Rodent Models for Testing Therapeutic Hypotheses in Treating Brain Tumors

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Rodent Models for Testing Therapeutic Hypotheses in Treating Brain Tumors

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Abstract

The development and application of rodent models for preclinical evaluation of novel therapeutics and approaches for treating brain tumors has been an area of intense interest for decades in neuro-oncology research. Notably, these models often serve as an important benchmarking tool for determining whether a therapeutic strategy is appropriate for consideration as a clinical trial. Since the year 2000, when the first genetically engineered mouse models for CNS cancer meeting was convened, preclinical rodent models for therapeutic testing have undergone substantial evolution. However, and even with this evolution, certain principles associated with these models have stood the test of time and form the basis of this review. Commensurate with the growth of rodent brain tumor modeling, some confusion can exist with respect to the appropriateness of individual models for addressing research project goals. Here we review the most common murine brain tumor paradigms, while directing specific attention to their usefulness in preclinical therapeutic testing. These models include: genetically engineered mice that spontaneously or inducibly develop brain tumors; syngeneic rodent models in which cultured tumor cells are engrafted into the same strain of rodent from which they were derived; and patient derived xenograft models in which human tumor cells are engrafted in immunocompromised rodents, most often mice. The basis for model selection from the extensive armamentarium of available models, for use in preclinical therapeutic testing can, be distilled into a few key considerations.
Syngeneic, immunocompetent mouse tumor engraftment models

The use of rodent brain tumor cell lines, developed as a consequence of animal treatment with chemical mutagens, generally nitrosoureas, has a long history in neuro-oncology research. Table 1 includes commonly utilized tumor cell line-host combinations including 9L, F98, and RG2 cells in Fisher rats, CNS1 cells in Lewis rats, GL261 and CT-2A cells in C57BL6 mice, SMA-560 cells in VM/Dk mice, and 4C8 cells in B6D2F1 mice (1,2). A survey of the literature indicates that the GL261-C57BL6 is the most extensively used model, and in general, mouse models have been favored, likely due in large part to the economy of purchasing and housing mice vs. rats. Although, in recent years, the neuro-oncology research community has directed more attention to the use of patient-derived xenograft models for therapeutic testing, the syngeneic, immunocompetent rodent models continue to serve a critically important role in brain tumor research, with current usage stimulated by heightened interest in preclinical testing of therapies that evoke an adaptive immune response against tumor. Notable therapeutic modalities of this type include IDO1, PD-1, PD-L1, CTLA-4, 4-1BB and/or OX-40 blockade (3-6).

Genetically Engineered Mouse (GEM) Models

During the 1990’s a new type of mouse model emerged for studying cancer that was based on the inactivation of tumor suppressor genes and/or introduction of activated oncogenes into the germline, such that the progeny of such genetically engineered mice would harbor genetic modifications favoring tumor development (Table 2). This movement caught hold early in the brain tumor research community and spawned a series of NCI-sponsored meetings for sharing information on the development of GEM models for CNS cancer (7). Early models were relatively unsophisticated with respect to the brain tumor relevance of oncogenic transgenes that promoted tumor formation. An example of such a model was presented by Ding et al. (8), and relies on glial fibrillary acid protein (GFAP) promoter to drive mutant Ras (V12Ha-ras). Despite the rarity of Ras mutations in glial tumors, this particular GEM has seen widespread use in brain tumor research, in large part because of its reproducible and consistent tumor development: symptomatic onset takes place ~ 12 weeks of age with 85% of mice presenting with low or high-grade astrocytoma (8). Tumors that develop in the V12Ha-ras model present with histologic and molecular characteristics consistent with those found in patient GBM, including mutation of TP53 and suppression of PTEN and CDKN2A expression, the latter of which encodes the p16 tumor suppressor. A
drawback to the V^{12}Ha-ras model, and demonstrated by other GEM models, is the frequent presentation of multifocal tumor development, which is not typical of GBM in patients.

GEM model sophistication increased rapidly during the 1990’s and early 2000’s, culminating with contemporary GEM possessing inducible tumor suppressor gene knockouts, oncogene knock-ins, and improved cell type-specificity control over genetic alteration induction (9). A prime example of a contemporary GEM model is based on GFAP-associated conditional inactivation of the NF1 tumor suppressor gene in mice that are constitutionally deficient in TP53 (10). Ras pathway activation, either by deregulated upstream receptor tyrosine kinase signaling, Ras mutation, or NF1 tumor suppressor inactivation, has been popular in GEM modeling of glial tumors. However, and in contrast to models based on the expression of mutant Ras, NF1 inactivating mutations occur frequently in malignant gliomas from patients. Humans with mutated NF1 have an increased risk of developing astrocytoma, and tumors with combined NF1 and TP53 inactivating mutations frequently manifest as GBM (11). GEM allowing for temporal, cell-type specific inactivation of NF1, in the context of a p53 null background, display high penetrance for NF1 gene inactivation causing tumor formation (>92%), with tumors showing many of the hallmark features of human GBM (10,12). A derivative of this model, involving the inclusion of constitutional PTEN haploinsufficiency, increases tumor formation to 100% when NF1 is inactivated, and decreases tumor latency (13).

The GEM models have addressed and continue to address needs associated with significant shortcomings of the engraftment models. They enable the analysis of events associated with early tumor development, provide opportunity to study tumor evolution and are not dependent on an invasive procedure, the intracranial injection of tumor cells, that disrupts the blood brain barrier and alters the tumor microenvironment. GEM models also are able to address potential brain tumor cell of origin identity(ies). Notably, the immunocompetent status of GEM is compatible with testing immunotherapies (14,15).

A weakness of GEM models is that they do not, in general, compare favorably with engraftment models for therapeutic testing capacity. Reasons for this include the extensive resources, time and costs associated GEM genotyping, breeding, and colony maintenance; asynchronous tumor development in age-matched mice of the
same strain; and the infrequent inclusion of a reporter transgene that can be used for monitoring intracranial tumor growth and response to therapy (16). However, and in contrast to GEM model tumors, established cell lines engrafted in rodent brains possess minimal heterogeneity, angiogenic potential, and often produce tumors that lack critical histopathological features in corresponding patient tumors, such as necrosis (8,17). Thus, while more cumbersome, GEM models are critically important experimental systems for testing therapies, and especially those that engage the host immune system for therapeutic effect.

**Patient-derived Xenograft (PDX) Models**

Human established cell lines (ECLs), continuously propagated as monolayer cultures in serum-supplemented media, such as the ubiquitous U87 line, have been used for establishing tumors in immunocompromised mice for nearly 30 years (18), and some of the earliest established lines continue to be a staple of laboratories conducting preclinical therapeutic testing in rodents. An extensive review of glioma ECL tumorigenicity was published by Ishii *et al.* (19), and this work continues to serve as a valuable reference for investigators engaged in human glioma research. Generally, xenografts established from ECLs are not referred to as patient-derived xenografts (PDX). The term, or the acronym, PDX, is usually applied to tumors that are propagated in mice, rather than in cell culture. Admittedly, however, any xenograft established from human tumor cells, regardless of method of tumor cell propagation, is a patient-derived xenograft.

With the intriguing potential and limited understanding of GEM model strengths and weaknesses at the outset of the transgenic mouse movement, interest in human tumor xenograft models became significantly decreased during the rapid expansion period of GEM research. However, two high impact studies prompted a resurgence of, and have sustained a high level of interest in brain tumor xenograft models. The first was presented by Singh *et al.* in 2004 (20), and demonstrated the existence of human tumor cell subpopulations within individual patient surgical specimens, having distinct tumorigenic potential in immunocompromised mice. This landmark publication was followed by the study of Bao *et al.* (21), which showed differential sensitivity of human glioma cell subpopulations to radiation treatment. The two studies, in combination, stimulated and have maintained a high level of interest in research directed at understanding the dynamics of intratumoral subpopulation
heterogeneity. Immunocompromised mice were, as well as continue to be, the tool with which to study key subpopulation biologic characteristics, namely successful engraftment and engrafted tumor growth rate.

The decade of 2000-2010 also proved to be a period of time during which there were substantial changes in approach to propagating human tumor tissues and cells. High resolution molecular profiling studies have clearly established that sustained in vitro propagation of patient tumor explant cultures, with cells grown as monolayers in medias supplemented with bovine sera, results in significant molecular and biologic changes to the tumor cells, in relation to the patient tumors from which they originated (22). Studies which emerged and that showed improved retention of patient tumor characteristics through direct surgical specimen engraftment and propagation in immunocompromised mice (23), as well as by growth and propagation of surgical specimen explant cultures in medias supplemented with specific amounts of defined growth factors that select for cancer stem cells (24), have had substantial influence on ways in which patient tumors and cells are sustained for ongoing use in research.

In addition to the discovery of new approaches for propagating tumor tissue and cells, there has been increased attention directed to the type of immunodeficient mouse host used for tumor tissue engraftment and propagation. The transplantation of xenogeneic tissue into mice requires neutralization and/or depletion of the adaptive immune response to avoid graft versus host immune-mediated tissue rejection. One of the most commonly utilized hosts for human tumor cell engraftment is the Foxn1-deficient nu/nu mouse strain, which is deficient for the thymus, a tissue required by lymphoid progenitor cells to undergo positive and negative selection that eventually produces naïve T cells and mature regulatory T cells (25). The preferential use of nu/nu mice in cancer research is due in part to historical rationale, as they were the first type of mouse to be widely available for human tumor xenograft establishment and propagation. Notably, they are relatively inexpensive, healthy (can survive as long as 2 years in an immunological barrier environment), and their lack of fur facilitates straightforward identification and quantification of tumors grown subcutaneously. Despite these attributes, athymic nu/nu mice likely introduce a bias for successful engraftment of surgical specimens, with successful engraftment mostly restricted to highly malignant variants within a histologic class of tumor. For brain tumors, this was indicated over a quarter of a century ago when it was shown that engrafted patient medulloblastomas frequently possess c-myc amplification (26). Based on contemporary molecular classification, these tumors represent a subset of group 3
medulloblastomas (27) and are associated with a relatively poor prognosis in patients. Similarly, molecular profiling of GBM xenografts, established in athymic nu/nu mice, suggests a selection bias against the neuronal subgroup of these tumors (28). However, with the significantly increased animal radiation sensitivity for many of the other immune-compromised models detailed below, athymic nu/nu mice are an important tool for pre-clinical testing of novel treatment regimens.

Motivated by the need to expand tumor subtypes that can be successfully engrafted and propagated, mice with more severe immunodeficiency have experienced increasing use in xenograft-associated research. Examples include Rag1 or Rag2 knockout mice that are unable to form mature T- and B-cells, NOD-scid mice that are impaired for T and B cell lymphocyte development and are variably defective in natural killer (NK) cell function, and the NOD-scid IL2rgnull (NSG) mice that lack mature T- and B-cells, are NK cell deficient, and are variably defective in macrophage and dendritic cell function (29). Potential barriers to working with severely immunodeficient mice is related to their high purchase price, their need for special care and housing, the increased incidence of immuno-proliferative responses to tissue engraftment and the presence of fur which, to an extent, obscures subcutaneous tumor cell engraftment. Despite these increased challenges, the more severely immunocompromised status of such mice has helped to create new models, such as serially transplantable IDH1-mutant PDX (30,31), established from lower grade gliomas and do not engraft well, if at all, in athymic nu/nu mice.

Choosing the most appropriate mouse host for patient tumor engraftment is a vital consideration with respect to successful engraftment, but as well with respect to testing therapies. Different strains of mice have inherent differences in chemotherapy and radiation sensitivity (32), which can be a limiting factor in the treatment regimen(s) that can be used in conducting anti-tumor efficacy studies. Regardless of the type of immunocompromised mouse one chooses, any intention for large scale engraftment-based research is well-served by directing special attention to comparing the costs of purchasing from a vendor vs. establishing and maintaining an in-house breeding colony, as the price for conducting large scale PDX research can be cost-prohibitive.
Another important consideration for xenograft establishment and propagation concerns anatomic location: intracranial (orthotopic) (33-35) vs. subcutaneous (heterotopic) (36). Whereas subcutaneous serial propagation of patient tumors has been demonstrated to maintain key molecular and biologic features of human brain tumors, as compared to propagation in vitro (23,37), the molecular and biologic characteristics of engrafted patient tumors diverge, to some extent, when propagating the same surgical specimen in heterotopic vs. orthotopic location. Orthotopic xenograft propagation has been confirmed to maximally retain corresponding patient tumor molecular characteristics (33,38,39). However, a notable weakness of orthotopic xenotransplantation is the uncertainty related to the length of time a mouse host can accommodate intracranial tumor before succumbing to tumor burden. Thus, orthotopically propagated tumors can be lost due to the unanticipated death of a tumor-bearing animal. Furthermore, orthotopic propagation is more limited with respect to the maximum size of tumor a single animal can yield, which is an important consideration for experiments requiring a large number of cells from a single engrafted animal. Heterotopic propagation has practical advantages that include the ability to directly visualize tumor growth, avoiding unexpected tumor-bearing animal deaths, and the generation of relatively large tumors that satisfy requirements for downstream experiments and further propagation. Heterotopic GBM PDX that have been used in support of studies published by multiple investigators are indicated in Table 3, along with some of the most commonly used and tumorigenic ECLs.

A brain tumor PDX concept that has generated recent discussion involves the consideration of a PDX that can be generated and therapeutically tested within a time frame relevant for informing the treatment strategy of a patient from which the PDX is derived. This personalized approach, often referred to as “Avatar” modeling (40), is unrealistic in the vast majority of instances given the latency period of initial PDX establishment, length of time required for PDX expansion, intracranial growth characterization, and subsequent therapeutic testing in vivo relative to the typical aggressive clinical course of brain tumors in patients. A more realistic alternative involves the development of PDX panels that provide representation of several molecularly defined subclasses of a specific tumor histologic classification, such as GBM, and that could be used to test pre-existing and/or novel therapies. The results of testing such panels could then be used to select therapies, which are effective against a specific
molecular subtype of PDX, in treating a corresponding patient whose tumor has a similar molecular profile as a responsive PDX (41,42).

Additional mouse brain tumor models

**Humanized mice.** Brain tumor initiation and progression not only reflects the occurrence and accumulation of mutations, but as well the coincident failure of the immune system to control tumor growth. Understanding how tumors affect host immunity is therefore a critical topic of investigation for achieving increased understanding of cancer immunobiology and for identifying therapeutic strategies that engage patient immune response against their cancer. Much of our understanding of interrelationships between brain cancer and immune response has stemmed from the results of studies utilizing syngeneic mouse brain tumor models. However, substantial differences exist between murine and human immune function, as well as cancer biology, so extrapolating from mouse to human may often carry with it a number of erroneous assumptions. The use of PDX models has largely precluded the study of immune response to tumor, due to the immunocompromised status of host mice. Recently, a humanized mouse model was described whereby NSG mice were engrafted with human fetal thymus and fetal liver-derived hematopoietic stem cells (43). Notably, the IL-2Rα− specific NOD−scid background supports human and murine hematopoietic cell engraftment, and suppresses human erythropoiesis, enhances human myelopoiesis, and reduces human B-lymphopoiesis in mice after transplant of bone marrow or liver cells (44), and HLA-matching can be provided for congruence with human tumor cell engraftment. NSG-SGM3-BLT mice possess a high level of human cell chimerism, and develop a mature immune system that includes human myeloid cells, T cells and B cells. Reports of humanized mouse models for studying human cancer are thus far infrequent, but seem likely to see substantial increase given the high level of interest in studying immunotherapies for treating cancer.

**RCAS-TVA.** The RCAS-TVA mouse model, though not so widely used, has nonetheless been influential in advancing understanding of brain tumor development, and for testing therapeutics for treating brain tumors (45,46). The fundamentals of this mouse model start with a GEM that has undergone modification for promoter-specific expression of a transgene encoding a retroviral receptor. Promoters for GFAP and nestin have been
frequently used in this regard for modeling brain cancer. Mice with brain tissue specific expression of the viral receptor either receive an intracranial injection with retrovirus or with cells that produce retrovirus. The virus used in this setting has typically been modified to introduce an activated oncogene and/or express an shRNA against a tumor suppressor gene. Viral uptake by cells expressing viral receptor and viral transgene expression causes tumor development for certain transgene combinations. In some instances, specific transgene combinations have been shown to cause consistent tumor formation, and in relatively short periods of time. In such instances, these models have proven useful for therapeutic testing (47).

**Sleeping Beauty.** A final model to mention involves use of the sleeping beauty approach, and in which virus is transduced into mouse cells for genomic insertion of a transposon, and expression of a transposase, which promotes transposon insertion at thousands of locations in recipient cells, ultimately aimed at the activation and/or inactivation of expressed sequences. This approach has been used almost exclusively for cancer gene discovery (48), and not for testing cancer therapies.

**Approaches for monitoring intracranial tumor growth and response the therapy.**

Survival analysis of orthotopically-injected rodents is the gold standard for conducting therapy-response studies with rodents bearing intracranial tumors, whether engrafted, induced, or spontaneously occurring. However, the time required for carrying out therapeutic efficacy studies based on survival endpoint criteria is often time consuming and provides a single metric from what is often a costly, and lengthy, experiment. Commonly used methods for obtaining in-experiment feedback, to complement survival results, include the timed euthanasia of animal subjects while on therapy, with subsequent analysis of brain tumor cell indicators of therapeutic activity, such as Ki-67 antibody staining for addressing proliferation effects of treatment, and TUNEL staining for determination of treatment effects on cell death. In immunocompetent animals undergoing immunotherapeutic evaluation, defined time point analyses are often used to examine brain tumor for immune cell infiltrates.

Tumor imaging methods, for obtaining in-experiment results on intracranial tumor response to treatment, have seen steadily-increasing use in recent years. Longitudinal tumor imaging methods in live animal subjects (49) include magnetic resonance imaging (MRI), fluorescent optical imaging, and positron emission tomography.
(PET). Additionally, bioluminescence imaging (BLI) is frequently used to detect the emission of photons from energy-dependent reactions involving the metabolism of exogenous luciferin substrate by cells that have been genetically modified to express luciferase. While D-luciferin has relatively poor distribution across an intact blood-brain barrier, D-luciferin imaging has been used successfully to evaluate response to therapies in orthotopic tumors in multiple studies, and a new generation of more brain penetrant synthetic luciferin will enhance the utility of this strategy. Notably, BLI studies have demonstrated a strong correlation between volumetric and treatment response (50), similar to MRI, with the benefit of a lower cost to operate, as well as lower overall labor requirement (51). Furthermore, the use of gadolinium-enhanced MRI normally requires the presence of specialized personnel for technical operation, which limits the analysis to individual mice and requires a several-fold increase in imaging time (51). Also, and unlike fluorescent imaging of GFP+ or RFP+ labeled tumor cells, which can cause indeterminate signal-to-noise ratios as a result of high normal tissue autofluorescence, photon scattering and fluorophore photo-bleaching, BLI possesses minimal background activity, facilitating a remarkably sensitive quantification of increasing, or decreasing tumor size (50,51). Regardless of the approach utilized, these methods can provide in-experiment feedback regarding therapeutic activity, or lack thereof.

Conclusions.

The investigation and benchmarking of novel therapeutics and administration strategies are likely to remain an essential part of preclinical research for translational bench-to-bedside laboratory-based discoveries. As reviewed above, a number of models are available for facilitating and promoting discovery leading to improved care and outcomes for brain tumor patients (Table 4). Rodent models are tools to be used for enabling discovery, and, as is the case for any tool, it is important that the “craftsman” knows which tools are most appropriate for a given circumstance. In this review we have provided an overview of available rodent models, or tools, and we look forward to reading of future discoveries from their application.
References


tumors and demonstrates sensitivity to a combination of MEK and PI3K pathway inhibitors. Disease Models & Mechanisms 8: 45-56 doi:10.1242/dmm.018168


Table 1. Common immunocompetent, syngeneic brain tumor engraftment models

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ψClassical Subtype  
*Proneural Subtype
Table 4. Advantages-Disadvantages* of Commonly Used Rodent Brain Tumor Models

**Syngeneic, Immunocompetent Engraftment Models**

**Advantages**
- enable immunotherapy studies
- numerous models
- ease of tumor cell propagation
- expandability/scalability
- availability of host animals
- synchronicity of tumor growth, within series of engrafted mice, is usually quite good
- consistency and reproducibility of results, both within and between laboratories
- cost

**Disadvantages:**
- cell heterogeneity diminished by extended culturing
- invasive process for tumor establishment
- do mutagen induced tumors have molecular profiles consistent with spontaneous tumors in patients?
- are there inherent differences in the therapeutic response of rodent tumor cells and human tumor cells?
- cell of origin?

**PDX Models**

**Advantages**
- improved retention of patient tumor molecular characteristics, relative to cell culturing
- numerous models have been developed, and model sharing is becoming more common
- expandability/scalability
- availability of animal hosts
- synchronicity of intracranial tumor growth, within series of engrafted mice, is usually quite good

**Disadvantages**
- fewer labs familiar with in vivo tumor propagation; use of transferred models may require training
- preparing cells from subcutaneous tumors for intracranial injection more complex and time consuming than harvesting cells from culture
- problem of decreased heterogeneity with increased passaging
- problem of changes to molecular and biologic properties with increased passaging
- more expensive than working with cultured cells

**Genetically Engineered Mice (GEM), Contemporary Models**

**Advantages**
- temporal as well as spatial/anatomic control of tumor development
- absence of invasive procedure to initiate tumor development
- tumor development is tissue and/or cell type restricted
- mice are immunocompetent

**Disadvantages**
- tumor development can be multifocal, and therefore not consistent with the presentation of tumor in most patients
- dependent upon the specific GEM, tumor development within a series of mice can be very asynchronous
- cost and complexity of developing and maintaining mice with multiple genetic alterations

* All models share a disadvantage of requiring the use of an imaging technique to monitor tumor growth and response to therapy.