Mutant Metabolic Enzymes Are at the Origin of Gliomas

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Abstract
Mutations of the isocitrate dehydrogenase (IDH) metabolic enzymes IDH1 and IDH2 have been found to be frequent and early genetic alterations in astrocytomas and oligodendrogliomas. All mutations identified to date affect a single amino acid located within the isocitrate binding site (R132 of IDH1 and the analogous R172 residue of IDH2). IDH1 and IDH2 mutations define a specific subtype of gliomas and may have significant utility for the diagnosis, prognosis, and treatment of patients with these tumors. [Cancer Res 2009;69(24):9157–9]

Identification of IDH1 as a Novel GBM Gene
Malignant gliomas are the most frequent and lethal tumors of the central nervous system, and glioblastoma multiforme [GBM; World Health Organization (WHO) grade IV astrocytoma] is the most biologically aggressive subtype. GBMs may arise de novo (primary GBM) or develop in the setting of a lower-grade glioma (secondary GBM; ref. 1). An unbiased, genome-wide analysis of the somatic mutations occurring in GBMs revealed recurrent mutations in R132, the active site of IDH1, a gene with no known link to gliomas, in 12% of tumors analyzed (2). Intriguingly, mutations of IDH1 predominantly occurred in younger patients, were associated with a better prognosis, and were preferentially found in tumors that possessed TP53 mutations but lacked other common GBM alterations; all characteristics of secondary GBMs. Additional studies have confirmed that IDH1 is mutated in >80% of secondary GBMs (3–8), whereas <10% of primary GBMs harbor these alterations.

Mutant IDH1 and IDH2 as Astrocytoma- and Oligodendroglioma-Specific Gatekeepers
To evaluate the timing of IDH1 alterations in glioma development, IDH1 mutations have been assessed in a large number of gliomas of various types, and the results (confirmed by multiple investigators) are striking: mutation of IDH1 occurs early in glioma progression, with somatic mutations of the R132 residue of IDH1 identified in the majority (>70%) of grades II and III astrocytomas and oligodendrogliomas, as well as in secondary GBMs that develop from these lower grade lesions (2–10). In addition, mutation analysis of the closely related IDH2 has revealed recurrent somatic mutations of IDH2 residue R172, with most mutations occurring in tumors lacking IDH1 mutations (5, 9). The R172 residue in IDH2 is the exact analog of the frequently mutated R132 residue of IDH1: Both are conserved in all known species and form hydrogen bonds with the isocitrate substrate (11).

Astrocytomas and oligodendrogliomas both contain frequent IDH1 or IDH2 mutations but do not share other genetic alterations that occur early in the development of these two glioma lineages. For example, the majority of low-grade diffuse astrocytomas contain both an IDH mutation and a TP53 mutation, whereas most oligodendrogliomas have both IDH mutations and 1p/19q loss (3, 5, 6, 8). One study revealed that, of 23 grade II astrocytomas with both IDH and TP53 mutations examined, 17 samples have both IDH and TP53 mutations, three have IDH mutations but do not contain TP53 mutations, and three have neither IDH nor TP53 mutations (5). In addition, Watanabe and colleagues dissected multiple biopsies from the same patients and found that IDH1 mutations always preceded the acquisition of a TP53 mutation or loss of 1p/19q (8). This genetic evidence suggests that IDH mutations are early genetic events in the development of a glioma from a cell-of-origin that can give rise to both astrocytes and oligodendrocytes. The fact that IDH mutations were not identified in any WHO grade I pilocytic astrocytomas (3, 5, 12), which rarely undergo malignant transformation, indicates that these tumors arise through a different mechanism.

IDH1 and IDH2 mutations are remarkably specific to grades II and III astrocytomas, oligodendrogliomas, and secondary GBMs. They are not found in ependymomas and are found only rarely in pilocytic (grade 1) astrocytomas (3, 5, 12). Most recently, IDH1 R132 mutation have been found in 15 of 187 acute myeloid leukemia samples (AML; ref. 13). The only tumors other than astrocytomas, oligodendrogliomas, and AMLs in which IDH1 mutations have been reported are a single case of colorectal cancer (14), two prostate carcinomas (7), and a minority of analyzed cases of adult supratentorial primitive neuroectodermal tumors (3, 10).

Gliomas with IDH Mutations Are a Different Subtype of Disease
The pattern of other genetic mutations found in gliomas with IDH mutations is entirely different from that in gliomas with wild-type IDH1 and IDH2 (Fig. 1). Nearly all of the anaplastic astrocytomas and GBMs with mutated IDH1 genes were also found to have a mutation of TP53 (80%), but only 5% had alterations in any of the common GBM genes PTEN, EGFR, or CDKN2A/CDKN2B. Conversely, anaplastic astrocytomas and GBMs with wild-type IDH1 and IDH2 had relatively few TP53 mutations (20%) and extremely frequent alterations of PTEN, EGFR, or CDKN2A/CDKN2B (74%). Similarly, loss of 1p/19q was observed in 85% of the oligodendrocytic tumors with mutated IDH but in none of the patients with wild-type IDH1 and IDH2 (3, 5, 6, 8).

Clinically, glioma patients with IDH mutations are also distinct from those with wild-type IDH1 and IDH2. For example, adult

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patients with anaplastic astrocytomas and GBMs with IDH mutations are significantly younger than those with wild-type IDH1 and IDH2 (median age of 34 versus 56 years for anaplastic astrocytomas and 32 versus 59 years for GBMs). However, no IDH mutations have been identified in pediatric glioblastomas, and children with IDH-mutated low-grade gliomas are older as well (median age 17 versus 5 years; refs. 5, 6, 15). GBM patients with IDH mutations have a median overall survival of 31 months, significantly longer than the 15-month survival in patients with wild-type IDH1 and IDH2 (5). Although both younger age and mutated TP53 are positive prognostic factors for GBM patients, this association between IDH1 mutation and improved survival is noted even in the subgroup of young patients with TP53 mutations (2). Mutations of IDH are also associated with improved prognosis in patients with anaplastic astrocytomas, whose median overall survival is 65 months for patients with mutations and 20 months for those without (5). A multivariate analysis has confirmed that IDH1 mutation was an independent favorable prognostic marker after adjustment for grade, age, MGMT status, genomic profile, and treatment (16).

**Functional Characterization of IDH Mutations**

The recurrence of mutations at a specific site on each gene in a heterozygous fashion is reminiscent of activating alterations in oncogenes such as BRAF, KRAS, and PIK3CA. However, biochemical studies have provided evidence that mutant IDH may act in a dominant-negative fashion to inhibit catalytic activity of the enzymes. The IDH1 and IDH2 enzymes catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate, generating NADPH from NADP+. IDH1 is localized in the cytoplasm and peroxisomes, whereas IDH2 is localized in the mitochondria and participates in the citric acid cycle for energy production. Modeling studies based upon the human cytosolic IDH1 crystal structure suggest that substitution of R132 with any one of the six amino acids observed thus far in gliomas would impair interactions of the enzyme with isocitrate (17). Biochemical analyses of recombinant IDH1 proteins revealed that mutant IDH1 had a dramatically reduced affinity for isocitrate (17). In addition, by assaying the reduction of NADP+ to NADPH, all IDH1 and IDH2 mutations identified in patients were found to abrogate their enzymatic activity (5, 17). Furthermore, the wild-type IDH1 and mutant (R132H) IDH1 was reported to form a heterodimer, which exhibited only 4% of the activity of the wild-type enzyme when assayed with limited isocitrate concentration and resulted in decreased α-ketoglutarate levels (17).

**Metabolic Enzymes as Cancer Genes and the Warburg Effect**

The biochemical studies of IDH1 have also shed some light on the possible underlying mechanism by which mutations in metabolic
pathways contribute to the pathogenesis of gliomas. Hypoxia-inducible factor (HIF) is a master regulator of genes that are activated by low oxygen levels and regulates the expression of genes implicated in glucose metabolism, angiogenesis, and other signaling pathways critical to tumor growth. Prolylhydroxylases hydroxylate and promote the degradation of HIF-1 in the presence of oxygen using α-ketoglutarate and iron as cofactors (18). Further, reduction in IDH1 activity produces a reduction in α-ketoglutarate levels that in turn can lead to stabilization of HIF-1α, activation of the HIF pathway, and contribution to gliogenesis (17). Nevertheless, this connection to HIF-1 may only partially account for the pathological function of IDH mutations in gliomas. Although the in vitro biochemical results show an effect of the mutations on IDH function, they do not necessarily mean that the mutations are inactivating. It is possible that the mutations have a different pathogenic effect, such as alteration of affinity for a substrate other than isocitrate.

In addition to reducing α-ketoglutarate levels, IDH1 and IDH2 contribute to NADPH production in cells. NADPH is required for the synthesis of glutathione, which protects cells from redox stress. There is still a paucity of biological data to support the hypothesis that decreased production of NADPH caused by IDH mutations may confer a survival advantage.

In addition to IDH mutations in gliomas, mutations in metabolic pathways occur in other cancers. Fumarate hydratase and succinate dehydrogenase have been shown to act as tumor suppressors of paraganglioma and leiomyoma, respectively (19). Accumulation of multiple genetic alterations in cancer cells results in dysregulation of various cellular pathways, some of which may modulate cellular metabolism (20). Otto Warburg observed that in most cancer cells, energy is produced predominantly by aerobic glycolysis in the cytosol, rather than by oxidation of pyruvate in mitochondria, as in most normal cells. He postulated that this change in metabolism is the fundamental cause of cancer (21). It has been proposed that aerobic glycolysis provides cancer cells with a growth advantage by supplying needed metabolites for incorporation into the biomass to produce a new cell (22).

One mechanism through which IDH mutations contribute to the Warburg phenomenon in glioma cells may be through changing metabolome or metabolite pools and thereby facilitating glycolytic flux. During glioma progression, the glycolytic traits found in these invasive cancer cells may arise as adaptive mechanisms to environmental constraints in which specific nutrients or oxygen may be limiting.

Conclusions

IDH mutations seem to play a central role in the pathogenesis of gliomas and define a specific subtype of glial tumors. This knowledge indicates opportunities to improve diagnostic and therapeutic strategies for gliomas, which are not currently targeted at the specific molecular alterations present in a particular tumor. The localization of IDH1 and IDH2 mutations to a single amino acid (R132 and R172, respectively) should simplify the use of this genetic alteration to distinguish IDH1- or IDH2-mutated and IDH1 and IDH2 wild-type gliomas and guide clinical treatment. Furthermore, metabolic profiling of glioma cells with IDH mutations could reveal new clues about the cancer-specific bioenergetics of these tumors and about novel strategies for therapeutic intervention.

However, any such improvements in the treatment for patients with IDH-mutated gliomas will hinge on a better understanding of the functional role of the mutant IDH in the pathogenesis of these tumors. Further analysis of IDH1 and IDH2 in glioma model systems will be necessary to clarify the genetic mechanisms involved in the initiation and malignant progression of this disease. These studies are anticipated to lead to an improved molecular classification of gliomas and should help establish these mutant genes and the related metabolic pathways as attractive targets for therapeutic intervention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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