**Current trends in mouse models of glioblastoma**

Masafumi Miyai¹,² · Hiroyuki Tomita¹ © · Akio Soeda² · Hirohito Yano² · Toru Iwama² · Akira Hara¹

Received: 31 March 2017 / Accepted: 1 October 2017 / Published online: 20 October 2017 © The Author(s) 2017. This article is an open access publication

**Abstract** Glioblastoma is the most deadly brain tumor type and is characterized by a severe and high rate of angiogenesis, remaining an incurable disease in the majority of cases. Mechanistic understanding of glioblastoma initiation and progression is complicated by the complexity of genetic and/or environmental initiating events and lack of clarity regarding the cell or tissue of origin. To determine these mechanisms, mouse models that recapitulate the molecular and histological characteristics of glioblastoma are required. Unlike in other malignancies, viral-mediated mouse models of glioblastoma rather than chemically induced mouse models have been developed because of its sensitivity to viruses. Based on recent molecular analyses reported for human glioblastoma, this review critically evaluates genetically engineered, xenograft, allograft, viral-mediated, and chemically induced mouse models of glioblastoma. Further, we focus on the clinical value of these models by examining their contributions to studies of glioblastoma prevention, tumorigenesis, and chemoresistance.

**Keywords** Glioblastoma · Mouse · Xenograft · Glioma

**Introduction**

Glioblastoma is the most common and deadly primary brain tumor [1], and the most aggressive type of glioma, a collection of tumors arising from glial cells. It is also termed glioblastoma multiforme because of its complex phenotype. The current standard of care is based on maximal safe surgical resection and concurrent chemoradiation with temozolomide, followed by maintenance chemotherapy, resulting in median survival rates of approximately 15 months [2].

Over the past few years, genomic and proteomic characterization along with robust animal models of glioblastoma have provided invaluable data. In addition, pre-clinical models and a better understanding of the core pathways disrupted in glioblastoma are providing renewed optimism for novel strategies targeting these devastating tumors. Here we discuss the current advances in rodent models, particularly mouse models, of glioblastoma and other gliomas, and how these developments have influenced strategies for therapeutic intervention.

**Pathological features of glioblastoma**

The 2016 World Health Organization Classification of Tumors of the Central Nervous System uses molecular parameters in addition to histology to define many tumor entities [3]. In this new classification, diffuse gliomas include WHO grade II and grade III astrocytic tumors, grade II and III oligodendrogliomas, and grade IV glioblastomas.

Glioblastomas are divided into three categories in the 2016 CNS WHO classification according to a key genetic prognostic marker, isocitrate dehydrogenase (IDH): glioblastoma, IDH-wildtype; glioblastoma, IDH-mutant; glioblastoma, NOS. IDH-wildtype (about 90% of cases) corresponds...
most frequently with clinically defined primary or de novo glioblastoma and predominates in patients over 55 years of age [4]. IDH-mutant glioblastoma (about 10% of cases) corresponds closely to so-called secondary glioblastoma with a history of lower-grade diffuse glioma, and preferentially arises in younger patients [4]. Glioblastoma NOS is a diagnosis that is reserved for tumors for which full IDH evaluation cannot be performed or for which test results remain inconclusive.

Pathological characteristics of glioblastoma is typically a highly cellular glioma, usually comprised of poorly differentiated, sometimes pleomorphic tumor cells with nuclear atypia and vigorous mitotic activity. Conspicuous microvascular proliferation and/or necrosis is an essential diagnosis characterization.

Molecular characteristics typical of IDH-wild type glioblastoma include TERT promotor mutations (present in ~ 80% of cases), homozygous deletion of CDKN2A/CDKN2B (~ 60%), loss of chromosomes 10p (~ 50%) and 10q (~ 70%), EGFR alterations (i.e. mutation, rearrangement, altered splicing, and/or amplification; ~ 50%), PTEN mutations (25–30%), and PI3K mutations (~ 25%) [4, 5].

Mouse models of glioblastoma

The use of mice to create suitable models for the study of specific tumors or to investigate the role of candidate genes has obvious advantages. Firstly, manipulation of the mouse genome to create specific genetic changes by microinjection of DNA into fertilized eggs or by homologous recombination in embryonic stem cells is relatively easy compared with other mammalian species such as rats. Another advantage is the availability of inbred strains of mice that are genetically identical, obtained by breeding sibling mice over 20 generations. Since these animals present the same genetic background, they can be compared for their response to a treatment or a genetic modification between different laboratories. The laboratory mouse shares extensive molecular and physiological similarities to humans and is a powerful tool for studying cancer.

Transgenic mouse models offer an opportunity to develop and utilize an easily replenished, reproducible, spontaneously manipulated, and more accurate pre-clinical model of human cancers, which we can use to extend our molecular knowledge and to test promising therapies. Therefore, mouse models that recapitulate human glioblastoma may be an invaluable tool. However, these conventional genetic approaches, such as transgenics and knockouts, are limited by the time and costs associated with extensive intercrossing of mouse lines. Several new viral vector-mediated genetic approaches offer the ability to directly modify the genome of somatic cells in mouse tissues and these have recently been applied to the rapid generation of complex mouse tumor models that harbor multiple genetic changes. On the other hand, xenograft and allograft models can be used to measure therapeutic responses to drugs more rapidly than genetically engineered or viral vector-mediated models. Chemical carcinogen-induced models are usually generated in rats, and only a small number of instances of chemical carcinogen-induced models are currently known.

Genetically engineered models

The molecular progression of gliomas, like many tumors, involves the accumulation of genetic and epigenetic alterations that result in the loss of tumor suppressor gene function (PTEN, TP53, CDKN2A, RB) or the activation of oncogenic pathways (p21–RAS, PI3K, EGFR, CDK4, MDM2) [6–8].

There are several examples of aberrant expression of relevant downstream signaling pathways in mouse glioma modeling. These include astrocytomas of varying grades resulting from glial fibrillary acidic protein (GFAP)-regulated expression of v-src [9]. Weissenberger et al. [9] generated a transgenic mouse model for low-grade astrocytoma (early) and high-grade astrocytoma (later) by expressing v-src kinase under the control of the GFAP gene regulatory elements in astrocytes.

Ding et al. [10, 11] utilized our initial observation of aberrant activation of the p21–RAS signaling pathway in astrocytomas to develop glioma models using ES transgenesis [11]. Since wild-type EGFR and mutant EGFRvIII are the most common gain-of-function alterations in malignant human astrocytomas, they reported the generation of mice expressing these proteins under the regulation of the GFAP promoter [10, 11].

When the glioblastoma-like tumors are examined in these mice, additional genetic alterations such as those found in human glioblastomas (overexpression of EGFR, CDK4, MDM2; decreased expression of CDKN2A, TP53, PTEN) are present [12].

Zhu et al. [13] reported that loss of TP53 and activation of the RAS pathway via NF1 inactivation in CNS cells is sufficient to cause malignant astrocytoma formation with 100% penetrance.

Overexpression of relevant oncogenic receptors or downstream signaling pathways has also been employed in the development of mouse glioma models. These have been either alone or in combination with mice harboring specific knockouts of relevant cell cycle regulatory proteins. For example, S100 glial precursor promoter-regulated v-ERBB (an activated member of the EGFR family) transgenic mice develop oligodendrogliomas, which are potentiated in terms of shorter latency and increased malignancy when initiated in mice deficient for both p16 and p19 (Cdkn2a-null mice) [14].
Briefly, Bardella et al. [15] have established small nodules like glioma in the subventricular zone. Nes-CreER(T2); Idh1fl(R132H)+ developed small nodules (up to 1 mm diameter) originating from the subventricular zone at 2–6 weeks after tamoxifen injection.

The nodules expressed proliferation markers, such as Ki67, and retained BrdU label, suggesting that they exhibited a variety of proliferative behaviors. Further, many nodule cells also expressed the astrocytic and NSC marker GFAP and in some lesions, a few cells expressed the neuroblast marker Doublecortin (Dcx).

Table 1 summarizes the currently used and relevant glioma mouse models that recapitulate the hallmarks of human glioblastoma.

**Viral vector-mediated transduction model**

In recent years, viral vectors have been extensively used for the generation of mouse models of interest in the study of brain tumors. Several routes for viral vector delivery to the brain are available: intracerebral stereotaxic injection, intrathecal and intraventricular injection, and intravascular infusion with or without modification of the blood–brain barrier. The choice of route for viral vector administration needs to be carefully considered since it affects neuronal cell transduction efficiency and spatial distribution, as well as the level of transgene expression in the infected cells [16]. Intracerebral injection offers the advantages of low toxicity, high local vector concentrations, and localized transgene delivery, but it does not allow wide viral vector distribution and requires invasive surgical intervention. Ubiquitous distribution of viral vectors in the CNS could be achieved by intrathecal or intraventricular injection but these methods do not permit spatial selectivity of delivery and require a large amount of vectors. Finally, intravascular viral vector applications do not require invasive surgical intervention but necessitate the use of high vector concentrations because of losses in peripheral organs such as the liver.

Virally transduced expression of relevant gain-of-function alterations, in combination with transgenic mouse technology, allows one to model such somatic alterations at a later stage in life, though it does not lead to germline colonies.

---

**Table 1** Genetically engineered and viral vector-mediated transduction mouse models of human glioma

| Tumor classification | Transgene | Knockout, knockin | Grade | Incidences | Study |
|----------------------|-----------|-------------------|-------|------------|-------|
| Small nodule like glioma | NES-CreER(T2) | IDH1 R132H knockin | NA* | 100% by 2–6 weeks | Bardella et al. [15] |
| Low-grade astrocytoma | Src transgene | | II | 14% by 2.5–65 weeks | Weissenberger et al. [9] |
| High-grade astrocytoma | GFAP-HRASV12 | Floxed NF1 + Trp53 knockout | III–IV | 100% by 2–16 weeks | Ding et al. [10] |
| Glioblastoma | NF1 + Trp53 cis | II–III | 100% by 4–32 weeks | Xiao et al. [55] |
| | HRASV12 and AKT | III | 40% by 16–20 weeks | Marumoto et al. [23] |
| | GFAP-Cre | II–IV | 100% by 10–45 weeks | Zhu et al. [13] |
| | PTENF/+ | IV | 42–49% by 12 weeks | Uhrbom et al. [18] |
| | GCN2a knockout | IV | 100% by 4–7 weeks | Hambardzumyan et al. [19] |
| | EGFRvIII(Ad-Cre virus) | PTENF/+ | II–IV | 93% by 6–15 weeks | Wei et al. [21] |
| | HRASV12 and AKT | Trp53 knockout | IV | 100% by 10–13 weeks | Marumoto et al. [23] |
| | NES-CreER | Floxed NF1, Floxed PTEN, Floxed Trp53 | III–IV | 100% by 24–56 weeks | Alcantara Llaguno et al. [24] |
| | EGFRvIII(Ad-Cre virus) | Cdkn2a, PTENF/+ | IV | 100% by 5–13 weeks | Zhu et al. [57] |
| | S100b-HERBB transgene | | II | 75% by 52 weeks | Weiss et al. [14] |
| | PDGFB (RCAS virus) | | II | 60% by 12 weeks | Dai et al. [20] |
| | High-grade oligodendrogliaoma | S100b-HERBB transgene | Cdkn2a knockout | III | 90% by 4–24 weeks | Weiss et al. [14] |
| | PDGFB (RCAS virus) | | III | 100% by 2–13 weeks | Ding et al. [11] |
| | Diffuse intrinsic pontine glioma | PDGFB + H3.3K27M (RCAS virus) | Trp53 knockout | II–IV | 73% by 5–12 weeks | Misuraca et al. [25] |

*not applicable
Although the link between a viral etiology and human gliomas is weak, retroviruses that have been engineered to express relevant gain-of-function genes have been used to create glioma models in mice and other mammals [17, 18]. This includes members of the Rous sarcoma virus family and simian sarcoma virus, whose transforming properties result from overexpression of the viral oncogene v-sis, the cellular counterpart of which is c-sis or PDGF-B.

Retroviruses carrying v-sis (PDGFB) injected into normal mice have yielded astrocytic tumors, with varying glioma types generated when injected in Cdkn2a-null mice. One of the best examples of coupling retroviruses to express somatically defined gain-of-function genes in varying cell lineages and genetic backgrounds to model gliomas is the RCAS-tva system [19]. This system results in focal gliomas, the subtype and grade of which varies with the injected retrovirally transduced gene (i.e. PDGFB, EGFVIII, activated p21–RAS, activated AKT), the lineage of the cell expressing the tva receptor (GFAP, NES) and underlying genetic cell cycle alterations in the mice (null for Cdkn2a, Trp53 etc.). For example, retrovirally transduced expression of v-sis or PDGFB in GFAP-tva mice resulted in oligodendrogliomas or mixed oligoastrocytomas in 40% of the mice, with 60% of NES-tva mice developing similar gliomas [10]. When these experiments were undertaken in Cdkn2a-null mice, the gliomas formed with a shorter latency and were of higher grade [20]. On the other hand, injection of adenovirus containing the EGFVIII mutant into mice harboring activated RAS led to efficient formation of glioblastoma [21].

Lentiviruses expressing oncopgenes such as HRAS or AKT were efficiently introduced into mice expressing Cre-recombinase under various promoters such as GFAP. Glioblastoma tumors were efficiently formed when lentiviruses harboring activated RAS and AKT were injected into mice expressing GFAP-Cre on a Trp53 heterozygous background [22, 23]. Current studies have allowed the determination that the gliomagenesis potential of mice is greater at a younger age with excision of glioblastoma-relevant genes such as PTEN, NF1, and TP53 [24]. Misuraca et al. [25] have established low- and high-grade glioma, which phenocopies diffuse intrinsic pontine glioma, resulting from injection of Pax3-Tv-a;Trp53fl/fl mice with RCAS-PDGFB and RCAS-Cre, with or without RCAS-H3.3K27M. In the RCAS/Tv-a glioma mouse model, avian retroviruses (produced from RCAS plasmids) infect mouse cells expressing Tv-a (the receptor for RCAS viruses) [26].

Xenograft and allograft models

For many years, immunodeficient rodents have been an important tool in modeling human glioblastoma. Propagation and testing of glioblastoma in such animals is most commonly accomplished in the subcutaneous flank location (heterotopic), although recent years have seen increased use of orthotopic (intracranial) xenograft models. For both heterotopic and orthotopic studies, xenograft and allograft tumors are usually established from permanent human glioblastoma cell lines.

Invasive orthotopic xenografts have also been established from surgical specimens that were first maintained as tissue spheroids in short-term culture [27, 28]. Finally invasive intracranial tumors have been established from heterotopic xenografts generated by direct transplantation of surgical specimens and subsequently sustained by serial passaging in the flanks of nude mice [29, 30].

Glioblastomas that have been continuously propagated as flank tumors recapitulate this very important and characteristic feature of human glioblastoma following intracranial transfer. Different from the heterotopic transplantation, the direct orthotopic transplantation denies the influences of in vitro culture, provides a proper microenvironment, and preserves the integrity of tumor-initiating cells [31, 32].

Many human and mouse cell lines have been used in xenograft and allograft models (Table 2). Tateishi et al. [33] used the SCID mouse to study the vulnerability of IDH1-mutant cancers to NAD+ depletion. Ashizawa et al. [34] used NOD-SCID mice and NOG mice to study the effect of the STAT3 inhibitor STX-0119 on the proliferation of cancer stem-like cells derived from recurrent glioblastoma. Other mouse strains such as athymic nude (Nu/Nu) mice [35–37], CD1 nude mice [38], and athymic nude Foxn1-nu mice have also been used [39–41].

A library of orthotopic glioblastoma xenograft models using surgical samples of glioblastoma patients has been established. These patient-derived glioblastoma xenograft (PDX) tumors recapitulated histopathological properties and maintained genomic characteristics of parental glioblastoma in situ [42, 43]. Soeda et al. [44] reported a glioblastoma xenograft model containing heterogeneous subclones derived from a single tumor of a patient. This model may be useful for evaluating cell- and patient-specific drug responses. Patient-derived primary glioma cells might be a good solution but they are sometimes unable to maintain for long in culture and finding an accessible cell type for gliomas might be problematic.

Patient-derived stem cells are used to identify cell functions that are altered by disease, such as Alzheimer’s and Parkinson’s disease and thereby provide a target for drug discovery. Patient-derived glioblastoma stem cells have been generated from xenograft tumors of the glioblastoma surgically resected [45, 46]. Patient-derived glioblastoma stem cells are in nature, formed much larger neurospheres in a short period of time rather than patient-derived glioblastoma cells (not stem cells) [47]. Further, Sancho-Martínez et al. [48] have recently established human induced pluripotent stem (iPS) cells based glioma models in vivo.
Table 2: Trends in xenograft and allograft mouse models of glioma

| No | Study Type | Cell line | Tumor histology | Genetic change | Animal model | Therapy | Drug administration method | Injection point of cells | Time of initiating the therapy | Duration of treatment | Observation period |
|----|-------------|-----------|----------------|---------------|-------------|---------|---------------------------|--------------------------|----------------------------|-------------------|------------------|
| 1  | Xenograft   | MGG152(TIC*), HT1080(Human cell line) | Recurrent glioblastoma | IDH1 mutant(MGG152), IDH1-R132C | SCID mice | NAMPT inhibitor | Oral administration (MGG152) intraperitoneal injection (HT1080) | Right striatum (MGG152) right flank (HT1080) | one week (MGG152) tumor diameters reached 5 mm (HT1080) | 1 x/week (MGG152): 17 days (HT1080) | About 30 days |
| 2  | Xenograft   | GB-SCC010 GB-SCC026 (primary glioblastoma stem cell lines from patients) | Primary glioblastoma | NOD-SCID**, mice, NGL**, mice 5–6 week old | STAT3 inhibitor | Oral administration | Subcutaneous | Bearing tumor of >35mm | Daily/three weeks | 28 days |
| 3  | Allograft   | Primary ink4a-arf-/- astrocyte (mouse cell line) | Similar to glioblastoma | ΔEGFR-expressing and PTEN wild-type | Athymic mice 6–8 week old female | EGFR inhibitor (gefitinib) | Oral gavage | Cerebral (2 mm lateral and 1 mm anterior to the bregma) | 20 days | 5 days per week | About 50 days |
| 4  | Xenograft   | SF-295(Human cell line) U251(Human cell line) | Glioblastoma | NA**** | Athymic mice | Temozolomide BCNU | Oral gavage(TMZ) tail vein injection | Right cerebral hemisphere | 1 day | day 1, day 5, day 9 | 90 days |
| 5  | Xenograft   | LNT-229 (Human cell line) LN-308(Human cell line) | Glioblastoma | Silencing microRNA-adapted shRNA | Neutralizing antibodies to VEGF or PI GF | Intraperitoneal injection | Right striatum | NA**** | Twice weekly | 60 days |
| 6  | Xenograft   | BT111(TIC*), BT116(TIC*) | Primary glioblastoma | Unmethylated MGMT(BT111) NA****(BT116) | NU-Foxn1** node mice | Monoamine oxidase B-activated pro-drug | Tail vein injection | Flank postglenoid foramen | 4 weeks (flank model): 90 days (intracranial model) | day 0, day 12, day 25 (flank model) day 115, day 123, day 131 (intracranial model) | 36 days (flank model): 307 days (intracranial model) |
| 7  | Allograft   | GL261-Luc(mouse cell line) | Glioblastoma | NA**** | C57BL/6J mice 6–8 week old | Radiation plus anti- PID-1 antibody | Intraperitoneal injection | Left striatum 1 mm lateral and 1 mm anterior to the bregma, 3 mm deep from the cortical surface | 10 days | day 10, day 12, day 14 | 90 days |
| 8  | Xenograft and allograft | LN-319(Human cell line), GL261(mouse cell line) | Glioblastoma | NA**** (LN-319) ErbB2 expression(GL261) | NSG*** mice (LN-319) C57BL/6 mice(GL261) | ErbB2/HER2-Specific NK Cells | Intratumoral injection | Subcutaneous right striatum (depth of 3 mm) | Weekly for 11 weeks (LN-319) weekly for 3 weeks(GL261) | 303 days (LN-319): 200 days (GL261) |
| No | Study | Type | Cell line | Tumor histology | Genetic change | Animal model | Therapy | Drug administration method | Injection point of cells | Time of initiating the therapy | Duration of treatment | Observation period |
|----|-------|------|-----------|-----------------|----------------|--------------|---------|--------------------------|--------------------------|-----------------------------|---------------------|-----------------|
| 9  | Parrish et al. [60] | Xenograft | GBM12(TIC*) | Primary glioblastoma | MGMT hypomethylated | NA**** | PARP inhibitor (rucaparib) | Intraperitoneal injection | Flank cerebral | NA**** | Days 1–5 every 28 days for 3 cycles | 121 days (flank model) 81 days (intracranial model) |
| 10 | Gupta et al. [61] | Xenograft | GBM12(TIC*) | Primary glioblastoma | TMZ-mgmt High TMZ-mgmt Low | Athymic mice | PARP inhibitor (veliparib) | Flank | Tumor of − 100 ± 15mm³ | 5 days every 28 days for 3 cycles | About 50 days |
| 11 | Garros-Regulez et al. [62] | Xenograft | U251 (Human cell line) | Glioblastoma | NA**** | Foxn1null- Foxn1null nude mice | mTOR inhibition (rapamycin) | Intraperitoneal injection | Flank | 1 week | Twice weekly for 12 weeks | About 60 days |
| 12 | Hashizume et al. [63] | Xenograft | SF7761(TIC*) | Primary pediatric human glioma adult glioblastoma | H3F3A K27M mutation H3F3A K27M mutation MGMT unmethylated | Athymic mice | Demethylase inhibitor | Intraperitoneal injection | Flank brain stem | SF7761: 50 days SF8628: 56 days GBM43: 5 days | SF7761: 160 days SF8628: 77 days GBM43: 18 days |
| 13 | Mathieu et al. [64] | Xenograft | Hs683 (Human cell line) U373 (Human cell line) | Glioma (Hs683) glioblastoma (U373) | NA**** | Nude mice (immuno-compromised) | Bevacizumab Temozolomide | Tail vein injection | Cerebral | 5 days | 3 times per week for 3 consecutive weeks | 80 days |
| 14 | Cho et al. [65] | Xenograft | L8443 (Human cell line) | Glioblastoma | Expressing EGFRvIII CT De11 mutant (by retroviral infection) | SCID mice | Cetuximab erlotinib | Intraperitoneal injection | Right striatum | 1 week | 3 times per week | 100 days |
| 15 | Yoshida et al. [66] | Xenograft | GBM39(TIC*) | glioblastoma | EGFRvIII amplified (GBM39) expressing EGFRvIII (by retroviral infection U87) wild-type EGFR (GBM12) | Athymic mice | Pan-ERBB inhibitor | Oral administration | Right caudate putamen | 14 days (GBM39) 11 days (U87) 6 days (GBM12) | 2 week (GBM39) 21 days (U87) day 10, day 13, day 16, day 20, day 23 (GBM12) | 66 days (GBM39) 32 days (U87) 70 days (GBM12) |
| 16 | Joo et al. [42] | Xenograft | Surgical specimens from glioblastoma patients | Glioblastoma | Depending on specimens | NOG**mice | NA**** | cerebral | With in 12 months | NA**** | About 200 weeks |

*Patient-derived tumor initiating cell
**NOD/Shi-Parkdcscid
***NOD/Shi-scid IL-2Rγ-null
****NOD-SCID IL2Rγnull
*****not applicable
Recent progress and expansion in next-generation sequencing (NGS) technologies enable to characterize the cancer genome in a time frame that is corresponding to treatment decisions, providing the chance to potentially increase the therapeutic effect by targeting the genomic alterations driving tumor behavior [49]. To challenge proposed therapy strategies on the patient of gliomas, the “Avatar mouse models”, which are based on the NGS data and generated as the individualized mouse xenografts by transplanting patient-derived tumor cells, have been investigated [50]. The development of PDX and patient-derived neurosphere/stem cell based xenograft models may approve bench testing of treatment strategies derived from the innovative genomic analysis.

There are important caveats to this approach that still need to be addressed in xenograft models. Firstly, the mice do not have an intact immune system. Inflammatory cells may be a critical component to the biology of the tumor and its response to certain drugs, particularly immunotherapy. Secondly, the surrounding stroma and microenvironment is of mouse origin, not human, and may interfere with drug response.

Chemical carcinogen-induced models

Previously, only a chemical carcinogen-induced mouse model, the GL261 model, have been derived from an intracranially-induced methylcholanthrene tumor in C57BL/6 mice [51]. Recently, Johanss et al. [52] have reported that, by the assessment of the ability of the epitopes predicted in silico to be the highest affinity binders to activate tumor-infiltrating T cells harvested from GL261, they have found the mechanisms of the T cell–activating immune-directed therapy, presumably due to its hypermutator phenotype. The ability of gliomas to induce local and systemic immunosuppression restrict the innate defense against tumor growth and the efficacy of adaptive immunotherapy and thus presents a significant challenge to the development of innovative therapies [53].

Spontaneous models

In 1971, H. Fraser has described the first incidence of a spontaneous glioma within the SMA-560 mouse strain. Initially, these tumors, which resembled human anaplastic astrocytomas, were restricted to in vivo studies only, as tumorigenicity was lost with repeated in vitro passaging of tumor explant cultures [54].

Conclusions

Glioblastoma is one of the most problematic cancers to treat. Despite advances in molecular profiling of the disease, information is still lacking, particularly regarding treatment.

As genome-wide sequencing efforts continue in humans, mouse glioma models that better recapitulate the complex genomic landscape of human glioma will be generated. These models will provide increasingly powerful tools for the validation of hypotheses engendered by human genomic data, such as confirming the driver mutations that are causal to oncogenesis, as well as for preclinical testing of personalized therapy.

Acknowledgements We thank all members of the Department of Tumor Pathology and Neurosurgery at Gifu University Graduate School of Medicine. This work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant Numbers 26430111 (H. T.) and 26670639 (A. S.)).

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Committee of Brain Tumor Registry of Japan: Report of Brain Tumor Registry of Japan (2001–2004). Neurol Med Chir (Tokyo) : 1-102, 2014. Neurol Med Chir (Tokyo) 13 (1):12

2. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO, European Organisation for R, Treatment of Cancer Brain T., Radiotherapy G, National Cancer Institute of Canada Clinical Trials G (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352 (10):987–996. doi:10.1056/NEJMoa043330

3. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW (2016) The 2016 World Health Organization Classification of tumors of the central nervous system: a summary. Acta Neuropathol 131(6):803–820. doi:10.1007/s00401-016-1545-1

4. Ohgaki H, Kleihues P (2013) The definition of primary and secondary glioblastoma. Clin Cancer Res 19(4):764–772. doi:10.1158/1078-0432.CCR-12-3002

5. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, Beroukhim R, Bernard B, Wu CJ, Genovese G, Shmulevich I, Barnholtz-Sloan J, Zou L, Vegesna R, Shukla SA, Ciriello G, Yung WK, Zhang W, Sougnez C, Mikkelsen T, Aldape K, Biggar DD, Van Meir EG, Prados M, Sloan A, Black KL, Eschbacher J, Finocchiaro G, Friedman W, Andrews DW, Guha A, Iacocca M, O’Nell BP, Foltz G, Myers J, Weisenberger DJ, Penny R, Kucherlapati R, Perou CM, Hayes DN, Gibbs R, Marra M, Mills
GB, Lander E, Spellman P, Wilson R, Sander C, Weinstein J, Meyerson M, Gabriel S, Laird PW, Haussler D, Getz G, Chin L, Network TR (2013) The somatic genomic landscape of glioblastoma. Cell 155(2):462–477. doi:10.1016/j.cell.2013.09.034
6. Cancer Genome Atlas Research N (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455 (7216):1061–1068. doi:10.1038/nature07385
7. Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, Chin L, DePinho RA, Cavenee WK (2007) Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev 21(21):2683–2710. doi:10.1101/gad.1596707
8. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Liu M, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Straub LG, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) An integrated genomic analysis of lung adenocarcinoma and lung carcinoma reveals convergent and divergent evolution. Science 321(5897):1803–1812. doi:10.1126/science.1164382
9. Weisenberger J, Steinbach JP, Malin G, Spada S, Rulicke T, Aguzzi A (1997) Development and malignant progression of astrocytomas in GFAP-v-src transgenic mice. OncoGene 14(17):2005–2013. doi:10.3824/ojn.1201168
10. Ding H, Roncari L, Shannon P, Wu X, Lau N, Karaskova J, Gutmann DH, Squire JA, Nagy A, Guha A (2003) Astrocyte-specific expression of activated p21-ras results in malignant astrocytoma formation in a transgenic mouse model of human gliomas. Cancer Res 63(9):3826–3836
11. Ding H, Shannon P, Lau N, Wu X, Roncari L, Baldwin RL, Takebayashi H, Nagy A, Gutmann DH, Guha A (2003) Oligodendrogliomas result from the expression of an activated mutant epidermal growth factor receptor in a transgenic mouse astrocytoma model. Cancer Res 63(5):1106–1113
12. Holland EC (2001) Gliomagenesis: genetic alterations and mouse models. Nat Rev Genet 2(2):120–129. doi:10.1038/35052535
13. Zhu Y, Guignard F, Zhao D, Liu L, Burns DK, Mason RP, Messing A, Parada LF (2005) Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. Cancer Cell 8(2):119–130. doi:10.1016/j.ccr.2005.07.004
14. Weiss WA, Burns MJ, Hackett C, Al-Dalahmah O, Krell D, Brazauskas P, Al-Qahtani K, Bardella C, Al-Dalahmah O, Krell D, Brazauskas P, Al-Qahtani K, Tomkova M, Adam J, Serres S, Lockstein H, Freeman-Mills L, Pfeffer I, Sibson N, Goldin R, Schuster-Boeckler B, Pollard PJ, Kriaucionis S, Ratcliffe PJ, Szele FG, Tomlinson I (2016) Expression of Idh1R132H in the murine subventricular zone stem cell niche recapitulates features of early gliomagenesis. Cancer Cell 30(4):578–594. doi:10.1016/j.ccell.2016.08.017
15. von Jouguieres G, Mersmann N, Klugmann CB, Harasta AE, Lutz B, Teahan O, Hsudisey GH, Frohlich D, Kramer-Elmers EM, Klugmann M (2013) Glial promoter selectivity following AAV-delivery to the immature brain. PLoS ONE 8(6):e65646. doi:10.1371/journal.pone.0065646
16. Holland EC, Celestino J, Dai C, Schaefer L, Sawaya RE, Fuller GN (2000) Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. Nat Genet 25(1):55–57. doi:10.1038/75596
17. Uhrbom L, Dai C, Celestino JC, Rosenblum MK, Fuller GN, Holland EC (2002) Ink4a-Arf loss cooperates with KRas activation in astrocytes and neural progenitors to generate glioblastomas of various morphologies depending on activated Akt. Cancer Res 62(19):5551–5558
18. Hambardzumyan D, Amankulor NM, Helmy KY, Becker OJ, Holland EC (2009) Modeling adult gliomas using RCAS-t-vA technology. Trans Oncol 2(2):89–IN86. doi:10.1593/doi.09100
19. Dai C, Celestino JC, Okada Y, Louis DN, Fuller GN, Holland EC (2001) PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. Genes Dev 15(15):1913–1925. doi:10.1101/gad.90301
20. Wei Q, Clarke L, Scheidenhelm DK, Qian B, Tong A, Sahna N, Karim Z, Bock NA, Reti R, Swohoda R, Purev E, Lavoie JP, Bajenaru ML, Shannon P, Herlyn D, Kaplan D, Henkeman RM, Gutmann DH, Guha A (2006) High-grade glioma formation results from postnatal pten loss or mutant epidermal growth factor receptor expression in a transgenic mouse glioma model. Cancer Res 66(15):7429–7437. doi:10.1158/0008-5472.CAN-06-0712
21. Ikawa M, Tanaka N, Kao WWY, Verma IM (2003) Generation of transgenic mice using lentiviral vectors: a novel preclinical assessment of lentiviral vectors for gene therapy. Mol Ther 8(4):666–673. doi:10.1016/s1525-0016(03)000240-5
22. Marumoto T, Tashiro A, Friedmann-Morvinski D, Scadeng M, Soda Y, Gage FH, Verma IM (2009) Development of a novel mouse glioma model using lentiviral vectors. Nat Med 15(1):110–116. doi:10.1038/nm.1863
23. Alcantara Llaguno S, Chen J, Kwon CH, Jackson EL, Li Y, Burns DK, Alvarez-Buylla A, Parada LF (2009) Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. Cancer Cell 15(1):45–56. doi:10.1016/j.ccr.2008.12.006
24. Misuraca KL, Hu G, Barton KL, Chung A, Becher OJ (2016) A novel mouse model of diffuse intrinsic pontine glioma initiated in Pax3-expressing cells. Neoplasia 18(1):60–70. doi:10.1016/j.neo.2015.12.002
25. Misuraca KL, Barton KL, Chung A, Diaz AK, Conway SJ, Corcoran DL, Baker SJ, Becher OJ (2014) Pax3 expression enhances PDGF-B-induced brainstem gliomagenesis and characterizes a subset of brainstem glioma. Acta Neuropathol Commun 2:134. doi:10.1186/s40478-015-0134-6
26. Engebraaten O, Hjortland GO, Hirschberg H, Fodstad O (1999) Growth of precultured human glioma specimens in nude rat brain. J Neurosurg 90(1):125–132. doi:10.3171/jns.1999.90.1.0125
27. Maheswaran R, Read TA, Lund-Johansen M, Skaffnesmo KO, Bjerkvig R, Engebraaten O (2003) Expression of extracellular matrix components in a highly infiltrative in vivo glioma model. Acta Neuropathol 105(1):49–57. doi:10.1007/s00401-002-0610-0
28. Antunes L, Angioi-Duprez KS, Bracard SR, Klein-Monhoven NA, Le Faou AE, Duprez AM, Plenet FM (2000) Analysis of tissue chimerism in nude mouse brain and abdominal xenograft models of human glioblastoma multiforme: what does it tell us about the models and about glioblastoma biology and therapy? J Histochem Cytochem 48(8):847–858. doi:10.1177/00221554004800613
29. Taillandier L, Antunes L, Angioi-Duprez KS (2003) Models for neuro-oncological preclinical studies: solid orthotopic and heterotopic grafts of human gliomas into nude mice. J Neurosci Methods 125(1–2):147–157. doi:10.1016/s0165-0270(03)90043-8
30. Shu Q, Wong KK, Su JM, Adesina AM, Yu LT, Tsang YT, Antaliff BC, Baxter P, Perlaky L, Yang J, Dauser RC, Chintagumpala M, Blaney SM, Lau CC, Li XN (2008) Direct orthotopic transplantation of fresh surgical specimen preserves CD133+ tumor cells in clinically relevant mouse models of medulloblastoma and glioma. Stem Cells 26(6):1414–1424. doi:10.1634/stemcells.2007-1009
32. Suggitt M, Bibby MC (2005) 50 years of preclinical anticancer drug screening: empirical to target-driven approaches. Clin Cancer Res 11(3):971–981
33. Tateishi K, Wakimoto H, Iafrate AJ, Tanaka S, Loebel F, Emery LV, Koutsoukos AD, Rubinstein LV, Moore RJ, Waud WR, Kisseleva E, Schneider H, Seystahl K, Rushing EJ, Herting F, Wei-Berg SR, Thorne AH, Chen CC, Mischel PS, Gonias SL, Cavezza EM, Reichel J, Porrati P, Pellegatta S, Qiu K, Gao Z, Ceccarelli M, Riccardi Br, Djahui A, Aldeape K, Golfinos JG, Zagg D, Mikkelsen T, Finocchiario G, Lasorella A, Rabandar M, Iavone A (2012) Transforming fusions of FGFR and TACC genes in human glioblastoma. Science 337(6099):1231–1235. doi:10.1126/science.1212728
34. Batchelor TT, Chiocca EA, Badr CE, Tannous BA (2016) Dissecting inherent intratumor heterogeneity in patient-derived glioblastoma culture models. Neuro Oncol 19(6):820–832. doi:10.1093/neuonc/now253
35. Plowman J, Waud WR, Kisseleva E, Schneider H, Seystahl K, Rushing EJ, Herting F, Wei-Berg SR, Thorne AH, Chen CC, Mischel PS, Gonias SL, Cavezza EM, Reichel J, Porrati P, Pellegatta S, Qiu K, Gao Z, Ceccarelli M, Riccardi Br, Djahui A, Aldeape K, Golfinos JG, Zagg D, Mikkelsen T, Finocchiario G, Lasorella A, Rabandar M, Iavone A (2012) Transforming fusions of FGFR and TACC genes in human glioblastoma. Science 337(6099):1231–1235. doi:10.1126/science.1212728
36. Jin J, Kim S, Nam DH (2013) Patient-specific orthotopic glioblastoma xenograft models recapitulate the histopathology and biology of human glioblastomas in situ. Cell Rep 3(1):260–273. doi:10.1016/j.celrep.2012.12.013
37. Castano A, Liu Y, Zhang M, Wang J, Chen J, Wang B, Sun T, Meerbrey KL, Schlabach MR, Sharpe MA, Livingston AD, Gist TL, Ghosh P, Han J, Baskin DS, Joo KM, Kim J, Jin J, Kim M, Seo H, Muradov J, Yang H, Choi YL, Park WY, Kong DS, Lee Ji, Ko YH, Woo HG, Lee J, Kim S, Nam DM (2013) Patient-specific orthotopic glioblastoma xenograft models recapitulate the histopathology and biology of human glioblastomas in situ. Cell Rep 3(1):260–273. doi:10.1016/j.celrep.2012.12.013
38. Waistworth D, Horbinski C, Hashizume R, James CD (2017) Therapeutic hypothesis testing with rodent brain tumor models. Neurotherapeutics 14(2):353–392. doi:10.1007/s13311-017-0523-1
39. Cooke A, Hara A, Kunisada T, Yoshimura S, Iwama T, Park DM (2015) The evidence of glioblastoma heterogeneity. Sci Rep 5:7979. doi:10.1038/srep07979
40. Teng J, Carla da Hora C, Kantar RS, Nakano I, Wakimoto H, Batchelor TT, Chiocca EA, Badr CE, Tannous BA (2017) Dissecting inherent intratumor heterogeneity in patient-derived glioblastoma culture models. Neuro Oncol 19(6):820–832. doi:10.1093/neuonc/now253
41. Olmez I, Shen W, McDonald H, Ozpolat B (2015) Differentiation of patient-derived glioblastoma multiforme cell lines results in a cancer stem cell-like state with mitogen-independent growth. J Cell Mol Med 19(6):1262–1272. doi:10.1111/jcmm.12479
42. Sancho-Martinez I, Nietv E, Xia Y, Hishida T, Aguirre A, Ocampo A, Ma L, Morey R, Krause MN, Zembrycki A, Ansorge O, Vazquez-Ferrer E, Dubova I, Reddy P, Lam D, Hishida Y, Wu MZ, Esteban CR, O’Leary D, Wahl GM, Verma IM, Laurent LC, Izpisua Belmonte JC (2016) Establishment of human iPSC-based models for the study and targeting of glioma initiating cells. Nat Commun 7:10743. doi:10.1038/ncomms10743
43. Baker M (2012) Genome interpreter vies for place in clinical market. Nature 490(7419):157. doi:10.1038/490157a
44. Garralda E, Paz K, Lopez-Casanp PP, Jones S, Katz A, Kann LM, Lopez-Rios F, Sarno F, Al-Shahour F, Vasquez D, Bruckheimer E, Angiuli SV, Colles A, Diaz LA, Velculescu VE, Valencia A, Sidransky D, Hidalgo M (2014) Integrated next-generation sequencing and avatar mouse models for personalized cancer treatment. Clin Cancer Res 20(9):2476–2484. doi:10.1158/1078-0432.CCR-13-3047
45. Ausman JL, Shapiro WR, Rall DP (2017) Studies on the chemotheraphy of experimental brain tumors: development of an experimental model. Cancer Res 30(9):2394–2400
46. Johanns TM, Ward JP, Miller CA, Wilson C, Kobayashi DK, Bender D, Fu Y, Alexandrov A, Mardis ER, Artyomov MN, Schreiber RD, Dunn GP (2016) Endogenous neoantigen-specific CD8 T Cells identified in two glioblastoma models using a cancer immunogenomics approach. Cancer Immunol Res 4(12):1007–1015. doi:10.1158/2326-6066.CIR-16-0156
47. Gousias K, Markou M, Arzoglou V, Voulgaris S, Vatholomatos G, Kostoulas A, Voulgaris P, Polyzois K, Kyriatis AP (2010) Frequent abnormalities of the immune system in gliomas and correlation with the WHO grading system of malignancy. J Neuroimmunol 226(1–2):136–142. doi:10.1016/j.jneuroim.2010.05.027
48. Fraser H (1971) Astrocytomas in an inbred mouse strain. J Pathol 101(3):266–270
49. Xiao A, Wu H, Pandolfi PP, Louis DN, Van Dyke T (2002) Astroglioma inactivation of the pRb pathway predisposes mice to malignant astrocytoma development that is accelerated by PTEN mutation. Cancer Cell 1(2):157–168
50. Reilly KM, Loisel DA, Bronson RT, McLaughlin ME, Jacks T (2000) Nf1;Trp53 mutant mice develop glioblastoma with frequent abnormalities of the immune system in gliomas and correlation with the WHO grading system of malignancy. J Neuroimmunol 226(1–2):136–142. doi:10.1016/j.jneuroim.2010.05.027
51. Charest A (2009) Oncogenic EGFR signaling cooperates with loss of tumor suppressor gene functions in gliomagenesis. Proc Natl Acad Sci USA 106(8):2712–2716. doi:10.1073/pnas.0813314106
58. Zeng J, See AP, Phallen J, Jackson CM, Belcaid Z, Ruzevick J, Durham N, Meyer C, Harris TJ, Albesiano E, Pradilla G, Ford E, Wong J, Hammers HJ, Mathios D, Tyler B, Brem H, Tran PT, Pardoll D, Drake CG, Lim M (2013) Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys 86(2):343–349. doi:10.1016/j.ijrobp.2012.12.025

59. Zhang C, Burger MC, Jennewein L, Genssler S, Schonfeld K, Zeiner P, Hattingen E, Harter PN, Mittelbronn M, Tonn T, Steinbach JP, Wels WS (2016) ErbB2/HER2-Specific NK Cells for Targeted Therapy of Glioblastoma. J Natl Cancer Inst 108(5):djv375. doi:10.1093/jnci/djv375

60. Parrish KE, Cen L, Murray J, Calligaris D, Kizilbash S, Mit-tapalli RK, Carlson BL, Schroeder MA, Sludden J, Boddy AV, Agar NY, Curtin NJ, Elmqquist WF, Sarkaria JN (2015) Efficacy of PARP inhibitor rucaparib in orthotopic glioblastoma xenografts is limited by ineffective drug penetration into the central nervous system. Mol Cancer Ther 14(12):2735–2743. doi:10.1158/1535-7163.MCT-15-0553

61. Gupta SK, Mladek AC, Carlson BL, Boakye-Agyeman F, Bakken KK, Kizilbash SH, Schroeder MA, Reid J, Sarkaria JN (2014) Discordant in vitro and in vivo chemopotentiating effects of the PARP inhibitor veliparib in temozolomide-sensitive versus -resistant glioblastoma multiforme xenografts. Clin Cancer Res 20(14):3730–3741. doi:10.1158/1078-0432.CCR-13-3446

62. Garros-Regulez L, Aldaz P, Arrizabalaga O, Moncho-Amor V, Carrasco-Garcia E, Manterola L, Moreno-Cugnon L, Barrena C, Villanua J, Ruiz J, Pollard S, Lovell-Badge R, Samprón N, García I, Matheu A (2016) mTOR inhibition decreases SOX2-SOX9 mediated glioma stem cell activity and temozolomide resistance. Expert Opin Ther Targets 20(4):393–405. doi:10.1517/14728222.2016.1151002

63. Hashizume R, Andor N, Ihara Y, Lerner R, Gan H, Chen X, Fang D, Huang X, Tom MW, Ngo V, Solomon D, Mueller S, Paris PL, Zhang Z, Petritsch C, Gupta N, Waldman TA, James CD (2014) Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma. Nat Med 20(12):1394–1396. doi:10.1038/nm.3716

64. Mathieu V, De Nève N, Le Mercier M, Dewelle J, Gaussian J-F, Dehoux M, Kiss R, Lefranc F (2008) Combining bevacizumab with temozolomide increases the antitumor efficacy of temozolomide in a human glioblastoma orthotopic xenograft model. Neoplasia 10(12):1383–1392. doi:10.1593/neo.08928

65. Cho J, Pastorino S, Zeng Q, Xu X, Johnson W, Vandenberg S, Verhaak R, Cherniack AD, Watanabe H, Dutt A, Kwon J, Chao YS, Onofrio RC, Chiang D, Yuza Y, Kesari S, Meyerson M (2011) Glioblastoma-derived epidermal growth factor receptor carboxyl-terminal deletion mutants are transforming and are sensitive to EGFR-directed therapies. Cancer Res 71(24):7587–7596. doi:10.1158/0008-5472.CAN-11-0821

66. Yoshida Y, Ozawa T, Yao TW, Shen W, Brown D, Parsa AT, Raizer JJ, Cheng SY, Stegh AH, Mazar AP, Giles FJ, Sarkaria JN, Butowski N, Nicolaides T, James CD (2014) NT113, a pan-ERBB inhibitor with high brain penetrance, inhibits the growth of glioblastoma xenografts with EGFR amplification. Mol Cancer Ther 13(12):2919–2929. doi:10.1158/1535-7163.MCT-14-0306

67. Giannini C, Sarkaria JN, Saito A, Uh JH, Galanis E, Carlson BL, Schroeder MA, James CD (2005) Patient tumor EGFR and PDGFRα gene amplifications retained in an invasive intracranial xenograft model of glioblastoma multiforme. Neuro Oncol 7(2):164–176. doi:10.1215/S1152851704000821

68. Sarkaria JN, Carlson BL, Schroeder MA, Grogan P, Brown PD, Giannini C, Ballman KV, Kitange GJ, Guha A, Pandita A, James CD (2006) Use of an orthotopic xenograft model for assessing the effect of epidermal growth factor receptor amplification on glioblastoma radiation response. Clin Cancer Res 12(7 Pt 1):2264–2271. doi:10.1158/1078-0432.CCR-05-2510

69. Pandita A, Aldape KD, Zadeh G, Guha A, James CD (2004) Contrasting in vivo and in vitro fates of glioblastoma cell subpopulations with amplified EGFR. Genes Chromosomes Cancer 39(1):29–36. doi:10.1002/gcc.10300