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**Central nervous system tumors and three-dimensional cell biology: Current and future perspectives in modeling**

Abou-Mrad Z *et al*. CNS tumors and 3D cell biology

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**Abstract**

Central nervous system (CNS) tumors are a variety of distinct neoplasms that present multiple challenges in terms of treatment and prognosis. Glioblastoma, the most common primary tumor in adults, is associated with poor survival and remains one of the least treatable neoplasms. These tumors are highly heterogenous and complex in their nature. Due to this complexity, traditional cell culturing techniques and methods do not provide an ideal recapitulating model for the study of these tumors’ behavior *in vivo*. Two-dimensional models lack the spatial arrangement, the heterogeneity in cell types, and the microenvironment that play a large role in tumor cell behavior and response to treatment. Recently, scientists have turned towards three-dimensional culturing methods, namely spheroids and organoids, as they have been shown to recapitulate tumors in a more faithful manner to their *in vivo* counterparts. Moreover, tumor-on-a-chip systems have lately been employed in CNS tumor modeling and have shown great potential in both studying the pathophysiology and therapeutic testing. In this review, we will discuss the current available literature on *in vitro* three-dimensional culturing models in CNS tumors, in addition to presenting their advantages and current limitations. We will also elaborate on the future implications of these models and their benefit in the clinical setting.

**Key Words:** Central nervous system tumors; Glioblastoma; Three-dimensional modelling; Spheroids; Organoids

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**Core Tip:** Central nervous system tumors present multiple challenges in treatment and patient prognosis. Glioblastoma, the most common adult brain tumor, remains one of the most lethal malignant brain tumors even at the current standard of care. Traditional *in vitro* culturing is limited in recapitulating the features of the *in vivo* tumors*.* We herein discuss recent advancements in three-dimensional culturing for tumor modeling, their advantages, and limitations, in addition to future perspectives.

**INTRODUCTION**

Central nervous system (CNS) tumors are distinct neoplastic entities that can either arise from different cells within the CNS (primary neoplasms) or metastasize to the brain from other body organs (secondary neoplasms). In the young population, primary brain tumors are more common than metastatic tumors, whereas metastasis accounts for more than half of those above 40 years of age[1]. The incidence rate of primary CNS tumors in adults in the United States is approximately 30 per 100000 persons[1]. Brain tumors are the most common and the most lethal tumor type among pediatric solid tumors[2]. Of all primary brain tumors, meningiomas and glial tumors (glioblastoma, astrocytoma, and oligodendroglioma among others) account for nearly two-thirds of adult intracranial tumors[3]. Glioblastoma is the most common malignant primary brain tumor in adults with a median age of 64 years[4,5]. Anaplastic astrocytoma and glioblastoma specifically have a 5-year survival rates of 30% and 5.6%, respectively[1].

Despite the advancement in surgical treatment, radiotherapy, and chemotherapy, many high-grade tumors recur and exhibit treatment failure causing high mortality among patients[6,7]. Recent development in cancer research has been revolving around the problem of therapy resistance and the emergence of recurrence in certain tumor types. Accordingly, the idea of cancer stem cells (CSCs) comes into play. Those cells are believed to arise from progenitors that reside within the tumor bulk and are responsible for tumor resistance and recurrence, adding to the complexity of cancer management[8,9]. Those subpopulations of cells are the main reason behind resistance to chemotherapy and radiotherapy. They have the ability to replenish the tumor after tumor resection and standard subsequent therapies[10]. Besides, the presence of CSCs per se might be a main reason behind tumor heterogeneity in different cancers, particularly CNS tumors[11]. Tumor heterogeneity refers to the presence of multiple subpopulations of cells with different genotypic and phenotypic profiles within the same tumor[12]. For example, in glioblastoma, CSCs have the capacity to differentiate into any of the cells that reconstitute the entire cell population that is found within the tumor, such as neurons, oligodendrocytes, and astrocytes[13].

Due to the complexity of CNS tumors, it is crucial to develop models that can recapitulate cancer development and progression and emulate better this disease process *in vitro.* While two-dimensional (2D) cell cultures have been the mainstay in cell cultures techniques for the past couple of decades, it remains challenging to use those models to decipher the *in vivo* behavior of CNS tumors[14]. This is particularly true since 2D cultures hinder the ability to study cell-cell interactions between different subpopulations of cells, as well as cell-extracellular matrix (ECM) interactions and other spatial relationships (Table 1). Moreover, testing therapeutics on models that do not recapitulate *in vivo* tumor behavior may not present the ideal reliable solution that can be applied to clinical settings. Implementing three-dimensional (3D) cell culturing techniques provides compelling evidence to perform more advanced experiments that can yield valuable insights to study human diseases[19,20]. In this review, we aim at recounting the current available literature on 3D culturing models in CNS tumors that allows us to surpass limitations of disease modeling. We also discuss the advantages and limitations of different 3D modeling techniques, particularly spheroid and organoid culturing, and elaborate on the future implication of those models in the clinical setting.

**3D SPHEROID MODELS**

Despite the advancement in medicine and development of new therapies for brain tumors, resistance to therapy and recurrence still represent main challenges. Heterogeneity and tumor initiating cells, known as CSCs, are considered to be the main reason behind cancer initiation, progression, metastasis, resistance, and recurrence[21]. Molecular interactions within the CSC niche are highly correlated with the treatment escape strategy. Immune reactions between CSCs and cytokines, such as interleukin-6, interleukin-4, and transforming growth factor-β along with epithelial-to-mesenchymal transition[22] and other processes implicated in cancer progression represent key players in tumor resistance to conventional treatments[23,24]. Similarly, CSCs rely on several dysregulated pathways to maintain stemness and differentiation into diverse cancerous cells. Such path ways include Wnt, Notch, Hedgehog, phosphatidylinositol 3 kinase/Akt, nuclear factor kappa B, and janus kinase/signal transducer and activator of transcription pathways among others, and are believed to instigate a major effect on tumor initiation and resistance[25]. Therefore, targeting this subpopulation of cells may reduce its stemness and hence the growth, migration, and resistance of the tumor, which will enable a better control over the outcome post-treatment[21]. In gliomas, the interaction of glioma stem cells with their surrounding environment, including the ECM, the disorganization of the brain vasculature, and the hypoxic and acidic milieu in necrotic areas, all combine to maintain the growth and self-renewal of glioma stem cells[26]. Interestingly, a subpopulation of stem/progenitor-like cells in human gliomas was identified as tumor-initiating cells expressing *A2B5*, a glial progenitor marker, and *CD133*, a stem cell marker[27].

To explore further the role of CSCs and eventually develop novel therapies targeting this subpopulation of cells in brain tumors, several methods of isolating them using specific biomarkers (Table 2) have been designed. Also, the need to mimic the ECM along with other factors found within the tumor niche became a necessity. More importantly, 2D assays failed to serve as screening tools for studying brain tumor growth and progression[34], particularly with the lack in the ability to assess cellular interactions using those models[35]. Therefore, 3D culture systems, including hanging drops, spheroids, and organoids among others (Figure 1), were developed to suit better research purposes in studying patient-derived samples[36] (Table 3).

The increasing demand for spheroid models may be attributed to the characteristics offered by these models, such as the relatively decreased cost when compared to animal models, in addition to the resolving of ethical issues attributed to animal use[18]. Heterogeneity of spheroid models, oxygen gradient, and cellular interactions are few common characteristics found between spheroid models and normal *in vivo* conditions[18]. For such reasons, culturing cells shifted from the conventional 2D systems to the upgraded 3D models as a promising tool to simulate the growth and interaction of tissues, whether cancerous or not, under natural conditions. As such, spheroid models became widely known for their role in research as tools for anti-cancer drug screening and discovery[18,42,43].

Several cancers are hard to culture using conventional methods due to their infiltrative and heterogenic characteristics. Therefore, recreating tumors by growing tumor spheroids from either cell lines or primary cells aims to solidify the efforts made in understanding cancer resistance[44], in addition to exploring molecular pathways contributing to tumor invasiveness, recurrence, and metastasis. Interestingly, the response of both cell lines and patient-derived tumor cells differs between 2D and 3D culture systems. Gomez-Roman *et al*[45] showed that three patient-derived glioblastoma cell lines had a similar radiosensitive result in 2D and 3D cultures. However, the effect of several drugs on the same cell lines differ on clonogenic survival in 2D *vs* 3D systems. In another study, when pediatric CNS tumor cells were cultured in neural stem cell media, generated cells expressing stem cell markers were able to undergo differentiation and when injected in immunocompromised mice and zebrafish, were able to initiate a tumor[46]. These findings suggest that 3D models represent an ideal *in vitro* culturing method to understand tumorigenic aspects of brain tumors, in addition to targeting CSCs.

Using 3D cultures has led to the discovery of several molecular pathways as novel therapeutic targets in brain tumors[19,20,47]. As such, Narayan *et al*[48]used 3D spheroid model to show that the inhibition of AKT using MK2206 reduced spheroid growth and sensitized spheroids as well to both radiation and chemotherapy (Temozolomide; TMZ), while the synergistic effect was not observed in 2D. Our group also evaluated the potential anti-tumor effect of Tideglusib, an irreversible glycogen synthase kinase-3β inhibitor drug, on human neuroblastoma cells showing that this drug significantly hindered the neurospheres formation eradicating the self-renewal ability of highly resistant CSCs[49]. We also demonstrated that Tideglusib alone and in combination with radiation significantly decreased the sphere formation of glioblastoma cell lines by targeting and reducing their CSC population[20]. On another note, since drug repurposing has recently emerged as a promising approach to target CSCs and overcome therapy resistance by identifying novel therapeutic strategies for cancer[47], we demonstrated that Metformin and Ara-a are both effective in the treatment of glioblastomas and neuroblastomas, *in vitro*, by targeting their cancer stem/progenitor cell population[47].

Glioma organotypic multicellular spheroid models were used to study lymphokine-activated killer (LAK) cell infiltration and toxicity using LAK cell therapy. Results showed that those cells caused intense cellular damage to the glioma spheroids, and hence, the model offered a solid base on which LAK cell therapy studies are based[50]. Moreover, MatrigelTM, a gel-like substance used as a 3D culture matrix to recapitulate the tumor microenvironment, was used to assess the invasiveness of embedded patient-derived glioblastoma cancer cells. Results conveyed that invasion in MatrigelTM increased in rigidity-independent cell lines[51]. Moreover, the use of spheroid culturing model enabled researchers to assess the effect of protein inhibition on the outcome of sphere formation ability. For instance, lentiviral short hairpin RNA knockdown of *PAR1* in a glioma cell subpopulation expressing *A2B5+* was able to reduce sphere formation, and consequently the growth and self-renewal as well[52]. Also, novel microRNAs were identified as molecular targets for glioblastoma by assessing the migration of patient-derived spheroids in serum-free conditions, where miR-32 and miR-222 were associated with decreased migration[53]. Generally, the spheroid model is widely used in cancer research to enrich the cancer stem/progenitor cell subpopulation and to enhance drug discovery using a realistic *in vitro* model before expanding to the relatively expensive *in vivo* research. Accordingly, a chemosensitivity assay has been developed for patient-derived hepatocellular carcinoma spheroids in which a 3D co-culture system was established to enable drug screening and hence an optimized treatment for every patient[54]. Likewise, patient-derived non-small cell lung cancer cells were expanded in culture for more than 120 d using the 3D spheroid model, in an attempt to establish a drug screening prototype[55]. Our group recently derived CSCs (as spheres in 3D cultures) from Kras-mutant lung adenocarcinoma cells and used whole-transcriptome sequencing to identify gene features differentially expressed in CSCs showing that those cells are associated with an augmented malignant phenotype including stemness, tumor-promoting inflammation, and anti-oxidant responses[56].

Although the 2D culture system was the predominant model in the previous research era, several attempts are taking place to shift from this conventional method towards a more advanced and realistic 3D model for culturing cell lines or patient-derived cells. In addition to the cell-cell interaction, cell-environment interactions and cellular heterogeneity represent an important aspect of the 3D culturing technique. Therefore, recapitulating the extracellular environment can provide better analysis of tumorigenic behavior to different treatment regimens[17]. However, even though 3D culture systems are more physiologically relevant than 2D and currently represent the transition between conventional culturing methods and *in vivo* experiments, several limitations still accompany those techniques. For instance, in spheroid formation, the distribution of oxygen, nutrients, and waste is still underdeveloped[14]. Also, cellular microenvironments are challenging to replicate in certain 3D methods, in addition to the difficulty in imaging that requires high standard and specialized microscopes, such as laser scanning confocal microscopes. Moreover, the dissociation of spheroids to single cells requires to proceed with several experiments, which represents one of the issues facing 3D cultures[17]. Last but not least, there is no unique and standard protocol for 3D culture formation, and there is a difficulty in obtaining spheres from cells extracted from certain tissues, while the most significant disadvantage remains the time consumption necessary to perform and analyze such assays[40].

**3D ORGANOID MODELS**

Basic science research on brain tumors is challenged mainly by the absence of reliable models that mimic human response. Organoids, which are 3D cell cultures grown *in vitro* to imitate organs, have emerged as promising tools for preclinical tumor modeling and experimentation[57] (Tables 4 and 5). Human neuroepithelial stem cells derived from induced pluripotent stem cells of patients with Gorlin syndrome were used to model medulloblastoma (MB) upon orthotopic transplantation in mice[66]. Genetically engineered cerebral organoids were used to model brain tumor formation and progression[38] and recapitulate the key aspects of malignancy in gliomas[58]. In cancerous organoids, oncogenes could be amplified using transposons, and mutations are induced in tumor suppressor genes using sequence editing techniques (CRISPR-Cas9). In a study by Bian *et al*[38], *MYC* gene amplification yielded CNS primary neuroectodermal tumor. Also, combinations of mutations associated with glioblastoma generated glioblastoma-like neoplastic cerebral organoids (neoCORs). These genetically engineered neoCORs allowed for the study of tumorigenesis and the different genetic aberrations that may be found within tumor cells[38]. In addition, neoCORs were found to emulate the structural organization of *in vivo* tumors while also containing both tumor cells and normal CNS cells.

Medulloblastoma, an aggressive brain tumor of childhood, causes the highest morbidity and mortality rates among cancer patients of the pediatric patient population[67,68]. Being a heterogeneous tumor, MB constitutes several subgroups: Wnt, Shh, Group 3, and Group 4[69]. Among the molecular subgroups, Group 3 MB known as the *MYC* group, is characterized by *c-MYC* amplification and is associated with a poor prognosis wherein approximately 50% of patients have metastatic disease at the time of diagnosis[59,69]. Differential expression of tumor oncogenes such as the overexpression of *c-MYC* with either *GFI1* overexpression or *p53* loss is shown to induce MB tumorigenicity[59]. Yet, neither patient-specific MB models nor specific therapy for Group 3 MB patients exists. Ballabio *et al*[59] showed that *OTX2/c-MYC* together constitute a novel driver gene combination required for MB tumorigenesis. In their study, human induced pluripotent stem cell-derived cancer organoids were established to model Group 3 MB. These organoids mimicked Group 3 genetic alterations as they overexpressed *GFI1/c-MYC* and *OTX2/c-MYC* gene combinations. Besides, DNA methylation signature and Group 3-specific markers analysis proved that the generated organoid-based MB model recapitulates several features of human Group 3 MB. In addition, SMARCA4, a chromatin modifier, reduced Otx2/c-MYC tumorigenesis *in vivo* and in human cerebellar organoids. On the other hand, treatment with Tazemetostat, a *EZH2*-specific inhibitor, reduced *OTX2/c-MYC* tumorigenesis in *ex vivo* cultures and the established human cerebellar organoids[59]. Therefore, human organoids-based models might truly represent useful tools to investigate the molecular mechanisms underlying cancer development, drug screening, and therapy.

Among the cell types essential for tumor pathogenesis are the endothelial cells. In glioblastoma, for instance, the tumor favors tissue invasion along with existing vasculature[70]. However, their absence limits glioma natural history reconstruction in organoids. Co-culturing with endothelial and mesenchymal progenitors would generate more reliable cerebral organoids[71,72]. Similarly, co-culturing with hematopoietic progenitors give rise to microglial cells that mediate brain inflammatory and injury responses[73,74]. In addition to the missing cell types, variability in the organoid generation is another limitation in human cerebral organoids. Brain organoids contain a subpopulation of non-CNS differentiated tissues and do not differentiate completely except for the deepest layers of the human cerebral cortex[58,75]. However, despite these existing limitations, tumors derived from cerebral organoids are like those generated in mice, transplantable like patient-derived tumors, and support both the proliferation and invasive behaviors upon using patient-derived primary tumor explants as well as established cancer cell lines[58]. Theoretically, to address host-tumor cell interaction in 2D culture, it is possible to co-culture brain cells with patient-derived glioma stem cells. Unfortunately, the resulting disordered mixture of normal cells would lack human ECM and would not represent the carefully organized arrangement of cells in the human brain[60]. However, human glioblastoma can be modeled in a primitive brain microenvironment using human embryonic stem cell-derived cerebral organoids and patient-derived glioblastoma stem cells. By growing patient-derived glioblastomas within the cerebral organoids, Linkous *et al*[60]established a clinically relevant cerebral organoid glioma GLICO model. In addition to being scalable for high-throughput drug screening, their *ex vivo* model closely phenocopies surgical and autopsy specimens. In addition to exhibiting high resistance to drug and radiation-induced genotoxic stress, glioma stem cells were reported to relocate to the human cerebral organoid where they invade and proliferate to form tumors that highly recapitulate patient glioblastomas[60].

On another note, one of the most recent advancements in CNS tumor modeling are the tumor-on-a-chip systems, which have been recently employed in glioblastoma (Table 4). Akay *et al*[61] developed a brain cancer chip able to culture primary tumor-derived human glioblastoma cells as 3D spheroids. In addition, this chip had a multi-channel system that allows for targeted drug delivery, which was upgraded from their previous model published back in 2016[61,76]. The authors hope that this new technology would provide clinical benefits to oncologists as a means of studying patient-specific drug responses, in addition to playing a crucial role in improving the current drug testing framework. This system showed a lot of potential; however, it harbors some limitations, as most spheroid systems do, due to the lack of the complete tumor microenvironment, which is known to influence glioblastoma’s drug sensitivity and general behavior[26,63,77,78].

In addition, microfluidic chip models were already being investigated as a potential biomimetic model for glioblastoma. Ayuso *et al*[62] developed a microfluidic chip that allowed them to simulate blood flow through the tumor in addition to vessel obstructing events that have been associated with glioblastoma. At around the same time, Cui *et al*[63] published findings on their microfluidic angiogenesis model chip, which allowed them to reconstitute organotypic glioblastoma models in a vascularized microenvironment that simulated immune and vascular conditions. This model allowed them to study the effects of immune cells and inflammation on the angiogenesis and tumor growth, in addition to possible therapeutic targets. Other researchers were also able to use microfluidic chips to study glioblastoma and the perivascular CSC niches as an important factor in tumor chemoresistance[64]. More recently, Yi *et al*[65] developed a bio-printed glioblastoma-on-a-chip model using patient-derived tumor cells co-cultured with vascular endothelial cells and a decellularized ECM from a porcine brain all compartmentalized in a way to simulate the complex tumor microenvironment. This compartmentalization helps create an oxygen gradient that recreates what is seen *in vivo* in glioblastoma with central hypoxia. Their model was shown to reproduce successfully the structural, biochemical, and biophysical qualities of the original *in vivo* tumor. The model’s strength comes with its ability to recreate a tumor with the same chemotherapy and radiotherapy resistance and susceptibility profile as its *in vivo* counterpart. These advancements have made the tumor-on-a-chip model, a fertile field for future studies in personalized medicine and the determination of the optimal treatment regimen for each patient. It may even be possible, soon, to conduct point-of-care testing using this system as it can be set up within 2 wk[65]. While more advanced than other 3D models that do not employ co-culturing, the glioblastoma-on-a-chip still lacks the immune cell component, which is an important aspect of cancer development and progression[79].

Organoids, while still a relatively new field, especially in neuro-oncology, have already proven extremely useful in the understanding of tumor pathophysiology, in addition to their practical clinical uses in testing therapeutics. Having provided a close simulation of the tumor microenvironment, organoids have allowed for the filling of many of the missing knowledge gaps in understanding tumor invasion[58,60,79,80], the role of the immune and vascular systems[10,63,81], the importance of intra-tumor oxygen gradients[77,82], and glioblastoma CSC behavior and role[10,60,83]. In addition, as organoids are becoming better models to recapitulate *in vivo* tumors, therapeutic testing may now provide more reliable and applicable results for clinical settings. They have also taken us a step further towards the use of patient-derived tumors for drug repurposing and drug combination testing to help in the clinical decisions pertaining to drug regimens, saving patients’ time and money that could have been wasted on ineffective treatments[38,59,61,80].

**CLINICAL IMPLICATIONS OF 3D SPHEROID AND ORGANOID MODELS**

Going from 2D to 3D culture allowed for better understanding of the pathology and the physiology of brain tumors[47]. Tiburcio *et al*[84] proved that it is better to study the effect of hemizygous/heterozygous *IDH1R132H* in 3D cultures*,* where they proved that in 3D culture, heterozygous cells for the *IDH1R132H* mutation had low malignancy whereas the hemizygous cells fell into a higher malignancy cluster. In contrast, among 2D cultures, both types were presented to have the same malignancy. In addition, the mesenchymal gene set in glioblastoma cells appears to be enhanced only in the 3D models, which is consistent with the fact that 3D cultures are more fit models to study glioma biology.

Moreover, brain tumors like glioblastoma can remodel their microenvironment and their ECM through interactions with basement membrane proteins in order to gain drug resistance against TMZ and increase their survival in its presence. Musah-Eroje and Watson[77] demonstrated that 3D models and assays could be used to characterize brain tumor cells and identify the pathways through which such cells gain their drug chemo-protection and metastasis abilities. However, it is not only the interaction with the microenvironment that makes tumor cells resistant to TMZ but also their interaction with nearby endothelial cells. Towards proving such correlation, a 3D model was used by Lin *et al*[64], where neurospheres formed from glioblastoma CSCs co-cultured with endothelial cells appeared to be more resistant than those not cultured with endothelial cells. A similar study was done by Civita *et al*[85] emphasized the role of astrocyte interaction with glioblastoma cells through the formation of nano tunnels that allows the exchange of undamaged mitochondria and other cellular contents with tumor cells under stress, which in turn promotes chemoresistance against anticancer drugs, including TMZ, vincristine, and clomipramine.

When it comes to establishing treatment protocols from a 2D model, heterogeneity and diversity between patients are some of the biggest challenges. Trying to solve such problem, Skaga *et al*[10] proved that patient-derived glioblastoma tumor spheres maintain the traits and characteristics of the parental tumor, which makes it a good model to test for patient-specific responses to drugs or to test the sensitivity of patient to a certain drug. However, such models do not account for the interaction between tumor cells and normal cells in the same culture. On the other hand, neoCORs created from induced pluripotent stem cells, have a more organ-like histology since they take into consideration such interactions between transformed and non-transformed cells[38]. In these models, it is possible to test for antitumor drug effect while running a safety test in the same system. In addition, neoCORs could be used to test the vulnerability of subjects to various combinations of driver mutations and improve the process of screening for efficient targeted therapy like immuno- and gene-therapy[38,86]. Furthermore, Malatesta *et al*[87] used organoids, composed of tumor cells cultured with astrocytes and normal neurons, as a model to prove that the knockdown of the primate-specific long noncoding RNA, *GTT1*, inhibits the growth of tumor cells without impacting normal cells and thus not affecting the organoids’ viability, making it a possible glioma-specific therapeutic target. However, most organoid models are limited by their lack of vasculature, and thus the study conducted may not account for glomeruloid microvascular proliferations and perivascular palisading necrosis[38]. Cui *et al*[63] avoided such limitations by producing a more complex 3D angiogenesis model that included immuno-vascular, cell-cell, and cell-matrix interactions with controllable immunosuppressive conditions. In their study, the authors emphasized the roles of macrophages in immunosuppression, in addition to the role of endothelial-macrophage and cell-ECM interactions in controlling malignant angiogenesis in glioblastoma tumors. Furthermore, a new 3D model was established mimicking the *in vivo* microenvironment of glioblastoma tumors, focusing on their potential in screening for novel therapeutics[63].

Brain cancer chips are another form of 3D modeling that have proven to be very effective when it comes to administrating multiple drugs simultaneously and testing their responses on patient-derived glioblastoma cells. In addition, they could be used to do high-throughput drug screening without any technician input, which renders it an efficient method to produce and generate personalized treatment plans in the near future[61,76].

**CONCLUSION**

In conclusion, recent advancements in cell culturing techniques have led to a trending gravitation away from 2D culturing models in favor of 3D models such as spheroids and organoids. While these new models have historically been more expensive, harder to maintain, and require more specialized skills and equipment[88], such limitations are becoming less relevant as these techniques are becoming more popular and mainstream. Tumor modeling is now a highly evolving field, and the development of 3D culturing techniques has opened the doors to fast-paced developments making this field one of the most dynamic currently in cellular biology. From the first reports of spheroid cell cultures in the 1980s to the recent breakthroughs in genetically-engineered brain organoids and patient-derived models, current tumor modeling techniques have brought us closer than ever to the era of personalized medicine and point-of-care tumor models for therapeutics testing. The benefit of 3D modeling and cell culturing does not stop only at oncological studies but has also been already used and paved the way for breakthroughs in other fields such as regenerative medicine[89,90]. It remains to be seen whether the benefits of 3D models could be harnessed in clinical scenarios soon; however, with the current pace of breakthroughs and new developments, this future may not be as far off as one might believe.

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**REFERENCES**

1 **Ostrom QT**, Gittleman H, Truitt G, Boscia A, Kruchko C, Barnholtz-Sloan JS. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2011-2015. *Neuro Oncol* 2018; **20**: iv1-iv86 [PMID: 30445539 DOI: 10.1093/neuonc/noy131]

2 **Smith MA**, Reaman GH. Remaining challenges in childhood cancer and newer targeted therapeutics. *Pediatr Clin North Am* 2015; **62**: 301-312 [PMID: 25435124 DOI: 10.1016/j.pcl.2014.09.018]

3 **Shamsdin SA**, Mehrafshan A, Rakei SM, Mehrabani D. Evaluation of VEGF, FGF and PDGF and Serum Levels of Inflammatory Cytokines in Patients with Glioma and Meningioma in Southern Iran. *Asian Pac J Cancer Prev* 2019; **20**: 2883-2890 [PMID: 31653130 DOI: 10.31557/APJCP.2019.20.10.2883]

4 **Ohgaki H**, Dessen P, Jourde B, Horstmann S, Nishikawa T, Di Patre PL, Burkhard C, Schüler D, Probst-Hensch NM, Maiorka PC, Baeza N, Pisani P, Yonekawa Y, Yasargil MG, Lütolf UM, Kleihues P. Genetic pathways to glioblastoma: a population-based study. *Cancer Res* 2004; **64**: 6892-6899 [PMID: 15466178 DOI: 10.1158/0008-5472.CAN-04-1337]

5 **Siegel RL**, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin* 2021; **71**: 7-33 [PMID: 33433946 DOI: 10.3322/caac.21654]

6 **Stupp R**, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO; European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups; National Cancer Institute of Canada Clinical Trials Group. Effects of radiotherapy with concomitant and adjuvant temozolomide *vs* radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* 2009; **10**: 459-466 [PMID: 19269895 DOI: 10.1016/S1470-2045(09)70025-7]

7 **Milano MT**, Okunieff P, Donatello RS, Mohile NA, Sul J, Walter KA, Korones DN. Patterns and timing of recurrence after temozolomide-based chemoradiation for glioblastoma. *Int J Radiat Oncol Biol Phys* 2010; **78**: 1147-1155 [PMID: 20207495 DOI: 10.1016/j.ijrobp.2009.09.018]

8 **Bradshaw A**, Wickremsekera A, Tan ST, Peng L, Davis PF, Itinteang T. Cancer Stem Cell Hierarchy in Glioblastoma Multiforme. *Front Surg* 2016; **3**: 21 [PMID: 27148537 DOI: 10.3389/fsurg.2016.00021]

9 **Bahmad HF**, Poppiti RJ. Medulloblastoma cancer stem cells: molecular signatures and therapeutic targets. *J Clin Pathol* 2020; **73**: 243-249 [PMID: 32034059 DOI: 10.1136/jclinpath-2019-206246]

10 **Skaga E**, Kulesskiy E, Fayzullin A, Sandberg CJ, Potdar S, Kyttälä A, Langmoen IA, Laakso A, Gaál-Paavola E, Perola M, Wennerberg K, Vik-Mo EO. Intertumoral heterogeneity in patient-specific drug sensitivities in treatment-naïve glioblastoma. *BMC Cancer* 2019; **19**: 628 [PMID: 31238897 DOI: 10.1186/s12885-019-5861-4]

11 **Bao B**, Ahmad A, Azmi AS, Ali S, Sarkar FH. Overview of cancer stem cells (CSCs) and mechanisms of their regulation: implications for cancer therapy. *Curr Protoc Pharmacol* 2013; **Chapter 14**: Unit 14.25 [PMID: 23744710 DOI: 10.1002/0471141755.ph1425s61]

12 **Gonçalves BÔP**, Fialho SL, Silvestrini BR, Sena IFG, Dos Santos GSP, Assis Gomes D, Silva LM. Central nervous system (CNS) tumor cell heterogeneity contributes to differential platinum-based response in an *in vitro* 2D and 3D cell culture approach. *Exp Mol Pathol* 2020; **116**: 104520 [PMID: 32828740 DOI: 10.1016/j.yexmp.2020.104520]

13 **Singh SK**, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature* 2004; **432**: 396-401 [PMID: 15549107 DOI: 10.1038/nature03128]

14 **Duval K**, Grover H, Han LH, Mou Y, Pegoraro AF, Fredberg J, Chen Z. Modeling Physiological Events in 2D vs. 3D Cell Culture. *Physiology (Bethesda)* 2017; **32**: 266-277 [PMID: 28615311 DOI: 10.1152/physiol.00036.2016]

15 **Costa EC**, Moreira AF, de Melo-Diogo D, Gaspar VM, Carvalho MP, Correia IJ. 3D tumor spheroids: an overview on the tools and techniques used for their analysis. *Biotechnol Adv* 2016; **34**: 1427-1441 [PMID: 27845258 DOI: 10.1016/j.biotechadv.2016.11.002]

16 **Huang YP**, Liu K, Wang YX, Yang Y, Xiong L, Zhang ZJ, Wen Y. Application and research progress of organoids in cholangiocarcinoma and gallbladder carcinoma. *Am J Cancer Res* 2021; **11**: 31-42 [PMID: 33520358]

17 **Jensen C**, Teng Y. Is It Time to Start Transitioning From 2D to 3D Cell Culture? *Front Mol Biosci* 2020; **7**: 33 [PMID: 32211418 DOI: 10.3389/fmolb.2020.00033]

18 **Pinto B**, Henriques AC, Silva PMA, Bousbaa H. Three-Dimensional Spheroids as In Vitro Preclinical Models for Cancer Research. *Pharmaceutics* 2020; **12** [PMID: 33291351 DOI: 10.3390/pharmaceutics12121186]

19 **Bahmad HF**, Mouhieddine TH, Chalhoub RM, Assi S, Araji T, Chamaa F, Itani MM, Nokkari A, Kobeissy F, Daoud G, Abou-Kheir W. The Akt/mTOR pathway in cancer stem/progenitor cells is a potential therapeutic target for glioblastoma and neuroblastoma. *Oncotarget* 2018; **9**: 33549-33561 [PMID: 30323898 DOI: 10.18632/oncotarget.26088]

20 **Bou-Gharios J**, Assi S, Bahmad HF, Kharroubi H, Araji T, Chalhoub RM, Ballout F, Harati H, Fares Y, Abou-Kheir W. The potential use of tideglusib as an adjuvant radio-therapeutic treatment for glioblastoma multiforme cancer stem-like cells. *Pharmacol Rep* 2021; **73**: 227-239 [PMID: 33140310 DOI: 10.1007/s43440-020-00180-5]

21 **Bajaj J**, Diaz E, Reya T. Stem cells in cancer initiation and progression. *J Cell Biol* 2020; **219** [PMID: 31874116 DOI: 10.1083/jcb.201911053]

22 **Cheaito KA**, Bahmad HF, Hadadeh O, Saleh E, Dagher C, Hammoud MS, Shahait M, Mrad ZA, Nassif S, Tawil A, Bulbul M, Khauli R, Wazzan W, Nasr R, Shamseddine A, Temraz S, El-Sabban ME, El-Hajj A, Mukherji D, Abou-Kheir W. EMT Markers in Locally-Advanced Prostate Cancer: Predicting Recurrence? *Front Oncol* 2019; **9**: 131 [PMID: 30915272 DOI: 10.3389/fonc.2019.00131]

23 **Rajayi H**, Tavasolian P, Rezalotfi A, Ebrahimi M. Cancer Stem Cells Targeting; the Lessons from the Interaction of the Immune System, the Cancer Stem Cells and the Tumor Niche. *Int Rev Immunol* 2019; **38**: 267-283 [PMID: 31578892 DOI: 10.1080/08830185.2019.1669593]

24 **Lytle NK**, Barber AG, Reya T. Stem cell fate in cancer growth, progression and therapy resistance. *Nat Rev Cancer* 2018; **18**: 669-680 [PMID: 30228301 DOI: 10.1038/s41568-018-0056-x]

25 **Makena MR**, Ranjan A, Thirumala V, Reddy AP. Cancer stem cells: Road to therapeutic resistance and strategies to overcome resistance. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165339 [PMID: 30481586 DOI: 10.1016/j.bbadis.2018.11.015]

26 **Manini I**, Caponnetto F, Bartolini A, Ius T, Mariuzzi L, Di Loreto C, Beltrami AP, Cesselli D. Role of Microenvironment in Glioma Invasion: What We Learned from In Vitro Models. *Int J Mol Sci* 2018; **19** [PMID: 29300332 DOI: 10.3390/ijms19010147]

27 **Ogden AT**, Waziri AE, Lochhead RA, Fusco D, Lopez K, Ellis JA, Kang J, Assanah M, McKhann GM, Sisti MB, McCormick PC, Canoll P, Bruce JN. Identification of A2B5+CD133- tumor-initiating cells in adult human gliomas. *Neurosurgery* 2008; **62**: 505-14; discussion 514-5 [PMID: 18382330 DOI: 10.1227/01.neu.0000316019.28421.95]

28 **Li Q**, Lin H, Rauch J, Deleyrolle LP, Reynolds BA, Viljoen HJ, Zhang C, Zhang C, Gu L, Van Wyk E, Lei Y. Scalable Culturing of Primary Human Glioblastoma Tumor-Initiating Cells with a Cell-Friendly Culture System. *Sci Rep* 2018; **8**: 3531 [PMID: 29476107 DOI: 10.1038/s41598-018-21927-4]

29 **Son MJ**, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell Stem Cell* 2009; **4**: 440-452 [PMID: 19427293 DOI: 10.1016/j.stem.2009.03.003]

30 **Bleau AM**, Hambardzumyan D, Ozawa T, Fomchenko EI, Huse JT, Brennan CW, Holland EC. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. *Cell Stem Cell* 2009; **4**: 226-235 [PMID: 19265662 DOI: 10.1016/j.stem.2009.01.007]

31 **Kondo T**, Setoguchi T, Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. *Proc Natl Acad Sci U S A* 2004; **101**: 781-786 [PMID: 14711994 DOI: 10.1073/pnas.0307618100]

32 **Rahman M**, Reyner K, Deleyrolle L, Millette S, Azari H, Day BW, Stringer BW, Boyd AW, Johns TG, Blot V, Duggal R, Reynolds BA. Neurosphere and adherent culture conditions are equivalent for malignant glioma stem cell lines. *Anat Cell Biol* 2015; **48**: 25-35 [PMID: 25806119 DOI: 10.5115/acb.2015.48.1.25]

33 **Pollard SM**, Yoshikawa K, Clarke ID, Danovi D, Stricker S, Russell R, Bayani J, Head R, Lee M, Bernstein M, Squire JA, Smith A, Dirks P. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. *Cell Stem Cell* 2009; **4**: 568-580 [PMID: 19497285 DOI: 10.1016/j.stem.2009.03.014]

34 **Del Duca D**, Werbowetski T, Del Maestro RF. Spheroid preparation from hanging drops: characterization of a model of brain tumor invasion. *J Neurooncol* 2004; **67**: 295-303 [PMID: 15164985 DOI: 10.1023/b:neon.0000024220.07063.70]

35 **Achilli TM**, Meyer J, Morgan JR. Advances in the formation, use and understanding of multi-cellular spheroids. *Expert Opin Biol Ther* 2012; **12**: 1347-1360 [PMID: 22784238 DOI: 10.1517/14712598.2012.707181]

36 **Bahmad HF**, Cheaito K, Chalhoub RM, Hadadeh O, Monzer A, Ballout F, El-Hajj A, Mukherji D, Liu YN, Daoud G, Abou-Kheir W. Sphere-Formation Assay: Three-Dimensional *in vitro* Culturing of Prostate Cancer Stem/Progenitor Sphere-Forming Cells. *Front Oncol* 2018; **8**: 347 [PMID: 30211124 DOI: 10.3389/fonc.2018.00347]

37 **Lv D**, Hu Z, Lu L, Lu H, Xu X. Three-dimensional cell culture: A powerful tool in tumor research and drug discovery. *Oncol Lett* 2017; **14**: 6999-7010 [PMID: 29344128 DOI: 10.3892/ol.2017.7134]

38 **Bian S**, Repic M, Guo Z, Kavirayani A, Burkard T, Bagley JA, Krauditsch C, Knoblich JA. Genetically engineered cerebral organoids model brain tumor formation. *Nat Methods* 2018; **15**: 631-639 [PMID: 30038414 DOI: 10.1038/s41592-018-0070-7]

39 **Weiswald LB**, Bellet D, Dangles-Marie V. Spherical cancer models in tumor biology. *Neoplasia* 2015; **17**: 1-15 [PMID: 25622895 DOI: 10.1016/j.neo.2014.12.004]

40 **Kapałczyńska M**, Kolenda T, Przybyła W, Zajączkowska M, Teresiak A, Filas V, Ibbs M, Bliźniak R, Łuczewski Ł, Lamperska K. 2D and 3D cell cultures - a comparison of different types of cancer cell cultures. *Arch Med Sci* 2018; **14**: 910-919 [PMID: 30002710 DOI: 10.5114/aoms.2016.63743]

41 **Pal R**. Key applications of organoids. [cited 10 Feb 2021]. In: Crown Bioscience [Internet]. Available from: https://blog.crownbio.com/key-organoid-applications

42 **Verjans ET**, Doijen J, Luyten W, Landuyt B, Schoofs L. Three-dimensional cell culture models for anticancer drug screening: Worth the effort? *J Cell Physiol* 2018; **233**: 2993-3003 [PMID: 28618001 DOI: 10.1002/jcp.26052]

43 **Sambale F**, Lavrentieva A, Stahl F, Blume C, Stiesch M, Kasper C, Bahnemann D, Scheper T. Three dimensional spheroid cell culture for nanoparticle safety testing. *J Biotechnol* 2015; **205**: 120-129 [PMID: 25595712 DOI: 10.1016/j.jbiotec.2015.01.001]

44 **Xiao W**, Zhang R, Sohrabi A, Ehsanipour A, Sun S, Liang J, Walthers CM, Ta L, Nathanson DA, Seidlits SK. Brain-Mimetic 3D Culture Platforms Allow Investigation of Cooperative Effects of Extracellular Matrix Features on Therapeutic Resistance in Glioblastoma. *Cancer Res* 2018; **78**: 1358-1370 [PMID: 29282221 DOI: 10.1158/0008-5472.CAN-17-2429]

45 **Gomez-Roman N**, Stevenson K, Gilmour L, Hamilton G, Chalmers AJ. A novel 3D human glioblastoma cell culture system for modeling drug and radiation responses. *Neuro Oncol* 2017; **19**: 229-241 [PMID: 27576873 DOI: 10.1093/neuonc/now164]

46 **Wenger A**, Larsson S, Danielsson A, Elbæk KJ, Kettunen P, Tisell M, Sabel M, Lannering B, Nordborg C, Schepke E, Carén H. Stem cell cultures derived from pediatric brain tumors accurately model the originating tumors. *Oncotarget* 2017; **8**: 18626-18639 [PMID: 28148893 DOI: 10.18632/oncotarget.14826]

47 **Mouhieddine TH**, Nokkari A, Itani MM, Chamaa F, Bahmad H, Monzer A, El-Merahbi R, Daoud G, Eid A, Kobeissy FH, Abou-Kheir W. Metformin and Ara-a Effectively Suppress Brain Cancer by Targeting Cancer Stem/Progenitor Cells. *Front Neurosci* 2015; **9**: 442 [PMID: 26635517 DOI: 10.3389/fnins.2015.00442]

48 **Narayan RS**, Fedrigo CA, Brands E, Dik R, Stalpers LJ, Baumert BG, Slotman BJ, Westerman BA, Peters GJ, Sminia P. The allosteric AKT inhibitor MK2206 shows a synergistic interaction with chemotherapy and radiotherapy in glioblastoma spheroid cultures. *BMC Cancer* 2017; **17**: 204 [PMID: 28320338 DOI: 10.1186/s12885-017-3193-9]

49 **Bahmad HF**, Chalhoub RM, Harati H, Bou-Gharios J, Assi S, Ballout F, Monzer A, Msheik H, Araji T, Elajami MK, Ghanem P, Chamaa F, Kadara H, Abou-Antoun T, Daoud G, Fares Y, Abou-Kheir W. Tideglusib attenuates growth of neuroblastoma cancer stem/progenitor cells *in vitro* and *in vivo* by specifically targeting GSK-3β. *Pharmacol Rep* 2021; **73**: 211-226 [PMID: 33030673 DOI: 10.1007/s43440-020-00162-7]

50 **Kaaijk P**, Troost D, Dast PK, van den Berg F, Leenstra S, Bosch DA. Cytolytic effects of autologous lymphokine-activated killer cells on organotypic multicellular spheroids of gliomas in vitro. *Neuropathol Appl Neurobiol* 1995; **21**: 392-398 [PMID: 8632834 DOI: 10.1111/j.1365-2990.1995.tb01076.x]

51 **Grundy TJ**, De Leon E, Griffin KR, Stringer BW, Day BW, Fabry B, Cooper-White J, O'Neill GM. Differential response of patient-derived primary glioblastoma cells to environmental stiffness. *Sci Rep* 2016; **6**: 23353 [PMID: 26996336 DOI: 10.1038/srep23353]

52 **Auvergne R**, Wu C, Connell A, Au S, Cornwell A, Osipovitch M, Benraiss A, Dangelmajer S, Guerrero-Cazares H, Quinones-Hinojosa A, Goldman SA. PAR1 inhibition suppresses the self-renewal and growth of A2B5-defined glioma progenitor cells and their derived gliomas in vivo. *Oncogene* 2016; **35**: 3817-3828 [PMID: 26616854 DOI: 10.1038/onc.2015.452]

53 **Munthe S**, Halle B, Boldt HB, Christiansen H, Schmidt S, Kaimal V, Xu J, Zabludoff S, Mollenhauer J, Poulsen FR, Kristensen BW. Shift of microRNA profile upon glioma cell migration using patient-derived spheroids and serum-free conditions. *J Neurooncol* 2017; **132**: 45-54 [PMID: 28091986 DOI: 10.1007/s11060-016-2356-x]

54 **Song Y**, Kim JS, Kim SH, Park YK, Yu E, Kim KH, Seo EJ, Oh HB, Lee HC, Kim KM, Seo HR. Patient-derived multicellular tumor spheroids towards optimized treatment for patients with hepatocellular carcinoma. *J Exp Clin Cancer Res* 2018; **37**: 109 [PMID: 29801504 DOI: 10.1186/s13046-018-0752-0]

55 **Zhang Z**, Wang H, Ding Q, Xing Y, Xu Z, Lu C, Luo D, Xu L, Xia W, Zhou C, Shi M. Establishment of patient-derived tumor spheroids for non-small cell lung cancer. *PLoS One* 2018; **13**: e0194016 [PMID: 29543851 DOI: 10.1371/journal.pone.0194016]

56 **Daouk R**, Hassane M, Bahmad HF, Sinjab A, Fujimoto J, Abou-Kheir W, Kadara H. Genome-Wide and Phenotypic Evaluation of Stem Cell Progenitors Derived From *Gprc5a*-Deficient Murine Lung Adenocarcinoma With Somatic *Kras* Mutations. *Front Oncol* 2019; **9**: 207 [PMID: 31001473 DOI: 10.3389/fonc.2019.00207]

57 **Neal JT**, Kuo CJ. Organoids as Models for Neoplastic Transformation. *Annu Rev Pathol* 2016; **11**: 199-220 [PMID: 26907527 DOI: 10.1146/annurev-pathol-012615-044249]

58 **Ogawa J**, Pao GM, Shokhirev MN, Verma IM. Glioblastoma Model Using Human Cerebral Organoids. *Cell Rep* 2018; **23**: 1220-1229 [PMID: 29694897 DOI: 10.1016/j.celrep.2018.03.105]

59 **Ballabio C**, Anderle M, Gianesello M, Lago C, Miele E, Cardano M, Aiello G, Piazza S, Caron D, Gianno F, Ciolfi A, Pedace L, Mastronuzzi A, Tartaglia M, Locatelli F, Ferretti E, Giangaspero F, Tiberi L. Modeling medulloblastoma *in vivo* and with human cerebellar organoids. *Nat Commun* 2020; **11**: 583 [PMID: 31996670 DOI: 10.1038/s41467-019-13989-3]

60 **Linkous A**, Balamatsias D, Snuderl M, Edwards L, Miyaguchi K, Milner T, Reich B, Cohen-Gould L, Storaska A, Nakayama Y, Schenkein E, Singhania R, Cirigliano S, Magdeldin T, Lin Y, Nanjangud G, Chadalavada K, Pisapia D, Liston C, Fine HA. Modeling Patient-Derived Glioblastoma with Cerebral Organoids. *Cell Rep* 2019; **26**: 3203-3211.e5 [PMID: 30893594 DOI: 10.1016/j.celrep.2019.02.063]

61 **Akay M**, Hite J, Avci NG, Fan Y, Akay Y, Lu G, Zhu JJ. Drug Screening of Human GBM Spheroids in Brain Cancer Chip. *Sci Rep* 2018; **8**: 15423 [PMID: 30337660 DOI: 10.1038/s41598-018-33641-2]

62 **Ayuso JM**, Monge R, Martínez-González A, Virumbrales-Muñoz M, Llamazares GA, Berganzo J, Hernández-Laín A, Santolaria J, Doblaré M, Hubert C, Rich JN, Sánchez-Gómez P, Pérez-García VM, Ochoa I, Fernández LJ. Glioblastoma on a microfluidic chip: Generating pseudopalisades and enhancing aggressiveness through blood vessel obstruction events. *Neuro Oncol* 2017; **19**: 503-513 [PMID: 28062831 DOI: 10.1093/neuonc/now230]

63 **Cui X**, Morales RT, Qian W, Wang H, Gagner JP, Dolgalev I, Placantonakis D, Zagzag D, Cimmino L, Snuderl M, Lam RHW, Chen W. Hacking macrophage-associated immunosuppression for regulating glioblastoma angiogenesis. *Biomaterials* 2018; **161**: 164-178 [PMID: 29421553 DOI: 10.1016/j.biomaterials.2018.01.053]

64 **Lin C**, Lin L, Mao S, Yang L, Yi L, Lin X, Wang J, Lin ZX, Lin JM. Reconstituting Glioma Perivascular Niches on a Chip for Insights into Chemoresistance of Glioma. *Anal Chem* 2018; **90**: 10326-10333 [PMID: 30094990 DOI: 10.1021/acs.analchem.8b02133]

65 **Yi HG**, Jeong YH, Kim Y, Choi YJ, Moon HE, Park SH, Kang KS, Bae M, Jang J, Youn H, Paek SH, Cho DW. A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. *Nat Biomed Eng* 2019; **3**: 509-519 [PMID: 31148598 DOI: 10.1038/s41551-019-0363-x]

66 **Huang M**, Tailor J, Zhen Q, Gillmor AH, Miller ML, Weishaupt H, Chen J, Zheng T, Nash EK, McHenry LK, An Z, Ye F, Takashima Y, Clarke J, Ayetey H, Cavalli FMG, Luu B, Moriarity BS, Ilkhanizadeh S, Chavez L, Yu C, Kurian KM, Magnaldo T, Sevenet N, Koch P, Pollard SM, Dirks P, Snyder MP, Largaespada DA, Cho YJ, Phillips JJ, Swartling FJ, Morrissy AS, Kool M, Pfister SM, Taylor MD, Smith A, Weiss WA. Engineering Genetic Predisposition in Human Neuroepithelial Stem Cells Recapitulates Medulloblastoma Tumorigenesis. *Cell Stem Cell* 2019; **25**: 433-446.e7 [PMID: 31204176 DOI: 10.1016/j.stem.2019.05.013]

67 **Rutkowski S**, von Hoff K, Emser A, Zwiener I, Pietsch T, Figarella-Branger D, Giangaspero F, Ellison DW, Garre ML, Biassoni V, Grundy RG, Finlay JL, Dhall G, Raquin MA, Grill J. Survival and prognostic factors of early childhood medulloblastoma: an international meta-analysis. *J Clin Oncol* 2010; **28**: 4961-4968 [PMID: 20940197 DOI: 10.1200/JCO.2010.30.2299]

68 **Northcott PA**, Jones DT, Kool M, Robinson GW, Gilbertson RJ, Cho YJ, Pomeroy SL, Korshunov A, Lichter P, Taylor MD, Pfister SM. Medulloblastomics: the end of the beginning. *Nat Rev Cancer* 2012; **12**: 818-834 [PMID: 23175120 DOI: 10.1038/nrc3410]

69 **Taylor MD**, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, Ellison DW, Lichter P, Gilbertson RJ, Pomeroy SL, Kool M, Pfister SM. Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol* 2012; **123**: 465-472 [PMID: 22134537 DOI: 10.1007/s00401-011-0922-z]

70 **Watkins S**, Robel S, Kimbrough IF, Robert SM, Ellis-Davies G, Sontheimer H. Disruption of astrocyte-vascular coupling and the blood-brain barrier by invading glioma cells. *Nat Commun* 2014; **5**: 4196 [PMID: 24943270 DOI: 10.1038/ncomms5196]

71 **Lauschke K**, Frederiksen L, Hall VJ. Paving the Way Toward Complex Blood-Brain Barrier Models Using Pluripotent Stem Cells. *Stem Cells Dev* 2017; **26**: 857-874 [PMID: 28398169 DOI: 10.1089/scd.2017.0003]

72 **Ibrahim M**, Richardson MK. Beyond organoids: In vitro vasculogenesis and angiogenesis using cells from mammals and zebrafish. *Reprod Toxicol* 2017; **73**: 292-311 [PMID: 28697965 DOI: 10.1016/j.reprotox.2017.07.002]

73 **Douvaras P**, Sun B, Wang M, Kruglikov I, Lallos G, Zimmer M, Terrenoire C, Zhang B, Gandy S, Schadt E, Freytes DO, Noggle S, Fossati V. Directed Differentiation of Human Pluripotent Stem Cells to Microglia. *Stem Cell Reports* 2017; **8**: 1516-1524 [PMID: 28528700 DOI: 10.1016/j.stemcr.2017.04.023]

74 **Takata K**, Kozaki T, Lee CZW, Thion MS, Otsuka M, Lim S, Utami KH, Fidan K, Park DS, Malleret B, Chakarov S, See P, Low D, Low G, Garcia-Miralles M, Zeng R, Zhang J, Goh CC, Gul A, Hubert S, Lee B, Chen J, Low I, Shadan NB, Lum J, Wei TS, Mok E, Kawanishi S, Kitamura Y, Larbi A, Poidinger M, Renia L, Ng LG, Wolf Y, Jung S, Önder T, Newell E, Huber T, Ashihara E, Garel S, Pouladi MA, Ginhoux F. Induced-Pluripotent-Stem-Cell-Derived Primitive Macrophages Provide a Platform for Modeling Tissue-Resident Macrophage Differentiation and Function. *Immunity* 2017; **47**: 183-198.e6 [PMID: 28723550 DOI: 10.1016/j.immuni.2017.06.017]

75 **Quadrato G**, Nguyen T, Macosko EZ, Sherwood JL, Min Yang S, Berger DR, Maria N, Scholvin J, Goldman M, Kinney JP, Boyden ES, Lichtman JW, Williams ZM, McCarroll SA, Arlotta P. Cell diversity and network dynamics in photosensitive human brain organoids. *Nature* 2017; **545**: 48-53 [PMID: 28445462 DOI: 10.1038/nature22047]

76 **Fan Y**, Nguyen DT, Akay Y, Xu F, Akay M. Engineering a Brain Cancer Chip for High-throughput Drug Screening. *Sci Rep* 2016; **6**: 25062 [PMID: 27151082 DOI: 10.1038/srep25062]

77 **Musah-Eroje A**, Watson S. A novel 3D *in vitro* model of glioblastoma reveals resistance to temozolomide which was potentiated by hypoxia. *J Neurooncol* 2019; **142**: 231-240 [PMID: 30694423 DOI: 10.1007/s11060-019-03107-0]

78 **Koh I**, Cha J, Park J, Choi J, Kang SG, Kim P. The mode and dynamics of glioblastoma cell invasion into a decellularized tissue-derived extracellular matrix-based three-dimensional tumor model. *Sci Rep* 2018; **8**: 4608 [PMID: 29545552 DOI: 10.1038/s41598-018-22681-3]

79 **da Hora CC**, Schweiger MW, Wurdinger T, Tannous BA. Patient-Derived Glioma Models: From Patients to Dish to Animals. *Cells* 2019; **8** [PMID: 31574953 DOI: 10.3390/cells8101177]

80 **Plummer S**, Wallace S, Ball G, Lloyd R, Schiapparelli P, Quiñones-Hinojosa A, Hartung T, Pamies D. A Human iPSC-derived 3D platform using primary brain cancer cells to study drug development and personalized medicine. *Sci Rep* 2019; **9**: 1407 [PMID: 30723234 DOI: 10.1038/s41598-018-38130-0]

81 **Nowogrodzki A**. How cerebral organoids are guiding brain-cancer research and therapies. *Nature* 2018; **561**: S48-S49 [PMID: 30258158 DOI: 10.1038/d41586-018-06708-3]

82 **Gomez-Roman N**, Chalmers AJ. Patient-specific 3D-printed glioblastomas. *Nat Biomed Eng* 2019; **3**: 498-499 [PMID: 31148599 DOI: 10.1038/s41551-019-0379-2]

83 **Nakod PS**, Kim Y, Rao SS. Biomimetic models to examine microenvironmental regulation of glioblastoma stem cells. *Cancer Lett* 2018; **429**: 41-53 [PMID: 29746930 DOI: 10.1016/j.canlet.2018.05.007]

84 **Tiburcio PDB**, Gillespie DL, Jensen RL, Huang LE. Extracellular glutamate and IDH1R132H inhibitor promote glioma growth by boosting redox potential. *J Neurooncol* 2020; **146**: 427-437 [PMID: 32020473 DOI: 10.1007/s11060-019-03359-w]

85 **Civita P**, M Leite D, Pilkington GJ. Pre-Clinical Drug Testing in 2D and 3D Human In Vitro Models of Glioblastoma Incorporating Non-Neoplastic Astrocytes: Tunneling Nano Tubules and Mitochondrial Transfer Modulates Cell Behavior and Therapeutic Respons. *Int J Mol Sci* 2019; **20** [PMID: 31795330 DOI: 10.3390/ijms20236017]

86 **Hess R**, Abi-Aad KR, Welz ME, Bendok BR. Genetically Engineered Neoplastic Cerebral Organoids - A New Frontier in Preclinical Cancer Research. *Neurosurgery* 2019; **85**: E172-E173 [PMID: 30860263 DOI: 10.1093/neuros/nyz028]

87 **Malatesta M**, Liu J, Pedraza L, Seo K, DiLullo E, Raleigh D, Kriegstein A, Ullian E, Lim D. Tmod-04. Targeting a glioma specific lncrna in a human brain organoid tumor model. *Neuro Oncol* 2018; **20**: vi269

88 **Byrne H**. Three-dimensional biological cultures and organoids. *Interface Focus* 2020; **10** [DOI: 10.1098/rsfs.2020.0014]

89 **Jeong HJ**, Jimenez Z, Mukhambetiyar K, Seo M, Choi JW, Park TE. Engineering Human Brain Organoids: From Basic Research to Tissue Regeneration. *Tissue Eng Regen Med* 2020; **17**: 747-757 [PMID: 32329023 DOI: 10.1007/s13770-020-00250-y]

90 **Chen HI**, Song H, Ming GL. Applications of Human Brain Organoids to Clinical Problems. *Dev Dyn* 2019; **248**: 53-64 [PMID: 30091290 DOI: 10.1002/dvdy.24662]

**Footnotes**

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**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

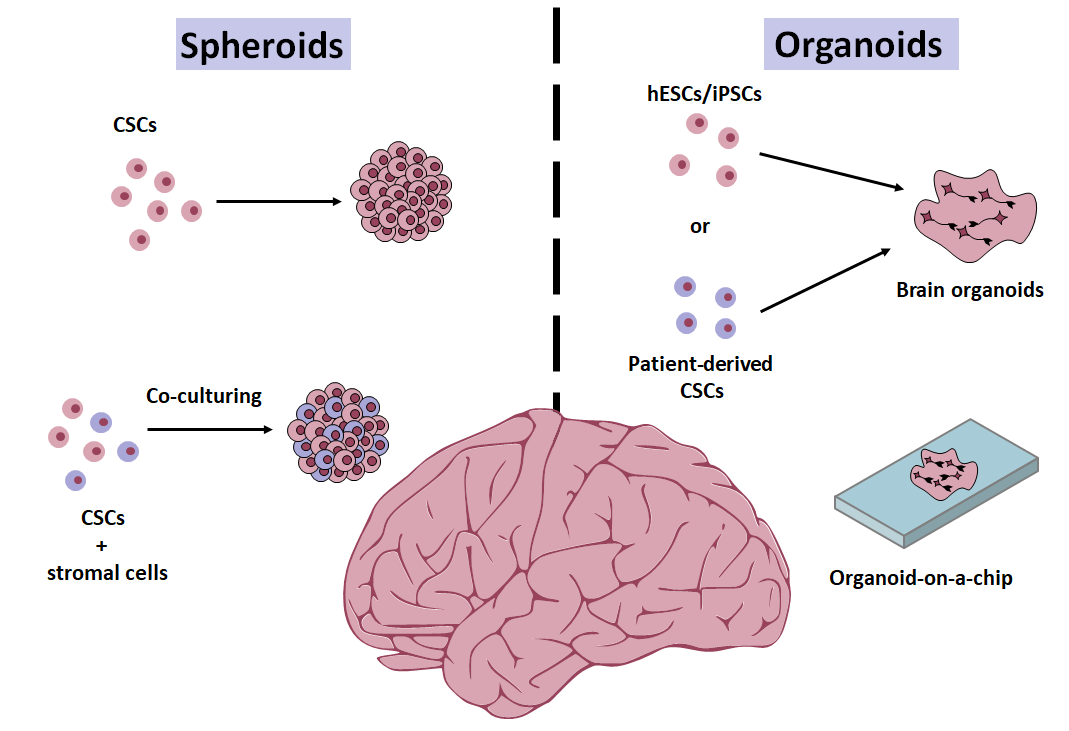
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**Figure Legends**



**Figure 1 Schematic illustrating the implication of three-dimensional cell culturing to model central nervous system tumors.** CSCs: Cancer stem cells; hESCs: Human embryonic stem cells; iPSCs: Induced pluripotent stem cells.

**Table 1 Comparison between two-dimensional and three-dimensional tumor cell culture systems[14-18]**

|  |  |  |
| --- | --- | --- |
|  | **2D tumor cell cultures** | **3D tumor cell cultures** |
| Time required | Few days | Few weeks |
| Physiological relevance | Does not simulate *in vivo* tumor | Simulates *in vivo* tumor |
| Cell-cell and cell-ECM interactions | Low to no interactions | High level of interactions |
| Cell morphology | Flat cells expanding on 2D surface | Preserved *in vivo* cell shapes and growth patterns; multilayer growth |
| Oxygen and nutrients perfusion | Homogeneous | Heterogeneous due to the three-dimensional geometry |
| Response to drugs | More susceptible to drug actions | More resistant to drugs with a similar drug penetration profile to *in vivo* tumor counterparts |
| Gene expression | Many differences compared to *in vivo* tumor counterparts | Similar to *in vivo* tumor counterparts |
| Differentiation | Poor | Well differentiated cells |
| Cost | Cheap | Expensive |
| Technique difficulty | Low | High |

2D: Two-dimensional; 3D: Three-dimensional; ECM: Extracellular matrix.

**Table 2 Cancer stem cells biomarkers in brain tumors**

|  |  |
| --- | --- |
| **Markers** | **Ref.** |
| CD133 | Gonçalves *et al*[12], Singh *et al*[13], Ogden *et al*[27], and Li *et al*[28] |
| A2B5 | Ogden *et al*[27], |
| CD24 | Gonçalves *et al*[12] |
| Aldehyde dehydrogenase (ALDH) | Gonçalves *et al*[12] |
| CD15 | Li *et al*[28], and Son *et al*[29] |
| ABCG2 | Li *et al*[28], Bleau *et al*[29], and Kondo *et al*[30] |
| Nestin | Rahman *et al*[32], and Pollard *et al*[33] |
| SOX2 | Rahman *et al*[32], and Pollard *et al*[33] |
| CD44 | Rahman *et al*[32], and Pollard *et al*[33] |
| OLIG2 | Rahman *et al*[32], and Pollard *et al*[33] |

**Table 3 Characteristics of three-dimensional modeling technologies**

|  |  |  |  |
| --- | --- | --- | --- |
| Modeling technology | Methods of generation | Applications | Limitations |
| Spheroids[37-39] | Static suspension; Hanging drops; Spinner and rotational bioreactor; Magnetic levitation; Microfluidic system; Gel embedding (Matrigel, *etc.*) | Radioresistance through hypoxia and cell-cell contacts; Chemosensitivity and drug screening; Migration and invasion; Propagation and analysis of CSCs | Tumor heterogeneity; Immune system response; Interaction with normal non-tumor cells; Lack organ-like histology |
| Organoids[14,17,37,40,41] | Culturing done on matrices (Matrigel, collagen I, hyaluronic acid, *etc.*); Addition of culture supplements including FGF, EGF, Noggin, N2, B27, *etc.* | Disease mechanism; Drug discovery and toxicology; Developmental, stem cell biology and regenerative medicine; Infectious disease | Oxygen and nutrient distribution underdeveloped; Cellular microenvironments are challenging to replicate; Imaging difficulties; Expensive and time consuming assay |

CSC: Cancer stem cell; EGF: Endothelial growth factor; FGF: Fibroblast growth factor.

**Table 4 Organoid and organoid-on-a-chip models**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Ref.** | **Model system** | **System cell origin** | **Tumor type** | **Relevant genes** | **Results summary** |
| Bian *et al*[38], 2018 | neoCOR | hESCs | CNS-PNET; GBM | Amplified expression of MYC; Differential expression of GBM associated gene aberrations (GBM-1, GBM-2, and GBM-3) | neoCOR used to test gain- and loss of-function mutations, singly or in combination; generation of CNS-PNET or GBM xeno-transplantable tumors |
| Ogawa *et al*[58], 2018 | Human cerebral organoids | hESC | GBM | RAS activation and TP53 deletion | Generation of tumors in cerebral organoids using CRISPR/Cas9 technology; tumors exhibited invasive phenotype and replicated the hallmarks of tumorigenesis *in vivo* |
| Ballabio *et al*[59], 2020 | Human cerebellar organoids | Human induced pluripotent stem cell (iPSC) | Medulloblastoma (MB) subgroup 3 | Overexpression of GFI1/c-MYC (GM) and OTX2/c-MYC (OM) gene  combinations | OM as a novel driver gene combination required for Group 3 MB tumorigenesis; GM and OM overexpression induces tumor formation in mouse cerebellum; SMARCA4 and Tazemetostat reduces OM tumorigenesis |
| Linkous *et al*[60], 2019 | GLICO | hESCs; iPSCs | GBM | - | GLICO recapitulate primary human GBM with in a primitive brain microenvironment; GSCs exhibit high resistance to drug and radiation-induced genotoxic stress; GSCs form tumor by relocating to the human cerebral organoid, invasion and proliferation within the microenvironment of the GLICO |
| Akay *et al*[61], 2018 | Microfluidic chip | Patient primary human GBM multiforme specimens | GBM | - | Generation of brain cancer chip that exhibit diffusion prevention mechanism to culture GBM-patient derived 3D spheroids; treatment with TMZ and bevacizumab (Avastin, BEV) in combination enhanced GBM cell death compared to TMZ alone |
| Ayuso *et al*[62], 2017 | Microfluidic chip | U-251 MG human GBM cell line | GBM | - | Generation of microfluidic device to behavior models that simulate blood flow through the tumor; deprivation of nutrients and oxygen induces pseudopalisade formation; pseudopalisading process renders GBM cells to become of more aggressive behavior |
| Cui *et al*[63], 2018 | Microfluidic chip | GL261 and CT-2A mouse glioma cell lines | GBM | - | Generation of microfluidic angiogenesis model that simulate GBM tumor angiogenesis and macrophage-associated immunosuppression within GBM tumor microenvironment; GL261 and CT-2A GBM-like tumors promote angiogenesis through driving M2-like macrophage polarization; TGF-b1, and surface integrin (avb3) endothelial-macrophage interactions regulates inflammation-mediated angiogenesis through Src-PI3K-YAP signaling; inhibition of integrin (avb3) and cytokine receptor (TGFb-R1) repress GBM tumor neovascularization |
| Lin *et al*[64], 2018 | Microfluidic chip | Patient derived GSCs | GBM | - | Generation of glioma perivascular niches on a chip; Perivascular niches maintain the pluripotent state of GSCs; Stronger chemoresistance of GSCs against TMZ associates with endothelial cell co-culturing, GSCs neurosphere formation and the expression of 6-O-methylguanine and Bmi-1 gene |
| Yi *et al*[65], 2019 | Bio-printed chip | Patient primary human GBM specimens | GBM | - | Generation of complex cancerous-tissue constructs constituting brain ECM composition, oxygen gradient-generating system, cancer-stroma structure; exhibited patient-specific response upon the treatment with drug combinations, chemoradiation and TMZ |

2-D: Two dimensional; 3-D: Three dimensional; CNS-PNET: Central nervous system primitive neuroectodermal; CSC: Cancer stem cell; GBM: Glioblastoma; GLICO: Cerebral organoid glioma; GSCs: Glioma stem cells; hESC: Human embryonic stem cells; NeoCOR: Neoplastic cerebral organoid; PI3K: Phosphatidylinositol 3 kinase; PNET: Primary neuroectodermal tumor; TGF: Transforming growth factor; TMZ: Temozolomide.

**Table 5 Features and characteristics comparison between spheroids and organoids**

|  |  |  |
| --- | --- | --- |
|  | **Spheroids** | **Organoids** |
| Cells used | Cell lines or CSCs | Embryonic stem cells, induced pluripotent stem cells or CSCs |
| Physiologic relevance | Lower | Higher |
| Tumor heterogeneity | Lower | Higher |
| Technique difficulty | Lower | Higher |
| Cost | Lower | Higher |
| Time | Weeks | 1-3 mo |
| Genetic manipulation | Moderately available | Moderately available |
| Biobanks | Not available (cells are difficult to maintain long-term) | Available |
| Advantages | Cost effective; Highly accessible; Good for high throughput drug testing | Retains tumor heterogeneity; Better simulation of the physiological environment |
| Disadvantages | Hard to maintain long-term; Not as representative of the physiologic environment | More complex; Higher failure rate; May give variable results |

CSC: Cancer stem cell.



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