7 Mouse Models of Glioblastoma

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Abstract: Glioblastoma is one of the most common malignant brain tumors. The prognosis for glioblastoma is still very poor despite intensive treatment by surgery, radiation, and chemotherapy. To develop new therapies for glioblastoma, preclinical mouse models are essential for analyzing the biology of glioblastoma, identifying new therapeutic targets, and evaluating the potential of new therapeutic strategies. Current preclinical glioblastoma models are classified into two categories: xenografts and genetically engineered mouse models. Xenografts are classified into two categories: glioblastoma cell-line xenografts and patient-derived xenografts. Glioblastoma cell-line xenografts generally have the advantages of high engraftment and growth rates, but it is doubtful whether glioblastoma cell-line xenografts reflect the true biological nature of glioblastoma. Patient-derived xenografts retain both the genetic and histological features of the primary tumor, and thus are expected to be good preclinical models in translational glioblastoma research. However, they cannot fully reflect the host's antitumor immunity in human glioblastoma. Glioblastoma genetically engineered mouse models make it possible to pinpoint genetic alterations involved in tumor initiation and progression, but tumors are usually composed of cells with specific, homogeneous genetic changes, and thus cannot completely reflect the intratumoral genomic and phenotypic heterogeneity of glioblastoma. Presently, patient-derived xenografts and glioblastoma

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genetically engineered mouse models are excellent glioblastoma mouse models for current use, but more work is needed to establish mouse models that fully recapitulate human glioblastoma.

Key words: Chemically induced mouse model; Genetically engineered mouse model; Glioblastoma; Preclinical model; Xenograft

Introduction

Glioblastoma is one of the most common malignant brain tumors. The prognosis for glioblastoma is still very poor; despite intensive treatment by surgery, radiation, and chemotherapy, the median survival is only about 15 months (1). Thus, there is an urgent need for more effective treatments, and various therapies for glioblastoma have been tested or are in development. To develop new therapies, preclinical mouse models are essential for analyzing the biology of glioblastoma, identifying new therapeutic targets, and evaluating the potential of new therapeutic strategies. Current preclinical glioblastoma models are classified into three categories: xenografts, genetically engineered mouse (GEM) models, and syngenic murine models (2, 3). In this chapter, we summarize the currently available mouse models of glioblastoma, the advantages and disadvantages of each model, and the prospects for developing better mouse models.

Xenografts

Glioblastoma xenografts are classified into two categories: glioblastoma cell-line xenografts and patient-derived xenografts.

GLIOBLASTOMA CELL-LINE XENOGRAFTS

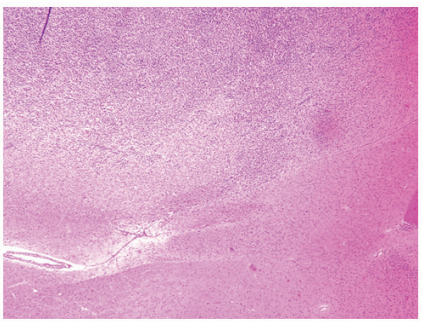
Commercially available glioblastoma cell lines include U87, U251, T98G, and A172, among others. These traditional glioblastoma cell lines are the most common models used in both *in vitro* and *in vivo* glioblastoma research. These glioblastoma cell lines, which were originally derived from glioblastoma patients, are usually cultured in serum-containing medium and xenografted into immunodeficient mice such as nude mice, NOD/SCID mice, and NOD/SCID gamma mice.

Glioblastoma cell-line xenografts generally have the advantages of high engraftment and growth rates, good reproducibility, and reliable disease growth and progression. Moreover, immortalized cell lines can be readily expanded for an unlimited number of passages *in vitro*, yielding a large number of tumor cells for experimental use (3). However, studies have reported that glioblastoma cell-line xenografts do not reflect the clinical characteristics of the original patient tumors (4); that is, the xenografted tumors are usually circumscribed and do not show single-cell invasion, tumor necrosis, or microvascular proliferation (5, 6). They also show differences in MHC (7) and integrin expression (3), suggesting that the xenografted tumors differ phenotypically from the original patient tumors. Genotypes of glioblastoma cell-line xenograft models also differ from the original

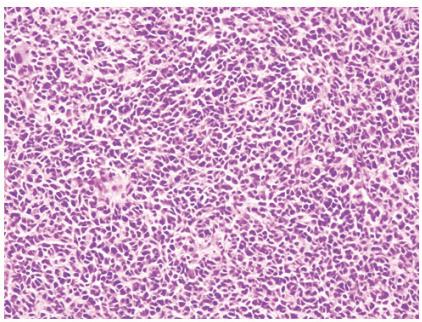
patient tumors (3); profiles from array-comparative genomic hybridization (aCGH) and whole-genome sequencing of glioblastoma cell lines are quite different from those typically found in primary glioblastoma (8, 9). Thus, it is doubtful whether glioblastoma cell-line xenografts reflect the true biological nature of glioblastoma, and this is a disadvantage in preclinical trials. Due to their genomic and transcriptomic deviations from glioblastoma *in situ*, glioblastoma cell lines are poor models for glioblastoma (3, 10).

PATIENT-DERIVED XENOGRAFTS

Patient-derived xenografts (PDX) (11, 12), a recent focus of glioblastoma research, are used extensively in translational research. The PDX model has the advantage of retaining both the genetic and histological features of the primary tumor from which it was derived (Figure 1). Because the tumors are propagated in successive generations of mice, PDX cells are not subjected to stresses that can arise in cell cultures (13, 14). There is some controversy as to whether PDX models are best established by injecting freshly biopsied tumor tissue (15, 16) or cultured tumor spheres into mice (17), and whether orthotopic or subcutaneous xenograft is preferable. PDX models are generally established by injecting glioblastoma tumor spheres produced under serum-free neurosphere-culture conditions, into immunodeficient mice. Tumor spheres have several advantages over serum-cultured glioblastoma cell lines: the tumor spheres retain a molecular profile similar to that of the patient's original tumor, thus maintaining tumor heterogeneity (18, 19); their molecular profile is stable over time, and they are both tumorigenic and phenotypically similar to the patient's original tumor, even in aspects such as single-cell invasion and tumor angiogenesis (20, 21). However, not all human gliomas are successfully cultured as tumor spheres; reported success rates vary from 10 to 20% (3, 22). Thus, one group took an alternative approach of using a serum-free cell-culture system to generate monolayer cultures on laminin-coated plates (23). At present, however, there is little molecular evidence for preferring adherent culture over sphere culture. The generation of tumorigenic cell populations



Representative picture of a H&E image from patient-derived orthotopic glioblastoma xenograft.(×40) day 70



Representative picture of a H&E image from patient-derived orthotopic glioblastoma xenograft.(×200) day 70

Figure 1 Representative picture of a H&E image from patient-derived orthotopic glioblastoma xenograft.

from human glioblastomas using neurosphere culture has significantly advanced our knowledge of specific subpopulations within human primary tumors. Even though their phenotypes *in vivo* are not necessarily predictable, these cell populations are an important tool for studying the tumorigenicity and progression of glioblastoma *in vivo*.

Another method for establishing PDX models is to inject tissues from fresh brain-tumor biopsies into immunodeficient mice. The biopsy tissue is generally minced with surgical blades and placed in flasks containing standard serum-supplemented tissue-culture medium (24). Under these conditions, tumor spheroids form quickly, and the spheroids maintain the architecture of the original tissue, including the endothelium, extracellular matrix components, and resident macrophages (24). PDX models from fresh brain-tumor biopsies display diffuse single-cell infiltration when implanted into the brain of immunodeficient rats or mice (15, 25, 26), and these biopsy xenograft models preserve other histological features of human glioblastoma. In one study, however, spheroids derived from a fresh brain-tumor biopsy failed to form tumors in the mouse brain (16). Thus, technological standardization is needed to establish highly reproducible PDX models from tumor spheroids.

Both cultured tumor spheres and tumor biopsy tissues maintain the genetic and phenotypical features of the original patient tumors when injected into immunodeficient mice. However, in theory, tumor biopsy tissues may have advantages over tumor spheres in that they maintain the original tissue architecture, complete with endothelium, extracellular matrix components, and resident macrophages. Thus, the injected biopsy tissue has a greater potential for reflecting the biological features of the original human glioblastoma. However, more studies are necessary to confirm the superiority of one method over the other.

Another controversy related to PDX models is whether orthotopic or subcutaneous xenografts are better. While orthotopic xenografts more closely mimic the clinical situation, subcutaneous xenografts, usually accomplished by transplanting the patient-derived tumor spheres or freshly biopsied tissue directly into the flanks of immunodeficient mice (27), are less technically challenging than orthotopic xenografts and are easily passaged *in vivo*. PDX is very useful not only in preclinical models of glioblastoma but also for verifying molecular changes and signaling pathways in various types of cancer. Thus, PDX models are expected to remain a mainstay in translational glioblastoma research.

Genetically Engineered Mouse Models

GEM preclinical models of glioblastoma have been reported to reflect the histology and biology of human glioblastoma. In many GEM glioblastoma models (3), gene expression is manipulated using Tet-regulation or Cre-inducible gene alleles to express or inactivate genes at a specific time or duration or in specific cells. GEM glioblastoma models can also be established by somatic-cell gene transfers using retroviral or adenoviral vectors to deliver Cre recombinase, such as in the RCAS/Tva system (28). Glioblastoma GEM models make it possible to pinpoint genetic alterations involved in tumor initiation and progression. These models are also useful for testing therapeutic strategies.

Syngenic Mouse Models

Syngenic mouse models of glioblastoma have long been used as indispensable tools for glioblastoma research. These models (GL261, GL26, CT-2A, and P560) (29) are established from spontaneous or chemically induced murine glioma. GL261, GL26, and CT-2A are from chemically induced mouse models of glioblastoma while P560 is from spontaneous mouse models of glioblastoma. GL261 models are perhaps the most extensively used syngenic mouse models of glioblastoma. These models are reported to recapitulate histologic and biological characteristics of glioblastoma. Furthermore, these models use immunocompetent mice, and thus are suitable for analyzing glioblastoma tumor immunology and immunotherapeutic research.

Advantages and Disadvantages of Each Type of Glioblastoma Mouse Model

GLIOBLASTOMA CELL-LINE XENOGRAFTS

Although cell lines in serum-containing media are readily established from human glioblastoma, it is difficult to establish cell lines from low-grade gliomas such as oligodendrogliomas (30, 31). More importantly, extensive clonal selection occurs after glioma cells are suspended in serum-containing medium, and further selection occurs during cell passaging. It is therefore highly doubtful that biological information obtained from glioblastoma cell-line xenografts can contribute to an accurate understanding of the biology of human glioblastoma. The glioblastoma cell lines are so different from the original patient tumors that it might be impossible to recapitulate the complex genetic and phenotypic traits of human gliomas with these cell lines.

PATIENT-DERIVED XENOGRAFTS

Unlike xenografts from glioblastoma cell lines, PDXs have the advantage of maintaining the histological and genetic features of the original tumor when engrafted into immunodeficient mice. It should also be emphasized that PDX models are highly variable, reflecting the inter-patient heterogeneity of glioblastoma, but are advantageous because of their clinical relevancy. However, PDX models also have shortcomings. They cannot be established from all patient tumors, especially from low-grade gliomas. Even if engraftment is successful, it usually takes between 2 and 11 months to obtain tumors (25). Furthermore, standardization and experimental planning may be difficult because PDX models are as variable as the glioblastomas they are derived from (3). However, once established, PDX models can contribute to the development of personalized treatment for individual patients. Another disadvantage of the PDX model is that it can only be established in immunodeficient mice such as nude, NOD-SCID, or NOD-SCID-gamma mice. The immune system in these mice differs innately from that of the host; thus, current PDX models do not represent the host immune system.

GENETICALLY ENGINEERED MOUSE MODELS

GEM models are particularly useful for identifying the molecular events responsible for tumor initiation and progression, and can also offer insight into the sequence of events underlying the genetic alterations occurring in response to specific mutations. GEM models are also useful for analyzing the role of the microenvironment in tumor biology (32).

However, it is not certain whether the gene changes involved in these models truly mirror the tumor-associated events in human glioblastomas. GEM tumors are usually composed of cells with specific, homogeneous genetic changes, and thus cannot completely reflect the intratumoral genomic and phenotypic heterogeneity of glioblastoma. In addition, GEM models are sometimes at a disadvantage in therapeutic studies because tumor initiation cannot be controlled, and thus the time of tumor formation is not highly reproducible.

SYNGENIC MOUSE MODEL

Syngenic mouse models use immunocompetent mice; thus, the greatest advantage of these models is that they recapitulate host immunity and are considered to be suitable for analyzing glioblastoma tumor immunology and immunotherapeutic research.

Preclinical findings from these mouse models have already been tested as clinical trials in human glioblastoma patients such as dendritic cell vaccines pulsed with whole tumor homogenate (33). These findings came from studies using GL261 models.

However, it remains to be seen whether murine glioma models faithfully reflect human glioblastoma; thus, further studies are needed to conclude on this.

Future Prospects for Mouse Models of Glioblastoma

Table 1 summarizes the characteristics of currently available mouse models of glioblastoma. At present, none of the animal models mentioned fully recapitulate human glioblastoma development and progression. Glioblastoma cell-line xenografts do not reflect the genetic background of human glioblastoma. PDX, GEM, and syngenic models better reflect phenotypic features of glioblastoma, and are thus the best of the currently available models for analyzing glioblastoma development and therapeutic strategies.

The value of PDX models in predicting human clinical-trial drug responses was recently highlighted by a study of 1000 PDX cancer models from various primary sites (34), and by the establishment of a large-scale breast-cancer PDX biobank (35). This type of large-scale PDX bank is likely to prove valuable for predicting human responses to clinical trials of new glioblastoma drugs, and should help make it possible to tailor therapy to the individual patient. However, since PDX models do not reflect the tumor microenvironment of the glioblastoma and are established in immunodeficient mice, they cannot fully reflect the host's antitumor immunity in human glioblastoma. Thus, PDX models can be improved by developing models that recapitulate human immunity and the human glioblastoma

TABLE 1 Characteristics of Each Glioblastoma Mouse Model

Model	Advantage	Disadvantage
Cell-line xenograft	High engraftment and growth rates Good reproducibility Reliable disease growth and progression	Does not recapitulate genetic and phenotypical feature of original tumor Need to use immunodeficient mice
Patient-derived xenograft	Recapitulate genetic and phenotypical feature of original tumor	Relatively low engraftment and growth rates Need to use immunodeficient mice
Genetically engineered mouse model	Identify the molecular events responsible for tumor initiation and progression Analyze the role of the microenvironment	Does not completely reflect the intratumoral genomic and phenotypic heterogeneity Tumor initiation cannot be controlled
Syngenic mouse model	Suitable for tumor immunity and immunotherapeutic research	Might be different from human glioblastoma

microenvironment. In the continued search for models that more fully reflect human glioblastoma, it will be particularly useful to compare the phenotypes developed in xenograft models with those obtained in various GEM models (3).

Conclusion

In this chapter, we summarized the currently available mouse models of glioblastoma. Each mouse model has its own advantages and disadvantages; thus, it is important to choose appropriate models depending on the purpose of the research. PDX, GEM, and syngenic models are excellent glioblastoma mouse models for current use and preclinical translational research for glioblastoma. However, further work is needed to establish mouse models that fully recapitulate human glioblastoma.

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