Isocitrate dehydrogenase status and molecular subclasses of glioma and glioblastoma

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Diffuse gliomas and secondary glioblastomas (GBMs) that develop from low-grade gliomas are a common and incurable class of brain tumor. Mutations in the metabolic enzyme glioblastomas (IDH1) represent a distinguishing feature of low-grade gliomas and secondary GBMs. IDH1 mutations are one of the most common and earliest detectable genetic alterations in low-grade diffuse gliomas, and evidence supports this mutation as a driver of gliomagenesis. Here, the authors highlight the biological consequences of IDH1 mutations in gliomas, the clinical and therapeutic/diagnostic implications, and the molecular subtypes of these tumors. They also explore, in brief, the non-IDH1–mutated gliomas, including primary GBMs, and the molecular subtypes and drivers of these tumors. A fundamental understanding of the diversity of GBMs and lower-grade gliomas will ultimately allow for more effective treatments and predictors of survival.

(http://thejns.org/doi/abs/10.3171/2014.9.FOCUS14505)

Key words • glioblastoma • IDH1 • tumor metabolism

Glioblastoma (GBM) is the most common brain tumor type, and despite surgery, radiation, and chemotherapy, it is incurable. 27,64 The majority of GBMs, 88%–90%, arise de novo and are classified as Grade IV gliomas according to WHO criteria. 16 These tumors are often present in elderly patients without any clinical evidence of a less malignant precursor tumor. Conversely, secondary GBMs progress from low-grade gliomas (WHO Grade II–III); these tumors arise in younger patients and are often associated with a better prognosis. Although histologically indistinguishable, primary and secondary GBMs greatly differ at the genetic and epigenetic levels. One such distinguishing feature is the identification of mutations in the metabolic enzyme isocitrate dehydrogenase 1 (IDH1). IDH1 mutations are thought to be one of the earliest detectable genetic alterations in low-grade diffuse gliomas. In this review, we highlight the biological consequences of IDH1 mutations in gliomas, the clinical and therapeutic/diagnostic implications, and the molecular subclasses of non-IDH1–mutated gliomas.

History of IDH1 Mutations

IDH1 mutations were initially reported in 2008; mutation of the arginine at codon 132 is the most frequent type, and these mutations are thought to lead to better overall survival. 51,77 The most common mutation is R132H (arginine to histidine), which is observed in 80%–90% of IDH1 mutations in astrocytic and oligodendrogial gliomas. 2,44 The most common mutation is R132H (arginine to histidine), which is observed in 80%–90% of IDH1 mutations in astrocytic and oligodendrogial gliomas. 2,44 Other mutations in IDH1 are rare, decreasing in frequency from R132C to R132G to R132S. 2,77 In cases in which IDH1 is not mutated, less frequent mutations at IDH2 have been reported at codon 172, with R172K being most frequent. 51,77 Interestingly, the R132C mutation in IDH1 is seen exclusively in patients with Li-Fraumeni syndrome. 75 Studies have shown that IDH1 mutations are predominant in secondary GBM (> 75%–80%) and are rare in primary GBM (5%). 44,51,76,77 It has been found that IDH1 is a marker of secondary GBM and that those primary GBMs diagnosed with IDH1 mutations may have been secondary gliomas that rapidly progressed to GBM with no early low-grade clinical symptoms experienced by patients, as elegantly reviewed by Ohgaki et al. 77

IDH1 mutations are frequent in diffuse astrocytoma
Metabolism in Brain Tumors and the Biological Roles of IDH1 Mutations

Metabolic alterations are highly recurrent in gliomas. This is exemplified by a switch in splice isoforms from the adult pyruvate kinase muscle 1 (PKM1) to the fetal PKM2, which is believed to promote aerobic glycolysis in several cancers by altering the rate of glycolysis and diverting glycolytic intermediates into biosynthetic processes that are essential for tumor growth. Pyruvate dehydrogenase kinase 1 (PDK1) is another perpetrator of aerobic glycolysis that is upregulated in most cancer cells, including GBMs. PDK can inhibit pyruvate entry into the tricarboxylic acid (TCA) cycle by phosphorylating and inactivating the enzyme pyruvate dehydrogenase (PDH). Inhibitors of PDK1 such as dichloroacetate (DCA) have been shown to have anticancer effects by shifting metabolism from glycolysis to glucose oxidation and inducing apoptosis in many cancers, including GBMs. Hexokinase II (HKII) has also been reported to be altered and plays an important role in glioma tumor metabolism. Perhaps the most studied metabolic alterations in gliomas, including GBMs, are mutations in IDH1 and IDH2. Mutations in IDH1 lead to neomorphic activity of IDH1, producing a different metabolite, 2-hydroxylutarate (2HG), that promotes a hypermethylator phenotype in addition to other phenotypes in gliomas and secondary GBMs (Fig. 1). IDH1 is an enzyme that participates in the citric acid (Krebs) cycle, converting isocitrate to α-ketoglutarate (α-KG), and is critical for generating adenosine triphosphate (ATP) for cellular energy. It has been shown that the mutant version of IDH1 interacts with and reduces the activity of the wild-type enzyme. IDH1 mutations are considered gain-of-function mutations, resulting in the production of the oncometabolite 2HG from α-KG.

IDH1 or IDH2 mutant gliomas contain higher levels of 2HG than do IDH wild-type tumors. In gliomas, the frequency of IDH1 mutations in codon 132 increases in the order R132L, R132S, R132G, R132C, to R132H, with R132H constituting more than 90% of all IDH1 mutations. It is surprising that a study demonstrated that the rare IDH1 R132 mutations produced more 2HG than did IDH1 R132H, and these findings may suggest natural selection against the rarer IDH1 R132 mutation in human glioma as a result of toxicity caused by high levels of 2HG.

IDH1 mutations and 2HG production have been shown to inhibit prolyl-hydroxylase (PHD) enzymes, which inhibit hypoxia-inducible factor 1α (HIF-1α), a major proangiogenic and proglycolysis transcription factor. IDH1 mutants in glioma and GBM cells have been shown to have elevated levels of HIF-1α and HIF-1β target genes, including GLUT1, VEGF, and PDK1. However, a study indicated that mutant IDH1 destabilizes HIF-1α in vitro, but the exact mechanism of how 2HG stabilizes HIF-1α is not fully understood. Recently, a transgenic mouse was generated using a conditional knock-in of IDH1 (R132H) in which activation was restricted to Nestin-expressing cells. This study demonstrated that these mice expressed 2HG and elevated HIF-1α levels and its gene transcriptional activity. Furthermore, these mice had defects in collagen and protein maturation, leading to a basement-membrane aberration and perinatal lethality. Whether IDH1 mutation is sufficient by itself to initiate glioma formation is still unknown.

Biological Roles of IDH1 Mutations: IDH1-Driven Hypermethylator Phenotype

A second alteration associated with IDH1 mutations is the presence of a CpG island hypermethylator phenotype (CIMP). From a genome-wide methylation profile analysis of astrocytomas and GBMs, it was reported that IDH1 mutations had unique CpG island methylation at a larger number of loci than did non-IDH1 mutant and primary GBMs. Several studies have shown that the introduction of mutant IDH1 into human astrocytes causes functional alterations of specific histone markers by impairing histone demethylation and the induction of DNA hypermethylation. Therefore, the IDH1 mutation is sufficient to establish a hypermethylation phenotype in gliomas. Furthermore, a single copy of the IDH1 R132H mutant was introduced into a human colon cancer cell line (HCT116) so that it was under the control of its endogenous promoter. This resulted in DNA methylation in more than 27,000 CpG dinucleotides. Collectively, these data provide insight into the epigenetic alterations induced by IDH1 mutations and support a causal role for IDH1 (R132H/wild type) mutants in driving epigenetic instability in human cancer cells. One study demonstrated mechanistically that 2HG is a competitive inhibitor of multiple α-KG-dependent dioxygenases, including histone demethylases and the ten-eleven translocation (TET)
family of 5-methylcytosine (5mC) hydroxylases. 2HG occupied the same space as \( \alpha \)-KG does in the active site of histone demethylases, and the expression of IDH1 and IDH2 mutants inhibited histone demethylation and 5mC hydroxylation (Fig. 1). In addition, in a study of a cohort of patients with acute myeloid leukemia (AML), IDH1/2 mutations were mutually exclusive, with mutations in the \( \alpha \)-KG–dependent enzyme TET2, and TET2 loss-of-function mutations are associated with epigenetic defects similar to those of IDH1/2 mutants. Consistent with these observations, the expression of IDH1/2 mutants impaired TET2 catalytic function in cancer cells and either expression of mutant IDH1/2 or Tet2 depletion–impaired hematopoietic differentiation, suggesting that IDH1 mutations and TET enzyme inactivation share proleukemogenic effects. 2HG has been shown to inhibit prolyl-hydroxylases, TET enzymes, and histone methylases, leading to the activation of HIF-1\( \alpha \) and aberrant epigenetic modifications in tumor cells.

**Fig. 1.** Functions of IDH1 mutations. Wild-type (wt) and mutant IDH1 form a heterodimer. Isocitrate is converted into \( \alpha \)-KG by wild-type IDH1 and generates nicotinamide adenine dinucleotide phosphate (NADPH), whereas mutant IDH1 converts \( \alpha \)-KG into 2HG and consumes the reducing agent NADPH in the reaction. 2HG has been shown to inhibit prolyl-hydroxylases, TET enzymes, and histone methylases, leading to the activation of HIF-1\( \alpha \) and aberrant epigenetic modifications in tumor cells.
Diagnostic and Prognostic Implications of IDH Mutations and Common Coexpressed Alterations

It was initially demonstrated that IDH1 mutation was a positive prognostic marker, because patients with this mutation had significantly better overall survival.\textsuperscript{34} Several studies also showed that patients with GBMs or diffuse gliomas with IDH1 mutation have better overall survival and progression-free survival.\textsuperscript{16,23,25,26,28,57,67} Furthermore, mutant IDH1/2 associated with 1p/19q deletion prognosticates good outcomes for those with Grade III oligodendrogial tumors.\textsuperscript{28,57,67}

Clinically diagnosed secondary GBM results in a median survival time of approximately 8 months, which is significantly longer than that for patients with primary GBM (approximately 5 months).\textsuperscript{45} Given the evidence that IDH1 mutations are restricted mostly to secondary GBMs and lower-grade gliomas, the IDH1 mutation as a prognostic marker of GBM may just reflect the 2 disease types. Although patients with the \textit{IDH1} mutation are generally younger, multivariate analysis has demonstrated that a patient’s \textit{IDH1} mutations are an independent prognostic factor even after adjusting for age, tumor grade, and \textit{MGMT} status.\textsuperscript{58} Two studies have demonstrated that patients with IDH1 mutations versus those with wild-type \textit{IDH1} had significantly better overall survival times when treated with surgery and radiotherapy (27 vs 11 months and 31 vs 16 months, respectively).\textsuperscript{44,77} In another study of patients with differentiated low-grade astrocytomas that progressed after radiotherapy, the responses to temozolomide did not differ between \textit{IDH1} mutant and wild-type tumors.\textsuperscript{16}

IDH mutations are thought to arise early in gliomagenesis and persist during progression to secondary GBM. Therapies for recurrent gliomas often fail, which may be partly because the genomic alterations that drive the growth of recurrences are distinct from those in the initial tumor. One group studied this recurrence by sequencing the exomes of 23 initial low-grade gliomas and matched recurrent tumors from the same patients.\textsuperscript{52} This study found that in 43% of the cases, at least half of the mutations in the initial tumor were undetected at recurrence, including driver mutations in \textit{TP53}, \textit{ATRX}, \textit{SMARCA4}, and \textit{BRAF}.\textsuperscript{32} Of great interest is that the \textit{IDH1} status never changed between primary and recurrent gliomas, which suggests that these tumors are initiated by the clonal expansion of cells with \textit{IDH1} mutant function.

Approximately 65% of diffuse astrocytomas carry mutations in \textit{TP53}, whereas oligodendrogliomas have frequent 1p/19q loss.\textsuperscript{39,49,55} IDH1/2 mutations are likely to occur before \textit{TP53} mutation or 1p/19q loss, because low-grade diffuse gliomas that have only an IDH1 mutation are more frequent than those that carry only a \textit{TP53} mutation or harbor 1p/19q loss.\textsuperscript{35,44,70} Therefore, an \textit{IDH1} mutation may be the initial alteration in a common cell of origin, with loss of 1p/19q driving the tumor toward an oligodendrogial lineage.\textsuperscript{44,47,60,70} Recently, a genome-wide sequencing project demonstrated that mutations in the CIC gene (homolog of the \textit{Drosophila} gene capicua) at 19q13 and in the FUBP1 gene at 1p are frequent in oligodendrogliomas but are rare or absent in diffuse astrocytomas, supporting the notion that the lp/19q with \textit{IDH1} mutation is essential for oligodendroglioma formation.\textsuperscript{3,29} Conversely, \textit{IDH1} mutations, followed by mutations in \textit{TP53} and \textit{ATRX} (thalassemia/mental retardation syndrome X-linked) and its binding partner DAXX (death-associated protein) may lead to diffuse astrocytoma formation. \textit{ATRX} and DAXX are involved in incorporating histones into chromosomal sites of heterochromatin, notably telomeres.\textsuperscript{23,38} Therefore, dysfunction of the ATRX/DAXX complex results in both widespread genomic instability and a phenomenon known as alternative lengthening of telomeres (ALT), a telomerase-independent mechanism of telomere maintenance that, analogous to telomerase activation, likely promotes cellular immortalization.\textsuperscript{38,40}

Treatment and Clinical Testing Implications of IDH1 Mutations in Gliomas

\textit{IDH1} mutation may lend itself as a diagnostic, predictive, or theranostic marker for treatments. To date, many clinical and molecular diagnostic laboratories sequence human tumors for \textit{IDH1} or \textit{IDH2} mutations. Furthermore, the generation of a monoclonal antibody against the most common \textit{IDH1} mutation (R132H) allows for immunohistochemical analysis of low-grade gliomas and GBMs.\textsuperscript{9,10} It is now possible, using MR spectrometry, to detect the presence of IDH1 mutant oncometabolite 2HG,\textsuperscript{11,12,18} which may allow for noninvasive detection of the grade or subtype of gloma and may be used as a method of evaluating responses to potential treatments against \textit{IDH1} mutant tumors.

The pharmaceutical company Agios has developed an \textit{IDH1} inhibitor (AGI-5198) that was shown in 2 separate studies to bind to and inhibit mutant \textit{IDH1} in a dose-dependent manner.\textsuperscript{15,56} Under conditions of 2HG inhibition, the inhibitor induced the demethylation of histone H3K9me3 and the expression of genes associated with gliogenic differentiation with appreciable changes in genome-wide DNA methylation.\textsuperscript{15} Therefore, it seems promising that these inhibitors may enter Phase I clinical trials of patients with glioma harboring \textit{IDH1} mutations. In addition to directly targeting \textit{IDH1}, there is growing evidence that disrupting protein function upstream or downstream of mutant \textit{IDH1} may yield promising novel therapeutic strategies. The targeting of glutaminase and glutamate carboxypeptidase II, an enzyme upstream of the mutated \textit{IDH1} pathway that produces glutamate from glutamine, yielded a strong antitumor response.\textsuperscript{52} \textit{IDH1} inhibition is also a driver of a hypermetlylator phenotype, and therefore, DNA methyltransferase (DNMT) inhibitors and histone deacetylase inhibitors may also have therapeutic efficacy. One study demonstrated that efficient induction of differentiation and growth inhibition was observed in \textit{IDH1} mutant glioma cells by targeting the DNMT with the DNMT inhibitor decitabine.\textsuperscript{15} It should be noted that many of these targeted enzymes are expressed in normal cells, and targeting these proteins in normal tissue may cause nonspecific toxicity. The

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Identification of IDH1 mutant synthetic lethal interactions using a genome-wide RNA interference screen or drug screen may reveal novel interactions with or regulators of mutant but not wild-type IDH1.

Non-IDH1–Mutated Glioma Molecular Classes

As mentioned earlier, IDH1 mutations are highly recurrent in low-grade gliomas and secondary GBMs (> 80%). However, the majority of GBMs are not secondary; what is the molecular profile of these tumors? Our latest insight into the molecular pathogenesis of GBMs comes from recent large-scale multi-institutional studies and collaborative projects investigating the molecular signature of GBMs. Integrated genomic/transcriptome and epigenomic analysis resulted in a gene expression–based molecular classification of GBMs into classical, mesenchymal (MES), proneural (PN), and neural subtypes, characterized by aberrations and gene expression of the epidermal growth factor receptor (EGFR), neurofibromatosis Type 1, and platelet-derived growth factor receptor α (PDGFRα). The studies have demonstrated that responses to aggressive chemotherapy and radiotherapy differ according to subtype.6,68

Subtype Switching in GBM

Unlike the IDH mutant PN CIMP-positive subtype, it has been observed that multiple samples from tumors within the same patient can harbor all of the molecular subtypes of GBM.63 These results reveal the genome-wide architecture of intratumor variability and heterogeneity that has not been accounted for in previous studies of GBM samples and warrant additional investigation. Several functional studies have also demonstrated that the molecular subtypes of GBM can undergo subtype switching in vitro and in vivo. One elegant study showed that patient-derived glioma sphere cultures that resemble either the PN or MES transcriptional subtypes differ significantly in their biological characteristics.4 It is surprising that the authors found that a subset of the PN glioma sphere cultures underwent transition to a MES state in a tumor necrosis factor–α/nuclear factor kB (NF-κB)–dependent manner. They were then able to demonstrate that the MES signature, CD44 expression, and NF-κB activation correlate with poor radiation response and shorter survival time in patients with GBM.4 A second study identified the transcriptional coactivator with a PDZ-binding motif (TAZ) to be highly associated with the MES network.5 TAZ expression was lower in PN GBMs and lower-grade gliomas, which correlated with CpG island hypermethylation of the TAZ promoter compared with that of MES GBMs. Silencing of TAZ in MES glioma stem cells decreased the expression of MES markers, invasion, self-renewal, and tumor formation.5 Conversely, the overexpression of TAZ in PN glioma stem cells and murine neural stem cells induced MES marker expression. Another study recently demonstrated that radiation induced a marked shift away from a PN expression pattern toward a MES pattern in a transgenic mouse model.24 Mechanistically, radiation activated STAT3 and CEBPB, which have been suggested to be master regulators of a MES shift.24 In summary, there is evidence to support that subtype switching can occur through transcriptional reprogramming or even by treatment.

IDH1 Mutations in Primary Versus Secondary GBMs

As mentioned throughout this review, IDH1 mutations are seen almost exclusively in low-grade gliomas and secondary GBMs. Primary GBMs have been reported to have very low mutation rates in IDH1 or IDH2 (< 3%–5%).8,47,68 Conversely, a small percentage of Grade II diffuse gliomas lack alterations in either IDH or TP53 or 1p/19q loss. Therefore, are these early-detected primary GBM lesions?

Low-grade gliomas and secondary GBMs lacking IDH1 mutations have infrequent TP53 mutations, and the patients with them have a shorter clinical history.24 One study demonstrated that secondary GBMs lacking IDH1 mutations had developed through progression from an anaplastic glioma (WHO Grade III), whereas the majority of secondary GBMs with IDH1 mutations had progressed from a WHO Grade II glioma.24 These results suggest the possibility that some tumors diagnosed as anaplastic as-
trocytoma may in fact be primary GBMs that were misdiagnosed because of a lack of GBM diagnostic hallmarks, including necrosis or microvascular proliferation. To test this hypothesis, we explored the Cancer Genome Atlas (TCGA) data set of 262 low-grade gliomas and found that 75% of them harbored IDH1 mutations (Fig. 3A).8 Of great interest was that patients with IDH1 wild-type low-grade gliomas showed enrichment of alterations in EGFR and loss of the CDKN2A locus, which are typically associated with primary GBMs. In support of this, EGFR and...
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CDKN2A were mutually exclusive from IDH1 mutations (Fig. 3A and B). Again, in the low-grade glioma data set, patients with IDH1 mutations had better overall survival than patients with wild-type IDH1 harboring EGFR amplifications and/or CDKN2A homozygous deletions (Fig. 3C). The median survival time of patients with low-grade gliomas with EGFR and CKN2A alterations was 8 months versus 16 months for those with low-grade gliomas with mutant IDH1. This observation suggests that IDH1 wild-type non-CIMP–positive gliomas with EGFR amplification/mutation and loss of CDKN2A may in fact represent early-detected primary lesions.

**Conclusions**

In the last decade, we have benefited from an explo-
sive wealth of knowledge identifying the molecular underpinnings of gliomas. We now know that the genetic landscape is diverse and carries unique alterations and different clinical histories. Emerging challenges will be the use of this wealth of data by biologists and clinicians to better understand gliomas and translate this knowledge into better prognostic, diagnostic, and treatment options for our patients.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: all authors. Acquisition of data: all authors. Analysis and interpretation of data: all authors. Drafting the article: Zadeh.

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