Isocitrate dehydrogenase mutations in gliomas

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Over the last decade, extraordinary progress has been made in elucidating the underlying genetic causes of gliomas. In 2008, our understanding of glioma genetics was revolutionized when mutations in isocitrate dehydrogenase 1 and 2 (IDH1/2) were identified in the vast majority of progressive gliomas and secondary glioblastomas (GBMs). IDH enzymes normally catalyze the decarboxylation of isocitrate to generate α-ketoglutarate (αKG), but recurrent mutations at Arg132 of IDH1 and Arg172 of IDH2 confer a neomorphic enzyme activity that catalyzes reduction of αKG into the putative oncometabolite D-2-hydroxyglutarate (D2HG). D2HG inhibits αKG-dependent dioxygenases and is thought to create a cellular state permissive to malignant transformation by altering cellular epigenetics and blocking normal differentiation processes. Herein, we discuss the relevant literature on mechanistic studies of IDH1/2 mutations in gliomas, and we review the potential impact of IDH1/2 mutations on molecular classification and glioma therapy.

Keywords: brain tumor metabolism, D-2-hydroxyglutarate, epigenetics, glioma genetics, isocitrate dehydrogenase mutations.

Normal Biochemistry of Isocitrate Dehydrogenases

Isocitrate dehydrogenase (IDH) enzymes catalyze the oxidative decarboxylation of isocitrate to form α-ketoglutarate (αKG), using NADP+ as a cofactor to generate NADPH during catalysis. IDH1 and IDH2 proteins share a high degree of sequence similarity (70% in humans) and are encoded by distinct genes (IDH1, 2q33; IDH2, 15q26). Although IDH1 and IDH2 are highly similar and catalyze identical, reversible reactions, IDH1 localizes to the cytosol and peroxisomes, while IDH2 localizes to the mitochondria. A third IDH enzyme, IDH3, catalyzes the forward decarboxylation of isocitrate to generate αKG for the tricarboxylic acid (TCA) cycle. IDH3 is an evolutionarily distinct multisubunit complex composed of 3 proteins encoded by distinct genes. In this review, we focus exclusively on the roles of IDH1 and IDH2 in cellular metabolism and glioma biology.

IDH1 and IDH2 are homodimeric enzymes that contain 2 active sites per dimer. Individual IDH subunits are composed of a large domain, small domain, and a clasp domain, and IDH2 contains an additional mitochondrial targeting sequence (Fig. 1A). Each active site contains binding sites for NADP(H), isocitrate, and a divalent cation1 (Fig. 1B). Catalysis proceeds by binding the NAPD+ cofactor in an inactive open conformation. This inactive conformation is characterized by a regulatory loop segment that prevents isocitrate binding to the active site by interacting with Ser94 of the IDH1 large domain. Isocitrate binding displaces the regulatory loop and is mediated by residues of both dimer subunits, including Ser94 and multiple conserved arginine residues in the active site (Fig. 1C). It is proposed that competitive binding of isocitrate to the catalytic cleft displaces the regulatory loop and induces a conformational change to a closed, catalytically active state that promotes decarboxylation of isocitrate to αKG. Interestingly, while IDH1/2 have no known allosteric regulators, bacterial IDH is inhibited by phosphorylation,2 suggesting that posttranslational modifications may modulate IDH1/2 activity.

IDH1 and IDH2 play important roles in a number of cellular functions, including glucose sensing, glutamine metabolism, lipogenesis, and regulation of cellular redox status. Although IDH1 and IDH2 catalyze identical reactions, these enzymes are thought to have distinct functions based primarily on their differential localizations and an altered catalytic flux between forward and reverse reactions. For example, IDHs are

Received 25 April 2014; accepted 16 June 2015
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reported to modulate the cellular response to glucose by positively regulating insulin secretion. More recently, IDH1 has been shown to play a critical role in lipid metabolism in a variety of cellular contexts. IDH1 promotes lipogenesis during hypoxia by catalyzing the reductive carboxylation of aKG to generate acetyl-CoA for lipid synthesis. Overexpression of IDH1 in liver and adipose tissue causes obesity, fatty liver, and hyperlipidemia in transgenic mice. Furthermore, IDH1 regulates phospholipid metabolism in astrocytes of developing mice. Similar to other roles of IDH1 in lipid metabolism, reverse flux of aKG through IDH2 has also been shown to promote synthesis of lipids by reductive glutamine metabolism. However, other studies have suggested that IDH2 may play a minor or more tissue-restricted role in reductive glutaminolysis.

IDH1 is the principal source of NADPH in the human brain and is thought to be a major source of NADPH in other tissues. NADPH protects against oxidative damage by reducing oxidized glutathione and thioredoxin. The generation of NAPDH by IDH1, via oxidative decarboxylation of isocitrate, has been shown to protect against lipid peroxidation and oxidative DNA damage. IDH1/2 activity has also been shown to protect against replicative senescence by reducing oxidative DNA damage and lipid peroxidation in cell culture. Consistent with an antioxidant role of IDH1, other studies have shown that the NAPDH-producing activity of IDH1 protects against UVB-induced phototoxicity, reduces reactive oxygen species, and limits ischemia-reperfusion injury in the kidney. Collectively, these studies suggest that wild-type IDH1 and IDH2 play significant roles in managing the extent of oxidative stress in response to various cellular insults.

**Discovery of IDH Mutations**

In 2008, exome-sequencing studies of glioblastoma (GBM) tumors identified recurrent missense mutations in the gene *IDH1*. Interestingly, *IDH1* mutations occurred in nearly all cases of secondary GBM in the study, (ie, tumors that had progressed from WHO grade II/III gliomas) but were rare in primary GBM cases. In subsequent studies, Yan et al reported that *IDH1* and *IDH2* mutations occur in a mutually exclusive manner in >80% of WHO grade II/III astrocytomas, oligodendrogliomas, and oligoastrocytomas. Numerous studies from our laboratory and others have now shown that *IDH* mutations occur...
in the vast majority of WHO grade II/III gliomas and secondary GBMs and occur in a number of other tumors, including acute myeloid leukemia (AML), intrahepatic cholangiocarcinoma, melanoma, and cartilaginous tumors. Additionally, somatic mosaic mutations in IDH1 cause Ollier disease and Maffucci syndrome, conditions that are characterized by the development of multiple cartilaginous tumors. The discovery of IDH mutations in glioma and other tumors revealed an unexpected role for IDH1 and IDH2 in the genesis and progression of human malignancies and prompted a series of studies to identify the mechanisms by which mutant IDH enzymes cause cancer.

In malignant glioma, mutant IDH proteins are almost ubiquitously expressed in tumor cells, and IDH mutations precede secondary and tertiary genetic lesions, suggesting that IDH mutations are an early causative event in the genesis of this brain tumor subset. Remarkably, mutations in IDH1 and IDH2 occur at specific arginine residues in the active sites of these enzymes (Table 1). For IDH1, the most common alteration is R132H (c.395G>A), comprising >80% of all IDH mutations. Other IDH1 mutations at Arg132 occur at lower frequencies, including R132S, R132C, R132G, and R132L. IDH2 mutations occur at Arg172, the analogous amino acid to IDH1 Arg132, and are most commonly an IDH2 R172K (c.515G>A) missense substitution. IDH1 Arg132 and IDH2 Arg172 are evolutionarily conserved residues in the active site of these enzymes and participate in isocitrate binding in the catalytic pocket (Fig. 1). Early reports suggested that IDH1/2 mutations caused a loss of normal oxidative catalytic function and dominant negative inhibition of the wild-type allele, which was supported by loss of NAPDH-producing activity and decreased affinity for isocitrate in mutant enzymes. However, in 2009, Dang et al reported the groundbreaking discovery that IDH1 mutations confer a gain-of-function neomorphic activity that reduces αKG to produce D-2-hydroxyglutarate (D2HG) in a manner that consumes NAPDH (Table 2). Subsequent studies confirmed this result and further demonstrated that leukemia-associated IDH2 mutations at Arg140, which is adjacent to IDH2 Arg172 in the active site, conferred an identical neomorphic function to produce D2HG from αKG.

The identification of recurrent IDH mutations in glioma and leukemia ultimately led to the discovery that IDH-mutant proteins harbor a unique gain-of-function activity to produce D2HG. These studies collectively demonstrated that IDH mutations are characteristic of a genetically and pathophysiologically distinct glioma subset. Accordingly, the following discussion will provide a detailed review of studies that have investigated the role of IDH mutations in glioma biology. In certain cases, experimental results from disease models other than glioma are included to highlight potential biological mechanisms underlying the role of IDH mutations in tumorigenesis.

**Table 1.** Frequency of specific IDH mutations in gliomas. Data are represented as the percentage of total IDH1/2 mutations in glioma patients according to Yan et al and Hartmann et al.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Amino Acid Change</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1</td>
<td>c.395G&gt;A</td>
<td>R132H</td>
<td>83.5–88.9</td>
</tr>
<tr>
<td></td>
<td>c.394C&gt;T</td>
<td>R132C</td>
<td>3.9–4.1</td>
</tr>
<tr>
<td></td>
<td>c.394C&gt;A</td>
<td>R132S</td>
<td>1.5–2.4</td>
</tr>
<tr>
<td></td>
<td>c.394C&gt;G</td>
<td>R132G</td>
<td>0.6–1.3</td>
</tr>
<tr>
<td></td>
<td>c.395G&gt;T</td>
<td>R132L</td>
<td>0.3–4.1</td>
</tr>
<tr>
<td>IDH2</td>
<td>c.515G&gt;A</td>
<td>R172K</td>
<td>2.4–2.7</td>
</tr>
<tr>
<td></td>
<td>c.515G&gt;T</td>
<td>R172M</td>
<td>0.8–1.8</td>
</tr>
<tr>
<td></td>
<td>c.514A&gt;T</td>
<td>R172W</td>
<td>0.0–0.7</td>
</tr>
<tr>
<td></td>
<td>c.514A&gt;G</td>
<td>R172G</td>
<td>0.0–1.2</td>
</tr>
</tbody>
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**Fig. 2.** Neomorphic enzyme activity of mutant IDH enzymes. IDH1 and IDH2 catalyze the oxidative decarboxylation of isocitrate to generate αKG, using NADP+ as a cofactor and producing NADPH and CO2. Recurrent mutations in the active site of IDH1 and IDH2 confer a gain-of-function activity that catalyzes the conversion of αKG into D2HG in a manner that consumes NAPDH.
Lys27 in immortalized human astrocytes. Both D2HG and L2HG are potent inhibitors of Jumonji-domain-containing family members of histone demethylases. Specifically, D2HG potently inhibits the histone demethylases JMJD2A, JMJD2C, and FBXL11, with IC50 values at or below \( \approx 100 \) \( \mu \)M for these enzymes. Because of the potential for D2HG to inhibit histone demethylases, recent studies have focused on the effects of IDH mutations and D2HG on cellular epigenetics and genome-wide DNA methylation. In glioma patients, IDH mutations are strongly associated with the glioma CpG island methylation phenotype (G-CIMP) in grade II/III gliomas and glioblastoma, and several lines of evidence now suggest that IDH mutations are sufficient to establish the G-CIMP phenotype in glioma cells. For example, Turcan et al reported that WHO grade II/III gliomas exhibit a distinct G-CIMP phenotype that can be recapitulated by expressing IDH1R132H in human astrocytes. A direct, mechanistic link between IDH mutations and DNA hypermethylation is provided by the observation that D2HG inhibits the activity of TET 5-methylcytosine hydroxylases. TETs are a family of \( \alpha \)KG-dependent enzymes that catalyze the first step in an active DNA demethylation process that converts 5-methylcytosine to its unmethylated form through a 5-hydroxymethylcytosine (5-hmC) intermediate. Several reports also suggest that D2HG can inhibit HIF hydroxylases, preventing HIF1\( \alpha \) degradation and increasing HIF1\( \alpha \)-dependent transcription.

Fig. 3. Cellular effects of elevated D2HG levels in glioma cells. IDH1 normally catalyzes the oxidative decarboxylation of isocitrate to generate \( \alpha \)KG. Mutant IDH enzymes generate D2HG, which can accumulate in glioma cells to levels 100-fold compared with normal tissue. \( \alpha \)KG functions as a cofactor for several cellular dioxygenases, including histone lysine demethylases, TET cytosine hydroxylases, and HIF prolyl hydroxylases. Excessive D2HG accumulation disrupts the normal function of \( \alpha \)KG-dependent enzymes causing increases trimethylation of multiple histone lysine residues and decreased 5-hydroxymethylcytosine abundance as well as a concomitant increased in global 5-methylcytosine levels. Several reports also suggest that D2HG can inhibit HIF hydroxylases, preventing HIF1\( \alpha \) degradation and increasing HIF1\( \alpha \)-dependent transcription.

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Studies on IDH mutations and D2HG production have led to a model in which the major oncogenic role of IDH mutations in glioma is altering DNA and histone methylation and inhibiting normal differentiation processes. Importantly, it has been reported that glioma stem-like cells with IDH mutations can be induced to differentiate by inhibiting the neomorphic activity...
of IDH1R132H or treating cells with the hypomethylating agent decitabine.\textsuperscript{55,57} However, in the first case, mutant IDH inhibition lowered D2HG levels in xenografts but did not reverse global DNA hypermethylation. In the second case, decitabine treatment induced differentiation while lowering DNA hypermethylation without affecting D2HG levels. Further work is therefore required to definitively identify an optimal strategy for targeting mutant IDH-dependent epigenetic effects in gliomas.

HIF1α is a hypoxia-inducible oncopgenic transcription factor that regulates the expression of important modulators of tissue oxygenation and vascularization, including VEGF and EPO.\textsuperscript{58} HIF1α protein stability and transcriptional activity are controlled by proline and asparagine hydroxylation mediated by PHD2 (EGLN1) and FIH, respectively.\textsuperscript{59} HIF1α hydroxylases are αKG-dependent enzymes and may therefore be modulated by cellular D2HG levels in a competitive inhibitory manner (Fig. 3B). However, evidence supporting this hypothesis is somewhat unclear. For example, expression of an IDH1 R132H transgene was initially reported to increase HIF1α protein expression due to IDH1 loss of function and decreased intracellular αKG levels.\textsuperscript{44} Expression of IDH1 R132H or treatment with cell-permeable D2HG has also been shown to increase HIF1α expression.\textsuperscript{47} Similarly, inducible expression of IDH1R132H in the brains of embryonic mice causes increased HIF1α protein expression and increased steady-state levels of IDH1α-inducible genes such as VEGF and GLUT-1.\textsuperscript{53} In contrast, 2HG stereoisomers, particularly D2HG, are relatively weak inhibitors of HIF1α prolyl and asparaginyl hydroxylases in vitro, with IC50 values > 1 mM.\textsuperscript{58} To further complicate the matter, D2HG has been reported to specifically increase the activity of the HIF1α hydroxylase EGLN in human astrocytes and colorectal cancer cells, leading to decreased levels of HIF1α protein.\textsuperscript{60} Further work is required to elucidate the relationship between elevated D2HG levels in glioma and the actions of HIF1α in brain tumors. It is possible that cell type and environmental factors play a dominant role in determining the extent to which D2HG accumulation can inhibit HIF hydroxylases. However, it is important to note that D2HG is known to accumulate in glioma tissue to concentrations that may exceed even the relatively high IC50 values associated with D2HG-mediated inhibition of FIH and EGLN.\textsuperscript{63}

Elevated D2HG levels have also been proposed as a biomarker to guide the noninvasive detection of IDH mutations. An in-depth discussion of these methods is beyond the scope and space limitations of this article, but these techniques have been reviewed in detail elsewhere.\textsuperscript{61}

Effects of IDH Mutations on Cellular Metabolism and Growth

IDH enzymes function in critical metabolic pathways in which they produce αKG and NADPH, metabolites that are required for normal macromolecule biosynthesis and redox balance. Therefore, in addition to D2HG-producing activity, mutations in IDH enzymes impair normal enzymatic function for converting isocitrate to αKG and generating NADPH. As a result, IDH mutations may have profound impacts on cellular metabolism by altering metabolic flux of αKG, depleting NADPH, and impairing normal biosynthetic pathways that utilize IDH activity. In their seminal study, Dang et al reported that D2HG levels reached remarkably high levels in glioma tissues harboring IDH mutations (ranging from 5 – 35 μmol D2HG/gram of tissue), but levels of other TCA cycle metabolites, including αKG, malate, fumarate, succinate, and isocitrate, were not significantly altered.\textsuperscript{45} This result suggested that IDH-mutant gliomas maintain normal levels of critical metabolites even in the presence of altered metabolic flux of αKG to D2HG. To investigate the effects of IDH mutations in cells, Reitman et al performed metabolomic profiling of human oligodendroglioma cells expressing IDH1R132H and IDH2R172K. In this study, mutant IDH expression caused widespread metabolic changes, including reduced levels of glutathione metabolites, alterations in free amino acids and TCA metabolite abundance, and significant reductions in N-acetyl-aspartyl-glutamate (NAAG).\textsuperscript{62} NAAG levels were also shown to be reduced in glioma tissues with IDH mutations compared with IDH\textsuperscript{WT} gliomas.\textsuperscript{62} A more recent study reported that IDH1R132H expression in U87 cells or human astrocytes caused similar decreases in glutamate and glutamine.\textsuperscript{61} A metabolomics study by Ohka et al showed that gliomas with IDH1 mutations had significantly decreased levels of glutamate and glutamine relative to IDH\textsuperscript{WT} gliomas.\textsuperscript{66} The study also demonstrated that TCA metabolites were not reduced in the context of IDH mutations. These findings suggest that increased glutaminolysis maintains normal levels of key TCA cycle metabolites to compensate for altered flux of αKG to D2HG in IDH-mutant gliomas.

Compensatory metabolic flux, including increased glutaminolysis, is a likely mechanism by which IDH-mutant tumors maintain normal metabolite abundance in biosynthetic pathways while producing extraordinary amounts of D2HG (Fig. 4). In agreement with this hypothesis, it has been reported that glioma cells expressing IDH1R132H are sensitive to inhibition of glutaminase, a key enzyme mediating the anaplerotic flux of glutamine to αKG.\textsuperscript{65} In addition to glutaminase, glutamate dehydrogenases (GDHs), enzymes that catalyze the oxidative deamination of L-glutamate to αKG, have also been shown to be critical for maintaining normal metabolic flux in the presence of IDH1R132H expression (Fig. 4). Chen et al recently reported that mRNA expression of GDH1 and GDH2 is significantly elevated in IDH mutant GBMs relative to IDH\textsuperscript{WT} GBMs.\textsuperscript{66} Additionally, they found that IDH1R132H expression in gliomagenic murine neural stem cells impaired the flux of glutamine and glucose to lipids and slowed growth of gliomas in a mouse model, suggesting that IDH1R132H-induced metabolic changes are growth-limiting in the absence of compensatory metabolic alterations. Importantly, transgenic expression of GDH2, but not GDH1, rescued glioma growth and promoted lipid synthesis in the presence of IDH1R132H expression.\textsuperscript{66} IDH1R132H lacks the reductive catalytic activity to generate citrate for lipid synthesis,\textsuperscript{67} yet IDH mutations increase the flux of glutamine to lipids, particularly under hypoxic conditions.\textsuperscript{64,66,68} It therefore seems likely that IDH1R132H-expressing tumors rely heavily on mitochondrial GDH2 and IDH2 for reductive flux of glutamine and glutamate to lipids (Fig. 4), which would explain the increased sensitivity of experimental IDH-mutant gliomas to glutaminase or GDH inhibition.\textsuperscript{65,66}

Collectively, studies on glioma metabolism have shown that IDH mutations induce widespread metabolic changes by altering critical metabolic pathways controlling macromolecule...
biosynthesis. Importantly, tumors harboring IDH mutations maintain normal levels of key metabolites by increasing the rate of glutaminolysis and increasing expression of enzymes involved in anaplerotic pathways, including GDH1 and GDH2 (Fig. 4). Future work should focus on the potential for therapeutically targeting compensatory metabolic pathways in IDH-mutant gliomas, including glutaminase and glutamate dehydrogenases.

**Immunotherapeutic Targeting of IDH Mutations**

Recent studies have begun to investigate the possibility of targeting IDH mutations by vaccination-based immunotherapy. In principle, IDH1/2 mutations are ideal tumor specific neo-antigens due to their uniform occurrence at specific codons in IDH1 and IDH2 and ubiquitous expression throughout all tumor cells. Schumacher et al recently showed that an IDH1R132H peptide vaccine is immunogenic in mice. Further, using MHC-humanized mice, they found that vaccination with a peptide containing IDH1R132H amino acids 123–142 elicited an MHC class II-specific antitumor response against IDH1R132H-expressing murine sarcomas. Additionally, another study recently demonstrated that immunization with an IDH1R132H-specific peptide vaccine slowed the growth of intracranial tumors expressing an IDH1R132H transgene. These promising results strongly suggest that mutant IDH-targeted immunotherapies can elicit potent antitumor immune responses.

**IDH1 and IDH2 Mutations as Glioma Biomarkers**

In addition to their role in gliomagenesis, IDH mutations have clear value in providing more accurate diagnostic information for patients. Currently, the diagnosis of brain tumor subtype and grade is based largely on histological criteria according to guidelines established by the World Health Organization. However, accurate diagnosis and grading can be challenging due to subjectivity in the interpretation of histological features. For example, observer-specific subjectivity is most evident in discriminating between cases of astrocytomas, oligodendrogliomas,
and mixed-histology oligoastrocytomas, all of which can share morphologic features. This lack of objectivity results in diagnostic inconsistencies, with one study showing concordance rates among neuropathologists as low as 52%.71 Accurate diagnosis is critical for clinical decision-making and optimizing therapy for patients as particular subtypes show increased radio and chemosensitivity, and dosages are determined by glioma type and grade.72 Genetic and molecular markers are therefore needed to supplement histological analyses to more clearly define distinct disease entities. This is perhaps best exemplified in the case of primary versus secondary GBM, which are histologically identical but have drastically different clinical courses and genetic profiles.73

In light of these diagnostic challenges and the need for clearer patient stratification, recent large-scale studies have aimed to identify genetic markers for glioma classification. These efforts have revealed clear molecular subtypes of glioma that correlate with histologic subtype and patient prognosis. In addition to IDH mutations, alterations such as 1p/19q loss of heterozygosity (LOH), EGFR amplification, MGMT promoter methylation, and mutations in TP53, ATRX, CIC, FUBP1, and the TERT promoter have contributed to identifying subtypes of glioma with distinct molecular signatures. This holds promise for more accurate diagnosis, improved clinical trial design, and tailored therapy. In this section, we will detail the diagnostic and prognostic value of IDH mutation and their roles in the molecular classification of glioma.

**Role of IDH Mutations in Glioma Classification and Prognosis**

The initial genomic studies that identified IDH mutations in gliomas also revealed that patients with IDH mutations exhibit distinct disease characteristics relative to IDHWT patients. Numerous studies reported that IDH1/2 mutations are associated with a younger age of diagnosis compared with IDHWT tumors in WHO grade II/III gliomas,22,26,74 and glioblastoma.75,76,77 IDH mutations are generally associated with a better prognosis, specifically among patients with glioblastoma (31 vs 15 months) and anaplastic astrocytoma (65 vs 38 months) when compared with IDHWT tumors of the same histologic type.21,22,26,74 –77 Interestingly, at least one study has reported that IDH status is only associated with longer survival in the context of adjuvant temozolomide therapy in WHO grade II gliomas,78 suggesting that IDH mutation may also be indicative of enhanced chemosensitivity. Furthermore, it is now well established that decreased MGMT expression, caused by MGMT promoter methylation, results in increased sensitivity to alkylating agents such as temozolomide.79,80 GBM patients with methylated MGMT promoter have a longer median survival of 21.7 months versus 15.3 months with the addition of temozolomide treatment.80 IDH mutations are strongly associated with the glioma G-CIMP in glioblastoma and WHO grade II/III gliomas,23,81 and the majority of G-CIMP GBMs and anaplastic gliomas also have MGMT promoter methylation,26,82 although methylation of this locus also exists to a lesser extent in IDHWT gliomas. Importantly, integrated analyses of DNA methylation, copy number variation, and mutation profiles have revealed that G-CIMP occurs in nearly all IDH-mutant WHO grade II/III gliomas and can be further defined into 2 distinct G-CIMP classes (CIMP-codeleted/CIMP-A or CIMP-non-codeleted/CIMP-B) based on the co-occurrence of IDH mutations with 1p/19q LOH.83,84 The CIMP-A DNA methylation profile is associated with 1p/19q codeletion and is a defining epigenetic feature of IDH-mutant gliomas harboring TERT promoter mutations and 1p/19q LOH, which are predominantly oligodendroglioma or oligoastrocytoma tumors. On the other hand, CIMP-B is a defining epigenetic feature of gliomas with IDH mutations that lack 1p/19q LOH and do not harbor TERT promoter mutations, which are predominantly astrocytomas.84

IDH mutations are thought to be the primary initiating event in WHO grade II/III gliomas and secondary GBMs. IDH-mutant tumors invariably acquire secondary lineage-defining genetic alterations that are closely related to histological subtypes (Table 2). For example, IDH-mutant astrocytomas frequently harbor mutations in the tumor suppressor TP53,85 –87 while TP53 mutations are largely absent in histologically defined oligodendrogliomas.87,88 On the other hand, IDH-mutant oligodendrogliomas frequently harbor mutations in the genes CIC/

**Table 2. Genetic classification of adult gliomas**

<table>
<thead>
<tr>
<th>Genetic Subtype</th>
<th>IDHWT TERTWT Oligodendroglioma</th>
<th>IDHWT TERTWT Astrocytoma</th>
<th>IDHWT TERTWT Astrocytoma</th>
<th>IDHWT TERTWT Astrocytoma</th>
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</thead>
<tbody>
<tr>
<td>Major genetic alterations</td>
<td>IDH mutation</td>
<td>TERT promoter mutation</td>
<td>TP53 mutation</td>
<td>EGFRT mutation</td>
</tr>
<tr>
<td>Associated histologies/classifications</td>
<td>IDH mutation</td>
<td>TERT promoter wild-type</td>
<td>TP53 mutation</td>
<td>EGFRT mutation</td>
</tr>
<tr>
<td>G-CIMP status</td>
<td>IDHWT TERTWT</td>
<td>Astrocytoma</td>
<td>IDHWT TERTWT</td>
<td>Astrocytoma</td>
</tr>
</tbody>
</table>

Abbreviations: Astro, astrocytoma; GBM, glioblastoma; Oligo, oligodendroglioma; OA, oligoastrocytoma; WHO, World Health Organization. The most frequent alterations within each subset are shown in bold text. CIMP-A86 (also CIMP-codeleted83) is tightly associated with 1p/19q codeletion and TERT promoter mutations, while CIMP-B84 (CIMP-non-codeleted83) is associated with intact 1p/19q and wild-type TERT promoter.
FUBP1 and exhibit LOH for chromosomal arms 1p/19q.\textsuperscript{76,89–92} Importantly, it has been shown that TPS3 mutations are exclusive of CIC/FUBP1 and 1p/19q alterations in mixed-histology astrocytomas.\textsuperscript{74,88} Therefore, the co-occurrence of secondary genetic alterations with IDH mutations may provide objective molecular biomarkers that clearly delineate mixed-histology oligoastrocytomas tumors into genetically distinct oligodendroglioma or astrocytoma subsets (Table 2).

In addition to the genetic alterations mentioned above, IDH-mutant gliomas exhibit distinct mutational patterns in the telomere maintenance genes ATRX and TERT. Inactivating mutations in ATRX are common in progressive astrocytomas (diffuse, anaplastic, and secondary GBM) and lead to alternative lengthening of telomeres.\textsuperscript{76,93} Mutations in the promoter region of TERT (ie, the catalytic subunit of telomerase) are common in primary GBM and oligodendrogliomas and lead to increased TERT expression and subsequent telomerase activation.\textsuperscript{76,76,86,94} Recent studies have demonstrated that TERT promoter mutations in oligodendroglioma and oligoastrocytoma are tightly linked to 1p/19q LOH, which is a defining lesion in these tumors and is associated with a better response to PCV (procarbazine, lomustine/CCNU, vincristine) treatment.\textsuperscript{95,96}

Most significantly, a large-scale study of 332 Japanese patients found that 144 of 147 participants who had IDH mutations and 1p/19q LOH also had TERT promoter mutations.\textsuperscript{86} The combination of TERT promoter mutations and IDH mutations may therefore provide an objective basis for genetically defining glioma subtypes and accurately identifying oligodendroglioma patients likely to benefit from PCV chemotherapy.

IDH-mutant tumors also exhibit distinct mRNA expression and copy-number alteration (CNA) profiles in comparison with IDH\textsuperscript{WTT} tumors.\textsuperscript{73,76} IDH-mutant tumors exhibit a proneural gene expression signature and lack EGFR amplifications that are characteristic of IDH\textsuperscript{WTT} tumors.\textsuperscript{76} Recently, a large-scale study from the German Glioma Network reported that WHO grade II/III gliomas with IDH mutations exhibited distinct CNA profiles.\textsuperscript{97} In this study, unsupervised clustering of IDH-mutant tumors revealed 3 distinct groups defined by (i) 1p/19q codeletion, (ii) 7q copy-number gain with minimal CNAs on other chromosomes, and (iii) an IDH-mutant subgroup with relatively frequent gains and losses on multiple chromosomes.\textsuperscript{97} Furthermore, stratification of patients in the study by CNA profiles yielded more distinct prognostic subgroups than stratification by histological subtypes. This finding is consistent with recent results showing that patient stratification by TERT promoter and IDH mutation status is superior to histological characterization in defining clinically distinct subgroups of malignant gliomas.\textsuperscript{74} A remaining genetic subtype of glioma is tumors that lack both TERT promoter mutations and IDH mutations. These TERT\textsuperscript{WTT}-IDH\textsuperscript{WTT} tumors consist largely of primary GBMs and anaplastic astrocytomas and harbor many of the genetic alterations seen in traditional primary GBMs (aside from TERT promoter mutation), including EGFR amplification and CDKN2A deletion.\textsuperscript{84} Importantly, WHO grade III and IV TERT\textsuperscript{WTT}-IDH\textsuperscript{WTT} gliomas exhibit a significantly worse prognosis than IDH-mutant astrocytomas of the same grade,\textsuperscript{74,98} and the median overall survival of grade IV TERT\textsuperscript{WTT}-IDH\textsuperscript{WTT} tumors is only several months longer than primary GBMs that harbor TERT promoter mutations.\textsuperscript{74,88,99}

Conclusion

The discovery of IDH1 and IDH2 mutations in gliomas is a seminal example of the power of unbiased genomic analyses to elucidate novel aspects of biology. The impact of this discovery has been far-reaching and continues to expand, with recent studies focusing on diverse fields ranging from peptide-mediated immunotherapy\textsuperscript{95,96} to mutation-guided enzyme redesign.\textsuperscript{100} Moreover, while the direct therapeutic targeting of IDH-mutant enzymes in leukemia has shown promise,\textsuperscript{95,101,102} the potential benefit of this strategy in gliomas is still unclear. Future work should comprehensively characterize the effects of IDH-mutant inhibitors on patient-derived intracranial xenografts to more thoroughly examine the therapeutic potential of IDH1/2 neomorphic enzyme inhibitors in the context of brain tumors. What is now clear, however, is that IDH mutations exert profound effects on epigenetics and cellular metabolism, and these glioma-associated alterations may serve as alternative targets for glioma therapy. Furthermore, IDH1 and IDH2 mutations are defining genetic markers at the apex of an emerging molecular classification scheme for distinct glioma subsets. In this way, IDH status provides outstanding utility as an objective biomarker to complement existing histological analyses and inform accurate diagnoses.

Funding

This work was funded by the V Foundation, the Accelerate Brain Cancer Cure Foundation, the Sloma and Cindy Silvian Foundation, the Voices Against Brain Cancer Foundation, the James S. McDonnell Foundation, American Cancer Society Research Scholar Award RSG-10-126-01-CCE, and National Cancer Institute Grants 5R01-CA140316, 1R01NS086943, and P50-CA190991. MSW is supported by the institutional training grant 5T32CA074736-15.

Conflict of interest statement. H.Y. receives royalties from Agios Pharmaceuticals and Personal Genome Diagnostics. H.Y. is a co-founder and owns stocks of Genetron Health (Beijing) Co. Ltd.

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