COMMENTARY

BRAF Mutations Open Doors for N-Ethyl-N-Nitrosourea—Induced Gliomagenesis

Robert S. McNeill,* David M. Irvin,1 and C. Ryan Miller*157

In this issue of the American Journal of Pathology, Wang et al1 identified recurrent Braf mutations in N-ethyl-N-nitrosourea (ENU)—induced rat gliomas by DNA sequencing. Their results provide a platform for preclinical development of novel targeted therapies for Braf-mutant gliomas. Precision oncology promises to revolutionize cancer therapy by stratifying tumors on the basis of their molecular characteristics and using rationally designed treatments in molecularly defined patient populations.2 Although the field of oncology is transitioning into the precision medicine era, conventional diagnostics, based on tissue type, tumor pathology, and patient demographics, remain essential to quality care. One natural pathological division for gliomas, the most common primary brain tumors in humans, is based on their invasion, with nondiffuse gliomas forming well-circumscribed tumors and diffuse gliomas invading the normal brain.3 Within these two broad diagnostic categories, gliomas are further subdivided on the basis of patient demographics into pediatric and adult diseases and further into specific diagnostic entities based on their histological appearance.

Human Gliomas Are Genomically Heterogeneous

Genomic analyses are necessary to clinically implement the concepts of precision medicine. Comprehensive genomic studies have revealed the genetic diversity of specific glioma entities and increased precision in defining relevant disease subtypes. For instance, integration of multiple genomic and molecular analyses determined that there are three molecular classes of adult lower-grade gliomas (grade II and III astrocytomas, oligoastrocytomas, and oligodendrogliomas, based on the World Health Organization 2007 classification scheme) that are more accurately represented by genetic testing for IDH1/2, ATRX, and TP53 mutations and chromosome 1p and 19q losses than by histological class.3,4 Large-scale genomic sequencing has also shown that pediatric gliomas are genomically distinct diseases compared to their histologically similar adult counterparts.5 Moreover, genomic analyses have shown that even within a single demographic group or histological entity, multiple molecular subtypes of disease exist.6,7 Our increased understanding of the molecular characteristics underpinning glioma pathogenesis and increased precision in defining disease subsets will hopefully lead to the development of treatments that target the molecular aberrations driving their genesis. However, to fully achieve the promise of precision medicine, it will be necessary to use preclinical models that match specific disease subsets not only in terms of pathology and demographics, but on genetic mechanisms as well.

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Address correspondence to C. Ryan Miller, M.D., Ph.D., University of North Carolina School of Medicine, 6109B Neurosciences Research Bldg, Campus Box 7250, Chapel Hill, NC 27599-7250. E-mail: rmiller@med.unc.edu

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Modeling Gliomas Using ENU

The first animal models of human gliomas were developed by treating either mice or rats with chemical carcinogens. One such carcinogen is ENU, a DNA ethylating agent that induces widespread DNA damage, resulting in single-nucleotide mutations, most commonly T:A to A:T transversions and T:A to C:G transitions. Rats in particular have been treated with ENU either during embryonic development or postnatally to induce gliomagenesis, and the resulting gliomas were either characterized within the intact brain or harvested to establish adherent cell lines cultured in vitro in serum-containing media.

Serum-cultured cell lines established from either chemically induced rodent gliomas or naturally occurring human tumors (established cell lines) have been the backbone of preclinical glioma research since the late 1960s. These models were widely disseminated and routinely used, but their genetic make-up was largely unknown until the advent of large-scale genomic analyses, such as microarray profiling and next-generation sequencing. These techniques have shown that established cell lines harbor more extensive genomic abnormalities than human gliomas and do not faithfully recapitulate the molecular profiles of patient samples. Reasons for these discrepancies include adaptation to nutrient-rich media and clonal selection. Nevertheless, gliomas that arise spontaneously within an intact rat brain after ENU treatment are not subject to the selection pressures of serum culture. Therefore, these models may more faithfully recapitulate the genomics of acutely isolated patient tumors. Whether mutations in certain genes are highly penetrant or whether multiple mutations converge on specific, common biological pathway(s) in ENU-induced gliomas remained unclear.

ENU-Induced Rat Gliomas Harbor Mutant Braf

The study by Wang et al determined the mutational profile of ENU-induced rat gliomas. Whole genome sequencing was performed on tumors from three BDIV and two BDIX rats induced with ENU at post-natal day 1. Somatic mutations ranged from 6354 to 13,807 per glioma, with a mean of 10,685, but recurrent copy number alterations were absent in the tumors examined. Consistent with ENU-induced mutagenesis, the most common single-nucleotide mutations were T:A to A:T transversions and T:A to C:G transitions. Between 35 and 93 of these somatic mutations were shown to be functional, causing amino acid changes (92%), introduction of stop codons (6%), or destruction of splice sites (2%). Somatic mutations in Srrm2, Olr158, Il12rb, Map1b, Rux2, Rsrc2, and Tcf21 occurred in two of the five gliomas analyzed. The only mutation unique to all five was an A to T missense mutation corresponding to BrafV545E. Wang et al found that this mutation was analogous to the BrafV600E mutation at both the nucleotide and amino acid levels in humans and mice. To confirm the occurrence of BrafV545E in ENU-induced rat gliomas, the authors expanded their cohort to another 33 BDIV and 12 BDIX gliomas. Sanger sequencing determined that all 45 also harbored the BrafV545E mutation. Moreover, BrafV545E mutant rat gliomas were immunoreactive to a human BRAFV600E antibody. Thus, they found that BrafV545E is a ubiquitous mutation in ENU-induced gliomas in rats.

Role of BRAF in Tumorigenesis

BRAF encodes a serine/threonine protein kinase that activates the mitogen activated protein kinase effector arm of receptor tyrosine kinase signaling. Activating BRAF mutations, particularly the most common V600E mutation, have been implicated in tumorigenesis in a variety of solid cancers, including melanoma (approximately 70%), papillary thyroid cancer (45%), and colorectal cancers (approximately 10%). This mutation lies within the kinase domain and leads to constitutive activation of the protein and hyperactivation of mitogen activated protein kinase signaling.

Genomic analyses have shown that BRAF mutations occur in nondiffuse human gliomas, including pilocytic astrocytomas, gangliogliomas, and pleomorphic xanthoastrocytoma. More important, all three tumor types develop most commonly in children and young adults and may display the cytological features of their diffuse oligodendroglioma counterparts, including perinuclear halos, crisp nuclear membranes, and prominent nucleoli. However, unlike diffuse oligodendrogliomas, each of these tumors are slow growing, benign (World Health Organization grade I, pilocytic astrocytomas and gangliogliomas) to mildly aggressive (World Health Organization grade II, pleomorphic xanthoastrocytoma), and have discrete tumor margins. They are thus generally surgically curable. Nevertheless, when location precludes complete resection, adjuvant therapies are required. Drugs targeting BRAFV600E (BRAFi) have been approved for treatment of metastatic melanoma. However, resistance inevitably occurs through a variety of mechanisms, including reactivation of mitogen activated protein kinase signaling or alternate activation of parallel pathways. Thus, rationally designed combination therapies that target multiple kinases within the same pathway and/or alternate pathways may be necessary to circumvent BRAFi resistance. Moreover, BRAFi have shown mixed results in the treatment of BRAF-mutant gliomas. Case reports suggest that BRAFV600E-mutant gliomas are sensitive to BRAFi, but this observation has not been confirmed in clinical trials. Developing more accurate preclinical models to determine the underlying biology of BRAF-mutant gliomas is necessary for efficient development of novel treatments.

McNeill et al
Historically, the histological and cytological features of ENU-induced rat gliomas, including the extent of brain invasion, have been variable, with most being diagnosed as oligodendrogliomas, astrocytomas, or mixed gliomas. The ENU-induced rat gliomas from the study by Wang et al showed cytological features similar to low-grade or anaplastic oligodendrogliomas, but lacked diffuse brain invasion. Taken together, the histopathology of these model tumors is consistent with the nondiffuse gliomas, and their lack of invasion further supports the use of this model for studying these entities. Indeed, a significant strength of this ENU-induced rat glioma model is the developmental timing of tumorigenesis that may mimic pediatric patients. ENU injections were performed in perinatal mice, mirroring the age at which \textit{BRAFV600E} gliomas might be initiated in humans. This timing, plus the ubiquitous penetrance of \textit{Braf} \textit{V545E} mutations, suggests that this preclinical model may prove beneficial in studying the basic biology of \textit{BRAF}-mutant pediatric gliomas and developing targeted therapies.

Using \textit{BRAF}-Mutant Murine Models to Study Gliomagenesis and Develop Targeted Therapies

The complete penetrance of \textit{BRAFV545E} mutations in the ENU-induced rat gliomas described by Wang et al suggests that this mutation drives tumorigenesis. However, the study was underpowered to identify other significantly mutated genes that may cooperate with mutant \textit{BRAF}. Future studies using whole exome sequencing, sophisticated statistical tools, and increased samples sizes could confirm the frequency of the \textit{BRAFV545E} mutation and identify potential cooperating mutations, such as \textit{CDKN2A} (\textit{Ink4a-Arf}) loss, that frequently co-occur in human gliomas.

In this regard, the role of \textit{BRAF} mutations in gliomagenesis has been explored experimentally. Overexpression of both wild-type \textit{BRAF} and \textit{BRAFV600E} in immortalized astrocytes induces senescence in vitro. One study using genetically engineered mice found that \textit{BRAFV600E} alone was not sufficient to induce gliomas, but cooperated with \textit{Cdkn2a} deletion to do so. Moreover, gliomas from these genetically engineered mice were well demarcated and noninfiltrative. In addition, the \textit{BRAFV600E} kinase domain alone was sufficient to generate pilocytic astrocytoma when expressed in situ. Both of these studies examined the role of \textit{BRAFV600E} in transformation of neonatal mouse neural stem cells. However, the role of \textit{BRAFV600E} in gliomagenesis could differ on the basis of the cell of origin. The cellular origins of \textit{Braf}-mutant, ENU-induced rat gliomas described in Wang et al remain unknown. Future studies should focus on elucidating the influence of cellular origin and cooperating mutations in \textit{BRAF}-driven gliomagenesis.

Genetically faithful, \textit{BRAF}-mutant preclinical glioma models will aid in the development of novel, targeted treatments. Preclinical data suggest that \textit{BRAF} is a viable target in \textit{BRAF}-mutant gliomas. Indeed, genetic ablation of \textit{BRAF} with shRNA reduces growth of \textit{BRAFV600E}-mutant glioma cell lines. This suggests that \textit{BRAF} mutations are involved in tumor maintenance as well as initiation. The role of \textit{BRAFV600E} in tumor maintenance has also been investigated pharmacologically. \textit{BRAFV600E}-mutant, patient-derived xenografts and established cell lines are sensitive to the \textit{BRAFi} PLX4720 in vitro. Moreover, intracranial genetically engineered mouse—derived allografts developed using \textit{BrafV600E} driven, \textit{Ink4a-Arf} null neural progenitor cells were also sensitive to PLX4720. These results implicate oncogene addiction to \textit{BRAFV600E} in gliomas, suggesting that the rat gliomas described by Wang et al may be addicted to \textit{BRAF} mutations as well.

Despite the promise of single agent—targeted therapies, drug resistance has limited their therapeutic benefits in melanoma patients. Given the resistance patterns seen clinically, it is unlikely that \textit{BRAF}-targeted therapies will be effective as single agents in gliomas. Therefore, the generation of rationally designed combination treatments is paramount. To this end, the use of genetically accurate preclinical model systems is necessary. Indeed, the \textit{BRAFi}, PLX4720, has been tested in vivo in combination with radiation in \textit{BRAFV600E} mutant patient-derived xenografts and in combination with the cyclin-dependent kinase 4/6 inhibitor PD0332991 in \textit{BRAFV600E} mutant, \textit{Ink4a-Arf} deleted genetically engineered mouse—derived allografts. These preclinical studies showed that combination treatment improved survival compared to untreated and PLX4720-treated mice. Nevertheless, all mice eventually succumbed to disease. Thus, more preclinical studies using experimentally tractable models are required to identify effective, rationally designed combination therapies. Should ENU-induced gliomas prove to have the same cooperating mutations as \textit{BRAF}-mutant human gliomas, use of tumor cells harvested from this model for orthotopic implantation in a manner similar to genetically engineered mouse—derived allografts may prove useful. Adapting the ENU model to culture and allografts will enable control of critical variables for preclinical drug studies, such as tumor latency and penetration. Moreover, leveraging a panel of \textit{BrafV545E}-mutant, ENU-induced rat gliomas harboring a variety of cooperating mutations will be useful in further elucidating \textit{BRAFi} resistance mechanisms in gliomas, determining how co-occurring mutations influence resistance, and evaluating novel combination therapies both \textit{in vitro} and \textit{in vivo}.

Conclusions

With the genomics revolution, development of models that faithfully recapitulate specific tumor subtypes have become increasingly important. The work of Wang et al thus opens the door to ENU-induced rat gliomas, firmly placing them...
as a viable model for BRAF-mutant human gliomas.1 These ENU-induced gliomas will provide a platform for improved preclinical modelling and drug development.

References


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2554