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Rat brain tumor models in experimental neuro-oncology: the C6, 9L, T9, RG2, F98, BT4C, RT-2 and CNS-1 gliomas

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Abstract

In this review we will describe eight commonly used rat brain tumor models and their application for the development of novel therapeutic and diagnostic modalities. The C6, 9L and T9 gliomas were induced by repeated injections of methylnitrosourea (MNU) to adult rats. The C6 glioma has been used extensively for a variety of studies, but since it arose in an outbred Wistar rat, it is not syngeneic to any inbred strain, and its potential to evoke an alloimmune response is a serious limitation. The 9L gliosarcoma has been used widely and has provided important information relating to brain tumor biology and therapy. The T9 glioma, although not generally recognized, was and probably still is the same as the 9L. Both of these tumors arose in Fischer rats and can be immunogenic in syngeneic hosts, a fact that must be taken into consideration when used in therapy studies, especially if survival is the endpoint. The RG2 and F98 gliomas were both chemically induced by administering ethylnitrosourea (ENU) to pregnant rats, the progeny of which developed brain tumors that subsequently were propagated in vitro and cloned. They are either weakly or non-immunogenic and have an invasive pattern of growth and uniform lethality, which make them particularly attractive models to test new therapeutic modalities. The CNS-1 glioma was induced by administering MNU to a Lewis rat. It has an infiltrative pattern of growth and is weakly immunogenic, which should make it useful in experimental neuro-oncology. Finally, the BT4C glioma was induced by administering ENU to a BD IX rat, following which brain cells were propagated in vitro until a tumorigenic clone was isolated. This tumor has been used for a variety of studies to evaluate new therapeutic modalities. The Avian Sarcoma Virus (ASV) induced tumors, and a continuous cell line derived from one of them designated RT-2, have been useful for studies in which de novo tumor induction is an important requirement. These tumors also are immunogenic and this limits their usefulness for therapy studies. It is essential to recognize the limitations of each of the models that have been described, and depending upon the nature of the study to be conducted, it is important that the appropriate model be selected.

Keywords

C6; 9L; T9; RG2; F98; BT4C; RT-2; CNS-1; Rat brain tumor models

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Introduction

There is a continuing need in experimental neuro-oncology for animal models that can be used to assess the efficacy of innovative approaches for the treatment of brain tumors. The rat has been one of the most widely used experimental animals, and rat brain tumor models have been used extensively since the mid 1970s. This review will focus on rat brain tumor models and their utility in evaluating the efficacy of various therapeutic modalities. Until recently, murine models [1] were used less frequently than rat models, but the ability to produce genetically engineered cell lines [2] has increased the use of murine models over the past five years. The relative advantages of rat and murine tumor models are summarized in Table 1. Feline and canine models have been used less frequently [3,4], but nevertheless, still provide an intermediate between rodent models and humans. It was first reported in the early 1970s that central nervous system (CNS) tumors could be induced reproducibly and selectively in adult rats that had been given repeated, weekly intravenous injections of *N*-methylnitrosourea (MNU) or a single dose of *N*-ethyl-*N*-nitrosourea (ENU). These studies led to the development of a number of rat brain tumor models that were highly reproducible and did not require the topical application of a chemical carcinogen to the brain [5]. In this review, we first will summarize some general principles relating to the use of brain tumor models. Although widely used, xenograft models based on the intracerebral (i.c.) implantation of human brain tumor cell lines into immunologically deficient rodents will not be discussed by us, and interested readers are referred to the recent review of Candolfi et al. [6]. The utility of these models notwithstanding, it is important to recognize that no currently available animal tumor model exactly simulates human high grade brain tumors such as glioblastoma multiforme (GBM) or anaplastic astrocytomas.

The cellular signaling pathways important for the genesis of brain tumor are multiple, with feedback mechanisms that can dramatically affect the efficacy of molecularly targeted therapeutic strategies. The heterogeneous composition of human high grade gliomas, which consists of tumor stem cells and differentiated tumor cells with varying characteristics, further complicates their susceptibility to treatment. Brain tumors also can evolve within their microenvironment, adapting to changes that produce epigenetic effects thereby altering their biology, but concomitantly providing additional targets for therapeutic intervention. Finally, genetic variations between individuals can dictate how tumors initiate, progress, and respond to treatment. Rat brain tumor models have provided a wealth of information on the in vitro and in vivo responses to various therapeutic modalities. The larger rat brain (~1200 mg) compared to that of the mouse (~400 mg) allows for more precise tumor cell implantation, and relatively larger volumes (~20 µl) that can be injected versus mice (5 µl; Table 1). Mouse models, on the other hand, have allowed researchers to rigorously test hypotheses developed from examining human tumors by genetically manipulating them and controlling specific variables such as environmental influences, in order to better understand the roles of different pathways, cell types, stromal factors and genetic variation [7]. Mouse tumor models (Table 1) also have allowed researchers to test hypotheses derived from examining human tumors, in a controlled environment with specific genetic alterations and controlled environmental influences [7].

There is a general consensus that valid brain tumor models should fulfill the following criteria: (1) they should be derived from glial cells; (2) it should be possible to grow and clone them in vitro as continuous cell lines and propagate them in vivo by serial transplantation; (3) tumor growth rates should be predictable and reproducible; (4) the tumors should have glioma-like growth characteristics within the brain including neovascularization, alteration of the blood-brain barrier (BBB), an invasive pattern of growth, and lack of encapsulation; (5) host survival time following i.c. tumor implantation should be of sufficient duration to permit therapy and determination of efficacy; (6) for therapy studies, the tumors should be either non or weakly immunogenic in syngeneic hosts; (7) they should not grow into the epidural space or extend

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beyond the brain and finally (8) their response or lack thereof to conventional treatment should be predictive of the response in human brain tumors.

In studies carried out prior to the 1970s, either cells or tumor fragments were injected i.c. using a free hand approach, which generally lacked reproducibility and precision. A stereotactic implantation procedure using suspensions of tissue-culture-derived brain tumor cells was more successful [8]. This procedure was further improved by the use of concentrated cell suspensions in small volumes, improved injection needles, better stereotactic localization to structures deeper in the white matter such as the caudate nucleus, the use of slower injection rates [9], 0.5–1.0% low gelling agarose to prevent backflow of tumor cells through the injection track [9] and cleansing of the operative field with a solution of Betadine. Finally, rinsing the surface of the brain with sterile water destroys extravasated tumor cells by osmosis prior to closure of the skull with bone wax has also been recommended [10]. This implantation procedure resulted in high success rates of i.c. tumor growth with the elimination of spinal and extracranial dissemination. The implantation of plastic [9] or metallic screws [11] with an entry port, which are permanently implanted in the skull to inject tumor cells, have been very useful [11,12]. Such devices can be left in place either at the time of or after tumor cell implantation in order to facilitate future administration of therapeutic agents at the same location without further stereotactic surgery. These are well tolerated and non-irritating in rats, but they cannot be as easily used in mice due to the thinness of their skulls [12]. Keeping these general principles of tumor cell implantation in mind, we will now discuss the currently available rat glioma models that have been used in immunocompetent animals.

C6 glioma

The C6 glioma was produced by Benda et al. [13] and Schmidek et al. [14], in Sweet's laboratory at the Massachusetts General Hospital (MGH) by repetitively administering MNU to outbred Wistar rats over a period of approximately 8 months. When animals developed neurological signs, they were euthanized, and the tumors were excised and explanted into tissue culture. Among these was a tumor designated as "#6", which was subsequently cloned by Benda et al. [13] and was shown to produce S-100 protein. Following cloning, it was re-designated "C6" [15]. The C6 glioma is composed of a pleomorphic population of cells with variably shaped nuclei. There is focal invasion into contiguous normal brain (Fig. 1a). Initially, the tumor was histopathologically classified as an astrocytoma, and eventually it was designated as a glial tumor following accession by the American Type Culture Collection, Rockville, MD (ATCC# CCL-107). The cells have been reported to have a mutant *p16/Cdkn2a/Ink4a* locus [16] with no expression of *p16* and *p19ARF* mRNAs, and a wildtype *p53* [17]. More recent molecular characterization, which compared changes in gene expression between the C6 glioma and rat stem cell-derived astrocytes, revealed that the changes in gene expression observed in the C6 cell line were the most similar to those reported in human brain tumors [18]. Compared to astrocytes, they also had increased expression of the *PDGFβ*, *IGF-1, EGFR* and *Erb3/Her3* genes, which are frequently overexpressed in human gliomas [19–21]. In a recent study, the significance of PDGF in gliomagenesis in adult rats was established by infecting white matter with a retrovirus encoding for PDGF and GFP. Within 2 weeks 100% of the animals had tumors derived from both infected and uninfected glial progenitors, thereby implicating PDGF in both autocrine and paracrine stimulation of glial progenitor cells [22]. Although, IGF-1 was overexpressed in C6 glioma cells, there was reduced expression of IGF-2, FGF-9 and FGF-10 relative to astrocytes. Similar to the increased activity of the *Ras* pathway observed in human gliomas [23], C6 cells also had an increase in both *Ras* expression and *Ras* guanine triphosphate activator protein [18]. However, contrary to what has been reported for human GBM, there was an increase in expression of Rb in these cells [18]. A subclone of C6 cells, stably expressing β-galactosidase, subsequently was described [24] and this has permitted in vivo immunohistochemical analysis of these tumors in the brain. This clone is

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available through the ATCC (# CRL-2303). However, it must be noted that the β-galactosidase marker protein itself can serve as a tumor antigen, and immunization of rats against the reporter gene protected the animals against tumor growth [24].

The C6 rat glioma model has been widely used in experimental neuro-oncology to evaluate the therapeutic efficacy of a variety of modalities, including chemotherapy [25], anti-angiogenic therapy [26], proteosome inhibitors [27], treatment with toxins [28], radiation therapy [29], photodynamic therapy [30], oncolytic viral therapy [31] and gene therapy [32]. Since this tumor arose in an outbred Wistar rat, however, there is no syngeneic host in which it can be propagated. This is a very serious limitation that diminishes its usefulness for survival studies since the tumor is immunogenic, even in Wistar rats. The C6 glioma has been demonstrated to be immunogenic in Wistar and BDX rats [33], and it therefore is not useful for evaluating the efficacy of immunotherapy. This problem is exemplified by prior studies in which C6 glioma cells were transfected with an antisense cDNA expression vector that downregulated the constitutive production of IGF-1 [34,35]. Not recognizing that the tumor was of Wistar origin, the authors unfortunately used BD IX rats, which they thought was the strain of origin, due to some ambiguity in the literature. Subsequently, it was reported that BD IX rats, which had been immunized with the C6 anti-sense IGF-1 transfected cells, were resistant to both s.c. and i.c. challenge of the C6 glioma. Similarly, Wistar rats, bearing C6 gliomas (s.c. or i.c.), developed potent humoral and cellular immune responses to the tumor, and rats challenged simultaneously with s.c. and i.c. tumors, had a survival rate of 100% [33]. Since C6 glioma cells are allogeneic in all inbred strains, this should provide a strong cautionary note for studies employing this tumor model and they should not be used for immunotherapy studies. Despite this limitation, the C6 glioma model continues to be used for a variety of studies related to brain tumor biology [36]. These have included studies on tumor growth, invasion, migration, BBB disruption, neovascularization, growth factor regulation and production, and biochemical studies [37–39]. Finally, single-cell clonal analysis has revealed that C6 cells also have cancer stem cell-like characteristics, including self-renewal, the potential for multi-lineage differentiation in vitro and tumor formation in vivo [40].

9L gliosarcoma

The 9L gliosarcoma has been the most widely used experimental rat brain tumor model. It was produced in Fisher 344 rats by the intravenous injection of 5 mg/kg of MNU for 26 weeks [14,41]. The original tumor was designated as tumor #9, which subsequently was cloned at the Brain Tumor Research Center, University of California, San Francisco, and then was designated “9L” [8,13,14]. These tumor cells could be propagated in vitro, which made them very useful for in vivo studies to investigate the effects of various therapeutic modalities on brain tumors. 9L cells can be implanted i.c. into syngeneic Fischer rats, following which they give rise to rapidly growing tumors. These are composed of spindle-shaped cells with a sarcomatoid appearance. The tumor margins are sharply delineated with little obvious invasion into the contiguous normal brain (Fig. 1b). The 9L gliosarcoma has a mutant *p53* gene [17], but there is normal expression of *p16* and *p19ARF* mRNAs, indicating that there is a wild type *p16/Cdkn2a/INK4α* locus [16]. Molecular characterization of the 9L relative to rat stem cell-derived astrocytes revealed an increased expression of the genes encoding *TGFα* and its receptor, *EGFR* [18]. Interestingly, decreased expression of *FGF-2, FGF-9*, and *FGFR-1* and *PDGFRβ* also was noted [18]. Recently, cancer stem-like cells (CSLCs) have been demonstrated in the 9L cell line. These CSLCs grow as neurospheres in chemically defined medium and express the neural stem cell markers Nestin and Sox2. They are self-renewable and differentiate in vitro into neuron- and glial-like cells [42]. The neurospheres have a lower proliferation rate and express several anti-apoptotic and drug related genes. Furthermore, these cells form tumors that are more aggressive than the parental 9L tumor [42], which could be an important property in future studies.

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The 9L gliosarcoma model has been used extensively to investigate mechanisms and development of drug resistance [43,44], transport of drugs across the blood-brain and blood-tumor barrier [45–48], imaging of brain tumors including radiological techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET) and imaging to evaluate tumor hypoxia and metabolism [49,50], pharmacokinetic studies of nitrosourea [48], mechanisms and effects of anti-angiogenic drugs [51,52], effects of radiation [53], chemotherapy [54,55], gene therapy [56–59], cancer stem cells [42], immunotoxin treatment [60], immunotherapy and cytokine therapy [57,61] and oncolytic viral therapy [62,63].

A number of these studies have yielded impressive therapeutic results, including apparent cures of tumor bearing animals. However, it must be emphasized that this tumor has been shown to be highly immunogenic. Animals immunized with X-irradiated 9L cells were resistant to both subcutaneous (s.c.) as well as i.c. tumor challenge, compared to 100% tumor takes in immunologically naïve animals [64]. This report was first published in the proceedings of a meeting, which did not receive wide circulation, but subsequent studies have confirmed the immunogenicity of this model [65,66]. Expression of the *s-Myc* gene under the control of a CMV promotor resulted in complete suppression of 9L tumor growth, as well as rejection of subsequent challenges of tumor cells. Histological examination of the tumors after s-*Myc* therapy revealed massive mononuclear cell infiltration with CD8 + T lymphocytes, which accounted for >70% of these infiltrating cells. These observations suggested that tumor rejection was due to a potent T-cell mediated, anti-tumor immune response. This, and several more recent studies, have underscored the significance of the anti-tumor immune response following gene therapy induced tumor eradication observed with 9L model. It is now recognized that in vivo bystander cell killing [67], which has been observed with the 9L gliosarcoma following delivery of the Herpes simplex virus thymidine kinase gene (*hsv-tk*), [62,68] followed by treatment with ganciclovir, was due in part to an anti-tumor immune response. The immunogenicity of the 9L glioma must be kept in mind when utilizing this model to evaluate the efficacy of novel therapeutic agents. Early studies employing radiation or chemotherapy alone were largely unsuccessful in curing the 9L tumor. However, the success obtained by boron neutron capture therapy and gene therapy highlights the importance of utilizing anti-tumor treatments that can destroy individual cancer cells and simultaneously spare host immune effector cells that can eradicate residual tumor cells [69–72].

Despite the fact that the 9L arose in a Fischer rat, 9L gliosarcoma cells also can form i.c. tumors in allogeneic Wistar rats [73]. Histopathological evaluation revealed that these tumors formed circumscribed masses that were not infiltrative and did not spread into the subarachnoid space or ventricles [73]. Immunostaining of the tumors revealed the presence of glial fibrillary acidic protein (GFAP-positive, infiltrating astrocytic cells, and activated ED1 positive macrophages/ microglia [73]. Higher numbers of K(ATP) and K(Ca) channels have been observed in 9L tumors grown in allogeneic Wistar rats compared to those grown in syngeneic Fischer rats. Furthermore, the allogeneic tumors showed a greater increase in brain tumor permeability upon treatment with potassium channel agonists, compared to those grown in syngeneic hosts [45]. The 9L tumor model also has been used following treatment to study the effect of BBB disruption [45], implantation of devices for repeated intratumoral delivery [12] and imaging [74].

The 9L gliomaosarcoma model also has been used to develop a model for brainstem tumors [75]. Progression to hemiparesis with the onset of symptoms occurred 17 days post-implantation into the brainstem. This model has been used to evaluate the efficacy of convection enhanced delivery (CED) of carboplatin to the brainstem, [75] and to study the response of recurrent, chemo-resistant gliomas. Two *bis*-chloroethyl nitrosourea (BCNU) resistant cell lines were derived from 9L cells by treating them with BCNU in vitro or in vivo. Both of these cell lines formed tumors in a 100% of the animals following i.c. implantation,

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and were much more invasive than the parental 9L cells [76]. The 9L gliosarcoma also has been used as a model to evaluate drug-resistant and invasive recurrent gliomas [77], but as previously indicated, caution must be used in evaluating results obtained with such a highly immunogenic tumor.

T9 glioma

Although not fully appreciated, the T9 glioma was at one time, and still may be the same as the 9L gliosarcoma [5]. The original stock of T9 cells was obtained from Sweet's laboratory at the MGH by Denlinger, and Koestner, and it was renamed T9 by them [65]. Similar to the immunogenicity of the 9L gliomosarcoma, [64], the T9 glioma also was found to be highly immunogenic [65]. Kida et al. found that rats immunized with irradiated T9 cells or T9 cells mixed with *C. parvum* rejected subsequent s.c. implants of T9 glioma cells [78]. However, in order to immunize against intracranial tumors, rats initially had to reject intradermal T9 cells [78]. As might have been predicted, these results indicated that, similar to the 9L gliosarcoma, the T9 glioma also was immunogenic. The T9 cell line subsequently has been shared among numerous investigators and has been used for many studies, including the evaluation of anti-angiogenic [79], and chemotherapeutic agents [80], immunotherapy [81], and gene therapy with interferon-β [82]. Although tumor specific or tumor associated antigens have yet to be identified, for the 9L gliosarcoma and T9 glioma, it is only a matter of time before they are identified.

RG2 glioma

The RG2 glioma (ATCC #CRL-2433) was produced in Koestner's laboratory at The Ohio State University by the i.v. administration of ENU (50 mg/kg body weight) to a pregnant Fischer 344 rat on the 20th day of gestation. Subsequently, the in vitro growth and morphology of the F98 glioma was described in detail [83], and based on its histopathology it was classified as an anaplastic or undifferentiated glioma [9]. The progeny of ENU-injected rats subsequently developed tumors, and following cloning by Wechsler in Germany, one of these clones was designated as "RG2" (rat glioma 2). The same clone was called the "D74-RG2" or "D74" in Koestner's laboratory at The Ohio State University. The RG2 glioma (Fig. 1c) is similar in microscopic appearance to the F98 glioma (Fig. 1d), and also has a highly invasive pattern of growth, which has made it a good representative model for GBM [84]. Gene expression profiling of these cells established that they had increased gene expression of *PDGFβ*, *IGF-1*, *Ras*, *Erb3/HER3* precursor mRNA and *cyclin D2* [18]. They express a wildtype *p53* and a concurrent loss in the expression of the *p16/Cdkn2a/Ink4* gene locus, [16]. It has been used for a variety of preclinical studies to evaluate changes in vascular permeability [85], disruption of the BBB [86,87], anti-angiogenic therapy [88], gene therapy [89], chemotherapy [90,91] and radionuclide therapy [92].

The RG2 glioma is non-immunogenic in syngeneic Fischer rats [84] and has low levels of MHC-1 expression compared to the C6 and 9L gliomas [93]. However, in vitro treatment with IFN-γ upregulated MHC class I antigen expression and also resulted in a significant in vivo anti-tumor immune response with increased survival of treated animals [93]. More recently, the RG2 glioma has been stably transfected with human Herpes virus Entry Mediator C (HveC) to facilitate HSV infection and has been used to study the therapeutic effects of oncolytic Herpes simplex virus-1 treatment [94]. The transfected cells retained their tumorigenicity following i.c. implantation in Fischer rats, and transfection of the HveC gene did not affect i.c. tumor growth [95]. However, it has not been determined if HveC can cause these cells to become immunogenic, and therefore, this must be taken into account when using the RG2 for immunotherapy studies.

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F98 glioma

The F98 glioma (ATCC # CRL-2397) was produced by Wechsler in Koestner's laboratory at the same time as the RG2 glioma. It is composed of a mixed population of spindle-shaped cells, the majority of which have fusiform nuclei, and a smaller number of polygonal cells with round to oval nuclei. There is extensive invasion of contiguous normal brain with islands of tumor cells at varying distances from the tumor mass, many of which form perivascular clusters (Fig. 1d). Similar to human GBM, these cells overexpress *PDGFβ*, and *Ras* along with an increase in *EGFR*, *cyclin D1* and *cyclin D2* expression relative to rat astrocytes [18]. Like the C6 glioma, they also have increased expression of Rb relative to rat astrocytes [18]. Immunofluorescence studies of F98 cells also revealed low expression of BRCA1, and a lack of radiation and cisplatin induced BRCA1 foci in these cells [54]. Usually, there is a necrotic core, scattered mitotic cells and non-glomeruloid neovascular proliferation [96]. The tumor is GFAP and vimentin positive with negligible staining for CD3 + T cells [96].

Since it simulates the behavior of human GBMs in a number of important ways, such as its highly invasive pattern of growth and low immunogenicity, it has been used to evaluate the efficacy of a variety of experimental therapeutic agents. It is refractory to a number of therapeutic modalities, including systemic chemotherapy with paclitaxel, and carboplatin [97], and it is poorly responsive to photon-irradiation alone [5], which in part may be related to its functionally impaired BRCA1 status that can favor genomic instability and impaired DNA repair [54]. Recently, it has been shown to be responsive to a combination of synchrotron radiation with cisplatin [98], and to convection enhanced delivery (CED) of carboplatin in combination with 6 MV photon-irradiation in rats bearing i.c. tumors [99,100]. This model has been used extensively by Barth et al. to evaluate the efficacy of boron neutron capture therapy (BNCT) [101,102]. Elleaume and her coworkers have evaluated cisplatin, carboplatin and iodine enhanced synchrotron stereotactic radiotherapy [103] in F98 glioma bearing rats [104]. It has also been used to evaluate non-invasive MRI to visualize tumor growth [105], diffusion tensor imaging [106], tumor angiogenesis [107] and the tumor tropism of mesenchymal stem cells [108].

The F98 glioma is very weakly immunogenic [109] and transfection with the gene encoding B7.1 co-stimulatory molecule [110], or syngeneic cellular vaccination combined with GM-CSF, did not enhance its immunogenicity [110,111]. This makes it a very attractive model to investigate the mechanisms underlying glioma resistance to immunotherapy. It has also been used to study the molecular genetic alterations in GBMs [112], effects of infusion rates on drug distribution in i.c. tumors [47], and for suicide gene therapy with Herpes simplex virus-1 thymidine kinase (HSV-TK) [113]. Like the 9L gliosarcoma, F98 cells also have been injected into the pontine tegmentum of the brainstem of Fischer rats to produce a model for brainstem tumors [75]. The histopathological and radiobiological characteristics of these tumors were comparable to aggressive, primary human brainstem tumors, which could facilitate preclinical testing of therapeutics to treat these lethal tumors.

F98 cells have been stably transfected with expression vectors encoding for wildtype EGFR and EGFRvIII, and the resulting cell lines have been designated $F98_{EGFR}$ (ATCC# CRL-2948) and $F98_{npEGFRvIII}$ (ATCC# CRL-2949). They each express ~10^5 non-functional (i.e. non-phosphorylatable) receptor sites per cell. This is below the threshold number of 10^6 sites per cell that can evoke a xeno-immune response against human EGFR in rats [114]. These cell lines have been used in Fischer rats for studies on molecular targeting [115] to evaluate the therapeutic efficacy of boronated mAbs and EGF for neutron capture therapy (NCT) [102, 116]. The boronated mAbs, L8A4, which is specific for EGFRvIII, and cetuximab, which recognizes wild type EGFR, specifically targeted their respective receptor positive i.c. tumors after CED and they were therapeutically effective following NCT [102,115–117].

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A bioluminescent F98 cell line recently was constructed by stably transfecting F98 cells with the luciferase gene. When implanted i.c. into the brains of Fischer rats, tumor size could be monitored by measuring luminescence. This model should permit rapid, non-invasive imaging of i.c. tumor growth to evaluate novel therapeutic modalities [118]. Finally, F98 cells also are capable of growing as i.c. xenografts in cats [119], but since these cells can evoke a xenoimmune response, this model is of limited usefulness. It is important to note that, what may be therapeutically effective in the rat, may not be in the human. However, it probably is safe to say that if a particular therapeutic approach is ineffective in a rat model, it is even more unlikely to be so in humans.

CNS-1 glioma

The CNS-1 glioma was derived from an inbred Lewis rat that had received weekly i.v. injections of MNU for 6 months [120]. Following i.c. implantation into Lewis rats, it demonstrated an infiltrative pattern of growth with leptomeningeal, perivascular, and periventricular spread and extension of the tumor into the choroid plexus [120]. Histologically, these tumors exhibited hypercellularity, nuclear atypia and pleomorphism, and had necrotic foci. These were surrounded by glioma cells arranged in a pseudopalisading pattern (Fig. 1e), although to a lesser extent than that seen in human GBM [6]. Like human GBMs, these tumors also were infiltrated with macrophages and T-cells, but did not have extensive glomeruloid endothelial/microvascular proliferation [6]. Kielian et al. identified the constitutive expression of monocyte chemotactic factor 1 (MCP-1) by CNS-1 cells [121]. In vivo, CNS-1 tumors also showed extensive infiltration by macrophages, which might confer a growth advantage [122]. This model has been useful to study glioma invasion [123], changes in the biology of glioma cells and their extracellular matrix [124–126], and gene therapy [127]. It also has been used to study the efficacy of immunotherapy as a potential treatment for human GBM [128] although its immunogenicity has not been studied in great detail.

BT4C glioma

The BT4C glioma was derived by giving a single transplacental administration of *N*-ethyl-*N*-nitrosourea (ENU) to pregnant BD IX rats. Dissociated brain tumor cells from one of these animals were propagated in vitro and after 200 days in culture they became tumorigenic [129]. The cells subsequently were implanted s.c. into BD IX rats and the resulting tumors contained a mixture of multipolar glia-like cells and flattened cells with fewer and shorter cytoplasmic processes and occasional giant cells [130]. BT4C glioma-derived tumors show high cellularity and have pleomorphic nuclei and numerous mitotic figures and the tumor blood vessels are irregular, dilated and show areas of proliferation (Fig. 1f) [131]. At the molecular level, BT4C cells express VEGF, tPA, uPA and MVD in the periphery of the growing tumor and are S100 positive by immunohistochemistry (M. Johansson, Personal communication). This model has been useful to test novel chemotherapeutic targeting strategies [132], antitumor effects of gene therapy [133], anti-angiogenic agents alone [134] and in combination with radiation and temozolomide [135]. BT4C gliomas also have been used to investigate the impact of hyperoxia on tumor bearing rats. This resulted in slower growth accompanied by increased apoptosis of tumor cells and reduced microvessel density (MVD). Apart from studies to evaluate therapeutic efficacy, the BT4C glioma model also has been used to study the molecular and biological changes induced by chemotherapy [136,137], radiation therapy [138] and suicide gene therapy [139]. BT4C cells, stably transfected with cDNA encoding *β*-galactosidase, have been used to evaluate the migration of single migrating tumor cell glioma spheroids and fetal brain aggregate coculture systems in vitro and in rat brains in vivo [140, 141].

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Avian sarcoma virus induced and RT-2 glioma

The induction of experimental brain tumors by the injection of Rous sarcoma virus has been described in canines, rats, and monkeys [5]. Tumors were induced by inoculating neonatal Fischer rats i.c. with purified avian sarcoma virus (ASV) suspensions [142]. All of the animals developed tumors within 2 weeks following ASV injection, 94% of which were anaplastic astrocytomas, and the remainder were low grade gliomas or sarcomas [143]. This model has been used to study the effects of chemo- and radiotherapy, BBB disruption, and tumor permeability [5]. The response to immunotherapy indicated that these tumors were immunogenic, and expressed a variety of virally encoded tumor specific antigens. A continuous cell line, designated “RT-2”, was derived from an ASV-induced Fischer rat tumor, and this has been used to study tumor growth [143], photochemotherapy [144], cytotoxic gene therapy [145] and radio-sensitization [146]. The RT2 tumor appears to be immunogenic, as evidenced by its ability to evoke a CD8 + T cell-mediated anti-tumor immune response [147], and this must be taken into account if it is used for immunotherapy studies. RT-2 cells expressing GFP have been used for quantitative assessment of glioma invasion in the rat brain [148]. The RT-2 glioma model also has been used to evaluate the therapeutic efficacy of oncolytic adenoviruses. Although they can be efficiently infected they do not permit efficient replication of E1-attenuated adenoviruses [63]. These cells also have been transfected with cDNA encoding heat shock protein 72 (HSP72), which was thought to be necessary for replication of E1 deleted adenoviruses [63]. These transfectants have been found to be permissive for replication of E1-deleted, conditionally replication-competent adenoviruses [63]. The inherent immunogenicity of the RT-2 glioma may limit its usefulness for survival studies, but nevertheless it still may be a useful model for other types of studies.

Concluding comments

Rat brain tumor models have provided a wealth of information on the biology, biochemistry, imaging and experimental therapeutics of brain tumors in experimental neuro-oncology, and there is every reason to believe that they will continue to do so. However, it is essential to recognize the limitations of each of the models that have been described, and depending on the nature of the study to be conducted, it is important that the appropriate model be selected. It now has become clear that immunogenic tumors such as the C6, 9L and T9 are not good choices for studies in immunocompetent rats, if the endpoint is prolongation of survival time or cure of the tumor. Destruction of tumor cells in these models, which have tumor infiltrating host immune effector cells within the tumor, can lead to significant amplification of an antitumor response. This may be the single most important in vivo contributor to the bystander effect that has been observed with gene therapy of the C6 and 9L gliomas following transfection with the *HSV-tK* gene and the lack of such immune amplification with the weakly immunogenic RG2 glioma. Anti-tumor immune response following transfection with suicide genes such as *HSV-tK* initially was unanticipated, but it is an important effect associated with both gene therapy and boron neutron capture therapy, but not with conventional chemo- and radiotherapy of the 9L gliosarcoma. Since human high grade brain tumors generally are regarded as being either non- or weakly immunogenic, therapeutic exploitation of this using modalities that spare tumor infiltrating host immune effector cells could have important therapeutic implications. Undoubtedly other rat brain tumor models will be developed, especially cell lines derived from genetically engineered rats that will expand the types of studies that can be carried out in this very important laboratory animal.

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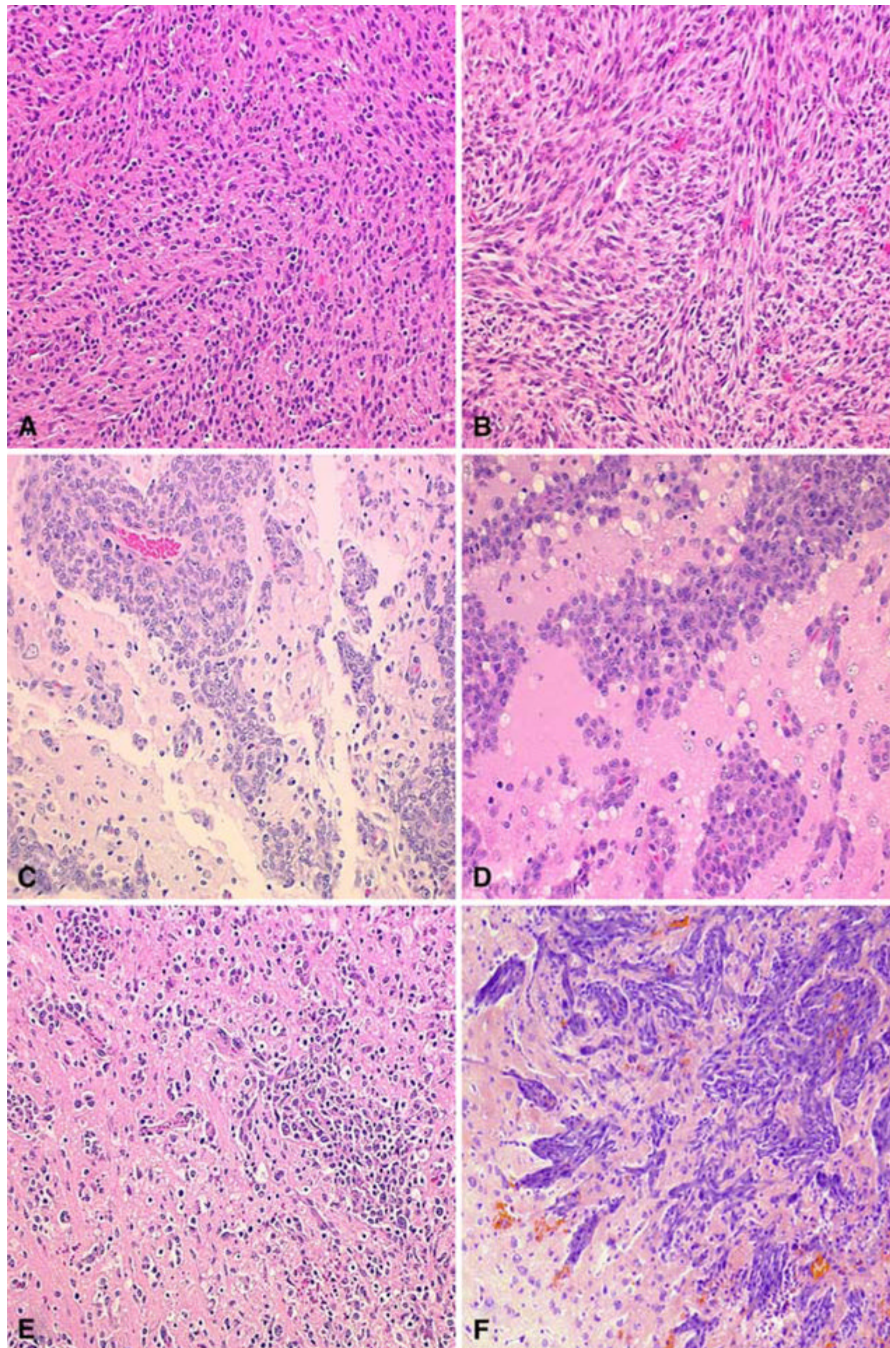

Fig. 1.

Histopathologic features of the C6, 9L, RG2, F98, CNS-1, and BT4C brain tumors. **A** The C6 glioma is composed of a pleomorphic population of cells with nuclei ranging from round to oblong. A herring-bone pattern of growth is seen in some areas and there is focal invasion of contiguous normal brain. There are scattered foci of necrosis with pseudo-palisading of tumor cells at the periphery. **B** The 9L gliosarcoma is composed of spindle-shaped cells with a sarcomatoid appearance. A whorled pattern of growth is seen with sharp delineation of the margins of the tumor with little invasion of contiguous normal brain. **C** The RG2 glioma is very similar in appearance to the F98 glioma and also has a highly invasive pattern of growth. **D** The F98 glioma is composed of a mixed population of spindle-shaped cells with fusiform

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nuclei, frequently forming a whorled pattern of growth, and a smaller subpopulation of polygonal cells with round to oval nuclei. There is extensive invasion of contiguous normal brain with islands of tumor cells at varying distances from the main tumor mass, which form perivascular clusters. Usually, there is a central area of necrosis filled with tumor cell ghosts. **E** The CNS-1 glioma is composed of a pleomorphic population of cells that show great variation in size and shape. There is extensive invasion of contiguous normal brain with dense infiltrates in some areas and in others, more circumscribed clusters of tumor cells. Small foci of hemorrhage are scattered through the tumor. **F** The BT4C glioma is composed of a pleomorphic population of tumor cells with a sarcomatous pattern of growth. Scattered tumor giant cells are seen and mitotic figures are frequent. The tumor grows expansively and invades the surrounding normal brain along perivascular tracts and occasional tumor cell nests are seen in the surrounding normal brain. There is neo-vascularization, especially in the tumor periphery, where microhemorrhages are frequent. Central necrosis is usually not present but occasionally scattered areas of necrosis may be seen in larger tumors. (Photomicrograph of the BT4C was kindly provided by M. Sandström and description by M. Johansson. Representative microscope slides of the CNS-1 glioma were kindly provided by Dr. Carol Kruse). All photomicrographs are at a magnification of 200×, except for **F**

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Table 1

Advantages and disadvantages of rat brain tumor models compared to mouse models

Advantages	Disadvantages
1. Larger size of the rat brain (compared to the mouse brain ~1200 mg vs ~ 400 mg) permits more precise stereotactic implantation than in mice, a longer interval of time until death and a thicker skull essentially eliminates osseous invasion and s.c. growth.	Rat brain tumor models cannot be as easily genetically engineered and manipulated as mouse models in order to elucidate the importance of genetic factors, signaling pathways, cell types and stroma in tumor growth and invasion.
2. Larger tumor size prior to death permits better in vivo localization and imaging by a variety of diagnostic modalities in the rat.	The potential to produce genetically engineered tumor cell lines is less in the rat than in the mouse.
3. Larger tumor size prior to death permits the administration of larger amounts of various therapeutic agents, especially if administered i.c. by CED and more critical evaluation of their effectiveness.	There are a smaller number of mAbs directed against rat surface antigens and chemokines compared to the mouse.
4. More extensive literature on in vitro and in vivo studies of rat brain tumors compared to mouse tumors.	Rats are more expensive to purchase and maintain than mice.

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Table 2

Comparison of various rat brain tumor models currently in use

Tumor	Strain of origin/Haplotype[*]	Mode of tumor induction[†]	Minimum i.c. inoculum	Immunogenicity	Pattern of growth	Molecular markers	Original reference
C6[**]	Outbred Wistar	MNU	10^4	Strong	Circumscribed	Deletion *p16/CDkn2a/Ink4a*; no expression of *p16* and *p19ARF* mRNAs or of wildtype *p53*; increased expression *of PDGFβ*, *IGF-1*, *EGFR and ErbB3/HER3* precursor proteins, decreased expression of *EGF-9 and 10 and IGF-II* genes	[41]
9L	Fischer/$RT1^{lv1}$	MNU	10^4	Strong	Circumscribed	Mutant *p53,* increased expression of *TGFα* and *EGFR*; decreased expression of *FGF-2, FGF-9, FGFR-1* and *PDGFRβ*	[41]
T9	Fischer/$RT1^{lv1}$	MNU	10^4	Strong	Circumscribed	Presumably similar to 9L	[41]
RG2 (D74)	Fischer/$RT1^{lv1}$	ENU	10^1–10^2	Weak	Infiltrative	Deletion of *p16/Cdkn2a/Ink4a* gene; increased expression of *PDGFβ*, *IGF-1*, *Ras*, and *Erb3/HER3* precursor mRNA and *cyclin D2*	[83]
F98	Fischer/$RT1^{lv1}$	ENU	10^1–10^2	Weak	Infiltrative	Deletion of *p16/Cdkn2a/Ink4a* gene; increased expression of *PDGFβ*, *RbRas*, *EGFR*, *cyclin D1* and *cyclin D2*	[83]
CNS-1	Lewis/$RT1^{l}$	MNU	5×10^3	Weak	Infiltrative	Expression of vimentin	[120]
BT4C	BD IX/$RT1^{dv1}$	ENU	10^4	Weak	Infiltrative	Expression of VEGF, tPA, uPA and MVD	[130]

[*] The haplotype information was kindly provided by Dr. Carol Kruse, Sidney Kimmel Cancer Center, San Diego, CA

[**] C6 cells express $RT1^{u}$, a haplotype of inbred Wistar-Furth rats [149]

[†] Abbreviations: *MNU*: methylnitrosourea; *ENU: N*-ethyl-*N*-nitrosourea