Over the past 4 years, our understanding of gliomagenesis and the practice of neuro-oncology have been radically changed by the discovery of mutations involving the isocitrate dehydrogenase (IDH) enzymes. IDH mutation has been found to be an inciting event in gliomagenesis and to have a profound effect on the molecular and genetic route of oncogenic progression and on clinical outcome.

To paraphrase Yang et al,\(^4\) oncogenic mutations involving the IDH1 and IDH2 genes share 4 distinct biochemical features. First, IDH1 and IDH2 mutations are predominantly somatic.\(^5\) Second, oncogenic IDH mutations in situ are universally heterozygous.\(^6,7\) Third, nearly all IDH mutations involve a single amino acid substitution affecting a residue in the enzyme active site: the arginine residue at codon 132 in IDH1 (Table 1) and the arginine residue at codon 172 or codon 140 in IDH2.\(^8\) Finally, mutations occur in a mutually exclusive manner in most cases, indicating a common underlying biochemical mechanism and common physiologic consequences.

In addition to these biochemical traits, mutations targeting the IDH1 and IDH2 genes exhibit 3 distinct clinical features. First, they occur in a restricted spectrum of tumors. In gliomas, they occur frequently in grade II and grade III oligodendrogliomas and astrocytomas, and in secondary glioblastomas, but not in primary glioblastoma.

<table>
<thead>
<tr>
<th>Amino Acid Substitution at Codon 132</th>
<th>Frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>R132H</td>
<td>92.4</td>
</tr>
<tr>
<td>R132C</td>
<td>3.2</td>
</tr>
<tr>
<td>R132G</td>
<td>~2.0</td>
</tr>
<tr>
<td>R132S</td>
<td>~2.0</td>
</tr>
<tr>
<td>R132L</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

* All described IDH1 mutations in glioma have been found to be point mutations of the arginine residue in codon 132.\(^8\)
Table 2. Frequency of IDH1 Mutations in Glioma Subtypes as Reported by Different Studies

<table>
<thead>
<tr>
<th>Type of Brain Tumor</th>
<th>Reported IDH Mutation Frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrocytoma</td>
<td></td>
</tr>
<tr>
<td>Diffuse (WHO grade II)</td>
<td>54, 68, 80, 72.7</td>
</tr>
<tr>
<td>Anaplastic (WHO grade III)</td>
<td>66.11</td>
</tr>
<tr>
<td>Oligodendroglioma (WHO grades II and III)</td>
<td>65 (grades II and III), 69 (grades II and III), 64.0 (grade II), 69.5 (grade III)</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>3</td>
</tr>
<tr>
<td>Secondary</td>
<td>50</td>
</tr>
<tr>
<td>Other primary brain tumors (WHO grade I tumors, ependymomas, etc)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Abbreviation: WHO, World Health Organization.

* For oligodendroglioma, the frequency based on grade II or III classification is reported in parentheses. An IDH mutation is exceedingly uncommon in primary glioblastoma and is absent in other primary brain tumors.

Clinical Relevance

Using whole-genome sequencing, Parson and colleagues first identified stereotypic somatic mutations at codon 132 of the IDH1 gene in a small proportion of patients with glioblastoma. Their finding was corroborated by the Cancer Genome Atlas Research Network, which described IDH1 as defining a clinically relevant subgroup of glioblastoma. IDH1 mutation has been found to be a frequent, likely early event in gliomagenesis, occurring in 70% to 80% of low-grade gliomas. Furthermore, mutation in the IDH1 genes predicted an alternative pathway of glioblastoma genesis. IDH-mutant tumors were more likely to harbor mutations in TP53 or to reveal a loss of chromosome 1p or 19q, and were less likely to have alterations in PTEN, EGFR, CDKN2A, or CDKN2B. As importantly, IDH mutation is associated with better outcomes in high-grade glioma. The median overall survival was 31 months for patients with an IDH-mutant glioblastoma and 15 months for patients with wild-type IDH1, whereas the median overall survival was 65 months for patients with an anaplastic astrocytoma harboring an IDH mutation and 20 months for those without mutations. Interestingly, IDH mutations were not found in pilocytic astrocytoma, suggesting that these tumors are biologically unrelated to infiltrative gliomas. A summary of IDH and other mutations in glioma is provided in Figure 2.

IDH mutation has also been found to be prognostic of longer survival for patients with low-grade glioma. These events are most common in cases of oligodendroglioma (94%), and less so in cases of astrocytoma (72%) or mixed tumors (83%). IDH mutation in low-grade glioma has a significant positive effect on overall survival (hazard ratio, 0.64), independent of histologic phenotype, and usually predicts the presence of MGMT promoter methylation (in 84% of IDH-mutant low-grade tumors). IDH mutation also appears to impart the benefit of progression-free survival (with a mean [SD] 3-year Kaplan-Meier estimated progression-free survival of 73.9 [4.5] years for patients with an IDH-mutant tumor vs 61.4 [6.9] years for patients with wild-type IDH). Conversely, the absence of IDH mutation in low-grade glioma has been found to be predictive of a briefer latency to malignant transformation and a shorter overall survival.

Houllier and colleagues have postulated that the survival benefit garnered by IDH1 mutation for patients with low-grade glioma is indicative of the effect of IDH mutation on the response to chemotherapy, rather than of a divergent biological behavior. IDH mutation does indeed appear to predict chemo-sensitivity in both low-grade and secondary high-grade gliomas. Regardless of the mechanism involved, the presence of IDH mutation is clearly associated with improved survival for patients with glioma. In the next
sections, we will further describe the biology of the IDH genes and review recent work explicating its role in disease.

**IDH, the Krebs Cycle, and 2-Hydroxyglutarate**

IDH1, IDH2, and IDH3 catalyze the reversible decarboxylation of isocitrate to α-ketoglutarate (α-KG, also known as 2-oxoglutarate [2OG]). IDH1 and IDH2 use NADP⁺ as a cofactor, producing NADPH in the process, whereas IDH3 uses NAD⁺ as a cofactor and so produces NADH. IDH1 is present in the cytosol and peroxisomes, whereas IDH2 and IDH3 are located in mitochondria. Under physiologic conditions, isocitrate and α-KG levels are balanced in a manner that reflects the cellular energy state.27-29

Recent work suggests that cells in a hypoxic environment rely almost exclusively on glutamine-derived α-KG for lipid synthesis; this shift of metabolism away from the Krebs cycle and glucose consumption to glutamine reduction is also characteristic of tumors, including gliomas.30 In glia, IDH also regulates aspects of glutamine and glutamate metabolism, as well as the synthesis of N-acetylated amino acids.31 The IDH enzymes further appear to play a crucial role in cellular protection and response to oxidative and energetic stress. NADPH plays a vital role in the regeneration of the antioxidant glutathione.32 In addition, α-KG itself functions as an antioxidant.33 In a recent study of HT-22 neurons,34 energetic challenge induced by substituting galactose for glucose resulted in increased expression of the IDH enzymes and cessation of proliferation, whereas induction of oxidative stress via glutathione depletion resulted in no alteration to IDH expression. Taken together, these findings indicate that moderate oxidative stress favors the production of NADPH and α-KG via IDH, but that IDH activity may be unaltered or even curtailed during periods of severe oxidative stress to minimize further production of oxygen-free radicals.

The effect of IDH mutation appears to be driven by both a loss of its normal catalytic function and a gain of function. IDH mutation decreases its binding affinity for isocitrate, while increasing its affinity for NADPH. This change abrogates the "forward" catalytic activity of IDH (ie, the conversion of isocitrate to α-KG), while limiting the "reverse" catalytic activity to a partial reaction in which α-KG is reduced but not carboxylated. As a result, cells harboring an IDH mutation experience a relative depletion of α-KG and NADPH and dysfunction of cell processes requiring these substrates.35 Just as, if not perhaps more, important, IDH1 mutation results in the aberrant production of 2-hydroxyglutarate (2-HG).36 Production of 2-HG by mutant IDH1 appears to depend on the presence of the wild-type enzyme, suggesting a mechanism underlying the uniform heterozygosity of an IDH mutation in order to achieve its metabolic effect successfully.37 Mutant IDH1 catalyzes the formation of the 2-HG enantiomer, D-2-HG, while tumor-derived IDH2 mutations produce an alternative 2-HG enantiomer, R-2-HG.38-40 It is unclear whether this structural difference is of functional consequence. D-2-HG and R-2-HG have both been postulated to act as an oncometabolite in glioma and leukemia cells.41

**IDH Mutation and Oncogenesis**

IDH mutation is thought to be an early if not the initial event in tumorigenesis in IDH-mutant gliomas and leukemias.42,43 IDH mutation appears to result in a cell state permissive of transformation. Mutation results in a profound change in the cell methylome; epigenetic changes are thought to be primary drivers of oncogenic evolution in some cancers and perhaps in gliomas.44 In addition, IDH mutation results in a block in cell differentiation and promotion of cell proliferation, 2 other frequent harbingers of carcinogenesis. We will review these hypotheses further in the next sections.
IDH and the Methylome

A recent study has shown that the mutant 2-HG-producing subunit of the IDH enzyme does not affect the normal reactions carried out by the wild-type α-KG-producing subunit. However, 2-HG exerts a profound effect on the function of α-KG–produced methyl groups, particularly affecting the dioxygenases, a family of enzymes involved in methylation of histones and DNA. The 2-HG binds to the dioxygenase catalytic core in a nearly identical orientation to α-KG and thereby inhibits binding of the normal substrate. 2-HG accumulation particularly affects the histone lysine dimethylation (H3K9me2 and H3K27me3) and TET family of DNA hydroxylases. Changes in the methylome that arise from inhibition of these 2 enzyme families are thought to underlie IDH-directed transformation. In support of this hypothesis, mutations of TET2 have been found in about 22% of cases but are mutually exclusive with IDH mutation. In addition, 2-HG accumulation has been shown to perturb collagen maturation and basement membrane function and thus may serve as a facilitator of further oncogenic change.

Expression of IDH1 R132H or IDH2 R172K mutants in immortalized astrocytes results in a significant increase in the repressive trimethylation of H3K9 (H3K9me3) and H3K27 (H3K27me3). The resulting methylation patterns mirror those seen in the histone lysine trimethylation (H3K9me3) and H3K27 trimethylation state and found in some low-grade gliomas and proneural glioblastomas, called the glioma CpG island methylator phenotype (G-CIMP). This concordance extends to astrocytes expressing mutant IDH1 and low-grade gliomas with G-CIMP. Furthermore, whole-genome sequencing of a low-grade glioma cohort set used to generate the methylome data showed that none of the G-CIMP-negative tumors possessed IDH mutation. These findings show that IDH1 mutation results in dramatic, widespread changes in histone methylation and gene expression, and that IDH mutation is likely the driver of the methylation changes that result in the G-CIMP phenotype in gliomas. Acquisition of a hypermethylated phenotype is also seen in endochondromas from patients with Ollier disease and Maffucci syndrome, nonfamilial diseases characterized by the development of multiple benign cartilaginous tumors. Interestingly, although mutant IDH1 is expressed in both normal and tumor cells in these patients, tumor development appears to be associated with higher expression levels of the mutant, suggesting that the risk of tumor development and acquisition of the hypermethylated phenotype is related to cellular levels of 2-HG.

IDH and Cell Differentiation

Interestingly, the expression of mutant IDH1 in immortalized astrocytes directed these cells toward a stem cell–like phenotype, characterized by decreased expression of the astrocyte marker, GFAP, and enhanced expression of the neural stem cell marker, nestin. Similarly, primary neurospheres infected with an IDH1 R132H mutant retrovirus failed to express markers of differentiation, despite exposure to retinoic acid and other culture conditions that induce astrocytic and neuronal differentiation in control cells. This inhibitory effect on cell differentiation was accompanied by a progressive remodeling of the methylome over successive passages, which could be reproduced by short interfering RNA–mediated inhibition of the H3K9-specific Jumonji-C histone demethylase, KDM4C (also known as JMJD2C). The acquisition of a progenitor phenotype is thought to be a necessary if not initiating step in gliomagenesis.

IDH and Hypoxia-Inducible Factor 1α

Proteosomal degradation of hypoxia-inducible factor 1α (HIF-1α) is mediated by its polyubiquitylation by the HIF prolyl 4-hydroxylases, EGLN1, EGLN2, and EGLN3. Koinunen et al found that 2-HG accumulation enhances EGLN activity and thus leads to decreased levels of HIF. Furthermore, the introduction of mutant IDH1 into human astrocytes results in a decrease in HIF-1α, parallel to the finding of decreased HIF-1α activity in proneural (IDH1-mutant) glioblastomas. The knockdown of HIF-1α or EGLN1 promotes the proliferation and colony formation of human astrocytes and suggests that EGLN might be a good target for glioma therapy.

Under normal physiological conditions, HIF-1α plays a central role in mediating mitochondrial oxygen consumption and limiting the production of reactive oxygen species. Loss of HIF-1α could expose a cell to increased risk of reactive oxygen species–mediated DNA damage and mutation. Conversely, HIF-1α activation in glioblastoma has been shown to enhance tumor cell proliferation and angiogenesis. It is interesting to postulate that the effect of IDH1 mutation on HIF-1α might be oncogenic by conferring an initial proliferative advantage and an increased risk of mutational burden to IDH1-mutant cells, but advantageous to outcome by conferring a less aggressive phenotype to the resulting cancer.

IDH-Directed Therapy

IDH Inhibitors

Zheng and colleagues developed a series of mutant IDH inhibitors that have little effect on the wild-type enzyme, based on their finding that isocitrate binding to the ligand binding site in wild-type IDH results in a conformational change in the enzyme that enables its catalytic activity, while, in the mutant enzyme, isocitrate binds to an alternative binding site that does not facilitate the necessary conformational change. The 2 candidate inhibitors were found to form hydrogen bonds and electrostatic interactions with a stronger affinity to the alternative isocitrate binding site than that of isocitrate, resulting in the stabilization of the mutant enzyme in its inactive, open conformational state.

Rohle and colleagues have developed a small-molecule IDH1 (R132H) mutation–specific inhibitor (AGI-5198) and studied its effects both in vitro and in vivo. Treatment of human glioma cells with AGI-5198 reversed the differentiation block associated with IDH mutation. Interestingly, reduction in tumor volume in mouse xenografts following treatment with AGI-5198 occurred despite only partial inhibition of mutant IDH1, a reduction in (but not elimination of) 2-HG, and a lack of a change in CIMP status. Upregulation of differentiation genes and downstream inhibition of histone demethylation required complete inhibition of mutant IDH1. Histopathological analysis of the tumors showed no changes in the concentration of cleaved caspase-3, indicating that the reduction in tumor volume was due to the inhibition of tumor proliferation rather than an increase in cell death.

To be effective, the molecules used in the treatment of IDH-mutant gliomas will need to be capable of penetrating the blood-
brain barrier and reaching therapeutic levels within the tumor. The work of Rohle and colleagues highlights the functional heterogeneity of the IDH mutation effectors, including the downstream effects of 2-HG, CIMP, and histone modifications; further investigation will be needed to clarify the role of each of these factors in tumorigenesis, the effects on the vulnerability of the tumor to other treatments such as chemotherapy, and their impact on other crucial aspects of cell metabolism. Furthermore, it is unclear whether the effects observed in studies using an oligodendroglioma cell line can be generalized to astrocytoma and glioblastoma cell lines; it has been postulated that the role of IDH may vary among these tumors. 62 An IDH-mutant inhibitor probe (ML309) capable of reducing 2-HG levels in a glioblastoma multiforme cell line has also been developed. 63

Wang and colleagues examined AGI-6780, a urea sulfonamide inhibitor of IDH2 (R140Q), in leukemia. AGI-6780 is a tight-binding allosteric noncompetitive inhibitor of isocitrate and an uncompetitive inhibitor of the NADPH cofactor. Binding of the inhibitor to the mutant enzyme holds the enzyme in an open conformational state, leaving it unable to perform catalysis. In IDH2 (R140Q) AML cells in vitro, AGI-6780 causes a dose-dependent reduction in 2-HG and cell differentiation. Surprisingly, however, and despite its effect on cell “stemness,” treatment with the IDH2 inhibitor in AML cells resulted in an increase in cell proliferation. These findings highlight the contextual specificity of IDH function in oncogenesis and speak to the need to investigate its role in each particular disease.

DNA Methyltransferase Inhibitors

DNA methyltransferases (DNMTs) are a family of enzymes that catalyze the addition of a methyl group to DNA. The use of DNMT inhibitors in IDH-mutated cancers is motivated by the hypothesis that the oncogenic effects of IDH mutation are driven by its effects on the methylome. Two groups have recently studied the DNMT inhibitors decitabine and 5-aza-cytidine in IDH-mutant glioma cell models.

Turcan and colleagues observed that the administration of decitabine to an IDH1 R132H mutant anaplastic astrocytoma cell line (TS603), while not affecting 2-HG, efficiently induced up-regulation of differentiation genes, an effect associated with a change in methylation markers leading to reexpression of Polycomb-controlled genes. Remarkably, the differentiation effect was maintained even after drug therapy was stopped. Of note, treatment with the IDH-mutant enzyme inhibitor AGI-5198 had no further benefit in cells pretreated with decitabine, implying that IDH inhibitors may be redundant when coupled with DNMT inhibitor therapy.

A concurrent study by Borodovsky and colleagues examined the DNMT inhibitor 5-aza-cytidine in an anaplastic astrocytoma xenograft (JHH-273). This inhibitor was able to reverse the G-CIMP hypermethylation state in a dose-dependent manner. As with decitabine, long-term low-dose treatment slowed tumor growth (determined by Ki-67 staining) even after the treatment was stopped, with a similar upregulation of differentiation genes.

Because decitabine and 5-aza-cytidine are already approved by the US Food and Drug Administration and can cross the blood-brain barrier effectively, they represent exciting new prospective drugs for clinical trial. Furthermore, their effects appear to be translatable to other IDH-mutated tumors such as AML and chondrosarcoma. Further study will be needed to determine the generalizability of these preliminary findings.

Other Targets

In addition to the IDH enzymes and DNMT, there may be other entities in the IDH effector scheme for which inhibitors can be developed that may result in a cumulative clinical benefit. Potential targets include the α-KG–dependent dioxygenases, such as TET2, TET3, and components of the glutamine/glutamate pathways. For example, suppression of BCAT1, an amino acid transferase involved in catabolism of branch-chain amino acids and mutated in glioma in a mutually exclusive fashion with IDH1 and IDH2, resulted in reduced invasiveness and proliferation in vitro and in a glioblastoma multiforme xenograft model.

Conclusions

Recent studies have broadened our still preliminary understanding of the consequences of a specific point mutation of a single gene for glioma biology. IDH mutation in glioma is an early event, which results in widespread changes to the methylome, the generation of the oncometabolite, 2-HG, and, ultimately, tumorigenesis.

The promise of IDH-directed therapy must be tempered by an appreciation that our understanding of their role in glioma biology is premature. For example, although IDH mutation is sufficient to induce cellular transformation in vitro, it does appear capable of driving transformation in vivo. The conditions necessary for IDH mutation to be permissive in the physiologic milieu are unclear. Similarly, although dysregulation of the cell redox state has been shown to contribute to transformation in other types of cancer, it is not clear whether NADPH and reactive oxygen species are in fact abnormal in IDH-mutant cancers. Finally, it must be remembered that the studies targeting IDH have been performed using chemical and small-molecule inhibitors rather than short interfering RNA-mediated knockdown and, therefore, cannot exclude the possibility that off-target effects might account for the identified effects. Therefore, the extent of the redundancy of IDH inhibitors when coupled with DNMT inhibitors is also presently unclear and requires further research.

Interestingly, the expression of mutant IDH1 R123H in established IDH wild-type glioma cell lines resulted in a marked decrease in the proliferation of these cells in vitro and increased latency of fatal tumor formation following intracranial xenotransplantation. This finding highlights a paradox of IDH biology: that a mutation that should appear to reduce cellular fitness could play a formative role in tumorigenesis. It also requires that the development and application of IDH-directed therapies be considered in a nuanced and complex manner.

At least 2 possible hypotheses can be entertained to explain the causative role of IDH and 2-HG in transformation. First, IDH mutation and 2-HG accumulation may be necessary both to promote transformation and to drive further mutation even after transformation has occurred. Conversely, the process of oncogenic progression may at some point become independent of the initial IDH mutation. Two corollaries result from these divergent hypotheses. In the first case, IDH inhibition would be an effective means of treating an IDH-mutant lesion because a reversal of the effect of IDH on the methylome or a decrease in 2-HG levels would relieve these cells of a persistent driving force for their progression. On the other hand, if the effects of IDH1 are important for initiation but not mainte-
nance or progression of the transformed state, then inhibition of IDH in a mature IDH-mutant cancer would likely be ineffective.

The results of early basic work investigating IDH and DNMT inhibitors suggest an intermediate conjecture situated somewhere between these 2 hypotheses. Future studies will be required to pinpoint the exact role of IDH mutation in glioma initiation and progression and to optimize the targeting of this pathway in cancer therapy.

REFERENCES


