REVIEW ARTICLE

C6 cell line: the gold standard in glioma research

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Abstract
Background: Glioblastoma multiforme is the most aggressive brain tumor with poor prognosis and an average survival of 1-2 years. Animal models that simulate the features of human glioma are the key to newer agents or therapeutic strategies. In order to establish such models, the C6 glioma cell line has been mostly used in neuro-oncology research.
Methods: In this narrative review, we systematically reviewed the international literature in order to retrieve and present the most important biological and molecular features of C6 cell line.
Results: Even though many cell lines have been developed, each cell line presents with slight differences from human glioma behavior. C6 cancer cell line is a rat glioma cell line, which can simulate in overall the high growth rate, the high vascularization, and the highly infiltrative character of glioblastoma multiforme.
Conclusions: Most of the C6 glioma research has been focused on testing a wide diversity of agents for their tumoricidal activity. C6 cell line is considered to be a safe and popular glioma model in the literature, providing a good simulation of glioblastoma multiforme. HIPPOKRATIA 2018, 22(3): 105-112.

Keywords: C6, cell line, rat glioma model, glioma, glioblastoma multiforme

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Introduction

Gliomas are tumors that derive from glial cells and are the most common tumors of the central nervous system (CNS). The classification of CNS tumors has recently been updated in the 2016 World Health Organization (WHO) classification, which is based on the type of the primary cell along with histological and molecular characteristics[1]. The most aggressive glioma tumor is glioblastoma multiforme (GBM), which has a poor prognosis with an average survival of 1-2 years depending on the isocitrate dehydrogenase (IDH) status[1]. Glioblastomas exhibit high growth rate, high vascularization, and are considered to be highly infiltrative. Unfortunately, in the last years, there has been only a few therapeutic advances on gliomas[2]. Even though researchers have proposed several molecular pathways and a variety of therapeutic targets, there was no success in the clinical trials[3,4]. This failure points out that as existing molecular knowledge for glioma tumors advances, it becomes clearer that in order to study innovative therapies, appropriate and predictive animal glioma models are of great importance. Animal glioma models are generated from glioma cell lines that are tumorigenic to laboratory animals and can simulate the fundamental biological properties of human gliomas. The key to a successive glioma model is the proper choice of a cancer cell line.

Methods

This review aims to present an update on the C6 glioma cell line and discuss newer therapeutic applications and effects using C6 glioma rat model. Therefore, we searched the Medline (PubMed) for C6 rat glioma model articles in English related to therapeutic applications in terms of tumor growth and proliferation, invasiveness, migration, immunogenicity, angiogenesis, and genetic profile published from 2000 to 2018. The following keywords were used in advanced search: (glioma models OR cell lines) AND rat AND C6 and the results were sorted by the most recent. We proceeded in discussing the genetic, morphologic, and angiogenic profile of C6 cell line, presenting newer aspects of its profile and the most recent therapeutic applications. Finally, we investigated the characteristics of C6 cell line that constitute it as the best glioma model for studying GBM. To answer these topics, a narrative but comprehensive review with systematic intent was conducted and is presented. The flow chart of the recovered and analyzed studies from PubMed is shown in Figure 1.

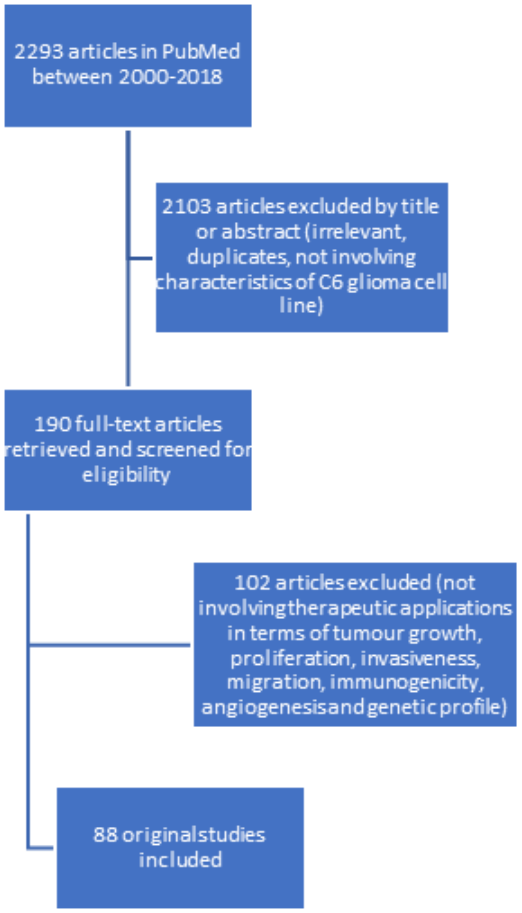

Figure 1: Flow chart of the recovered and analyzed studies in PubMed regarding articles involving therapeutic applications in terms of tumor growth, proliferation, invasiveness, migration, immunogenicity, angiogenesis, and genetic profile, focusing on literature of the period 2000-2018.

Results

Genetics

C6 cell line was developed in adult Wistar-Furth rats in the late 1960s after the rats were repetitively exposed to *N*-Nitroso-*N*-methylurea[5]. This glioma cell line is composed of pleomorphic cells with variably shaped nuclei. Genetically, the cells are reported to have a wild type of p53 gene, an increase in the expression of the Rb gene and mutant p16/Cdkn2a/Ink4a locus but without the expression of p16 and p19ARF mRNAs[6]. They also overexpress the same genes that are expressed in human gliomas: the PDGFβ, insulin-like growth factor (IGF)-1, epidermal growth factor receptor (EGFR), and Erb3/Her3 precursor proteins[7,8]. Furthermore, there is a reduced expression of IGF-2, FGF-9, and FGF-10, while there is no change in the expression of MMP-7 gene. As human gliomas exhibit increased activity of genes of Ras pathway[9], C6 cells also exhibit upregulation of Ras pathway. Nevertheless, increased expression of Ras guanine triphosphate activator protein keeps the Ras pathway under control. In addition, increased expression of TGFα precursor was also reported[10].

Mutations in genes encoding IDH1 and IDH2 in gliomas have been linked to patient's prognosis[11], but they are not detected in C6 cells[12]. However, researchers have proved that artificial mutagenesis of IDH2 in C6 cells increases their sensitivity to chemotherapy and promotes cell migration and tumor growth[12,13]. IDH2 mutated C6 cells could be a new promising proliferation and migration glioma model for the development of new agents[14,15]. Cell adhesion and signal transduction are essential features of tumors, regulated by cell surface antigens. CD9 is a cell surface antigen that is typically expressed in the myelin sheath of nerves. Its increased expression is found in high-grade gliomas, and it has been proposed as a marker for the degree of glioma malignancy. In C6 glioma cell line, CD9 has a significant increase[10]. Finally, it should be noted that there is a sub-clone of C6 cell line that expresses β-galactosidase marker protein, which acts as a tumor antigen. Even though this marker protein can help at *in vivo* immunohistochemical analysis of C6-derived tumors, it must be taken into consideration that immunization of rats against the gene of β-galactosidase can protect against tumor growth[16].

Morphology and mechanisms of development

C6 cells are spindle-like cells that simulate human GBM when they are injected in the brain of neonatal rats[17,18]. Glioma models have been developed in Wistar rats[18,19] and exhibit the same histological features as human GBM, such as foci of tumor necrosis, nuclear polymorphism and high mitotic index[17,20,21]. The main histological differences are that C6 does not express glial fibrillary acidic protein (GFAP), whereas vimentin is variably expressed[22]. The tumor doubling times can be evaluated by experimental volume data after fitting in a modified Gompertz function[20]. The brain tumor C6 model has been shown to occur as early as 5-7 days post-implantation after magnetic resonance imaging (MRI) tumor detection and growth monitoring with volumetric analysis[23-25]. Even though immune microenvironment of C6 gliomas resembles that of a human GBM[21], the cell line is capable of producing an immune response in Wistar and BDX (inbred rat strain X) rats[26], and therefore, it cannot be used for assessing immunotherapy. However, several studies report significant tumor growth ranging from 70 % to 91 % after the implantation of C6 in Wistar rats[19,27]. Initial assessments on C6 implantation in Long-Evans and Sprague-Dawley rats do not support simulation of a human GBM model. In these animals, C6 formulates in most cases a rounded and well-demarcated brain tumor without evidence of parenchymal invasion resembling more in brain metastasis than GBM[28,29]. In general, C6 glioma model has been used to study several biological features of brain tumors, such as tumor growth, tumor invasion and migration, angiogenesis, growth factor production and regulation, and blood-brain barrier disruption[30-34].

C6 glioma cells invade and migrate in cerebral cortex post-implantation by attaching to the endothelial base-

ment membrane[18,35]. This route of migration resembles the human xenografted cell lines in a rat brain, and it simulates the migration of malignant gliomas in humans[36]. Tumor invasion is achieved through the degradation of the basement membrane as well as of the extracellular matrix. Tumors can be developed due to the invasion of a single C6 cell into the surrounding brain tissue, depending mainly on metalloprotease activity, but not on cell proliferation itself[37,38]. Metalloprotease activity includes factors that comprise a family of endopeptidases that are metal ion-dependent. They are responsible for the degradation process but are also needed for angiogenesis. Orthotopic C6 brain gliomas have been found to exhibit high amounts of matrix metalloprotease (MMP) proMMP2 and its activated form, which is usually found only in tumoral brain tissue[39]. Activated MMP2 is detected as part of collagenase activity and the basement membrane degradation process[40]. The matrix metalloprotease activity of C6 cells can transform CNS myelin into a substrate for cell migration[41]. Myelin degradation enables C6 cells to invade and migrate through white matter; this process is attributed to membrane type 1 MMP (MT1-MMP), found on the cytoplasmic membrane of the C6 cells[42]. Furthermore, other molecules, which are overexpressed in C6 cells, play an essential role in the invasion and particularly in the adhesion of the C6 cells at the surrounding tissue. These include the intercellular adhesion molecule (ICAM) and the cell surface antigen CD9, which is usually found in the myelin sheaths[43-45].

Angiogenesis in C6 derived tumors

The development of the C6 tumor is also associated with the vascular status of the C6-derived gliomas. The lack of oxygen in the center of the tumor is an essential factor of neovascularization. C6 cells secrete several angiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)[46,47], that contribute to tumor growth via tyrosine kinase receptors and MMPs, respectively[48,49]. C6 implanted cells present with several growth phases related to the vascular status of the tumor. Therefore, there is the lag phase, the proliferative phase, and the exponential phase of tumor growth, which is associated with the vascularization process[50]. The latter includes three stages: the avascular, the early vascular, and the late vascular stage. During the vascularization process, MRI and pathology study of an orthotopic C6 glioma model in Sprague-Dawley rats revealed four patterns of neovascularization. Two of them are found inside the tumor and include: i) splitting angiogenesis, also known as intussusception angiogenesis, in which the extent of the capillary wall inside the lumen splits the single vessel in two, and ii) sprouting angiogenesis, in which activated endothelial cells of the existing vessels release proteases that degrade the basement membrane and allow the endothelial cells to escape their site and proliferate into the surrounding matrix in a tandem way. The third pattern refers to vascular co-option found in the tumor margin, and the last is vascular mimicry, which is recognized in the surrounding necrotic area[51].

Discussion

The ideal glioma model should be similar to human GBM in terms of morphological characteristics, its invasive pattern and ability, its vascular behavior, and its immune microenvironment. There is a plethora of cell lines that simulate human GBM and are used in research. The most commonly used include human-derived cell lines such as U251 and U87, murine cell line GL261, and rat cell lines 9L/LacZ, F98, RG2, CNS-1, and C6. U251 and U87 are xenograft models that can be developed only in immunocompromised rodents, whereas the rest cell lines can be developed in immunocompetent syngeneic models. This restriction constitutes human-derived lines inadequate for a tumor-immune microenvironment study. All models exhibit similar morphological characteristics except 9L/LacZ and F98, which resemble gliosarcoma and anaplastic glioma respectively. All models share similar to GBM nuclear pleomorphism and high mitotic index, while F98 and 9L/LacZ present a low percentage of tumor necrotic foci and C6 moderate. The most aggressive and invasive models are RG2, F98, and 9L/LacZ, whereas C6, GL261, and CNS-1 have a moderate invasive ability and poor invasiveness is a feature of human cell lines U87 and U251[52]. Each glioma model is able to create its own vascular network with vessels of different length and diameter. All models exhibit high neovascularization but either by neovascularization or recruitment of existent vasculature. U87 has been shown to exhibit profuse neovascularization and has been widely used to study GBM angiogenesis[53]. Half of the cell lines mentioned above can cause an immune response of the host. The most vigorous immune response is reported in 9L/LacZ, whereas C6, U87, U251 cause a moderate response. Non-immunogenic cell lines are considered to be GL261, CNS-1, F98, and RG2. However, when a study of the tumor-immune microenvironment is of need, C6 is well studied and resembles human GBM immune infiltrates[21]. A series of markers and most common genetic mutations complete the profiling of the cell lines. S100 protein is expressed in all cell lines except U87, F98, and RG2, whereas GFAP is expressed in U251, GL261, CNS-1, and F98. A wild type p53 can be found in C6, U87, F98, and RG2, while EGFR overexpression is a common feature in all cell lines except U87 and RG2. A summary of the most important characteristics of the above cell lines is presented in Table 1.

The C6 rat glioma model is one of the commonest experimental models used in neuro-oncology in order to study the growth and the invasion of high-grade gliomas. A recent MRI and magnetic resonance angiography (MRA) *in vivo* study has reported that C6 resembles human GBM better than other rodent glioma models[20,54]. The most common host of C6 cell line for an *in vivo* study is immunocompetent Wistar rats, but other species such as Sprague-Dawley and Long-Evans have also been used. However, Long-Evans rats have not been extensively used in glioma models, and studies using this species are very scarce in the literature[55,56]. On the contrary,

Table 1: A comparative profile of the most important features of the commonest used in research cell lines.

Profile	C6	U251	U87	GL261	9L/LacZ	CNS-1	F98	RG2
Invasiveness	moderate	low	low	moderate	high	moderate	high	high
Nuclear pleomorphism	+	+	+	+	+	+	+	+
High mitotic index	+	+	+	+	+	+	+	+
Foci of tumour necrosis	moderate	high	high	high	low	moderate	low	high
Angiogenic	high	high	high	high	high	moderate	high	moderate
Immunogenic	+	+	+	-	+	-	-	-
GFAP	-	+	-	+	-	+	+	-
S100	+	+	-	+	+	+	-	-
Vimentin	-	+	+	variable	N/A	+	+	+
Syngeneic model existance	+	-	-	+	+	+	+	+
$p14^{ARF}$ mutation	-	+	+	+	-	N/A	N/A	N/A
p16 mutation	+	+	+	+	-	N/A	+	+
PTEN mutation	-	+	+	+	-	N/A	N/A	N/A
p53 mutation	-	+	-	+	+	N/A	-	-
kRAS mutation	N/A	+	+	+	N/A	N/A	+	+
EGFR overexpression	+	+	-	+	+	N/A	+	-

GFAP: glial fibrillary acidic protein, PTEN: phosphatase and tensin homolog, EGFR: epidermal growth factor receptor, +: presence, -: absence, N/A: not available.

although it has been primarily reported that implantation of C6 in Sprague-Dawley rats does not produce a similar invasion pattern as human GBM[28,29], recent studies state otherwise and have used this model to investigate tumor growth or the extent of resection[57,58]. C6 glioma model has been used to test variable treatment modalities, including newer and more effective drugs against glioma, radiation therapy, photodynamic therapy, or even gene therapy[59-62]. Despite the fact that it has been extensively used, allogeneic proteins of the major histocompatibility complex (MHC) found in the C6-derived tumors can cause an immune response. These proteins are up-regulated in the C6 glioma model and simulate a therapeutic response[63]. The immunological reaction is present in both intracranial C6 glioma models and subcutaneous flank C6 models of Wistar rats[26]. Even though the cell implantation protocol affects tumor formation, allogenicity is the main problem for low rates of tumor intake. Nevertheless, it has been documented that C6 presents a similar composition of the immune infiltrates to human GBM. The invasion and immunosuppression-related genes in C6 gliomas produce similar immune evasion pattern as in human GBM. Therefore, C6 glioma model is considered to be a good model of an immunocompetent host for in vivo studies[21] and has been widely used in research studying tumor growth and invasion as well as anti-tumor drug effectiveness (Table 2).

C6 glioma model has been proved to express a diversity of proteins, growth factors and/or their receptors, which constitute targets for tumor research. Among most popular targets are angiogenic factors such as VEGF and bFGF and its receptors vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR) respectively[80,81]. Other factors targeted are platelet-derived growth factor and its receptors platelet-derived growth factor receptor (PDGFR)[82], that regulate cell growth and division, and factors that stimulate cell growth and differentiation such as epidermal growth factor (EGF) and its receptor EGFR[83]. More complexed targets are IGF and its receptors[84] that are responsible for cell proliferation and inhibition of cell death.

Tumor growth inhibition remains the primary target of C6 glioma model research. Several popular drugs such as ibuprofen, dopamine, and aspirin have been tested on such models. The first two have succeeded in that direction[64,74], whilst aspirin reduced the glioma invasion[85]. C6 glioma models have also been a preferred model for experimental therapies with nanoparticles. Transferrin (Tf)-modified polyethylene glycol-polylactic acid (PEG-PLA) nanoparticles conjugated with resveratrol as well resveratrol-loaded lipid-core nanocapsules have been shown to reduce tumor growth[67,68]. Furthermore, nanoparticles with a fusion protein derived from factor VII facilitated anti-glioma delivery of paclitaxel. In that way, they targeted both neovascular and glioma cells leading to cell apoptosis and tumor necrosis[70]. Combined therapeutic approaches have also been tested against C6 glioma models. Hyperbaric oxygen, as well as photodynamic therapy, have been used against glioma, in combination with temozolomide, which is an approved by The Food and Drug Administra-

Table 2: Most prominent therapeutic applications and effects using C6 glioma model.

Therapy	In vitro/ vivo	Experimental design	Effects	Reference
Ibuprofen	In vivo	C6/LacZ rat glioma cells into the Wistar rats brain – two treatment groups	Growth inhibition	Dagestan et al. 2012[64]
Hyperbaric oxygen (HBO) and temozolomide (TMZ)	In vivo	stereotactic injection of C6/LacZ rat glioma cells into the Wistar rats brain – treatment with HBO, TMZ and a combination of them. Intra-/peri-tumoural vessels, microendothelial proliferations, immunohistochemistry and necrotic area, were evaluated.	Growth inhibition	Dagistan et al. 2012[65]
Serine protease urokinase plasminogen activator (uPA), and matrix metalloproteases (MMP-2 / MMP-9)	In vivo/in vitro	Determination of MMP-2, MMP-9, uPAR and uPA in the tumour core and of infiltration zone in vitro C6 glioma cells and in an in vivo orthotopic C6 glioma model in Sprague Dawley rats	Growth and invasion inhibition	Schuler et al. 2012[66]
Resveratrol-loaded lipid-core nanocapsules	In vitro/in vivo	RSV-LNC (5 mg/kg/day, i.p.) for 10 days in rats with orthotopic C6 tumours	Growth inhibition	Figueiró et al. 2013[67]
Transferrin (Tf)-modified poly ethyleneglycol-poly lactic acid (PEG-PLA) nanoparticles conjugated with resveratrol	In vivo/in vitro	Tf-PEG-PLA-RSV administered in vitro and in vivo in C6 orthotopic glioma model of Wistar rats	Growth inhibition	Guo et al. 2013[68]
Diruthenium-Ibuprofen compound	In vivo	The compound was tested in the rat C6 orthotopic glioma model in vivo	Growth inhibition	Benadiba et al. 2014[69]
EGFP-EGF1-conjugated nanoparticles (ENPs)	In vitro and in vivo	Balb/c mice –nanoparticles with a fusion protein derived from factor VII facilitate anti-glioma delivery of paclitaxel by targeting both neovascular and glioma cells	cell apoptosis and tumour necrosis	Zhang et al. 2014[70]
diruthenium-GLA complex (Ru2GLA)	In vivo/in vitro	Administration of Ru2GLA in an orthotopic C6 glioma model in Wistar rats	C6 cell proliferation in vivo and the changes in tumour morphology	Miyake et al. 2014[71]
Photodynamic therapy (PDT) and temozolomide	In vivo	The expression of P-glycoprotein (P-gp) in endothelial cells was investigated after treating glioma bearing Wistar rats with temozolomide, PDT or a combination of them	Growth inhibition	Zhang et al. 2014[72]
Resveratrol	In vivo / in vitro	Oral administration of resveratrol in orthotopic glioma model of Wistar rats. The expression of EGFR, GFAP, PCNA, MMP-9, NF-κB, COX-2 and VEGF was investigated	Growth inhibition	Wang et al. 2015[73]
Dopamine	In vivo	reprogramming M2-polarized macrophages	Growth inhibition and vascular normalization	Qin et al. 2015[74]
Dimethylaminomicheliolide (DMAMCL)	In vivo/in vitro	Oral administration of DMAMCL in a subcutaneous glioma model in Wistar rats	Growth inhibition	An et al. 2015[75]
Lapachol	In vivo / in vitro	Intragastric administration of lapachol in Wistar rats with C6 orthotopic glioma model. Proliferation, apoptosis, DNA damage, topoisomerase I (TOP I) and topoisomerase II (TOP II) activities were detected	Growth inhibition, possibly through inhibiting TOP I and TOP II expression	Xu et al. 2016[76]
Anti-vascular endothelial growth factor receptor-1 monoclonal antibody	In vivo	Influence of D16F7 on glioma growth and angiogenesis in vivo using C6 glioma cells transfected with the human VEGFR-1	Growth inhibition and anti-angiogenetic effect	Atzori et al. 2017[77]
Flavonoid FLA-16	In vivo / in vitro	Intraperitoneal administration of FLA-16 in an intracranial and subcutaneous C6 glioma model in Wistar rats or BALB/c nude	Growth inhibition through CYP4A inhibition by flavonoid FLA-16. Normalization of tumour vasculature through down-regulation of TAMs and EPCs-derived VEGF and TGF-b via PI3K/Akt signaling	Wang et al. 2017[78]
Lactoferrin modified daunorubicin plus honokiol liposomes	In vivo / in vitro	Action mechanism studies were performed on BBB model, brain glioma cells and glioma-bearing mice	Growth and invasion inhibition	Liu et al. 2017[79]

HBO: Hyperbaric oxygen, TMZ: temozolomide, uPA: urokinase plasminogen activator, MMP: matrix metalloproteases, RSV-LNC: resveratrol-loaded lipid-core nanocapsules, Tf: transferrin, PEG-PLA: modified polyethylene glycol-polylactic acid, ENPs: EGFP-EGF1, Ru2GLA: diruthenium- gamma-linolenic acid complex, PDT: photodynamic therapy, P-gp: P-glycoprotein, GFAP: glial fibrillary acidic protein, PCNA: proliferating cell nuclear antigen, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, COX-2: cyclooxygenase-2, VEGF: vascular endothelial growth factor, VEGFR-1: vascular endothelial growth factor receptor-1, DMAMCL: dimethylaminomicheliolide, TOP: topoisomerase, FLA-16: flavonoid-16, CYP4A: cytochromes P450 family, TAMs: tumor-associated macrophages, EPCs: endothelial progenitor cells, TGF-b: transforming growth factor beta, PI3K/Akt signaling: phosphatidylinositol-4,5-bisphosphate 3-kinase/ protein kinase B signaling, BBB: blood-brain barrier.

tion (FDA) chemotherapeutic agent for glioblastoma[65,72]. These combinations have shown to augment growth inhibition of gliomas. Among newer therapeutic techniques, clinical research offers gene therapy. C6 glioma cells have been used to explore the limits of gene therapy in glioblastomas. An example of gene therapy was the introduction of INF-γ gene in C6 cells by retroviral delivery, which led to tumor growth inhibition by B- and T-cells activation and by inhibition of angiogenesis[86].

Another promising field of research is that of chemokines and their receptors that are involved in proliferation and migration of glial precursor cells. Chemokines are also involved in tumor metastasis, tumor growth and progression[87,88]. More specifically, tumor research has shown great interest in the chemokine CXCL12 and the axis CXCL12-CXCR7[89]. CXCL12 chemokine is found in necrotic areas, as well as in areas of neo-angiogenesis, and are responsible for the proliferation of glioblastoma progenitor cells[90]. CXCR7 can be found in capillaries of the neo-angiogenetic tumor tissue of human glioblastoma[91]. Furthermore, CXCR7 is localized in the adult rat brain and particularly in astrocytes, and Schwann cells[92]. It is noteworthy that the axis CXCL12-CXCR7 has been proven not only to mediate migration but also to reduce apoptosis induced by the chemotherapeutic agent temozolomide[91,93].

Additionally, recent studies have highlighted the potential role of activated macrophages and microglia in glioma development. The endothelial monocyte-activated polypeptide II (EMAPII) is a cytokine expressed by macrophages and microglia. It plays a role against angiogenesis and acts as a pro-inflammatory cytokine[94]. It is also responsible for the activation and infiltration of macrophages and induces endothelial apoptosis. ED1 is a lysosomal protein found both in macrophages, who underwent phagocytosis and microglia. Another marker which is observed in several CNS pathologies such as ischemia and Wallerian degeneration is CD8[95,96]. The early accumulation of the aforementioned markers was found in C6 rat glioma models[97], and this could prove a promising research field for studying either the development of the glioma or test new tumoricidal agents in gliomas.

Conclusions

C6 cell line is quite popular within glioma research. It has been widely used in order to establish a rat glioma model, which simulates human glioblastoma. Even though literature supports low tumor development rates in rats and mice due to C6 allogenicity, literature is also very rich regarding *in vivo* studies, as this review demonstrated. C6 cell line gives the advantage of a syngeneic model without the need of immunocompromised rodents in comparison to xenograft models. It can produce a highly angiogenic, invasive glioma model with distinct peritumoral environment altering pre-existing vasculature for its needs and with many of the human GBM morphological characteristics. It can be developed as an orthotopic model in rats which offers easier MRI study investigation in comparison to murine models due to the size of the animal. MRI and MRA studies have shown the superiority of C6 cell line in GBM similarity in comparison to other rodent models. Furthermore, its expressed markers facilitate an immunohistochemical investigation as in other rat cell lines, but its genetic profile resembles better human GBM than the rest rat glioma models. Finally, C6 is preferred in tumor-immune microenvironment studies since it employs similar immune infiltrates and evasion strategies as does human GBM. Therefore, C6 cell line offers a wide variety of therapeutic studies including growth and invasive pattern studies, angiogenic and immune models, and a plethora of molecular and genetic targets for newer pharmacological agents. Most of the C6 glioma research has been focused on testing a wide diversity of agents for their tumoricidal activity. Moreover, C6 rat glioma models have also been extensively used to analyze glioma characteristics such as development, invasion, migration, and angiogenesis. In general, the C6 rat glioma model is thought to be a quite safe model, and it has been widely used throughout the timeline. Nevertheless, a researcher should not forget to take into consideration the pros and cons of every cell line, in accordance with the appropriate model to be studied.

Conflicts of interest

Authors declare no conflicts of interest.

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