

Review

Metabolism, Activity, and Targeting of D- and L-2-Hydroxyglutarates

Dan Ye,^{1,2,*} Kun-Liang Guan,^{1,3} and Yue Xiong^{1,4,*}

Isocitrate dehydrogenases (*IDH1/2*) are frequently mutated in multiple types of human cancer, resulting in neomorphic enzymes that convert α -ketoglutarate (α -KG) to 2-hydroxyglutarate (2-HG). The current view on the mechanism of *IDH* mutation holds that 2-HG acts as an antagonist of α -KG to competitively inhibit the activity of α -KG-dependent dioxygenases, including those involved in histone and DNA demethylation. Recent studies have implicated 2-HG in activities beyond epigenetic modification. Multiple enzymes have been discovered that lack mutations but that can nevertheless produce 2-HG promiscuously under hypoxic or acidic conditions. Therapies are being developed to treat *IDH*-mutant cancers by targeting either the mutant *IDH* enzymes directly or the pathways sensitized by 2-HG.

Production of Oncometabolite 2-HG by Cancer-Associated Mutations in *IDH1* and *IDH2*

The metabolic genes encoding *IDH1/2* were found to be mutated by two genomic studies in human glioma and acute myeloid leukemia (AML) in 2008 and 2009 [1,2]. These seminal, and surprising, findings set off intense efforts to determine the biochemical mechanisms and clinical implications of *IDH* mutations. We now know that *IDH1* and *IDH2* are the most frequently mutated metabolic genes in human cancer [3,4]. *IDH1* and *IDH2* mutations occur frequently in low-grade glioma (~80%), AML (~12%), cartilaginous tumors (~75%), intrahepatic cholangiocarcinoma (ICC) (15–20%), and angioimmunoblastic T cell lymphoma (AITL) (30–40%), sporadically in melanoma (6%), prostate cancer (3%), hepatocellular carcinoma (HCC) (1%), and medulloblastoma (1%), and infrequently in thyroid, pituitary, stomach, breast, and pancreatic cancers. Genomic studies have also established that *IDH* mutations are early events, perhaps the first genetic lesions that occur during tumorigenesis [5–7] (see Outstanding Questions). Importantly, *IDH* mutations define distinct subtypes of tumors within otherwise heterogeneous glioma [7–9], AML [10], ICC, and HCC cancers [11], and these mutations exhibit unique ages of onset, clinical behaviors, and responses to therapy. Thus, *IDH* mutations appear to initiate pathogenesis by a common mechanism.

Mutations targeting *IDH1* and *IDH2* in different types of tumors share four features, providing initial insights into the mechanism of *IDH* mutations. First, *IDH1* and *IDH2* mutations are somatic, not germline. Second, all tumors with *IDH1/2* mutations are heterozygous, suggesting a gain-of-function and dominant effect over the remaining wild-type allele. Third, nearly all *IDH1/2* mutations occur in a few hotspots in the enzymes' active sites – Arg132 in *IDH1* and correspondingly Arg172 in *IDH2*, plus Arg140 in *IDH2*—suggesting a direct impact on the catalytic properties of the enzymes. Fourth, *IDH1* and *IDH2* mutations occur in a mutually exclusive manner, indicating a common biochemical mechanism targeting the same pathway by either mutant protein.

Highlights

Many enzymes can promiscuously produce 2-HG by side reactions, causing pathologically significant concentrations of 2-HG in cells lacking *IDH* mutations.

Metabolic and stress conditions such as T cell activation or hypoxia can promote enzyme promiscuity to produce 2-HG.

In addition to histone and DNA demethylases, 2-HG can impair the activity of other α -KG-dependent dioxygenases and their associated cellular pathways, such as DNA repair.

¹Molecular and Cell Biology Lab, Institute of Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai 200032, China

²Department of General Surgery, Huashan Hospital, Fudan University, Shanghai 200040, China

³Department of Pharmacology and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

⁴Department of Biochemistry and Biophysics, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

*Correspondence: yedan@fudan.edu.cn (D. Ye) and yxiong@email.unc.edu (Y. Xiong).

During the past 8 years, we have gained extensive mechanistic understanding of how *IDH* mutations contribute to tumorigenesis. Soon after their discovery, it was demonstrated that all tumor-derived mutations in *IDH1* and *IDH2* disrupt their normal catalytic activity; that is, converting isocitrate to α -KG [also known 2-oxoglutarate (2OG)] [12–14]. However, the most remarkable feature of *IDH* mutations is the **neomorphic enzymatic** (see **Glossary**) activity acquired by the mutant enzymes, which can convert α -KG to a previously little-known metabolite, D-2-hydroxyglutarate (D-2-HG), now referred to as an oncometabolite [14,15]. Although long recognized as a key nexus for multiple metabolic pathways, α -KG is also a co-substrate for **α -KG/Fe(II)-dependent dioxygenases** [16,17]. This non-metabolic function of α -KG and the fact that D-2-HG differs from α -KG by only an oxygen in place of a hydroxyl group (Figure 1) have led to the discovery that D-2-HG is an antagonist of α -KG, competitively inhibiting α -KG/Fe(II)-dependent dioxygenases, including the JmjC-domain family of histone demethylases and the **TET** family of DNA dioxygenases [18,19]. This antagonist property provides a biochemical basis for, and is supported by, the genetic observations that *IDH1/2* mutations are associated with the CpG island methylator phenotype (G-CIMP) in glioma [20] and ICC [21]. It is further supported by the observation that G-CIMP can be established in primary astrocytes when mutant *IDH1* is ectopically expressed [22]. This property is also consistent with the fact that *IDH1/2* mutation occurs in a mutually exclusive manner with *TET2* mutations in AML [23]. Co-crystal structural studies reveal that 2-HG occupies the same space as α -KG in the active site of histone demethylases [19]. High 2-HG concentration is associated with increased histone methylation in primary glioma and induces cell differentiation *in vitro* [19,24]. Thus, by impairing histone and DNA methylation, thereby altering gene expression, *IDH* mutations block or skew progenitor cell differentiation, promoting tumorigenesis in conjunction with subsequent oncogenic mutations.

In this review we recap early investigations on 2-HG before the discovery of its production by mutant *IDH* enzymes. We then discuss recent developments regarding the metabolism, biochemical targets, and cellular functions of 2-HG and therapeutic strategies to treat mutant *IDH*-associated tumors.

Evolutionary Conservation and Functional Importance of 2-HG Removal

2-HG was first described in 1868 by the German biochemist Karl Heinrich Ritthausen [25], who was better known for his discovery of glutamic and aspartic acids. In 1872, Ritthausen determined the optical rotation of 2-HG [26]. In 1920, Swedish physiologist Torsten Thunberg described 2-HG as a substrate of intermediary metabolism in frog muscle, and he also demonstrated that 2-HG can be oxidized to α -KG [27]. Not until 17 years later did Weil-Malherbe characterize an **enantiomer**-specific L-2-HG dehydrogenase that oxidizes L-2-HG to α -KG in many animal tissues [28]. However, 2-HG did not attract much interest until recently because no physiological function of 2-HG was described in any organism in which it was studied, including bacteria, yeast, plants, *Drosophila*, other animals, and humans. This lack of interest changed in 1980 with the discovery that 2-HG accumulation is linked to so-called **2-hydroxyglutaric aciduria (2HGA)** diseases. 2-HG can be detected in the urine of healthy humans at a concentration of 20 mg/g (23 μ mol/mmol creatinine) [29,30]. In 1980, D-2-HG and L-2-HG were found at 60- to 100-fold and 11- to 25-fold higher concentrations in two unrelated children [31,32]; these phenotypes define two clinically related, biochemically distinct diseases, D- and L-2-hydroxyglutaric aciduria (D2HGA and L2HGA) [33]. 2-HG aciduria begins during infancy or early childhood and worsens over time, usually leading to severe disability by early adulthood. Both diseases are rare and they share many clinical features, including delayed development, seizures, and, most notably, abnormalities in the cerebrum that affect muscle

Glossary

2-Hydroxyglutaric aciduria

(2HGA): a rare neurometabolic disorder (only several hundred cases reported worldwide) characterized by significantly elevated concentrations of 2-HG in plasma, cerebrospinal fluid, and urine. There are three types of 2HGA – D2HGA, L2HGA, and combined D,L-2HGA – depending on whether the excess 2-HG is the D or L isomer or both. A detailed description of 2HGA, including clinical diagnosis and management, patient support, and resources can be found at <https://ghr.nlm.nih.gov/condition/2-hydroxyglutaric-aciduria>.

α -Ketoglutarate (α -KG)/Fe(II)-dependent dioxygenases:

enzymes that catalyze the transfer of both oxygen atoms from molecular oxygen (O_2) to a substrate (hydroxylation). Dioxygenases differ from monooxygenases (e.g., cytochrome P450 oxidases), which transfer one oxygen atom from O_2 to a substrate and reduce the other oxygen atom to water. Dioxygenases are distributed widely in all kingdoms of life and more than 60 dioxygenases occur in humans [122]. Dioxygenases catalyze the hydroxylation of lysine, proline, and asparagine in proteins and of nucleotides in DNA and RNA. Members of this enzyme family regulate multiple cellular pathways, including collagen maturation, fatty acid metabolism, oxygen sensing, histone and DNA demethylation, DNA repair, and demethylation of tRNA and mRNA [16,17].

ALKBH: the bacterial family of α -KG/Fe(II)-dependent dioxygenases. The prototypical member of the family is the *Escherichia coli* AlkB, which was discovered by its activity in affording resistance to alkylating agents [126]. Nine ALKBH enzymes exist in mammalian cells (ALKBH1–8, FTO). Originally thought to be limited to repairing DNA alkylation, ALKBH has recently been found to function in RNA metabolism [127,128].

Collagen hydroxylase: also known as prolyl 4-hydroxylase; the first α -KG/Fe(II)-dependent dioxygenase to be identified [130], controlling the biogenesis and stability of collagen. There are three types of collagen hydroxylase, catalyzing the

function, speech, vision, thinking, emotion, and memory. In addition, brain malignancies have been reported in some individuals with L2HGA, but not with D2HGA [34].

The causes and mechanisms of 2HGA diseases have been identified in 2004. Separate biochemical purification of the enzymes acting on L-2-HG and mapping of L2HGA DNA led to the simultaneous discovery of L-2-hydroxyglutarate dehydrogenase (L2HGDH) [35,36]. The same purification revealed a separate enzyme, D-2-hydroxyglutarate dehydrogenase (D2HGDH), that acts specifically on D-2-HG [37]. The *D2HGDH* gene was found to be mutated in about half of individuals with D2HGA [38,39] and, recently, in a small fraction of diffuse large B cell lymphomas [40]. These studies established that L2HGDH and D2HGDH localize in the mitochondrion and, when homozygously mutated in the germline, cause L2HGA and D2HGA, respectively (Figure 1). Subsequently, sequence comparisons and biochemical studies have characterized a single FAD-dependent L2HGDH enzyme in *Escherichia coli* (*YgaF*) [41] and *Drosophila* (*CG10639*) [42] and two yeast D2HGDH enzymes (*Dld2* and *Dld3*) [43]. Thus, removal of 2-HG is an evolutionarily conserved, and likely to be functionally important, activity. However, only recently has the source of 2-HG production being identified.

In 2010, the puzzle presented by the half of individuals with D2HGA that lack *D2HGDH* mutations was explained by the finding of *IDH2* mutations in these individuals (now referred to as type II D2HGA) [44]. A third type of 2HGA, combined D,L-hydroxyglutaric aciduria, was reported in neonates with encephalopathy and accumulation of both D- and L-2-HG [45]. This phenotype is caused by mutations in the mitochondrial citrate carrier *SLC25A1* [46]. It is currently unclear how *SLC25A1* deficiency causes elevated D- and L-2-HG. Possibly, the phenomenon may be explained by mitochondrial accumulation of citrate and other TCA intermediates, including α -KG, the precursor of both 2-HG enantiomers whose production is catalyzed promiscuously by various enzymes (see below).

2-HG Production by Promiscuous Enzymatic Reactions

In contrast to the long history of discovery of 2-HG and enzymes for its metabolic removal, only recently have we understood how 2-HG is produced. The first reported 2-HG-producing activity in mammalian cells was an oxidoreductase in rat kidney, liver, and brain mitochondria that catalyzes the interconversion of several oxoacids, including α -KG, and hydroxy-acids, including D-2-HG [41]. As determined by mass isotopomer distribution analysis, cultured lymphoblasts from individuals with D2HGA or L2HGA rapidly interconverted α -KG to either D-2-HG or L-2-HG by an activity in the mitochondrion [47,48]. Thus, α -KG is the apparent direct precursor of D- and L-2-HG in mammals and other organisms. In 2007, Schaftingen and colleagues identified the elusive mitochondrial ' α -KG reductase'. Surprisingly, a previously well-characterized enzyme, mitochondrial malate dehydrogenase (MDH2/mMDH) and its cytoplasmic counterpart (MDH1/cMDH) can catalyze NAD⁺-dependent α -KG reduction to L-2-HG [49]. MDH normally catalyzes interconversion of malate and oxaloacetate. Thermodynamically, MDH favors oxaloacetate-to-malate reduction, but the reaction may be driven in reverse by oxaloacetate and NADH (Figure 2). MDH-catalyzed α -KG-to-D- or -L-2-HG reduction would be coupled with NADH oxidation to NAD⁺ as in the oxaloacetate-to-malate reduction reaction, but MDH conversion of α -KG to 2-HG was 10⁷–10⁸ less efficient catalytically [49]. Thus, the investigators proposed that L-2-HG is formed by a 'side reaction' of MDH, L-2-HG serves no particular physiological purpose, and L-2-HG is removed by L2HGDH during 'metabolite repair'.

Although enzymes are remarkably efficient and specific, many enzymes can exhibit **enzyme promiscuity** in catalyzing a fortuitous side reaction in addition to their primary reactions [50].

hydroxylation of C-3 of proline (C-P3H), C-4 of proline (C-P4H), and C-5 of lysine (PLOC1, 2, and 3) [17].

EGLN/PHD hydroxylase: refers to the Egl-9 family of hypoxia-inducible factors or prolyl hydroxylase domain-containing proteins. The family includes three members in human cells: EGLN1 (PHD2), EGLN2 (PHD1), and EGLN3 (PHD3). EGLN/PHD catalyzes proline hydroxylation of HIF-1 α under normoxia conditions (10–21% oxygen); hydroxylation of HIF-1 α promotes its degradation by the ubiquitin proteasome pathway. A decrease in oxygen concentration to 1–5% (hypoxia) diminishes EGLN/PHD activity, thereby limiting the hydroxylation and degradation of HIF-1 α and promoting HIF-1 α activation of hypoxia adaptation genes. EGLN/PHD-mediated regulation of HIF-1 α is the core of ancient oxygen-monitoring machinery used by metazoans from the nematode *Caenorhabditis elegans* to humans [129].

Enantiomers: two molecules that have the same chemical formula but are structurally mirror images (like the left and right hands). D and L isomers are determined by the configuration about an asymmetric carbon atom (a carbon having four different substituents), using the enantiomeric glyceraldehydes as a reference. D and L come from the Latin words *dexter* (on the right) and *laevus* (on the left). This old and more commonly used D/L nomenclature accidentally, but not always, correlates with the modern R/S system of nomenclature, wherein (R) comes from *rectus* (right-handed) and (S) from *sinister* (left-handed). Enantiomers often have different chemical reactions with other enantiomer substances; enantiomers sometimes display biochemical and biological differences, such as the different inhibitory potencies of D- and L-2-HG toward α -KG/Fe(II)-dependent dioxygenases.

Enzyme promiscuity: activity of an enzyme that is distinguished from, and unrelated to, the primary activity and that is not involved in an organism's physiology. The catalytic efficiency of an enzyme's promiscuity is often several orders of magnitude lower than the efficiency of its

While normally physiologically irrelevant, under certain environmental conditions these promiscuous activities may affect enzyme evolution and may benefit or harm cell function. Besides MDH, at least two additional metabolic enzymes, 3-phosphoglycerate dehydrogenase (PHGDH) and lactate dehydrogenase A (LDHA), have been reported to promiscuously produce 2-HG. PHGDH catalyzes the first step of serine biosynthesis, converting 3-phosphoglycerate (3PG) to 3-phosphohydroxypyruvate (3PHP) coupled with NAD⁺ reduction, a pathway that is conserved in all organisms (Figure 2). Unexpectedly, *E. coli* PHGDH (SerA) was found to be capable of catalyzing the NADH-dependent reduction of α -KG to both L- and D-2-HG [51]. A similar promiscuous reductase activity was detected for PHDGH, which converts α -KG to D-2-HG in human breast cancer cell lines [52] and in yeast [43]. The affinity of *E. coli* and human PHGDH for α -KG is 30- to 40-fold lower than the affinity for its primary substrate 3PG, an effect that may explain why the α -KG reductase activity of PHDGH was not detected in a different study [53].

In a study to determine how hypoxic cells maintain the synthesis of citrate, it was found that mitochondrial IDH2 can catalyze the reverse reaction (reductive carboxylation) in hypoxic cells, converting α -KG to isocitrate and then citrate and, surprisingly, with a concomitant increase in D-2-HG [54]. It is unknown whether wild-type IDH2 can directly produce 2-HG. Subsequent metabolomics studies discovered that hypoxia increased α -KG and L-2-HG in a broad range of transformed human cell lines [55,56]. Three different mechanisms were proposed for the hypoxia-induced L-2-HG elevation: reduced malate and increased NADH favoring the promiscuous reaction of MDH2, reduced *D2HGDH* gene expression [56], and promiscuous reaction by LDHA [55] (Figure 2). L-2-HG accumulation in hypoxic cells can be efficiently reduced by the single knockdown of *LDHA* and L-2-HG accumulation was modestly decreased further by the double knockdown of *LDHA* and *MDH2*, suggesting that LDHA is the major producer of L-2-HG in hypoxic cells [55]. Similarly, knockdown of *Ldha*, but not *Mdh1* or *Mdh2* alone, decreased L-2-HG in hypoxic primary mouse T cells in response to T cell receptor stimulation [57]. This finding is consistent with a *Drosophila* genetic study showing that dLDH is necessary and sufficient to produce L-2-HG from α -KG in rapidly growing larvae [42] (see Outstanding Questions).

Metabolic acidosis is a common feature of hypoxia and this has prompted investigators to test the effect of pH on 2-HG production by different enzymes. It was found that acidity stimulates purified recombinant LDHA, and to a lesser extent MDH, to produce L-2-HG by several orders of magnitude *in vitro* and accumulates L-2-HG in cultured cells by several-fold in a manner correlating with pH decrease [58,59]. α -KG is a weak acid that equilibrates between protonated and deprotonated forms. Low pH drives the equilibrium toward protonation. According to molecular modeling, LDHA prefers the protonated form of α -KG. When the pH was decreased from 7.4 to 6.0, a pH near that in hypoxic cells, the Michaelis–Menten constant (K_m ; an inverse measure of affinity) of LDHA for α -KG was reduced by about fourfold [58].

Pathological Significance of 2-HG Accumulation in Cells Lacking IDH Mutation

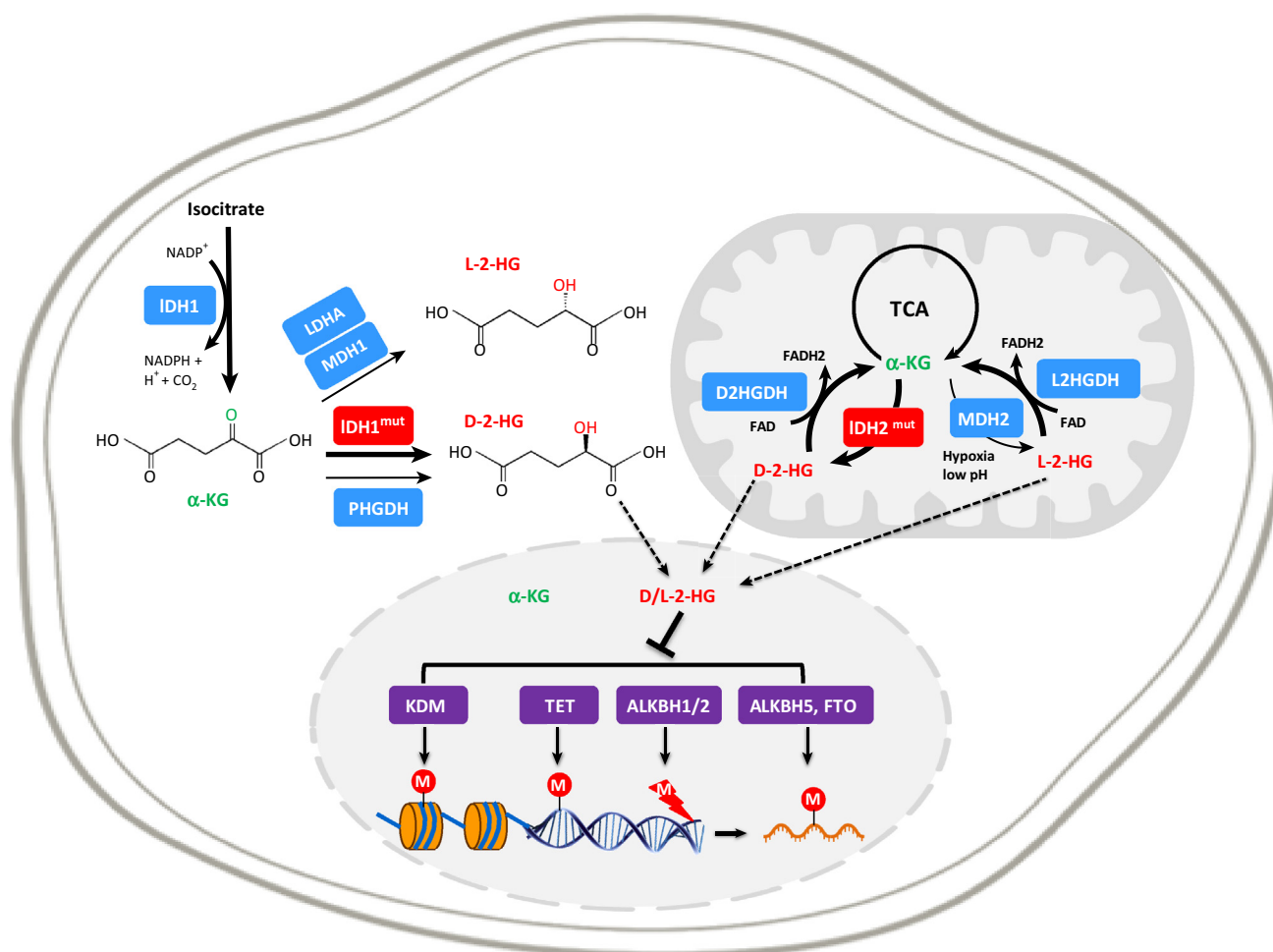
Elevated 2-HG has now been observed *in vitro* in many tumor cells lacking an *IDH* mutation. These include DLD1 and RKO colorectal cancer cells [60], papillary thyroid carcinoma [61], renal cell carcinoma [62], Myc-driven ER-negative breast cancer [63], and AML [64]. Elevated 2-HG is observed even in non-tumorigenic heart during ischemic preconditioning [65] and hematopoietic stem cells after disruption of the respiratory chain [66]. Are there additional enzymes, besides PHDGH, MDH1/2, and LDHA, and other conditions besides hypoxia and acidity, that can promiscuously produce or stimulate 2-HG production? In human cells both

primary activity. Enzyme promiscuity should not be confused with enzymes that have multiple substrates. It is believed that primordial enzymes possessed broad specificity and converted multiple substrates with similar efficiencies. Although contemporary enzymes have become specialized in catalyzing one reaction, many enzymes can promiscuously catalyze reactions other than those for which they have evolved.

KDM: refers to the JmjC-domain family of histone lysine (K) demethylases. These demethylases are evolutionarily conserved in eukaryotes and include 18 genes in humans. Except KDM1/LSD1, all KDMs belong to the α -KG/Fe(II)-dependent dioxygenase family, and they use α -KG as a co-substrate to oxidize the methyl group of lysine residues in histones, leading to eventual demethylation [123].

Neomorphic enzyme: an enzyme that has gained a novel function different from the wild-type gene by mutation.

TET: refers to the ten-eleven translocation family of DNA methylcytosine dioxygenases. Single TET enzymes are found in insects and there are three such enzymes in humans: TET1, TET2, and TET3. TET enzymes belong to the α -KG/Fe(II)-dependent dioxygenase family; they oxidize the methyl group on carbon 5 (C-5) of cytosine (5mC) in DNA. However, instead of a single demethylation reaction, TET enzymes catalyze three stepwise oxidations: (i) 5mC to 5-hydroxymethylcytosine (5hmC); (ii) 5hmC to 5-formylcytosine (5fC); and (iii) 5fC to 5-carboxylcytosine (5caC). DNA repair enzymes such as glycosylases remove 5caC, which is then replaced by unmethylated cytosine [124,125].



Trends in Cancer

Figure 1. Metabolism and Targets of 2-Hydroxyglutarate (2-HG). The thick and thin arrows represent the primary and promiscuous reactions, respectively. FAD, flavin adenine dinucleotide; FADH₂, reduced form of FAD; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of NADP. Additional abbreviations are listed in Table 1.

D2HGDH and L2HGDH are localized in the mitochondrion. Thus, the mitochondrion is probably the primary site that accumulates toxic concentrations of 2-HG, and this organelle is likely to have needed to evolve a mechanism for 2-HG removal (see Outstanding Questions). However, PHGDH, LDHA, and MDH1 are all localized in the cytoplasm. Is MDH2 the only enzyme producing 2-HG in mitochondria or are other enzymes (e.g., IDH2) involved? Enzyme promiscuity can be affected by reaction conditions (e.g., solvent, pH, temperature), substrate similarity (coenzyme, enantiomers), the structure of the substrate-binding pocket, and other conditions that relax active site stringency [67]. An oxidase or reductase whose active site can accommodate α-KG could theoretically produce 2-HG. The neomorphic activity acquired by IDH1/2 mutations is an illuminating example of enzyme promiscuity: a single point mutation at R132 in IDH1 or R172 in IDH2 reorganizes the active site, causing reduced affinity for its principal substrate (isocitrate) and increased affinity for NADPH to support α-KG reduction [14,68].

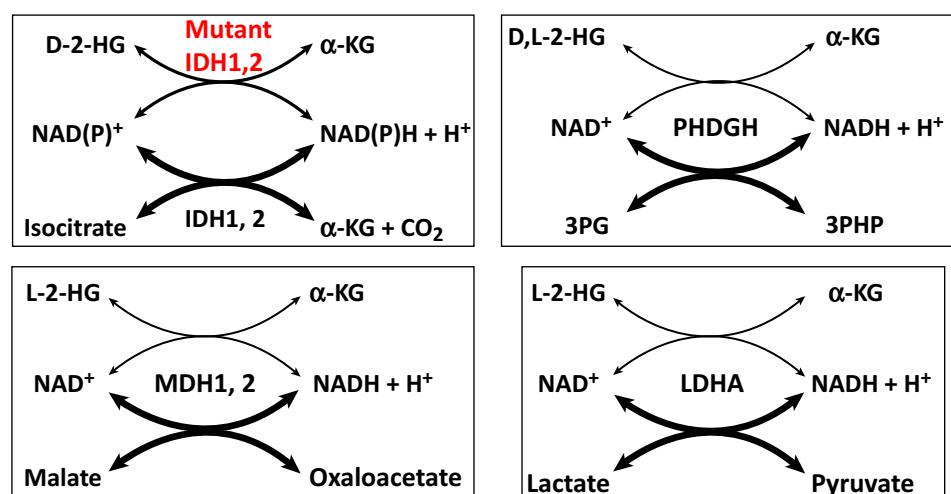
Can cells lacking IDH mutations acquire pathological concentrations of 2-HG? Hypoxia and an acidic extracellular environment are common features of tumor tissues, suggesting that 2-HG

Table 1. Abnormal Accumulation of D- and L-2-HG Impairs Multiple Cellular Pathways

2-HG enantiomer	Producing enzyme	Molecular target	Affected cellular pathway	Associated disease	Refs
D-2-HG	Mutant IDH1, 2	PHD/EGLN	HIF-1 α	Glioma	[12,73,74]
D-2-HG	Mutant IDH1, 2	TET	DNA demethylation	Glioma, AML	[19,23]
D-2-HG	Mutant IDH1, 2	KDM	Histone demethylation	Glioma, AML	[18,19]
D-2-HG	Mutant IDH1	ALKBH1, 2	DNA repair	Glioma	[18,79,80]
D-2-HG	Mutant IDH2	FTO	RNA demethylation	AML	[131]
D-2-HG	Mutant IDH2	N.D.	?	D-2HG aciduria type II	[44]
D-2-HG	Mutant IDH1, 2	N.D.	STAT1 pathway; T cell function and infiltration	Tumor growth	[92,93]
D-2-HG	Mutant IDH2	N.D.	N.D.	Cardiomyopathy	[115,132]
D-2-HG	Mutant IDH1, 2	KDM4A DEPTOR	mTOR pathway	N.D.	[94]
D-2-HG	D2HGDH mutation	N.D.	N.D.	D-2HG aciduria type I	[38,123,133]
D-2-HG	<i>In vitro</i> addition		PIN1, NF- κ B pathway and stromal cells	AML	[95]
D-2-HG	<i>In vitro</i> addition	Cytochrome c oxidase	Cell respiration		[134]
L-2-HG	LDHA ^a	KDM	Hypoxia	L-2HG aciduria	[49,55,56,58,59]
L-2-HG	MDH ^a	KDM	Hypoxia	L-2HG aciduria	[49,55]
L-2-HG	L2HGDH mutation	AASS		L-2HG aciduria	[103,135]
L-2-HG	LDHA	KDM	T cell function and infiltration	Tumor suppression	[57]
L-2-HG	L2HGDH low expression	N.D.	N.D.	Kidney cancer	[136]

AASS, aminoacidate-semialdehyde synthase (lysine- α -KG reductase); DEPTOR, DEP domain containing mTOR-interacting protein; FTO, fat mass and obesity-associated, α -KG-dependent dioxygenase; KDM, lysine demethylase; PHD, prolyl hydroxylase domain-containing (also EGLN, Egl-9-family hypoxia inducible factor); PIN1, peptidylprolyl *cis/trans* isomerase, NIMA-interacting 1; TET, ten-eleven translocation family of methylcytosine dioxygenase.

^aUnder acidic or hypoxia conditions.



Trends in Cancer

Figure 2. Production of 2-Hydroxyglutarate (2-HG) by Promiscuous Enzymatic Reactions. The thick and thin arrows represent the primary and promiscuous reactions, respectively. PHDGH, phosphoglycerate dehydrogenase; 3PG, 3-phosphoglycerate; 3PHP, 3-phosphohydroxypyruvate. Additional abbreviations are listed in Figure 1.

may accumulate to a pathologically significant concentration in cells lacking *IDH* mutations. 2-HG has been reported to reach millimolar concentrations comparable with concentrations found in *IDH1*-mutated cells [57,60]. Because L-2-HG is five- to tenfold more potent than D-2-HG in inhibition of some α -KG-dependent dioxygenases [18,19], it seems plausible that 2-HG elevation in IDH wild-type cells would have a pathological effect. The human *PHGDH* gene is genomically amplified in a subset of breast cancer [69] and melanoma [70] and its knockdown decreases cell proliferation but not intracellular serine concentration; thus, increased D-2-HG, but not altered serine biosynthesis, may contribute to tumorigenesis in these tumors. More broadly, hypoxia and an acidic environment resulting from lactate accumulation (the Warburg effect) are commonly associated with tumorigenesis. Because only a limited number of tumor samples has been analyzed for 2-HG accumulation, we do not know whether moderate 2-HG accumulation contributes to tumor development. A recent study found that tumor hypoxia broadly reduced the activity of TET dioxygenases and caused DNA hypermethylation [71]. Reduced TET activity resulted from a deficient supply of oxygen, a co-substrate of α -KG-dependent dioxygenases, but it remains to be determined whether 2-HG was elevated in these tumors to a concentration sufficiently high to have inhibited TET activity. An intriguing, still untested possibility is whether 2-HG elevation broadly impairs the DNA methylation landscape and thereby contributes to tumorigenesis.

2-HG Activity beyond Epigenetic Alteration

Most efforts to understand the mechanisms of IDH mutations and 2-HG in tumorigenesis have thus far focused on the **KDM** and TET enzymes and alterations of histone and DNA methylation, which have been extensively reviewed recently (e.g. [4,72]). Recent studies have revealed that D-2-HG can also antagonize other α -KG-dependent dioxygenases that are not involved in epigenetic alteration (Table 1).

2-HG Can Inhibit EGLN/PHD and Collagen Hydroxylases

An early study found that D-2-HG has the lowest half-maximal inhibitory concentration (IC_{50} = 24–106 μ M) toward KDM members, followed by ABH2 (424 μ M), FIH (1500 μ M), PHD2 (7300 μ M), and BBOX1 (13 200 μ M) [18]. Because D-2-HG accumulates to as high as 5–35 μ mol/g (5–35 mM) in *IDH*-mutated glioma [68], D-2-HG in *IDH*-mutant tumors could inhibit other α -KG-dependent dioxygenases and thereby affect cellular pathways besides epigenetic control. The first reported instance of an IDH mutation was a non-epigenetic α -KG-dependent dioxygenase – the **EGLN/PHD hydroxylase** that catalyzes proline hydroxylation of hypoxia-inducible transcription factor 1 α (HIF-1 α). Hydroxylation of HIF-1 α promotes its binding to the cullin2-VHL E3 ubiquitin ligase and subsequent degradation. In cultured cells, expression of mutant IDH1 increased HIF-1 α , an effect that was reversed by the addition of cell-permeable α -KG [12]. HIF-1 α accumulation was found in the brain tissues of mice expressing a brain-specific *Idh1* R132H conditional allele [73]. Whether PHD is a major target of mutant IDH is uncertain given the finding that D-2HG has a lower IC_{50} toward PHD than to other α -KG-dependent dioxygenases and that HIF could also be stabilized by other conditions, such as oxygen deprivation. A surprising finding was that D- but not L-2-HG stimulates PHD activity and reduces HIF levels, leading to enhanced proliferation of astrocytes [74]. Later, a separate study found that both enantiomers of 2-HG can be non-enzymatically oxidized to α -KG to concentrations that support *in vitro* catalysis by α -KG-dependent dioxygenases, including PHD2 [75]. L-2-HG is more potent than D-2HG in inhibiting α -KG-dependent dioxygenases and was found to also inhibit PHD and stabilize HIF-1 α *in vitro* and in cells [58,59,75]. In addition to EGLN/PHD hydroxylase, the activity of **collagen hydroxylase** was inhibited in *Idh1*-mutant brain tissues, as seen by the perturbation of collagen maturation and basement membrane function [73].

Thus, D-2-HG, when accumulated to a high level, may inhibit both groups of prolyl hydroxylases and impairs HIF-1 α signaling and collagen biogenesis.

2-HG Causes Genetic Instability

Disruptions in DNA repair pathways cause cells to accumulate DNA damage and become predisposed to oncogenic transformation. An ancient and critical DNA repair enzyme is *E. coli* AlkB, which was discovered by its activity to confer resistance to alkylating agents. AlkB repairs the cytotoxic lesions 1-methyladenine (1 mA) and 3-methylcytosine (3mC) in DNA, in a reaction requiring oxygen, α -KG, and Fe(II) [76,77]. Nine AlkB homolog (**ALKBH**) genes exist in mammalian cells (*ALKBH1–8*, *FTO*) and ALKBH2 and ALKBH3 repair alkylating DNA damage by a mechanism similar to that of *E. coli* AlkB [78]. D-2-HG has been demonstrated to directly inhibit ALKBH2 and ALKBH3 *in vitro* [18,79]. Cells expressing mutant IDH display reduced repair kinetics and accumulate double-strand DNA breaks (DSBs). These cells are sensitized to alkylating agents that depend on the catalytic activity of mutant IDH to produce D-2-HG, thus linking D-2-HG accumulation to DNA damage [79]. A more detailed kinetic analysis showed that when the ratio of D-2-HG to α -KG was set to mimic their average concentrations in *IDH*-mutated glioma (3.73 mM vs 100 μ M), the repair efficiencies of ALKBH2 and ALKBH3 were 73–88% inhibited and were restored by addition of α -KG [80]. These findings suggested that the inhibition of ALKBH observed *in vitro* and in cultured cells can occur in *IDH*-mutated tumors.

Besides ALKBH inhibition, D-2-HG can also impair DNA repair and cause genetic instability indirectly, by altering the expression of genes involved in DNA repair. In HCT116 colon cancer cells and HeLa cervical cancer cells engineered to express R132H mutant IDH1 in the endogenous locus, there was a significant increase in DSBs and sensitivity to inhibitors of poly(adenosine 5'-diphosphate-ribose) polymerase (PARP) [81]. These increases were attributed to a marked deficiency in homologous recombination (HR), a major pathway of DSB repair. An siRNA screen targeting 64 known α -KG-dependent dioxygenases identified two closely related H3K9 histone demethylases, KDM4A and KDM4B, that link DSB repair to the recruitment of repair factors to sites of DNA damage [81]. A possible link between KDM inhibition and increased DNA damage was suggested in a study of myeloid lineage-specific *Idh1* R132H conditional knock-in mice, which exhibited diminished DNA repair, age-dependent accumulation of DNA damage, and impaired self-renewal of long-term hematopoietic stem cells [82]. These defects were linked to an increase in repressive histone methylation (e.g., H3K9, H3K27), both globally and in the promoter of the DNA damage sensor ATM, and to decreased ATM expression in *IDH1*-mutated mouse hematopoietic stem cells and human AML samples [82].

In sum, these studies suggest that *IDH* mutation and D-2-HG accumulation cause genetic instability and contribute to tumorigenesis, either directly by inhibiting ALKBH repair enzymes or indirectly by altering the expression of DNA repair genes. These studies also provide a molecular explanation for the finding that chemotherapy with a combination of DNA alkylating agents benefits glioma patients with *IDH1* mutations [83,84]. Similarly, daunorubicin, an inhibitor of topoisomerase II, sensitizes *Idh1* hematopoietic stem cells [82] and is more effective in treating *IDH1*-mutated AML [85]. These findings may be relevant for the design of treatment strategies for *IDH*-mutated tumors with regard to whether the use of inhibitors of mutant IDH might reduce the efficacy of DNA-damaging chemotherapy agents (see discussion below).

2-HG Affects T Cell Differentiation and Tumor Immunity

Leukemic IDH mutations result in 2-HG accumulation and disrupt TET2 function, effects that have been shown to promote reversible leukemogenesis in cultured hematopoietic cells [23,86,87]. An intriguing new pathway linked to 2-HG is T cell differentiation and tumor

immunity. T cell activation is associated with dramatic metabolic reprogramming, notably the switch from oxidative phosphorylation to glycolysis [88]. These metabolic changes support T cell proliferation, differentiation, and function. T-helper 17 (T_H17) and T regulatory (T_{reg}) cells are T cell subtypes that share similar differentiation precursors from naive CD4⁺ T cells but perform different functions in immune homeostasis (i.e., promotion and suppression of inflammation, respectively). 2-HG was found to be five- to tenfold higher in T_H17 cells compared with T_{reg} cells and elevated 2-HG was associated with increased promoter methylation and transcriptional inhibition of Foxp3, the master regulatory transcription factor of T_{reg} cells [89]. Treatment of naive CD4⁺ T cells with 2-HG or knockdown of *Tet* genes stimulated T_H17 cell differentiation. The origin of 2-HG in T_H17 cells was traced to glutamate, which can be converted to α -KG by glutamic-oxaloacetic transaminase (GOT). In mice, inhibition of GOT by a pan-transaminase inhibitor, aminooxyacetic acid (AOA), increased Foxp3 expression, reprogrammed T_H17 to T_{reg} cells *in vitro*, and caused autoimmune encephalomyelitis. We do not yet know which enzyme produces 2-HG in T_H17 cells.

CD8⁺ T cells, the cytotoxic T lymphocytes that kill cancer cells, traffic to hypoxic areas in tumors and inflamed tissues, and HIF-1 α mediates this migration [90,91]. Activation of HIF-1 α in CD8⁺ T cells increased LDHA, which converts glutamine-derived α -KG to L-2-HG and causes L-2-HG concentrations to be as high as 1.5 mM [57]. Increased L-2-HG altered the gene expression and differentiation pathway of CD8⁺ T cells, leading to enhanced antitumor capacity in mice when adoptively transferred with tumor cells. This enhanced antitumor immunity function could be recapitulated by simply soaking CD8⁺ T cells with cell-permeable L-2-HG. Both KDM and TET have been implicated as targets of L-2-HG [57]. In contrast to enhancing the function of immune cells, accumulation of 2-HG in tumors has been linked to resistance to tumor immunity. Human *IDH1*-mutant gliomas have reduced expression of chemokines and less immune infiltration compared with wild-type *IDH1* gliomas [92,93]. Analysis in syngeneic mice showed that, compared with glioma with wild-type *IDH1*, *IDH*-mutated gliomas have fewer immune cells, reduced expression of chemokine genes, neutrophil chemotaxis, and accumulation of T cells in tumors. Treatment of *IDH1* wild-type cells *in vitro* with cell-permeable D-2-HG reduced chemokine production and, conversely, treatment of *IDH1*-mutated cells with an inhibitor of mutant IDH1 blocks D-2-HG-increased chemokine production and T cell infiltration [93]. It will be important to establish the target enzymes of 2-HG and downstream genes responsible for the enhancement and resistance to immunity in immune and tumor cells (see Outstanding Questions).

Does 2-HG Target Other Proteins Besides α -KG-Dependent Dioxygenases?

2-HG accumulation has also been linked to several additional cellular pathways. Prompted by the mutually exclusive mutation between *IDH1* and *PETN* in low-grade glioma, it was found that D-2-HG activates mechanistic target of rapamycin (mTOR) signaling partly by inhibiting the activity of KDM4A, which promotes the stability of DEPTOR, a negative regulator of mTORC1/2 [94]. Accumulation D-2-HG in colorectal cancer cells lacking *IDH* mutation was associated with increased H3K4 trimethylation in the promoter of ZEB1, a regulator of epithelial–mesenchymal transition (EMT), and increased ZEB1 expression and EMT [60]. In addition, D-2-HG has been linked to the activation of nuclear factor kappa B (NF- κ B) signaling [95] and altered metabolism [96–99], although we do not know the direct targets of 2-HG in these processes.

In addition to α -KG, 2-HG is structurally similar to other TCA metabolites, including succinate. This has led to studies to determine the effect of D-2-HG toward the enzyme that uses succinate as a substrate, succinate dehydrogenase (SDH). D-2-HG was found to be able to competitively inhibit SDH resulting in the accumulation of succinate, increased protein

hypersuccinylation, and impairment of mitochondrial respiration [100]. In addition, 2-HG has been reported to bind the DNA methyltransferase DNMT1 and the small GTPase Cdc42, although neither enzyme binds α -KG [101,102]. 2-HG stimulated DNMT1 to bind the promoter and repress the expression of receptor-interacting protein kinase 3 (RIP3), a regulator of programmed necrosis/necroptosis, suggesting that impaired RIP3-dependent necroptosis may contribute to tumorigenesis driven by *IDH* mutation [102]. Binding of 2-HG to Cdc42 disrupted Cdc42's association with the effector mixed lineage kinase 3 (MLK3), leading to suppression of MLK3 activation and MLK3-mediated apoptosis in serum-deprived mutant IDH1 cells [101]. Future genetic and biophysical characterizations are necessary to understand the significance and affinities of 2-HG binding to DNMT1 and Cdc42.

Therapeutic Targeting of *IDH* Mutant Tumors

Two unique aspects make mutant IDH1 and IDH2 attractive targets for the treatment of *IDH*-mutated tumors. First, mutant IDH1/2 acquires a neomorphic activity distinct from the wild-type IDH1/2. An inhibitor specific to a mutant enzyme should not interfere with wild-type enzyme activity. Second, the product of mutant IDH1, D-2-HG, apparently lacks any physiological function. Complete inhibition of D-2-HG synthesis, unlike inhibition of other gain-of-function oncogenes, should have no or minimal toxicity. There is great interest in developing small-molecule inhibitors of mutant IDH enzymes. However, we do not yet have definitive evidence of 2-HG addiction by IDH-mutant tumors. Although several investigators have reported that inhibiting 2-HG production resulted in reduced tumor growth [103–105], one study reported that, in some contexts, cell transformation by mutant IDH1 was not easily reversed by 2-HG depletion [106]. Presently, there are 19 registered clinical trials (see ClinicalTrials.gov) testing the responses of advanced hematological malignancies (AML, MDS/MPN, and AITL) and advanced solid tumors (glioma, cholangiocarcinoma, and chondrosarcoma) to six inhibitors of mutant IDH enzymes, four targeting mutant IDH1 (AG-120, IDH-305, FT-2102, and BAY-1436032), one targeting mutant IDH2 (AG-221), and one pan-inhibitor targeting both mutant IDH1 and mutant IDH2 (AG-881).

Monotherapy Targeting Mutant IDH Enzymes

The first reported small-molecule inhibitor of mutant IDH, compound 35 (later referred to as AGI-5198), was identified in a high-throughput screen against the IDH1(R132H) homodimer. AGI-5198 inhibited both R132H- and R132C-mutant IDH1 with similar potencies (IC_{50} = 70 and 160 nM, respectively) and AGI-5198 did not inhibit wild-type IDH1 or mutant IDH2 (IC_{50} > 100 μ M) [104,107]. Using patient-derived IDH1 (R132H) TS603 glioma cells, this compound was shown to inhibit D-2-HG production and induce histone methylation and gliogenic differentiation. Also, AGI-5198 reduced colony formation in soft agar and tumor growth in a mouse xenograft model [104]. When tested in *IDH1*-mutated cholangiocarcinoma cells, this compound exhibited greater potency, it inhibited colony formation and cell migration and induced apoptosis [108]. Several additional inhibitors have since been reported to potently inhibit mutant IDH1 with no apparent effect on wild-type IDH1 or IDH2 [109–113]. These latter inhibitors are structurally different and inhibit mutant IDH1 enzymes by different mechanisms, such as competing with α -KG for binding to the active site [107], blocking magnesium binding [112], and locking the enzyme in a catalytically inactive conformation [111]. There are only two reports on the effect of mutant IDH1 inhibitors in patient-derived *IDH1*-mutant glioma cells in an orthotopic mouse model and presently the results are inconclusive [105,106].

Inhibitors of mutant IDH2 have also been developed and advanced to clinical trials. The first inhibitor, AGI-6780, was a slow tight binder (causing inhibition at a concentration comparable with that of the enzyme) discovered in high-throughput screening against IDH2(R140Q) [114].

AGI-6780 binds allosterically to the dimer interface and induces the differentiation of leukemia cells expressing mutant IDH2. An optimized inhibitor, AG-221, displayed nanomolar potency against the IDH2(R140Q) homodimer ($IC_{50} = 0.1 \mu M$), the IDH2(R140Q/WT) heterodimer ($IC_{50} = 0.03 \mu M$), and the IDH2(R172 K/WT) heterodimer ($IC_{50} = 0.01 \mu M$) and is much less inhibitory toward the wild-type IDH2 homodimer ($IC_{50} = 1.8 \mu M$). AG-221 inhibited D-2-HG production and induced the differentiation of *IDH2*-mutated primary leukemia cells; it suppressed D-2-HG production and extended survival in tumor xenograft models [113]. An inhibitor of mutant IDH2, AGI-026, has also been shown to provide survival benefit in a D2HGA mouse model with an R140Q knock-in mutation in the *Idh2* locus [115].

The only reported human clinical trials targeting mutant IDH thus far are two Phase I/II studies of relapsed or refractory AML patients treated with AG-221 (enasidenib) [116,117]. In August 2017, AG-221 was approved by the FDA as the first drug targeting a mutant IDH enzyme. Continuous daily treatment with enasidenib was generally well tolerated and induced hematological responses with an overall response rate of 40.3% and a median response duration of 5.8 months in patients in whom prior AML therapy had failed. Induction of differentiation, not cytotoxicity, appears to drive the clinical efficacy. Suppression of 2-HG alone, however, did not predict response, as most non-responding patients also exhibited 2-HG suppression. Co-occurring mutations in NRAS and other MAPK pathway effectors were enriched in non-responding patients, suggesting that RAS signaling contributes to therapeutic resistance.

Combination Therapy and Novel Strategies Targeting IDH-Mutant Tumors

Combination therapy, a treatment using more than one medication or modality, is a traditional cornerstone of cancer therapy. The design of combination therapies is being significantly facilitated by cancer genomics, which has identified specific driver mutations and addicted oncogenes in various types and subtypes of tumors, revealing tumor vulnerabilities and novel targets. The first such vulnerability of *IDH*-mutant tumors was recognized retrospectively from the treatment of low-grade glioma patients with a chemotherapy known as PCV that includes a combination of two DNA alkylating agents, procarbazine and CCNU/lomustine, and a microtubule poison, vincristine. Following successful chemotherapy for recurrent malignant oligodendroglioma [118], randomized controlled trials confirmed the benefits of PCV for patients with low-grade glioma. PCV benefit was subsequently linked to *IDH1* mutations with an overall survival of 9.4 years for patients with *IDH* mutation versus 5.7 years for patients with wild-type *IDH* [83,84]. One probable mechanism is that the D-2-HG accumulated in *IDH1*-mutated glioma inhibited the activity of ALKBH2 and ALKBH3 to repair the alkylating lesions, sensitizing *IDH1*-mutant cells to PCV [79]. Temozolomide (TMZ), another alkylating agent, is a more easily administered, less toxic alternative to PCV. It is still being debated whether TMZ confers survival benefit on patients with *IDH*-mutant glioma [43].

IDH-mutant tumors are also vulnerable to inhibitors of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in the NAD^+ salvage pathway [106]. This vulnerability was discovered because mutant *IDH1* reduces the NAD^+ concentration and NAD^+ is a critical coenzyme for many enzymes. Depletion of NAD^+ in *IDH1*-mutated cells was attributed to increased promoter methylation and decreased expression of *NAPRT1*, the rate-limiting enzyme in NAD^+ biosynthesis, and NAD^+ depletion rendered cells sensitive to inhibition of the NAMPT-catalyzed NAD^+ salvage pathway [106]. Tateishi *et al.* showed that TMZ caused a burst of consumption of NAD^+ and further sensitized *IDH1*-mutant cells to NAMPT inhibitors [119]. In addition to sensitizing cells to alkylating agents and NAMPT inhibitors, 2-HG can sensitize mutant *IDH1*-engineered cells to inhibitors of PARP [81,120], a family of proteins that catalyze polymerization of ADP-ribose derived from NAD^+ and that is involved in the repair of

various types of DNA damage. We currently do not know the molecular pathway underlying 2-HG-induced sensitivity to PARP1 inhibitors. There are conflicting reports on whether a decreased concentration of NAD⁺ sensitizes *IDH1*-mutated cells to PARP inhibitors [81,119,121]. Therefore, it appears that 2-HG accumulation can cause genetic instability by inhibiting the activity of ALKBH2/3 [18,79], decreasing the expression of *ATM* [82], and impairing the function of PARP, sensitizing *IDH*-mutant cells to multiple DNA-damaging agents and inhibitors of DNA repair pathways.

Concluding Remarks

Both D- and L-2-HG enantiomers were considered dead-end metabolites without physiological functions and remained largely unnoticed for more than a century. The origin of their production was a mystery and the mechanism of their toxicity at elevated concentrations was unknown (see Outstanding Questions). The discovery of 2-HG production and accumulation in *IDH*-mutated tumors transformed 2-HG into the 'poster child' for oncometabolites. The major targets of 2-HG, α -KG-dependent dioxygenases, have been identified, including the KDM family of histone demethylases and the TET family of DNA demethylases. Recent studies have revealed activities of 2-HG beyond epigenetic control and have even linked 2-HG to the normal process of T cell regulation. Investigators have also identified additional sources of 2-HG from promiscuous enzyme reactions in cells lacking *IDH* mutations. These studies have raised many questions regarding the metabolism and activity of 2-HG. *IDH* mutation is one of the earliest genetic alterations during gliomagenesis, suggesting a function for D-2-HG in tumor initiation. What role does D-2-HG play in tumor progression and maintenance? Does 2-HG play different roles in tumors and T cells during tumorigenesis *in vivo*? Are there other enzymes that promiscuously produce 2-HG? What metabolic and stress conditions affect the promiscuity of these enzymes? 2-HG cannot diffuse across the cell membrane. Do specific transporters move 2-HG between different subcellular compartments or across the plasma membrane? D2HGDH and L2HGDH are localized in the mitochondrion. Do D-2-HG and L-2-HG actively accumulate in this compartment and affect mitochondrial respiration?

Answers to these and other questions will lead to new mechanistic and clinical understanding of 2-HG metabolism and activity and how 2-HG metabolism affects gene expression and other nuclear activities.

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References

1. Parsons, D.W. *et al.* (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* 321, 1807–1812
2. Mardis, E.R. *et al.* (2009) Recurring mutations found by sequencing an acute myeloid leukemia genome. *N. Engl. J. Med.* 361, 1058–1066
3. Yang, H. *et al.* (2012) *IDH1* and *IDH2* mutations in tumorigenesis: mechanistic insights and clinical perspectives. *Clin. Cancer Res.* 18, 5562–5571
4. Dang, L. and Su, S.M. (2017) Isocitrate dehydrogenase mutation and (R)-2-hydroxyglutarate: from basic discovery to therapeutics development. *Annu. Rev. Biochem.* 86, 305–331
5. Watanabe, T. *et al.* (2009) *IDH1* mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am. J. Pathol.* 174, 1149–1153
6. Brat, D.J. *et al.* (2015) Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N. Engl. J. Med.* 372, 2481–2498
7. Suzuki, H. *et al.* (2015) Mutational landscape and clonal architecture in grade II and III gliomas. *Nat. Genet.* 47, 458–468
8. Ceccarelli, M. *et al.* (2016) Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. *Cell* 164, 550–563

Outstanding Questions

What enzymes produce 2-HG in cells lacking *IDH* mutations?

What metabolic and stress conditions affect 2-HG production in cells lacking *IDH* mutations?

Does 2-HG bind to and affect the activities of proteins besides α -KG-dependent dioxygenases?

Does 2-HG function in any normal cellular process?

9. Eckel-Passow, J.E. *et al.* (2015) Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. *N. Engl. J. Med.* 372, 2499–2508
10. Ley, T.J. *et al.* (2013) Genomic and epigenomic landscapes of adult *de novo* acute myeloid leukemia. *N. Engl. J. Med.* 368, 2059–2074
11. Chaisaingmongkol, J. (2017) Common molecular subtypes among asian hepatocellular carcinoma and cholangiocarcinoma. *Cancer Cell* 32, 57–70 e3
12. Zhao, S. *et al.* (2009) Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1 α . *Science* 324, 261–265
13. Yan, H. *et al.* (2009) IDH1 and IDH2 mutations in gliomas. *N. Engl. J. Med.* 360, 765–773
14. Ward, P.S. *et al.* (2010) The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting α -ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* 17, 225–234
15. Dang, L. *et al.* (2010) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 465, 966
16. Loenarz, C. and Schofield, C.J. (2011) Physiological and biochemical aspects of hydroxylations and demethylations catalyzed by human 2-oxoglutarate oxygenases. *Trends Biochem. Sci.* 36, 7–18
17. Martínez, S. and Hausinger, R.P. (2015) Catalytic mechanisms of Fe(II)- and 2-oxoglutarate-dependent oxygenases. *J. Biol. Chem.* 290, 20702–20711
18. Chowdhury, R. *et al.* (2011) The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep.* 12, 463–469
19. Xu, W. *et al.* (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α -ketoglutarate-dependent dioxygenases. *Cancer Cell* 19, 17–30
20. Noushmehr, H. *et al.* (2010) Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* 17, 510–522
21. Wang, P. *et al.* (2013) Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. *Oncogene* 32, 3091–3100
22. Turcan, S. *et al.* (2012) IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 483, 479–483
23. Figueroa, M.E. *et al.* (2010) Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* 18, 553–567
24. Lu, C. *et al.* (2012) IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature* 483, 474–478
25. Ritthausen, H. (1868) Ueber die glutansäure, das zersetzungsprodukt der glutaminsäure durch salpetrige säure. *J. Prakt. Chem.* 103, 239–242 (in German)
26. Ritthausen, H. (1872) Ueber das drehungsvermögen von glutan- und aepfelsäure. *J. Prakt. Chem.* 5, 354–355 (in German)
27. Thunberg, T. (1920) Zur kenntnis des intermediären stoffwechsels und der dabei wirksamen enzyme. *Skand. Arch. Physiol.* 40, 1–91 (in German)
28. Weil-Malherbe, H. (1937) The oxidation of l(-)- α -hydroxyglutaric acid in animal tissues. *Biochem. J.* 31, 2080–2094
29. Lawson, A.M. *et al.* (1976) Urinary organic acids in man. I. Normal patterns. *Clin. Chem.* 22, 1283–1287
30. Gregersen, N. *et al.* (1977) Low molecular weight organic acids in the urine of the newborn. *Acta Paediatr. Scand.* 66, 85–89
31. Chalmers, R.A. *et al.* (1980) D-2-Hydroxyglutaric aciduria: case report and biochemical studies. *J. Inher. Metab. Dis.* 3, 11–15
32. Duran, M. *et al.* (1980) L-2-Hydroxyglutaric aciduria: an inborn error of metabolism? *J. Inher. Metab. Dis.* 3, 109–112
33. Kranendijk, M. *et al.* (2012) Progress in understanding 2-hydroxyglutaric acidurias. *J. Inher. Metab. Dis.* 35, 571–587
34. Aghili, M. *et al.* (2009) Hydroxyglutaric aciduria and malignant brain tumor: a case report and literature review. *J. Neurooncol.* 91, 233–236
35. Rzem, R. *et al.* (2004) A gene encoding a putative FAD-dependent L-2-hydroxyglutarate dehydrogenase is mutated in L-2-hydroxyglutaric aciduria. *Proc. Natl. Acad. Sci. U. S. A.* 101, 16849–16854
36. Topcu, M. *et al.* (2004) L-2-Hydroxyglutaric aciduria: identification of a mutant gene *C14orf160*, localized on chromosome 14q22.1. *Hum. Mol. Genet.* 13, 2803–2811
37. Achouri, Y. *et al.* (2004) Identification of a dehydrogenase acting on D-2-hydroxyglutarate. *Biochem. J.* 381, 35–42
38. Struys, E.A. *et al.* (2005) Mutations in the D-2-hydroxyglutarate dehydrogenase gene cause D-2-hydroxyglutaric aciduria. *Am. J. Hum. Genet.* 76, 358–360
39. Struys, E.A. *et al.* (2005) Mutations in phenotypically mild D-2-hydroxyglutaric aciduria. *Ann. Neurol.* 58, 626–630
40. Lin, A.P. *et al.* (2015) D2HGDH regulates α -ketoglutarate levels and dioxygenase function by modulating IDH2. *Nat. Commun.* 6, 7768
41. Kalliri, E. *et al.* (2008) Identification of *Escherichia coli* YgaF as an L-2-hydroxyglutarate oxidase. *J. Bacteriol.* 190, 3793–3798
42. Li, H. *et al.* (2017) *Drosophila* larvae synthesize the putative oncometabolite L-2-hydroxyglutarate during normal developmental growth. *Proc. Natl. Acad. Sci. U. S. A.* 114, 1353–1358
43. Becker-Kettern, J. *et al.* (2016) *Saccharomyces cerevisiae* forms D-2-hydroxyglutarate and couples its degradation to D-lactate formation via a cytosolic transhydrogenase. *J. Biol. Chem.* 291, 6036–6058
44. Kranendijk, M. *et al.* (2010) IDH2 mutations in patients with D-2-hydroxyglutaric aciduria. *Science* 330, 336
45. Muntau, A.C. *et al.* (2000) Combined D-2- and L-2-hydroxyglutaric aciduria with neonatal onset encephalopathy: a third biochemical variant of 2-hydroxyglutaric aciduria? *Neuropediatrics* 31, 137–140
46. Nota, B. *et al.* (2013) Deficiency in SLC25A1, encoding the mitochondrial citrate carrier, causes combined D-2- and L-2-hydroxyglutaric aciduria. *Am. J. Hum. Genet.* 92, 627–631
47. Struys, E.A. *et al.* (2004) Investigations by mass isotopomer analysis of the formation of D-2-hydroxyglutarate by cultured lymphoblasts from two patients with D-2-hydroxyglutaric aciduria. *FEBS Lett.* 557, 115–120
48. Struys, E.A. *et al.* (2007) Novel insights into L-2-hydroxyglutaric aciduria: mass isotopomer studies reveal 2-oxoglutaric acid as the metabolic precursor of L-2-hydroxyglutaric acid. *J. Inher. Metab. Dis.* 30, 690–693
49. Rzem, R. *et al.* (2007) L-2-Hydroxyglutaric aciduria, a defect of metabolite repair. *J. Inher. Metab. Dis.* 30, 681–689
50. Khersonsky, O. and Tawfik, D.S. (2010) Enzyme promiscuity: a mechanistic and evolutionary perspective. *Annu. Rev. Biochem.* 79, 471–505
51. Zhao, G. and Winkler, M.E. (1996) A novel α -ketoglutarate reductase activity of the serA-encoded 3-phosphoglycerate dehydrogenase of *Escherichia coli* K-12 and its possible implications for human 2-hydroxyglutaric aciduria. *J. Bacteriol.* 178, 232–239
52. Fan, J. *et al.* (2015) Human phosphoglycerate dehydrogenase produces the oncometabolite D-2-hydroxyglutarate. *ACS Chem. Biol.* 10, 510–516
53. Achouri, Y. *et al.* (1997) Cloning, sequencing and expression of rat liver 3-phosphoglycerate dehydrogenase. *Biochem. J.* 323, 365–370
54. Wise, D.R. *et al.* (2011) Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α -ketoglutarate to citrate to support cell growth and viability. *Proc. Natl. Acad. Sci. U. S. A.* 108, 19611–19616
55. Intlekofer, A.M. *et al.* (2015) Hypoxia induces production of L-2-hydroxyglutarate. *Cell Metab.* 22, 304–311

56. Oldham, W.M. *et al.* (2015) Hypoxia-mediated increases in L-2-hydroxyglutarate coordinate the metabolic response to reductive stress. *Cell Metab.* 22, 291–303
57. Tyrakis, P.A. *et al.* (2016) S-2-Hydroxyglutarate regulates CD8⁺ T-lymphocyte fate. *Nature* 540, 236–241
58. Intlekofer, A.M. *et al.* (2017) L-2-Hydroxyglutarate production arises from noncanonical enzyme function at acidic pH. *Nat. Chem. Biol.* 13, 494–500
59. Nadochiy, S.M. *et al.* (2016) Acidic pH is a metabolic switch for 2-hydroxyglutarate generation and signaling. *J. Biol. Chem.* 291, 20188–20197
60. Colvin, H. *et al.* (2016) Oncometabolite D-2-hydroxyglutarate directly induces epithelial–mesenchymal transition and is associated with distant metastasis in colorectal cancer. *Sci. Rep.* 6, 36289
61. Rakheja, D. *et al.* (2011) Papillary thyroid carcinoma shows elevated levels of 2-hydroxyglutarate. *Tumour Biol.* 32, 325–333
62. Shim, E.H. *et al.* (2014) L-2-Hydroxyglutarate: an epigenetic modifier and putative oncometabolite in renal cancer. *Cancer Discov.* 4, 1290–1298
63. Terunuma, A. *et al.* (2014) MYC-driven accumulation of 2-hydroxyglutarate is associated with breast cancer prognosis. *J. Clin. Invest.* 124, 398–412
64. Wang, J.H. *et al.* (2013) Prognostic significance of 2-hydroxyglutarate levels in acute myeloid leukemia in China. *Proc. Natl. Acad. Sci. U. S. A.* 110, 17017–17022
65. Nadochiy, S.M. *et al.* (2015) Metabolomic profiling of the heart during acute ischemic preconditioning reveals a role for SIRT1 in rapid cardioprotective metabolic adaptation. *J. Mol. Cell. Cardiol.* 88, 64–72
66. Anso, E. *et al.* (2017) The mitochondrial respiratory chain is essential for haematopoietic stem cell function. *Nat. Cell Biol.* 19, 614–625
67. Hult, K. and Berglund, P. (2007) Enzyme promiscuity: mechanism and applications. *Trends Biotechnol.* 25, 231–238
68. Dang, L. *et al.* (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 462, 739–744
69. Possemato, R. *et al.* (2011) Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 476, 346–350
70. Locasale, J.W. *et al.* (2011) Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. *Nat. Genet.* 43, 869–874
71. Thienpont, B. *et al.* (2016) Tumour hypoxia causes DNA hypermethylation by reducing TET activity. *Nature* 537, 63–68
72. McKenney, A.S. and Levine, R.L. (2013) Isocitrate dehydrogenase mutations in leukemia. *J. Clin. Invest.* 123, 3672–3677
73. Sasaki, M. *et al.* (2012) D-2-Hydroxyglutarate produced by mutant IDH1 perturbs collagen maturation and basement membrane function. *Genes Dev.* 26, 2038–2049
74. Koivunen, P. *et al.* (2012) Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. *Nature* 483, 484–488
75. Tarhonskaya, H. *et al.* (2014) Non-enzymatic chemistry enables 2-hydroxyglutarate-mediated activation of 2-oxoglutarate oxygenases. *Nat. Commun.* 5, 3423
76. Treweek, S.C. *et al.* (2002) Oxidative demethylation by *Escherichia coli* AlkB directly reverts DNA base damage. *Nature* 419, 174–178
77. Farnes, P.O. *et al.* (2002) AlkB-mediated oxidative demethylation reverses DNA damage in *Escherichia coli*. *Nature* 419, 178–182
78. Duncan, T. *et al.* (2002) Reversal of DNA alkylation damage by two human dioxygenases. *Proc. Natl. Acad. Sci. U. S. A.* 99, 16660–16665
79. Wang, P. *et al.* (2015) Oncometabolite D-2-hydroxyglutarate inhibits ALKBH DNA repair enzymes and sensitizes IDH mutant cells to alkylating agents. *Cell Rep.* 13, 2353–2361
80. Chen, F. *et al.* (2017) Oncometabolites D- and L-2-hydroxyglutarate inhibit the AlkB family DNA repair enzymes under physiological conditions. *Chem. Res. Toxicol.* 30, 1102–1110
81. Sulkowski, P.L. *et al.* (2017) 2-Hydroxyglutarate produced by neomorphic IDH mutations suppresses homologous recombination and induces PARP inhibitor sensitivity. *Sci. Transl. Med.* 9, eaa12463
82. Inoue, S. *et al.* (2016) Mutant IDH1 downregulates ATM and alters DNA repair and sensitivity to DNA damage independent of TET2. *Cancer Cell* 30, 337–348
83. Cairncross, J.G. *et al.* (2014) Benefit from procarbazine, lomustine, and vincristine in oligodendroglial tumors is associated with mutation of IDH. *J. Clin. Oncol.* 32, 783–790
84. Buckner, J.C. *et al.* (2016) Radiation plus procarbazine, CCNU, and vincristine in low-grade glioma. *N. Engl. J. Med.* 374, 1344–1355
85. Patel, J.P. *et al.* (2012) Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N. Engl. J. Med.* 366, 1079–1089
86. Gross, S. *et al.* (2010) Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *J. Exp. Med.* 207, 339–344
87. Losman, J.A. *et al.* (2013) (R)-2-Hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science* 339, 1621–1625
88. Machver, N.J. *et al.* (2013) Metabolic regulation of T lymphocytes. *Annu. Rev. Immunol.* 31, 259–283
89. Xu, T. *et al.* (2017) Metabolic control of TH17 and induced Treg cell balance by an epigenetic mechanism. *Nature* 548, 228–233
90. Doedens, A.L. *et al.* (2013) Hypoxia-inducible factors enhance the effector responses of CD8⁺ T cells to persistent antigen. *Nat. Immunol.* 14, 1173–1182
91. Finlay, D.K. *et al.* (2012) PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8⁺ T cells. *J. Exp. Med.* 209, 2441–2453
92. Amankulor, N.M. *et al.* (2017) Mutant IDH1 regulates the tumor-associated immune system in gliomas. *Genes Dev.* Published online May 2, 2017. <http://dx.doi.org/10.1101/gad.294991.116>
93. Kohanbash, G. *et al.* (2017) Isocitrate dehydrogenase mutations suppress STAT1 and CD8⁺ T cell accumulation in gliomas. *J. Clin. Invest.* 127, 1425–1437
94. Carboneau, M. *et al.* (2016) The oncometabolite 2-hydroxyglutarate activates the mTOR signalling pathway. *Nat. Commun.* 7, 12700
95. Chen, J.Y. *et al.* (2016) The oncometabolite R-2-hydroxyglutarate activates NF- κ B-dependent tumor-promoting stromal niche for acute myeloid leukemia cells. *Sci. Rep.* 6, 32428
96. Reitman, Z.J. *et al.* (2011) Profiling the effects of isocitrate dehydrogenase 1 and 2 mutations on the cellular metabolome. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3270–3275
97. Izquierdo-Garcia, J.L. *et al.* (2015) IDH1 mutation induces reprogramming of pyruvate metabolism. *Cancer Res.* 75, 2999–3009
98. Grassian, A.R. *et al.* (2014) IDH1 mutations alter citric acid cycle metabolism and increase dependence on oxidative mitochondrial metabolism. *Cancer Res.* 74, 3317–3331
99. Chesnelong, C. *et al.* (2014) Lactate dehydrogenase A silencing in IDH mutant gliomas. *Neuro Oncol.* 16, 686–695
100. Li, F. *et al.* (2015) NADP⁺-IDH mutations promote hypersuccinylation that impairs mitochondria respiration and induces apoptosis resistance. *Mol. Cell* 60, 661–675
101. Jiang, B. *et al.* (2017) IDH1 mutation promotes tumorigenesis by inhibiting JNK activation and apoptosis induced by serum starvation. *Cell Rep.* 19, 389–400
102. Yang, Z. *et al.* (2017) 2-HG inhibits necroptosis by stimulating DNMT1-dependent hypermethylation of the RIP3 promoter. *Cell Rep.* 19, 1846–1857

103. Ma, S. *et al.* (2017) *L2hgdh* deficiency accumulates L-2-hydroxyglutarate with progressive leukoencephalopathy and neurodegeneration. *Mol. Cell. Biol.* Published online January 30, 2017. <http://dx.doi.org/10.1128/MCB.00492-16>
104. Rohle, D. *et al.* (2013) An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* 340, 626–630
105. Pusch, S. *et al.* (2017) Pan-mutant IDH1 inhibitor BAY 1436032 for effective treatment of IDH1 mutant astrocytoma *in vivo*. *Acta Neuropathol.* 133, 629–644
106. Tateishi, K. *et al.* (2015) Extreme vulnerability of IDH1 mutant cancers to NAD⁺ depletion. *Cancer Cell* 28, 773–784
107. Popovici-Muller, J. *et al.* (2012) Discovery of the first potent inhibitors of mutant IDH1 that lower tumor 2-HG *in vivo*. *ACS Med. Chem. Lett.* 3, 850–855
108. Li, L. *et al.* (2015) Treatment with a small molecule mutant IDH1 inhibitor suppresses tumorigenic activity and decreases production of the oncometabolite 2-hydroxyglutarate in human chondrosarcoma cells. *PLoS One* 10, e0133813
109. Zheng, B. *et al.* (2013) Crystallographic investigation and selective inhibition of mutant isocitrate dehydrogenase. *ACS Med. Chem. Lett.* 4, 542–546
110. Davis, M.I. *et al.* (2014) Biochemical, cellular, and biophysical characterization of a potent inhibitor of mutant isocitrate dehydrogenase IDH1. *J. Biol. Chem.* 289, 13717–13725
111. Okoye-Okafor, U.C. *et al.* (2015) New IDH1 mutant inhibitors for treatment of acute myeloid leukemia. *Nat. Chem. Biol.* 11, 878–886
112. Deng, G. *et al.* (2015) Selective inhibition of mutant isocitrate dehydrogenase 1 (IDH1) via disruption of a metal binding network by an allosteric small molecule. *J. Biol. Chem.* 290, 762–774
113. Yen, K. *et al.* (2017) AG-221, a first-in-class therapy targeting acute myeloid leukemia harboring oncogenic IDH2 mutations. *Cancer Discov.* 7, 478–493
114. Wang, F. *et al.* (2013) Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science* 340, 622–626
115. Wang, F. *et al.* (2016) A small molecule inhibitor of mutant IDH2 rescues cardiomyopathy in a D-2-hydroxyglutaric aciduria type II mouse model. *J. Inher. Metab. Dis.* 39, 807–820
116. Maurino, V.G. and Engqvist, M.K. (2015) 2-Hydroxy acids in plant metabolism. *Arabidopsis Book* 13, e0182
117. Hudig, M. *et al.* (2015) Plants possess a cyclic mitochondrial metabolic pathway similar to the mammalian metabolic repair mechanism involving malate dehydrogenase and L-2-hydroxyglutarate dehydrogenase. *Plant Cell Physiol.* 56, 1820–1830
118. Cairncross, J.G. and Macdonald, D.R. (1988) Successful chemotherapy for recurrent malignant oligodendroglioma. *Ann. Neurol.* 23, 360–364
119. Tateishi, K. *et al.* (2017) The alkylating chemotherapeutic temozolomide induces metabolic stress in IDH1-mutant cancers and potentiates NAD⁺ depletion-mediated cytotoxicity. *Cancer Res.* 77, 4102–4115
120. Bleiweis, A.S. *et al.* (1967) Formation of α -hydroxyglutaric acid by *Aspergillus glaucus*. *J. Bacteriol.* 94, 1560–1564
121. Lu, Y. *et al.* (2017) Chemosensitivity of IDH1-mutated gliomas due to an impairment in PARP1-mediated DNA repair. *Cancer Res.* 77, 1709–1718
122. Rose, N.R. *et al.* (2011) Inhibition of 2-oxoglutarate dependent oxygenases. *Chem. Soc. Rev.* 40, 4364–4397
123. Klose, R.J. *et al.* (2006) JmjC-domain-containing proteins and histone demethylation. *Nat. Rev. Genet.* 7, 715–727
124. Pastor, W.A. *et al.* (2013) TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. *Nat. Rev. Mol. Cell Biol.* 14, 341–356
125. Wu, X. and Zhang, Y. (2017) TET-mediated active DNA demethylation: mechanism, function and beyond. *Nat. Rev. Genet.* 18, 517–534
126. Samson, L. and Cairns, J. (1977) A new pathway for DNA repair in *Escherichia coli*. *Nature* 267, 281–283
127. Alemu, E.A. *et al.* (2016) ALKBHs-facilitated RNA modifications and de-modifications. *DNA Repair (Amst.)* 44, 87–91
128. Fedeles, B.I. *et al.* (2015) The AlkB family of Fe(II)/ α -ketoglutarate-dependent dioxygenases: repairing nucleic acid alkylation damage and beyond. *J. Biol. Chem.* 290, 20734–20742
129. Kaelin, W.G., Jr and Ratcliffe, P.J. (2008) Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell* 30, 393–402
130. Hutton, A. *et al.* (1967) Cofactor and substrate requirements of collagen proline hydroxylase. *Arch. Biochem. Biophys.* 118, 231–240
131. Elkashef, S.M. *et al.* (2017) IDH mutation, competitive inhibition of FTO, and RNA methylation. *Cancer Cell* 31, 619–620
132. Karlstaedt, A. *et al.* (2016) Oncometabolite D-2-hydroxyglutarate impairs α -ketoglutarate dehydrogenase and contractile function in rodent heart. *Proc. Natl. Acad. Sci. U. S. A.* 113, 10436–10441
133. Allis, C.D. *et al.* (2007) New nomenclature for chromatin-modifying enzymes. *Cell* 131, 633–636
134. Chan, S.M. *et al.* (2015) Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nat. Med.* 21, 178–184
135. Rzem, R. *et al.* (2015) A mouse model of L-2-hydroxyglutaric aciduria, a disorder of metabolite repair. *PLoS One* 10, e0119540
136. Shim, E.H. and Sudarshan, S. (2015) Another small molecule in the oncometabolite mix: L-2-hydroxyglutarate in kidney cancer. *Oncoscience* 2, 483–486