

REVIEW

What a difference a hydroxyl makes: mutant IDH, (*R*)-2-hydroxyglutarate, and cancer

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Mutations in metabolic enzymes, including isocitrate dehydrogenase 1 (IDH1) and IDH2, in cancer strongly implicate altered metabolism in tumorigenesis. IDH1 and IDH2 catalyze the interconversion of isocitrate and 2-oxoglutarate (2OG). 2OG is a TCA cycle intermediate and an essential cofactor for many enzymes, including JmjC domain-containing histone demethylases, TET 5-methylcytosine hydroxylases, and EglN prolyl-4-hydroxylases. Cancer-associated IDH mutations alter the enzymes such that they reduce 2OG to the structurally similar metabolite (*R*)-2-hydroxyglutarate [(*R*)-2HG]. Here we review what is known about the molecular mechanisms of transformation by mutant IDH and discuss their implications for the development of targeted therapies to treat IDH mutant malignancies.

Cellular metabolism has been hypothesized to play a central role in cancer since the observation made almost a century ago by Otto Warburg (Warburg 1956) that cancer cells preferentially generate energy by metabolizing glucose to lactate. Even in the presence of oxygen, cancer cells switch from generating ATP by the highly energy-efficient process of oxidative phosphorylation to the much less efficient process of glycolysis (Vander Heiden et al. 2009; Dang 2012). Why this switch occurs has long been a mystery, although the observation that normal cells use “aerobic glycolysis” during periods of increased proliferation supports the hypothesis that this metabolic switch is an important feature of rapidly dividing cells (Locasale and Cantley 2011; Lunt and Vander Heiden 2011). Recent work suggests that aerobic glycolysis facilitates cellular transformation by producing the high levels of glycolytic intermediates that proliferating cells need for the biosynthesis of lipids, amino acids, and nucleic acids. Moreover, metabolic reprogramming appears to be sufficient to mediate tumorigenesis in some systems (Lyssiotis and Cantley 2012; Sebastian et al. 2012). Nevertheless,

whether altered cellular metabolism is a cause of cancer or merely an adaptive response of cancer cells in the face of accelerated cell proliferation is still a topic of some debate.

The recent identification of cancer-associated mutations in three metabolic enzymes suggests that altered cellular metabolism can indeed be a cause of some cancers (Pollard et al. 2003; King et al. 2006; Raimundo et al. 2011). Two of these enzymes, fumarate hydratase (FH) and succinate dehydrogenase (SDH), are bone fide tumor suppressors, and loss-of-function mutations in FH and SDH have been identified in various cancers, including renal cell carcinomas and paragangliomas. The third mutated enzyme, isocitrate dehydrogenase (IDH), is a more complicated case. Mutations in two isoforms of IDH, IDH1 and IDH2, are common in a diverse array of cancers, including gliomas and acute myelogenous leukemia (AML) (Dang et al. 2010). The mutant enzymes are not catalytically inactive. Rather, the cancer-associated mutations alter the catalytic activity of the enzymes such that they produce high levels of a metabolite, (*R*)-2-hydroxyglutarate [(*R*)-2HG], which is normally found at very low levels in cells (Dang et al. 2009; Ward et al. 2010). How this aberrant enzymatic activity contributes to cellular transformation is an area of intense interest and some controversy, and several hypotheses have been proposed for how IDH mutations promote tumorigenesis. It has been suggested that (*R*)-2HG functions as an “oncometabolite” to promote cellular transformation, perhaps by altering the redox state of cells or modulating the activity of metabolic and epigenetic tumor suppressor enzymes that use the structurally similar metabolite α -ketoglutarate as a cosubstrate (Reitman and Yan 2010; Yen et al. 2010; Kaelin 2011). Others have posited that loss of IDH activity from either loss of the wild-type allele or gain of a dominant-negative mutant allele alters normal mitochondrial function and promotes the metabolic switch of cancer cells to glycolysis (Leonardi et al. 2012; Oermann et al. 2012).

Here, we review the spectrum of tumors that harbor IDH mutations and discuss what has been learned to date about how mutant IDH and (*R*)-2HG contribute to

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tumorigenesis. We also discuss the potential of mutant IDH as a therapeutic target in cancer and discuss some of the outstanding questions about mutant IDH biology that warrant further investigation.

Normal functions of the IDH family of metabolic enzymes

Eukaryotic cells express three different isoforms of IDH (Fig. 1; Dalziel 1980). IDH1 and IDH2 are homodimeric NADP⁺-dependent enzymes that catalyze the oxidative decarboxylation of isocitrate to produce α -ketoglutarate (also known as 2-oxoglutarate [2OG]), NADPH, and CO₂ (Fig. 2). IDH3 is a structurally unrelated heterotetrameric NAD⁺-dependent enzyme that similarly decarboxylates isocitrate and produces 2OG, NADH, and CO₂. The reactions catalyzed by IDH1 and IDH2 are reversible, and the directionality of the reactions therefore depends in large part on the relative K_m values of the forward and reverse reactions and the relative levels of isocitrate and 2OG in the cell (Lemons et al. 2010). The reaction catalyzed by IDH3, on the other hand, is irreversible under physiologic conditions and is principally regulated by substrate availability and positive and negative allosteric effectors. Calcium, ADP, and citrate activate IDH3, whereas ATP, NADH, and NADPH inhibit this enzyme (Gabriel et al. 1986).

The different IDH isoforms have overlapping, but non-redundant, roles in cellular metabolism (Fig. 1; Mailloux et al. 2007; Reitman and Yan 2010). IDH1 localizes to the cytoplasm and peroxisomes and plays a role in promoting the activity of the numerous cytoplasmic and nuclear dioxygenases that require 2OG as a cosubstrate (Hausinger 2004). Another important function of IDH1 is the generation of nonmitochondrial NADPH. NADPH provides critical reducing equivalents needed for lipid biosynthesis and is also an essential antioxidant that protects cells from oxidative stress and radiation damage (Jo et al. 2002; Lee et al. 2002; Kim et al. 2007). Finally, as the reaction catalyzed by IDH1 is reversible, IDH1 is capable of catalyzing the reductive carboxylation of 2OG to isocitrate, which can then be further metabolized to acetyl-CoA to support lipid biosynthesis (Koh et al. 2004; Filipp et al. 2012; Metallo et al. 2012).

IDH2 and IDH3, in contrast, localize to the mitochondrial matrix (Fig. 1). IDH3 plays a central role in mitochondrial respiration by catalyzing one of the rate-limiting steps of the TCA cycle. The 2OG produced by IDH3 is further metabolized to succinate, and the NADH is used by the electron transport chain to generate ATP (Barnes et al. 1971). It is not clear whether IDH2 likewise contributes to flux through the TCA cycle. Rather, IDH2 is thought to regulate energy metabolism by modulating the relative abundance of isocitrate and 2OG in the mitochondria

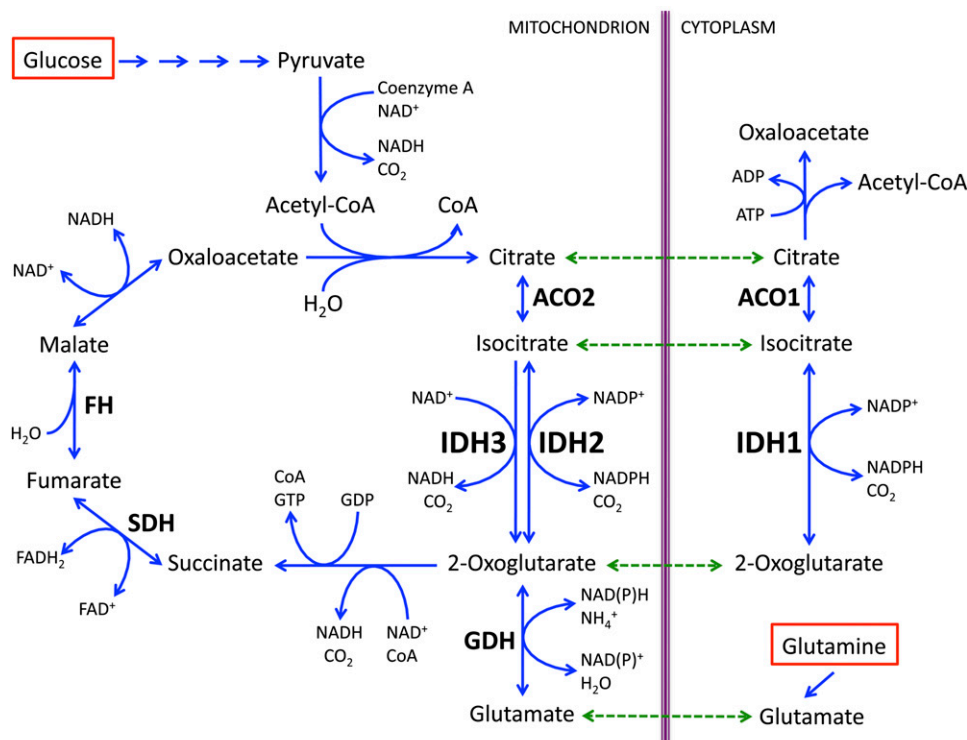


Figure 1. The role of the IDH family of enzymes in the TCA cycle. Shown here are the mitochondrial and cytoplasmic reactions that involve IDH; also shown are the principal mechanisms of entry of glucose and glutamine-derived carbon molecules into the TCA cycle. IDH1 reversibly catalyzes the NADP⁺-dependent decarboxylation of isocitrate to 2OG in the cytoplasm, whereas IDH2 mediates the same reaction in the mitochondria. IDH3 catalyzes the NAD⁺-dependent conversion of isocitrate to 2OG in the mitochondria in a reaction that is irreversible under physiologic conditions. (ACO) Aconitase.

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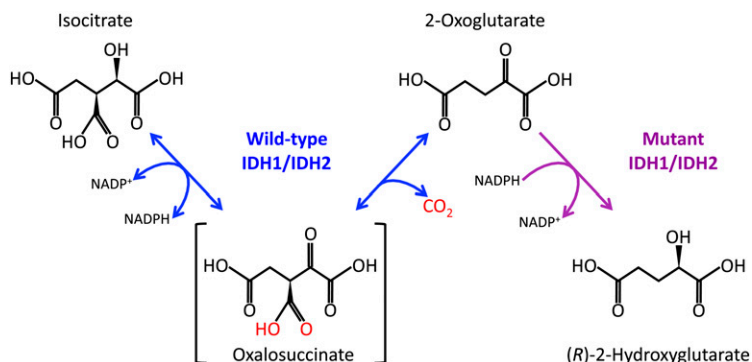


Figure 2. Reactions catalyzed by wild-type and mutant IDH1 and IDH2. Wild-type IDH1 and IDH2 catalyze a two-step reaction (indicated by blue arrows). The first step involves the oxidation of isocitrate to the unstable intermediate oxalosuccinate. In this reaction, NADP⁺ acts as the hydrogen acceptor and is reduced to NADPH. In the second step of the reaction, the β -carboxyl group is released as carbon dioxide (CO₂), resulting in production of 2OG. Mutant IDH1 and IDH2 catalyze a single-step reaction (indicated by the purple arrow). In this reaction, NADPH is oxidized to NADP⁺, with concomitant reduction of 2OG to (R)-2HG. The mutant enzymes are unable to catalyze the carboxylation of (R)-2HG and therefore cannot generate 2OG. 2OG and 2HG are structurally very similar. They differ only in the replacement of the ketone group in 2OG with a hydroxyl group in 2HG.

(DeBerardinis et al. 2008). Under conditions where levels of mitochondrial glutamate are high or under hypoxic conditions when glucose-dependent citrate production is compromised, glutamate dehydrogenase (GDH) converts glutamate to 2OG (Sazanov and Jackson 1994; Comte et al. 2002). The resultant increase in the 2OG:isocitrate ratio favors the reverse IDH2 reaction, which results in conversion of 2OG and NADPH to isocitrate and NADP⁺. This isocitrate can then be fed into the TCA cycle by IDH3, which converts the isocitrate back to 2OG and produces additional NADH, or isomerized by aconitase to produce citrate. Citrate, in addition to being a TCA cycle intermediate, is a critical metabolite required for fatty acid biosynthesis. Accordingly, IDH2-mediated reductive carboxylation of 2OG plays an important role in sustaining energy production and promoting cell proliferation during periods of hypoxia (Wise et al. 2011; Filipp et al. 2012). IDH2, as an important source of mitochondrial NADPH, also plays a critical role in the protection of cells from mitochondrial-specific oxidative stress, such as that generated by the electron transport chain (Lee et al. 2004, 2007).

IDH mutations in human cancer

Glioblastoma multiforme (GBM) is a highly invasive brain tumor that is notoriously refractory to chemotherapy and radiation therapy. In 2008, in an effort to identify novel therapeutic targets in GBM, Parsons et al. (2008) undertook a landmark genomic sequencing effort of 22 adult primary and secondary GBM tumors. They identified recurrent somatic mutations at Arg132 of IDH1 that were present in five of the six secondary GBMs but none of the 16 primary GBMs included in the study. Several follow-up sequencing studies of malignant gliomas confirmed that IDH1 Arg132 mutations are very common in specific types of adult brain tumors, occurring in >70% of adult grade II and grade III gliomas and >80% of adult secondary GBMs (Balss et al. 2008; Bleeker et al. 2009). Moreover, many of the grade II/III gliomas and secondary GBMs that are IDH1 wild type harbor mutations at the analogous codon, Arg172, of IDH2 (Hartmann et al. 2009; Yan et al. 2009). Overall, 80%–90% of adult grade II/III

gliomas and secondary GBMs harbor mutations at either Arg132 of IDH1 or Arg172 of IDH2 (Table 1; Chang et al. 2011). Conversely, <10% of primary GBMs and pediatric GBMs harbor IDH mutations, and no IDH mutations have been identified to date in brain tumors of nonglial subtypes (Balss et al. 2008; Yan et al. 2009; Capper et al. 2010; Byeon et al. 2012).

In 2009, Mardis et al. (2009) performed whole-genome sequencing of a case of normal karyotype AML (NK-AML) and identified a mutation at Arg132 of IDH1. Further sequencing efforts confirmed that IDH mutations are highly recurrent in clonal myeloid disorders. Mutations in IDH1 and IDH2 are present in 5%–20% of cases of de novo NK-AML and in 10%–20% of cases of secondary AML that result from leukemic transformation of “pre-malignant” myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN) (Kosmider et al. 2010; Pardanani et al. 2010; Tefferi et al. 2010; Patnaik et al. 2012; Rakheja et al. 2012; Zhou et al. 2012). IDH mutations are also present, albeit at a lower frequency (5%–10%), in chronic-phase MDS and MPN but are rare in translocation-positive AML (Mardis et al. 2009; Abbas et al. 2010). IDH2 mutations are also present in 10%–40% of cases of angioimmunoblastic T-cell lymphoma (AITL) but are uncommon in other T- or B-cell lymphoid malignancies (Cairns et al. 2012; Zhang et al. 2012).

IDH mutations have been found in a number of other solid tumors besides gliomas. Over 50% of chondrosarcomas harbor IDH mutations (Amary et al. 2011a; Arai et al. 2012; Meijer et al. 2012), and IDH mutations have been linked to the pathogenesis of the enchondromatosis syndromes Ollier disease and Maffucci syndrome (Pansuriya et al. 2010). Ollier disease and Maffucci syndrome are rare nonhereditary pediatric cancer predisposition syndromes in which affected children develop cartilaginous tumors as their developing bones undergo endochondral ossification. Recently, sequencing analysis has determined that 40%–90% of the tumors in these patients harbor mutations at Arg132 of IDH1 or Arg172 of IDH2 (Amary et al. 2011b; Pansuriya et al. 2011). Interestingly, many of these patients develop multiple independent IDH mutant tumors but express wild-type IDH1 and IDH2 in their normal tissues. This suggests that their IDH mutations are occurring

Table 1. Frequency of *IDH1* and *IDH2* mutant alleles in human cancers

Tumor type	IDH mutated	IDH1 ^{mut} :IDH2 ^{mut}	Mutant alleles	Allele frequency	Reference
Grade II/III glioma 2° GBM	80%–90%	20:1	IDH1 R132H	85%–90%	Chang et al. 2011 Pusch et al. 2011
			<i>IDH1</i> R132C/S/L/G/V	5%–8%	
			<i>IDH1</i> R100Q	<1%	
			<i>IDH2</i> R172K/M/W/S/G	3%–5%	
NK-AML secondary AML	10%–30%	1:1 - 1:2	<i>IDH1</i> R132H	10%–20%	Abbas et al. 2010 Marcucci et al. 2010 Paschka et al. 2010 Koszarska et al. 2012
			<i>IDH1</i> R132C/S/L/G/P	15%–30%	
			IDH2 R140Q	30%–50%	
			<i>IDH2</i> R140W/L	3%–5%	
			<i>IDH2</i> R172K/G/M/Q	10%–15%	
AITL	10%–40%	IDH2 only	IDH2 R172K	60%	Cairns et al. 2012
			<i>IDH2</i> R172G/T/S	35%	
			<i>IDH2</i> R140G	< 5%	
Chondrosarcoma	50%–70%	20:1	IDH1 R132C	40%–50%	Amary et al. 2011a Pansuriya et al. 2011 Arai et al. 2012
			<i>IDH1</i> R132G/H/L/S	30%–50%	
			<i>IDH2</i> R172S/T	5%–10%	
Cholangiocarcinoma	10%–20%	10:1	IDH1 R132C	50%–60%	Borger et al. 2012
			<i>IDH1</i> R132L/G	30%–40%	
			<i>IDH2</i> R172W	10%	

Listed are the frequencies of the different *IDH* mutant alleles in IDH mutant malignancies. The most frequent alleles are indicated in bold. The results outlined are from meta analyses (Chang et al. 2011) and studies that included large numbers of patients (Abbas et al. 2010; Marcucci et al. 2010; Paschka et al. 2010; Amary et al. 2011a; Pansuriya et al. 2011; Pusch et al. 2011; Arai et al. 2012; Borger et al. 2012; Cairns et al. 2012; Koszarska et al. 2012).

during embryonic development and that their tumors are a consequence of somatic mosaicism of mutant IDH-expressing cells. Interestingly, Ollier disease and Mafucci syndrome appear to be associated with an increased risk of secondary neoplasms; in particular, gliomas and AML. IDH mutations have also been found in 10%–20% of cholangiocarcinomas as well as a few rare cases of paraganglioma, colon cancer, prostate cancer, and lung cancer (Sjoblom et al. 2006; Bleeker et al. 2009; Kang et al. 2009; Gaal et al. 2010; Sequist et al. 2011; Borger et al. 2012).

Among the cancers associated with IDH mutations, the mutations appear to have a predilection for certain tumor subtypes or locations. In cholangiocarcinoma, the frequency of IDH mutations in intrahepatic tumors is 28%, whereas the frequency is only 7% in extrahepatic tumors (Borger et al. 2012). In chondrosarcomas, IDH mutations appear to be exclusive to central, periosteal, and dedifferentiated tumors, with no IDH mutations having been identified to date in peripheral chondrosarcomas or other cartilaginous tumors (Amary et al. 2011a; Arai et al. 2012).

IDH1 and IDH2 mutations occur at different frequencies in different tumor types (Table 1). In gliomas, >80% of IDH mutations occur at Arg132 of IDH1 and result in replacement of the arginine residue with a histidine (IDH1 R132H) (Balss et al. 2008; Yan et al. 2009; Chang et al. 2011). In cholangiocarcinomas and chondrosarcomas, IDH1 Arg132 mutations are also far more common than IDH2 Arg172 mutations, although the IDH1 mutations in these tumors most often result in an arginine-to-cysteine substitution (IDH1 R132C) (Amary et al. 2011a; Arai et al. 2012; Borger et al. 2012). This is in contrast to AML, where IDH1 and IDH2 mutations occur at similar

frequencies (Abbas et al. 2010; Marcucci et al. 2010; Paschka et al. 2010; Koszarska et al. 2012). The single most common IDH mutation in AML is the replacement of Arg140 of IDH2 with a glutamine (IDH2 R140Q). Interestingly, IDH2 Arg140 mutations have not been found in gliomas, cholangiocarcinomas, or chondrosarcomas. However, an IDH1 mutation analogous to Arg140 of IDH2, IDH1 Arg100, has been described in rare cases of glioma (Pusch et al. 2011). In AITL, all of the IDH mutations reported to date are IDH2 mutations, with >90% occurring at IDH2 Arg172 (Cairns et al. 2012). Why the frequency of these different amino acid substitutions is so variable in different tumors is not known. It is also worth noting that IDH1 and IDH2 mutations appear to be mutually exclusive. There are no reported cases of brain tumors harboring both mutations, and although rare cases of IDH1/IDH2 double mutant AML have been reported, it is not clear whether these mutations occur in the same or different leukemic subclones (Paschka et al. 2010).

The prognostic significance of IDH mutations is not consistent across all tumor types. In the case of GBM, IDH mutations appear to confer a more favorable prognosis, with a median survival for patients with IDH mutant GBM of 31 mo, as compared with a median survival of 15 mo for patients with IDH wild-type GBM (Yan et al. 2009; SongTao et al. 2012). Whether this difference is driven by the IDH mutational status of the tumors or reflects other fundamental biological differences between primary and secondary GBM is as yet unclear. In AML, the prognostic significance of IDH mutations is still somewhat ambiguous. Several studies have reported that IDH mutational status does not impact prognosis in AML, whereas other studies have found that IDH mutations are associated with

an increased or decreased risk of disease relapse when compared with IDH wild-type disease, depending on the specific patient population examined (Abdel-Wahab et al. 2011; Rakheja et al. 2012; Zhou et al. 2012). In MDS and MPN, IDH mutations have been consistently found to be markers of poor prognosis. Furthermore, the observation that the frequency of IDH mutations is higher in late stage MPN and high-risk MDS (20%) than in early stage and low-risk disease (<4%), coupled with the observation that IDH mutations are frequently present in secondary AML, has led to speculation that IDH mutations are involved in the progression of chronic MDS and MPN to full-blown leukemia. (Tefferi et al. 2010, 2012; Thol et al. 2010; Patnaik et al. 2012).

Catalytic activity of mutant IDH

When IDH mutations were first identified in cancer, they were hypothesized to contribute to tumorigenesis by causing loss of function and dominant-negative inhibition of wild-type IDH activity (Yan et al. 2009; Zhao et al. 2009). However, subsequent studies found that, on the contrary, tumor-associated IDH mutations cause a gain of function (Dang et al. 2009; Ward et al. 2010). The mutations are all located in the active sites of the enzymes. Arg100 and Arg132 in IDH1 and Arg140 and Arg172 in IDH2 form hydrogen bonds with the α -carboxyl and β -carboxyl groups of isocitrate and mediate isocitrate binding (Xu et al. 2004). Mutations at these residues decrease the binding affinity of the enzyme active sites for isocitrate and increase their binding affinity for NADPH, which significantly abrogates the normal oxidative decarboxylation activity of the enzymes (Dang et al. 2009). In addition to abrogating the “forward” catalytic activity of IDH, the mutations change the conformation of the enzyme active site such that the “reverse” reaction is only a partial reaction, one in which 2OG is reduced but not carboxylated. This results in conversion of 2OG to (R)-2HG rather than isocitrate (Fig. 2). In short, IDH mutations cause the reverse IDH reaction to be favored but partially disrupted, leading to high-level production of (R)-2HG.

Interestingly, although IDH1 and IDH2 mutants both produce (R)-2HG, they appear to have somewhat distinct enzymatic properties. Cells that express mutant IDH1 accumulate less (R)-2HG than do mutant IDH2-expressing cells (Ward et al. 2013). Moreover, (R)-2HG production by mutant IDH1 is enhanced by coexpression of wild-type IDH1, whereas mutant IDH2-expressing cells accumulate high levels of (R)-2HG independent of wild-type IDH2 activity. It has been hypothesized that the lower production of (R)-2HG by mutant IDH1 is due to the fact that cytoplasmic 2OG is limiting in cells, whereas mitochondrial 2OG is not. Consistent with this model, the addition of a mitochondrial localization signal to mutant IDH1 results in enhanced (R)-2HG production. Interestingly, mutant IDH1 forms heterodimers with wild-type IDH1, but mutant IDH2 does not bind wild-type IDH2 (Dang et al. 2009; Ward et al. 2013). It has been hypothesized that heterodimerization of wild-type and mutant

IDH1 in the cytoplasm provides the mutant subunit with a local source of 2OG that can then be metabolized to (R)-2HG. Consistent with this model, it has recently been reported that loss of the wild-type *IDH1* allele in IDH1 mutant gliomas is associated with significantly lower tumor levels of (R)-2HG than are seen in heterozygous *IDH1* mutant tumors (Jin et al. 2013).

Mutant IDH is an oncogene

In a number of different in vitro transformation assays, expression of mutant IDH promotes the proliferation and inhibits the differentiation of cells. Expression of a tumor-derived IDH1 mutant (IDH1 R132H), but not a catalytically inactive form of the mutant enzyme, enhances the proliferation and soft agar colony formation of immortalized human astrocytes and confers growth factor independence and blocks the erythropoietin-induced differentiation of TF-1 cells, a human erythroleukemia cell line (Koivunen et al. 2012; Losman et al. 2013). Expression of mutant IDH is also able to inhibit the differentiation of murine 3T3-L1 fibroblasts and the differentiation of primary murine hematopoietic stem cells and immortalized murine myeloid progenitor cells (Figueroa et al. 2010; Lu et al. 2012; Losman et al. 2013).

Although mutant IDH is able to promote cellular transformation in vitro, the mutant enzyme does not appear to be sufficient to transform primary cells in vivo. Sasaki et al. (2012a,b) have characterized the phenotype of a conditional knock-in mouse model in which they inserted *IDH1* R132H into the endogenous *IDH1* locus and expressed mutant IDH in either the brains or the hematopoietic systems of mice. Brain-specific expression of IDH1 R132H during embryogenesis results in perinatal lethality of the mice from cerebral hemorrhage without evidence of underlying malignancy (Sasaki et al. 2012a). Although it is possible that the mice would develop brain tumors if they lived longer, the hematopoietic-specific *IDH1* R132H knock-in mice, which have a normal life span, similarly do not develop malignancy (Sasaki et al. 2012b).

Despite the lack of a cancer phenotype in the *IDH1* R132H knock-in mice, the hematopoietic stem cell phenotype of the mice does provide some insights into how mutant IDH contributes to tumorigenesis. Although IDH1 R132H-expressing bone marrow cells are not immortalized, the mice do show selective expansion of their hematopoietic stem and early progenitor compartments but not their committed myeloid and lymphoid progenitor compartments. This observation is quite interesting given the distinct self-renewal properties of these different cell types. Normal committed progenitors are unable to self-renew and are consigned to undergo further differentiation, whereas stem cells and early progenitors have inherent self-renewal capabilities (Chao et al. 2008; Orford and Scadden 2008; Seita and Weissman 2010). When stem cells and early progenitors divide, they make the decision to undergo either symmetric division, in which both daughter cells are identical to the original cell, or asymmetric division, in which one daughter cell is identical to the original cell and the other is committed to undergoing

further differentiation. The balance between symmetric and asymmetric cell division is crucial to cellular homeostasis. Excessive symmetric division causes inappropriate expansion of stem cell compartments, whereas excessive asymmetric division results in stem cell exhaustion (Jacob and Osato 2009; Marciniak-Czochra et al. 2009; Matsumoto and Nakayama 2013). The observation that only stem and early progenitor cells are expanded in the *IDH1* R132H knock-in mice suggests that mutant IDH promotes leukemogenesis by increasing symmetric division within a population of cells that have inherent self-renewal capacity but that mutant IDH is not, by itself, able to immortalize self-renewing cells or bestow self-renewal upon more differentiated cells. This observation has important biological implications. The limited ability of mutant IDH to promote the proliferation of primary cells suggests that the specific cell lineage in which the IDH mutation occurs significantly influences the ability of the mutation to contribute to transformation. An IDH mutation in a committed progenitor cell would likely not confer a proliferative advantage to that cell, whereas the same mutation in a more primitive cell would contribute to clonal expansion.

It is also notable that hematopoietic stem cells isolated from *IDH1* R132H knock-in mice are able to differentiate normally *in vitro* and are able to competitively repopulate the bone marrow of transplanted recipient mice. This suggests that expression of mutant IDH has little if any effect on cellular differentiation in otherwise normal hematopoietic cells. This limited ability of mutant IDH to block the differentiation of normal primary cells suggests that other specific mutations that cooperate with mutant IDH to block cellular differentiation are likely to be required to bring about the maturation arrest that is characteristic of leukemia cells.

Mutant IDH-derived (*R*)-2HG is an oncometabolite

2HG is a five-carbon dicarboxylic acid with a chiral center at the second carbon atom (Fig. 2). There are therefore two possible enantiomers of 2HG: (*R*)-2HG [otherwise known as (*D*)-2HG] and (*S*)-2HG [otherwise known as (*L*)-2HG]. (*R*)-2HG and (*S*)-2HG are both byproducts of normal mitochondrial metabolism (Kranendijk et al. 2012). The principal source of (*R*)-2HG in IDH wild-type cells is a reaction catalyzed by hydroxyacid-oxoacid transhydrogenase (HOT) (Struys et al. 2005b). HOT converts γ -hydroxybutyrate (GHB) to succinic semialdehyde (SSA), with concomitant reduction of 2OG to (*R*)-2HG. (*S*)-2HG is generated during conversion of oxaloacetate to (*L*)-malate by (*L*)-malate dehydrogenase, an enzyme of the TCA cycle (Rzem et al. 2007).

(*R*)-2HG and (*S*)-2HG are believed to be unwanted byproducts of cellular metabolism, and their intracellular levels in normal cells are maintained at <0.1 mM. 2HG is prevented from accumulating in cells by the actions of two enzymes—(*D*)-2HG and (*L*)-2HG dehydrogenase (2HGDH)—that convert (*R*)-2HG and (*S*)-2HG, respectively, back to 2OG (Struys et al. 2005a; Steenweg et al. 2010). There is currently no known physiologic role for either enantiomer in normal metabolism.

IDH mutants exclusively produce the (*R*) enantiomer of 2HG, and the levels of (*R*)-2HG in IDH mutant tumors can be extremely elevated, ranging from 1 mM to as high as 30 mM (Dang et al. 2009; Gross et al. 2010; Choi et al. 2012). These high levels of (*R*)-2HG appear to be a consequence of a significant imbalance between (*R*)-2HG production and metabolism in tumor cells. IDH1 R132H has an estimated catalytic rate (K_{cat}) of $1.0 \times 10^3 \text{ sec}^{-1}$ (Dang et al. 2009), whereas recombinant D2HGDH has an estimated K_{cat} of 0.8 sec^{-1} (Engqvist et al. 2009). It is therefore likely that mutant IDH overwhelms the capacity of D2HGDH to oxidize the excess (*R*)-2HG back to 2OG. This model is supported by the observation that in patients with type I (*D*)-2-hydroxyglutaric aciduria (D2HGA), an inborn neurometabolic disorder caused by germline mutations in D2HGDH, levels of (*R*)-2HG are elevated but to a much lesser extent than is observed in IDH mutant tumors (Wickenhagen et al. 2009). Type II D2HGA, which is caused by germline IDH2 R140Q and IDH2 R140G mutations, is associated with higher levels of (*R*)-2HG and a more severe clinical course (Kranendijk et al. 2010, 2011). Interestingly, D2HGA is not associated with an increased incidence of cancer. It is important to note, however, that many patients with severe D2HGA die in infancy and early childhood, and it is therefore difficult to make definitive conclusions about an association between high levels of (*R*)-2HG and long-term susceptibility to cancer.

High intracellular concentrations of (*R*)-2HG are sufficient to mediate the *in vitro* transforming effects of mutant IDH. Treatment of TF-1 leukemia cells with concentrations of a cell-permeable form of (*R*)-2HG that achieve tumor-relevant intracellular levels is able to recapitulate the effects of mutant IDH expression (Losman et al. 2013), and treatment of cells with cell-permeable (*R*)-2HG is able to inhibit the differentiation of murine 3T3-L1 fibroblasts and immortalized murine myeloid progenitor cells (Lu et al. 2012; Losman et al. 2013). Furthermore, tumor cells that harbor IDH mutations and produce high levels of (*R*)-2HG appear to require continuous (*R*)-2HG to remain transformed. Withdrawal of (*R*)-2HG from TF-1 leukemia cells transformed by cell-permeable (*R*)-2HG reverses their growth factor independence and restores their ability to differentiate (Losman et al. 2013), and inhibition of mutant IDH1 expression in glioma cell lines that harbor naturally occurring IDH1 R132H mutations impairs *in vitro* colony formation and *in vivo* tumor engraftment by the cells (Rohle et al. 2013). Finally, highly potent and specific inhibitors of mutant IDH have been developed that are able to reverse the transformation of TF-1 cells expressing IDH1 R132H and IDH2 R140Q (Popovici-Muller et al. 2012; Losman et al. 2013; Wang et al. 2013). These inhibitors also induce the differentiation of primary IDH mutant human leukemia cells *in vitro* and recapitulate the effects of suppression of IDH1 R132H expression in IDH mutant glioma cell lines *in vitro* and *in vivo* (Rohle et al. 2013; Wang et al. 2013). Taken together, these findings suggest that the transforming activity of mutant IDH is mediated by (*R*)-2HG and that

(*R*)-2HG is necessary to maintain the transformed phenotype of tumor cells that harbor IDH mutations.

Mechanisms of transformation by (*R*)-2HG

Several mechanisms of mutant IDH-mediated transformation have been proposed. One model for how mutant IDH contributes to tumorigenesis, and the one that has perhaps gained the most traction since the discovery of (*R*)-2HG, is transformation by 2OG antagonism. (*R*)-2HG is structurally and chemically very similar to 2OG (Fig. 2), and it has been proposed that (*R*)-2HG transforms cells by competitively inhibiting 2OG-dependent enzymes that function as tumor suppressors (Chowdhury et al. 2011; Xu et al. 2011; Koivunen et al. 2012; Lu et al. 2012). There are ~70 known and putative 2OG-dependent dioxygenases in the GenBank DNA database that could be relevant targets of (*R*)-2HG in mutant IDH-mediated transformation (Table 2).

TET2

The myeloid tumor suppressor *TET2* is a particularly intriguing candidate to be a pathogenically relevant target of (*R*)-2HG in IDH mutant tumors. *TET1*, *TET2*, and *TET3* are members of a family of 2OG-dependent DNA-modifying enzymes that hydroxylate 5-methylcytosine (5mC) to generate 5-hydroxymethylcytosine (5hmC) (Tahiliani et al. 2009; Ito et al. 2010). The *TET* enzymes are also able to further oxidize 5hmC to generate 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) (He et al. 2011; Ito et al. 2011). Somatic mutations in *TET2*, including chromosomal microdeletions and missense, nonsense, and frame-shift mutations, are common in clonal myeloid disorders, occurring in 10%–40% of cases of AML, MDS, MPN, and CMML (chronic myelomonocytic leukemia) (Abdel-Wahab et al. 2009; Delhommeau et al. 2009; Tefferi et al. 2009; Couronne et al. 2010; Chou et al. 2011). Interestingly, *TET2* appears to be a haploinsufficient tumor suppressor. Although a subset of cases of MDS and AML present with biallelic *TET2* loss, the majority of cases

present with heterozygous *TET2* mutations (Abdel-Wahab et al. 2009; Bejar et al. 2011; Gaidzik et al. 2012). The other *TET* family members are not recurrently mutated in hematopoietic malignancies, although *TET1* is an infrequent MLL fusion partner in rare cases of MLL translocation-positive AML (Lorsbach et al. 2003).

TET enzymes are thought to play an important role in the epigenetic regulation of gene expression by mediating the demethylation of DNA. The conversion of 5mC to 5hmC and then to 5fC and 5caC by *TET* enzymes results in the generation of guanine:oxidized cytosine base-pair mismatches. It has been hypothesized that these mismatches are substrates for base excision repair by thymine–DNA glycosylase (TDG) (He et al. 2011; Raiber et al. 2012). It has also been suggested that 5caC residues are substrates for an as yet unidentified enzyme that can decarboxylate 5caC to generate unmethylated cytosine (Ito et al. 2011). In either case, oxidation of 5mC by *TET* enzymes, followed by TDG-mediated base excision or 5caC decarboxylation, would result in DNA demethylation. Indeed, overexpression of *TET2* in HEK293 cells results in decreased 5mC levels and increased 5hmC, 5fC, and 5caC levels, whereas knockdown of *TET1* in mouse embryonic stem cells results in decreased 5hmC, 5fC, and 5caC levels (Ito et al. 2011).

Given the putative role for *TET2* in DNA demethylation, it would be expected that loss of *TET2* activity should impair DNA demethylation and result in DNA hypermethylation. However, different studies have found inconsistent and even contradictory associations between *TET2* mutational status and DNA methylation status in myeloid diseases. One study reported that *TET2* mutant AML has a DNA hypermethylation signature (Figueroa et al. 2010). Two other studies found that genomic DNA is hypermethylated in *TET2* wild-type myeloid diseases and is hypomethylated in *TET2* mutant myeloid diseases (Ko et al. 2010; Perez et al. 2012). In yet another study, 5hmC levels were reported to be lower in *TET2* mutant CMML than in *TET2* wild-type disease, but global DNA methylation was the same in both cohorts (Yamazaki et al. 2012). In this last study, when locus-

Table 2. List of known and putative 2OG-dependent dioxygenases in the in the GenBank DNA database

DNA/RNA-modifying enzymes	JmjC domain-containing enzymes		Proline/lysine hydroxylases	Other hydroxylases
<i>TET1</i>	KDM2A	KDM7A	EGLN1	ASPH
<i>TET2</i>	KDM2B	KDM8	EGLN2	ASPHD1
<i>TET3</i>	KDM3A	HR	EGLN3	ASPHD2
<i>ABH1</i>	KDM3B	JARID2	P4HA1	BBOX1
<i>ABH2</i>	KDM4A	JHDM1C	P4HA2	FIH1
<i>ABH3</i>	KDM4B	JMJD1C	P4HA3	HSPBAP1
<i>ABH4</i>	KDM4C	JMJD4	P4HB	OGFOD1
<i>ABH5</i>	KDM4D	JMJD6	P4HTM	OGFOD2
<i>ABH6</i>	KDM5A	JMJD7	PLOD1	PAHX-API
<i>FTO</i>	KDM5B	JMJD8	PLOD2	PHYH
	KDM5C	MINA	PLOD3	PHYHD1
	KDM5D	NO66	LEPRE1	
	KDM6A	PHF2	LEPREL1	
	KDM6B	PHF8	LEPREL2	
		UTY	BBOX2	

specific CpG island methylation was analyzed, only 11 of 27,578 CpG sites were differentially methylated between 5hmC-low and 5hmC-high patient samples. In the 5hmC-low patient samples, of the differentially methylated CpG sites, two were hypermethylated and the other nine were hypomethylated, suggesting that 5hmC levels do not correlate with 5mC levels at all. Some of these inconsistencies may be due to technical differences in experimental approach. It is also possible that TET2 mutations alter the methylation state of DNA at specific loci and only variably alter global DNA methylation.

Although 5mC and 5hmC are the only known substrates of TET2, TET2 may have other hydroxylation substrates besides DNA that mediate its tumor suppressor functions. Moreover, the hydroxylation of 5mC and the oxidation of 5hmC by TET2 might have other functions besides being intermediate steps in DNA demethylation. It has been observed that, in some cells, 5hmC modifications are copied to daughter strands during DNA replication (Szulwach et al. 2011). This requires a considerable expenditure of energy, as the daughter strands would first need to be methylated and then hydroxylated in order to maintain parental DNA 5hmC status. It seems unlikely that there would be significant selective pressure to maintain a nonfunctional transitional state of DNA demethylation. Rather, it seems more likely that 5hmC modifications and perhaps also 5fC and 5caC modifications play specific roles in the regulation of 5mC in the regulation of gene expression, perhaps by serving as recognition sites for the binding of distinct epigenetic and transcriptional regulators. Consistent with this hypothesis, specific “readers” of 5mC, 5hmC, and oxidized forms of 5hmC have recently been identified (Mellen et al. 2012; Spruijt et al. 2013). These include the transcriptional repressor MeCP2, which preferentially binds 5mC and 5hmC; the cell cycle regulator p53, which preferentially binds 5fC; and the DNA methyltransferase DNMT1, which preferentially binds 5caC. How the binding of these “readers” to modified cytosine residues contributes to the regulation of gene expression is not well understood, but it is likely that any perturbation of these interactions through imbalances in hydroxylation and oxidation of cytosine residues would have profound effects on cellular homeostasis.

There are several lines of evidence to suggest that TET2 is an important target of (R)-2HG in mutant IDH-mediated transformation. The catalytic activity of TET2 is potently inhibited by (R)-2HG in vitro (Xu et al. 2011; Koivunen et al. 2012). Furthermore, IDH mutant brain tumors and leukemias frequently display global DNA hypermethylation signatures (Figuroa et al. 2010; Ko et al. 2010; Turcan et al. 2012). Perhaps the most persuasive evidence for a link between IDH and TET2 mutations is the observation that IDH mutations are mutually exclusive with TET2 mutations in AML (Figuroa et al. 2010; Gaidzik et al. 2012). This suggests that the two mutations act on the same leukemogenic pathways and have redundant effects on cellular transformation. Indeed, (R)-2HG enhances the transforming effects of partial inhibition of TET2. In TF-1 cells, depletion of TET2 induces growth factor independence and blocks

cellular differentiation similarly to expression of mutant IDH, and this transformation by TET2 depletion is potentiated by cell-permeable (R)-2HG (Losman et al. 2013).

It is unclear whether TET2 and IDH are similarly linked in other tumors. Depletion of TET2 in immortalized human astrocytes phenocopies the transforming effects of mutant IDH expression, but TET2 mutations have not been described in brain tumors (Koivunen et al. 2012). Interestingly, *TET2* promoter methylation is increased in some cases of IDH wild-type grade II/III glioma (Kim et al. 2011). Given that promoter methylation is frequently associated with transcriptional silencing, this suggests that TET2 expression and activity are lost in these IDH wild-type gliomas. Taken as a whole, these observations suggest that loss of TET2 activity by either a loss-of-function mutational event, epigenetic silencing, or inhibition of TET2 activity by (R)-2HG is a frequent and important pathogenic event in brain tumors and myeloid diseases.

The evidence that IDH and TET2 mutations function in the same oncogenic pathway notwithstanding, it is worth noting that the phenotype of the conditional *TET2* knockout mouse is quite distinct from that of the *IDH1* R132H knock-in mouse described above (Moran-Crusio et al. 2011; Sasaki et al. 2012b). Homozygous deletion of *TET2* in the hematopoietic compartment of mice does not cause leukemia but does result in a more significant expansion of hematopoietic stem and early progenitor cells than is seen in *IDH1* R132H knock-in mice. Furthermore, *TET2*-null hematopoietic stem cells have a marked competitive repopulating advantage in transplanted recipient mice when compared with *TET2* wild-type stem cells. This increased repopulating activity is not seen with *IDH1* R132H knock-in stem cells, suggesting that self-renewal is more markedly enhanced by loss of TET2 than by expression of mutant IDH. *TET2*-null mice also show granulocyte and monocyte precursor lineage expansion and a skewing of myeloid differentiation toward monocyte/macrophage lineages, phenotypes that are not observed in *IDH1* R132H knock-in mice.

There appears to be a dose-dependent effect of loss of TET2 activity on murine hematopoiesis. Mice with heterozygous loss of *TET2* show an intermediate hematologic phenotype, with a less dramatic increase in stem cell self-renewal and a less dramatic dysregulation of myeloid differentiation than is seen in the *TET2*-null mice. This observation raises an interesting point. If transformation by TET2 suppression is indeed gene dosage-dependent, then it is reasonable to assume that selective pressure exists for heterozygous *TET2* mutant cells and *IDH* mutant cells to further inhibit TET2 activity. A heterozygous *TET2* mutant clone could do this by mutating the remaining wild-type *TET2* allele or acquiring an *IDH* mutation. An IDH mutant clone could do this by acquiring a *TET2* mutation. Why, then, are *IDH* mutations and heterozygous *TET2* mutations mutually exclusive? One possible explanation is that the clonal advantage conferred by loss of TET2 is less dose-dependent in human myeloid cells than it is in mice. A cell that has already partially inhibited TET2 activity by either losing one allele of *TET2* or acquiring an

IDH mutation might not benefit significantly from further *TET2* inhibition. With this in mind, it is interesting to note that, although biallelic *TET2* loss is relatively common in MDS, occurring in >25% of cases, complete loss of *TET2* is not associated with a more aggressive clinical disease phenotype (Jankowska et al. 2009; Mohamedali et al. 2009; Bejar et al. 2011).

Another possible explanation for the mutual exclusivity of *TET2* and *IDH* mutations in leukemia is that acquisition of an *IDH* mutation confers a clonal disadvantage to *TET2* mutant cells. Cells expressing mutant *IDH* grow more slowly under optimal culture conditions than their parental counterparts (Seltzer et al. 2010; Bralten et al. 2011; Losman et al. 2013). This suggests that mutant *IDH* and (*R*)-2HG have growth-suppressive effects that coexist with their transforming, growth-promoting effects. Perhaps in cells that do not require mutant *IDH* for cell growth and survival (for example, cells in which *TET2* is already mutated), the negative effects of (*R*)-2HG on proliferation overwhelm the positive effects of (*R*)-2HG on transformation. This explanation could also account for the less pronounced phenotype of the *IDH1* R132H knock-in mice as compared with the *TET2* conditional knockout mice. Perhaps the mild phenotype of the *IDH1* R132H knock-in mice is not the result of inadequate inhibition of *TET2* but rather is a reflection of the balance between the protransforming effects of (*R*)-2HG inhibition of *TET2* and the anti-transforming effects of (*R*)-2HG inhibition of other, growth-promoting enzymes.

JmjC histone demethylases

The *JmjC* family of histone lysine demethylases is another family of enzymes that may be a pathogenically relevant target of (*R*)-2HG. Histone demethylases play an important role in the epigenetic regulation of gene expression (Klose et al. 2006; Shilatifard 2006). Histone methylation defines the structure of chromatin, and methylation of some histone residues (H3K4, H3K36, and H3K79) is often associated with transcriptionally active euchromatin, whereas other histone methylation marks (H3K9, H3K27, and H4K20) are usually associated with transcriptionally silent heterochromatin. The patterns of histone methylation at different genetic loci are a function of the balance between the activities of histone methyltransferases and histone demethylases, each of which binds and alters the methylation of specific histone lysine residues. Disruption of histone methylation or demethylation can have profound effects on gene expression, and *JmjC* histone demethylases have been linked to the pathogenesis of a number of different cancers (Cloos et al. 2008; Varier and Timmers 2011).

Several *JmjC* histone demethylases appear to function as tumor suppressors. The H3K36 *JmjC* demethylase *KDM2B* (*JHDM1B*/*FBX10*) is a transcriptional repressor that regulates ribosomal gene expression and represses c-Jun-mediated transcription (Frescas et al. 2007; Koyama-Nasu et al. 2007). Consistent with its putative role as a tumor suppressor, *KDM2B* expression is significantly lower in brain tumors than in normal brain tissue. The

H3K9 *JmjC* demethylase *KDM3B* (*JMJD1B*) is frequently deleted in 5q⁻ MDS and AML (Hu et al. 2001), and the H3K4 *JmjC* demethylase *KDM5C* (*JARID1C*) is occasionally mutated and inactivated in clear cell renal carcinoma (Dalglish et al. 2010; Hakimi et al. 2013). The H3K27 *JmjC* demethylase *KDM6A* (*UTX*) and its paralog, *KDM6B* (*JMJD3*), can also function as tumor suppressors. *KDM6A* cooperates with the Retinoblastoma tumor suppressor protein to enforce cell cycle blockade and also negatively regulates the activity of NOTCH (Herz et al. 2010; Terashima et al. 2010; Wang et al. 2010). *KDM6B* is involved in the regulation of p53 and has been shown to promote the terminal differentiation of glioblastoma cells (Barradas et al. 2009; Sola et al. 2011).

It is possible that inhibition of tumor suppressor histone demethylases by (*R*)-2HG contributes to transformation by mutant *IDH*. In fact, (*R*)-2HG has been shown to inhibit the activity of many 2OG-dependent dioxygenases *in vitro*, including the *JmjC* histone demethylases *KDM2A*, *KDM4A*, *KDM4C*, and *KDM7A* (Chowdhury et al. 2011; Xu et al. 2011; Lu et al. 2012). Although the IC₅₀ values for inhibition of 2OG-dependent enzymes by (*R*)-2HG vary considerably (ranging from 25 μM to >10 mM), it is important to remember that (*R*)-2HG can accumulate to millimolar levels in tumors. Moreover, overexpression of mutant *IDH* and treatment of cells with cell-permeable (*R*)-2HG has been reported to increase H3K4, H3K9, H3K27, H3K36, and H3K79 histone methylation marks in cell culture models (Lu et al. 2012).

The biological significance of the histone methylation changes induced *in vitro* by (*R*)-2HG is not clear, and it is not yet known whether (*R*)-2HG affects histone methylation in primary human *IDH* mutant tumors. H3K9 trimethylation is frequently increased in *IDH* mutant gliomas (Vanneti et al. 2013). However, many *IDH* wild-type gliomas also display H3K9 hypermethylation, suggesting that aberrant histone methylation is a common finding in brain tumors irrespective of *IDH* mutational status. In the hematopoietic-specific *IDH1* R132H knock-in mice, bone marrow stem cells show a modest increase in methylated H3K4 levels but no change in other histone methylation marks (Sasaki et al. 2012b). Furthermore, no differences in global histone methylation are apparent in the brains of the brain-specific *IDH1* R132H knock-in mice (Sasaki et al. 2012a). Although this could be interpreted as evidence that (*R*)-2HG does not significantly affect histone methylation *in vivo*, it is also possible that (*R*)-2HG induces biologically important changes in histone methylation only at specific genetic loci that promote tumor formation. Histone methylation changes that are beneficial could be preferentially retained in mutant *IDH*-expressing cells, whereas deleterious changes could be reversed by compensatory changes such as down-regulation of specific histone methyltransferases.

As described above, several *JmjC* histone demethylases can function as tumor suppressors. However, some histone demethylases are also known to promote tumor growth. Moreover, there are multiple examples of chromatin-modifying enzymes—including histone demethylases and their opposing methyltransferases—that can act

as either oncoproteins or tumor suppressors in different cellular contexts (Cloos et al. 2008). It is interesting to speculate that the different functions of the JmJc histone demethylases in different tissues could explain, at least in part, the tissue specificity of IDH mutations in cancer. If the proliferation and survival of a particular cell were dependent on the activity of JmJc histone demethylases that are potently inhibited by (R)-2HG, that cell would likely be relatively resistant to transformation by mutant IDH. Conversely, a cell in which those same JmJc histone demethylases are dispensable for proliferation or have tumor suppressor functions might be particularly vulnerable to transformation by mutant IDH.

EglN prolyl-4-hydroxylases

EglN1, EglN2, and EglN3 are members of a family of 2OG-dependent dioxygenases that regulate the activity of HIF, a heterodimeric transcription factor that mediates the cellular response to hypoxia (Kaelin and Ratcliffe 2008; Semenza 2012). In the presence of oxygen, EglN hydroxylates specific proline residues on HIF α , and these hydroxylated proline residues are then recognized by the von Hippel-Lindau (VHL) E3-ubiquitin ligase complex. The VHL complex polyubiquitylates HIF α and targets it for proteasomal degradation. Under hypoxic conditions, when intracellular levels of oxygen are low, the activity of EglN is inhibited. This permits accumulation of HIF α , which can then heterodimerize with HIF β , translocate to the nucleus, and activate the transcriptional response of cells to hypoxia. EglN1 is believed to be the principal HIF prolyl-hydroxylase, with EglN2 and EglN3 playing variable compensatory roles in the regulation of HIF in different tissues under specific cellular conditions.

EglN enzymes were initially reported to be inhibited by (R)-2HG (Zhao et al. 2009). However, further study has shown that, on the contrary, (R)-2HG potentiates EglN activity in vitro and in vivo and blunts the induction of HIF α in response to hypoxia in cell culture models (Koivunen et al. 2012; Losman et al. 2013). Consistent with this observation, IDH mutant brain tumors display decreased HIF activation compared with their wild-type counterparts (Williams et al. 2011; Koivunen et al. 2012). It should be noted that HIF α levels are elevated in the brains of the brain-specific *IDH1* R132H knock-in mice (Sasaki et al. 2012a). However, these mice succumb at birth to cerebral hemorrhage, suggesting that their brain perfusion during development is abnormal. It is possible that tissue hypoxia, rather than a direct effect of (R)-2HG on EglN activity, is the cause of increased brain levels of HIF α in these mice.

Likewise, there is no evidence that HIF is activated in human IDH mutant leukemias. On the contrary, there is evidence to suggest that activation of HIF inhibits myeloid leukemia. A number of human myeloid leukemia cell lines have been reported to proliferate more slowly and undergo terminal differentiation and apoptosis upon exposure to hypoxia and upon overexpression of HIF α (Huang et al. 2003; di Giacomo et al. 2009; Zhang and Chen 2009; He et al. 2013), and inhibition of HIF α

expression by RNAi blocks the differentiation of leukemia cells in response to all-*trans* retinoic acid (ATRA) (Zhang et al. 2008; Zhang and Chen 2009). Moreover, in a mouse model of acute promyelocytic leukemia, exposure of diseased mice to intermittent hypoxia induces leukemia cell differentiation and prolongs survival of the mice (Liu et al. 2006). These observations suggest that HIF may be a tumor suppressor in myeloid leukemia. However, it is not known whether the HIF response to hypoxia is truly blunted in IDH mutant tumors in vivo, as one would predict given that (R)-2HG can serve as an EglN agonist.

The ability of (R)-2HG to function as an EglN cosubstrate appears to play an important role in mutant IDH-mediated cellular transformation. In immortalized human astrocytes, either overexpression of EglN1 or depletion of HIF1 α is sufficient to promote soft agar colony formation (Koivunen et al. 2012). Conversely, depletion of EglN1 in mutant IDH-transformed astrocytes inhibits the growth and soft agar colony formation of the cells. Intriguingly, (S)-2HG, which potently inhibits EglN in vitro, is unable to promote transformation of TF-1 leukemia cells. In fact, (S)-2HG antagonizes TF-1 cell transformation induced by TET2 loss (Losman et al. 2013). This is despite the fact that (S)-2HG is a more potent inhibitor of TET2 than is (R)-2HG (Xu et al. 2011; Koivunen et al. 2012). Depletion of EglN1 in transformed TF-1 cells reverses transformation induced by expression of mutant IDH or by depletion of TET2, suggesting that the anti-leukemic properties of (S)-2HG relate to its ability to inhibit EglN1 (Losman et al. 2013). In short, inhibition of EglN1 by (S)-2HG reverses the leukemic transformation that would otherwise ensue as a result of its inhibition of TET2 activity.

Other candidate targets of (R)-2HG

A number of other 2OG-dependent enzymes might be inhibited by (R)-2HG in IDH mutant tumors (Table 2). Three types of collagen hydroxylases—the Leprecan family of prolyl-3-hydroxylases, the P4HA family of prolyl-4-hydroxylases, and the PLOD family of lysyl-5-hydroxylases—all require 2OG for activity, and P4HA1 has been found to be inhibited by (R)-2HG in vitro (Koivunen et al. 2012). The collagen prolyl-hydroxylases mediate the hydroxylation of proline residues required for formation of the collagen triple helix (Gorres and Raines 2010), and the collagen lysyl-hydroxylases mediate the hydroxylation of lysine residues required for collagen cross-linking into stable fibrils (Bank et al. 1999). Interestingly, collagen maturation has been found to be impaired in the brain-specific *IDH1* R132H knock-in mice, suggesting that these enzymes are targets of inhibition by mutant IDH in vivo (Sasaki et al. 2012a). Although a role for these enzymes in cancer has not been established, the observation that expression of collagen prolyl-3-hydroxylases and prolyl-4-hydroxylases is down-regulated in many B-cell lymphomas suggests that these enzymes function as tumor suppressors in some tissues (Teodoro et al. 2006; Hatzimichael et al. 2012).

Other potential 2OG-dependent targets of (R)-2HG include FIH1 (factor inhibiting hypoxia-inducible factor

1), an asparaginyl hydroxylase that regulates the transcriptional activity of HIF (Mahon et al. 2001); the ABH family of DNA demethylases that are involved in DNA damage repair (Lee et al. 2005); and the RNA demethylase FTO (fat mass and obesity-associated), which is believed to be important for the regulation of cellular metabolism (Jia et al. 2008; Berulava et al. 2013).

Other possible mechanisms of transformation by mutant IDH

Another proposed mechanism of transformation by mutant IDH harkens back to Warburg's mitochondrial model of oncogenesis. Warburg (1956) hypothesized that the primary defect in cancer cells is impaired mitochondrial function, which causes cells to switch from oxidative phosphorylation to aerobic glycolysis. Warburg's model has been somewhat discredited by the observation that mitochondrial function is not impaired in most cancer cells (Wallace 2012). However, there are data to suggest that (*R*)-2HG directly interferes with normal mitochondrial function. Some D2HGA patients have elevated levels of urinary lactate, suggesting that they have some degree of mitochondrial dysfunction (Kranendijk et al. 2012). Furthermore, the activity of two enzyme complexes in the electron transport chain, complex IV (cytochrome *c* oxidase) and complex V (ATP synthase), can be inhibited by (*R*)-2HG in vitro (Kolker et al. 2002; Latini et al. 2005). Inhibition of ATP synthase expression and activity has been observed in some cancers and is believed to directly promote cellular transformation by causing the accumulation of excess electrons that are then transferred to molecular oxygen to generate superoxide (O_2^-) (Willers and Cuezva 2011).

Mutant IDH-expressing cells may have an impaired ability to neutralize reactive oxygen species (ROS) (Latini et al. 2003). Conversion of 2OG to (*R*)-2HG by mutant IDH consumes NADPH, and the high catalytic rate of the mutant IDH reaction could conceivably result in depletion of cellular NADPH (Yan et al. 2009; Bleecker et al. 2010). NADPH is the electron donor used by glutathione reductase to regenerate reduced glutathione, which is the principal cellular and mitochondrial antioxidant that regulates cellular redox (Kirsch and De Groot 2001; Block and Gorin 2012). Depletion of NADPH might therefore decrease the ability of cells to neutralize DNA-damaging oxygen-free radicals. NADPH also has other regulatory functions in cells that could contribute to cellular transformation. NADPH plays a role in nutrient sensing and participates in the regulation of the cellular metabolic rate (Newsholme et al. 2010; Newsholme and Krause 2012), and NADPH is also involved in the regulation of thio-redoxins, enzymes that regulate the thiol-disulphide redox state and activity of several transcription factors, including NF- κ B and AP1 (Arner and Holmgren 2000; Kabe et al. 2005).

Although dysregulation of cellular redox has been shown to contribute to transformation in other types of cancer, it is not clear whether NADPH and ROS are in fact dysregulated in IDH mutant tumors. Consistent with

the notion that mutant IDH can perturb cellular NADPH levels, the embryonic brains of the brain-specific *IDH1* R132H knock-in mice have a slight increase in their $NADP^+$:NADPH ratio and slightly decreased levels of reduced glutathione (Sasaki et al. 2012a). However, the brains of the *IDH1* R132H mice have lower, not higher, levels of ROS than control mice. In the hematopoietic-specific *IDH1* R132H knock-in mice, the $NADP^+$:NADPH ratio and the levels of ROS are normal in the bone marrow (Sasaki et al. 2012b). Despite the apparent lack of increased ROS in the *IDH1* R132H knock-in mice, it is nonetheless possible that cellular redox dysregulation plays a role in transformation by mutant IDH. The mice may have subtle or compartmentalized, and yet pathophysiologically relevant, perturbations in their brain and bone marrow redox states that only become apparent under conditions of oxidative stress. It is also possible that otherwise normal cells have compensatory mechanisms that largely maintain redox homeostasis in the presence of mutant IDH and that these mechanisms are defective in cancer cells.

Another potential contributor to mutant IDH-mediated transformation is deficiency of wild-type IDH activity. Although most tumor-associated mutations in IDH result in (*R*)-2HG production, a few rare *IDH1* and *IDH2* mutants that do not produce (*R*)-2HG have recently been identified in lymphoid and thyroid tumors (Ward et al. 2012). These mutations are monoallelic and result in either decreased enzymatic activity or loss of expression of the mutant enzyme. It has not yet been determined whether these loss-of-function mutations contribute to cellular transformation or are simply passenger mutations, but they do raise the possibility that *IDH* haploinsufficiency in IDH mutant cells could be tumorigenic.

Therapeutic targeting of IDH mutant tumors

The observation that (*R*)-2HG is sufficient to promote cellular transformation and that transformation by (*R*)-2HG is reversible suggests that inhibiting (*R*)-2HG production by mutant IDH would have efficacy in the treatment of IDH mutant cancers. In this regard, first-generation mutant IDH inhibitors capable of suppressing the production of (*R*)-2HG in vitro and in vivo have recently been reported (Popovici-Muller et al. 2012; Losman et al. 2013; Rohle et al. 2013; Wang et al. 2013).

As described above, EglN activation plays a causal role in the transformation of astrocytes by mutant IDH in cell culture models and appears to play a permissive role in the transformation of TF-1 leukemia cells by mutant IDH. These findings, if proven robust, warrant further exploration of the potential of EglN inhibitors for the treatment of IDH mutant neoplasms. A number of EglN inhibitors have been developed for the treatment of anemia and tissue ischemia, some of which have advanced to human clinical trials (Robinson et al. 2008; Miyata et al. 2011; Sen Banerjee et al. 2012; Forristal et al. 2013).

Finally, it seems likely that the millimolar levels of (*R*)-2HG that accumulate in IDH mutant tumors also create certain liabilities for tumor cells. For example, inhibition

of certain enzymes by (R)-2HG, whether they are pathogenic targets or “bystanders,” might decrease cellular fitness and hence decrease the resistance of cells to certain forms of therapeutic attack. There is some indirect evidence to support this idea. For example, introduction of mutant IDH into TF-1 cells confers a growth disadvantage to the cells under cytokine-rich conditions even though mutant IDH confers a growth advantage to the cells under cytokine-poor conditions (Losman et al. 2013). Moreover, stable expression of IDH1 R132H impedes the growth and migration of human IDH1 wild-type GBM cell lines both in vitro and in mouse xenografts models (Bralten et al. 2011). The relatively good prognosis and enhanced sensitivity to chemotherapy and radiation therapy of IDH mutant brain tumors might also reflect deleterious effects of (R)-2HG in tumor cells (Houillier et al. 2010; SongTao et al. 2012; Li et al. 2013). Therefore, another strategy for tackling IDH mutant tumors could be to systematically identify and target the unique dependencies that are created in the presence of millimolar concentrations of (R)-2HG, thereby converting the oncometabolite into an Achilles’ heel.

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