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REVIEW

Recent *in vitro* models and tissue engineering strategies to study glioblastoma

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Abstract

Glioblastoma is a highly malignant brain tumor classified as grade IV with a poor prognosis and approximately a year of survival rate. The molecular changes that trigger primary glioblastoma are usually epidermal growth factor receptor mutations and amplifications, *Mouse Double Minute* and *TP53* mutations, *p16* deletion, *phosphatase and tensin homolog* and *telomerase* promoter mutations. In the vast majority of glioblastomas, altered signaling pathways were identified as receptor tyrosine kinase/Ras/PI3K, p53. Isocitrate dehydrogenase 1/2 mutations have also been associated with poor prognosis in glioblastoma The treatment options are very limited and complicated because of the diverse composition and heterogeneity of the tumors and unresponsiveness to the treatments with the existence of barriers reaching the brain tissue. Despite new trials, drug candidates that appeared effective in cell culture or mouse models failed in the clinic. Recently, new sophisticated experimental systems, including the those that mimic the tumor microenvironment, have started being used by several research groups, which will allow accurate prediction of drug efficacy. Tissue engineering strategies are also being combined with innovative cancer models, including spheroids, tumorspheres, organotypic slices, explants, tumoroids, and organoids. Such 3D systems provide powerful tools for studying glioblastoma biology by representing the dynamic evolution of the disease from the early to the metastatic stages and enabling interaction with the microenvironment. In this review, we both enlighten the molecular mechanisms that lead to glioblastoma development and detailed information on the tissue engineering approaches that have been used to model glioblastoma and the tumor microenvironment with the advantages and disadvantages. We anticipate that these novel approaches could improve the reliability of preclinical data by reducing the need for animal models.

Introduction

Glioblastoma

Glioblastoma (GBM) is a rare primary fatal brain cancer type that originates from glial cells, which exist in the central nervous system (CNS) (Uddin et al., 2020). GBM, usually occurring in adults (constituting more than 60% of all brain tumors), is the most aggressive tumor of the CNS, with a low survival rate and poor prognosis even approximately 15 months after adjuvant chemotherapy following surgical resection of current therapy (Montemurro, 2020; Rock et al., 2012). There is a relationship between age and the incidence of the disease since the research show that most GBM patients are generally 65 years of age and older (Sasmita et al., 2018). The estimated prevalence among all primary brain tumors is 4-8 per 100,000 people while this prevalence is 250,000 cases globally (Nejo et al., 2020). According to statistics from 2011 to 2015, the estimated

yearly age-adjusted incidence of GBM in the United States is 3.21 per 100,000 people, with the prevalence depending on age and gender. It was determined that men are 1.58 times more likely than women to develop GBM. In respect of race or ethnicity, white people have the highest incidence. The total incidence of GBM is reported to be 9.23 cases per 100,000 population, with the prevalence in the United States (Tan et al., 2020a).

GBM has malignant tumor characteristics such as atypical cells, nuclear hyperchromasia, increased mitotic figures, angiogenesis, and necrotic areas with high vascularity. The infiltrative nature of GBM complicates treatment and reduces the effect of chemical agents. In addition, its direct effects on the neurological function of the brain, psychological health, and quality of life also cause problems in treatment (Reardon & Wen, 2006). GBM is known to be derived from glial cells; however, neural stem cells, at the stage of differentiation into glia, may also give rise to cancer development. Because of the active DNA repair and regeneration features, GBM stem cells are hard to treat (Stoyanov et al., 2018a). As of 2016, GBM tumors are classified by the WHO as 90% Isocitrate dehydrogenase (IDH)-wild type and 10% IDH 1 and 2 mutants, when compared to the wild type, mutant *IDH 1 and 2* have a better prognosis (Batash et al., 2017).

GBM tumors can be found in any region of the CNS as primary and secondary types of malignant and non-malignant tumors (Nejo et al., 2020). Those types have different genetic pathways, so their influence on patients varies as to patients' ages (Sasmita et al., 2018). Primary GBM accounts for more than 80% of GBM and arises from neural stem cell precursors, whereas secondary GBM arises from mutations of grown neural cells (Stoyanov et al., 2018a). Primary GBM has been linked to *epidermal growth factor (EGF*) overexpression, *phosphatase and tensin homolog on chromosome ten* (*PTEN)* mutation, *cyclin-dependent kinase inhibitor 2A (CDKN2A)* deletion, and, less frequently, *murine double minute 2* (*MDM2)* amplification (Ohgaki et al., 2004). The *tumor protein 53 (p53)* mutation is commonly found as a precursor in secondary GBM (Kleihues & Ohgaki, 1999). While primary GBMs are seen in older patients with an average age of 62, secondary GBMs originate from lower-grade astrocytoma or oligodendroglioma generally indicate in the frontal lobe and are seen in younger patients with an average age of 45 (Shah et al., 2021). *IDH1* and *IDH2* are found in approximately 70% of secondary GBM and low-grade glioma, although they occur in less than 10% of primary GBM (Zeng et al., 2015). The prognosis of primary GBM is not as good as that of secondary GBM. The standard care for GBM is to apply surgery following radiotherapy in combination with concomitant and up to six maintenance cycles of temozolomide chemotherapy to the majority of newly diagnosed patients (le Rhun et al., 2019) (**Figure 1**).

Glioblastoma Molecular Mechanisms

70% of IDH-wild-type GBMs carry *EGFR* amplification and *Telomerase Reverse Transcriptase (TERT)* promoter mutations (Brennan et al., 2013). *TERT* promotor mutations result in the creation of new ETS (Erythroblast Transformation Specific) transcription factor binding sites and increased TERT activity, promoting TERT transcription and, thereby, tumor cell immortalization (Horn et al., 2013). *TERT* mutations that reduce survival probability increase TERT expression and are exclusive to *ATRX* mutations found in IDH mutant astrocytic gliomas. The *TERT* promoter mutation is found in oligodendrocytic tumors with the 1p/19q deletion in IDH-mutant GBM. Eribulin, a tubulin polymerization inhibitor, has been shown to reduce TERT activity in GBM models, justifying its clinical exploration (Takahashi et al., 2019). Furthermore, in adult GBM, proto-oncogenes have impressed on the *EGFR*, *platelet-derived growth factor receptor A (PDGFRA)*, and *hepatocyte growth factor receptor (HGFR)* genes, as well as the cyclin-dependent kinase genes *CDK4* and *CDK6*, and the murine double minute genes *MDM2* and *MDM4*. Overexpression, amplification, and mutation can cause EGFR phenotypic alterations in GBM, and nearly 50% of EGFR-enhanced GBM have the potential to carry a deletion mutation. *EGFR* amplification can occur via transcription or RNA insertion and correlates with the presence of EGFR protein variants. One specific variant of EGFR, EGFRvIII, has a deletion in the N-terminal ligand binding site between amino acids 6 and 273 and leads to ligand-independent activation of EGFR and is a constitutively active potential neoantigen. The therapeutic usage of conventional tyrosine kinase inhibitors like gefitinib is

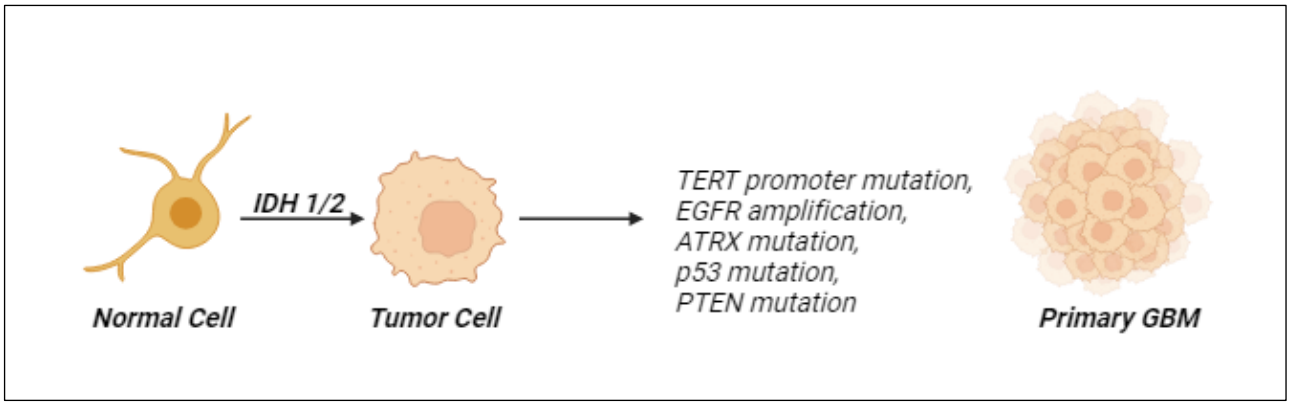

Figure 1. Mutations observed in glioblastoma.

limited due to the particular character of EGFRvIII, and it is therapeutically important that EGFRvIII has a therapeutic effect against malignancies. Protein Kinase A (PKA)-dependent phosphorylation of DOCK180, a Rac1 guanine exchange factor, is mediated by EGFRvIII. In a cell line expressing EGFRvIII, overexpression of mutant DOCK180 lacking the S1280 phosphorylation site reduced receptor-stimulated proliferation and survival. Although EGFRvIII-specific PKA phosphorylation may be a good therapeutic target if it can be inhibited by the EGFRvIII/PKA/DOCK180 interaction, EGFRvIII is not associated with overall median survival, except in cases with survivors of more than one year, which limits the therapeutic value of this target (Carlsson et al., 2014). The failure of EGFR tyrosine kinase inhibitors to show single-agent action has been reviewed in numerous publications, and it is not certain that the medications will limit the pathway's activity even if they reach the tumor site (Peralta-Arrieta et al., 2022). In another study, expression of EGFR or EGFRvIII was targeted in EGFRvIII-positive recurrent GBM within the vaccine called rindopepimut, which produces a viability signal when combined with bevacizumab, but failed in phase III in newly diagnosed patients (NCT01498328) (Weller et al., 2017). Also, when combined with temozolomide, depatuxizumab mafodotin, an antibody-drug combination consisting of an EGFR antibody ABT-806 linked to monomethyl auristatin F, was expected to be active in GBM with EGFR amplification, but it was ineffective (NCT02573324). While EGFR amplification is maintained throughout the disease, the loss of *EGFRvIII* expression observed as a result of phase III indicated that EGFRvIII expression may not be stable suggesting that chimeric antigen receptor (CAR) T cells or bispecific T-cell-binding antibodies targeting EGFRvIII may not work as well (Gedeon et al., 2018; O'Rourke et al., 2017; Van Den Bent et al., 2015).

p53 is a tumor suppressor protein and initiates apoptosis when DNA damage cannot be repaired. The *p53* mutations lead to the transition from low-grade astrocytoma to high-grade GBM. Induction of apoptosis and enhanced survival in a mouse model after normal chemotherapy have been demonstrated in recent gene therapy research with nanoparticle delivery of the *p53* gene targeting GBM and cancer stem cells. However, this has not been evaluated in human trials. The fact that *PAX3*, a member of the *PAX* gene family and acting in a *p53*-dependent manner to inhibit apoptosis, is up-regulated in many cancer types, including GBM, suggesting that it might be a potential oncogene. *PAX3*, which is an important factor in the differentiation of NSCs into astrocytes, can be considered as a diagnostic marker in GBM treatment (Zhu et al., 2018). *PTEN* mutations or deletions were discovered in more than half of the samples in primary tumors expressing mutant *p53*, indicating that GBM development is characterized by several concurrent tumor suppressor alterations (Zheng et al., 2008). Neutralization studies focused on *MDM2* or *MDM4* gene amplification are ongoing for patients with impairments in *p53* function (NCT03107780) (le Rhun et al., 2019). *PTEN* is a tumor suppressor of phosphatase that is essential for cellular homeostasis. Mutations in *PTEN* are found in 5 to 40% of GBM cases and may be a prognostic indicator in patients over the age of 45 (Srividya et al., 2011). PTEN protects the neural stem cell population by blocking cell cycle entry under normal conditions, while *PTEN* null mutants are more sensitive to growth stimuli and more prone to proliferation than wild-type neural stem cells (Groszer et al., 2006). PTEN levels are positively connected with patient survival; hence, it could be a useful diagnostic tool (Ermoian et al., 2002). The loss of *PTEN* can be seen in the IDH-wild-type of GBM tumors, resulting in excessive activation of the PI3K/AKT and mammalian target of rapamycin (mTOR) signaling. By suppressing protein metabolism, the PI3K/AKT/mTOR pathway regulates anabolic pathways in the cell and controls tumor formation. mTOR controls *PTEN* loss by phosphorylating p70S6 kinase 1 (S6K1) and eIF4E binding protein (4EBP), which are activated and inactivated, respectively. Accordingly, mutated oncogenic PI3K subunits increase. The PI3K/mTOR pathway is unavoidably changed as a result of the loss of tumor suppressor phosphatase and *PTEN* mutation. Activation of the PI3K/mTOR pathway suppresses autophagy and impairs proteasome function (Benitez et al., 2021). PI3K/AKT/mTOR pathway activates mutations in phosphatidylinositol- 4,5-bisphosphate 3-kinase catalytic subunit alpha *(PIK3CA),* which encodes the catalytic subunit p110 alpha (p110α) and in phosphoinositide- 3-kinase regulatory subunit 1 (PIK3R1), which encodes the p85α regulatory subunit. This pathway has been used with standard TMZ and chemotherapy treatment or in place of TMZ in patients with *MGMT* promoter-unmethylated GBM. As a result, no efficacy was observed, but mTOR inhibitors were slightly tolerated despite TMZ (Ma et al., 2015; Wick et al., 2016).

The *MET, FGFR,* and *AXL* genes are three independently acting receptor tyrosine kinases (RTKs) that are linked to cancer cell proliferation. *MET* gene encoding the hepatocyte growth factor receptor plays an important role in the migration and invasion of glioma cells in response to the inhibition of angiogenesis and hypoxia (Li et al., 2011). However, inhibition of MET, whose amplification has been proven in crizotinib treatment, does not affect the disease (Chi et al., 2012; Wen et al., 2011). Some IDH-wild-type GBM cases have oncogenic fusions between *fibroblast growth factor receptor (FGFR)* and *transforming, acidic, coiled-coil-containing protein (TACC)* genes, serving constitutive kinase activity. This fusion could be a target for drugs that inhibit FGFR (Perry & Wesseling, 2016).

Vascular endothelial growth factor (VEGF or VEGF-A) is an important signalling molecule of the nervous system and is responsible for GBM angiogenesis. Glioblastoma stem-like cells (GBSCs) are micrometastases that are formed after primary GBM

lesions are surgically removed. These small tumor cells have the potential to be used as therapeutic targets. GBSCs lead to tumor formation as a result of up-regulated signal pathways to protect NSC characteristics. Cellular responses are mediated by VEGF Receptor 1 (VEGFR1, Flt1) and VEGFR2 (KDR/Flk1) expressed on the surface of GBSCs. Cytokines (e.g., HGF, VEGF, PDGF, and PIGF) produced by endothelial cells can alter the biology of cancer stem cells by stimulating the survival of the adjacent cancer stem cells. Simultaneously, as GBM grows rapidly, it begins to be deprived of oxygen, resulting in hypoxia. During hypoxia, inducible transcription factors like hypoxia-inducible factors (HIFs) can stimulate VEGF secretion, and VEGF upregulation has a negative impact on therapy. An increase in VEGF has been shown to promote tumorigenesis in human GBSCs (Xu et al., 2013). Under hypoxic conditions, HIFs could be a potential upstream regulator of PAX3 in differentiated GSCs (Zhu et al., 2018). Bevacizumab, a drug approved by the Food and Drug Administration (FDA), is still being studied for its effect on tumor dynamics, despite showing good survival results. Subgroups of patients who benefited from bevacizumab's long survival were difficult to identify, and researchers were unable to develop a model in which VEGF could be targeted. Other VEGF inhibitors, such as cediranib, have also been shown in randomized clinical trials to be ineffective (Batchelor et al., 2013; le Rhun et al., 2019).

Transforming growth factor-β (TGF-β) regulates cell proliferation, differentiation, and apoptosis, and binds and activates a membrane receptor serine/threonine kinase complex that, when activated, phosphorylates several Smad family proteins, such as Smad2, which prognostically adversely affects GBM. TGF-β stimulates the expression of genes that control the cell cycle and the extracellular matrix (ECM), such as plasminogen activator inhibitor (PAI)-1 and PDGF. TGF-β also activates important tyrosine kinase receptor (TKR) effector pathways, such as PKB/AKT and ERK, independently of Smad. TGF-β is thought to be a tumor suppressor factor, and the mutations it acquires as a result of antiproliferative effects facilitate its pro-tumorigenic activity (Frei et al., 2015). TGF-β 1/2 proteins have been identified as key molecules in the immunosuppression of GBM. Although TGF-β inhibition has shown promising results in animal studies, clinical translation of TGF-β targeting using TGF-2 specific antisense oligonucleotides or tyrosine kinase inhibitors targeting TGF-β receptor II has been unsuccessful with galunisertib. The limited dose limit of TGF-β receptor inhibitors due to toxicity makes them difficult to use in clinical studies (le Rhun et al., 2019).

Human *Ras genes (Rat Sarcoma)*, such as *H-Ras, N-Ras,* and *K-Ras*, are oncogenes, and their activation and deactivation are regulated by binding to guanosine triphosphate (GTP) or guanosine diphosphate (GDP), as it is a G protein. Activation of RAF kinase by Ras regulates some signaling pathways, including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathways, and is an effective factor in the regulation of cell proliferation, signal transduction, apoptosis, and tumorigenesis. Raf is activated after growth factor signalling driven by EGFR (epithelial growth factor receptor) and PDGFR (platelet-derived growth factor receptor), and by this way it regulates Ras activity. The Ras/MAPK pathway disruption has been shown to cause aberrant cell proliferation and, ultimately, cancer. The increase in the expression levels of Ras, EGFR, PDGFR, and other receptor tyrosine kinases. Therefore, it was concluded that the Ras/MAPK pathway can be targeted for the treatment of GBM (Mao et al., 2012).

Signal transducers and activators of transcription (STAT) protein complexes, a family of Src Homology-2 (SH2)-dependent proteins involved in the function of transcription factors, activate transcription of target genes having roles in proliferation and apoptosis. STAT3, one of the STAT proteins activated by EGF, is upregulated in GBM and has an important role in the development of astrocytes. STAT3 may also function as a tumor suppressor in GBM. Numerous metalloenzymes and zinc-dependent transcription factors use zinc as a catalytic/structural component. Research has established a connection between zinc levels and the risk of cancer, and it has been observed that the zinc transporter (ZIP4) is upregulated in cases of human pancreatic cancer. Upregulation of ZIP4 in cancer cells enhances cell proliferation, and overexpression of ZIP4 increases Interleukin 6 (IL-6) transcription via cyclic adenosine monophosphate response element binding (CREB), which activates STAT3 and raises cyclin D1 production. Studies have shown that ZIP4 is overexpressed in GBM and that new therapeutic targets may emerge in the control of malignancy by targeting relevant molecular activities (Mao et al., 2012).

Secondary GBM is defined by mutations in the metabolic enzymes isocitrate dehydrogenase 1 and 2 (IDH1/2), which are also genetic markers for GBM. IDH-1 mutations are found in the active site, where somatic point mutations prohibit the enzyme from successfully converting isocitrate to alpha-ketoglutarate and can cause a drop in enzyme efficiency or an increase in enzymatic performance depending on the substrate. Furthermore, in 90% of cases, the arginine at codon 132 is replaced by a histidine (Yan et al., 2009). The R132H mutation allows IDH-1 to convert alpha-ketoglutarate to 2-hydroxyglutatate (2HG), an onco-metabolite (Jin et al., 2013). 2HG levels can be identified using magnetic resonance thus it could be a good biomarker for IDH-1 mutations. The studies about the IDH inhibitors were proven to be successful in glioma xenografts; IDH-1 is now being targeted for therapeutic usage. Drug candidate AG-120 is currently undergoing a phase II trial (clinicaltrials.gov; NCT04056910). In patients over the age of 55, IDH-R132H mutation is used to differentiate between IDH-wild-type and IDH-mutant GBMs. Sequencing is usually recommended if the result is

negative in young patients. Copy number gains on chromosome 7, monosomy of chromosome 10, mutations in the phosphatase and *PTEN* tumor suppressor gene homozygous deletion of the cyclin-dependent kinase inhibitor 2A and 2B *(CDKN2A/p14ARF and CDKN2B)* loci on 9p21, and *TERT* promoter mutations are all common in IDH wild-type tumors. A phenotype of CpG island hypermethylation may also characterize a subset of IDH-mutant glioblastomas, with promoter methylation at numerous loci. Under grade IV gliomas, the WHO 2016 classification added a new subtype: H3F3A or HIST1H3B/C K27M (H3-K27M)-mutant diffuse midline gliomas. They are most common in children and young adults and have an extremely poor prognosis. These tumors were previously classified as glioblastomas, but they are now considered a distinct entity (Tan et al., 2020a). Advances in genomic sequencing are helping to shape personalized treatment for GBM. To create integrated analysis on a shared dataset, The Cancer Genome Atlas (TCGA) has made public genomic databases of more than 20 tumors. In GBM, a *Neurotrophic Tyrosine Kinase Receptor Type 1–Neurophacin* gene fusion was discovered using TCGA RNA-Seq data. This gene fusion boosted the proliferation of 3T3 cells in vitro, implying a carcinogenic role (J. Kim et al., 2014). The utilization of huge databases like TCGA has made oncogenic gene fusion analysis a burgeoning subject. However, the TCGA GBM dataset provides potential prognostic utility in addition to oncogenesis targets (Q. Zhao et al., 2015). The histological grade is critical in determining postoperative management. Adjuvant radiotherapy and/or chemotherapy are used to treat grade III gliomas. Treatment may be postponed in patients with grade II lesions until the disease progresses. Furthermore, patients with IDH mutant astrocytic gliomas and *IDH* and *TERT* promoter mutant oligodendrogliomas had different overall survival rates (Masui et al., 2016).

Some methylated promoters in GBM cause changes in the expression of tumor suppressor genes such as *PTEN*, *pRB*, and *p53*. *O(6)-methylguanine-DNA methyltransferase (MGMT)*, which is seen in 40% of primary GBMs, is one of the important markers for GBM. Silencing of the DNA repair enzyme *MGMT* gene promoter is associated with being a marker of DNA methylation, and *MGMT* predicts a favorable outcome in patients with GBM due to its sensitivity to alkaline chemotherapy agents. The reinstatement of guanine from O-6-methylguanine, the type of genomic lesion induced by alkylating agents used for chemotherapy drugs such as temozolomide, may be explained by the sensitivity of *MGMT* to alkaline chemotherapy agents. Studies have shown that treatments with alkaline agents give more positive results. Poor survival on treatment is associated with unmethylated tumors, confirming the predictive value of *MGMT* promoter methylation for response to chemotherapy in IDH1/2 wild-type GBMs. *MGMT* promoter methylation used as a predictive marker in elderly patients determines the best therapy and inclusion of TMZ. Patients with *MGMT* methylation are divided into those who require only radiotherapy (patients with *MGMT* promoter unmethylated tumors) and those who require TMZ chemotherapy or a combination of TMZ and radiotherapy (patients with *MGMT* promoter-methylated tumors). Treatment with TMZ separately from the detecting *MGMT* methylation in non-elderly GBM patients remains controversial, and patients' pseudoprogression (PsPD) may be beneficial for their *MGMT* methylation status. PsPD appears as an increase in tumor size on radiological imaging with standard (radiotherapy and TMZ) therapy. While not triggering any symptoms, it is found in 91% of patients with a methylated *MGMT* promoter and 41% of patients with an unmethylated *MGMT* promoter (Aldape et al., 2015).

Treatment Strategies

Although there is a lot of information about the molecular mechanism of GBM, there is still no definitive and effective treatment technique due to its localization, complexity, and heterogeneity (molecular subtypes: neural, proneural, classical, and mesenchymal) (Alifieris & Trafalis, 2015b; Bruns et al., 2021). While radiation applied five times a week for six weeks and the daily oral chemotherapy drug TMZ are the most effective standard treatment options after surgical resection, targeting the pathways specified in its molecular mechanism may create better treatment options for GBM. Individualization of treatment will produce the best positive outcome, as treatment depends on several factors, such as time of diagnosis, new onset or relapse, performance status, and age of the patient. Among the chemotherapy agents used in GBM are the combination of carboplatin, irinotecan, carmustine (BCNU), etoposide and procarbazine, lomustine, and vincristine regimen (PCV). Based on the outcomes of this phase II phase of GBM therapy, it has been shown that certain combinations represent an improvement in some GBM inhibitors, such as EGFR, mTOR, and angiogenesis medicines (Alifieris & Trafalis, 2015a; Mao et al., 2012). Surgery is an essential component of standard care as it overcomes many things such as reducing tumor burden, controlling seizures, reversing neurological deficits, introducing local therapeutic agents, and improving quality of life (Kardan & Satter, 2016). Surgical resection is divided into two classes as gross total resections, which is generally recommended, and subtotal resection. Since GBM is a locally invasive tumor, it cannot be completely cured by surgical resection, and is observed that 80% of the disease recurs approximately in seven months (Scott et al., 2011). Patients with a better prognosis may be younger, have a lower tumor volume, and have acceptable functional status prior to surgery (Nam & De Groot, 2017). The effect of surgical resection depends on the location of the tumor in the brain and regions such as the cortex, brain stem or basal ganglia are not

suitable for surgical resection. Such dangerous areas have a negative impact on prognosis (Scott et al., 2011).

Radiotherapy is one of the treatment methods used to destroy the remaining tumor cells after surgical resection and has been found to correlate with the increased median survival rates, especially when GTR (gross total resection) could not be performed. In standard therapy, 60 Gy is given in 2-Gy fractions five times a week for six weeks. Hypofractionated radiotherapy is administered at a biologically equivalent dose of 40 Gy, given in fractions of 2.67 Gy for three weeks, because long-term radiation is not suitable for patients aged 70 years and older with a poor prognosis. Hypofractionated radiotherapy results in better survival when administered with an alkylating agent usually preferred in the first-line therapy, temozolomide (TMZ). In elderly patients with *MGMT* promoter methylation, radiation-free temozolomide alone is used, and re-irradiation is an option in selected situations at relapse (Tan et al., 2020b). Because EGFRvIII upregulates DNA double-strand break repair machinery, imparting cellular resistance to such treatments, EGFRvIII inhibitors may increase overall tumor susceptibility to radiation therapy, which can be an issue in GBM. Gamma knife therapy brings stereotactic high doses of radiation to the targeted GBM area, but it is considered ineffective in the treating primary tumors due to the excessively large tumor volume (Carlsson et al., 2014). Radiation therapy has some limitations and risks associated with its invasive nature, necrosis, permanent neuron damage, and radioresistance (Smith et al., 2001). Recent radiation-based therapies to be evaluated in patients with malignant gliomas include intensity-modulated radiation therapy and boron neutron capture therapy (Norden & Wen, 2006).

Various chemotherapeutic agents have been tested to improve the survival rate of GBM patients and have evolved, mostly with the approval of TMZ, an alkylating agent for newly diagnosed GBM. Apart from TMZ, active alkylating agents such as carmustine (BCNU) and lomustine (CCNU) have also been tested (Alifieris & Trafalis, 2015b). BCNU and CCNU are very cytotoxic and have many side effects. Drugs such as carboplatin, oxaliplatin, etoposide, and irinotecan are known as second-line drugs. Other chemotherapeutic drugs of GBM include anti-VEGF monoclonal antibodies (Bevacizumab), anti-FGF antibodies, monoclonal antibodies targeting EGFR (Erlotinib and Gefitinib), and tyrosine kinase inhibitors (Iacob & Dinca, 2009).

A more effective treatment could not be developed after the standard GBM treatment (TMZ + radiation therapy) was introduced in 2005. The anti-VEGF antibody bevacizumab has been approved by the FDA for recurrent GBM, but survival has not improved in phase III studies. One of the challenging aspects of establishing a treatment for GBM is the physical barrier, the blood-brain barrier (BBB). The blood-brain barrier (BBB) consists of various proteins, including claudins, occludins, and junctional adhesion molecules, which form tight junctions that connect capillary endothelial cells. The BBB only allows the passage of molecules <500 Da and <400 nm, and provides passive diffusion of lipophilic molecules. Other molecules can cross the BBB via pinocytosis, receptor or carrier proteins. BBB and its homeostatic balance are supported with ATP-binding cassette transporters (e.g., multidrug resistance-1 (MDR1), P-glycoprotein, breast cancer resistance protein, and numerous other drug resistance proteins) that are expressed on vessel walls. In high-grade neural cancers such as GBM, the BBB is heterogeneously disrupted. With heterogeneity, tumor vessels form niches with different permeability to oxygen, nutrients, and drugs. GSCs are located in the perivascular hypoxic niches of the brain and are important for cytotoxic therapies. Many drugs do not pass the BBB adequately. For example, the PI3K/AKT/mTOR pathway is one that is activated in approximately 30% of GBMs. However, a certain amount of the developed drugs can pass through the BBB, which makes the treatment unsuccessful. While clinical trials are ongoing for inhibitors of the PI3K pathway, GDC-0068, and GDC-0084 (NCT02430363 and NCT03522298), it is not yet clear whether these failed results are due to poor BBB penetration or tumor heterogeneity (Ou et al., 2021).

Temozolomide (TMZ) is the most preferred oral drug in the chemotherapy of GBM as a pro-drug capable of crossing the BBB with an alkyl group. TMZ initiates apoptosis by adding methyl groups to bases in DNA, but more than half of patients with GBM are resistant to TMZ because they have the O6-methylguanine methyltransferase (MGMT)-based repair system. In this defense mechanism, damaged alkylated guanine nucleotides are repaired by transferring the methyl at the O6 site of guanine to its cysteine residues. Thus, TMZ might fail to kill cancer cells due to elevated DNA repair. In recurring GBM, TMZ also fails as there is acquired resistance (Karachi et al., 2018). The side effects of TMZ, such as toxicity in the blood and nausea, are milder than the side effects of other tested drugs (Chua et al., 2019). TMZ is stable at acidic pH levels, while it is unstable at basic pH levels. It is easily absorbed in the circulation due to its 194 Da weight and spontaneously decomposed to generate monomethyl triazene 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC). Activated MTIC methylates DNA in guanine-rich regions (Denny et al., 1994; Tsang et al., 1990).

In a controlled study for Lomustine (CCNU; chloroethyl cyclohexyl nitrosourea), another FDA-approved drug for the treatment of GBM, the median survival time was noted as 11.5 months. CCNU has been shown to induce apoptosis by cross-linking DNA and RNA, and is currently used for patients with recurrent GBM and patients with the unmethylated MGMT repair system. Its combination with bevacizumab did not provide an extra advantage in terms of patient survival. When the PVC (P: procarbazine, C: lomustine, V: vincristine) treatment determined by the FDA for GBM

was compared with TMZ, survival rates were found to be similar (Fisher & Adamson, 2021).

Carmustine (BCNU; bis-chloroethyl nitrosourea) is another FDA-approved alkylating agent with an average survival of 11.75 months and is used in the treatment of recurrent GBM. In addition to cross-linking of DNA and RNA, it also binds to lutathione reductase, inducing apoptosis. Among the toxicities it shows, the most common ones are pulmonary, ocular, and bone marrow toxicities. It is administered intravenously (dose ratio: 150-200 mg/m2) every 6 weeks. Like carmustine, carmustine wafer implants are FDA-approved for GBM and contain 7.7 mg of BCNU per wafer (8 doses recommended). The aim of the treatment, which is applied directly to the tumor resection cavity, is to reduce toxicity, and it has been observed to significantly improve survival. BCNU wafers are not used as standard care because of their high cost and high complication rates (Fisher & Adamson, 2021).

Bevacizumab (BVZ) received FDA approval in 2009 following favorable outcomes in Phase II trials for the treatment of recurrent GBM. VEGF produced in tumor cells regulates blood vessels and cell growth. BVZ inhibits the binding of VEGF-A to the cell *surface VEGF* receptor tyrosine kinases VEGFR1 and VEGFR2, thereby arresting GBM progression. BVZ shows anti-vascular and anti-edema effects by reducing vascularity (M. M. Kim, Umemura, and Leung 2018). When BVZ and TMZ were compared with TMZ alone, no extra benefit was seen, on the contrary, side effects were increased. In addition, cytotoxic drugs such as etoposide and carboplatin are not FDA-approved, although they show benefits for recurrent GBM when administered with BVZ. BVZ, which is still used to treat symptomatic edema and radiation necrosis, also reduces the need for steroid medications and their negative effects (Fisher and Adamson 2021).

Tissue Engineering Strategies to Study Glioblastoma

Tissue engineering (TE) aims to create new tissue or organ by combining a large number of cells together with biocompatible materials and cell transplantation fields for the treatment of damaged tissue or organ (Duvall et al., 2013a, 2013b; Enderle & Bronzino, 2011). The fields of biomaterials, three-dimensional (3D) printing technologies, nanotechnology, induced pluripotent stem cells (iPSCs), and gene editing technologies (such as clustered regularly interspaced short palindromic repeats, CRISPR) are technologies that TE benefits from and are important for modeling disease and treatment modalities. In this way, organoids and 3D tissue studies are carried out, the control and manipulation of cells in the environment are taken under control, and developments in the field of personalized therapy are experienced. Thus, serious diseases such as cancer became more understandable, and improved treatments were found for many diseases. The use of 3D models is also advantageous in terms of reducing animal experiments (Figure 2) (Chandra et al., 2020).

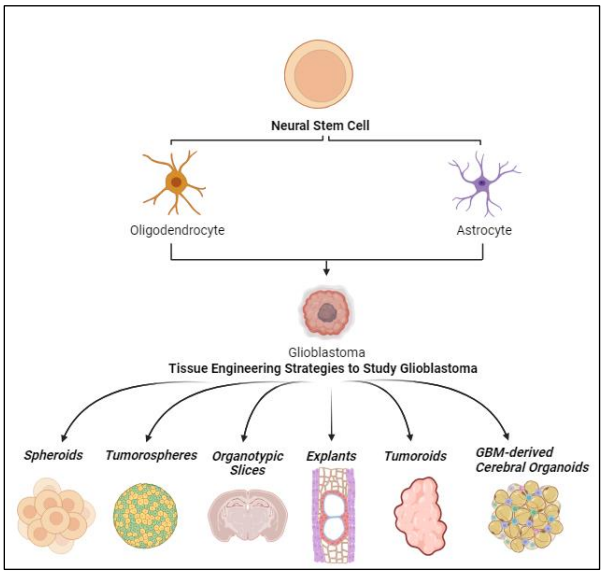

Figure 2. 3D in vitro culture strategies for glioblastoma research.

Numerous novel antineoplastic medications have been used in the realm of cancer biology. The resistance mechanisms of various cancer types have been elucidated, aided by bioinformatics profiling. Additionally, the effectiveness of anticancer drugs has been assessed in vitro using single-layer cancer cell lines. However, the monolayer does not provide a result beyond prediction as to whether the findings will be conclusive information for the clinic. The obvious reason for this is that cells are pulled out of their existing microenvironment, such as the ECM, soluble signals, 3D structure, stromal cells, and disordered microvasculature that surrounds tumor cells *in vivo*. Tumor growth in humans is complex, and the microenvironment of the tumor is better understood in animal models. However, animal models and the results of *in vitro* tests may not provide definitive solutions. In this direction, 3D *in vitro* cancer models have been developed, and the most widely used model is the human tumor spheroid (**Figure 2**). The purpose of using spheroids with features such as cell morphology and gene expression is for preclinical testing of anticancer drugs. Biological gels exhibit the microenvironment of the 3D cancer model to a greater extent than spheroids. Polymer matrices have been used recently and emerged as a technique by which the 3D cancer environment can be better adjusted as a substrate. Polymer matrices 3D development confers treatment resistance to cells in this system (Burdett et al., 2010). In conclusion, being able to mimic the GBM microenvironment is a crucial point for *in vitro* models and the generation of new treatments. The microenvironment of GBM includes astrocytes and oligodendrocytes mentioned in the previous sections, as well as ECM proteins, glucosaminoglycans, soluble signals, and extracellular vesicles that trigger ECM release and cell migration. During tumor formation, low levels of fibrous proteins (collagen, laminin, and hyaluronic acid) are upregulated.

Thus, the ECM concentration rises from 20% to 48% (Bruns et al., 2021).

There are successful 3D models for GBM that have been developed over time. Tumoroids developed in GBM tissue or cancer stem-like cells (CSLCs), organotypic slices developed with cells isolated from the brain or grafting spheroids, cerebral organoids, and tumorospheres emerging with the discovery of CSLCs are among these models (Soubéran & Tchoghandjian, 2020) (Figure 2).

Spheroids

As one of the most widely used 3D models, spheroids are more advantageous in terms of eliminating cost and ethical concerns, and they are a very important need in terms of producing more effective drugs by modeling tumor lesions *in vivo* for cancer treatment. Tumor lesions in suspension spheroids can be obtained with cell culture approaches as well as bioreactor usage. However, the characteristics of the ECM of the native tumor are not similar; however only the morphological and functional features can be mimicked. Matrix-grown spheroids are a more advantageous alternative to suspensions, as they better mimic the microenvironment and stroma and produce more cancer stem cells. Clonal expansion is one of the techniques used in tumor spheroids for drug screening and is formed by dividing a single cell into spheroids over several weeks after immobilization of the matrix (matrigel, polyethylene glycol [PEG], fibrin, etc.). An FDA-approved panel (NCI-60) of 60 cell lines representing cancer types is used in the production of the spheroids. Yet, this does not reflect well the characteristics of primary cells and tumor heterogeneity, resulting in a different cell-matrix interaction. Simple GBM spheroid models that allowed cell-matrix interaction and accurately reflected heterogeneity, as well as the physical and chemical aspects of GBM, provided a wealth of information about treatment responses (Bruns et al., 2021). As an example, U87-originated GBM spheroids multiply quicker in soft gels and their infiltration rises, whereas tumor spheroids' invasive capacity is affected by matrix thickness. In addition, drug response was examined in U87 GBM spheroids encapsulated in fibrin gels and infiltrated into the matrix, and as a result, applied atorvastatin caused decreased infiltration capacity and increased apoptosis. Besides, chitosan/PEG hydrogels have been proven to increase drug resistance more than Matrigel-formed spheroids. Studies have shown that different drug responses can be observed for different GBM subtypes and unique, varying microenvironments (Bruns et al., 2021).

Tumorospheres

Tumorospheres formed by symmetric or asymmetric division by taking advantage of the self-renewal property of stem cells grown by clonal expansion were first developed for normal neural stem cells grown as neurospheres. Surface indicators such as A2B5, L1CAM, integrin6, CD15, CD44, and CD133, which were found to increase in the population of CSLC, have been identified thanks to tumorospheres that enable a better understanding in brain tissue. Tumorospheres have been generated in many different cancer types in the following years, however, a single proven marker to identify CSLCs with sufficient sensitivity and specificity has not proposed yet (Soubéran & Tchoghandjian, 2020; Weiswald et al., 2015). Primary tumorospheres are formed by the mechanical and enzymatic degradation of GBM tissue. Besides cell culture medium, growth factors such as EGF or FGF support the proliferation and maintenance of observed gene expression traits. The various neural cells (neurons, astrocytes, or oligodendrocytes) that are formed after differentiation in the tumorospheres reflect the heterogeneity and organization of tumor cells. While tumorospheres are an important tool for studying CSLC differentiation and migration in GBM, their lack of GBM microenvironment cells is a severe drawback (Soubéran & Tchoghandjian, 2020).

Organotypic Slices

Transferring a 200-400 µm-thick slice of GBM cells or spheroids/tumoroids from a healthy rodent brain into cell culture and supplementing with cell culture media, or neurobasal media yields the organotypic slice model. Organotypic slice models, as they preserve vasculature, reflect heterogeneity better, and allow GBM to be studied in the native microenvironment without tissue thawing or culture migration. Organotypic slices bridge the gap between ex vivo and *in vivo* research, allowing researchers to manipulate tumor cells as well as the brain microenvironment. Depletion of microglia over time can also be utilized to explore the role of immune cells in tumor growth and therapeutic response (Parker et al., 2017; Soubéran & Tchoghandjian, 2020).

Explants

Explants are models created by growing small tumor parts with the logic of placement in conjunction with the cancer cells' surroundings. The quality of the selected tumor fragments is crucial to the explants' success; thus the resected sample with surgery should be cleaned and filtered with phosphate-buffered saline before being cut into pieces and covered with glass coverslips. The cells are cultured on the explant, in addition to cancer cells and CSLCs, as well as vessels, fibroblasts, and immune cells, and begin to migrate after being cultured. While explants are used to detect the proliferation, differentiation and growth of the tumor by preserving the microenvironment, the absence of healthy tissue can be considered as a limitation (Soubéran & Tchoghandjian, 2020).

Tumoroids

Tumoroid models are tumor reconstitutions from a small tumor piece in a culture medium or with

dissociated CSLCs for long-term use. To make tumoroid models, certain culturing procedures are used. Based on MRI imaging, tumors can be formed from multiple tumor areas of the parental tumor. Although their proliferation rate slows down after a few months in culture, tumors can stay stable and viable for more than a year. The success rate of this technique is between 30-90% and can be frozen for later use. Tumoroids also retain tumor features and tissue architecture, as well as glial, ECM material and immune cells such as astrocytes, oligodendrocytes, neurons, fibroblasts, striated collagen fibers, macrophages, and T cells. The tumoroid model constitutes a suitable approach for GBM research because it accurately reflects heterogeneity. The model represents a fast growth rate that allows the identification of mixed cellular responses, and may be used to create tailored assays. However, the disadvantage might be that the results are not reproducible because of the lack of heterogeneity of the microenvironment in tumoroids derived from CSLCs (Soubéran and Tchoghandjian 2020).

GBM-derived cerebral organoids

Human embryonic stem cells (hESCs) and induced pluripotent stem cells can be used to create GBM models obtained from cerebral organoids (hiPSCs) (Soubéran & Tchoghandjian, 2020). Patient-derived primary cultures, xenografts, and genetically modified glioblastomas were used to construct one of the first models to be cultivated organoids utilizing matrigel-based 3D culture methods. The cellular shape of these organoids has been shown to help monitor radiation resistance and GBM metastasis. GBM organoids can also be cryopreserved and define the histological characteristics, cellular diversity, and transcriptional profiles. Patient responses to chemotherapy and tumor development were examined by exposing GBM organoids to several treatment options. The lack of a normal brain microenvironment and vascular system, however, is one of the cons. Neoplastic cerebral organoids (neoCORs) were created to alter cerebral organoids genetically to promote GBM tumor growth. To develop tumors in iPSC-derived brain organoids, CRISPR-based genome editing techniques were applied. GBM organoids are primarily cancer cells, whereas neoCORs are tumors that are formed within cerebral organoids produced from iPSCs. As a result, neoCOR models can be used to study tumors in their early stages. Although its application is limited, CRISPR may be able to broaden its application because it effectively summarizes the heterogeneity of GBM. The glioblastoma cells used to create GLICOs (Glioblastoma Co-cultures) were cultured from patient tumor tissue under defined conditions to promote the maintenance of a stem cell phenotype. Although the GLICO model incorporates the advantages of the GBM organoids and neoCOR models, it suffers from the same flaws as other organoid-GBM models in terms of vascularization and immune cells (Zhang et al., 2020).

CSLCs became a focal point in GBM research after studies on mice revealed that they are more cancer-prone. In the 2D culture medium, primary CSLC spheroids do not properly reflect tumor invasion, microenvironment, interactions, or shape (Rybin et al., 2021). 3D organoid models were used to solve these flaws and provide a better comprehension of the cellular connections of GBM. In 3D organoid models, genetically modified transgenic mice, murine models, and patient-generated xenografts (PDX) are employed. PDX models that accurately reflect patient cancers, such as histological markers and invasiveness, are employed since transgenic models do not portray tumor complexity and heterogeneity well. PDX models using freshly resected tumors or CSLCs cultivated at different stages with a fluorescent marker protein or other genetic alteration accurately mimic 3D growth and GBM phenotyping. Despite these benefits, PDX has drawbacks such as excessive time, cost, and a lack of the microenvironment. To address these issues, some sophisticated organoid-GBM culture systems that are compatible with heterogeneity and microenvironment have been designed. Lancaster et al. were the first to try to make a cerebral organoid from hiPSCs or hESCs by producing embryoid bodies (EBs). The ectoderm germ layer is found in neural tissue, and EBs with neuroectoderm development were cultivated and placed in matrigel to achieve organoid structure. Next, the bioreactor was used to preserve and mature the organoid oxygen and nutrient absorption. Cerebral organoids differentiated over a period of 1-2 months and formed different parts of the brain. The progenitor region has been demonstrated by immunofluorescence staining using the neuron-specific class III beta-tubulin (TUJ1), which is used as a marker of neurons in the central and peripheral nervous systems from the early stage of neural differentiation, and the sex-determining region Y box 2 (SOX2), a well-known marker of neural stem and progenitor cells, and their function is self-renewal of these cells (Sun et al., 2021).

Cerebral-derived organoids can be used to examine the chronology of mutational steps, to investigate the developmental natural history of cancer ex vivo, to make more general analyses such as tumor proliferation, invasion and progression, as well as to investigate the interactions between tumor cells and non-neoplastic cells because they are cultured in the same culture dish. Due to the paucity of vascular structures and other cells in the microenvironment, these models lack histological characteristics such as microvascular growth and necrosis that are typical of GBMs (Soubéran & Tchoghandjian, 2020).

Discussion

GBM is the most common fatal brain tumor of the central nervous system, known for its poor prognosis and survival rate. GBMs, which can be pathologically primary and secondary, are derived from astrocytoma

or oligodendroglioma. This grade IV tumor is more likely to occur with age, and the average survival time is 15 months (Rock et al., 2012). GBM stem cells are difficult to treat as they enable more mutations to occur, and thus maintaining resistance to therapy and exhibit active DNA repair and regeneration properties (Stoyanov et al., 2018b). The classification by WHO as IDH-wild-type (90%) and IDH-mutant (10%) with a better prognosis was defined in 2016 (Batash et al., 2017). The typical treatment for this condition is TMZ and radiation, with MRI and CT used for diagnostic and treatment monitoring (Ali et al., 2020).

Looking at the molecular mechanism of GBM, *EGFR* amplification and *TERT* promoter mutations are found in 70% of IDH-wild-type tumors (Brennan et al., 2013). *PTEN* and *p53* mutations have also been found (Zheng et al., 2008). *PTEN* mutations are seen in 5-40% of GBMs, and they're more common in patients over 45 years old (Srividya et al., 2011). *PTEN* mutations decrease autophagic induction by activating the *PI3K/AKT/mTOR* pathway (Benitez et al., 2021). *Retinoblastoma (RB)* gene is mutated in most other cancers but only 6%–11% in GBM has been observed; therefore, therapy for *RB* mutations is not a common path. Furthermore, RTKs that enhance cancer cell aggressiveness, such as *MET, FGFR,* and *AXL*, or increased levels of growth hormones like VEGF, which promote the growth of GBSC-derived tumors, also raise the risk of GBM formation (Batchelor et al., 2013; Li et al., 2011). *TGF-β*, a tumor suppressor gene that regulates numerous biological processes and phosphorylates the prognostic Smad family protein, boosts the expression of *PDGF*, which controls the cell cycle, and ECM, which governs gene expression (Frei et al., 2015). Clinical trials, however, have failed due to the toxicity of TGF- β receptor inhibitors (le Rhun et al., 2019). Up-regulation of STAT3 protein from the STAT protein family, *IDH1* and *IDH2* mutations are also seen in the molecular mechanism of GBM. *MGMT*, which is found in 40% of GBMs and induces alterations in the expression of tumor suppressor genes like *PTEN, pRB,* and *p53*, is clearly one of the most important targets in GBM therapy (Aldape et al., 2015).

GBM is challenging to treat because of its various molecular subtypes and complexity. Although the outcome of treatment is dependent on several aspects such as the time of diagnosis and the patient's resistance, establishing a treatment is extremely challenging (Alifieris & Trafalis, 2015b). Although surgical resection is indicated in people under the age of 70, the tumor's location may influence the resection possibility (Gilard et al., 2021; Scott et al., 2011). Radiotherapy is used to eradicate residuals following surgical resection; however, it is not indicated for people over the age of 70 (Tan et al., 2020b). In addition to these treatments, various chemical compounds that might impact the GBM molecular mechanism have been explored. TMZ, CCNU, BCNU, and BVZ are FDA-approved and clinically available drugs. TMZ is the most favored chemotherapeutic drug in GBM treatment, notwithstanding its resistance to GBMs with *MGMT* activity (Karachi et al., 2018).

TE is a discipline that uses biocompatible treatments, including 3D printing biomaterials, nanotechnology, iPSCs, and gene editing approaches, to better understand and treat the disease. Although TE studies are beneficial because they minimize animal experiments in 3D use, make many cancer mechanisms more understandable, and the 3D structure more closely resembles the microenvironment of the real structure than cell culture, 3D mimicry of complicated tumors is extremely difficult (Burdett et al., 2010; Chandra et al., 2020). Many 3D models have been generated as a result of TE, including tumoroids, spheroids, organotypic slices, cerebral organoids, and tumorospheres, which are all commonly utilized in GBM treatment (Soubéran & Tchoghandjian, 2020).

Spheroids are a low-cost and morally favorable 3D model for preclinical testing of platinum-based antineoplastic medicines. Matrix-grown spheroids better reflect the microenvironment than suspension spheroids (Soubéran & Tchoghandjian, 2020). Using three different techniques: hanging drop, liquid overlay, and suspension culture, Froehlich et al. attempted to create tumor spheroids in three different mammary cell lines. According to the research, the hanging drop spheroid creation methodology is the preferred way since pellet formation in the liquid overlay technique is dependent on the kind of well, and the suspension culture technique results in spheroid size variance (Froehlich et al., 2017). In another study, a spheroid model of HA was co-cultured with tumor and healthy pancreatic cells in another investigation, because HA is known to be increased in tumors. As a result, the rate of cancer cell migration in the spheroids increased, and the cells became more sensitive to pharmacological treatment (Wong et al., 2019). Tumorospheres, another model for neural stem cells based on the self-renewal ability of stem cells, began to be developed and gave a better knowledge of CSLCs (Weiswald et al., 2015). In the research conducted by Zhao and colleagues, an elevated CLSC rate was observed when lung CLSCs were cultured to evaluate their lung cancer tumorosphere capacity. Additionally, lung tumorospheres demonstrated increased levels of proliferation, invasion, and drug resistance. In a prior in vivo study of GBM, CD133, a marker for neural stem cells, was similarly found to be elevated in tumorospheres (Salmaggi et al., 2006; W. Zhao et al., 2016). However, the other 3D model, organotypic slices, effectively captures the heterogeneity of GBM but does not fully replicate the tumor microenvironment (Soubéran & Tchoghandjian, 2020). The study of Marques-Torrejon et al. utilized organotypic slices, a method developed because completing the GBM model with *in vivo* transplantation is time- and money-inefficient. In this study, starting from the subependymal region where CLSCs are located, it has been shown that human CLSCs can be grafted into the mouse subependymal region. CD9 was also found to

be coupled with CD133, an astrocyte marker that has been shown to be raised in earlier research. As a result, distinct tumor behaviour in different brain regions have been mentioned. Organotypic slices, on the other hand, cannot be preserved for more than 3 weeks and can activate their immunity (Angeles et al., 2018). On the one hand, the approach of Sidorcenco et al. established an ex vivo GBM tissue slice tandem co-culture to test particular inhibitors. This method avoids the use of animals by using organotypic tissue fused with a tumor in the host microenvironment and entire tumor tissue from mice xenografts. For future GBM analysis, this study is preferable to spheroids (Sidorcenco et al., 2020). Explant modeling is made by culturing selected high-quality tumor cells and is utilized to detect parameters such as microenvironment, tumor profile, and differentiation; however, it lacks healthy tissue (Soubéran & Tchoghandjian, 2020). Because of the considerable molecular alterations occurring in GBM and the recent importance of CLSC vasculature in tumor growth, it does not adapt data from cell culture to the patient. A new 3D explant system was established in a study that enhanced the explant procedure by keeping the original structure of the tumor components, and it was highlighted that this system considerably improved the cytoarchitecture of the tumor stroma (Shimizu et al., 2011). Tumoroids and GBM-derived from cerebral organoids models are very popular because they accurately depict tumor features (Soubéran & Tchoghandjian, 2020). The heterogeneity of GBM makes it challenging to treat, as current *in vitro* models struggle to sustain mutational variety. GBOs (glioblastoma organoids) can be generated by analyzing the parent tumors particular to each patient and taking an intrusive approach to transplantation. They also underline that a biobank is required for this to be more basic (Jacob et al., 2020). Tatla et al., on the other hand, created an *in vitro* vascularized tumoroid model in order to study GBM angiogenesis. The model consists of a fibrin gel filled with easily produced and cost-effective endothelial cells (HUVEC) and GBM. Despite the model's lack of vascularity and BBB, the complexities of angiogenesis were accurately summarized, and CLSCs were discovered to enhance angiogenic sprouting when cultivated (Tatla et al., 2021).

Herein, a fatal primary brain tumor GBM and the possible TE applications are summarized. Many 3D models used are still under development and have provided important information about GBM. Organotypic slices reflect heterogeneity well, explants reflect many aspects of GBM, and tumoroids imitate the milieu well. Models constructed in the matrix, such as spheroids or tumorspheres, better reflect the 3D structure, giving us information about tumor spread. The development of these 3D models and the discovery of a treatment will take time. The ability of tumoroids to imitate the microenvironment, which is one of the most attractive 3D applications, as well as newly developed advanced models (for example, Organ-on-a-Chip and Four-Dimensional Bioprinting), may lead to increased interest in this sector.

Conclusion

GBM is a lethal primary brain tumor that has a poor prognosis and no treatment. The heterogeneous nature of GBM, its tendency for mutation, and the fact that it does not manifest itself in the same way in every patient make it challenging to develop a standard treatment. There are numerous studies on GBM treatment accessible. Tumoroids, spheroids, organotypic slices, cerebral organoids, scaffolds, organ-on-a-chip, and tumorospheres are examples of TE structures that have lately been used to better comprehend complicated diseases like cancer. The use of preclinical 3D models in these 3D approaches allows researchers to learn more about the GBM microenvironment by reducing *in vivo* approaches.

Author Contributions

MK: Investigation, Methodology, Writing; POY: Supervision, writing, review and editing.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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