

Mouse Models of Human Cancers Consortium Workshop on Nervous System Tumors

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Abstract

Nervous system tumors are clinically challenging neoplasms that form within the central and peripheral nervous system. Although there have been many clinical trials using novel agents for the treatment of primary brain tumors, there have been few advances that positively affect overall patient survival. Over the past several years, there has been significant progress in the development of accurate small-animal spontaneous brain tumor models, small-animal neuroimaging, and tools for the bioinformatic analysis of complex molecular data sets, all of which have contributed to an improved understanding of the pathogenesis of human brain tumors. Whereas these models will continue to be of great value in basic science investigations, they can also be used to identify and validate potential therapies for brain tumors and to evaluate these drugs in preclinical trials. The National Cancer Institute recently convened a workshop to review the current state of small-animal brain tumor modeling and to make recommendations about the use of these models to improve the clinical outcome for patients with brain tumors. In this meeting report, we outline the current state of small-animal models for brain tumors, the potential applications of these models, and the recommendations made by the workshop participants for the use of mouse models in the preclinical evaluation of potential brain tumor therapies. (Cancer Res 2006; 66(1): 10-3)

Workshop Goals

Tumors affecting the brain, spinal cord, and peripheral nerves are unique neoplasms, owing to their specialized microenvironment, proximity to eloquent tissue, and relative resistance to conventional antitumor therapies. In this regard, brain tumors arise within the highly organized central nervous system and their treatment invariably results in both short- and long-term damages to normal brain. Gliomas, the most common primary brain tumor, account for only 2% of all cancers, yet rank among the deadliest of cancers: patients harboring the most malignant form of glioma have a median survival of <12 months. The clinical challenges are to understand the molecular and cellular basis for clinical responses when they occur, to improve on the delivery of therapies to the brain, to identify and validate new

drugable targets, and to develop combinations of agents that are directed at specific pathways essential for ongoing tumor growth. With the generation of accurate small-animal brain tumor models that recapitulate critical aspects of the human disease, it is now possible to address these pressing problems. To galvanize efforts in this direction, the National Cancer Institute sponsored the Mouse Models of Human Cancers Consortium (MMHCC) Workshop on Nervous System Tumors in St. Louis, Missouri on June 17-18, 2005. During this meeting, the current state of small-animal brain tumor modeling was reviewed and recommendations were made to develop collaborative, cross-disciplinary strategies that would facilitate the translation of basic science discoveries to the clinic.

Current State of Mouse Models of Human Nervous System Tumors

Nervous system tumors can be divided into those tumors that arise from glial lineage cells (glioma, astrocytoma, and oligodendroglioma), external granule cells in the developing cerebellum (medulloblastoma), peripheral nerve Schwann cells (schwannoma, neurofibroma, and malignant peripheral nerve sheath tumor), ependymal cells lining the cerebrospinal fluid ventricular system (ependymoma), and leptomeningeal cells encasing the brain and spinal cord (meningioma). Whereas excellent small-animal models exist for schwannoma, neurofibroma, malignant peripheral nerve sheath tumor, and oligodendroglioma, this workshop focused primarily on astrocytoma, medulloblastoma, and meningioma.

The current brain tumor models, generated by experimental manipulation of the mouse, have been constructed to recapitulate both the genetics and biology of the human diseases. Several brain tumor models have been established using germ-line genetic modification (genetically engineered mice or GEM), in which constitutive or inducible genetic changes are targeted to specific cell types (e.g., astroglial cells). Unlike xenograft models, in which human tumor cell lines are introduced into immunocompromised mice, tumors in the engineered models evolve spontaneously within the natural brain microenvironment. These GEM models either involve the conditional expression of oncogenes or the conditional inactivation of tumor suppressor genes responsible for inherited brain tumor predisposition syndromes (e.g., neurofibromatosis 1 and 2). Robust models of neurofibromatosis 1-associated low-grade and high-grade gliomas as well as neurofibromatosis 2-associated meningioma have been generated using this approach. Another method for targeting genetic lesions to specific brain cells involves the somatic introduction of viral vectors carrying specific genes, such as oncogenes. Retroviruses have also been employed to identify novel genes implicated in brain tumor formation and progression using random proviral insertional mutagenesis strategies.

Note: National Cancer Institute-sponsored symposium held at the E.P. Newman Education Center, Washington University School of Medicine, June 17-18, 2005, St. Louis, Missouri.

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Despite the advantages of each of the mouse modeling approaches above, it is not yet clear that all clinical aspects of the human tumors can be modeled by targeting genetic changes to mouse cells. In this regard, there are likely differences between rodent and human brain tumors with respect to growth and susceptibility to anticancer treatment. For this reason, several groups have begun to develop human orthotopic brain tumor models in which surgically resected human brain tumors are directly explanted into rodent brains. As a complementary approach, normal human fetal astrocytes have been engineered to harbor genetic changes that mimic those found in high-grade glioma to generate malignant gliomas that are histologically identical to their human counterparts when grown in mouse brain. In addition, isolated human brain tumor stem cells have been used to generate gliomas and medulloblastoma tumors in mice. Collectively, these models have immediate applicability to the study of brain tumor formation and therapeutic drug design.

What Can We Do with Small Animal Models of Brain Tumors?

Understanding the molecular and cellular pathogenesis of brain tumors. Unlike cancers of the skin, colon, breast, and prostate, there are no approaches to early cancer detection for brain tumors. In this regard, brain tumors are usually malignant at initial clinical presentation and often widely invasive, and do not afford us the opportunity to study the evolution of these cancers from preneoplastic stages. Indeed, for most types of brain tumor, there is considerable debate about the neoplastic cell of origin, the role of the brain environment in promoting neoplastic transformation, and the precise genetic changes required for tumorigenesis. Using currently available GEM brain tumor models, these critically important questions are being addressed with rapid progress.

Cell of origin. Although it is often presumed that astrocytomas (gliomas) arise from astrocytes, it is not known whether these tumors originate from stem cells, immature progenitors, or differentiated astrocytes. By using cell-specific promoters to direct genetic changes to specific cell types in GEM, the critical target cell(s) can be identified. A strategy that is especially powerful for target cell identification involves engineering a gene that can be conditionally activated or inactivated upon the expression of Cre recombinase in a specific cell type or at a particular developmental stage. For glioma modeling, there are currently several transgenic Cre lines that are being used to define the cell of origin and to determine the effect of timing of gene inactivation/activation on tumorigenesis. In an effort to develop a toolbox of genetic markers for the different developmental stages of astroglial cell types in the nervous system, Bachoo and colleagues integrated microarray data sets across several *in vitro* model systems of astrocyte differentiation, primary astrocyte cultures, and various astrocyte-rich brain structures. This annotated data set provides new markers for the molecular classification of brain tumors as well as identifies additional genes of which promoter sequences can be usurped to develop additional Cre transgenic mice for conditional brain tumor-associated gene manipulation.

The importance of understanding the cell of origin of brain tumors and its relationship to normal brain development is underscored by the findings of Wechsler-Reya and associates. In their genetic model of medulloblastoma in mice, tumors arise from

mislocalized preneoplastic nests of immature progenitor-like cells that parallel aspects of normal cerebellar development. Given that neuroglial progenitors have different cellular and biochemical properties than differentiated brain cells, identifying the cell of origin has considerable effect on the design of future biologically based brain tumor treatments.

Brain microenvironment. Brain tumors arise in the context of a developing and constantly adapting brain environment. In the case of pediatric brain tumors, the brain tumor microenvironment is changing as a function of normal brain maturation, with myelination and synapse formation/remodeling occurring for most of the first two decades of life. In addition, in both children and adults, there is a complex relationship between the evolving tumor and the normal brain environment that involves new blood vessel formation and infiltration of microglia. The response of the brain to the developing tumor creates a changing microenvironment, which can positively or negatively affect tumor growth. The contribution of this adapting cellular microenvironment to tumor formation as well as to specifying tumor location could lead to the development of additional therapies and strategies to treat tumor progression and minimize brain injury from therapy. In this regard, recent studies by several laboratories modeling neurofibromatosis 1 (NF1)-associated central and peripheral nervous system tumors have shown that the *Nf1* heterozygous environment (brain and peripheral nerve) plays a critical role in initiating tumorigenesis. Moreover, Rubin and colleagues have recently shown that the localized and developmentally regulated expression of a specific chemokine may uniquely dictate the growth of gliomas along the optic pathway in children with neurofibromatosis 1.

Genetic modifiers. There is emerging evidence that genetic loci exist that function as modifiers in the pathogenesis of glioma in mice. Reilly and colleagues have shown that *Nf1*^{+/-};*p53*^{+/-} mice with specific genetic backgrounds have different susceptibilities to glioma formation. One such locus is located on mouse chromosome 11. Future studies of these loci in mice might help to uncover similar susceptibility genes in humans useful for predicting the risk of brain tumor formation and/or the response to therapy.

Development and evaluation of brain tumor therapies. Given the poor success rate in treating brain tumors, there is a great need to develop and optimize new therapies. Small-animal models can serve as platforms for anticancer therapeutic target identification and validation. In this regard, tumors and derivative cells can be used in drug discovery programs and resulting candidates can be evaluated for their ability to “hit” the desired target while minimizing off-target effects. One of the most frequent genetic changes seen in malignant glioma is amplification and mutational activation of the epidermal growth factor receptor (EGFR). Based on this observation, the common EGFR mutation (EGFRvIII) has been targeted in recent preclinical studies of glioma therapy.

In addition, Van Dyke and colleagues used astrocytes derived from their GEM model of anaplastic astrocytoma to identify pathways downstream of *Pten* inactivation important for tumor cell invasion and found that an atypical protein kinase C was required for tumor invasion. Similarly, Gutmann and colleagues have used their GEM model of *Nf1* low-grade optic glioma to identify new molecular targets for therapeutic drug design, including hyperactivation of the mammalian target of rapamycin pathway, and showed that inhibition of mammalian target of rapamycin pathway activation in *Nf1*-deficient astrocytes

restores normal growth *in vitro*. Studies such as these and others, along with preclinical testing in the appropriate mouse models, provide an avenue for identifying and validating potential drug targets.

Small-animal models also provide a unique opportunity to determine why conventional therapies fail. Studies aimed at optimizing drug delivery to the brain, identifying critical brain tumor growth control pathways, and targeting brain microenvironment cells involved in tumorigenesis (e.g., microglia and endothelial cells) will prove critical to the design of future therapeutics for brain tumors. In this regard, studies by Eric Holland and associates have begun to employ their virally induced glioma models to evaluate both conventional human chemotherapies as well as novel biologically based treatments in preclinical therapeutic trials.

Development of biomarkers. It would be clinically useful to be able to predict which patients are likely to respond to specific therapies early in the course of brain tumor treatment. The identification of serum or cerebrospinal fluid biomarkers that can provide prognostic information about the clinical course or response to therapy would be invaluable. Using a combination of converging genetic and genomic approaches, recent studies from a number of different research groups have identified the serum marker YKL-40 as a potential biomarker for malignant glioma survival and response to radiation therapy.

Similarly, the ability to provide predictive information from brain imaging would represent a major advance in brain tumor treatment. High field strength magnetic resonance imaging (MRI) now provides unparalleled visualization of brain tumors as well as the ability to study their biological properties. Because MRI is highly sensitive to water movement, this modality has been used to correlate MRI signal features with the growth and response of brain tumors to chemotherapy. In this fashion, it is conceivable that MRI might be used to develop biomarkers for brain tumor growth, beyond its ability to provide anatomic detail. In addition to MRI, micro-computerized tomography, micro-positron emission tomography using radiolabeled [¹¹C]methionine, and bioluminescence imaging have all been used to provide biological information about brain tumor growth and response to therapy. The application of bioluminescence to study the intracellular properties of tumor cells affords a unique opportunity to validate the ability of a given therapy to reach and hit its intended target.

Network analysis of brain tumors. The last several years has witnessed an explosion in high-throughput global analyses of chromosomal losses and gains as well as gene and protein expression. Each of these technologies has been used to define the molecular changes important for brain tumor formation and progression as well as to identify specific targets for therapeutic drug design. Comprehensive genomic, genetic, and proteomic profiling efforts have been initiated by Howard Fine and associates at the National Cancer Institute to develop a biologically significant pathologic classification system for glioma (Glioma Molecular Diagnostic Initiative) as part of the Repository of Molecular Brain Neoplasia Data project.⁴ The availability of these vast data sets for both mouse and human brain tumors requires sophisticated bioinformatic network capabilities, as have been pioneered by Mark Ellisman, Robert Williams, and colleagues for the study of the normal brain

[Biomedical Informatics Research Networks].⁵ The ability of the brain tumor clinical trials consortia and Specialized Programs of Research Excellence (SPOREs) to contribute tissue samples with detailed clinical information to these repositories, combined with the availability of suitable preclinical mouse brain tumor models, is likely to significantly accelerate progress in brain tumor treatment.

Summary and Recommendations

Building on the recommendations of the two earlier MMHCC workshops on nervous system tumors, the meeting participants recommended several significant conceptual and systematic changes to the study of brain tumors. First, it is likely that brain cancer actually represents a distortion or disturbance of normal brain development. In this respect, brain development is a highly orchestrated event, composed of cell proliferation, programmed cell death, migration, and differentiation. The signals for each of these processes are developmentally and temporally regulated by cues present in the brain microenvironment. If brain tumors represent developmental anomalies, a preneoplastic brain lesion could be likened to populations of cells present during a normal developmental stage, but now occurring at the wrong time or in the wrong location. It was recommended that investigations of the genetic and cellular determinants that govern normal brain development be applied to the study of brain tumors.

Second, the analysis of brain tumors requires a cross-disciplinary approach. Collaborative research efforts that combine the expertise of cancer biologists with developmental neurobiologists, systems biologists, and bioinformatics researchers will be necessary to begin to unravel the complexities of brain tumor formation and treatment. In this regard, it was recommended that specialized programs be developed that are specifically dedicated to the training of scientists in collaborative group science focused on brain tumors.

Lastly, as a field, we are fortunate to have both GEM models and orthotopic human model systems for the preclinical evaluation of brain tumor therapies. It was recommended that both GEM and human tumor orthotopic models be melded as complementary approaches because each provides unique information. Moreover, small-animal preclinical trials should be embedded within the current human clinical trial consortium studies with the identical design and outcome measures. The availability of information from both human and small-animal studies will allow for key comparisons and will likely afford critical insights into the genetic and cellular targets for the next generation of human brain tumor therapies.

Meeting Participants

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⁴ <http://rembrandt.nci.nih.gov/>.

⁵ <http://www.nbirn.net/>.

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