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Current review of *in vivo* GBM rodent models: emphasis on the CNS-1 tumour model

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ABSTRACT

GBM (glioblastoma multiforme) is a highly aggressive brain tumour with very poor prognosis despite multi-modalities of treatment. Furthermore, recent failure of targeted therapy for these tumours highlights the need of appropriate rodent models for preclinical studies. In this review, we highlight the most commonly used rodent models (U251, U86, GL261, C6, 9L and CNS-1) with a focus on the pathological and genetic similarities to the human disease. We end with a comprehensive review of the CNS-1 rodent model.

Key words: U251, U87, GL261, C6, 9L, CNS-1.

INTRODUCTION

GBM (glioblastoma multiforme) is the most common and aggressive primary brain tumour, with an extremely poor prognosis and very few therapeutic advances in the last decade (Wen and Kesari, 2008). Patients have an average survival of 1–2 years after diagnosis with current available treatments (Wen and Kesari, 2008). Recently, research has advanced our understanding of the genetics and molecular pathology of the disease and has identified several therapeutic targets, such as inhibitors of VEGF (vascular endothelial growth factor) and EGFR (endothelial growth factor receptor) (Van Meir et al., 2010). Unfortunately, these targeted therapies have not shown

success in clinical trials, despite strong preclinical data (Furnari et al., 2007; Krakstad and Chekenya, 2010; Van Meir et al., 2010). Although these factors developed for targeted therapy (e.g. VEGF and EGFR) are known to be overexpressed in GBM, the failure of successful clinical outcomes reveals the complex nature of the disease. The dichotomy between what should be successful therapeutic targets and the ineffectiveness of drugs directed towards them underscores the critical importance of appropriate and predictive rodent models in the study of innovative therapies for the treatment of gliomas. As researchers begin to better understand the complex interaction between glioma cells and their CNS (central nervous system)/immune microenvironment, a shift away from xenograft tumour models towards non-immunogenic models is now at the forefront of GBM research. This review will first briefly discuss the most commonly used rodent models of GBM, with a focus on the potential for these models to recapitulate the human brain neoplasm on genetic, imaging, pathophysiology and therapeutic levels. Finally, we present a comprehensive review of the non-immunogenic CNS-1 rodent model for GBM.

U251 GLIOMA MODEL

General protocol

The U251 malignant glioma cell line was originally established from a 75-year-old male with GBM by Ponten and others

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Abbreviations: Akt, protein kinase B; BBB, blood–brain barrier; BEHAB, brain-enriched hyaluronan-binding; CNS, central nervous system; CXCR4, CXC chemokine receptor 4; EGFR, endothelial growth factor receptor; GBM, glioblastoma multiforme; GFAP, glial fibrillary acidic protein; H/E, haematoxylin/eosin; HIF-1 α , hypoxia-inducible factor-1 α ; IGF-1, insulin-like growth factor 1; IL-1 α , interleukin-1 α ; MMP, matrix metalloproteinase; MNU, methylnitrosourea; MRI, magnetic resonance imaging; NCAM, neural cell adhesion molecule; PI3K, phosphoinositide 3-kinase; TNF α , tumour necrosis factor α ; VEGF, vascular endothelial growth factor; PTEN, phosphatase and tensin homologue deleted on chromosome 10.

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(Ponten, 1975; Houchens et al., 1983). This GBM cell line is known to mimic the salient features of human GBM and as such has received significant attention over the last four decades in xenogeneic mouse models of cancer (Houchens et al., 1983; Harada et al., 1994; Husain et al., 1998; Candolfi et al., 2007; Radaelli et al., 2009). The U251 cell line has been used both in subcutaneous and intracranial mouse models (Camphausen et al., 2005). For example, the intracranial model involves injections of 1×10^5 – 1×10^6 of U251 cells suspended in 5–25 ml (Houchens et al., 1983; Candolfi et al., 2007), approx. 2 mm anterior to the bregma and 2 mm to the left (or right) of the midline inserted 2–3 mm deep into the striatum of the brain (Houchens et al., 1983; Valdes et al., 2010) of athymic nude mice.

Pathology

This intracranial mouse model recapitulates most of the key salient features of GBMs at the histopathological level (Candolfi et al., 2007; Wen and Kesari, 2008; Radaelli et al., 2009). Under H/E (haematoxylin/eosin) staining, U251 cells show an infiltrative pattern of invasion into normal brain parenchyma, significant foci of palisading necrosis, a tortuous pattern of microvascular proliferation, hypertrophic endothelium, cellular pleomorphism, giant multinucleated cells, atypia including mitotic figures and irregular nucleoli, and foci of oedema and haemorrhage (Candolfi et al., 2007; Radaelli et al., 2009). While the invasion pattern is not strongly diffused along white matter tracks, it does present perivascular satellitosis and subpial spread. However, this rodent model lacks perineuronal satellitosis and invasion along white matter tracks. Immunohistochemical analysis of U251 xenografts shows striking similarities to GBMs, with neoplastic cells positive for GFAP (glial fibrillary acidic protein), vimentin and S100B staining (Kleihues and Cavenee, 2000; Brat and Van Meir, 2004; Homma et al., 2006; Radaelli et al., 2009). Tumour cells also show high levels of cellular proliferation, with over 50% of tumour cells staining positive for Ki-67. At regions of pseudo-palisading necrosis and perinecrotic areas, these tumours display positive caspase 3 and HIF1- α (hypoxia-inducible factor-1 α) staining (Radaelli et al., 2009). Finally, in recent years, interest has arisen regarding tumour stem cells (Van Meir et al., 2010). This tumour model contains a CD133⁺ subpopulation of cells able to form neurosphere aggregates with self-propagating potential (Qiang et al., 2009).

Genetics

The U251 xenograft model also displays similarities at the genetic level to human GBM (Louis, 1994; Louis et al., 2001; Furnari et al., 2007; Krakstad and Chekenya, 2010; Van Meir et al., 2010), with the identification of non-functional mutant tumour suppressor p53 (Radaelli et al., 2009) as well as mutant PTEN (phosphatase and tensin homologue deleted on chromosome 10). Deletions of p14^{ARF} and p16, two cell cycle suppressor genes whose function is as critical negative

regulators of Cdks (cyclin-dependent kinases), and whose deletion leads to loss of activity of the retinoblastoma protein (Furnari et al., 2007), are observed in U251 (Fueyo et al., 1996; Ishii et al., 1999) (Table 2). The PI3K (phosphoinositide 3-kinase)/Akt (also known as protein kinase B) pathway shows up-regulation in U251 as a result of high Akt expression. This has been shown to be a contributing factor in the increased survival, proliferation, migration, angiogenesis and resistance to apoptosis observed in GBM (Koul et al., 2006; Furnari et al., 2007; Radaelli et al., 2009). A study by Camphausen et al. (2005) reported gene expression profile differences between subcutaneous and intracranial tumour models, noting the importance of different *in vivo* growth conditions and the role of the microenvironment in U251.

Summary

In summary, U251 recapitulates the salient histological and immunohistochemical features of human GBM. In addition, a number of genetic alterations show similarities to human GBM, including alterations in key tumour suppressors and oncogenic pathways. Further, MRI (magnetic resonance imaging) features of the U251 mouse model correlate with human GBM, including a necrotic centre, poorly demarcated, infiltrative tumour borders and an enhanced rim on T2-weighted imaging; on post-contrast T1-weighted images an intense rim is observed similar to human GBM (Radaelli et al., 2009). Brain volume is a limiting factor for imaging, as more powerful, expensive magnets are required to achieve proportionately better imaging of anatomical structures. Equivalent images of whole mice brains compared with rat brains require a more expensive, powerful MRI magnet to obtain similar resolution of anatomical structures, which may be unavailable to the investigator. Finally, this xenogeneic mouse model is criticized for not reproducing the tumour–host immune response. Future work requires full genome sequencing of U251 and comparison with GBM.

U87 GLIOMA MODEL

General protocol

The U87 GBM model was originally established by Ponten and colleagues from a female with GBM (Ponten, 1975). This tumour model shows significant dissimilarities when compared with the U251 model and human GBM, but has nevertheless received significant attention, especially for assessing tumour angiogenesis and anti-angiogenic therapies (Candolfi et al., 2007; de Vries et al., 2009; Radaelli et al., 2009). Similar to the U251 model, this model shows significant gene expression profile differences between differing *in vivo* growth conditions (e.g. subcutaneous and intracranial) (Camphausen et al., 2005). Similar to the U251 murine model, the intracranial model

involves concentrations of 1×10^5 – 1×10^6 of U87 cells suspended in 5–10 ml, and injections at approx. 1 mm anterior and 3 mm lateral to the bregma inserted 3–4 mm deep into the brain (Roberts et al., 1998; Rubin et al., 2003; Moore et al., 2004; Candolfi et al., 2007) or right striatum (Roberts et al., 1998) of athymic nude mice.

Pathology

The U87 model displays key dissimilarities to the U251 model and human GBM at the histopathological level (Kleihues and Cavenee, 2000; Brat and Van Meir, 2004; Homma et al., 2006; Rong et al., 2006; Candolfi et al., 2007; de Vries et al., 2009; Radaelli et al., 2009). U87 tumours are highly cellular with atypia such as mitotic figures and irregular nucleoli, and profuse neovascularization (Candolfi et al., 2007). Unlike GBM, these tumours show a non-diffusely infiltrative growth pattern, with a well-demarcated tumour mass surrounded by reactive astrocytes (de Vries et al., 2009; Radaelli et al., 2009). Tumour vasculature shows significantly more homogeneous and leaky vessels, which means greater access by systemic drugs, in contrast with GBM (de Vries et al., 2009). Further, necrotic foci are rare in number with features differing from GBM, including no pseudo-palisading patterns and neutrophil infiltration (Candolfi et al., 2007; Radaelli et al., 2009). U87 tumour cells stain negative for GFAP and S100, but positive for vimentin and over 40% positive nuclei for Ki-67. GFAP positive staining is only observed at the reactive astrocyte border surrounding the well-demarcated tumour mass (Candolfi et al., 2007; Radaelli et al., 2009). The necrotic foci also display areas of positive caspase 3 and HIF1- α staining (Radaelli et al., 2009). Finally, similar to the U251 model, U87 tumours show a CD133⁺ subpopulation of cells able to form neurosphere aggregates with self-propagating potential, of interest to the tumour stem cell community (Qiang et al., 2009).

Genetics

U87 cells show several similarities as well as some key differences to human GBM (Louis, 1994; Louis et al., 2001; Furnari et al., 2007; Krakstad and Chekenya, 2010; Van Meir et al., 2010). Unlike the U251 model, U87 demonstrates a wild-type tumour suppressor p53 (Radaelli et al., 2009). U87 shows a mutant PTEN, deletion of p14^{ARF} and p16 (Fueyo et al., 1996; Ishii et al., 1999), and PI3K/Akt pathway up-regulation as a result of high Akt expression (Koul et al., 2006; Furnari et al., 2007; Radaelli et al., 2009) (Table 2). The study by Camphausen et al. (2005) mentioned above found significant differences between U87 and U251 when grown *in vitro* or under subcutaneous conditions, but found that both lines displayed similar gene expression patterns when grown intracranially. A recent whole genome sequencing of U87 was performed (Clark et al., 2010). This study examined the various mutations, homo/heterozygous deletions and intra/interchromosomal events, which included over 140 genes affected by complete deletion (e.g. CDKN2A) and common mutations found in gliomas (e.g. PTEN).

Summary

Unlike the U251 xenogeneic model, U87 shows significantly less similarity to human GBM and as such caution must be used when extrapolating conclusions from studies using the U87 cell line. Further, a key feature of human GBM, which significantly contributes to its resistance to therapy and high recurrence rate, is the diffusely invasive infiltration pattern into normal brain parenchyma of tumour cells (Louis et al., 2001). In the U87 model, tumours show clear demarcation from normal parenchyma without the diffusely infiltrative pattern of GBM and lack GFAP or S100B immune staining. At the genetic level, there are key differences and similarities to human GBM, which include a wild-type p53 and aberrant PI3K/Akt respectively. Finally, MRI features of the U87 mouse model including a homogeneous and enhanced, well-demarcated tumour nodule on T2-weighted imaging, which does not correlate well with GBM (Radaelli et al., 2009). Furthermore, investigators should be aware that the xenograft models can incorporate host (non-human) glycolipids, possibly altering the biochemical and immunogenic properties of the model (Ecsedy et al., 1999). Overall, this tumour model has been used to test anti-angiogenic therapies, but limitations of extrapolating conclusions from this model system include the aforementioned dissimilarities to human GBM, as well as the usual concerns with xenogeneic models and limitations with MRI of small rodents.

GL261 GLIOMA MODEL

General protocol

GL261 is a syngeneic mouse model of GBM in C57BL/6 mice that do not require a deficient immune system. As a result this model may mimic more closely the growth and immune response of human GBM. Seligman and Shear originally developed the model in 1939 through intracranial implantation of 20 methylcholanthrene pellets into mice brains (Seligman and Shear, 1939; Newcomb and Zagzag, 2009). In general, 1×10^5 GL261 tumour cells are injected in a volume of 2–4 μ l, 2 mm from the sagittal suture at a depth of 3 mm (Zhu et al., 2010). Several advances in experimental immunotherapy for GBM have been achieved using this model given this is an immune competent model and there are a vast number of antibodies and immune markers available for mice (Maes and Van Gool, 2011).

Pathology

Early descriptions of GL261 cells describe this tumour as displaying ependyoblastoma characteristics. Recent work further describes poorly differentiated cells with morphology similar to human GBM cells (Shapiro et al., 1970; Zagzag et al., 2000). A key feature of this model is the diffusely

infiltrating and invasive characteristics of GL261 cells into normal brain. Histopathological examination demonstrates individual cell invasion several millimetres away from the tumour margin (Zagzag et al., 2003; Newcomb and Zagzag, 2009). These invasive cells display several of the 'secondary structures of Scherer': (i) perineuronal satellitosis, (ii) perivascular satellitosis, (iii) subpial spread and (iv) invasion along the white matter tracts (Burger et al., 2002). Furthermore, GL261 tumour cells express CXCR4 (CXC chemokine receptor 4), which has been linked to the invasive and migratory properties of GBM (Zagzag et al., 2008). GL261 also displays endothelial thickening based on increased CD31 expression [30]. Similar to GBM, GL261 tumours have areas of pseudo-palisading necrosis. Along with an invasive phenotype, the GL261 mouse model shares many histopathological markers with human GBM. GL261 tumours stain positive for GFAP and S100 and endothelial cells stain positive for CD31 antigen (Newcomb and Zagzag, 2009). The tumours express areas of angiogenesis and hypoxia, which correlate with expression of VEGF and HIF-1 α (Zagzag et al., 2003). Both of these markers have been shown to correlate with an increase in growth and decreased survival in patients with GBM (Zagzag et al., 2006).

Genetics

Genetically, GL261 cells share many key mutations with human GBM. Point mutations in the K-ras oncogene and p53 tumour suppressor gene have been identified (Szatmari et al., 2006; Candolfi et al., 2007) (Table 2). GL261 cells also demonstrate increased activation of the PI3K pathway and phosphorylation of Akt, a serine/threonine protein kinase, leading to downstream signalling both *in vitro* and *in vivo* (Choe et al., 2003; Furnari et al., 2007). The PI3K/Akt signalling pathway is often dysregulated in human GBM tumour cells, leading to EGFR expression, increased proliferation, survival and cellular migration observed in GBM (Choe et al., 2003; Furnari et al., 2007; Van Meir et al., 2010).

Summary

Several aspects of GL261 allow for an excellent model of human GBM, particularly when compared with xenograft models (U251 and U87) in immune suppressed mice. GL261 tumour cells share several important markers and mutations with human GBM. The diffusely infiltrative property of GBM is one of the major reasons for failure of current treatment (Wen et al., 1990; DeAngelis, 2001; Furnari et al., 2007; Van Meir et al., 2010), and the GL261 model recapitulates this feature on microscopic examination. As mentioned previously, one of the major challenges in using a mouse model is the size of the brain, particularly in studies that require imaging techniques like MRI. Equivalent images of whole mice brains compared with rat brains require a more expensive, powerful MRI magnet to obtain similar resolution of anatomical structures, which may be unavailable to the investigator. In conclusion, when imaging is not

a limitation, GL261 is a strong model for studying GBM therapies, particularly immune-based therapies.

C6 GLIOMA MODEL

General protocol

The C6 glioma cell line was developed in the late 1960s in Sweet's laboratory by repetitive administration of MNU (methylnitrosourea) in adult Wistar-Furth rats (Benda et al., 1968). Although originally developed in Wistar rats, C6 can be implanted in Sprague-Dawley and Long-Evans rats without rejection (Nagano et al., 1993; Whittle et al., 1998). Tumour cells are usually implanted into the fronto-parietal lobe at 1×10^5 cells in 5 μ l, with variable rates of tumour take (Parsa et al., 2000; Branle et al., 2002). This tumour can also be implanted on the right flank for easier measurements in growth studies and associated limitations in extrapolating from subcutaneous studies.

Pathology

C6 glioma cells share several general histopathological and specific tumour markers with human GBM. C6 glioma tumours demonstrate regions of focal invasion into brain tissue when implanted in Wistar rats, similar to the diffuse infiltrating pattern seen in GBM (Chicoine and Silbergeld, 1995). At the cellular level, C6 tumours do display areas of necrosis, nuclear polymorphism and high mitotic rates (Auer et al., 1981). In terms of histopathological markers, C6 cells express S100B protein, but do not express GFAP, and variable levels of vimentin (Pfeiffer et al., 1970; Whittle et al., 1998; Chou et al., 2003).

Genetics

The genetics of the C6 cell line offer further comparison with human GBM. A common tumour suppressor gene, p16, is known to have a high mutation rate in GBM and mutations in the *p16/CDKN2A/NK4A* locus are frequent occurrences in C6 tumour cells (Ueki et al., 1996; Ishii et al., 1999; Schlegel et al., 1999; Furnari et al., 2007) (Table 2). The tumour suppressor, p53 is one of the most frequently mutated genes in human GBM, and the C6 cell line differs from humans in their expression of the tumour suppressor protein p53 with a wild-type p53 along with minimal PTEN expression (Asai et al., 1994).

Summary

A clear advantage in using the C6 glioma model is the extensive characterization and the literature produced using this model, including genetic analysis (Grobben et al., 2002).

However, the immunogenicity of this model is an important factor that must be considered even when studying non-immune based therapies. When placed in a Sprague–Dawley or Long–Evans rats, the tumour loses its invasive characteristics and grows in an encapsulated manner, no longer resembling the diffusely infiltrative nature of GBM (San-Galli et al., 1989). For example, Parsa et al. (2000) demonstrated strong antibody responses to C6 even when grown in Wistar rats. In conclusion, any therapeutic development and testing in this model must be interpreted with caution.

9L GLIOMA MODEL

General protocol

The 9L glioma model is historically one of the most widely used glioma tumour models. It has been extensively used to study chemotherapy and radiation effects (Henderson et al., 1981; Wen et al., 1990; Kimler et al., 1992; Kruse et al., 1993). Recently, it has also been used to study tumour stem cells (Ghods et al., 2007). The 9L gliosarcoma cell line was originally developed by Brenda, Schmidek and colleagues in the 1970s through repetitive administration of MNU in Fischer 344 rats (Benda et al., 1971; Schmidek et al., 1971). Most research in this cell line has been conducted with the syngeneic Fischer rats; however, it has also been characterized in allogenic Wistar rats (Stojiljkovic et al., 2003). Tumour cells are usually injected into the striatum at a volume of 1×10^5 cells in 5 μ l of cell suspension, showing high rates of tumour take after implantation (Stojiljkovic et al., 2003).

Pathology

It is important to note that 9L tumour cells have a sarcomatous appearance under histopathological examination, differentiating this model from other glioma models (Barth, 1998). 9L cells share some histological similarities with GBM tumour cells. For example, they are S100 β -positive. However, they do not express GFAP and do not show the typical GBM pattern of diffusely invasive cells (Benda et al., 1971). In Wistar rats, tumours are infiltrated by macrophages and microglia (ED₁ positive) and CD4⁺/CD8⁺ T-cells after approx. 2 weeks of growth (Stojiljkovic et al., 2003). Despite evidence of a T-cell immunological response, tumour growth is not inhibited in this model when compared with other rodent models with immune responses.

Genetics

Similar to human GBM cells, 9L cells have a mutant p53 gene (Asai et al., 1994). They also over express EGFR (epithelial growth factor receptor), which has been extensively studied as a therapeutic target in human GBM (Sibenaller et al., 2005;

Lo, 2010). 9L differs from humans in their expression of p16, FGFR-1 (fibroblast growth factor receptor-1) and PDGFR β (platelet-derived growth factor receptor β ; Sibenaller et al., 2005), all of which are mutated or highly expressed in GBM, but not in 9L. The 9L tumour cells also fail to express other commonly mutated tumour suppressor genes such as PTEN.

Summary

Despite the wide use of the 9L glioma model, it is important to note the significant differences when compared with human GBM. Currently, the WHO (World Health Organization) classifies gliosarcoma as a subset of grade IV gliomas (Louis et al., 2001). However, careful consideration should be implemented when using a gliosarcoma model as a general model for GBM. Recent review of the literature has identified unique histological and genetic characteristics of gliosarcoma compared with GBM (Han et al., 2010). Some of these differences include infrequency of EGFR mutations and temporal lobe predilection, an implication that this subset of tumours may respond uniquely to various therapies (Han et al., 2010). 9L is considered an immunogenic tumour, since it is possible to immunize syngeneic rats with irradiated 9L tumour cells (Denlinger et al., 1975). Furthermore, the circumscribed growth pattern serves as a poor model to study the invasive properties of gliomas.

CNS-1 GLIOMA MODEL

General protocol

In 1990, Dr Michael C. Molleston, working in the laboratory of one of the authors (W.F.H.) sought to develop a syngeneic astrocytoma model. Each week for 36 weeks, he intravenously injected young rats with 5.0 mg/kg of MNU. Approx. 7 months after the start of treatment, rats began to die of tumours. One rat was found moribund and it was possible to obtain an MRI scan (Figure 1a). The study showed a well-developed neoplasm in the basal ganglia. From this tumour, the CNS-1 tumour line was established. CNS-1 tumour cells are usually injected into the striatum at a volume of 1×10^5 cells in 5 μ l of cell suspension. Original studies with this cell line indicate median survival time of 30 days with high tumour take (Kruse et al., 1994).

Pathology

At the time that the CNS-1 cell line was developed, the currently available GBM models failed to recapitulate many of the key characteristics of tumour growth *in vivo*. CNS-1 tumour cells were unique in their ability to express several glioma markers. These include positive staining for GFAP, S100 β , vimentin, RAR- α (retinoic acid receptor α), intracellular adhesion molecule-1 and neuron-specific enolase (Kruse

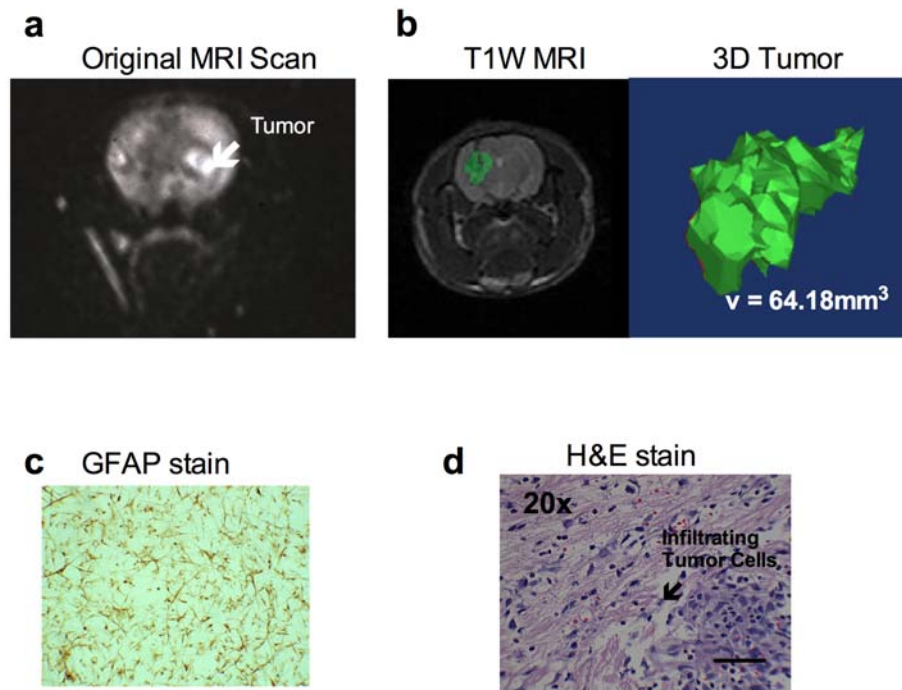


Figure 1 Characteristics of the CNS-1 rat glioma model

(a) MRI of the original tumour in which the CNS-1 tumour cell line was derived. (b) T1-weighted MRI of 1×10^5 CNS-1 cells in Lewis rat on day 15. Tumour is highlighted in green. To the right is a 3D reconstruction of the tumour using Mimics Software (Materialise, Leuven, Belgium) by summing the number of voxels that exceeded the intensity threshold in each plane on the T1-weighted subtraction images and multiplying by the appropriate spatial scaling factor ($0.35 \text{ mm} \times 0.35 \text{ mm} \times 1.00 \text{ mm}$ per voxel). (c) GFAP staining of the first cell line of CNS-1. (d) An H/E staining of infiltrating tumour cells into normal parenchyma of a Lewis rat brain (scale bar indicates $100 \mu\text{m}$).

et al., 1994) (Table 1). A key feature of the CNS-1 model is also the diffusely infiltrative and invasive pattern of growth *in vivo*. Unlike the 9L glioma model, CNS-1 cells demonstrate perivascular spread and individual tumour cell infiltration into normal parenchyma (Kruse et al., 1994). CNS-1 cells demonstrate periventricular spread and leptomeningeal spread (Kruse et al., 1994).

The tumour microenvironment is known to promote tumour cell growth and invasion. This should be taken into consideration when choosing an appropriate animal model for therapeutic studies. Several immunological studies have been conducted with the CNS-1 model to elucidate the micro-environment's role in tumour growth (Owens et al., 1998; Regina et al., 2003; Demeule et al., 2004). In the presence of a brain tumour, endothelial cells may undergo phenotypic and functional changes, which result in promotion of tumour growth (Demeule et al., 2004). In the presence of CNS-1 tumour, endothelial cells undergo hyperplasia and display a pseudo-palisading pattern accompanying necrosis (Kerbel, 2008), both of which are indications of increased angiogenesis mimicking human GBM (Beranek, 2002).

The angiogenic nature of GBM is well established and has historically been a target for therapeutic development (Kerbel, 2008). Endothelial cells are also involved in drug resistance across the BBB (blood-brain barrier), by helping to

prevent leakiness of the BBB. For example, P-glycoprotein is an efflux transporter in endothelial cells, which prevents significant accumulation of hydrophobic drugs (van Asperen et al., 1997). Histopathologic examination of human GBM shows a high expression of P-glycoprotein, possibly offering an explanation for clinical drug resistance in these tumours (Sawada et al., 1999).

In addition to endothelial cells, microglia and macrophages also support tumour growth and invasion. In human GBM, these cells account for as much as 10–34% of the tumour mass (Roggendorf et al., 1996). There is a strong positive correlation between the number of macrophages and microglia and the grade of tumour (Kielian et al., 2002). Similar to human GBM, CNS-1 brain tumours demonstrate infiltration of macrophages and microglia, constituting a major proportion of the tumour cell mass (Kielian et al., 2002). As a result, the CNS-1 model has been used to investigate the relationship between tumour cells, microglia and macrophages. MCP-1 (monocyte chemoattractant protein-1) was identified as a chemoattractant secreted by tumour cells, promoting the migration of microglia and macrophages to the tumour site (Kielian et al., 2002). Several tumour-promoting cytokines have been detected at the tumour site *in vivo*, but not by CNS-1 cells *in vitro*, further supporting the role of immune cells in the tumour microenvironment (Kielian et al., 2002). These

Table 1 Characteristic histological markers and growth of tumour cells in GBM rodent models

Expression of histological markers and growth characteristics are indicated by (+), while failure of expression is indicated by (-).

Rodent and model	Markers			Growth	
	GFAP	S100 β	Vimentin	Invasive	Immunogenic
Mouse					
U251	+	+	+	-	+
U87	-	-	+	-	+
GL261	+	+	+/-	+	-
Rat					
C6	-	+	N/A	+/-	+
9L	-	+	N/A	-	+
CNS-1	+	+	+	+	-

cytokines include IL-1 α , IL-1 β , IL-10, TNF α (tumour necrosis factor α), TNF β and IFN γ (interferon- γ ; Kielian et al., 2002).

As previously mentioned, the CNS-1 model may be used to study the invasive properties of tumour cells. Plasminogen activators are traditionally involved in the breakdown of blood clots. However, they are also highly expressed in GBM and involved in degradation of surrounding tissue, promoting tumour invasion (Demeule et al., 2004). Endothelial cells surrounding CNS-1 tumours up-regulate urokinase plasminogen activators [tPA (tissue plasminogen activator), PA (plasminogen activator), PAI-1 (plasminogen-activator inhibitor) and uPAR (urokinase-type plasminogen activator receptor)] (Demeule et al., 2004). MMPs (matrix metalloproteinases) are another class of enzymes involved in degradation and tumour invasion. MMPs have been implicated as a crucial target in GBM cell invasion and as a result have been the focus for therapeutic development (Furnari et al., 2007). CNS-1 cells express MMP-2, but do not express MMP-9. However, expression of MMP-9 has been detected in endothelial cells and microglia found within the tumour microenvironment (Regina et al., 2003).

Unlike some of the other tumour models, the genetics of CNS-1 have not been extensively studied. Here, we briefly discuss the molecular work that has been undertaken and molecular players identified in the CNS-1 cell line. The karyotype of CNS-1 cells demonstrates one or more trisomies often of chromosomes 11 and 13 (Kruse et al., 1994). In terms of protein expression, CNS-1 cells were used to correlate decreased activity of L-isoaspartyl methyltransferase (a repair enzyme) in gliomas (Lapointe et al., 2005). Most of the research surrounding CNS-1 proteins has focused on its

invasive phenotype. BEHAB (brain-enriched hyaluronan-binding) protein is a chondroitin sulfate proteoglycan found in the ECM (extracellular matrix). BEHAB expression is increased in gliomas and has been associated with glioma cell migration and proliferation (Jaworski et al., 1996). BEHAB/brevin cDNA was transfected into CNS-1 cells and implanted into Lewis rats. Tumours with BEHAB/brevin cDNA had a decreased survival and increased invasion and vasculature on histology (Nutt et al., 2001). The CNS-1 rodent model was then used to identify ADAMTS5 (a disintegrin and metalloproteinase with thrombospondin motifs 5) as the MMP required for proteolytic cleavage of BAHAB/brevin (Viapiano et al., 2008). Another protein characterized in CNS-1 involved in migration and invasion is the NCAM (neural cell adhesion molecule; Owens et al., 1998). CNS-1 cells transfected with NCAM demonstrated increased invasion *in vitro*. However, under *in vivo* conditions, NCAM increases local infiltration but decreases long-range invasion and migration. These results help to differentiate the mechanisms and signalling involved in glioma invasion. Many of the therapeutic studies investigated using the CNS-1 cell model involve gene therapy. For a summary of therapeutic studies in the CNS-1, we refer readers to Table 3.

Summary

The CNS-1 rodent model shares several key features with human GBM, including histological markers and growth pattern. The role of the immune environment has also been extensively investigated in this model. Although there has

Table 2 Genetic characteristics of rodent GBM models

The presence of a genetic mutation is indicated by (+), while overexpression is indicated by (++)

Rodent and model	Genetic mutation or overexpression					
	p14	p16	PTEN	p53	kRAS	EGFR
Mouse						
U251	+	+	+	+	+	++
U87	+	+	+	-	+	-
GL261	+	+	+	+	+	++
Rat						
C6	-	+	-	-	N/A	++
9L	-	-	-	+	N/A	++
CNS-1	N/A	N/A	N/A	N/A	N/A	N/A

Table 3 Therapeutic studies conducted in the CNS-1 GBM rodent model

Category	Year	Therapy	References
Virus	2004, 2008 and 2010	Adenovirus vectors encoding Flt3L adenovirus vector expressing HSV1-TK (Herpes simplex virus type I thymidine kinase) and FLT3L	(Ali et al., 2004; King et al., 2008; Puntel et al., 2010)
Vectors	2004	Block TGF β with transfected decorin gene	(Biglari et al., 2004)
Gene therapy	2000 and 2003	CpG oligodeoxynucleotides blockage of IGF-1 (insulin-like growth factor 1) with antisense and triple helix IGF-1 expression	(Carpentier et al., 2000; Trojan et al., 2003)
Imaging	2002 and 2007	BOLD imaging of O ₂ detection PPIX fluorescence	(Dunn et al., 2002; Bisland et al., 2007)
Small molecule	2011	Propentofylline	VL Jacobs, RP Landry, Y Liu, EA Romero-Sandoval and SA De Leo, unpublished work

been some work, an extensive understanding of the genetics in CNS-1 cells is lacking. Despite this, CNS-1 cells share several histological features and diffuse growth pattern with human GBM and is one of the few models that also recapitulates the tumour microenvironment.

CONCLUSION

Rodent models of GBM have been available for decades; however, very few new therapies have successfully translated into the clinic during this time (Louis et al., 2001). The extent to which these models recapitulate the human disease remains debatable. GBM is one of the most challenging tumours to treat with approx. 1 year median survival following diagnosis. Despite aggressive surgery, chemotherapy and radiation treatments, the tumours virtually always relapse (Van Meir et al., 2010). An ideal model should recapitulate the key histopathological, genetic and imaging features encountered in GBM's aggressive growth as well as being a reproducible, reliable model.

As ongoing research continues to shed light on the genetic and cellular mechanisms involved in GBM, these new advances must be taken into consideration when choosing an appropriate model. Currently available rodent models can be classified into spontaneous oncogenesis, allograft or xenograft. For this review, we focused on engrafted models. However, we direct the reader to Sonabend et al. (2007) for a more extensive review on the several GBM genetic mouse models available. Also, our review provides only a general overview on the histopathological comparison of the rodent tumour cells to human GBM, since this has been reported previously (Radaelli et al., 2009).

We summarize herein some of the most commonly used rodent models of GBM with a focus on the CNS-1 rat model. Two of the most commonly used mouse models presented herein, U251 and U87 are xenograft models used in athymic nude mice. Comparing these cell lines, U251 is histologically most similar to human GBM. While these models enable the use of human GBM tumour cell lines, they are in an immune-compromised rodent, which does not allow for adequate

study of the tumour-CNS/immune microenvironment. The mouse GL261 model, however, is a syngeneic model in C57BL/6 mice. This murine model demonstrates several of the critical GBM characteristics such as secondary structures of Scherer and tumour suppressor gene mutations. It is important to mention a more recent mouse model of GBM that has not been extensively reported in the literature but does share several key phenotypes with GBM. The VM-M3 model is derived from a spontaneous astrocytoma in the VM/dK strain of mice. These tumour cells are highly infiltrative, demonstrating all four secondary structures of Scherer and expression of CXCR4 (Shelton et al., 2010). Since this rodent model was only recently introduced, there are no extensive data pertaining to its histological and genetic markers. It is known that they do not express GFAP. However, this presents yet another promising model for the study of glioma invasion. Both 9L and C6 in rats (when grown in Sprague-Dawley rats) as well as U87 in mice demonstrate well-circumscribed tumour growth patterns *in vivo*. C6 can grow invasively in Wistar-Furth rats. However, rats do produce strong immune responses to C6 tumour cells. The CNS-1, U251 and GL261 models, in comparison, share several histological surface markers with human GBM and grow in a very similar pattern (i.e. diffusely infiltrative) *in vivo*. An advantage of using a rat rodent model compared with mouse is the ease in performing imaging studies in a rat due to its larger size.

Advancements have been made in the understanding of how GBM tumour cells invade and interact with their immune environment. As we move towards developing new therapeutics and testing in animal models, researchers must ensure rodent models are incorporating our new understandings of GBM. Failure to do this will only elicit further therapeutic developments that will fail in clinical trials. Both the CNS-1 model in rats and the GL261 model in mice have been used to investigate the role of the tumour microenvironment. This review presented the most common rodent glioma models, highlighting their differences when compared with the human disease. In addition, we presented the first comprehensive review of the CNS-1 glioma model. An understanding of how glioma models recapitulate the disease will enable scientists to choose the best model for preclinical studies, hopefully translating to more successful clinical trials in the future.

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