The Evolving Role of Molecular Markers in the Diagnosis and Management of Diffuse Glioma

Jason T. Huse1 and Kenneth D. Aldape2

Abstract

While the classification of diffuse gliomas has relied on the examination of morphologic features supplemented with techniques such as immunohistochemistry, there is an increasing recognition of substantial biologic diversity within morphologically defined entities. High-throughput technologies, in particular studies that integrate genome-wide data from diverse molecular platforms, increasingly identify the existence of robust and distinct glioma subtypes. While treatment advances and improvement of outcomes for patients with diffuse glioma have been modest, there may be benefit to integrate findings from biologic studies into clinical practice to enhance the precision of treatment for these diseases. Recent examples such as the identification of mutations in IDH1 and IDH2 as an early genetic event that is predominantly in lower-grade gliomas (grades 2 and 3) underscore the importance of molecular discovery leading to the ability to develop subclassifications with prognostic and potentially therapeutic implications. In contrast, glioblastoma (grade 4), the most common and aggressive glioma, typically arises without IDH mutation, supporting the need for different therapeutic approaches. Additional genomic and epigenomic signatures are generally nonoverlapping between IDH-mutant and IDH wild-type diffuse glioma, and despite comparable histopathology, IDH-mutant gliomas can be considered as biologically distinct from IDH wild-type gliomas. In this CCR Focus article, we highlight and summarize the current understanding of recent molecular findings and the relationships of these findings to clinical trials and clinical management.

See all articles in this CCR Focus section, "Discoveries, Challenges, and Progress in Primary Brain Tumors."

Clin Cancer Res; 20(22); 5601–11. ©2014 AACR.

Introduction

Diffuse gliomas are the most common intrinsic tumors of the central nervous system and consist of neoplasms that exhibit a wide range of biologic and clinical features. From a long-range perspective, glial tumors are broadly defined as either circumscribed or diffuse. Circumscribed gliomas include entities such as pilocytic astrocytoma, World Health Organization (WHO) grade 1. These tumors are, in principle, potentially curable by surgical resection and will not be considered further. In contrast, diffuse gliomas are by definition incurable by surgery due to their infiltrative nature, are more common, especially in adults, and are the subject of this review. Diffuse gliomas are categorized by malignancy grade with a range of WHO grades 2, 3, and 4 (glioblastoma) and exhibit a wide range of clinical behavior, ranging from slow clinical progression in lower grade tumors, to very short median survival times for patients with WHO grade 4 tumors (glioblastoma). Glioma is unusual, if not unique, as a malignant tumor with little propensity for distant metastasis, and prognosis is a function almost entirely of grade rather than stage. Malignancy grade, and to a lesser extent morphologic determinations of presumed histogenesis (astrocytoma vs. oligodendroglioma vs. mixed oligoastrocytoma) accounts for some of the variation in patient outcome, but substantial and extensive within-grade and within-entity clinical and biologic variability exists, prompting investigation into molecular factors which may better account for such variation. While in the past the molecular basis that underlay these contrasts between glioma variants was not well understood, recent efforts at molecular characterization, especially through high-throughput genomic and epigenomic screens have, in their identification of distinctive and highly recurrent genomic and epigenomic abnormalities, clarified some of this diversity and have provided the beginnings of new concepts in tumor classification. In addition, insights gained from these studies have pointed to viable avenues for therapeutic development.

As stated above, diffuse gliomas of adulthood are unified by a shared propensity to widely infiltrate surrounding normal brain parenchyma, a property that effectively renders them incurable by resection. As such, a major goal of neurosurgical resection in cases of diffuse glioma includes
accurate material for diagnosis and classification as well as cytoreduction when clinical indicated; but the goal of complete resection with curative intent is essentially unrealistic with diffuse glioma, as patients almost universally undergo tumor recurrence even after substantial cytoreductive surgery. That said, the range of diffuse glioma behavior exhibits considerable clinical heterogeneity. More specifically, patients with glioblastoma (WHO grade 4) demonstrate overall survival times of approximately 15 months while those affected by low-grade (WHO grade 2) astrocytomas and oligodendrogliomas frequently exhibit prolonged clinical courses lasting years to even decades (1, 2). Another feature of diffuse gliomas (again, generally in contrast to circumscribed variants) is the phenomenon of tumor progression/malignant transformation, where lower grade gliomas (WHO grades 2 and 3) over time not only recur but also progress to higher malignancy grade (WHO grades 3–4), with associated rapidity of clinical deterioration.

Historically, gliomas were classified largely on morphologic and histopathologic characteristics, forming the foundation for further investigations. Morphologic classification is necessarily based on interpretation of microscopic features using routinely available tools. While clinical and radiologic features are at times taken into account, the WHO-sanctioned classification is at its core, histology-based. The value of this remains within its clinical utility by virtue of the association of tumor grade with patient outcome (3). However, many aspects of the histologic-based classification (which is not outlined in detail here) fall short with respect to potential personalization of care, with respect to matching of the biologic foundations of each tumor with appropriately targeted treatment regimens.

Large-scale molecular profiling of diffuse gliomas is occurring both by individual laboratories as well as national consortia such as The Cancer Genome Atlas (TCGA) network. Glioblastoma was an early tumor type in the TCGA armamentarium, with the recent addition of grade 2–3 gliomas (referred to by TCGA as the “lower grade glioma” effort). These efforts and others have led to an enhanced understanding of the molecular makeup of diffuse gliomas and have uncovered a number of recurring aberrations in genes and pathways, including mutations/abnormalities in specific genes, multicomponent expression signatures, and DNA methylation patterns (4–7). While a number of these markers and pathways have been previously implicated in glioma biology, some represent new discoveries. Moreover, the multidimensional datasets provided by TCGA and related efforts allow for the integration of findings from orthogonal platforms (e.g., gene mutation, mRNA, epigenetics) within a large set of tumor samples. Such analyses will lead to new opportunities for the identification of robust biologic subtypes. In turn, patterns of aberrations are becoming evident from these data that point to the need for new approaches to biomarker analysis that go beyond both routine procedures that are in the comfort zone of current clinical laboratories (e.g., immunohistochemistry) as well as beyond single marker analyses. While efforts such as TCGA and other genome-wide studies have been helpful, it is important to put these efforts in perspective, including limitations such as the retrospective nature of the findings, the fact that findings from these studies are often not transformative and, in the future, require prospective evaluation in uniformly treated patients (Fig. 1). Efforts to include clinically relevant molecular alterations into the formal, WHO classification scheme for brain tumors are underway (8). In addition, while some prognostic markers are emerging, an unmet need in the field are true predictive markers for diffuse glioma, that when implemented, inform real-time treatment decisions for the individual patient.

Established Oncogenes and Cancer Pathways in Glioma

Early work, beginning several decades ago, identified recurring molecular alterations characterizing diffuse gliomas. A large number of such genetic events have been characterized to date, and patterns have emerged pointing to key pathways likely driving these tumors, some of which have been causally verified using mouse models (9, 10). Among these are losses of the RB1 and p53 tumor suppressor genes or, alternatively, alterations in genes involved in pathways related to these tumor suppressors. Mutations in the TP53 gene have long been considered molecular hallmarks of lower grade astrocytic tumors and the secondary glioblastomas into which they evolve (11–13). While mutations in RB1 itself have been known for some time (14) not to be common in gliomas, genes encoding its upstream regulators are frequently altered (5, 14, 15). In particular, CDKN2A, which encodes both the INK4A and ARF genes, crucial activators of RB1 and p53, respectively, is deleted in a large percentage of diffuse gliomas (16), particularly glioblastomas (15, 17–19). In addition, upstream repressors such as CDK4 and D-type cyclins (RB inhibitors) and MDM2 (p53 inhibitor), are frequently upregulated, often by gene amplification, providing alternative mechanisms for p53 and RB1 pathway silencing. Together, the sheer frequency of these abnormalities underscores the importance of the RB1 and p53 pathways as tumor-suppressive molecular networks in glioma biology. This concept was shown quite clearly in the initial TCGA publication on glioblastoma, which found that there were some alterations expected to inactivate the p53 and Rb signaling networks in at least 87% and 78% of all cases, respectively (5).

In addition to tumor-suppressive pathways, perhaps the most well-known and common activating oncogenic changes in glioma are those involving receptor tyrosine kinases (RTK). Mouse modeling studies have underscored the importance of these events as gliomagenic drivers. The EGFR frequently undergoes high-level genomic amplification in adult glioblastoma (~40%), often in conjunction with constitutively activating mutations in the ectodomain of the protein that include, but are not limited to, the variant III (vIII) deletion event (5, 15, 20–22). While the expression of EGFRvIII is complex and heterogeneous (23), it is detected in approximately 30% to 50% of cases in which EGFR amplification is present. In addition, high-level
amplification of the platelet-derived growth factor receptor gene (PDGFRA) is also present, although in a smaller proportion (~13%) of adult glioblastoma (5). Analogous to EGFR, constitutively activating deletion mutants in PDGFRA have been described in receptor-amplified tumors (24). PDGFRA amplification also appears to be a common genomic RTK alteration impacting pediatric glioblastoma as well as diffuse intrinsic pontine glioma (18, 25, 26). Though much less common, high-level MET amplification also occurs in glioblastomas (5, 15, 18). More importantly, individual glioblastoma tumors can display activating genomic alterations in multiple RTKs, simultaneously, with amplified receptors often segregating to distinct cellular subpopulations (27, 28). This finding has implications on the likely efficacy of drugs targeting single RTKs in these tumors. Finally, while lower-grade (grade 2–3) gliomas infrequently harbor high-level amplification in RTK genes, enhanced PDGF signaling and PDGFRA phosphorylation has been routinely implicated in their pathogenesis (29–31). Together, the high degree of heterogeneity and complexity of RTK biology in glioma may account for some of the lack of success of anti-EGFR trials in glioblastoma.

Genomic alterations involving core components of oncogenic signaling pathways situated downstream of RTKs are common findings in glioblastoma. Integrated analysis in the initial TCGA glioblastoma report described activation of the extended PI3K–AKT–mTOR and RAS–MAPK molecular pathways in nearly all glioblastoma samples evaluated (9). Dysregulating alterations include activating mutations in either the catalytic (PIK3CA) or regulatory (PIK3R1) domains of PI3K and are found in approximately 15% of adult glioblastomas, as well as deletions and/or silencing mutations in PTEN, the primary negative regulator of PI3K–AKT signaling. (~1/3 of cases). Beyond genetic alterations of PTEN, additional epigenomic and microRNA (miRNA)-based mechanisms of PTEN repression have also been described in diffuse gliomas with the former operative in significant numbers of adult lower-grade gliomas (WHO grades 2 and 3, hereafter referred to as LGGs: 50%–60%; refs. 30, 32–35). Mutations in the RAS antagonist neurofibromin (NF1) have long been known to cause neurofibromatosis type 1, a cancer predisposition syndrome characterized by frequent neurofibromas and, to a lesser extent, astrocytomas (36). However, more recent studies have demonstrated NF1 mutation/deletion in 15% to 18% of primary glioblastomas (5, 15), where it appears to represent a key molecular alteration of the mesenchymal glioblastoma subclass (see below).

Gene Expression Signatures

Gene expression profiling was the first high-throughput technology to be broadly applied to human cancer. Largely because of its early adoption, gene expression technology remains a standard approach enabling the identification and characterization of biologic subclasses in clinicopathologic tumor entities. Initial successful applications of expression profiling to identify tumor subclasses included adenocarcinoma of the breast and diffuse large B-cell lymphoma. In early work on gliomas focused primarily on high-grade tumors like glioblastoma, comprehensive transcriptional analysis was initially used to establish molecular correlates for known clinical and/or histopathologic distinctions,
such as WHO grade, astrocytic versus oligodendrogial morphology, and primary versus secondary glioblastoma status (37–45). Subsequent studies have focused more on identifying natural molecular distinctions within diffuse gliomas and, despite some differences, have revealed robust subclasses within glioblastoma. The first of these studies examined prognostically relevant gene signatures in WHO grade 3 and 4 diffuse gliomas, revealing three major subclasses, termed proneural, mesenchymal, and proliferative. This was followed by additional investigations, most notably that of TCGA, which delineated four transcriptional subclasses, termed proneural, neural, classical, and mesenchymal (7).

In this latter study, genomic associations were also identified, with classical, proneural, and mesenchymal tumors strongly enriched for abnormalities in EGFR, PDGFRA and IDH1 or IDH2, and NF1, respectively. Incorporation of these findings, in the context of the fundamental differences between IDH-mutant and IDH wild-type glioma leads to the concept of IDH as the first "split" into two groups with additional subtypes within each of these groups (Fig. 2). These and other analyses support the notion that malignant gliomas can be loosely classified into two subgroups, corresponding to proneural and mesenchymal gene signatures (Fig. 3). Analyses from both studies found that the large majority of IDH wild-type diffuse gliomas are glioblastomas, IDH-mutant/G-CIMP- positive gliomas represent the majority of grade 2 and 3 diffuse gliomas (top) and are within the proneural expression subclass. These can be further subclassified on the basis of 1p/19q codeletion (frequently showing oligodendroglioma histology) or TP53 mutation (frequently showing astrocytic histology). IDH-mutant glioblastoma is rare (5%–10% of glioblastomas) but is well recognized, especially in younger adults. IDH wild-type gliomas (bottom) show characteristic changes, including as possible driver events loss of 10, gain of 7, and loss of 9p/TP53 mutation (56). These can be then subdivided into additional expression subclasses. The majority of IDH wild-type diffuse gliomas are glioblastomas, although grade 2–3 tumors are also represented.

Clinically Relevant Classification Markers

IDH mutation

The discovery of IDH mutations in diffuse glioma represents a major paradigm shift in our understanding and integrating mRNA, miRNA, gene copy number, and global DNA methylation data, reported five and three glioblastoma subclasses, respectively, emerging from unsupervised analysis (47, 48). Nevertheless, in both studies, tumors defined as either proneural or mesenchymal by mRNA profiling tended to colocalize within the new subclassification scheme. While these two subclasses appear to be reproducibly defined and characterized, and the relationship of IDH-mutant and G-CIMP tumors (see below) to the proneural expression class appears stable, the ability of gene expression signatures to reliably classify gliomas in a clinically relevant fashion remains uncertain. Indeed, subclass assignment has been shown to change following treatment (4, 49). Moreover, a recent study analyzing expression signatures of single cells within glioblastoma samples showed substantial intratumoral heterogeneity of expression subclasses within each tumor (50), suggesting that bulk mRNA profiling of a glioma simply represents an average of a heterogeneous mix of transcriptional signatures, likely influenced by multiple factors (genetic changes, microenvironment, treatment, etc.). These considerations suggest that while gene expression is a critical component of glioma biology, alternative cancer-specific alterations in the genomic/epigenomic space may represent more stable metrics for tumor classification.

Figure 2. Subtypes and key molecular signatures in diffuse glioma. IDH-mutant/G-CIMP- positive gliomas represent the majority of grade 2 and 3 diffuse gliomas (top) and are within the proneural expression subclass. These can be further subclassified on the basis of 1p/19q codeletion (frequently showing oligodendroglioma histology) or TP53 mutation (frequently showing astrocytic histology). IDH-mutant glioblastoma is rare (5%–10% of glioblastomas) but is well recognized, especially in younger adults. IDH wild-type gliomas (bottom) show characteristic changes, including as possible driver events loss of 10, gain of 7, and loss of 9p/TP53 mutation (56). These can be then subdivided into additional expression subclasses. The majority of IDH wild-type diffuse gliomas are glioblastomas, although grade 2–3 tumors are also represented.
Classification of these tumors. The finding of mutations in IDH1 and IDH2 in glioma was completely unexpected and made using a genome-wide pure discovery approach (15), as compared with an approach taken by groups that focused on a candidate mutation, indicating the value of discovery science to generate novel and important findings. IDH1 and IDH2 in glioma was completely unexpected and made using a genome-wide pure discovery approach (15), indicating the value of discovery science to generate novel and important findings. The finding of mutations in glioma suggests that this is an early event in the pathogenesis of a subset of diffuse glioma. The finding that both astrocytoma and oligodendroglioma harbor IDH mutations at high frequency suggests that this is an early event in molecular pathogenesis, as well as suggesting that astrocytoma and oligodendroglioma have a similar cell of origin. IDH-mutant gliomas will typically exhibit additional alterations, namely mutations in TP53 (corresponding to histologically diagnosed astrocytoma) or, conversely, codeletion of chromosomes 1p and 19q (resulting from an unbalanced t(1;19)(q10;p10) translocation (refs. 52, 53; corresponding to oligodendroglioma histology). Second, the clinicopathologic distinction between the de novo primary versus secondary pathways of glioma development can largely be accounted for by the absence and presence, respectively, of IDH mutations. Most gliomas that are identified at a lower grade (grade 2 or 3) are IDH-mutant, whereas most cases of de novo glioblastoma (short clinical presentation, glioblastoma histology at initial diagnosis) are IDH-wild-type. There are signature DNA copy number changes that are nearly synonymous with the molecular definitions of primary glioblastoma include gains of chromosome 7 and loss of chromosome 10. These occur in 80% to 90% of glioblastoma at initial diagnosis and are essentially mutually exclusive with IDH mutation. In addition, amplification at 7p, harboring EGFR, occurs in 40% to 50% of glioblastoma at initial diagnosis, but again does not occur in the setting of IDH-mutant diffuse glioma. Conversely, patterns of recurrent copy number alterations in IDH-mutant diffuse gliomas, including 1p/19q codeletion, consist largely of events not frequently encountered in primary glioblastoma. Moreover, the glioma CpG island methylator phenotype (G-CIMP), characterized by stereotypic and concordant methylation of a set of CpG sites, is almost entirely restricted to IDH-mutant tumors. This finding was functionally clarified to show that mutant IDH can cause changes corresponding to G-CIMP in model systems (54). Molecular changes that occur between IDH-mutant and wild-type diffuse gliomas are to a large extent unshared, suggesting distinct biology despite histologic similarity (see Fig. 4). There is mutual exclusivity of 1p/19q codeletion and TP53 mutation among IDH-mutant glioma, suggesting a hierarchy of genomic events and divergent subpathways within IDH-mutant glioma. While IDH/G-CIMP status can distinguish two major subtypes of diffuse glioma, there are some correlates with current histopathologic classification. For example, at initial diagnosis, among lower grade gliomas (grade 2–3), the majority are IDH-mutant/G-CIMP positive, with only 10%–20% in the IDH wild-type/G-CIMP− negative genomic class. Alternatively, among newly diagnosed glioblastoma/grade 4 tumors, IDH mutation/G-CIMP positivity is uncommon (5%–10%), with the majority of glioblastoma...
as IDH wild-type. Nevertheless, both genomic subtypes are observed in all grades and serve to distinguish diffuse glioma into distinct biologic subtypes. The presence or absence of IDH mutation provides the molecular correlate for the previously established concepts of “secondary” or “progressive” (left) pathways to glioblastoma, absent/presence of IDH mutation provides the molecular correlate of this long established paradigm. With the identification of IDH mutation/G-CIMP as present in a subset of diffuse glioma, a distinction can be made into two fundamentally distinct glioma types that cannot be resolved histologically. Left, the IDH-mutant/G-CIMP-positive molecular changes in glioma are illustrated. Right, IDHwt/G-CIMP-negative gliomas are illustrated. In the IDH-mutant subset, presentation as a lower grade (WHO grade 2–3) is uncommon, whereas presentation as glioblastoma is uncommon. The opposite is true for IDH wild-type glioma (right), but these differences are relative. The lower aspect of the figure illustrates the point that routine histology, on which current classification is based, cannot reliably distinguish these important subtypes of diffuse glioma. Question marks indicate changes that have not been fully elucidated or are not well characterized.

In addition to delineating a large subclass of diffuse glioma, IDH mutation status serves as a useful classification marker to distinguish LGCs from histologically similar entities, whether neoplastic or not. For example, oligodendroglioma can occasionally be difficult to distinguish from morphologically similar entities (e.g., central neurocytoma, clear cell ependymoma), and the presence of an IDH mutation essentially rules out these alternative possibilities. Occasionally, a diffuse glioma can be difficult to distinguish from a circumscribed glioma (e.g., pilocytic astrocytoma, pleomorphic xanthoastrocytoma, etc.) and again the presence of an IDH mutation resolves this differential diagnosis. Finally, the presence of IDH-mutant tumor cells again mitigates the distinction between diffuse glioma and reactive conditions (astrogliosis). In this regard, the availability of the R132H-specific anti-IDH1 mutant antibody is especially helpful, as tumor cells are often in the minority in these cases. However, the ease of mutant IDH1 IHC should not preclude the need for a full workup and/or interpretation of results, as 10% to 20% of all IDH mutations (non-R132H IDH1 mutations and all IDH2 mutations) are...
been postulated (64). Loss of ATRX function impairs the and IDH1 are almost invariably associated with mutations of chromosomal arm 1p are also present in 1p/19q codeleted tumors. 1p/19q codeletion is almost invariable associated with IDH mutation, and within the setting of IDH-mutant glioma, 1p/19q codeleted tumors carry a better prognosis than non-codeleted tumors when matched for malignancy grade. While it is not clear whether the natural history of codeleted tumors differs from that of IDH-mutant/non-codeleted tumors in the absence of therapy, it does appear that codeletion is a marker of improved response to cytotoxic chemotherapy. Long-term results of two large randomized clinical trials, European Organization for Research and Treatment of Cancer Radiation Therapy Oncology Group 9402 (59, 60), showed that adjuvant PCV chemotherapy confers a survival advantage in the setting of 1p/19q codeletion. These findings demonstrate that, for diffuse gliomas in general, codeletion can be viewed as a predictive marker for PCV chemotherapy. However, codeletion defines what is essentially a subset of IDH-mutant glioma and the degree to which codeletion serves as a predictive chemotherapeutic marker in the context of IDH-mutant gliomas alone remains less clear. An additional unanswered question relates to the fact that PCV chemotherapy has since been subsumed with temozolomide therapy, raising the issue as to whether molecular correlates related to PCV chemotherapy will correspond to patients treated with temozolomide. Defining predictive markers are one of the most important goals of biomarker characterization and personalized medicine and with activity in antiangiogenic therapy, immunotherapy, and therapies for pediatric tumors (all addressed in this edition of CCR Focus; refs. 61–63).

**1p/19q loss**

The finding of combined 1p/19q codeletion and its correlation with oligodendroglioma has been known for some time (17) and its characteristic presence in a subset of gliomas has spurred efforts to identify is biologic and clinical significance. Most oligodendrogliomas with 1p and 19q codeletions also carry mutations in the ***CIC*** gene, a homolog of the *Drosophila* gene *capicua*, on chromosomal band 19q13.2, (57, 58). In addition, although perhaps less frequently, mutations in *FUBP1* on chromosomal arm 1p are also present in 1p/19q codeleted tumors. 1p/19q codeletion is almost invariable associated with IDH mutation, and within the setting of IDH-mutant glioma, 1p/19q codeleted tumors carry a better prognosis than non-codeleted tumors when matched for malignancy grade. While it is not clear whether the natural history of codeleted tumors differs from that of IDH-mutant/non-codeleted tumors in the absence of therapy, it does appear that codeletion is a marker of improved response to cytotoxic chemotherapy. Long-term results of two large randomized clinical trials, European Organization for Research and Treatment of Cancer Radiation Therapy Oncology Group 9402 (59, 60), showed that adjuvant PCV chemotherapy confers a survival advantage in the setting of 1p/19q codeletion. These findings demonstrate that, for diffuse gliomas in general, codeletion can be viewed as a predictive marker for PCV chemotherapy. However, codeletion defines what is essentially a subset of IDH-mutant glioma and the degree to which codeletion serves as a predictive chemotherapeutic marker in the context of IDH-mutant gliomas alone remains less clear. An additional unanswered question relates to the fact that PCV chemotherapy has since been subsumed with temozolomide therapy, raising the issue as to whether molecular correlates related to PCV chemotherapy will correspond to patients treated with temozolomide. Defining predictive markers are one of the most important goals of biomarker characterization and personalized medicine and with activity in antiangiogenic therapy, immunotherapy, and therapies for pediatric tumors (all addressed in this edition of CCR Focus; refs. 61–63).

**ATRX mutations and TERT alterations**

Recent work has shown that mutations in the SWI/SNF chromatin regulator *ATRX* frequently occur in gliomas, and are almost invariably associated with mutations of *TP53* and *IDH1* genes across glioma entities (58, 64, 65). A strong correlation between inactivation of ATRX and the phenomenon known as alternative lengthening of telomeres has been postulated (64). Loss of ATRX function impairs the heterochromatic state of the telomeres and leads to telomere destabilization, which facilitates the development of alternative lengthening of telomeres. These same studies showed that ATRX mutations were a very specific marker for astrocytic lineage tumors, including diffuse and anaplastic astrocytomas and most oligoastrocytomas. This evidence makes ATRX mutations an appealing counterpart for 1p and 19q codeletions, which seem to be mutually exclusive with ATRX mutations. As most mutations detected to date are truncating and thus lead to a reduction in protein concentrations, immunohistochemical assessment of loss of ATRX could be a reasonable surrogate marker of ATRX mutations. Moreover, combining 1p/19q codeletion and ATRX assessments in the clinical setting could help guide diagnosis within the spectrum of IDH-mutant gliomas and, in the long-term, to stratify patients for specific treatments. TERT promoter mutations are also characteristic of diffuse glioma and are mutually exclusive with ATRX mutation in IDH-mutant diffuse glioma (66). Promoter mutations result in increased TERT expression, with one study showing that expression level of TERT in tumors carrying those mutations was on average 6.1 times higher than that of TERT wild-type tumors (67). Within IDH-mutant glioma, TERT promoter mutations are observed in almost all tumors harboring concurrent 1p/19q loss, while IDH-mutant non-1p/19q codeleted tumors tend to have ATRX mutation (67). The mutations are stereotypic, with the C228T mutation within the TERT promoter occurring 146 bp upstream of the ATG start codon of TERT, whereas the C250T mutation occurs 126 bp upstream. Both mutations generate a de novo sequence, which contains the ETS transcription factor binding motif, allowing recruitment of transcription factors and increased expression (68).

**MGMT promoter methylation**

A number of clinical trials and cohort studies have shown that promoter methylation and silencing of the MGMT gene, which codes for O6-methylguanine-DNA methyltransferase, a DNA repair enzyme, is associated with prolonged progression-free and overall survival in patients with glioblastoma who are being treated with alkylating agent chemotherapy (69–73). A landmark practice-changing trial compared concurrent/adjuvant temozolomide during and after radiotherapy versus radiotherapy alone for glioblastoma patients (71). Subset analysis of samples from this trial showed that the benefit from chemotherapy was almost exclusively attributable to patients with MGMT-methylated tumors (71). Further work in elderly patients, in whom aggressive treatment is a clinical issue, showed improved outcome in the setting of chemotherapy for MGMT-methylated tumors and, interestingly, worse survival associated with unmethylated tumors (75, 76), indicating that in this setting MGMT methylation is not prognostic, but rather predictive. Additional work supports the predictive value of MGMT methylation and there is evidence to suggest that its predictive status is conditional on IDH mutation status (predictive only in IDH-mutant glioma; refs. 77, 78). To date, the literature on
MGMT methylation in glioblastoma would suggest that overall, it is at least partially a predictive (as opposed to merely prognostic) marker for chemotherapy. This concept has led to patient selection for clinical trials omitting TMZ in patients with glioblastoma without MGMT methylation to find treatments for this patient group with no real treatment options at present. Work in this area, to date presented in abstract form, awaits further maturity.

However, several important considerations and caveats deserve mention. First, while MGMT methylation is not necessarily a defining marker of G-CIMP, there is clear concordance and overlap between these two biomarkers. Specifically, while MGMT methylation is clearly present in a subset of G-CIMP–negative glioblastomas, MGMT methylation appears to be present in nearly all cases of G-CIMP–positive glioblastomas (79). As G-CIMP itself is a favorable prognostic marker, the effect of MGMT methylation in IDH wild-type/G-CIMP–negative glioblastoma needs to be fully defined. Second, methodology varies with respect to MGMT methylation detection and results are not completely uniform, warranting some caution in the interpretation of studies across laboratories (80). Third, determination of MGMT status by immunohistochemistry has notable interobserver variability and is not reliably associated with promoter methylation or outcome. A recent study in which four observers examined MGMT expression showed suboptimal interobserver agreement, as well as only modest correlation with MGMT promoter methylation status and no significant association of MGMT expression with patient outcome (81). This may call into question, to some extent, whether the favorable outcome experienced in MGMT-positive glioblastomas (79) with MGMT unmethylated tumors limits the use of this marker to actually personalize therapy for glioblastoma. Future work to optimize laboratory detection of MGMT methylation, reduce interlaboratory variability, and clearly define the relationship of MGMT methylation to patient outcome and IDH/G-CIMP status is warranted to fully evaluate this promising biomarker.

Concluding Remarks

Substantial progress has been made in the molecular classification of primary brain tumors. The recent molecular characterization of diffuse glioma has provided not only a clarified framework for the conceptualization of these tumors, but also revealed pathways for the development of more effective targeted therapeutics. Experience with several markers is at the point where clinical integration is becoming standard of care. For MGMT promoter methylation in glioblastomas, including those arising in elderly patients, and 1p and 19q codeletions in anaplastic oligodendrogial tumors, molecular markers now play a major role in clinical decision making (59, 60, 75, 76). Dependent on the outcome of ongoing phase II and III trials, biomarkers to predict resistance or sensitivity to angiogenesis inhibition could also prove useful. Meanwhile, high-throughput analyses at the genetic, epigenetic, and expression levels have shown their value in refining the classification of brain tumors and prognostication of outcome. These techniques might soon become more widely available, easier to standardize, and have less bias than single marker assessments—e.g., current methods for MGMT methylation assessment—and might soon become more cost effective. Accordingly, we predict that the current histology-dominated diagnostic assessment of brain tumors will be increasingly supplemented by molecular diagnostic tests, which eventually might be gradually replaced by high-throughput profiling techniques, including array-based approaches and next-generation sequencing. However, for those treating patients with these tumors, there is the feeling that translation of molecular findings into meaningful improvements in patient outcomes has been slow. A recent study underscores some of the challenges in translating molecular findings into clinical trials. Using matched primary–recurrent matched pairs. The study showed that recurrences did not exhibit the full set of mutations found in the initial tumor, suggesting that the mutational profile of recurrent glioma cannot be completely deduced from the tumor at initial diagnosis (82). Combined with the finding that temozolomide therapy can result in mutations in the DNA mismatch repair pathway that result in a hypermutator phenotype (83). These findings emphasize the need for tumor resampling (Fig. 1), when possible, in the setting of clinical trials for recurrent glioma (emphasized in Fig. 1). Advances in minimally invasive means to sample and detect key genomic aberrations in glioma, such as cell-free nucleic acid (84, 85) or circulating tumor cells (86). As the process of therapeutic refinement moves forward, more effective preclinical models and optimal clinical trial design will be absolutely crucial, as will the ready availability of sophisticated genomic technology in the clinical environment, starting with the use of relevant molecular markers as an objective means for tumor classification.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: K.D. Aldape
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.D. Aldape
Writing, review, and/or revision of the manuscript: J.T. Hue, K.D. Aldape

Received August 21, 2014; revised September 22, 2014; accepted September 24, 2014; published online November 14, 2014.

References


The Evolving Role of Molecular Markers in the Diagnosis and Management of Diffuse Glioma

Jason T. Huse and Kenneth D. Aldape


<table>
<thead>
<tr>
<th>Updated version</th>
<th>Access the most recent version of this article at: <a href="http://clincancerres.aacrjournals.org/content/20/22/5601">http://clincancerres.aacrjournals.org/content/20/22/5601</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cited articles</td>
<td>This article cites 85 articles, 39 of which you can access for free at: <a href="http://clincancerres.aacrjournals.org/content/20/22/5601.full#ref-list-1">http://clincancerres.aacrjournals.org/content/20/22/5601.full#ref-list-1</a></td>
</tr>
<tr>
<td>Citing articles</td>
<td>This article has been cited by 3 HighWire-hosted articles. Access the articles at: <a href="http://clincancerres.aacrjournals.org/content/20/22/5601.full#related-urls">http://clincancerres.aacrjournals.org/content/20/22/5601.full#related-urls</a></td>
</tr>
<tr>
<td>E-mail alerts</td>
<td>Sign up to receive free email-alerts related to this article or journal.</td>
</tr>
<tr>
<td>Reprints and Subscriptions</td>
<td>To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at <a href="mailto:pubs@aacr.org">pubs@aacr.org</a>.</td>
</tr>
<tr>
<td>Permissions</td>
<td>To request permission to re-use all or part of this article, use this link <a href="http://clincancerres.aacrjournals.org/content/20/22/5601">http://clincancerres.aacrjournals.org/content/20/22/5601</a>. Click on &quot;Request Permissions&quot; which will take you to the Copyright Clearance Center's (CCC) Rightslink site.</td>
</tr>
</tbody>
</table>