IDH1 and *IDH2* Mutations in Tumorigenesis: Mechanistic Insights and Clinical Perspectives

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Abstract

Genes encoding for isocitrate dehydrogenases 1 and 2, *IDH1* and *IDH2*, are frequently mutated in multiple types of human cancer. Mutations targeting IDH1 and IDH2 result in simultaneous loss of their normal catalytic activity, the production of α-ketoglutarate (α-KG), and gain of a new function, the production of 2-hydroxyglutarate (2-HG). 2-HG is structurally similar to α-KG, and acts as an α-KG antagonist to competitively inhibit multiple α-KG–dependent dioxygenases, including both lysine histone demethylases and the ten-eleven translocation family of DNA hydroxylases. Abnormal histone and DNA methylation are emerging as a common feature of tumors with *IDH1* and *IDH2* mutations and may cause altered stem cell differentiation and eventual tumorigenesis. Therapeutically, unique features of *IDH1* and *IDH2* mutations make them good biomarkers and potential drug targets. *Clin Cancer Res; 18(20); 5562–71.* ©*2012 AACR.*

Introduction

Altered metabolic regulation in tumor cells was observed more than 80 years ago. Tumor cells, despite having an increased uptake of glucose, produce much less ATP than expected from complete oxidative phosphorylation and accumulate a significant amount of lactate (1–3). This phenomenon, representing arguably the first molecular phenotype characterized in cancer, is commonly known as Warburg Effect. The Warburg Effect's most notable clinical application is in 2[18F]fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET), where it provides the theoretical basis for the detection of tumors because of their increased glucose uptake relative to surrounding normal tissues. Despite its long history and broad clinical application, however, relatively little progress has been made over past 4 decades in understanding how altered metabolic regulation contributes to tumorigenesis. This is largely because of the fact that cancer research during this period has focused on genetic mutations in human cancer that, until very recently, were not known to include metabolic enzymes. The recent discovery of mutations targeting metabolic genes in cancer has renewed interest in cancer metabolism. Eight genes: *FH*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *IDH1*, and *IDH2*, encoding for 4 different metabolic enzymes: fumurate hydratase (FH), succinate dehydrogenase (SDH), and isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are frequently mutated. These mutations are both germinal and somatic, and occur in a wide range of human cancers (4). In this review, we will focus the discussion on the mechanisms and the translational research of *IDH1* and *IDH2*, 2 of the most frequently mutated metabolic genes in human cancer.

IDH1 and *IDH2* genes are mutated in gliomas, acute myeloid leukemia, and multiple other types of human cancers

The mutation targeting *IDH1* was first discovered in 2008 by a cancer genome project that systematically sequenced 20,661 genes in 22 human glioblastoma multiforme (GBM) samples and discovered 5 instances same heterozygous Arg132-to-His (R132H) point mutation (5). This finding was quickly confirmed by multiple studies through directed sequencing of *IDH1* and its homologue *IDH2* which cumulatively established that *IDH1* or, less frequently, *IDH2* genes are mutated in more than 75% of grade 2 to 3 gliomas and secondary glioblastomas (6–15). A separate cancer genome project in 2009 compared the genomes from tumor and normal cells in an individual patient with acute myeloid leukemia (AML) and identified a mutation in the *IDH1* gene that was subsequently found in additional AML samples (16). Further directed sequencing established that the *IDH1* or *IDH2* genes are mutated in close to 20% of AML (17–24). Following the discovery in glioma and AML, mutations targeting *IDH1* and *IDH2* genes were found in multiple additional types of human tumors, including thyroid carcinomas (16%; refs. 25, 26), cartilaginous tumor (75%; refs. 27–29), intrahepatic cholangiocarcinoma (10% to 23%; refs. 30, 31), as well as several other types of tumors at lower frequency (refs. 31–34; Table 1).

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doi: 10.1158/1078-0432.CCR-12-1773

Table 1. *IDH1* and *IDH2* mutations in multiple human solid tumors

Tumor types	Total mutation frequency	IDH1	Mutation number	Percentage (%)	2HG production	IDH2	Mutation number	Percentage (%)	2HG production	Reference
		R132H	1,705	91.32	Yes	R172K	24	60.00	Yes	
		R132C	76	4.07	Yes	R172M	9	22.50	Yes	
		R132S	31	1.66	Yes	R172W	5	12.50	Yes	
Glioma	75%	R132G	35	1.87	Yes	R172G	2	5.00	Yes	5–15, 42
		R132L	18	0.96	Yes					
		R132V	1	0.05	Yes					
		R132P	1	0.05	Yes					
		R132H	68	40.00	Yes	R140Q	89	76.72	Yes	
		R132C	46	27.06	Yes	R140W	2	1.72	Yes	
AML	20%	R132S	23	13.53	Yes	R140L	3	2.59	Yes	16–24
		R132G	15	8.82	Yes	R172K	21	18.10	Yes	42, 43
		R132L	4	2.35	Yes	R172G	1	0.86	Yes	
		V71I	14	8.24	No					
		R132C	63	77.78	Yes	R172S	1	100	Yes	
		R132H	14	17.28	Yes					
Cartilaginous tumors	75%	R132S	1	1.23	Yes					27–29, 42
		R132G	2	2.47	Yes					
		R132L	1	1.23	Yes					
		G70D	6	31.58	No					
		V71I	1	5.26	No					
		I130M	1	5.26	No					
Thyroid carcinomas	17%	H133Q	1	5.26	No					25, 26, 45
		A134D	2	10.53	No					
		V178I	8	42.11	No					
		R132C	5	62.50	Yes	R172W	1	100	Yes	
Cholangiocarcinoma	9/62	R132L	2	25.00	Yes					30, 31, 42
		R132G	1	12.50	Yes					
		R132H	1	50	Yes					
Prostate cancers	2/79	R132C	1	50	Yes					32, 42
Acute B-lymphoblastic leukemia	1/60	R132C	1	100	Yes					32, 42
Paragangliomas	1/131	R132C	1	100	Yes					33, 42
Colorectal carcinoma	2/180									31, 42
Melanoma	1/78	R132C	1	100	Yes					34, 42

IDH1 and *IDH2* mutations exhibit distinct biochemical and clinical features

Mutations targeting *IDH1* and *IDH2* genes in different types of tumors share 4 distinct biochemical features. First, *IDH1* and *IDH2* mutations in tumors are predominantly somatic and rarely germline (35). Second, all tumors with *IDH1/2* mutations are heterozygous. This is consistent with both a gain of function and dominant effect over the remaining wild-type allele. Third, nearly all *IDH1/2* mutations cause a single amino acid substitution, Arg132 in IDH1 (to 1 of 6 amino acid residues—His, Cys, Leu, Ile, Ser, Gly, and Val), or corresponding Arg172 in IDH2 (to 1 of 4 different residues—Lys, Met, Gly, and Trp), and Arg140 in IDH2 to either Gln or Trp. These 3 residues are located in the enzymes' active sites, suggesting a direct impact of mutation on the catalytic properties of the enzymes. Infrequently IDH1 mutations also include R100A in adult glioma, and G97D in colon cancer cell lines and a pediatric glioblastoma line (36). Finally, *IDH1* and *IDH2* mutations occur in a mutually exclusive manner in most cases, indicating a common underlying biochemical mechanism and physiologic consequence. Only rarely, individual tumors have been found to sustain mutations in both the *IDH1* and *IDH2* genes (8).

Mutations targeting *IDH1* and *IDH2* genes also exhibit 3 distinct clinical features. First, they occur in a highly restricted tumor spectrum. For example, they occur frequently in grade 2 to 3 gliomas and secondary glioblastomas, but not in primary GBM. Similarly, they are frequently found in cytogenetically normal AML, but not other subtypes of AML. This pattern suggests that the contribution of *IDH1/2* mutations to tumorigenesis may be linked to cell fate determination at a specific stage of stem or progenitor cell differentiation. Second, *IDH1/2* mutations occur at an early stage of tumorigenesis, and represent the earliest known mutation in glioma. This is consistent with the notion that *IDH1/2* mutations may impair cell fate determination and subsequent differentiation. Finally, in glioma (13), AML (37), and intrahepatic cholangiocarcinoma (37) where a sufficient number of samples have been analyzed,

IDH1 or *IDH2* mutations alone or in combination with other gene mutations (in the case of AML) are associated with better prognosis. These findings, together with results showing that ectopic expression of tumor-derived mutant IDH1 reduces the proliferation of established glioma cells *in vitro* (38, 39), suggest that mutant IDH enzymes, although promoting tumorigenesis in the long run, may also cause growth inhibition resulting from 2-HG toxicity.

These unique properties of *IDH1/2* mutations not only raise important mechanistic, biologic, and clinical questions about the role of this metabolic pathway in tumorigenesis, but also provide a unique opportunity to develop a strategy for therapeutic intervention.

Mutant IDH1 and IDH2 lose their normal activity to produce α-KG and gain a new activity producing 2-HG

The first biochemical alteration that is associated with tumor-derived IDH1 or IDH2 mutants is the loss of their normal activity in catalyzing the $NADP^+$-dependent oxidative decarboxylation of isocitrate into α-ketoglutarate (α-KG, also known as 2-oxyglutarate or 2OG) and NADPH (Fig. 1; refs. 13, 40). In cultured cells, ectopic expression of tumor-derived IDH1 mutant was found to result in inhibition of the activity of prolyl hydroxylase (PHD), a member of the α-KG–dependent dioxygenase family of enzymes (see below), which can be restored by feeding cells with cell-permeable α-KG (40). This finding provided early evidence linking the mutation in IDH1 or IDH2 to the function of a specific metabolite, α-KG.

A subsequent study found that, surprisingly, the mutant IDH1 not only abolished its normal activity, but also gained a new function: catalyzing the α-KG to D-2-hydroxyglutarate (D-2-HG, also known as *R*-2-HG; ref. 41; Fig. 1). Further studies found that all of the tumor-derived IDH2 mutants targeting either Arg140 or Arg172 also gained this new activity (42–44). In addition to glioma and AML, the accumulation of D-2-HG has been confirmed in enchondroma (45), indicating a cell-autonomous nature to the 2-HG production and accumulation in IDH1/2-mutated cells.

Astonishingly, D-2-HG accumulates to as high as 5 to 35 μmol/g (or 5–35 mmol/L) in the case of gliomas. Taking advantage of these high metabolite levels, efforts are currently underway to develop magnetic resonance spectroscopy (MRS) techniques to noninvasively detect the accumulation of D-2-HG in glioma patients (46–50). This MRS-based brain imaging for D-2-HG is still very experimental, and is not yet ready for routine clinical application.

Beside mutant IDH1/2, there are several additional enzymes in mammalian cells, such as 2-hydroxyglutarate dehydrogenase, hydroxyacid-oxoacid transhydrogenase, and L-malate dehydroxygenase, which are also involved in 2-HG metabolism, suggesting the possibility that their alteration could lead to 2-HG accumulation as well (51–54). L-2HG and D-2HG aciduria (L-2HGA and D-2HGA) are autosomal recessive neurometabolic disorders which were first described in 1980. They are characterized by the significant elevation (by 10- to 100-folds) of urinary levels of D-2-HG or L-2-HG (55, 56). D-2HGA is rare, with symptoms including epilepsy, hypotonia, and psychomotor retardation. L-2HGA is more prevalent and severe, and mainly affects the central nervous system in infancy leading to progressive hypotonia, tremors, epilepsy, leukoencephalopathy, mental retardation, psychomotor regression, and occasionally brain tumors (57).

IDH1 and IDH2 enzymes produce NADPH and α-KG

The IDH family includes 3 distinct enzymes in human cells: IDH1, IDH2, and IDH3. All the 3 enzymes catalyze the same enzymatic reaction: oxidative decarboxylation of isocitrate to produce α-KG, but each has its own unique features (Fig. 1). IDH1 is located in the cytosol and the peroxisomes, whereas IDH2 and IDH3 are located in the mitochondria. IDH1 and IDH2 use $NADP^+$, whereas IDH3 uses NAD^+ as electron acceptors to produce NADPH or NADH, respectively. While both IDH1 and IDH2 form a homodimer, IDH3 is a heterotetrameric enzyme formed by 2 α subunits, 1 β subunit, and 1 γ subunit and is the principle IDH enzyme involved in the tricarboxylic acid

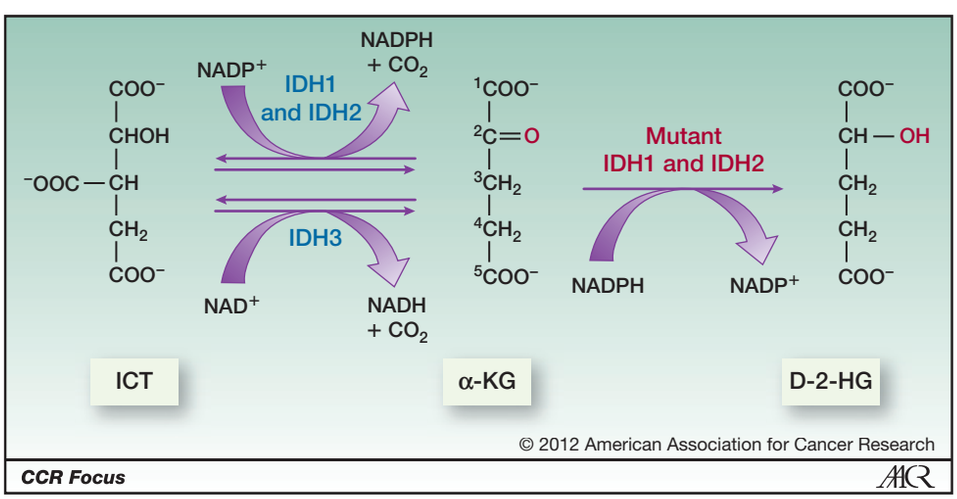

Figure 1. Chemical reactions catalyzed by the wild-type IDH enzymes and tumor-derived IDH1/2 mutants. The only structural difference between α-KG and D-2-HG is the replacement of the 2-ketone group in α-KG by a hydroxyl group in 2-HG and is indicated in red.

(TCA) cycle. Mutations have thus far only been found to target either *IDH1* or *IDH2* genes in human tumors, but not *IDH3*. The basis for this prevalence is not entirely clear, but likely relates to the facts that loss of function of IDH3, unlike that of IDH1 and IDH2, may be detrimental to cell growth because of disruption of the TCA cycle. In addition, Arg132, which is conserved in both IDH1 and IDH2 and is the principle site of mutation, is not conserved in any of the 3 IDH3 subunits.

Two products of IDH1 and IDH2 enzymes, NADPH and α-KG, play broad functions in cell regulation. NADPH is involved in many cellular processes including defense against oxidative stress, fatty acid synthesis, and cholesterol biosynthesis. As reducing oxidative stress and increasing fatty acid synthesis are required for cell division, NADPH is an important metabolite for the proliferation of both normal and tumor cells. IDH1 mutation was previously found to result in lowered NADPH tissue levels (58), although no difference in NADPH levels was observed in another study (59). Whether reduced NADPH production by the mutations targeting IDH1/2 causes decreased cell proliferation, thus contributing to relatively slower tumor growth, or is being compensated by the increased activity of other NADPH producing enzymes has not been determined.

α-KG plays critical roles in 4 different metabolic and cellular pathways. First, α-KG is a key intermediate in the TCA/Krebs cycle for energy metabolism. Second, α-KG is an entry point for several 5-carbon amino acids (Arg, Glu, Gln, His, and Pro) to enter the TCA by GDH after they are first converted into glutamate. Metabolism of glutamate to α-KG is a major step in anaplerosis whereby TCA cycle intermediates are replenished after being extracted for biosynthesis. Third, α-KG can be reduced back to isocitrate and then citrate for the eventual synthesis of acetyl CoA, the central precursor for fatty acid synthesis and protein acetylation. Recent studies have shown that α-KG can be reductively carboxylated by the NADPH-linked cytosolic IDH1 or mitochondrial IDH2 to form isocitrate that can then be isomerized to citrate (60, 61) under hypoxic conditions (62). These findings support the notion that IDH1 and IDH2 are bidirectional enzymes under physiologic conditions that can both produce and consume α-KG to meet cellular demands. Fourth, α-KG is used as a cosubstrate for multiple α-KG–dependent dioxygenases involved in the hydroxylation of various protein and nucleic acid substrates (Fig. 2). This last function of α-KG, although less known, is emerging as the main target of *IDH1* and *IDH2* mutations in human tumors.

α-KG–dependent dioxygenases hydroxylate diverse substrates and regulate many cellular pathways

Dioxygenases (also known sometimes as oxygen transferases) refer to the enzymes that incorporate both atoms of molecular oxygen (O_2) into their substrates. Dioxygenases whose activity requires Fe(II) and α-KG as cofactors are often referred to as Fe(II)- and α-KG–dependent dioxygenases. In the reactions catalyzed by these enzymes, both α-KG and O_2 can be considered to be cosubstrates with 1 oxygen atom being attached to a hydroxyl group in the substrate (hydroxylation) and the other taken up by α-KG leading to the decarboxylation of α-KG and subsequent release of carbon dioxide (CO_2) and succinate (Fig. 3).

The first identified α-KG–dependent dioxygenase was collagen prolyl hydroxylase, discovered in 1967 (63). After this pioneering work, the α-KG–dependent dioxygenases have been established as a widely distributed and continuously expanding family. The most notable new addition is the ten-eleven translocation (TET) family of DNA hydroxylases (64). The α-KG–dependent dioxygenases are present in all living organisms and catalyze hydroxylation reactions

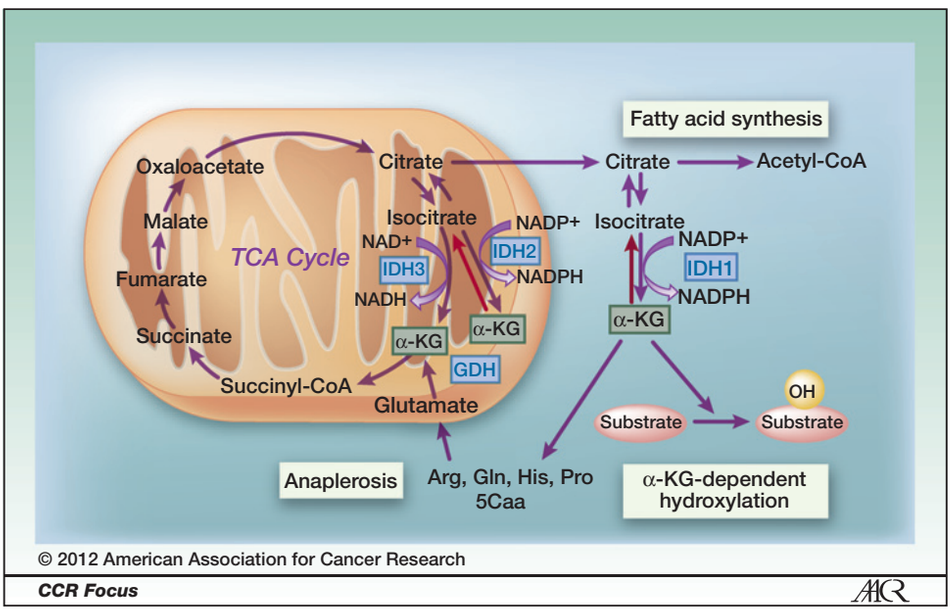

Figure 2. Production and utilization of α-KG in human cells. Four enzymes—IDH1, IDH2, IDH3, and GDH—can produce α-KG, which is used for 4 separate pathways: TCA cycle, anaplerosis, fatty acid synthesis, and protein and nucleic acid hydroxylation. Red colored arrows indicate reducing reactions catalyzed by either IDH1 or IDH2. 5Caa, 5-carbon amino acids.

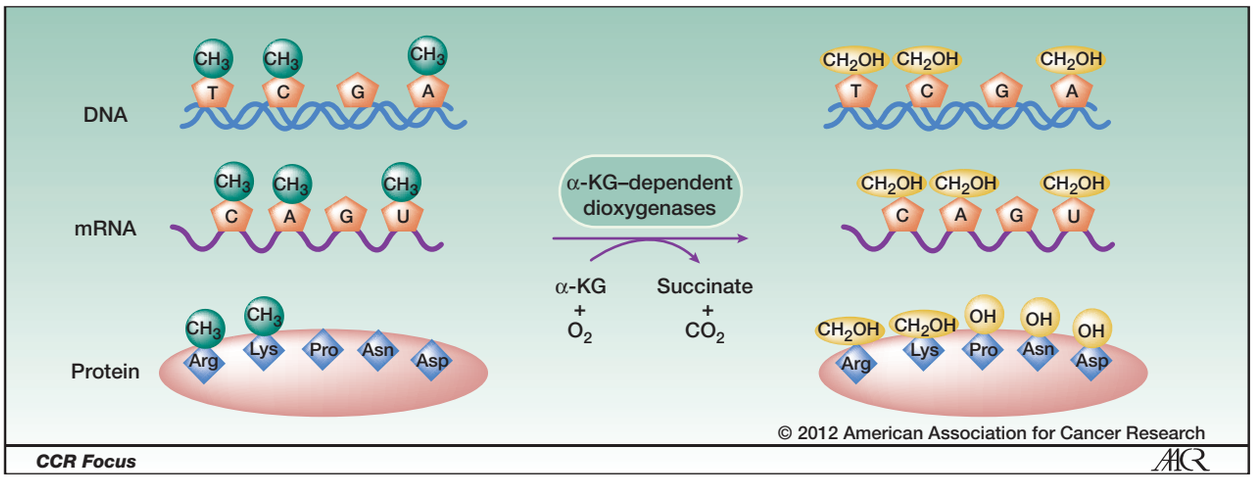

Figure 3. α-KG–dependent dixoygenases hydroxylate diverse substrates. Greater than 60 α-KG–dependent dixoygenases are estimated to be present in human cells. They hydroxylate many different substrates, including proteins, DNA, and RNA.

on a diverse set of substrates. They are involved in various pathways involving collagen, histones, and transcription factors, alkylated DNA and RNA, lipids, antibiotics, and the recently discovered 5-methylcytosine of genomic DNA and 6-methyadennine of RNA (refs. 65, 66; Fig. 3). It is estimated that there are more than 60 α-KG–dependent dioxygenases in humans based on sequence homology at the active site (67). As the result of such a broad spectrum of substrates, the change in the activity of α-KG–dependent dioxygenases resulting from *IDH1/2* mutation is expected to potentially affect multiple cellular pathways.

2-HG is structurally similar to and acts as an antagonist of α-KG

The catalytic core of α-KG–dependent dioxygenases consists of a conserved double-stranded β-helix fold (67, 68). In the active site of Fe(II)/α-KG–dependent dioxygenases, α-KG uses 2 oxygen atoms from the α-keto carboxyl end—1 from its C-1 carboxylate and 1 from C-2 ketone—to coordinate Fe(II) and 2 oxygen atoms linked to C-5 at the acetate end to interact with conserved residues in the dioxygenases. Both enantiomers of 2-HG are similar in structure to α-KG with the exception of the oxidation state on C-2 whereby the 2-ketone group in α-KG is replaced by a hydroxyl group in 2-HG. This suggests that 2-HG may act as a competitive antagonist of α-KG to interfere with the function of α-KG–dependent dioxygenases (Fig. 1). This hypothesis was experimentally shown for multiple α-KG–dependent dioxygenases, in particular histone lysine demethylases (KDMs) and the TET family of DNA hydroxylases both *in vitro* and *in vivo* (42, 69). In gliomas with IDH1 mutation, both histone and DNA methylation are higher than those in gliomas with wild-type IDH1. Perhaps the most direct evidence supporting this hypothesis was a structural analysis that showed 2-HG bonding to the catalytic core of α-KG–dependent dioxygenases and adopted a nearly identical orientation as α-KG, thereby preventing the binding of α-KG to the enzyme active site (42, 69).

α-KG–dependent histone and DNA demethylases are two main targets of IDH mutations

Not all α-KG–dependent dioxygenases are expected to be inhibited equally by 2-HG. The ones which have higher affinities with 2-HG would be more sensitive to the accumulation of 2-HG in IDH1/2 mutated cells. In fact, Chowdhury and colleagues found that D-2-HG inhibits different α-KG–dependent dioxygenases *in vitro* with a wide range of potencies (69), with histone H3K9 and H3K36 demethylase KDM4A/JMJD2A being the most sensitive (IC_{50} = 24 μmol/L), followed by H3K9/H3K36 demethylase KDM4C/JMJD2C (79 μmol/L), H3K36 demethylase KDM2A/FBXL11 (106 μmol/L), DNA repair enzyme ALKBH2 (424 μmol/L), FIH (1.5 mmol/L), prolyl hydroxylases (7.3 mmol/L), and γ-butyrobetaine dioxygenase BBOX-1 (13 mmol/L). This finding suggests that the KDM family of histone demethylases, which includes as many as 32 distinct enzymes in human cells and controls nearly all histone demethylation, is a major target of IDH1/2 mutation. This notion is supported by *in vivo* studies in both cultured cells and in human tumors. The levels of multiple histone methylations, including H3K4, H3K9, H3K27, and H3K79, were elevated in cells expressing tumor-derived IDH1/2 mutant or treated with cell-permeable 2-HG and in glioma with mutated IDH1 (42). More recent researches confirmed these findings and showed further that depletion of H3K9 demethylase KDM4C/JMJD2C blocked cell differentiation (70).

The second major target of IDH1/2 mutations is the TET family of DNA hydroxylases which catalyze 3 sequential oxidation reactions, converting 5-methlycytosine first to 5-hydroxymethylcytosine, then to 5-formylcytosine, and finally to 5-carboxylcytosine which can then be converted to unmethylated cytosine by thymine DNA glycosylase (12, 71–73). Three lines of genetic evidence support TET DNA hydroxylases as being pathologically relevant targets of IDH1/2 mutations. First, promoter DNA methylation profiling analysis has revealed that a subset of

glioblastomas, known as the proneural subgroup (74), is enriched for *IDH1* mutation and displays hypermethylation at a large number of loci (75) that is known as the glioma-CpG island methylator phenotype. These findings suggest a potential link between *IDH1* mutation and increased DNA methylation. Second, inactivating mutations of the *TET2* gene were found in about 22% of AML cases, notably occurring in a mutually exclusive manner with that of *IDH1/2* genes in AML (43). Third, ectopic expression of $IDH1^{R132H}$ mutant in immortalized primary human astrocytes, a cell type from which glioblastoma is believed to develop, induce extensive DNA hypermethylation and reshaped the methylome in a fashion that mirrors the changes observed in *IDH1*-mutated low-grade gliomas (76), supporting the notion that *IDH1* mutation alone is sufficient to cause the hypermethylation phenotype. Finally, direct biochemical evidence supporting TET as a target of IDH1/2 mutation is that D-2-HG inhibits TET activity *in vitro* and that the inhibition can be overcome by the addition of α-KG (42).

IDH1 and *IDH2* mutations are good biomarkers

Four features make *IDH1/2* mutations easily detectable, reliable, and specific biomarkers. First, *IDH1* and *IDH2* mutations occur in a highly restricted tumor spectrum and cell type. Second, nearly all tumor-derived mutations target IDH1 at a single residue, Arg132, and IDH2 at 2 residues, Arg140 and Arg172, which are located in a single exon 4 and can be simply identified through PCR-based amplification and sequencing using small amounts of tumor samples (e.g., 1 section of paraffin embedded tissue or a few cells). Third, antibodies specifically recognizing mutant $IDH1^{R132H}$ protein have been developed, making it possible to identify IDH1 mutation through conventional immunohistochemistry (77, 78). Fourth, MRS-based brain imaging technology, although still experimental and not yet ready for routine clinical application, has been developed that can noninvasively detect the accumulation of 2-HG in glioma patients (46–50).

In brain tumors, *IDH1/2* mutations occur frequently (>75%) in grade 2 to 3 gliomas and secondary glioblastomas, but much less frequently in primary GBM and other brain tumors. As such, *IDH1/2* mutations can be used to distinguish between primary and secondary GBM that are pathologically indistinguishable but clinically distinct entities with different prognoses. In addition, other reports suggest that *IDH1/2* mutation can be used to distinguish oligodendroglioma from morphological mimics such as dysembryoplastic neuroepithelial tumors (79), infiltrative gliomas from nonneoplastic reactive gliosis (80, 81) or other noninfiltrative neoplasms like gangliogliomas (82), or pilocytic astrocytomas from other astrocytomas (83). However, it remains to be proven whether *IDH1* mutation is a prognostic factor *per se* or a predictor of response to treatment. One study noted that *IDH1* mutation is closely linked to prognosis in grade 2 to 4 gliomas (84); however, another recent study suggested that *IDH* mutation status may not have significant prognostic impact in grade 2 gliomas (85). In leukemia, *IDH1/2* mutations were found frequently in cytogenetically normal adult AML, but not other subtypes of pediatric AML. Mutation of *IDH2* alone, but not *IDH1*, is associated with a slightly favorable prognosis (86). Patients with cooccurring *NPM1* and either *IDH1* or *IDH2* mutations have significantly better overall survival (37). Similarly, in intrahepatic cholangiocarcinoma, mutations in *IDH1* or *IDH2* gene were associated with longer overall survival and were independently associated with a longer span of time to tumor recurrence after resection (30). Efforts are currently underway to prospectively study the treatment responses in tumor patients with *IDH1/2* mutations and provide further therapeutic insights.

Are mutant IDH1 and IDH2 good drug targets?

The question on whether mutant IDH1/2 is a good drug target can be more specifically framed as to whether IDH1/2-mutated tumors are addicted to 2-HG. A unique feature of *IDH1/2* mutations is that mutants of IDH1/2 actively produce a new metabolite, 2-HG, that does not have an apparent physiologic function. Therefore, small molecules that selectively inhibit the 2-HG producing activity of mutant IDH1/2 would expect to have a marginally toxic effect toward normal cells. Given that multiple α-KG–dependent dioxygenases are inhibited by 2-HG in IDH1/2-mutated cells, a sudden withdrawal of 2-HG, if achieved, could conceivably cause a detrimental effect to the survival of IDH1/2-mutated cells. However, the direct evidence showing 2-HG addiction by the IDH1/2-mutated tumor cells has not been reported at present.

Conclusions and Perspectives

Several critical questions concerning the mechanisms and therapeutic targeting of IDH1/2-mutated tumors remain unanswered. First, what genetic alterations collaborate with *IDH1/2* mutations in promoting tumorigenesis? Given its broad inhibitory activity toward multiple α-KG–dependent dioxygenases, the accumulation of 2-HG is expected to be toxic to the IDH1/2-mutated cells. In fact, ectopic expression of tumor-derived mutant IDH1 decreased the proliferation of D54 glioblastoma cells while overexpression of wild-type IDH1 stimulated D54 cell proliferation (38). One hypothesis explaining the tumorigenic activity of mutant IDH1/2 would be that there is an additional genetic alteration that offsets or alleviates the toxicity of 2-HG. In low-grade glioma and secondary GBM, *p53* mutations cooccur early and frequently with *IDH1* mutation (87, 88). Furthermore, recurrent losses of chromosomes 1p and 19q have long been observed to associate with the development of glioma, in particular oligodendroglioma (refs. 87, 89; Fig. 4). Two poorly characterized genes, human homolog of Drosophila capicua (*CIC*) located in chromosome 19q and far upstream element binding protein (*FUBP1*) located on chromosome 1p, have recently been identified as leading candidates for the 1p and 19q tumor suppressor genes which are mutated in an almost exclusive cooccurring manner with the *IDH1/2* mutation (90–92). In AML, *IDH1* and *IDH2*

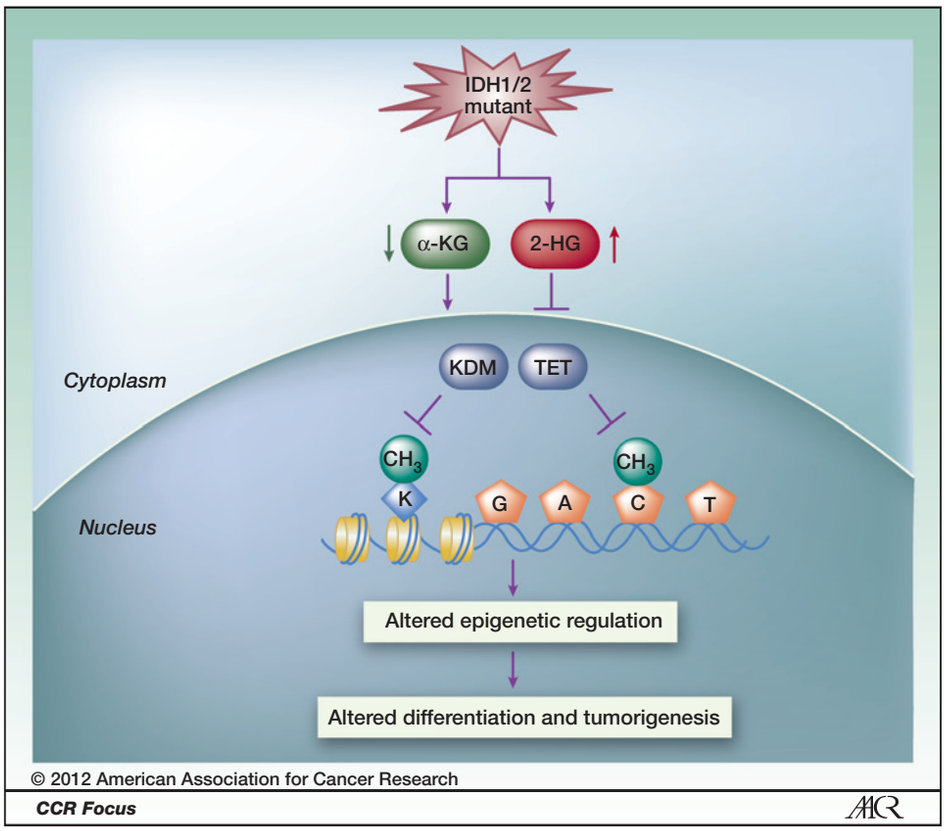

Figure 4. IDH1/2 mutations inhibit both histone and DNA demethylation and alter epigenetic regulation. Tumor-derived IDH1 and IDH2 mutations reduce α-KG and accumulate an α-KG antagonist, 2-HG, leading to the inhibition of both KDMs and the TET family of DNA hydroxylases. These inhibitions alter the epigenetic control of stem and progenitor cell differentiation.

genes are most frequently comutated with nucleophosmin *NPM1* gene, followed by DNA (cytosine-5)-methyltransferase 3A (*DNMT3A*; refs. 37, 93, 94). Whether *p53*, *CIC*, *FUBP1*, *NPM1*, and *DNMT3A* mutations collaborate with *IDH1/2* mutation remains to be determined.

Second, what are the downstream target genes of *IDH1/2* mutation? In AML, mutations targeting *IDH1/2* and *TET2* occur mutual exclusively (43), suggesting that, genetically, IDH1/2 and TET2 may function in the same, linear IDH-TET pathway. This hypothesis was supported by the finding that coexpression of wild-type and mutants of IDH1/2 resulted in the stimulation and inhibition of TET activity, respectively, in cultured cells and that 2-HG directly inhibited TET activity *in vitro* (42). The downstream targets of the IDH-TET pathway have not been identified. There are 2 competing hypotheses concerning the nature of the IDH-TET targets. One possibility is that a specific small set of genes, yet to be identified, are normally activated by TET-mediated DNA demethylation and control the fate of stem and progenitor cell differentiation. Inhibition of TET activity, by either mutation in a *TET* gene or the inhibition of TET activity in IDH1/2-mutated cells alters their expression and consequently restricts cellular differentiation. Alternatively, impairment of the IDH-TET pathway may not selectively impede the expression of a small group of genes to contribute tumorigenesis. Rather, DNA and histone methylation are altered widely in IDH1/2 and TET2 mutated cells that increases epigenetic plasticity analogously to the case of increased mutation rates and genomic plasticity in cells with impaired DNA repair pathways. Subsequent selection of cells that have acquired proliferative and survival advantages, in a context-dependent manner, would lead to clonal expansion and eventual tumorigenesis.

Third and urgently, mouse models for mutant IDH1/2, whether transgenic, xenograft or ultimately knock-in IDH1/2 mutant mice, are not only needed to obtain direct genetic evidence for the oncogenic activity of 2-HG, but more importantly for testing the effects of small molecule inhibitors of mutant IDH1/2. The challenges of generating the IDH1/2 mouse model likely reflect the strong toxicity of 2-HG produced by the tumor-derived mutant IDH1/2 that may severely block normal mouse development. This is also reflected in the fact that despite the establishments of many cell lines from glioma, AML, chondrosarcoma, and thyroid carcinomas, only one, HT1080 chondrosarcoma (reclassified by the Wellcome Trust Sanger Institute from previously fibrosarcoma), has been found to contain a mutation in IDH1 (R132C). Compounding the difficulty is the possibility that mutant IDH1/2 alone may not be sufficient to cause tumorigenesis and combination with a yet-to-be identified collaborating genetic mutation may be necessary. Recent isolation of a glioma stem cell line, BT142, containing heterozygous $IDH1^{R132H}$ mutation and establishment of a BT142 orthotopic xenograft mouse provide the first mouse model for investigating the oncogenic activity of 2-HG (95). More recently, haematopoietic and myeloid-specific conditional $IDH1^{R132H}$-knock-in mice were generated (96), which, although not developing

spontaneous tumors, are characterized with induction of a leukemic DNA methylation signature. The availability of these mouse models will advance our understanding of the mechanistic links between *IDH1* mutations and tumorigenesis and develop therapeutics against IDH1/2-mutated tumors.

In conclusion, the discovery and subsequent investigation of *IDH1/2* mutations in tumors have renewed interest into the research of tumor metabolism, identified a good biomarker for early detection and prognosis of several types of tumors, and led to the elucidation of the IDH-TET pathway in the epigenetic control of cell differentiation and tumor suppression. The extensive efforts that are currently underway should lead to a better understanding of the role of altered metabolic enzymes and metabolites in tumorigenesis, and novel strategies for therapeutic intervention in IDH1/2-mutated tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K.-L. Guan, Y. Xiong
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.-L. Guan
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Xiong
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Acknowledgments

The authors thank previous and current members of the Fudan MCB laboratory for the discussion. The authors thank Eric Oermann for reading the manuscript.

Grant Support

This work was supported by MOST 973 (nos. 2012CB910300, 2012CB910101, 2011CB910600, and 2009CB918401) and NSFC (grant nos. 30600112, 30871255, 31071192, and 81120108016). This work was also supported by NIH grants to Y. Xiong (CA163834) and K.L. Guan (R01CA108941), and James McDonnell Foundation and Samuel Waxman Foundation (to Y. Xiong).

Received May 30, 2012; revised August 8, 2012; accepted August 9, 2012; published online October 15, 2012.

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