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Re-thinking Brain Tumors: The Fourth Mouse Models of Human Cancers Consortium Nervous System Tumors Workshop

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Introduction

Despite increased understanding of molecular changes in brain tumorigenesis and successful establishment of mouse brain tumor models, the prognosis for brain cancer has improved only slightly over the past several decades. In November 2007, members of the brain tumor mouse models community convened to discuss how to effectively leverage mouse models to better understand brain tumorigenesis and discover targets for therapy. Discussions focused on identification of molecular targets and cell lineage, microenvironment, and genomic contributions to tumor development and maintenance. Herein, we present recommendations for optimizing mouse models to achieve better outcomes for brain tumor patients.

Since the first National Cancer Institute (NCI) Mouse Models of Human Cancer Consortium (MMHCC) meeting in 2000 (1), many mouse models of brain cancer have been developed, and the field has progressed from generating models to using models to gain insights into the cellular and molecular pathogenesis of brain tumors. In November 2007, an international meeting sponsored by the MMHCC and the Office of Rare Diseases convened to discuss the current status of mouse brain tumor modeling and make recommendations for exploiting these models to address fundamental questions in brain tumor biology (for participants, see Supplemental Appendix).

A major theme of the meeting was the growing appreciation of the complexity of brain tumors (Supplementary Fig. S1). Development of the central nervous system (CNS) requires ordered and tightly regulated signals that instruct cells to grow, die, mature, and move at the right time and place. Tumors of the nervous system form as a result of mutations that co-opt these signals to promote inappropriate proliferation, survival, differentiation, and migration – the hallmarks

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of cancer (2). Because brain tumorigenesis may reflect improper responses to instructions important for normal brain development, a more integrated view of brain cancer is needed. A central focus of the meeting was how to apply lessons and techniques from developmental biology to the study of brain tumorigenesis.

At the molecular level, the signaling pathways important for brain tumorigenesis are multiple, not necessarily linear, with feedback mechanisms that must be taken into account when designing molecularly targeted interventions. At the cellular level, brain tumors are heterogeneous, composed of tumor stem cells and differentiated tumor cells with different characteristics and susceptibilities to therapy. At the level of the organ, brain tumors co-evolve with their environment, with stromal cells and factors in the surrounding microenvironment being important for tumor maintenance and progression, offering additional targets for intervention. Finally at the organism level, genetic variations between individuals can dictate how tumors initiate, progress, and respond to treatment. Mouse models allow researchers to rigorously test hypotheses developed from examining human tumors using genetic manipulation and controlling specific variables (*e.g.*, environmental influences) to better understand the roles of different pathways, cell types, stromal factors, and genetic variation.

Searching for Achilles' Heel in the Brain -- Targeting Molecular Pathways in Tumors

The topic of molecular targets for brain tumors was led by Drs. Marco Giovannini (INSERM, Paris, France) and Martine Roussel (St. Jude Children's Research Hospital, Memphis, TN), and focused on the characterization of specific molecular targets and signaling pathways, as well as development of effective drug delivery methods in preclinical mouse models. Several experts discussed loss or constitutive activation of key tumor suppressors and oncogenes, respectively, with an emphasis on PI3K/PTEN and RB/TRP53/INK4A-ARF tumor suppressor pathways.

Dr. Suzanne Baker (St. Jude Children's Research Hospital, Memphis, TN) discussed the importance of feedback inhibition in signaling pathways, and the need to test putative targets, such as intermediates in the PI3K/PTEN/S6/mTOR pathway, in different contexts to determine whether targeting the pathway is likely to be universally successful, or successful only in certain contexts. Because molecular signaling pathways can branch and activate compensatory signaling pathways, inhibition of specific targets (*e.g.*, mTOR) can actually lead to release of inhibition of certain branches of the pathway, culminating in increased tumor growth. Similarly, Dr. Terry Van Dyke (National Cancer Institute-Frederick, MD/University of North Carolina, Chapel Hill, NC) presented intriguing data on the levels of EGFR expression in accelerating or inhibiting Ras-driven brain tumors.

In addition to their use in identifying important molecular targets and understanding interactions between signaling pathways, mouse models can be powerful tools for developing drug delivery approaches and high-throughput evaluation of targets and therapies. Dr. Martine Roussel discussed micro-RNA as an additional layer of complexity, whereby many pathways may be altered by loss of expression or misexpression of a single micro-RNA (3). Dr. John Ohlfest (University of Minnesota, Minneapolis, MN) presented a novel approach to generate humanized spontaneous gliomas using transposons expressing human proteins in neonatal mouse brains (manuscript submitted). Because the resulting tumor-bearing mice are immune-competent expressing human proteins, this model can be used to test immunotherapies. Dr. Alain Charest (Tufts University, Boston, MA) discussed the use of nanoparticles for effective delivery of siRNAs to treat GBM in mice.

Defining the Players –Cell Types Involved in Initiation and Maintenance of Tumors

There has been considerable interest in cancer stem cells (CSCs) as the population of cells within the tumor that are resistant to therapy and lead to recurrence of tumors. This has led to a blurring of the lines between neural stem cells (NSCs) and CSCs. Dr. Luis Parada (University of Texas-Southwestern, Dallas, TX) and Dr. Richard Gilbertson (St. Jude Children's Research Hospital, Memphis, TN) led a session focused on defining different cell types important in brain tumorigenesis and determining which types are most critical to target therapeutically. Human cancers contain a small number of stem-like cells (CSCs) that are able to propagate tumors in immunocompromised mice. However, the existence of the CSC has been argued on the basis of observations made following xenografting of human CSCs into mice. Since species differences might account for the selective growth of certain cell types in these models, demonstrating the existence of CSCs in genetically-engineered mouse (GEM) brain tumors would provide important support for the CSC hypothesis.

An important topic of discussion was the terminology used in the literature relevant to interpreting brain CSC studies. Although the terms CSC and tumor-initiating cell (TIC) are often used interchangeably, CSCs are isolated from end-stage malignant tumors, harboring numerous mutations supporting tumor growth in xenotransplantation assays. These cells should be differentiated from true TICs *in vivo*, which often have a small number of mutations, possess limited tumorigenic capacity, and are identified in the early stages of tumor development. It remains unclear whether TICs are themselves NSCs, other progenitor cells, or differentiated cells.

Dr. Robert Wechsler-Reya (Duke University, Durham, NC) reported that both granule neuron precursors (GNPs) and ventricular zone NSCs in the cerebellum could serve as TICs for medulloblastoma. However, tumors initiating in NSCs grew more rapidly, suggesting that the cell in which tumors initiate has an important impact on tumorigenesis. Similarly, Dr. David Rowitch (University of California-San Francisco, CA) demonstrated that both multipotent and unipotent cells within the GNP lineage are capable of forming medulloblastoma, and the survival time of mice varied with the cell type targeted. These results suggest that differences in tumor development might relate to the number of susceptible cells targeted by particular genetic changes at specific developmental stages.

Dr. Wechsler-Reya highlighted the issue of CSC markers, showing that *Ptch1*^{+/-} mouse medulloblastomas contain a rare fraction of GNP-like *Math1*⁺CD15⁺ cells that propagate the tumor through serial transplantation. CD15⁺ cells can be found in a subset of human medulloblastoma, distinct from CD133⁺ CSCs. These findings suggest that there may be distinct tumor-propagating cells for different subtypes of brain tumors.

Dr. Cynthia Wetmore (Mayo Clinic, Rochester, MN) presented intriguing data that Shh pathway activation may be tumor-initiating, but not tumor-propagating, in medulloblastoma cells with stem cell-like properties (5). Dr. Silvia Marino (Barts and London School, London, England) presented a different approach to examine the role of brain NSCs in tumorigenesis by introducing oncogenic mutations in NSCs from postnatal mouse brain and magnetic sorting according to cell surface markers, then testing their tumorigenic potential *in vivo*.

Finally, it was concluded that we require a much broader arsenal of lineage-specific promoters and markers to study brain tumorigenesis. Lineage-specific transcription factors, such as *Olig2* and *Math1*, represent promising sources of markers and promoters that could be powerful tools for better understanding brain tumor biology. The discussants agreed that the development of standard antibody panels for the characterization of tumors and tumor intermediates would greatly facilitate our ability to test hypotheses regarding the origin and maintenance of brain tumors.

Location, Location, Location – Targeting the Microenvironment

Clinical observations strongly suggest that patient age and tumor location affect brain tumorigenesis and therapeutic response. These temporal and spatial differences in tumor biology are hypothesized to reflect variations in TICs and/or microenvironment influences on tumor initiation, proliferation, apoptosis, differentiation and migration, as well as protection by the microenvironment against cytotoxic treatments. In the session on tumor microenvironment led by Dr. Eric Holland (Memorial Sloan Kettering Cancer Center, New York, NY) and Dr. Joshua Rubin (Washington University, St. Louis, MO), participants discussed the cell types and conditions defining the microenvironment, how these factors may influence response to therapy, and how these factors can be used for future drug design.

The molecular basis for microenvironment influences on tumor formation and growth may mimic the relationships between stroma and neural progenitor cells during normal brain development. They are likely to involve cell-cell, cell-matrix and paracrine interactions. The cellular components of microenvironment include endothelial cells, non-neoplastic brain cells (astrocytes, entrapped axons), as well as inflammatory cells of the brain (activated astrocytes, microglia) and bone marrow (macrophages, neutrophils and lymphocytes). The matrix and soluble factor components are likely to include a myriad of secreted growth factors (*e.g.*, chemokines) and matrix components, such as hyaluronic acid, as well as heparan and chondroitin sulfate proteoglycans. The data presented made it clear that brain tumors can co-opt developmental paracrine signals to support glioma growth (6–8). In addition, tumors are dependent on metabolic pathways that allow for adaptation to hypoxic conditions through altered expression of glycolytic pathway enzymes (the “Warburg effect”) (9).

Collectively, these studies support the multi-faceted role of tumor stroma in regulating tumor formation and progression. The challenge in the immediate future for researchers will be generating testable hypotheses regarding specific stromal elements and factors in mouse brain tumor models that promote or attenuate tumorigenesis. This may require the development of additional lineage- and region-specific promoter elements as well as viral delivery systems that support the manipulation of stromal function in an age- and region-specific manner. In addition, it will be important to consider the tumor as a system under selective pressures, co-evolving with its environment. Spontaneous mouse tumor models in which this evolutionary process can be followed and experimentally manipulated will lead to a better understanding of the causative factors for end-stage human tumors.

Considering the Whole –Tumors and Individual Variation

With the appreciation of the complexity of signaling pathways, cell types, and environmental factors in brain tumors comes an appreciation that individual variation in any of these factors may influence tumorigenesis and response to therapy. In this respect, the group recognized that no single mouse model of brain cancer is sufficient to explain the heterogeneous nature of human brain tumors. The more we compare different models to identify common underlying mechanisms and targets, the closer we will get to effective therapies. It is therefore important to characterize and compare both mouse and human tumors at the genomic and systems levels to find commonalities in tumorigenesis. In this regard, mouse models of brain tumors provide tractable systems for directly studying the genetic susceptibility to brain tumors relevant to disease susceptibility, progression, and response to therapy (10).

Warning—Disk Full!

With the advent of new initiatives such as the Allen Brain Atlas and The Cancer Genome Atlas, the volume of genetic, genomic, imaging and anatomical data has grown exponentially, necessitating the development of powerful user interfaces for mining these complex datasets. In the session led by Drs. Maryann Martone (University of California-San Diego, CA) and

Robert Williams (University of Tennessee-Memphis, TN), the need to efficiently integrate and analyze bioinformatic and anatomical data was discussed. This will require standardization of semantic and bioinformatic frameworks, crossing scales from molecular to cellular and anatomical levels, and developing tools to facilitate cross-species analyses. A number of websites are being designed to help researchers to find tools and contribute data, such as the Neuroscience Information Framework (NIF)¹, a centralized catalog of web neuroscience resources, GeneNetwork,² a site cataloging normal brain expression data, and the Cell Centered Database,³ an online repository of image data for data management and analysis.

Recent advances in small-animal imaging and the desire to incorporate imaging into preclinical studies have resulted in challenges in both throughput and analysis. Dr. Mark Henkelman (University of Toronto, Ontario, Canada) presented a system for high spatial resolution magnetic resonance imaging (MRI) in which 16 mice can be imaged simultaneously in a large-bore 7-Tesla MR scanner with essentially no loss in either sensitivity or image resolution (11). Dr. Joel Garbow (Washington University, St. Louis, MO) discussed the challenges associated with processing and analysis of high-density MRI data. A significant impediment is the absence of a standardized format for small-animal imaging data akin to the DICOM (Digital Imaging and Communications in Medicine) standard adopted for clinical imaging data (12).

Bridging the Gap from Bench to Bedside – Taking Advantage of Mouse Models to Further Clinical Research

The mouse modeling community recognizes that models are only as good as the ultimate benefit they provide to patients. An informal discussion led by Dr. W. Alfred Yung (MD Anderson Cancer Center, Houston, TX) and Dr. Elizabeth Maher (University of Texas-Southwestern, Dallas, TX) included clinicians involved in brain tumor clinical trials and researchers, addressing how mouse models could be better used to benefit clinical research. The discussion focused on the importance of mouse models for understanding fundamental biology of tumorigenesis, studying progression, and defining resistance to therapy. In addition, there is a need to meld human xenografts with GEM models. Dr. Van Dyke described a new pilot program at NCI, the Center for Applied Preclinical Research, aiming to develop standardized methods for testing therapies in GEM models. Although it is clear that past models of brain cancer have been poorly predictive of therapeutic success in humans, the newer GEM models are still relatively untested. Mouse modelers and drug developers will use the center to test experimental therapies in a variety of models in a standardized manner. This should help to validate models and determine how targeted therapies could be tested on patients with particular molecular profiles.

Summary

As we move closer to the promise of personalized and targeted therapies for brain tumors, models that reflect the complexity and heterogeneity of brain tumors are expected to lead to more predictive models and new approaches for therapy. As tumors may commandeer the cues that govern normal cell growth during development, the next generation of therapies is likely to result from an improved understanding of developmental neurobiology. Armed with robust preclinical GEM brain tumor models, we are now uniquely poised to discover and evaluate new therapies.

¹<http://neurogateway.org/catalog/goto.do?page=.home>

²<http://www.genenetwork.org>

³<http://ccdb.ucsd.edu/CCDBWebSite/index.html>

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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