Molecular features assisting in diagnosis, surgery, and treatment decision making in low-grade gliomas

**Ricky Chen, MD,^1** Vijay M. Ravindra, MD,^2** Adam L. Cohen, MD,^2** Randy L. Jensen, MD, PhD,^2** Karen L. Salzman, MD,^4** Andrew P. Prescott, PhD,^5** and Howard Colman, MD, PhD^2**

^1Department of Neurology, Clinical Neurosciences Center; ^2Department of Neurosurgery, Huntsman Cancer Institute and Clinical Neuroscience Center; ^3Division of Oncology, Huntsman Cancer Institute; ^4Department of Radiology, Division of Neuroradiology; and ^5Department of Radiology and Brain Institute, University of Utah, Salt Lake City, Utah

The preferred management of suspected low-grade gliomas (LGGs) has been disputed, and the implications of molecular changes for medical and surgical management of LGGs are important to consider. Current strategies that make use of molecular markers and imaging techniques and therapeutic considerations offer additional options for management of LGGs. Mutations in the isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) genes suggest a role for this abnormal metabolic pathway in the pathogenesis and progression of these primary brain tumors. Use of magnetic resonance spectroscopy can provide preoperative detection of IDH-mutated gliomas and affect surgical planning. In addition, IDH1 and IDH2 mutation status may have an effect on surgical resectability of gliomas. The IDH-mutated tumors exhibit better prognosis throughout every grade of glioma, and mutation may be an early genetic event, preceding lineage-specific secondary and tertiary alterations that transform LGGs into secondary glioblastomas. The O6-methylguanine-DNA-methyltransferase (MGMT) promoter methylation and 1p19q codeletion status can predict sensitivity to chemotherapy and radiation in low- and intermediate-grade gliomas. Thus, these recent advances, which have led to a better understanding of how molecular, genetic, and epigenetic alterations influence the pathogenicity of the different histological grades of gliomas, can lead to better prognostication and may lead to specific targeted surgical interventions and medical therapies.

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2-hydroxylglutarate (2HG) rather than the normal products of NADPH (nicotinamide adenine dinucleotide phosphate [reduced form]) and α-ketoglutarate.\textsuperscript{12} It is thought that 2HG alters cellular genetic and epigenetic programs, such as histone demethylation, hypoxia sensing, and induction of DNA hypermethylation, leading to tumorigenesis.\textsuperscript{5,10,16,35} Methylation of the DNA repair protein O6-methylguanine-DNA-methyltransferase (MGMT) gene promoter is correlated with IDH mutation, and this may perhaps be attributed to the global DNA hypermethylation effects of the 2HG oncometabolite.\textsuperscript{24}

A better understanding of these and other molecular changes in gliomas and their oncometabolic sequelae has changed the way in which various histological grades of gliomas are diagnosed and treated. Laboratory testing of molecular features including IDH status, 1p19q codeletion, and MGMT promoter methylation has offered new insights on prognosis and management, including predicted response to chemotherapy and radiation treatment. Recently, 2 separate groups have reported results on the successful noninvasive detection of 2HG.\textsuperscript{9,14} Use of MR spectroscopy (MRS) can provide preoperative detection of IDH-mutated gliomas and affect surgical planning. In addition, the impact of IDH1 and IDH2 mutation status on surgical resectability of gliomas has been explored recently.\textsuperscript{4} Previous reviews have addressed the evidence behind 1p19q codeletion, and MGMT promoter methylation has offered new insights on prognosis and management, including predicted response to chemotherapy and radiation treatment. Recently, 2 separate groups have reported results on the successful noninvasive detection of 2HG.\textsuperscript{9,14} Use of MR spectroscopy (MRS) can provide preoperative detection of IDH-mutated gliomas and affect surgical planning. In addition, the impact of IDH1 and IDH2 mutation status on surgical resectability of gliomas has been explored recently.\textsuperscript{4} Previous reviews have addressed the evidence behind IDH1 and 1p19q codeletion as major prognostic features for gliomas. In this article, we review these key concepts and provide a general summary of the molecular implications for medical and surgical management of LGGs.

**Diagnostic and Prognostic Implications of IDH Mutation**

In contrast to glioblastoma multiforme (GBM), the majority (65%–90%) of LGGs, which include astrocytoma, oligodendroglioma, and oligoastrocytoma, harbor mutations in IDH, with most of these being IDH1 mutations.\textsuperscript{33} This strong association between LGGs and IDH mutation has several diagnostic implications. The first is the plausible hypothesis that IDH1-mutant GBMs represent malignant transformation of undiagnosed LGGs, and IDH1 wild-type GBM is, in many ways, a different disease from GBM. This theory is supported by studies of serial biopsy samples obtained in patients with glioma, in which the IDH1 mutation appears to be a relatively early genetic event,\textsuperscript{11,33,37} preceding other lineage-specific mutations, such as TP53 and ATRX mutations in astrocytomas and 1p19q codeletion, CIC, and telomerase reverse transcriptase (TERT) mutations in oligodendrogliomas.\textsuperscript{13,34,37}

In The Cancer Genome Atlas studies, IDH1-mutant GBMs cluster by gene expression in the proneural subtype.\textsuperscript{5,11} The IDH1 mutation initiates a cellular program through the effects of the oncometabolite 2HG, which appears to modulate cellular programs including hypoxia sensing, histone demethylation, and induction of a globally hypermethylated state of DNA and angiogenesis.\textsuperscript{3} This hypermethylated state of DNA, known as glioma CpG island methylator phenotype (G-CIMP), is prognostically favorable.\textsuperscript{5,11} By gene expression, all GBMs with the G-CIMP phenotype are proneural, but not all proneural GBMs are G-CIMP.\textsuperscript{32} The IDH mutation is thought to play a causal role in creation of the G-CIMP phenotype and may be responsible for much of its survival advantage.\textsuperscript{31} Thus, IDH1 genetic mutation is thought to be an early and perhaps the initiating event leading to tumor development. Based on these insights, IDH1 wild-type GBMs are now seen as primary GBMs that probably do not share the same pathophysiological features as secondary GBMs that arise from LGGs with IDH mutation\textsuperscript{11} and associated subsequent secondary and tertiary mutations that cause their malignant transformation.\textsuperscript{37} Therefore, IDH1 wild-type GBMs behave much differently than their IDH1-mutant counterparts, with a later age of onset, worse prognosis, and distinct imaging characteristics.\textsuperscript{13,34,35}

A second diagnostic implication of the relationship between tumor grade and IDH status is that LGGs that are IDH wild type clinically tend to behave as aggressively as GBM.\textsuperscript{13} Among gliomas of the same histopathological grade, the presence of IDH1 or IDH2 mutation confers a significantly better prognosis. A meta-analysis of 937 patients showed that among patients with LGG, those whose lesions had IDH mutation had a more favorable prognosis (pooled hazard ratio [HR] of 0.585, p = 0.025) compared with IDH wild-type LGGs.\textsuperscript{33} In the NOA-04 trial of 318 patients with anaplastic glioma, multivariate analyses also showed that IDH1 mutation was an independent prognostic factor that conferred a longer progression-free survival (PFS) irrespective of the treatment received, and this effect was statistically greater than the risk reduction seen with 1p19q codeletion, MGMT promoter methylation, or histological features.\textsuperscript{39} Conversely, the absence of IDH mutation in an intermediate- or even low-grade glioma portends a potentially worse prognosis than that of a GBM that has IDH1 mutation.\textsuperscript{33} Codeletion of 1p19q and MGMT promoter methylation are also independent markers of a favorable prognosis that appear to increase the sensitivity of tumors to radiation and chemotherapy.\textsuperscript{39} Silencing of the MGMT promoter by methylation has been shown in prior trials to benefit patients with GBM who receive treatment with temozolomide.\textsuperscript{17} Thus, the use of molecular markers provides important prognostic information that can guide decision making in the treatment of LGGs and intermediate-grade gliomas.

**Relationship of IDH, 1p19q, MGMT, and Other Markers**

In 2013, Leu et al.\textsuperscript{24} analyzed the interrelationship of molecular markers in LGG by assessing their correlation with each other and their independent and combined prognostic predictive value. They found that MGMT promoter methylation and IDH mutation are strongly correlated, with nearly all MGMT methylated tumors having IDH mutation, but only about half of MGMT unmethylated tumors having IDH mutation. 1p19q codeletion and IDH mutation also have a strong correlation, and essentially all tumors with 1p19q deletion also have IDH mutation; however, the relationship between IDH and p53 is less correlated. In terms of survival associations in their study, IDH mutation in an intermediate- or even low-grade glioma portends a potentially worse prognosis than that of a GBM that has IDH1 mutation.\textsuperscript{33}
mutation combined with MGMT methylation had a favorable impact on overall survival (OS) compared with IDH wild type (HR 0.33, p < 0.01). Further combination of IDH mutation and MGMT methylation with 1p19q codeletion (triple combination) resulted in an even greater survival benefit (HR 0.18, p < 0.001). The results of this study suggest not only that these molecular markers exhibit varying degrees of correlation and prognostic influence, but also that their effects can be independent and additive.

These specific combinations of alterations are also changing the current view of pathological diagnosis, and future WHO classifications are expected to include some of these markers. In addition, a number of novel alterations have been associated with specific histopathological–molecular subtypes. For example, most infiltrating astrocytomas and secondary GBMs have mutations in TP53 and ATRX in addition to IDH. Oligodendrogiomas have IDH mutation and 1p19q codeletion, but also have high frequencies of mutations in CIC, FUBP1, and TERT promoter. In contrast, the vast majority of primary GBMs lack IDH mutations, but instead are characterized by mutations or copy number alterations in EGFR, PTEN, TP53, PDGFRA, NF1, and CDKN2A/B and the TERT promoter. Some of these markers also carry prognostic significance within subgroups. For example, 1 study identified PDGFRA amplification as an independent prognostic factor within IDH1-mutated GBMs that is associated with a worse prognosis.

Work with orthotopic xenografts of IDH1-mutated gliomas has identified tertiary alterations, including the PIK3CA and KRAS mutations as well as PDGFRA, MET, and N-Myc amplification, which appear to be involved in the eventual malignant transformation of LGGs. Retrospective analysis of 149 patients with gliomas showed that tertiary alterations were present in 13.4% of the sample and were found exclusively in high-grade gliomas or progressive LGGs. The development of tertiary alterations after progression in LGGs was associated with significantly shorter subsequent PFS (median 9 vs 36.1 months, p = 0.0011), whereas there was no significant difference in PFS from the time of initial diagnosis. Again, these data support the fundamental tenet of cancer biology that “stepwise acquisition of distinct classes of mutations results in more aggressive disease.” In the “molecular evolutionary tree of gliomas,” IDH1/2 mutation represents an early “trunk mutation” that precedes the “lineage-defining” secondary alterations of TP53 mutation and ATRX mutation in astrocytomas and 1p19q codeletion with CIC and FUBP1 mutation in oligodendrogiomas, and “tertiary alteration detected at progression [is] the driver of malignant degeneration.” These authors observed that the ability to form xenografts was enhanced by the presence of these tertiary alterations and that selective inhibitors of their downstream pathways were able to suppress proliferation in vitro, suggesting that these mutations are oncogenic drivers of transformation.

**Novel Preoperative Molecular Testing and Imaging for Surgical Planning**

The strong link between LGGs and IDH mutation can be exploited to improve the accuracy of tumor diagnosis, which traditionally has relied solely on histopathological analysis of resection. For example, in a prospective analysis comparing 246 patients with GBM and 157 with anaplastic astrocytomas, Kim et al. found that histopathological diagnosis of GBM was highly dependent on the volume of resection. Smaller resections that were < 20 ml resulted in a much lower rate of diagnosis of GBM than was seen with larger resections > 20 ml. In contrast, IDH1 mutation status did not correlate with the size of resection. This indicates a greater likelihood of underdiagnosis of GBM with smaller resections, and molecular genotyping for IDH1 mutation could be used to increase the diagnostic accuracy.

In 2012, Elkhalel et al. described a technique of ex vivo detection of 2HG in which the nuclear MR technique of proton high-resolution magic angle spinning (1H HR-MAS) spectroscopy was used for patients with recurrent LGGs. The investigators collected a total of 104 tissue samples from 52 patients; assessment of the IDH1 status yielded 40 patients classified as positive for IDH1 mutations and 12 as IDH1 negative. In addition, 26 of 31 patients whose lesions had converted to a higher grade at the time of recurrence were classified as IDH1 mutated compared with 14 of the 21 whose lesions had remained Grade II. There was an 86.4% concordance rate between the presence of 2HG, as detected by 1H HR-MAS spectroscopy, and IDH1 mutation status, as determined by antibody staining and genetic sequencing.

In 2012, Choi et al. similarly reported the noninvasive detection of 2HG by proton MRS. They used a method of spectral fitting to estimate the concentrations of 2HG in the tumors of 30 patients. Indeed, they correlated the detection of 2HG with mutations in IDH1 and IDH2 and with increased levels of 2HG by mass spectrometry of resected tumor.

These 2 studies identified a direct metabolic consequence of a genetic mutation in a cancer cell through noninvasive imaging. Although there are technical limitations to be considered prior to bringing these technologies to everyday practice, including signal overlaps with γ-amino butyric acid, glutamate, and glutamine, and increased water concentration from edema, which could underestimate the concentrations of metabolites, these parameters may be adjusted for using MRS sequence optimization and through the use of a phantom, respectively. The use of 2HG detection as an adjuvant in assessing the presence of IDH1 and IDH2 mutation when evaluating a brain lesion may have future implications for surgical planning. Using this novel imaging technique not only enables clinicians to distinguish tumor from normal brain or nonneoplastic process (such as demyelinating disease), but the presence of 2HG spectra in vivo may make possible presurgical detection of IDH1 mutation in a suspected glioma (Fig. 1).

In 2012, Pope et al. demonstrated in 27 patients that MRS detected elevated 2HG levels in gliomas with IDH1 mutations compared with wild type (p = 0.003) and, when measured in vivo, these levels were significantly correlated with those measured via MRS (p = 0.0001). These findings can provide essential preoperative information about IDH1 status to neurosurgeons, which may impact the extent of resection that is desired.
Surgical Planning and Resection

Surgical intervention for LGGs is highly debated. A 2012 study from Norway sought to determine whether diagnostic biopsy followed by a “wait and scan” approach or early resection would affect OS in patients with LGGs. The results showed a significantly better OS in the early resection group (p = 0.01). The estimated 5-year survival was 60% for biopsy and watchful waiting versus 74% for early resection.18

In 2014, Béiko et al.4 reported that IDH1 status was independently associated with complete resection of enhancing disease (93% complete resection in the mutant group vs 67% in the wild type, p < 0.001) in a series of 335 patients with malignant gliomas (either Grade III or Grade IV), suggesting that IDH1-mutant gliomas are more amenable to complete resection. IDH1 status was also associated with survival difference between wild-type and mutant tumors. Complete resection of enhancing disease in patients with IDH1 wild-type tumors was associated with a median survival of 19.6 months versus 10.7 months for incomplete resection, thus supporting complete resection for Grade III and IV tumors.4 In this study, no survival benefit was observed with further resection of nonenhancing disease. In contrast, patients with an IDH1 mutation have an additional survival benefit with maximal resection of total tumor volume (median survival 9.75 years for > 5-ml residual vs not reached for < 5 ml, p = 0.025).18 Although no similar study has been performed to examine LGGs, perhaps some inference can be made about the contrast-enhancing portion of tumors and accessibility based on IDH1 status in planning and carrying out resection.

Implications for Chemotherapy and Radiation

There is evidence that the presence of 1p19q codeletion is not only a positive prognostic indicator but also a strong predictor of chemosensitivity. In the Radiation Therapy Oncology Group (RTOG) 9402 trial, a prospective study of 291 patients with anaplastic oligodendroglioma and mixed anaplastic oligoastrocytomas, patients with tumors in which 1p19q was codeleted had a 2-fold longer survival (14.7 vs 7.3 years median OS, p = 0.03; and 8.4 vs 2.9 years median PFS, p = 0.001) with the addition of procarbazine, lomustine, and vincristine (PCV) chemotherapy to radiation compared with radiation therapy alone.6 Even with radiation alone, the presence of 1p19q codeletion was associated with longer OS (7.3 vs 2.7 years, p < 0.001). For noncodeleted tumors, and for all patients combined, there was no survival difference in adding the chemotherapy to radiation. Interestingly, the Kaplan-Meier survival curves of codeleted and noncodeleted tumors did not diverge for 5 years, leaving us to wonder whether there are other factors complicating the role of 1p19q codeletion in the chemosensitivity of these tumors. In the previously mentioned NOA-04 study, MGMT promoter methylation was also found to improve prognosis and render tumors more sensi-

FIG. 1. In vivo 1H MRS brain spectra from a patient presenting with an IDH-mutated glioma. All data were obtained from 2 × 2 × 2–cm³ voxels positioned within the lesion (right) as seen on the FLAIR and T1-weighted MR images (insets) and normal-appearing brain tissue contralateral to tumor location (left) as seen on the T1-weighted MR image (inset), by using a point-resolved spectroscopy sequence at 3 T with TE 97 msec, according to the technique developed by Choi et al. Both panels display the spectral fits (red line spectra) overlaid on the raw spectral data (black line spectra), and fit residuals (raw data minus fit) are presented above both spectral fits. Normal brain spectra show significant singlet resonances attributable to N-acetylaspartate (NAA) (2 ppm), creatine (Cre) (3 ppm), and choline (Cho) (3.2 ppm) methyl protons, and glutamic acid (Glu) and glutamine (Gln) form a spectral complex displaying a dispersive character between 2.2 and 2.45 ppm. In contrast, the ultimate fitting of 1H MRS data recorded from the IDH-mutated glioma clearly identifies the significant presence of 2HG accompanied by elevated Cho concentration, reduced NAA, and lower levels of Glu and Gln.
tive to radiation, which suggests the possibility that methylation inactivates genes responsible for radiosensitivity.29

The EORTC trial,36 which parallels RTOG 9402, also showed a significant advantage in OS (42.3 vs 30.6 months, p = 0.018) and PFS (24.3 months vs 13.2 months, p = 0.0003) to using upfront combination PCV chemotherapy and radiation in anaplastic oligodendrogliomas (HR 0.75, 95% CI 0.60–0.95). Although the difference in OS was borderline significant in the Ipi19q codeleted radiotherapy and PCV treatment group (HR 0.56, 95% CI upper limit of 1.03, p = 0.0594), there was a much higher increase in PFS in codeleted tumors (106 months—from 50 months with radiotherapy alone to 156 months; HR 0.42, 95% CI 0.24–0.74, p = 0.002) with radiotherapy and PCV. In comparison, the PFS of noncodeleted tumors was 9 months in the radiotherapy-only group and 15 months in the radiotherapy and PCV group, an increase of only 6 months (HR 0.73, 95% CI 0.56–0.97, p = 0.026). Although all Ipi19q codeleted tumors are IDH mutated, not all IDH-mutated tumors are Ipi19q codeleted. The EORTC subset analysis, however, did demonstrate an independent predictive effect of IDH mutation separate from Ipi19q codeletion, suggesting that either Ipi19q codeletion or IDH mutation is predictive of benefit from chemotherapy in anaplastic oligodendrogliomas.15 Together, the RTOG 9402 and EORTC studies reveal that the standard of care for oligodendrogliomal tumors that are Ipi19q codeleted should be combination chemotherapy and radiation.

Several open questions remain. First, given the biological similarities between IDH wild-type Grade III gliomas and GBMs, it is tempting to extrapolate that temozolomide should be beneficial in IDH wild-type GBMs, but neither RTOG 9402 nor EORTC 26951 saw a benefit to PCV in this subgroup. Second, although temozolomide is much more tolerable than PCV, it is not known whether it is as effective in Grade II and III gliomas. One retrospective study suggests that it is not, although it is more commonly used.23 Two ongoing studies, the CODEL and CATNON trials, will probably answer the question of whether temozolomide can be used in combination with radiation to achieve similar effects to PCV, with less toxicity.

Conclusions

The preferred management of LGGs has been controversial, but recent advances are elucidating how molecular, genetic, and epigenetic alterations influence the pathogenicity of the different histological grades of gliomas. Better understanding of these mechanistic processes has led to a better understanding of LGGs that aids in diagnosis and planning for surgery, as well as promotion and creation of specific targeted therapies.

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References


Author Contributions

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Correspondence

Ricky Chen, Department of Neurology, Clinical Neurosciences Center, University of Utah, 175 N. Medical Dr. E, Salt Lake City, UT 84132. email: ricky.chen@hsc.utah.edu.