarram N, Saxena T, Carroll S, Bellamkonda R. Engineering challenges for brain tumor immunotherapy. *Adv Drug Deliv Rev*. 2017;114:19-32. doi:10.1016/j.addr.2017.06.006
84. Zhao R, Li B, Zhang S, et al. The N-methyladenosine-modified pseudogene HSPA7 correlates with the tumor microenvironment and predicts the response to immune checkpoint therapy in glioblastoma. *Front Immunol*. 2021;12:653711. doi:10.3389/fimmu.2021.653711
85. Nickl V, Schulz E, Salvador E, et al. Glioblastoma-derived three-dimensional ex vivo models to evaluate effects and efficacy of tumor treating fields (TTFields). *Cancer*. 2022;14:5177. doi:10.3390/cancers14215177
86. Abdullah K, Buehler J, Bird C, et al. Establishment of patient-derived organoid models of lower grade glioma. *Neuro Oncol*. 2021;23:vi216-vi217. doi:10.1093/neuonc/noab273
87. Chan H, Ng HK, Chan AK, et al. Establishment and characterization of meningioma patient-derived organoid. *J Clin Neurosci*. 2021;94:192-199. doi:10.1016/j.jocn.2021.10.035
88. Yamazaki S, Ohka F, Hirano M, et al. Newly established patient-derived organoid model of intracranial meningioma. *Neuro Oncol*. 2021;23:1936-1948. doi:10.1093/neuonc/noab155
89. Frisira E, Rashid F, Varma SN, et al. NPI-0052 and γ-radiation induce a synergistic apoptotic effect in medulloblastoma. *Cell Death Dis*. 2019;10:785. doi:10.1038/s41419-019-2026-y
90. Neal J, Li X, Zhu J, et al. Organoid modeling of the tumor immune microenvironment. *Cell*. 2018;175:1972-1988.e1916. doi:10.1016/j.cell.2018.11.021
91. Pine A, Cirigliano SM, Nicholson JG, et al. Tumor microenvironment is critical for the maintenance of cellular states found in primary glioblastomas. *Cancer Discov*. 2020;10:964-979. doi:10.1158/2159-8290.Cd-20-0057
92. D'Alessandris QG, Pallini R, Gopalakrishnan J. Rapid and efficient invasion assay of glioblastoma in human brain organoids. *Cell Rep*. 2020;31(10):107738. doi:10.1016/j.celrep.2020.107738
93. Linkous A, Balamatsias D, Snuderl M, et al. Modeling patient-derived glioblastoma with cerebral organoids. *Cell Rep*. 2019;26:3203-3211.e3205. doi:10.1016/j.celrep.2019.02.063
94. Pasqualini C, Kozaki T, Bruschi M, et al. Modeling the interaction between the microenvironment and tumor cells in brain tumors. *Neuron*. 2020;108:1025-1044. doi:10.1016/j.neuron.2020.09.018
95. Krieger T, Tirier SM, Park J, et al. Modeling glioblastoma invasion using human brain organoids and single-cell transcriptomics. *Neuro Oncol*. 2020;22:1138-1149. doi:10.1093/neuonc/noaa091
96. Broekman M, Maas SL, Abels ER, Mempel TR, Krichevsky AM, Breakefield XO. Multidimensional communication in the microenvirons of glioblastoma. *Nat Rev Neurol*. 2018;14:482-495. doi:10.1038/s41582-018-0025-8
97. Venkataramani V, Tanev DI, Strahle C, et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. *Nature*. 2019;573:532-538. doi:10.1038/s41586-019-1564-x
98. Venkatesh H, Morishita W, Geraghty AC, et al. Electrical and synaptic integration of glioma into neural circuits. *Nature*. 2019;573:539-545. doi:10.1038/s41586-019-1563-y
99. Zeng Q, Michael IP, Zhang P, et al. Synaptic proximity enables NMDAR signalling to promote brain metastasis. *Nature*. 2019;573:526-531. doi:10.1038/s41586-019-1576-6
100. Osswald M, Jung E, Sahm F, et al. Brain tumour cells interconnect to a functional and resistant network. *Nature*. 2015;528:93-98. doi:10.1038/nature16071
101. Chen Q, Boire A, Jin X, et al. Carcinoma-astrocyte gap junctions promote brain metastasis by cGAMP transfer. *Nature*. 2016;533:493-498. doi:10.1038/nature18268
102. Choe M, Kim JS, Yeo HC, et al. A simple metastatic brain cancer model using human embryonic stem cell-derived cerebral organoids. *FASEB J*. 2020;34:16464-16475. doi:10.1096/fj.202000372R
103. Magill S, Vasudevan HN, Seo K, et al. Multiplatform genomic profiling and magnetic resonance imaging identify mechanisms underlying intratumor heterogeneity in meningioma. *Nat Commun*. 2020;11:4803. doi:10.1038/s41467-020-18582-7
104. Liu S, Malatesta M, Lien BV, et al. CRISPRi-based radiation modifier screen identifies long non-coding RNA therapeutic targets in glioma. *Genome Biol*. 2020;21:83. doi:10.1186/s13059-020-01995-4
105. Lawler S, Speranza M, Cho C, Chiocca E. Oncolytic viruses in cancer treatment: a review. *JAMA Oncol*. 2017;3:841-849. doi:10.1001/jamaoncol.2016.2064
106. Melo AO, Malinger G, Ximenes R, Szejnfeld PO, Sampaio SA, De Filippis AB. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? *Ultrasound Obstet Gynecol*. 2016;47:6-7. doi:10.1002/uog.15831
107. Ferreira R, Granha I, Ferreira RS, et al. Effect of serial systemic and intratumoral injections of oncolytic ZIKV in mice bearing embryonal CNS tumors. *Viruses*. 2021;13:2103. doi:10.3390/v13102103
108. Zhu Z, Mesci P, Bernatchez JA, et al. Zika virus targets glioblastoma stem cells through a SOX2-integrin αβ axis. *Cell Stem Cell*. 2020;26:187-204.e110. doi:10.1016/j.stem.2019.11.016
109. Zhu Z, Gorman MJ, McKenzie LD, et al. Zika virus has oncolytic activity against glioblastoma stem cells. *J Exp Med*. 2017;214:2843-2857. doi:10.1084/jem.20171093
110. Sarkar A, Hochedlinger K. The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. *Cell Stem Cell*. 2013;12:15-30. doi:10.1016/j.stem.2012.12.007
111. Mansouri S, Nejad R, Karabork M, et al. Sox2: regulation of expression and contribution to brain tumors. *CNS Oncol*. 2016;5:159-173. doi:10.2217/cns-2016-0001
112. Magge R, Barbaro M, Fine H. Innovations in neuro-oncology. *World Neurosurg*. 2021;151:386-391. doi:10.1016/j.wneu.2021.02.093
113. Brown P, Jaeckle K, Ballman KV, et al. Effect of radiosurgery alone vs radiosurgery with whole brain radiation therapy on cognitive function in patients with 1 to 3 brain metastases: a randomized clinical trial. *JAMA*. 2016;316:401-409. doi:10.1001/jama.2016.9839
114. Ye E, Lee J, Lim Y, Yang S, Park S. Effect of duty cycles of tumor-treating fields on glioblastoma cells and normal brain organoids. *Int J Oncol*. 2022;60:8. doi:10.3892/ijo.2021.5298
115. Jermakowicz A, Rybin MJ, Suter RK, et al. The novel BET inhibitor UM-002 reduces glioblastoma cell proliferation and invasion. *Sci Rep*. 2021;11:23370. doi:10.1038/s41598-021-02584-6
116. Bojcevski J, Wang C, Liu H, Abdollahi A, Dokic I. Assessment of normal tissue radiosensitivity by evaluating DNA damage and repair kinetics in human brain organoids. *Int J Mol Sci*. 2021;22:13195. doi:10.3390/ijms222413195
117. Das D, Li J, Cheng L, Franco S, Mahairaki V. Human forebrain organoids from induced pluripotent stem cells: a novel approach to model repair of ionizing radiation-induced DNA damage in human neurons. *Radiat Res*. 2020;194:191-198. doi:10.1667/rr15567.1

118. Vinel C, Rosser G, Guglielmi L, et al. Comparative epigenetic analysis of tumour initiating cells and syngeneic EPSC-derived neural stem cells in glioblastoma. *Nat Commun*. 2021;12:6130. doi:10.1038/s41467-021-26297-6
119. Skardal A, Aleman J, Forsythe S, et al. Drug compound screening in single and integrated multi-organoid body-on-a-chip systems. *Biofabrication*. 2020;12:025017. doi:10.1088/1758-5090/ab6d36
120. Qian X, Su Y, Adam CD, et al. Sliced human cortical organoids for modeling distinct cortical layer formation. *Cell Stem Cell*. 2020;26:766-781.e769. doi:10.1016/j.stem.2020.02.002
121. Daviaud N, Garbayo E, Schiller P, Perez-Pinzon M, Montero-Menei C. Organotypic cultures as tools for optimizing central nervous system cell therapies. *Exp Neurol*. 2013;248:429-440. doi:10.1016/j.expneurol.2013.07.012
122. Yuki K, Cheng N, Nakano M, Kuo C. Organoid models of tumor immunology. *Trends Immunol*. 2020;41:652-664. doi:10.1016/j.it.2020.06.010
123. Zhang I, Lépine P, Han C, et al. Nanotherapeutic modulation of human neural cells and glioblastoma in organoids and monocultures. *Cell*. 2020;9:2434. doi:10.3390/cells9112434
124. Li X, Ootani A, Kuo C. An air-liquid interface culture system for 3D organoid culture of diverse primary gastrointestinal tissues. *Methods Mol Biol*. 2016;1422:33-40. doi:10.1007/978-1-4939-3603-8_4
125. Maloney E, Clark C, Sivakumar H, et al. Immersion bioprinting of tumor organoids in multi-well plates for increasing chemotherapy screening throughput. *Micromachines*. 2020;11:208. doi:10.3390/mi11020208
126. Yi H, Jeong YH, Kim Y, et al. A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. *Nat Biomed Eng*. 2019;3:509-519. doi:10.1038/s41551-019-0363-x
127. Trujillo C, Muotri A. Brain organoids and the study of neurodevelopment. *Trends Mol Med*. 2018;24:982-990. doi:10.1016/j.molmed.2018.09.005
128. Thomas C, Tejwani L, Trujillo CA, et al. Modeling of TREX1-dependent autoimmune disease using human stem cells highlights L1 accumulation as a source of neuroinflammation. *Cell Stem Cell*. 2017;21:319-331.e318. doi:10.1016/j.stem.2017.07.009
129. Trujillo C, Gao R, Negraes PD, et al. Complex oscillatory waves emerging from cortical organoids model early human brain network development. *Cell Stem Cell*. 2019;25:558-569.e557. doi:10.1016/j.stem.2019.08.002
130. Quadrato G, Nguyen T, Macosko EZ, et al. Cell diversity and network dynamics in photosensitive human brain organoids. *Nature*. 2017;545:48-53. doi:10.1038/nature22047
131. Jang H, Kim S, Koh Y, Yoon K. Engineering brain organoids: toward mature neural circuitry with an intact cytoarchitecture. *Int J Stem Cells*. 2022;15:41-59. doi:10.15283/ijsc22004
132. Cho A, Jin Y, An Y, et al. Microfluidic device with brain extracellular matrix promotes structural and functional maturation of human brain organoids. *Nat Commun*. 2021;12:4730. doi:10.1038/s41467-021-24775-5
133. Giandomenico S, Mierau SB, Gibbons GM, et al. Cerebral organoids at the air-liquid interface generate diverse nerve tracts with functional output. *Nat Neurosci*. 2019;22:669-679. doi:10.1038/s41593-019-0350-2
134. Mansour A, Gonçalves JT, Bloyd CW, et al. An in vivo model of functional and vascularized human brain organoids. *Nat Biotechnol*. 2018;36:432-441. doi:10.1038/nbt.4127
135. Yakoub A, Sadek M. Development and characterization of human cerebral organoids: an optimized protocol. *Cell Transplant*. 2018;27:393-406. doi:10.1177/0963689717752946
136. Ostrom Q, Adel Fahmideh M, Cote DJ, et al. Risk factors for childhood and adult primary brain tumors. *Neuro Oncol*. 2019;21:1357-1375. doi:10.1093/neuonc/noz123
137. Hargreave M, Mørch L, Winther J, Schmiegelow K, Kjaer S. Association between maternal hormonal contraception use and central nervous system tumors in children. *JAMA*. 2022;327:59-66. doi:10.1001/jama.2021.22482
138. Kim H, Lee S, Lim J, Yoo J, Hwang D. The epidermal growth factor receptor variant type III mutation frequently found in gliomas induces astrogenesis in human cerebral organoids. *Cell Prolif*. 2020;54(2):e12965. doi:10.1111/cpr.12965
139. Schönrock A, Heinzelmann E, Steffl B, et al. MEOX2 homeobox gene promotes growth of malignant gliomas. *Neuro Oncol*. 2022;24(11):1911-1924. doi:10.1093/neuonc/noac110
140. Lenin S, Ponthier E, Scheer KG, et al. A drug screening pipeline using 2D and 3D patient-derived in vitro models for pre-clinical analysis of therapy response in glioblastoma. *Int J Mol Sci*. 2021;22(9):4322. doi:10.3390/ijms22094322
141. Darrigues E, Zhao EH, De Loose A, et al. Biobanked glioblastoma patient-derived organoids as a precision medicine model to study inhibition of invasion. *Int J Mol Sci*. 2021;22(19):10720. doi:10.3390/ijms221910720
142. Golebiewska A, Hau A-C, Oudin A, et al. Patient-derived organoids and orthotopic xenografts of primary and recurrent gliomas represent relevant patient avatars for precision oncology. *Acta Neuropathologica*. 2020;140(6):919–949. https://doi.org/10.1007/s00401-020-02226-7.

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