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REVIEW

Oncogenic Isocitrate Dehydrogenase Mutations: Mechanisms, Models, and Clinical Opportunities

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ABSTRACT Heterozygous mutations in catalytic arginine residues of isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) are common in glioma, acute myeloid leukemia, chondrosarcoma, cholangiocarcinoma, and angioimmunoblastic T-cell lymphoma. The mutant enzymes acquire a neomorphic activity that converts α-ketoglutarate (α-KG) to D-2-hydroxyglutarate (D2HG), a rare metabolite. In cells and tissues expressing mutant IDH, D2HG concentrations are highly elevated. D2HG may act as an "oncometabolite" by inhibiting a class of α-KG–dependent enzymes involved in epigenetic regulation, collagen synthesis, and cell signaling. Knock-in mouse models of IDH1 mutations have shed light on these mechanisms and will provide valuable animal models for further investigation.

Significance: Mutations in *IDH1* and *IDH2* promote the development of a number of malignancies. These active site mutations cause a gain-of-function leading to the accumulation of the rare metabolite D2HG. Mouse models of these mutations should provide insights into the mechanisms driving tumorigenesis and facilitate evaluation of new treatments. *Cancer Discov; 3(7); 730–41. ©2013 AACR.*

INTRODUCTION

In 2008 and 2009, two independent cancer genome sequencing projects identified mutations in isocitrate dehydrogenase 1 (*IDH1*) in both glioblastoma multiforme (GBM; ref. 1) and acute myeloid leukemia (AML; ref. 2). Further investigation revealed that mutations of the homologous enzyme isocitrate dehydrogenase 2 (*IDH2*) were present in other cases of these diseases (3). In IDH1, the disease-associated alterations were all missense mutations confined to a single arginine residue, R132, in the enzyme active site. An R132H substitution constituted the vast majority of mutational events. In IDH2, the disease-associated mutations were present at the homologous R172 residue (with the alterations being predominantly R172K), and at another active site arginine R140 (with the alterations being predominantly R140Q; ref. 4). In all cases, the mutations in *IDH1* and *IDH2* were mutually exclusive and present in the hemizygous state. More than 75% of grade 2/3 gliomas and secondary glioblastomas, and more than 20% of cytogenetically normal AML cases, were found to harbor an *IDH1* or *IDH2* mutation. At the time, this discovery represented a major success for cancer genome sequencing projects and sparked a flurry of investigations aimed at understanding the role that *IDH* mutations play in tumorigenesis.

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BIOCHEMISTRY OF *IDH* MUTATIONS

Normal IDH1/2 Reaction

IDH1 is localized in the cytoplasm and peroxisomes, whereas IDH2 is found in the mitochondrial matrix. These homodimeric enzymes both catalyze a redox reaction that converts isocitrate to α-ketoglutarate [α-KG; also known as 2-oxoglutarate (2-OG)] while reducing NADP to NADPH and liberating CO_2 (Fig. 1). IDH1 and IDH2 are highly homologous to each other but are structurally, functionally, and evolutionarily distinct from the NAD-dependent, heterotrimeric IDH3 enzyme that functions in the tricarboxylic acid (TCA) cycle to produce the NADH required for oxidative phosphorylation. The physiologic function of the NADP-dependent IDH1/2 enzymes has not been well characterized, but they are thought to play roles in the metabolism of glucose, fatty acids, and glutamine, and to contribute to the maintenance of normal cellular redox status.

The reaction catalyzed by IDH1 and IDH2 is thought to be one of only three major mechanisms of NADPH production in mammalian cells (5). NADPH is the major carrier of reducing equivalents in cells. It supplies reducing power to key reactions

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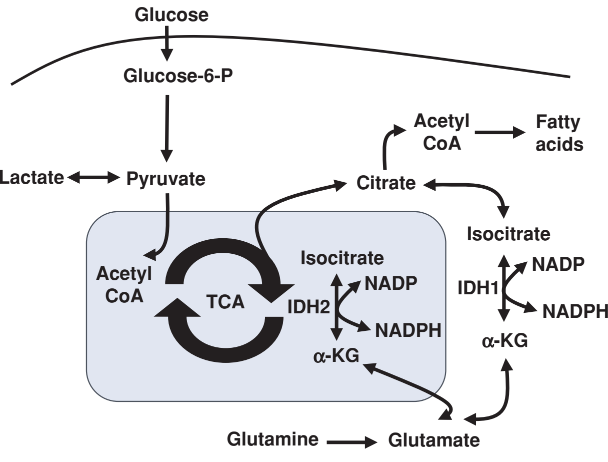

Figure 1. Biochemical pathways proximal to the IDH1 and IDH2 reactions. IDH1 is located in the cytoplasm and peroxisomes, whereas IDH2 is located in the mitochondrial matrix. The reactions catalyzed by these enzymes are linked to metabolites involved in amino acid metabolism, particularly that of glutamine and glutamate, and in fatty acid synthesis and the TCA cycle. IDH1 and IDH2 activities also affect the cytoplasmic and mitochondrial ratio of NADP/NADPH, a major source of reducing potential.

Isocitrate — Wild-type IDH1/IDH2 (NADP → NADPH) → α-KG + CO_2

α-KG — Mutant IDH1/IDH2 (NADPH → NADP) → D2HG

Figure 2. Enzymatic reactions catalyzed by wild-type and mutant IDH enzymes. Top: wild-type IDH1 and IDH2 convert isocitrate to α-KG and CO_2, with concomitant production of NADPH from NADP. Bottom: tumor-associated mutant IDH1 and IDH2 enzymes convert α-KG to D2HG, with concomitant production of NADP from NADPH. D2HG is a chiral molecule very similar in structure to α-KG. The chiral center in D2HG is denoted by *.

in a number of macromoecular biosynthetic pathways, and to systems that defend against the oxidative stress imposed by reactive oxygen species (ROS). For example, both the glutathione and thioredoxin antioxidant systems rely on the reducing power of NADPH to regenerate their capacity to detoxify oxidative damage. Therefore, mutations that disrupt the normal functions of IDH1 and IDH2 may have significant consequences for cellular redox balance. In fact, the initial examinations of this question produced data suggesting that the tumor-associated mutations in IDH1 acted via dominant negative inhibition of the wild-type enzyme's activity (6). It was proposed that this inhibition could lead to a drop in 2-OG levels and disrupt intracellular signaling processes sensitive to the concentration of this metabolite (6). However, this loss-of-function explanation did not fit well with the very restricted spectrum of observed mutations in the IDH enzymes or their heterozygosity.

The key breakthrough in understanding the effects of *IDH1* and *IDH2* mutations was a discovery made by scientists at Agios Pharmaceuticals in late 2009. Using a metabolite profiling strategy, they discovered that the mutant IDH enzymes acquire a neomorphic activity in which the normal product α-KG is converted to 2-hydroxyglutarate (2-HG) in a reaction that consumes, rather than produces, NADPH (ref. 7; Fig. 2). Structural data revealed that these mutations not only alter the affected arginine but also substantially reorganize several other key residues within the active site. As a result, the affinity of the mutant enzymes for isocitrate is reduced and their affinity for α-KG and NADPH is increased, allowing the neomorphic reaction to proceed.

2-HG in Tumors

The product of the novel reaction, 2-HG, is a poorly understood metabolite that does not participate in any known productive metabolic pathway. This chiral molecule is very similar in structure to the achiral 2-OG and is normally present at low levels in both its D- (or R-) and L- (or S-) enantiomeric forms. (This article will use the D/L rather than the R/S nomenclature to describe these molecules.) Several wild-type metabolic enzymes produce both 2-HG enantiomers as low efficiency by-products, and both are maintained at low concentrations in normal cells and tissues by 2-HG dehydrogenase housekeeping enzymes that recycle these compounds back to α-KG (Fig. 3). *In vitro* and *in vivo* experiments have shown that the mutant IDH enzymes exclusively produce the D- form of 2-HG (D2HG). In patients whose tumors bear somatic *IDH1* or *IDH2* mutations, D2HG production outstrips its elimination such that it builds up to concentrations that are orders of magnitude higher than normal, in some cases to millimolar levels (4, 7, 8). This outcome prevails no matter what amino acid substitution occurs at any of the 3 mutated arginine residues in IDH1/2. Although the detailed biochemical mechanism of catalysis by mutant IDH enzymes is not yet fully understood, it seems that the presence of the wild-type enzyme derived from the normal IDH allele increases the capacity of the mutant IDH enzyme to produce

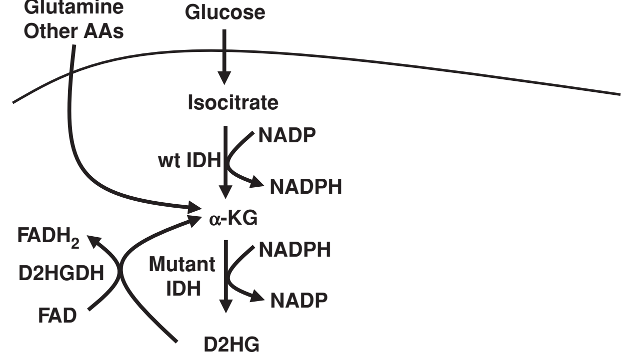

Figure 3. Generation and catabolism of D2HG. α-KG derived from isocitrate via the wild-type (wt) IDH reaction, or from the metabolism of amino acids (AA) via several different pathways, is converted to D2HG by the mutant IDH enzyme activity in a reaction that consumes NADPH. D2HG dehydrogenase (D2HGDH) converts some D2HG back to α-KG in a reaction that produces $FADH_2$ from FAD. However, when this system is overwhelmed, D2HG accumulates to high levels and contributes to tumorigenesis.

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2-HG (9). These findings, combined with the pattern of mutation in these genes, support the concept that the production of D2HG by the mutant enzyme is responsible for driving tumor progression. Hence, D2HG has been described as an "oncometabolite" in glioma and AML.

2-HG Acidurias

There is another clinical situation that results in high levels of 2-HG. A group of rare, inherited, neurometabolic disorders called 2-hydroxyglutaric acidurias are characterized by dramatically elevated D2HG or L2HG levels in the central nervous system (CNS), serum, and urine of affected patients (10, 11). All L2HG acidurias, and half of D2HG acidurias, are caused by homozygous loss-of-function mutations in the 2-HG dehydrogenase enzymes responsible for converting L2HG and D2HG back into α-KG. Subsequent to the discovery of the D2HG-producing *IDH* mutations, the approximately 50% of D2HG aciduria patients for whom the underlying genetic defect was unknown were found to harbor germline *IDH2* mutations (12).

The phenotypes of 2-HG acidurias are diverse, with patients exhibiting a range of developmental CNS symptoms, neurologic deficits, and a reduced lifespan. Some of these patients are at higher risk of developing brain tumors, consistent with the proposed effects of the 2-HG produced by mutant IDH enzymes in gliomas. However, malignant brain tumors have been described only in patients with L2HG acidurias. Furthermore, the majority of these tumors were medulloblastomas or pediatric neuroectodermal tumors, which is inconsistent with the histologic spectrum of IDH-related brain tumors (13). Early mechanistic studies in rats aimed at understanding the pathology of these diseases suggested that 2-HG can increase ROS levels in the CNS, but few additional details have emerged (14). Patients with a 2-HG aciduria therefore provide a unique physiologic model of the effects of long-term exposure to 2-HG, and suggest that 2-HG alone is not sufficient to drive tumorigenesis. This lack of tumorigenicity seems to be especially true for the hematopoietic system, as no hematologic malignancies have been observed in 2-HG aciduria patients. Therefore, although *IDH* mutations appear to be major contributors to tumorigenesis in glioma and AML, additional cooperating mutations must be required for complete transformation and disease progression.

IDH Mutations in Protein Engineering

As an interesting aside, the discovery of the unique gain-of-function resulting from these specific arginine mutations in the IDH enzymes has had implications beyond the field of medicine. The industrial production of certain classes of synthetic polymers, including nylon and some pharmaceuticals, relies on adipic acid, which is currently produced from fossil fuels. Biochemical engineering approaches have identified an alternative method of adipic acid production from simple sugars that involves a series of enzymatic steps. However, the efficient production of a key enzyme activity, 2-hydroxyadipate dehydrogenase, required for this process has been lacking. By translating the cancer-associated gain-of-function mutations in the IDH enzymes to the related *Saccharomyces cerevisiae* homoisocitrate dehydrogenase enzyme, investigators were able to reengineer this protein to catalyze the required reaction and produce chirally pure (R)-2-hydroxyadipic acid (15). It remains to be seen whether this approach will enable the cost-effective and scalable fossil fuel–independent production of this key chemical.

CLINICAL SIGNIFICANCE OF *IDH* MUTATIONS

Focused genotyping efforts across all major human cancer types in 2009 and 2010 revealed that *IDH1* and *IDH2* mutations were not present to any significant extent in most common tumors (16, 17). These data have been corroborated more recently by results from various cancer genome projects, in that only a few cases of sporadic *IDH* mutations have been observed. However, examinations of rarer tumor types have revealed a significant number of *IDH* mutations in chondrosarcoma (18), including enchondromas and hemangiomas associated with Ollier disease and Maffuci syndrome (19, 20), cholangiocarcinoma (21, 22), a small proportion of prostate cancers (23), and angioimmunoblastic T-cell lymphomas (AITL; ref. 24). As in glioma and AML, these mutations were all heterozygous events involving the R132 codon in IDH1 or the R140 or R172 codon in IDH2. Even though the incidence of these diseases is rare, and *IDH* mutations are relevant in only a small proportion of each patient population, there is much to be learned by studying them in concert with the more common IDH-associated cancers. The next few sections of this article describe the consequences of *IDH* mutation in various tumor types and the mechanistic insights derived from each.

Glioma

A wealth of clinical data related to *IDH1* and *IDH2* mutations in glioma has been generated by numerous groups worldwide, and has been comprehensively reviewed elsewhere (25, 26). *IDH* mutations occur in 70% to 90% of all adult grade 2/3 astrocytomas and oligodendrogliomas, and in the secondary glioblastomas to which these tumors progress. Interestingly, *IDH* mutations are not a common event in primary glioblastoma (5% to 15%), suggesting that the molecular events leading to these diseases are distinct. It has even been suggested that secondary glioblastomas lacking *IDH* mutations would be more accurately characterized as primary glioblastomas, as their molecular features and natural history are most similar to primary GBM. *IDH* mutations are not common in any other CNS tumor, with the exception of a few primitive neuroectodermal tumors (27).

The molecular detection of *IDH* mutations has become standard practice in many institutions, and has proven useful in the clinical management of glioma. In some situations, the differential diagnosis of CNS lesions based on histology alone can be difficult. For example, distinguishing between reactive gliosis and diffuse glioma, or between oligodendroglioma and other similar entities, can be a challenge. In some of these cases, *IDH* status has proven extremely useful as a diagnostic biomarker (28). Furthermore, *IDH* mutations are associated with longer survival of patients with glioma or secondary glioblastoma and thus represent an important prognostic indicator. Although *IDH* mutations are associated with younger patient age and other positive prognostic features, they are independent predictors of a favorable outcome in multivariate analyses. It is not yet clear whether

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IDH status is also a predictive marker of response to chemotherapy or radiation treatment, as a number of studies have shown equivocal or conflicting results (26).

As noted above, *IDH* mutation alone is insufficient for tumorigenesis. Analysis of various CNS tumors has shown that *IDH* mutations are significantly associated with O-6-methylguanine-DNA methyltransferase promoter methylation (26); with mutations in *TP53*, commonly found in astrocytic tumors (3); and with the 1p19q codeletion event that arises from an unbalanced translocation in oligodendrogliomas (29). In fact, almost all gliomas with an *IDH* mutation also harbor either a *TP53* mutation or a 1p19q codeletion (25, 29). Taken together, the collective data from a large number of clinical studies support a model in which *IDH* mutations occur early during gliomagenesis, and subsequent *TP53* or 1p19q deletion events determine the progression of the disease to astrocytoma or oligodendroglioma, respectively (25, 30).

Myeloid Neoplasia

The role of *IDH* mutations in myeloid neoplasia has also been extensively investigated since the discovery of these mutations in AML, and comprehensive clinical reviews are available (31, 32). Unlike in glioma, where mutations are overwhelmingly R132H substitutions in IDH1, *IDH* mutations in AML are more diverse. Approximately half of all such mutations occur in IDH2, and both R172K and R140Q are common. The same *IDH* mutations have also been observed in myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN), both of which are precursor conditions that can progress to AML (33). However, the acquisition of an *IDH* mutation does not seem to be associated with progression to AML in patients with myeloproliferative disease. Again, additional tumorigenic events seem to be required.

IDH mutations do not cluster within any of the subtypes of AML as defined by the French-American-British classification scheme, except that they are absent in acute promyelocytic leukemias driven by the 15;17 translocation that leads to *PML-RARA* fusions. There is a significant association of *IDH* mutations with mutations in nucleophosmin mutations in patients with AML with a normal cytogenetic profile, placing them within a group of patients with an intermediate risk profile. Unlike in glioma, *IDH* mutations do not provide any clinically significant prognostic information in AML, especially when examined in the context of the most recent molecular prognostic models. However, D2HG is highly elevated in cells and serum of patients with *IDH*-mutant AML irrespective of the specific mutation. Thus, D2HG concentration may serve as a biomarker of response to treatment and disease progression (8).

At the genetic level, *IDH* mutations in AML are mutually exclusive with mutations in tet methylcytosine dioxygenase 2 (*TET2*), which is altered in 10% to 20% of AML cases. This observation suggests that *IDH1/2* and *TET2* mutations may act via the same mechanism (34). The mechanism of tumorigenesis associated with *IDH* and *TET* mutations will be discussed later in this article.

AITL

AITL is one of the three most common peripheral T-cell lymphoma (PTCL) subtypes, along with anaplastic large cell lymphoma and PTCL-not-otherwise-specified (35). AITL normally presents as a systemic disease, with polyadenopathy and a variety of immunologic abnormalities, and carries a poor prognosis. On the basis of molecular marker expression and microarray expression profiling, AITL is thought to arise from the follicular T-helper (T_{FH}) cells that are present in germinal centers and cooperate with B cells in mounting an effective immune response (35, 36). Recurrent mutations in the *TET2* gene are associated with AITL, but the molecular pathogenesis and other underlying genetic events driving AITL are largely unknown (37). *IDH2*^{R172} mutations are present in approximately 20% to 45% of AITL cases, but not in other types of PTCL (24). However, there are no clinical or pathologic differences among patients with AITL based on IDH2 status, and their prognoses are the same. *IDH1* mutations have not been detected in patients with AITL, supporting the concept that *IDH1* and *IDH2* mutations can have different effects depending on the cellular context.

As in AML, the identification of *IDH2* mutations in AITL presents an opportunity to develop a mechanistic model of the disease, develop clinically useful biomarkers, and evaluate novel therapeutic strategies. Of note, although there are no AITL cell lines currently available, the first AITL xenografts have recently been reported (38), opening up opportunities for further mechanistic study. Characterization of these model tumors should soon yield fresh insights into *IDH2*-mutant AITL.

Chondrosarcoma

Cartilaginous tumors range from benign enchondromas through low-grade chondrosarcomas to highly malignant chondrosarcomas, and represent a significant proportion of human mesenchymal tumors. Ollier disease and Maffucci syndrome are noninherited conditions characterized by multiple benign enchondroma tumors whose underlying genetic lesions are largely unknown. Because patients with these conditions are also at higher risk of developing glioma and AML, a large survey of *IDH* mutation status in chondrosarcoma, osteosarcoma, and other mesenchymal tumors was conducted (18), as well as a focused assessment of patients with Ollier disease and Maffucci syndrome (19, 20). Approximately 50% of sporadic central and periosteal chondrosarcomas were found to have hemizygous *IDH1* or *IDH2* mutations, with *IDH1* mutations predominating. No *IDH1* mutations were identified in any other mesenchymal tumor type (18). In patients with Ollier disease or Maffuci syndrome, a high proportion of the enchondroma tumors present also harbored *IDH* mutations. Interestingly, a low level of *IDH* mutation was also found in nontumor DNA in these patients, suggesting that postzygotic *IDH* mutations, and the resulting somatic mosaicism, can lead to these diseases (19, 20). In all cases investigated, elevated D2HG levels were detected in these patients. Similar to D2HG aciduria patients, further investigation of the distribution of *IDH*-mutant cells in these individuals should provide information regarding the susceptibility of various cell lineages to the effects of *IDH* mutations and elevated D2HG levels.

Cholangiocarcinoma

Cholangiocarcinomas are a relatively rare subset of gastrointestinal malignancies involving the gall bladder and bile duct. Patients with these cancers have a poor prognosis, with a

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median survival of approximately 1 year (39). The spectrum of genetic events associated with cholangiocarcinoma is not completely characterized, but two studies examining mutations in the tumors of several hundred patients with cholangiocarcinoma have been conducted (21, 22). *IDH* mutations were identified in 10% to 23% of intrahepatic cholangiocarcinomas, but not in cholangiocarcinomas of extrahepatic origin. Elevated levels of D2HG were detected in tumor tissues, and *IDH1* mutations were more common than *IDH2* mutations. Curiously, the pattern of amino-acid substitutions in the mutant IDH enzymes was significantly different than that observed in glioma or AML. For example, the R132H mutation so common in glioma and AML was significantly under represented in cholangiocarcinoma. As in glioma, the presence of an *IDH* mutation seems to predict better overall patient survival.

Prostate Cancer

Three independent studies have investigated *IDH1* mutations in prostate cancer. An initial survey of a broad variety of tumor types identified a small number of prostate cancers with *IDH1* mutations (17). A follow-up study focused specifically on prostate tumors identified *IDH1* mutations in approximately 2% of patients (23), a result confirmed in a third study (40). No *IDH2* mutations have been reported in prostate cancers to date. Although 2% is a low incidence rate, prostate cancer is highly prevalent, meaning that even a small mutation rate represents a significant number of patients with *IDH1*-mutant prostate cancer. Unfortunately, even considering these three studies, too few patients have been profiled thus far to draw any meaningful conclusions about the relationship between *IDH1* mutations and the pathophysiology of this disease. A greater understanding of the role of *IDH* mutation in this setting may eventually offer additional tools for the management of prostate cancer.

MECHANISTIC EFFECTS OF *IDH* MUTATIONS DURING TUMORIGENESIS

2-OG Dioxygenase Inhibition

The currently available evidence suggests that the ability of mutant IDH enzymes to promote tumorigenesis is due to their neomorphic enzyme activity and D2HG production. In principle, the acquisition of an *IDH* mutation could impair a cell's ability to regulate its redox state, defend against ROS, and/or metabolize several classes of macromolecules. However, to date, the majority of work investigating the effects of *IDH* mutations has focused on the role of D2HG as a competitive inhibitor of a class of enzymes dependent on α-KG as a cosubstrate. These enzymes are most commonly referred to as 2-OG–dependent dioxygenases, and this nomenclature will be used in this article. D2HG and α-KG are structurally identical except that the C2 carbonyl group of α-KG is replaced by a hydroxyl group in D2HG. Not surprisingly, considering the high levels of D2HG generated in *IDH*-mutant cells, this molecule indeed competitively inhibits this class of 2-OG–dependent enzymes. More than 80 2-OG–dependent dioxygenases exist in humans and are involved in diverse biologic processes. In general, these enzymes require oxygen, ascorbate, and iron as cofactors, and convert α-KG to succinate and CO_2 while carrying out their specific enzymatic activities. Several members of this family have been studied in depth, and the mechanisms of their catalysis and inhibition have recently been reviewed (41).

In vitro biochemical studies have shown that both D2HG and L2HG can act as competitive inhibitors of 2-OG–dependent dioxygenases by binding to the α-KG–binding pocket in the enzyme's active site (42). Although the ability of 2-HG to compete with α-KG varies depending on the specific enzyme, it appears that L2HG is consistently a better inhibitor than D2HG. However, even for D2HG, the K_m values for enzyme inhibition are well within the range of concentrations observed in cells and tissues of IDH-mutant tumors, suggesting that D2HG can impair the activity of this class of enzymes *in vivo*. To date, the enzymes that have received the most attention as potential targets of D2HG inhibition include the TET proteins involved in DNA methylation, the JumonjiC domain-containing histone demethylases, the prolyl hydroxylases (PHD) and lysyl hydroxylases (LHD) required for collagen folding and maturation, and the PHDs that regulate hypoxia-inducible factor (HIF) signaling (Fig. 4).

Epigenetic Regulation

Epigenetic regulation of gene expression occurs via chemical modification of histone proteins and of DNA itself. Disruption of normal patterns of epigenetic regulation is known to play a major role in tumor progression. Methylation of cytosine residues in DNA and methylation of lysine residues in histone proteins are key epigenetic events that are both controlled in part by 2-OG–dependent dioxygenases. Accordingly, D2HG-mediated inhibition of these enzymes has been proposed as a means by which *IDH* mutations could contribute to tumorigenesis.

Changes to DNA methylation are known to influence both neuronal and hematopoietic cell differentiation by regulating stem cell self-renewal and directing commitment to cell fates. The TET family of dioxygenases (TET1, TET2, and TET3) was recently shown to catalyze the hydroxylation of 5-methylcytosine residues in DNA (43), resulting in an accumulation of 5-hydroxymethylcytosine. This hydroxylation event is the first step in a pathway leading to the demethylation of these cytosine residues. Although the precise mechanisms regulating the activity and specificity of the TET enzymes are not fully understood, it is clear that they play important roles in maintaining appropriate DNA methylation patterns across the genome during key stages of development (43). Interest in TET enzymes recently surged with the discovery of *TET2* mutations in cases of AML, MPN, and MDS that lacked *IDH* mutations (34, 44). *In vitro*, *IDH* mutations interfere with TET-driven demethylation of DNA, resulting in elevated 5-methylcytosine levels and generating a hypermethylation signature characteristic of less differentiated cells. Accordingly, samples from patients with *IDH*-mutant AML or *TET2*-mutant AML show similar patterns of global DNA hypermethylation with characteristic epigenetic changes (34). In glioma, a tumor phenotype known as the CpG island methylator phenotype is highly associated with *IDH1* mutations (45). Although *TET* loss-of-function mutations have not been observed in gliomas, both silencing of *TET2* by promoter hypermethylation (46) and nuclear exclusion of TET1 (47) have been found in tumors with wild-type IDH enzymes. These observations suggest that, in gliomas lacking *IDH* mutations, alternative mechanisms inhibiting

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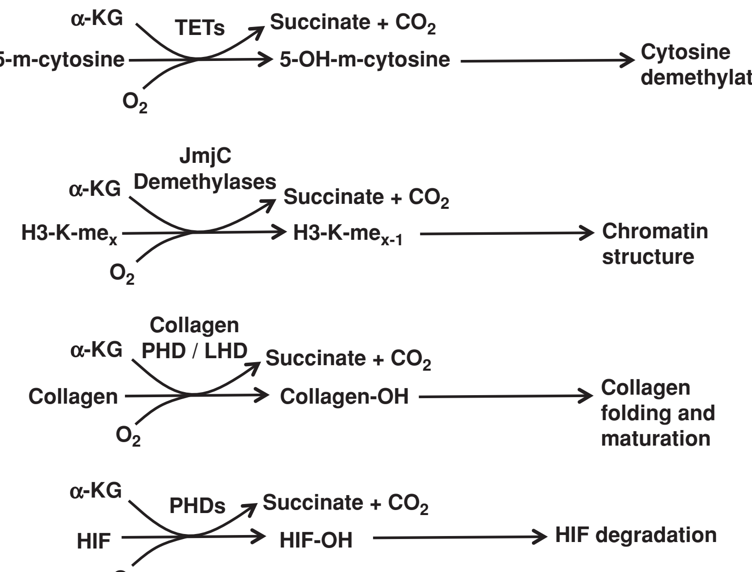

Figure 4. Reactions of 2-OG–dependent dioxygenases inhibited by D2HG. Reactions are shown for four classes of 2-OG–dependent dioxygenases potentially inhibited by D2HG. All four reactions convert α-KG to succinate and CO_2, incorporate O_2, and require iron and ascorbate as cofactors. The TET enzymes hydroxylate 5-methylcytosine, which initiates demethylation of these sites. The Jumonji C (JmjC) domain–containing histone demethylases remove methyl groups from the lysine residues of histone proteins, affecting the histone code and altering chromatin structure and transcription. The collagen PHD and LHD hydroxylate proline and lysine residues of collagen during its maturation in the endoplasmic reticulum. These modifications are required for proper folding and glycosylation of collagen. The PHDs controlling the stability of the HIF transcription factors hydroxylate the HIF1α and HIF2α proteins on specific proline residues, targeting them for degradation.

the TET enzymes may play a role in disease pathogenesis. In cholangiocarcinomas and chondrosarcomas, *IDH* mutations are associated with DNA hypermethylation of CpG islands in a manner also seen in glioblastomas and AML (20, 21). *In vitro* experiments have shown that *IDH* mutations are sufficient to establish this methylation signature and thereby influence cellular differentiation (48, 49).

Histone Methylation

In addition to DNA methylation, the methylation of lysine residues on histone proteins also contributes to alterations to chromatin structure and transcriptional regulation. Histone methylation events combine with other posttranslational modifications, including acetylation, phosphorylation, and ubiquitination, to establish a histone code that is responsible for recruiting the multiprotein complexes which epigenetically control transcription. A major family of enzymes responsible for the regulation of histone methylation is the Jumonji C (JmjC)-domain–containing histone demethylases, which are also members of the class of 2-OG–dependent dioxygenases potentially inhibited by D2HG. A member of the JmjC family has been crystallized in a complex with D2HG, clearly showing that D2HG can occupy the α-KG–binding site (50). There is also experimental evidence from cell culture systems showing that the introduction of *IDH* mutations can alter histone methylation patterns (51). Because of the complex combinatorial nature of the histone code, and technical challenges in specific and quantitative measurement of histone modifications, predictions regarding the effects and importance of these methylation changes are difficult to make. However, the genetic and epigenetic characterization of patient samples combined with *in vitro* biochemistry results and data from cell culture systems strongly implicate D2HG-driven epigenetic alterations as an important feature of *IDH*-mutant cancers.

Collagen Synthesis

Hydroxylation of proline and lysine residues in collagen is critical for reinforcing this protein's helical structure and thermal stability, and is required for certain glycosylation events (52). These posttranslational modifications are carried out by 2-OG–dependent dioxygenases residing in the endoplasmic reticulum (ER). These enzymes require ascorbate as a cofactor, and a lack of sufficient ascorbate levels causes the loss of activity of these enzymes that is responsible for the symptoms of scurvy. Hydroxylated collagen is a key component of the extracellular matrix (ECM), and interaction between the ECM and neurons or hematopoietic cells regulates their proliferation and differentiation in the brain or bone marrow, respectively. Therefore, altered ECM structure and function due to D2HG-mediated inhibition of 2-OG–dependent dioxygenase activity could contribute to tumor progression in both glioma and AML (53). In addition, disruption of collagen folding by suppression of PHD activity leads to ER stress due to the accumulation of misfolded collagen protein in the ER, and ER stress is closely associated with transformation. Although patients with D2HG aciduria do not exhibit scurvy symptoms, the local inhibition of collagen hydroxylase enzymes by D2HG may contribute to the phenotype of patients with *IDH*-mutant tumors.

HIF Signaling

Another group of enzymes potentially inhibited by D2HG are the PHDs that regulate the protein stability and transcriptional activity of the HIF1 and HIF2 transcription factors. Under normoxic conditions, these enzymes hydroxylate the HIF1α and HIF2α proteins on key proline residues such that they are recognized by Von Hippel Lindau (VHL) protein, a ubiquitin E3 ligase. This ubiquitination targets HIF1α and HIF2α for rapid proteasomal degradation. In the absence of oxygen, these hydroxylation events cannot proceed efficiently and HIF1α and HIF2α are free to dimerize with HIF1β, forming complete transcription factors that coordinate the response to hypoxia. HIF stability is also influenced by intracellular concentrations of α-KG, ROS, and the activity of other signaling pathways (54). Importantly, the PHDs responsible for HIF regulation have additional cellular targets, and therefore

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the inhibition of these enzymes by D2HG could have HIF-independent effects (55). This regulatory complexity means that HIF stability and target gene activation are cell type- and context-dependent. Direct *in vitro* studies examining the effects of 2-HG on PHDs and HIF activity have yielded conflicting results. Some reports show that D2HG acts as an activator rather than an inhibitor of PHDs, leading to decreased levels of HIF (56, 57). However, other studies have observed the expected inhibitory effect of D2HG on the PHD2 enzyme and increased HIF levels (50). The explanation for these different findings and the molecular mechanism by which D2HG activates PHDs are yet to be determined. In addition, *in vitro* biochemical data have shown that the affinity of D2HG for the HIF PHDs is lower than its affinity for other 2-OG–dependent dioxygenases, further calling into question the involvement of the HIF PHDs in IDH-associated tumorigenesis. More accurate *in vivo* models and detailed molecular characterization of patient samples will be required to define the role of the HIF PHDs in IDH-mutant diseases.

Metabolic Alterations

Little is known about the metabolic requirements of hematopoietic and neural stem and progenitor cells, or how metabolism is altered during their differentiation. Given the positioning of IDH1 and IDH2 in the cellular metabolic network, and their ability to produce NADPH, mutations in these enzymes may perturb cellular metabolism in profound ways that contribute to tumorigenesis. Moreover, a relevant phenomenon initially reported in the 1960s has recently been confirmed: that wild-type IDH enzymes are able to catalyze the reverse reaction in which α-KG is carboxylated to form isocitrate while consuming NADPH (58–60). This reverse reaction seems to be particularly important for the synthesis of fatty acids under conditions of mitochondrial dysfunction or hypoxic stress. Therefore, mutation of an IDH enzyme could alter either the forward reaction or the reverse reaction or both, as well as upset the NADP/NADPH ratio. Initial metabolic profiling of the effects of *IDH* mutations has been conducted *in vitro* using overexpression systems, and the introduction of a mutant IDH enzyme seems to alter several metabolic pathways (61). However, it is difficult to predict whether these particular changes also occur *in vivo* in the cell types of interest, because the metabolic microenvironment of the affected tissues will also affect intracellular metabolic regulation. Further investigations of the metabolic effects of *IDH* mutations are required to fully understand their effects at the levels of the tissue and the organism.

MOUSE MODELS OF *IDH* MUTATION

IDH1 R132H Knock-in Mouse

Mechanistic studies of *IDH* mutations have been hampered by a lack of appropriate model systems. Initial surveys of existing cell lines and attempts to derive *IDH*-mutant cell lines were largely unsuccessful, suggesting that these mutations are not generally compatible with viability under standard cell culture conditions. More recently, the use of alternative techniques has led to the successful derivation of several *IDH*-mutant cell lines, including a glioma cell line and xenograft (62), three chondrosarcoma lines (63), and a set of astrocytoma lines (9). The commonly used fibrosarcoma cell line HT-1080 also harbors an *IDH1*^{R132C} mutation and produces D2HG in culture (64). *In vitro* analyses of these lines have shown that cell membranes are not permeable to D2HG and that this metabolite is not readily taken up by cells in culture, compromising the direct investigation of the effects of *IDH* mutations and D2HG in controlled experimental systems (50). A further complication is that the metabolic phenotype of cells is heavily influenced by local microenvironmental conditions, which can vary widely in different tissues and are not accurately modeled by standard *in vitro* cell culture. Thus, the effects of *IDH* mutations on cellular metabolism have been difficult to explore in cultured cells.

Genetically engineered mouse models have provided more relevant systems for understanding the pathophysiologic consequences of *IDH* mutations. In two recent articles (65, 66), our group described the generation and initial characterization of a conditional knock-in (KI) mouse that was constructed using the lox-stop-lox (LSL) system and bears the most common *IDH1* mutation, R132H. In the absence of Cre-recombinase (Cre) expression, the LSL *IDH1*^{R132H} mutant allele is null, but when Cre is expressed, the stop codon upstream of exon 4 is excised and the mutant IDH1 protein is expressed from the endogenous locus. Interestingly, mice homozygous for the null *Idh1* allele (*Idh1* knockout mice) are viable and fertile, indicating that wild-type IDH1 function is dispensable in mice under standard laboratory conditions. However, mice heterozygous for the LSL IDH1^{R132H} allele crossed with a mouse constitutively expressing Cre are embryonic lethal at an early stage. This observation supports the hypothesis that the gain-of-function of the mutant enzyme and its production of D2HG are critical for the tumor-promoting effects of mutated IDH1.

Hematopoietic System IDH1 Mutants

To examine the effects of the *IDH1*^{R132H} mutation on the myeloid compartment, LSL IDH1^{R132H} mice were crossed to Vav-Cre or LysM-Cre mice, resulting in the expression of the mutant IDH1 protein in the entire hematopoietic system or early during myeloid development, respectively. In both cases, the progeny were born at the expected Mendelian ratio and displayed no gross phenotype early in life. However, by 6 to 12 months of age, both mutants exhibited decreased bone marrow cellularity, splenomegaly, and evidence of extramedullary hematopoiesis indicative of a dysfunctional bone marrow niche. Levels of D2HG were highly elevated in the hematopoietic tissues, serum, and urine of these animals. Although they did not develop frank myeloid leukemias, these mice had elevated numbers of Lin^{-}Sca1^{+}cKit^{+} (LSK) cells in the bone marrow and spleen. The LSK population includes long-term hematopoietic stem cells (HSC), short-term HSCs, and lineage-restricted progenitor cells (LRP). In contrast, there were no significant alterations to the more committed progenitor cell populations. This phenotype is reminiscent of human MDS (33).

To examine the epigenetic effects of the conditional *IDH1*^{R132H} mutation, a preliminary analysis of the methylation signature of LSK cells was conducted. LysM-Cre IDH1^{R132H} KI mice showed a greater proportion of highly methylated DNA fragments whose genomic regional distribution resembled that in human AML (34). Histone methylation at some key H3 lysine residues was also increased, consistent with

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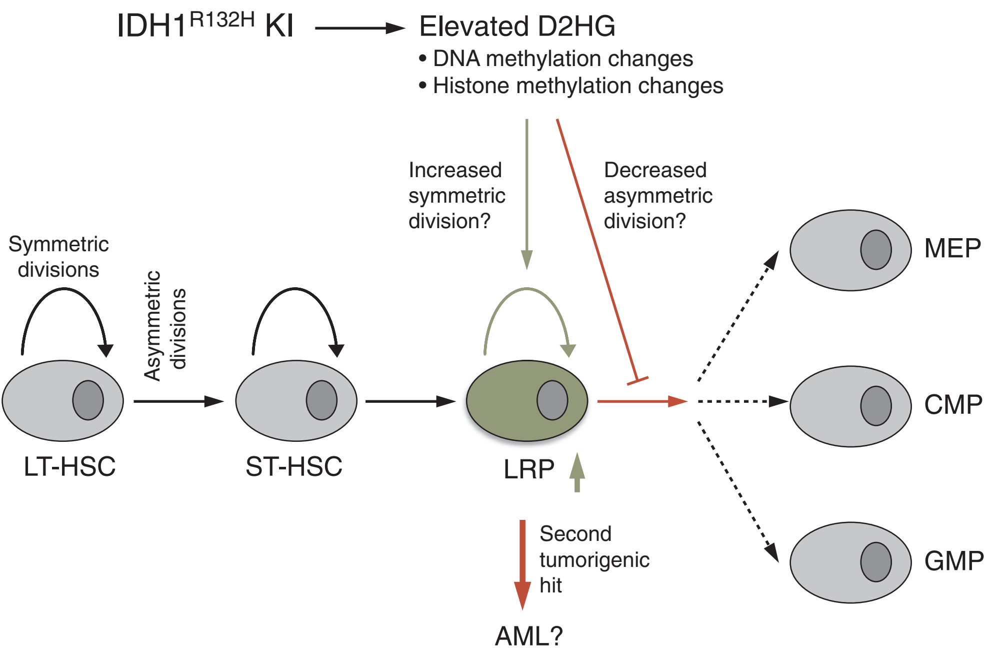

Figure 5. Model of the effects of *IDH1*^{R132H} mutations on the murine hematopoietic system. Hematopoietic cell differentiation is regulated via a balance between symmetrical and asymmetrical cell division in hematopoietic stem and progenitor populations in the bone marrow. Symmetric cell division maintains stem and progenitor populations, whereas asymmetric division leads to differentiation. Long-term hematopoietic stem cells (LT-HSC) differentiate into short-term hematopoietic stem cells (ST-HSC), which in turn differentiate into lineage-restricted progenitor cells (LRP). Mutant IDH activity generates increased levels of D2HG, which alter the methylation of DNA and histones. Symmetric division of LRPs is consequently increased and/or their asymmetric division is decreased, leading to an expansion of this population. However, differentiation is not completely blocked, and normal proportions of megakaryocyte/erythroid progenitors (MEP), common myeloid progenitors (CMP), and granulocyte/macrophage progenitors (GMP) are found in these KI mice. It is likely that this expanded pool of LRP cells later acquires a second tumorigenic mutation that leads to further progression toward AML.

D2HG-mediated inhibition of histone demethylases. A partial block in cellular differentiation and/or an increase in symmetric cell division within the LRP pool may be responsible for the LRP accumulation observed in *IDH1*^{R132H} mice. In the current model, as the size of the LRP pool expands, the number of cells at risk for acquiring a second tumorigenic event increases, thereby raising the risk that these hematopoietic progenitors may give rise to AML (Fig. 5).

CNS IDH1 Mutant

To examine the effects of the *IDH1*^{R132H} mutation in the CNS, heterozygous IDH1^{R132H} KI mice were crossed with Nestin-Cre (Nes-Cre) mice to activate expression of the mutant enzyme during neuronal development (65). Under the control of Nestin, Cre is expressed as early as E10.5 in neural stem cells, and nearly complete recombination in all CNS cells is achieved by E12.5 (67). Nes-Cre IDH1^{R132H} mice were born at the expected Mendelian ratio but died shortly after birth due to massive cerebral hemorrhage, which could be observed as early as E14.5. Metabolic analysis revealed that α-KG levels were slightly reduced and D2HG levels dramatically increased in the brains of E14.5 Nes-Cre IDH1^{R132H} mice. Preliminary assessment of epigenetic differences showed that Nes-Cre IDH1^{R132H} mice exhibit reduced levels of 5-hydroxymethylcytosine in their DNA, consistent with D2HG-mediated inhibition of TET hydroxylases. These mutants also showed increased levels of HIF1α protein and upregulation of a set of HIF1 target genes, including *VEGF*. These results are consistent with D2HG-mediated inhibition of the HIF PHDs responsible for targeting HIF1α for degradation. In contrast, HIF1 levels and HIF1 target genes were not altered in hematopoietic cells in the LysM-Cre IDH1^{R132H} or Vav-Cre IDH1^{R132H} mice discussed above, suggesting that effects on HIF signaling may be context dependent (4).

An intriguing observation in Nes-Cre IDH1^{R132H} mice was that collagen synthesis and maturation are impaired in these mutants, as measured by the level of soluble collagen present in the developing brain. Type IV collagen is a crucial basement membrane component in the CNS and promotes appropriate interactions between astrocytes and endothelial cells. Basement membrane architecture was disrupted in Nes-Cre IDH1^{R132H} brains, especially around blood vessels. This disruption likely contributes to the brain hemorrhage in these mutants. In addition, it has been previously shown that disruption of collagen folding by suppression of PHD activity leads to ER stress (26). Indeed, the levels of several ER stress–responsive genes were elevated in the brains of Nes-Cre *IDH1*^{R132H} mice. Taken together, these D2HG-dependent alterations to developing neural stem and progenitor cells, and to the microenvironment-mediated interaction between astrocytes and endothelial cells, may be responsible for the brain hemorrhage phenotype observed in these mice (Fig. 6).

Future Mouse Models

Mouse models should continue to prove useful in defining the tumor-promoting events associated with IDH mutations.

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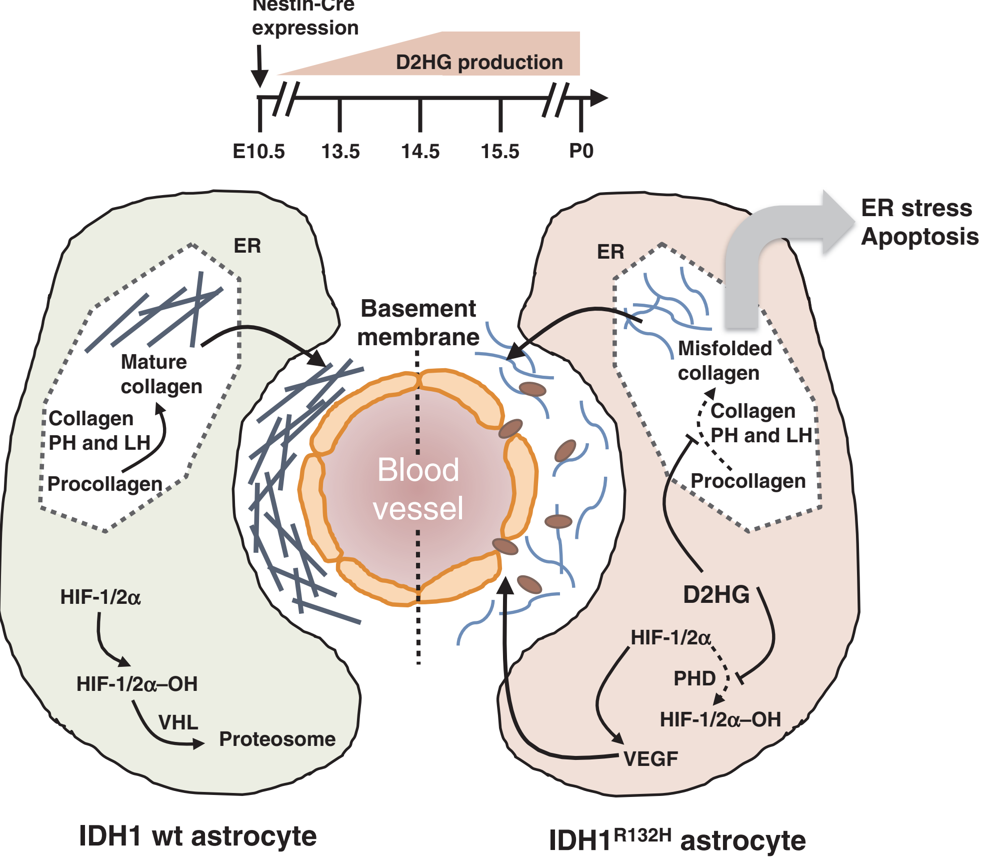

Figure 6. Model of the effects of *IDH1*^{R132H} mutations on the developing murine brain. Expression of mutant IDH1 in the developing brain causes D2HG to rise to high levels between day 10.5 and day 14.5 of embryonic development. Elevated D2HG inhibits appropriate hydroxylation of collagen, leading to alterations in the ECM. In addition, the accumulation of unfolded proteins in the ER produces ER stress, leading to apoptosis. High D2HG levels also inhibit HIF degradation, resulting in increased expression of HIF target genes such as *VEGF*. The combination of abnormal ECM and increased VEGF may impair vascular structure and function, precipitating the brain hemorrhage observed in these animals. wt, wild-type.

It will be interesting to examine the effects of combining these *IDH* mutations with other oncogenic mutations relevant to AML and glioma. Furthermore, the use of alternative promoters to drive Cre expression should allow the examination of the effect of *IDH* mutations on the cell types and tissues relevant to cholangiocarcinoma, chondrosarcoma, prostate cancer, and AITL. All these mouse models could also serve as accurate preclinical models for the evaluation of new therapeutic strategies targeting *IDH*-mutant disease in humans.

POTENTIAL CLINICAL APPLICATIONS

Direct Approaches

Although our understanding of the biology of *IDH* mutations is incomplete, researchers are already exploring several opportunities for clinical application. Sensitive and specific identification of *IDH* mutations by detection of mutant DNA, detection of mutant protein by immunohistochemistry (68), and measurement of D2HG levels are now all clinically feasible. The D2HG molecule itself can also be directly imaged using nuclear magnetic resonance spectroscopy, allowing for noninvasive mapping of D2HG accumulation in an affected tissue (69, 70). Although technically challenging, this approach may allow *IDH* status to be determined when biopsies are difficult to obtain. Once known, the *IDH* mutation status of a patient's tumor may serve as an important diagnostic and prognostic factor. For example, in glioma, knowledge of *IDH* mutation status complements histologic analysis and allows a pathologist to distinguish among tumors with similar pathologies. Furthermore, because *IDH* mutation status is a strong predictor of progression and outcome in glioma, its incorporation into prognostic models could improve clinical management of this disease. Similarly, for PTCLs, *IDH* mutation status may aid in distinguishing AITL from other PTCL diseases. In AML, D2HG is being evaluated as a biomarker of disease burden and therapy response in the hope that it may prove to be a more accurate and sensitive indicator of disease activity than currently available approaches (71).

It may also soon be possible to develop therapeutic strategies that specifically target *IDH*-mutant disease. Inhibition of the abnormal enzymatic activity of mutant IDH1 or IDH2 could permit very specific targeting of the tumor (as opposed to normal tissue), with the therapeutic window limited only by the specificity of a compound for the mutant enzyme. Studies on the first generation of compounds targeting the neoactivity of mutant IDH enzymes have been reported,

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and these agents seem to display exquisite specificity for the mutant IDH activity (72). Moreover, these compounds have shown biochemical activity *in vivo*, in that they are able to reduce D2HG production in IDH1-overexpressing xenograft tumors (72). Recent reports have also shown that IDH1 and IDH2 small-molecule inhibitors can reverse the transforming effects of *IDH1* mutations, and promote cellular differentiation in leukemia and glioma model systems (57, 73, 74). What now remains is to determine whether inhibition in established tumors results in an antitumor effect or increases tumor sensitivity to other therapies. Testing of these novel compounds in *IDH*-mutant mouse models should provide direct answers to some of these questions. The presence of D2HG should serve as a powerful predictive biomarker to identify those who could potentially benefit from IDH-targeted therapies. D2HG may also be a useful pharmacodynamic biomarker that can be used to monitor therapy response. Finally, an effective and specific IDH2 inhibitor could provide an effective therapy for the 50% of patients whose D2HG aciduria is caused by a germline *IDH2* mutation.

Indirect Approaches

Beyond direct targeting of mutant IDH enzymatic activity, there may be other opportunities to successfully treat *IDH*-mutant tumors. Because of the highly elevated levels of D2HG in these patients and its pleiotropic effects, *IDH*-mutant tumors may be particularly sensitive to other therapeutic approaches. For example, the dramatic effects of *IDH* mutations on the epigenetic state of tumor cells may present options for effective epigenetic-targeted therapy. The identification of a number of recurrent mutations in MPN, MDS, and AML that alter epigenetic regulation (including *IDH* mutations) has led to increased interest in therapeutically targeting DNA and histone methylation in these diseases. The DNA-demethylating agents 5-azacytidine (5-Aza-CR) and 5-aza-2′-deoxycytidine (5-Aza-CdR, decitabine) act as DNA methyltransferase inhibitors and are already used in treating patients with high-risk MDS and AML (75). Further development of more specific epigenetic-modulating agents that can serve as useful tools in the management of *IDH*-mutant diseases is underway. A greater understanding of the effects of *IDH* mutations on tumor metabolism and physiology may reveal other potential opportunities to treat patients bearing these mutations.

CONCLUSION

It has been less than 5 years since *IDH1* mutations were first discovered in glioblastoma. In this relatively short time, clinical evaluation of thousands of patient samples has defined the set of neoplastic diseases in which *IDH* mutations play an important role (Fig. 7). Mechanistic studies have discovered that these mutations cause the IDH protein to acquire a unique enzymatic activity that generates high levels of a previously little-studied metabolite, D2HG. Strong biochemical, cell culture, and clinical evidence has been gathered that has defined the effects of D2HG on the 2-OG–dependent dioxygenases, and has implicated alterations to a number of biologic processes as contributors to malignant progression in *IDH*-mutant tumors. The first mutant IDH KI and IDH loss-of-function mouse strains have been developed and

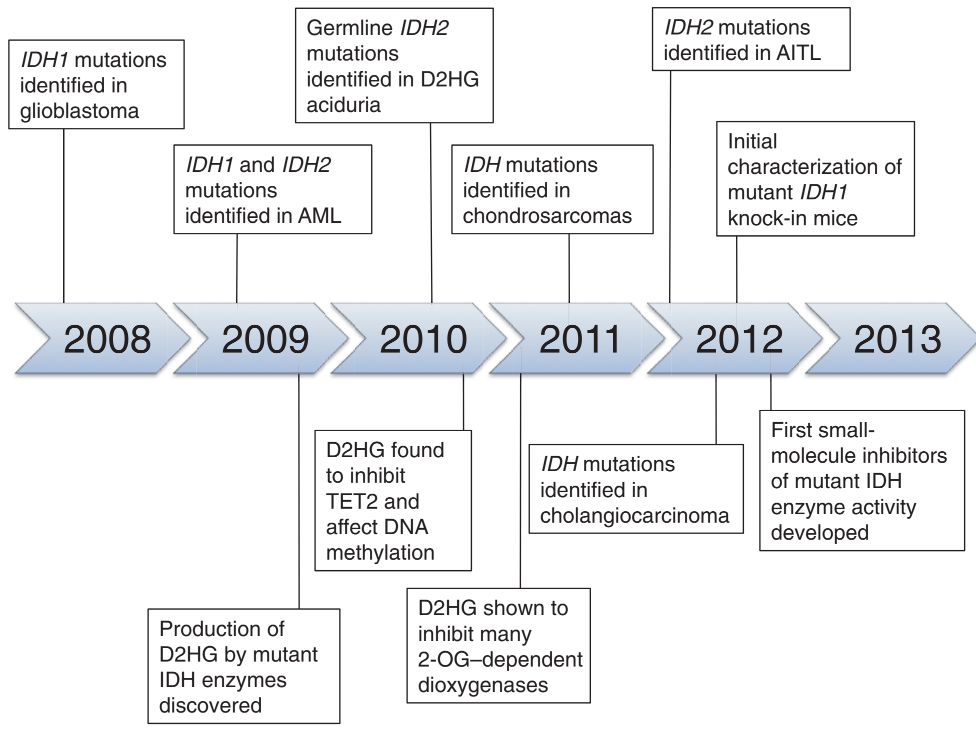

Figure 7. Time line illustrating many of the key clinical and mechanistic discoveries made during the investigation of cancer-associated *IDH* mutations.

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characterized, and will continue to serve as powerful experimental models of these diseases. *IDH* mutation detection and D2HG measurement have already been incorporated into clinical practice in a number of settings and are under evaluation for their use as tools in the management of several diseases. Finally, the first specific small-molecule inhibitors of mutant IDH enzymes have been developed, raising hope for a new and effective treatment strategy for tumors bearing these mutations. While many questions remain regarding the role of *IDH* mutations in tumorigenesis, the stage is set for rapid progress in getting answers in the years to come.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R.A. Cairns, T.W. Mak
Writing, review, and/or revision of the manuscript: R.A. Cairns, T.W. Mak

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