Impending Impact of Molecular Pathology on Classifying Adult Diffuse Gliomas

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Background: Progress in molecular oncology during the last decade has enabled investigators to more precisely define and group gliomas. The impacts of isocitrate dehydrogenase (IDH) mutation (mut) status and other molecular markers on the classification, prognostication, and management of diffuse gliomas are likely to be far-reaching.

Methods: Clinical experience and the medical literature were used to assess the current status of glioma categorization and the likely impact of the pending revision of the classification scheme of the World Health Organization (WHO).

Results: IDH-mut is a defining event in most adult fibrillary astrocytomas (FAs) and nearly all oligodendrogliomas (ODs). The IDH-mut status of most gliomas can be established by immunohistochemistry for the most common mutant of \( IDH1 \) (R132H). IDH wild-type (wt) diffuse gliomas include several familiar entities — in particular, glioblastoma (GBM) and most pediatric gliomas — as well as an assortment of less well-defined entities. The codeletion of 1p/19q distinguishes OD from FA, which, by contrast, shows frequent loss of the \( \alpha \)-thalassemia/mental retardation syndrome X-linked protein. Mixed oligoastrocytomas are typically classifiable as either OD or FA using molecular testing.

Conclusions: The current practice of designating IDH-mut WHO grade 4 astrocytoma as secondary GBM will likely be discouraged, and primary or de novo GBM, which is always IDH-wt, may lose this qualification. Histologically, low- or intermediate-grade IDH-wt gliomas with molecular changes characteristic of GBM might justify the designation of GBM WHO grade 3. Mixed oligoastrocytoma is losing popularity as a diagnostic term because most cases will fall into either the FA or OD category. Distinguishing IDH-mut from IDH-wt tumors in clinical trials is likely to clarify sensitivity rates or tumor resistance among subgroups, thus suggesting opportunities for targeted therapy.

Introduction

Insight into the pathobiology of primary brain tumors has accompanied technological and analytic discoveries in molecular biology during the last decade. Important genetic, epigenetic, and proteomic alterations have been documented in a variety of low- and high-grade gliomas with clinical implications. Gliomas, which constitute the majority of tumors derived from intra-axial elements, may arise from undifferentiated or partially differentiated astrocytes, oligodendrocytes, or ependymal cells. Although histology remains a mainstay of diagnosis, overlapping morphological features can often be resolved without subjectivity; for example, mixed oligoastrocytoma (MOA) vs either fibrillary astrocytoma (FA) or oligodendroglioma.
(OD), small cell glioblastoma (GBM) vs anaplastic OD, and ependymoma (EP) vs FA.

Many gliomas exhibit characteristic genetic, chromosomal, and/or biochemical signatures indicative of distinct pathophysiology, presaging a reproducible and reliable molecular overlay to histopathological diagnosis. Pertinent ancillary studies beyond histopathology may include immunohistochemical testing, chromosomal assessment, traditional and array-based genetics, epigenetic analysis, and ribonucleic acid (RNA) profiling (both messenger RNA [mRNA] and noncoding micro-RNA [miRNA]). The World Health Organization (WHO) classification of central nervous system tumors will likely incorporate these findings in its soon-to-be released update. Such an improved classification system may contribute to the improved clinical investigation of new and targeted therapies to combat the continued guarded outlook for most primary brain neoplasms. Of particular interest are the implications of isocitrate dehydrogenase (IDH) mutation (mut) status.

**Two Groups of Gliomas**

The traditional categorization of gliomas into their morphological subgroups (astrocytic, oligodendrogial, and ependymal) typically precedes textbook discussions of particular entities. This is likely to change. In a seminal, genome-wide study, Parsons et al documented IDH1 mutations in subsets of glial neoplasms. It was soon recognized that mutations of IDH1 are frequent in FAs, ODs, and MOAs, particularly at amino acid residue 132 (arginine), which is replaced by histidine in nearly 90% of tumors bearing an IDH1 mutation. This residue may also be replaced by cysteine, serine, or other amino acids, and mutations may rarely occur at other loci. IDH2 may also be affected, albeit at a different locus (residue 172). Mutations of other IDH isoforms have not been reported to accompany gliomagenesis. Thus, the finding of an IDH1 or IDH2 mutation can be termed IDH-mut, and the absence of a mutation in either gene is designated as IDH wild type (wt). Acute myeloid leukemia, chondrosarcoma, giant cell tumor of the bone, and brainstem astrocytoma, EP, medulloblastoma, and meningioma, diffuse astrocytomas, including de novo GBM, gliomatosus cerebri, thalamic, and brainstem astrocytoma, harbor a variety of genetic changes, and they should be regarded as distinct from their IDH-mut counterparts despite being morphologically indistinguishable. Conversely, cases of OD are almost always IDH-mut. Rare IDH-wt exceptions, including most pediatric cases, should be segregated within this diagnostic category until their pathogenesis is resolved. Morphologically MOAs are frequently IDH-mut and subsequently classifiable as either FA or OD following further molecular testing. The appropriate pathological diagnosis for the rare IDH-wt MOA remains unsettled; however, such tumors should still be segregated from the IDH-mut categories.

**Accumulation of 2-Hydroxyglutarate**

Early events in developing neoplasms involve the acquisition of aberrations in proliferation-related intracellular signaling pathways. IDH normally participates in the oxidative decarboxylation of isocitrate to α-ketoglutarate. The mutant form lacks this important enzymatic property and instead generates an abundance of 2-hydroxyglutarate (2HG). Excess 2HG interferes with chromatin-modifying activity, thus leading to global DNA hypermethylation and the glioma-CpG island methylation phenotype, which is linked to tumorigenesis. Therefore, 2HG can be designated as an oncometabolite. Because IDH alterations are fundamental to pathobiology, disease progression, diagnosis, response to adjuvant treatment, and clinical outcome, the classification of gliomas should first acknowledge IDH-mut vs IDH-wt status. This approach would solidify the analysis of subtypes of IDH-mut tumors, allowing more objective classification of IDH-wt entities, and highlight the molecular distinctions between pediatric and adult tumors exhibiting similar morphological features.

**Common Morphological Entities**

The 3 morphological entities that exhibit IDH-mut are FA (grades 2–4), OD (grades 2 and 3), and MOA (grade 2 or 3). All other brain tumors are IDH-wt, including most pediatric cases, pilocytic astrocytoma of any age, pleomorphic xanthoastrocytoma, subependymal giant cell astrocytoma, EP, medulloblastoma, and meningioma. IDH-wt diffuse astrocytomas, including de novo GBM, gliomatosus cerebri, thalamic, and brainstem astrocytoma, harbor a variety of genetic changes, and they should be regarded as distinct from their IDH-mut counterparts despite being morphologically indistinguishable. Conversely, cases of OD are almost always IDH-mut. Rare IDH-wt exceptions, including most pediatric cases, should be segregated within this diagnostic category until their pathogenesis is resolved. Morphologically MOAs are frequently IDH-mut and subsequently classifiable as either FA or OD following further molecular testing. The appropriate pathological diagnosis for the rare IDH-wt MOA remains unsettled; however, such tumors should still be segregated from the IDH-mut categories.

**Immunohistochemistry**

Once assigned to the diffuse glioma category by routine pathological assessment, testing for the IDH1 R132H mutation may be easily conducted using a commercially available antibody test that recognizes the altered epitope. A positive result has high sensitivity and specificity rates for IDH-mut R132H, although reports exist of cross-reactivity with R132L and R132M mutations. A total of 10% to 15% of IDH-mut cases are immunonegative, and such cases can be sent for direct sequencing or hotspot analysis for both IDH1 and IDH2. IDH1 R132C is the most common immunonegative mutation.

**IDH1/2 Mutations Account for Most Fibrillary Astrocytomas**

A patient with low-grade astrocytoma typically presents with new-onset seizures, headaches, focal neurological deficits, or all of these symptoms. Neuroimaging typically shows a hemispheric hypointense, nonenhancing mass that — if followed without sur-
TP53 Mutations Common in IDH-mut Astrocytomas

Mutations in TP53 have been recognized in astrocytoma, ranging from 30% to 50% of studied cases, and alterations in related genes are frequently detected in all grades of diffuse astrocytomas. Interactions between a p53 alteration and IDH-mut in the earliest stages of tumor initiation have been suggested by the predilection for the uncommon R132C mutation to occur in Li–Fraumeni syndrome (germline mutation of p53 with tumor predisposition). Nuclear p53 overexpression is common in astrocytoma, is readily detected with immunohistochemistry (Fig E), and often correlates with TP53 mutation; however, as a diagnostic or prognostic marker, its utility is limited, and it has been supplanted by IDH, α thalassemia/mental retardation syndrome X-linked (ATRX), and 1p/19q testing.

ATRX Loss Characterizes IDH-mut Astrocytomas

Mutations in the telomere maintenance protein ATRX appear to distinguish IDH-mut FA from tumors that lack this change—in particular, OD. Absence of ATRX protein (Fig F) by immunohistochemistry is evident in 25% to 30% of adult and pediatric high- and low-grade astrocytomas. Loss of ATRX expression and IDH-mut typifies more than 95% of adult diffuse astrocytomas, and it is almost mutually exclusive with 1p/19q codeletion. Classification based on IDH-mut status and ATRX expression will likely be a key recommendation in the next WHO classification update.

WHO Grade 4 IDH-mut Fibrillary Astrocytoma

WHO grade 4 FA with an IDH-mut accounts for fewer than 10% of WHO grade 4 gliomas, typically prompting the designation of secondary GBM, however, the weight of evidence, both molecular and clinical, suggests that this term is outdated. It is likely that IDH-mut WHO grade 4 astrocytoma arises from a different precursor pool than IDH-wt GBM. IDH-mut gliomas follow a distinct molecular trajectory from IDH-wt tumors. The ambiguity of the term “primary GBM” has been emphasized by the occasional de novo presentation of an IDH-mut WHO grade 4 astrocytoma or conversely by IDH-wt low-grade glioma progressing quickly to GBM. In addition, compared with those with IDH-wt GBM, patients with IDH-mut WHO grade 4 FA are younger and have longer survival times. Therefore, it is clear that IDH1-mut FAs—whatever the grade—and IDH1-wt gliomas are different diseases. Because GBM is a familiar term for pathologists and clinicians, it will likely be retained, but this term may be restricted to morphologically appropriate gliomas.
that lack IDH-mut. It will be interesting to determine whether the classic histopathological WHO grading system developed for diffuse astrocytoma when IDH status was not being tested would require modification for IDH-mut astrocytomas.

**Characterizing Oligodendroglioma**

The classic OD appearance of small round cells arranged in honeycomb-like nests with “chicken wire”-like coarse, branching vasculature, microcalcifications, and perinuclear haloes has withstood the scrutiny of advancing technologies. 1p/19q codeletion is prognostic, predictive, and diagnostic. In addition, more than 90% of 1p-/19q- ODs are also IDH-mut. Among IDH-mut gliomas, mutations in the promoter of TERT are confined to OD; conversely, OD lacks ATRX mutations. For practical purposes, grading of OD is restricted to grades 2 and 3 because patient outcomes are considerably better for OD than for IDH-wt gliomas. Surgical cases with ambiguous features can be initially designated as MOA or simply as diffuse glioma. Support is growing for the notion that most MOAs can be placed into either the FA or OD category using IDH status, ATRX expression, and 1p/19q codeletion testing, and that a genuine mixture of the 2 is a rare occurrence.

**Diffuse IDH-wt Gliomas in Adults**

About 30% of WHO low-grade adult diffuse astrocytomas and fewer than 10% of morphologically diagnosed OD are IDH-wt; by contrast, the majority of newly encountered GBM is IDH-wt. Among the IDH-wt low-grade astrocytoma group, some of these cases harbor chromosomal and molecular alterations, thus aligning them with de novo GBM. The remaining cases possess a variety of genetic and biochemical alterations, some of which are more akin to pediatric gliomas, particularly BRAF alterations and histone modifications, facilitating further subclassification.

**IDH-wt Glioblastoma**

The most common primary brain tumor, GBM, is also the most lethal. Histologically, GBM is populated by anaplastic astrocytes with mitotic activity, glomeruloid vascular proliferation, and geographic necrosis with (or without) peripheral pseudopalisading. The molecular landscape of GBM is complex and likely accommodates several distinct entities, as well as considerable intratumoral heterogeneity and overlap. Most GBMs arise rapidly and are clinically designated as being primary or de novo, whereas individuals with a lower-grade IDH-mut astrocytoma may progress to grade 4 (secondary GBM). Future classification may eliminate the “secondary” GBM designation because progression-free and overall survival rates as well as responses to adjuvant chemotherapy or radiotherapy are improved. If IDH status is unknown, then array-based genetic testing for IDH1 and IDH2 can be considered in patients for whom an increased likelihood of a positive result exists, particularly for those tumors that exhibit features such as oligodendrogial morphology or loss of ATRX expression.

**Complex Molecular Signature of IDH-wt GBM**

Among the myriad molecular alterations in GBM, EGFR mutations, deletions, and protein overexpression are common. Clinical trials targeting the epidermal growth factor receptor VIII mutant form, in which exons 2 to 7 are lost, have shown promise, but detecting the mutation, either by immunohistochemistry, reverse transcription–polymerase chain reaction, or multiplex ligation-dependent probe amplification, is of dubious significance in multivariate models. WTI is variably expressed in gliomas, and although it correlates with worse outcome, its effect is overshadowed by age and IDH-mut status. The significance of the loss of PTEN is unsettled in GBM, but it may be a poor prognostic indicator in diffuse gliomas, particularly in tumors lacking 1p/19q codeletion. TERT promoter mutations are detectable in about one-half of IDH-wt GBMs and only those lacking ATRX mutation; TERT promoter mutations in IDH-mut tumors are restricted to OD. Other changes, such as p16, NF, RB, or CDKN2A, have yet to achieve diagnostic or therapeutic significance. Therefore, the molecular pathogenesis of sporadic GBM in adults remains uncertain. Sophisticated expression profiling, which allows grouping into proneural, neural, mesenchymal, and classical phenotypes, has yet to reach the point of diagnostic utility, possibly due to considerable intratumoral heterogeneity.

**Intraoperative Diagnosis: High-Grade Glioma**

A pathologist handling an aggressive neoplasm with ring enhancement and central necrosis may provide an intraoperative diagnosis of GBM if morphologically appropriate. However, IDH-mut FA may undergo anaplastic transformation following disease progression, or it may have histological features similar to IDH-wt GBM at presentation; alternatively, OD may acquire mitotic activity, vascular proliferation, and necrosis and yet still follow a clinical course more consistent with WHO grade 3. IDH-mut tumors maintain their molecular signatures over time and subsequent resections, and they have improved outcomes relative to IDH-wt GBM with similar grading features. Because these cases are morphologically indistinguishable on intraoperative smears and frozen sections, an intraoperative diagnosis of high-grade glioma for practical purposes would ensure the appropriate handling of diagnostic material. The final diagnosis could range from FA grades 3 or...
4, OD grade 3, EP grade 3, GBM, or primitive neuroectodermal tumor/medulloblastoma (depending on location). It remains to be determined whether the preoperative demonstration of a 2-hydroxyglutarate peak on magnetic resonance spectroscopy is sufficient evidence of IDH-mut or conversely whether its absence is sufficient to imply IDH-wt.52

**Glioblastoma WHO Grade 3**

Considerable interest exists in the biology and clinical behavior of low- or intermediate-grade diffuse astrocytic IDH-wt neoplasms in adults. Some examples that share molecular features with GBM can be considered as being “missed” GBM due to undersampling. Other patients may present during the early phase of tumor progression and before florid mitotic activity, vascular proliferation, and necrosis have developed; biopsy findings may indicate FA WHO grade 2 or 3.40 The tumors with molecular findings of GBM will more rapidly advance than their IDH-mut counterparts, although their overall outcome is still better than GBM.51 In the future, such examples may be categorized as GBM WHO grade 3, and the designation of FA WHO grade 3 might be reserved for IDH-mut cases.

**IDH-mut Is Uncommon in Pediatric Diffuse Glioma**

IDH-mut glial neoplasms do not manifest before adolescence41; conceptually, this can be attributed to a slow accumulation of 2HG and a delayed development of the glioma-CpG island methylation phenotype. Molecular alterations predominantly observed in children and young adults correlate with patient age and tumor location.27,62 Recent studies have emphasized the prognostic significance of detecting the mutated form of histone 3 and will likely lead to its routine testing in midline pediatric high-grade astrocytoma.55,64 By contrast to the tumors seen in adults, pediatric OD is rarely accompanied by IDH-mut or 1p/19q codeletion.23,65 This indicates at least 2 forms of OD: the adult IDH-mut and pediatric IDH-wt types. Whether rare IDH-mut pediatric diffuse gliomas represent an early occurrence of their adult counterparts has not yet been established.61,66

**Conclusions**

Isocitrate dehydrogenase (IDH) mutation (mut) is an early and likely initiating event in the development of many gliomas. This provides an opportunity to more precisely classify the 2 IDH-mut tumor types, fibrillary astrocytoma and oligodendroglioma. Distinguishing IDH-mut from IDH wild-type (wt) tumors may improve our understanding of tumor biology, prognostication, patient stratification in clinical trials, and the selection of potential therapeutic targets.67 Failure to appreciate this fundamental dichotomy from the outset of study design risks a misinterpretation of results; for example, a companion of micro-ribonucleic acid (RNA) in low- compared with high-grade gliomas may be better interpreted as comparing IDH-mut vs IDH-wt tumors.3,8 By contrast, a trial of the intratumoral heterogeneity of messenger RNA expression identified IDH-wt status as a fundamental study inclusion criterion.5

Glioblastoma remains an essential diagnostic category and will likely be subdivided on a molecular basis, but, ideally, it would exclude IDH-mut tumors. The diagnostic term secondary glioblastoma might be replaced with astrocytoma World Health Organization (WHO) grade 4 and restricted to IDH-mut tumors. The diagnostic term de novo glioblastoma could then be shortened to glioblastoma WHO grade 4 and restricted to IDH-wt tumors. Histologically lower-grade neoplasms of evidently similar biology may qualify for the designation of glioblastoma WHO grade 3 if they possess the molecular signature of glioblastoma but lack vascular proliferation and necrosis.

**References**


