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Review

Mouse models of glioma

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ABSTRACT

Gliomas are the most common primary tumour in the central nervous system in adults. The pathological hallmark of gliomas is their propensity for extensive infiltration into the surrounding brain parenchyma which results in tumour recurrence. Despite the use of optimal surgical removal and adjuvant therapies the most aggressive of these tumours, glioblastoma multiforme, has a poor patient prognosis, with median survival of less than 15 months. In this review, we discuss mouse glioma models that have been utilised to advance our basic knowledge of the processes involved in gliomagenesis and their use in the testing of novel therapies and treatment regimens in the preclinical setting.

1. Introduction

Gliomas are the most prevalent malignant brain tumour, causing approximately 2.42% of cancer-related deaths in the USA in 2013 and with over 23,000 new patients expected annually [1]. They account for 32% of all central nervous system (CNS) tumours and 80% of malignant primary CNS tumours [2]. There are a number of histological subtypes, as stratified by the World Health Organization (WHO) [3]. Grade I and II low-grade astrocytomas are slow-growing, less aggressive tumours, while Grade III and IV are the more aggressive malignant tumours. The most common form, with the worst prognosis, is glioblastoma multiforme (GBM) (WHO Grade IV) [4–6]. The current standard treatment for GBM includes surgery to maximally debulk the tumour and post-operative fractionated radiation therapy with concomitant chemotherapy with temozolomide and subsequent adjuvant temozolomide [7]. The median survival for GBM patients treated with this protocol is 14.6 months, with only 26.5% of individuals surviving for 2 years. Similar median survival figures (14.8 months) have also been achieved with alternative treatments, including photodynamic therapy [8–10]. The infiltrative nature of gliomas inevitably results in incomplete surgical removal of all the tumour cells. Despite recent advances in the field of neuro-oncology, the continued dismal prognosis for glioma patients demonstrates the need for efficacious tumour-specific therapies.

Modelling the formation of gliomas in mouse models is essential for the development of more effective treatments. A number of core signalling pathways are known to be involved in gliomagenesis (Fig. 1). As with many cancers, gliomas undergo a considerable range of genetic, histological and physiological changes that contribute to their malignant and invasive phenotype [11,12]. The use of models allow for the systematic identification of signals and pathways that contribute to tumour initiation, maintenance of tumourigenic phenotype and resistance to therapeutic intervention. The information gathered from the biological pathway investigations allows for the evaluation of novel experimental targeted therapeutic strategies in preclinical studies. Whilst many of the biological pathways involved in cancer have been studied extensively in cell culture, and the results have been informative, the complex biology of the *in vivo* environment is not entirely approachable within a cell culture system. Therefore, there are innate limitations for modelling invasion, angiogenesis and metastasis in a cell culture system. Also, there is increasing evidence that the surrounding stroma and multiple local growth factors found in an *in vivo* environment may promote cancer progression and therapeutic resistance [13–15]. In addition, with the development of the cancer stem cell field [16,17], xenograft models have yielded important information on therapeutic resistance from the small population of self-renewing stem-like cells that may be responsible for tumour recurrence [17,18].

An ideal mouse glioma model should possess a number of features to allow for an accurate prediction of clinical outcome of novel therapeutic strategies. The model must show histological resemblance (cellular heterogeneity) to the human glioma (Fig. 2), it must bear a genetic similarity to the human glioma, must be orthotopic and show intraparenchymal growth providing the full range of complex *in vivo* tumour–stromal interactions, be non-immunogenic in a host with an intact immune system, must

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show invasive and angiogenic-like growth with no encapsulation, be highly reproducible with predictable growth, and must imitate the therapeutic response of the human glioma.

2. Glioma models

A number of different approaches have been utilised for developing glioma models in animals over several decades. Primarily, these include chemically induced models, xenograft transplantation models and genetically engineered models (GEM) – see Table 1 for a representative cross-section of these models [19–23]. Due to the extensive number of models utilised for the various approaches, examples representing each of these strategies will be discussed below.

2.1. Chemically induced models

The first successful chemical induction of brain tumours was carried out by Seligman and Shear in 1939 with the implantation of methylcholanthrene pellets into the brains of mice [24]. A number of the mice developed gliomas, which were subsequently evaluated through subcutaneous passage [25]. Gliomas have been induced by treating animals, most commonly, with the DNA alkylating agents known as the nitrosoureas (for a review see Barth [26]). These models are of undefined genetics, in that tumour inductions occur through the non-random alkylation of bases which gives rise to base mis-pairing and point mutations. As a result, the time of induction, incidence, malignancy type and location of tumours varied greatly within each study [27–30]. As proposed in the landmark papers outlining the hallmarks of cancer by Hanahan and Weinberg [31,32], the progressive evolution of normal cells to a neoplastic state is a multistep process. The cells undergo a series of genetic events and through signalling interactions with the surrounding microenvironment, they acquire the traits that enable them to become tumourigenic. Induction of gliomas based on exposure to chemical agents, such as the nitrosoureas, are more likely a result of a neurotropic or teratogenic effect than a true representation of the processes involved in the human counterpart.

Chemically induced glioma models include 9L, C6, GL261 and CNS-1 [33,34]. An advantage of these models is that the tumours present in a syngeneic immunocompetent host allowing the immune system to interact with the developing tumour. The C6 glioma cell line was developed by the recurring administration of methylnitrosourea (MNU) in adult Wistar rats [35]. The cells share several histopathological and tumour markers with GBM [36,37], but differ from human GBM in the expression profile of the tumour suppressor p53, coupled with reduced phosphatase and tensin homolog (PTEN) expression [38,39]. The 9L glioma cell line was also created with the use of MNU, but has a sarcomatous appearance, does not express glial fibrillary acidic protein (GFAP) or show the diffuse infiltrative pattern of GBM cells. However, they do possess a mutant p53 gene and overexpress the epidermal growth factor receptor (EGFR) as seen in many human GBM [38,40]. The CNS-1 glioma cell line was established from a rat injected weekly with MNU. It expresses several glioma markers, including GFAP, S100β, vimentin and intracellular adhesion molecule-1, whilst displaying a diffuse and infiltrative pattern of growth [41]. The GL261 model is syngeneic and was created by the intracranial implantation of methylcholanthrene tablets into mouse brains [42]. The cells show a poorly differentiated morphology similar to GBM, and demonstrate a diffuse and infiltrative pattern into surrounding normal brain [43,44]. As seen in human GBM, this model exhibits point mutations in the p53 tumour suppressor gene [45] and K-ras oncogene [46], whilst also showing increased activation of the phosphoinositide 3-kinase (PI3K) pathway and phosphorylation of Akt [47,48].

But there have been observations of spontaneous tumour rejection in studies using the C6 and 9L cell line models which may mimic therapeutic efficacy, ultimately leading to a biased outcome [49]. However, the interaction between the tumour and the immune system is an important consideration, especially for invading tumour cells that migrate from the main tumour towards the normal brain environment. This factor may account for the disappointing translation of many successful preclinical studies in immunocompromised mice to humans. But histological descriptions of these models have been varied. GL261 cells were initially described as displaying ependymoblastoma characteristics [50], whereas more recent work further describes the cells as poorly differentiated, with morphology similar to GBM cells [43] and individual cell invasion several millimetres from the tumour margin [51]. C6 glioma tumours exhibit a diffuse infiltrating pattern similar to GBM when they are implanted into Wistar rats [52], but when placed in Sprague-Dawley or Long-Evans rats, they grow as a discrete mass lacking an invasive edge [53].

Table 1
A selection of reported mouse models of human glioma

Model	Tumour grade	Strategy	Study
9L	GS	Xenograft	Schmidek et al. [28]
C6	GBM	Xenograft	Benda et al. [39]
CNS-1	GBM	Xenograft	Kruse et al. [41]
F98	GBM	Xenograft	Ko et al. [124]
GL261	GBM	Xenograft	Seligman and Shear [24]
RG2	GBM	Xenograft	Swenberg et al. [125]
U251MG	GBM	Xenograft	Houchens et al. [59,126]
U87MG	GBM	Xenograft	Ponten et al. [59]
v-Src	A, AA	Transgenic/GFAP promoter	Wee et al. [127]
V^{12}Ha-ras	A, AA, GBM	Transgenic/GFAP promoter	Ding et al. [128]
V12Ha-ras and EGFRvIII	OA	Transgenic/GFAP promoter	Ding et al. [102]
GFAP-Cre	GBM	Flox *Nfl* + p53 knockout	Reilley et al. [129]
GFAP-T121 tg	GBM	PTEN ± knockout	Xiao et al. [130]
NESTIN-Cre^{ER}	GBM	Flox *NF1*, Flox *PTEN*, Flox *p53*	Alcantara Llaguno et al. [131]
PTEN−/− and K-ras	GBM	RCAS/tv-a; cre-lox system – deletion of PTEN/nestin	Yan et al. [132]
p53−/− and Nf1−/−	A, AA, GBM, lymphoma, sarcoma	GFAP-driven cre-lox system - deletion of Nf1	Dai et al. [133]
PDGFB and Ink4a-Arf−/−	A, AA, GBM, OA	RCAS/tv-a; cre-lox system – deletion of PTEN	Hambardzumyan et al. [134]
PDGFB and p53−/−	GBM, OA	Transgenic mice/GFAP	Weiss et al. [103]

A = astrocytoma, AA = anaplastic astrocytoma, EGFR = epidermal growth factor receptor, GBM = glioblastoma multiforme, GFAP = glial fibrillary acidic protein, GS = gliosarcoma, OA = oligoastrocytoma, OG = oligodendroglioma, PDGFB = platelet-derived growth factor B-chain, PTEN = phosphatase and tensin homolog, RCAS/tv-a = replication competent avian leucosis virus splice acceptor/receptor for avian leukosis virus subgroup A.