Isocitrate dehydrogenases (IDHs) catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG). Somatic mutations in genes encoding IDH1 and IDH2 were first identified in glioma and subsequently in acute myeloid leukemia and other solid tumors. These heterozygous point mutations occur at the arginine residue of the enzyme’s active site and cause both loss of normal enzyme function and gain of function, causing reduction of α-KG to D-2-hydroxyglutarate, which accumulates. D-2-hydroxyglutarate may act as an oncometabolite through the inhibition of various α-KG-dependent enzymes, stimulating angiogenesis, histone modifications and aberrant DNA methylation. Possibly, IDH mutations may also cause oncogenic effects through dysregulation of the tricarboxylic acid cycle, or by increasing susceptibility to oxidative stress. Clinically, IDH mutations may be useful diagnostic, prognostic and predictive biomarkers, and it is anticipated that a better understanding of the pathogenesis of IDH mutations will enable IDH-directed therapies to be developed in the future.

The homodimeric enzymes, IDH1 and IDH2, catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) and reduce NADP+ to NADPH. They share considerable sequence similarity and an almost identical protein structure [1], but function in different subcellular locations.

IDH1 is localized to the cytoplasm and peroxisome [2], where it serves as a major source of cytosolic NADPH. It plays a role in cellular metabolic processes such as lipid and glucose metabolism [3,4], protects against reactive oxygen species (ROS) and radiation [2,5,6] and mediates de novo lipogenesis under hypoxic conditions through the reductive carboxylation of α-KG [7].

IDH2 is localized to the mitochondria [2] where its main function is to regulate the tricarboxylic acid (TCA) cycle. IDH2 also plays an important role in maintaining cellular citrate levels under hypoxic conditions [8] and in protecting against oxidative stresses [5,9,10].

IDH3 is a NAD+-dependent isocitrate dehydrogenase enzyme also present in the mitochondrial matrix of the eukaryotic cells, where it is thought to play a central role in the TCA cycle. IDH3 is a tetrameric protein and it does not share sequence similarity with IDH1 and IDH2. Moreover, while IDH1 and IDH2 catalyze reversible reactions with no known allosteric modifiers, the reaction catalyzed by IDH3 is irreversible and regulated by different positive and negative allosteric effectors [2].

Somatic mutations in IDH1 were initially found through genome-wide sequencing studies of patients with glioblastoma (GBM) [11]. It was then discovered that mutations in IDH1 and IDH2 also occurred in patients with grade 2 and 3 gliomas and that the two mutations were mutually exclusive of each other [12]. Both IDH1 and IDH2 mutations occur more frequently in grade 2–3 gliomas and secondary GBMs (70–75%) than in primary GBMs (5%) [13]. They occur at higher frequencies in younger patients and are associated with a relatively favorable prognosis [12,14]. Serial biopsy studies have demonstrated that IDH mutations are an early event in gliomagenesis [15], and occur in association with TP53 mutation, or with loss of heterozygosity of 1p/19q [2], as well as MGMT methylation and alternative telomere lengthening [16]. In addition, IDH mutations are inversely associated with genetic changes common to primary GBM, such as EGFR amplification and CDKN deletion [12].

To date no mutations in any of the genes encoding the IDH3 subunits have been identified in
Studies performed on mitochondrial fractions of rat kidney, liver and brain showed that D-2HG is produced from the reaction of α-KG and NADPH, and reduced affinity for isocitrate being a dominant-negative effect due to the formation of catalytically inactive heterodimers of mutant and wild-type proteins [31].

Subsequent studies, however, demonstrated that mutant IDH1/2 are not in fact inactive enzymes, but instead possess novel enzymatic activity resulting from their increased affinity for NADPH, and reduced affinity for isocitrate [28,29,32]. This novel enzyme function results in the NADPH-dependent reduction of α-KG to D-2-hydroxyglutarate (D-2HG), which has been shown to accumulate at high levels in IDH1- and IDH2-mutated tumors [28,29].

Since the formation of a protein dimer is a dynamic process, it is probable that wild-type–wild-type and mutant–mutant IDH homodimers are also present in cells, but it appears that the peculiarity of mutant IDH is to function as a heterodimer consisting of one wild-type and one mutant protein, each functioning independently. The activity of the wild-type protein is partially inhibited by the mutant protein, which itself exhibits novel enzymatic activity, resulting in a simultaneous decrease in α-KG production, and increase in D-2HG formation, respectively [33]. Studies of the IDH1 wild-type–mutant heterodimer demonstrate that both wild-type and mutant subunits contribute to the production of D-2HG from isocitrate or α-KG, respectively [33]. Furthermore, the genetic knockdown of wild-type IDH1 function in IDH1-mutant cell lines can significantly decrease 2-hydroxyglutarate (2HG) production [34], and intratumoral D-2HG is significantly reduced in IDH1 mutant GBMs that have lost the wild-type IDH1 allele, compared with GBMs with heterozygous IDH1 mutations, demonstrating the importance of wild-type as well as mutant IDH function in D-2HG production [34].

The excess production of D-2HG, and/or inhibition of α-KG production via the mechanisms outlined above are thought to give IDH mutant cells protumorigenic potential.

### Effects of D-2HG on the activity of α-KG-dependent enzymes

Our current understanding of the exact cellular role of D-2HG is limited [35], although it may function as an intermediate in the production of 5-aminolevulinate and porphyrin in heme synthesis [36,37]. Studies performed on mitochondrial fractions of rat kidney, liver and brain demonstrated that D-2HG is produced from the activity of the HOT enzyme, which catalyzes the conversion of γ-hydroxybutyrate to succinic acid.
semialdehyde, with a stoichiometric production of D-2HG from α-KG [38] (for a review see [39]). To maintain carbon balance, D-2HG is subsequently interconverted to α-KG via the activity of D-2HGDH.

It has been proposed that accumulated D-2HG acts as an oncometabolite, with numerous potential protumorigenic effects. One of the rationales for the role of 2HG in tumorigenesis is derived from the observation of patients with rare inherited neurometabolic disorders, the D-2- and L-2-hydroxylglutaric acidurias (D-2- and L-2-HGA). These two distinct syndromes result from germline homozygous inactivating mutations in the genes that encode for D-2- and L-2-HGDH [40,41]. Mutations in D-2- and L-2-HGDH result in the accumulation of D-2HG or L-2HG enantiomeric acids in patients’ physiological fluids, and some L-2HG affected individuals have an increased incidence of brain tumors [42–44]. Interestingly, D-2HGA has also been found to be associated with germline IDH2 R140 mutations in a cohort of patients, who do not have alterations in the D-2HGDH [45]. Thus D-2-HGA has been distinguished in two different subtypes, type one that has been associated with mutations in D-2HGDH and type two that has been associated with mutations in IDH2. However, patients with type one and type two D-2-HGA have no higher risk of developing brain tumors [46]. Although this contrasts with the findings observed in IDH-mutated tumors, where an accumulation of D-2HG [28,29], but not L-2HG is thought to promote tumorigenesis, it could be hypothesized that this discrepancy may be due to the ability of mutant IDH to produce D-2HG at a significantly higher level to that seen in D-2-HGA, and that the levels produced in the latter disorder are simply insufficient to promote tumorigenesis. Indeed, D-2HG levels are higher in fluids and lymphoblastic cells from patients with D-2-HGA associated with IDH2 mutations than from those with mutations in D-2HGDH, with the mean serum levels approximately five-times higher in type two than in type one D-2-HGA patients (e.g., 14,663–112,122 ng/ml range values, 54,210 ng/ml mean value vs 3851–18,218 ng/ml range values, 10,072 ng/ml mean value, respectively) [46,47]. Since the clinical outcome of patients affected by type two D-2-HGA is more severe compared with that of patients affected by type one D-2HGA, it is plausible to establish a correlation between D-2HG concentration and the severity of the disorder. However, D-2HG levels present in the serum of patients affected by type two D-2HGA are also higher than those measured in IDH1 and IDH2 mutant AML patients, where pretreatment D-2HG levels were measured to be as high as 30,000 ng/ml (median 3004 ng/ml) [48] and 66,207 ng/ml (median 1863 ng/ml) [49] compared with 61 ng/ml (median) [48] and 87 ng/ml (median) [49] in IDH wild-type patients, suggesting there may be no direct correlation between the level of D-2HG concentration and the risk of tumor development in D-2-HGA [48]. The association between brain tumors and L-2-HGA, but not D-2-HGA may also be explained by the fact that L-2HG inhibits certain enzymes, whose activity have been implicated in tumorigenesis, more potently than D-2HG [50,51]. An alternative theory could be that because patients with D-2-HGA have a more severe clinical course compared with L-2-HGA patients (they often die in infancy or early adulthood [46]), they simply do not live long enough for brain tumors to develop. Perhaps more compelling evidence to support the role of D-2HG in promoting tumorigenesis is illustrated by the findings observed in IDH-mutated tumors showing that patients with IDH2 R140-mutant AML produce lower levels of D-2HG and have a better prognosis than patients IDH2 R172 mutant AML [25], suggesting a correlation between the amount of D-2HG produced by mutant IDH, and the characteristics of the resulting tumor.

The most notable attribute of D-2HG is its ability to competitively inhibit α-KG-dependent enzymes. This inhibition occurs owing to the significant structural similarity between D-2HG and α-KG, which are identical except that a hydroxyl group in D-2HG replaces the C2 carbonyl group in α-KG [50,52]. Mammalian cells express over 60 α-KG-dependent enzymes [53,54] and the inhibition by 2HG of several of these enzymes, namely HIF-PHD, JHDMs, the TET family of 5-methylcytosine hydroxylases and C-P4H could lead to tumor formation by stimulating angiogenesis, histone modifications, aberrant DNA methylation and abnormal collagen maturation, respectively.

HIF is a transcription factor that, when overexpressed, is associated with malignant progression and a poor outcome in several cancers [55]. HIF has a wide range of target genes that in physiological conditions promote adaptation of cells to low oxygen tension (hypoxia). In pathological conditions their activation can be critical to tumor growth, by inducing a hypoxic response under normoxic conditions (pseudohypoxia). HIF target genes include those
that modulate angiogenesis, glycolysis, growth factor signaling, apoptosis and metastasis [56,57]. In normoxia, HIF is labile, because the hydroxylat-
ion by HIF-PHD of two proline residues in its oxygen-dependent degradation domain triggers its association with the pVHL E3 ligase com-
plex and the subsequent degradation via the ubiquitin-proteasome pathway [58,59]. It has been demonstrated that D-2HG [50], and reduced lev-
els of α-KG [31] can inhibit HIF-PHD enabling HIF to become stable in normoxic conditions, and leading to the inappropriate activation of the target genes described above. HIF1α is in fact upregulated in cells that overexpress mutant IDH1, in cells treated with exogenous 2HG, in tumors harboring IDH1 R132H mutations and in the brains of idh1 R132H knock-in Nestin-Cre (idh-knock-in) mice [31,50,60]. Furthermore, the expression of HIF1α target genes Glut1, VEGF, and PGK1 is increased in the brains of idh-knock-in mice and in IDH1 knockdown cell lines [31,60].

However, D-2HG has been shown to be only a relatively weak inhibitor of HIF-PHD [51] and, furthermore, studies by both Metellus et al. [55] and Williams et al. [61] found no correlation between IDH mutation status and the expression of HIF1α in patients with glioma. Additionally recent studies demonstrated that in contrast to L-2HG, which acts as an inhibitor of HIF-PHD, D-2HG can actually stimulate rather than inhibit the activity of this enzyme, and in some cellular contexts, such as human astrocyte, colorectal and erythroleukemia cell lines, the expression of IDH1 R132H reduced HIF levels [62,63]. Moreover in erythroleukemic cells it has been demonstrated that transformation is pro-
moted specifically by D-2HG and not by L-2HG and surprisingly the loss of HIF-PHD activity by shRNAs was able to block leukemic transfor-
mation by mutant IDH [63], suggesting that in this context HIF or other specific targets of hydrox-
ylation by HIF-PHD could suppress the onco-
genric potential of D-2HG. These findings are interesting given that they conflict with those of earlier studies, and also because, although HIF is traditionally viewed as being oncogenic, it may also act as a tumor suppressor under certain con-
ditions [64,65]. Consequently it remains unclear whether D-2HG has an agonistic or antagonistic effect on HIF-PHD, and whether HIF1α plays a definitive role in promoting tumorigenesis in IDH-mutated tumors.

Histone demethylases remove methyl groups from methylated lysine, and regulate gene expression by modulating chromatin structure and transcription factor binding [66]. The inhibition of histone demethylation by D-2HG could promote tumorigenesis by many mechanisms, possibly by blocking cellular differentiation [67]. Exogenous 2HG and knockdown of wild-type IDH1 function, inhibit the activity of multiple histone demethylases in vitro and in vivo, and the levels of multiple histone methylation mark-
ers are elevated in mutant IDH1- and IDH2-
expressing cells and in human IDH1-mutant gliomas [50,51,67]. Furthermore, hypermethylated histones were observed in myeloid cells extracted from mice in which mutant idh1 was specifically targeted in the hematopoietic system [68].

TET catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine (5hmC) [69], the latter of which plays a role in passive DNA demethylation by preventing DNMT mainte-
nance of methylation, and induces active DNA demethylation by DNA repair mechanisms [70]. 5hmC also controls transcriptional regulation by preventing the binding of CpG binding proteins to methylated DNA [70]. TET itself controls cellular differentiation and embry-
onic stem cell maintenance by regulating the expression of genes that maintain pluripotency [52,71]. Expression of mutant IDH1 and IDH2 and administration of exogenous 2HG inhibit TET in vitro and in vivo, resulting in reduced 5hmC production, a process that is reversible by the addition of exogenous α-KG [50]. Fur-
thermore, IDH1-mutated gliomas accumulate significantly lower levels of 5hmC and higher levels of 5-methylcytosine than IDH1 wild-type tumors [50], although 5hmc is also significantly depleted in several human cancers independent of IDH mutation status [72].

A CpG island methylator phenotype (G-CIMP) was recently identified in a subgroup of gliomas that correlated strongly with IDH1 and IDH2 mutations [73]. Patients with tumors exhibiting this phenotype presented at a younger age and experienced better survival, features that are also associated with IDH mutant glioma. Two subsequent studies identified a distinct homogenous hypermethylation profile in IDH mutant gliomas that was similar to G-CIMP [74,75]. This hypermethylation profile remained stable from low- to high-grade gliomas, suggest-
ing that these epigenetic changes occur early in gliomagenesis [74], and in view of the fact that IDH1 mutations also occur as an early event in gliomagenesis [15] it seems plausible that these two observations are linked. The introduction of IDH1 R132H into primary human astro-
cytes using lentiviral vectors, induces significant
DNA hypermethylation [67,68], which mirrors the changes seen in G-CIMP-positive gliomas. Conversely, hypomethylation of DNA is observed at specific loci when wild-type IDH1 is overexpressed using lentiviral vectors, suggesting that αKG levels may also alter the methylene [76].

Hypermethylation is also a dominant feature of IDH mutant AML cells, which display a global hypermethylation and gene-specific methylation profile similar to the one present in TET-mutant AML cells [52]. Global changes in DNA methylation patterns were also observed in DNA analyzed from hematopoietic-specific conditional idh R132H knock-in mice, which paralleled those observed in human IDH1- and IDH2-mutant AML, and included signaling pathways responsible for stem cell maintenance, hematopoietic cell proliferation and differentiation, and leukemogenesis [68]. It seems likely, therefore, that these genetic observations are linked, and possibly result from the inhibition of TET and/or histone demethylases.

The histone modifications and DNA methylation changes outlined above have the potential to affect the expression of many oncogenes, tumor suppressor genes and key signaling pathways providing a plausible mechanistic link between IDH mutation and tumorigenesis. Further correlation between methylation and gene-expression profiles in IDH mutant tumors may help to identify the causative genes and pathways involved.

C-P4H hydroxylates proline residues in collagen and catalyzes the production of endostatin [50]. In the brains of Nestin-idh1 R132 knock-in mice, D-2HG inhibited the hydroxylation of collagen, causing defective collagen protein maturation and basement membrane abnormalities [66]. Endostatin inhibits angiogenesis and tumor growth [77] and its expression is reduced in IDH1 knockdown cells, in cells overexpressing IDH1 R132H and in IDH1 mutant gliomas [50]. C-P4H inhibition by D-2HG may, therefore, promote tumor angiogenesis and growth.

**IDH mutation effects on TCA function & glucose sensing**

The TCA cycle is a central pathway in oxidative metabolism. It functions to generate NADH and FADH, which feed electrons into the respiratory chain, and is involved in the cellular metabolism of carbohydrates, lipids and amino acids [78]. The ‘Warburg effect’ is a paradoxical phenomenon that describes a situation whereby malignant cells shift their energy production from oxidative phosphorylation towards glycolysis under aerobic conditions. It is considered to be one of the metabolic hallmarks of cancer [79], involving the diversion of the malignant cell’s metabolism, so that survival, growth and division are favored over cellular function [80]. The identification of mutations in genes encoding the TCA cycle enzymes SDH and FH led to renewed interest in Warburg’s hypothesis and the potential link between metabolic dysregulation and cancer [11,81]. Mutations in SDH are associated with the development of pheochromocytomas and paragangliomas, while mutations in FH predispose to the development of leiomyomas of the skin and uterus, and renal tumors.

Different mechanisms have been proposed to explain the tumorigenic process associated with the loss of activity of SDH or FH enzymes. Indeed SDH and FH deficiencies support tumor formation by increasing the levels of their respective TCA cycle substrates, succinate or fumarate, which leads to oncogenic signaling in cells [66,82]. Among the proposed mechanisms, different studies have shown that SDH- and FH-associated tumors are highly vascular and are characterized by a strong hypoxic signature, likely resulting from the inhibition of HIF-PHD by fumarate and succinate [83,84]. FH, SDH and IDH are all enzymes with an essential role in cellular metabolism, and although the underlying mechanism of tumorigenesis in the associated tumors seems to be caused by the accumulation of the respective oncometabolites fumarate, succinate and 2-hydroxyglutarate, the mechanisms of tumorigenesis and the pattern of tumor predisposition conferred by mutations in these genes look very different. This could be explained by the fact that the main feature of these oncometabolites is the inhibition of the α-KG-dependent enzyme family (to whom HIF-PHD also belongs), with different specificities to different enzymes and thus with different biological consequences [84].

Several studies have been performed to analyze the possibility that IDH mutations can cause changes in global cellular metabolism to favor tumor pathogenesis. Initial studies of the effect of IDH mutations on TCA cycle function failed to demonstrate significant alterations in TCA cycle metabolites [28,29]. However, a subsequent study by Reitman et al. did demonstrate a depletion of the TCA cycle metabolites citrate, cis-aconitate, α-KG, malate and fumarate and an accumulation of biosynthetic precursors, in cell lines that expressed IDH1 R132H and IDH2R172K mutations, suggesting that IDH1 and IDH2 mutations can result in TCA cycle downregulation [85]. The fact that...
IDH1 mutant cells shared multiple metabolic changes with 2HG-treated cells, but not with IDH1 wild-type knockdown cells, suggests that 2HG production is responsible for the metabolic effects observed [85]. Furthermore 2HG may impair the mitochondrial respiratory chain, through inhibition of cytochrome C oxidase and ATP synthase [86,87]. Alterations in the cellular NADPH: NADP+ ratio, may also contribute to the metabolic effects observed [29]. Acetyl-CoA, which is generated from citrate in the cytoplasm, has been shown to regulate the acetylation of cytoplasmic proteins [88]. Alterations in TCA cycle metabolite levels associated with IDH mutations could affect cytosolic acetyl-CoA concentrations and, therefore, regulate the acetylation and activity of many tumorigenic proteins [85].

The depletion of glutamate in IDH mutant cells observed in Reitman’s study is also potentially relevant. Glutamate is a major excitatory neurotransmitter in the CNS, and the glutamatergic pathway is associated with tumor development, proliferation, survival and metastasis in gliomas as well as other tumors [89,90]. Glioma cells actively secrete glutamate, by increasing cystine–glutamate exchange activity, and this markedly elevates extracellular glutamate levels, and stimulates glutamate receptors on surrounding brain cells, promoting their cell death and encouraging glioma cell invasion and migration [91]. Interestingly, an elevated flux of glutamine to 2HG via glutamate and α-KG is observed in IDH1 mutant cells [29]. Furthermore, inhibition of glutaminase, which converts glutamine to glutamate, slows the growth of IDH1 mutant glioma cells [92]. These findings suggest that IDH1 mutant tumors may rely on glutamine and glutamate to maintain cellular α-KG levels, or possibly as an alternative energy source to glycolysis, although it is also possible that these changes simply illustrate the importance of glutamate generally in glioma development and progression as described above.

IDH mutations may also lead to aberrant glucose sensing. Abnormal glucose control has been linked to the development of colon, breast, prostate, liver and bladder cancers [93]. Furthermore, it has been suggested that the use of the antidiabetic drug metformin decreases the risk of specific cancers in patients with Type 2 diabetes [94,95]. IDH1 has been shown to play a role in glucose sensing by controlling glucose-stimulated insulin secretion by pancreatic β-cells, and suppression of IDH1 activity impairs glucose-stimulated insulin secretion and pyruvate cycling flux [96]. The mechanism by which altered glucose sensing would lead to tumorigenesis is unclear, although it has been suggested that low cytosolic NADPH levels resulting from mutations in IDH1 may play a role by aberrantly signaling a low nutrient state to downstream effectors in the glucose-sensing pathway, leading to increased cellular nutrient consumption and providing a selective growth advantage [2].

IDH mutation & cellular response to oxidative stress
IDH1 and IDH2 act in a protective role against various cellular insults, such as ROS and radiation, and IDH activity increases in response to such insults [2,5,6,9,10]. Conversely, knockdown of wild-type IDH1 and IDH2 has been shown to enhance cellular oxidative damage and exacerbate the induction of apoptosis by agents such as selenite and staurosporine [6,30,97]. NADPH, a product of IDH1 and IDH2 wild-type activity, is important in this protective process, by acting directly as a reducing agent and by interacting with the glutathione and thioredoxin systems [55]. α-KG is also able to function as an antioxidant [55]. Furthermore, D-2HG has been demonstrated to induce oxidative stress in animal models [98]. However, ROS production was not found to be significantly altered in IDH1 mutant cell lines [62], and ROS levels were actually reduced in the brains of IDH1 R132H knock-in NestinCre mice, although the intracellular NADPH:NADP+ ratio and glutathione levels were increased and decreased, respectively [60]. Furthermore, in hematopoietic-specific conditional idh+ R132H-knock-in mice no alterations in the levels of either ROS, NADP+ or NADPH were observed [68]. Consequently, although a depletion of cellular NADPH, α-KG, and the accumulation of D-2HG may hypothetically lead to increased oxidative stress, resulting in oxidative DNA damage and tumorigenesis in IDH mutant tumors, current evidence from in vivo studies is conflicting and does not entirely support this theory.

Clinical relevance
IDH mutations may have a role as prognostic biomarkers in several cancers. In patients with intrahepatic cholangiocarcinoma, mutations in IDH1 and IDH2 are associated with a longer time to tumor recurrence after surgical resection and a longer overall survival [99]. In AML IDH2 but not IDH1 is associated with a better prognosis [100], while in grade 2–4 gliomas, IDH1
mutation is associated with a better prognosis than \textit{IDH} wild-type disease [104].

\textit{IDH} mutations may also act as predictive biomarkers in gliomas. Patients with \textit{IDH}1 mutant GBMs have been demonstrated to exhibit a better response to temozolomide than patients with \textit{IDH} wild-type disease [102]; however, \textit{IDH} mutation status does not predict response to procarbazine, lomustine and vincristine chemotherapy in patients with anaplastic oligodendroglioma [103]. As new treatments emerge, a greater understanding of the predictive value of \textit{IDH} mutation status in glioma, as well as in other tumors associated with \textit{IDH} mutations, may enable therapies to be tailored more appropriately to patients in the future.

\textit{IDH} mutation testing is a useful diagnostic tool in glioma and its use has become part of standard clinical practice [104]. \textit{IDH} mutations can be detected in gliomas through direct sequencing-based techniques, or by immunohistochemistry (IHC) [105], and can help to distinguish gliomas from benign CNS lesions [106], astrocytomas and oligodendrogliomas from other CNS tumors [106,107], and primary GBM from secondary GBM [108]. Furthermore, identification of mutant \textit{IDH} protein by IHC in pathological specimens, can enable the differentiation of tumor cells from reactive glial cells [108], which may help postoperatively to determine the completeness of surgical resection, and aid in the analysis of post-therapy biopsy samples [109]. Direct sequencing-based techniques used to detect \textit{IDH} mutations may give false-negative results if samples are insufficient or are contaminated with normal tissue [104]. IHC is advantageous clinically, as methods are time and cost efficient, and relatively simple to implement in hospital pathology laboratories [110]. Furthermore, antibodies have been developed that are specific to the most frequently occurring \textit{IDH} mutation status in glioma, \textit{IDH1 R132H} [111,112]. These antibodies have been demonstrated to be 100% sensitive and 100% specific [108,113] in detecting \textit{IDH1 R132H}, and this method appears to be more sensitive than direct sequencing, especially in cases of low-grade diffuse astrocytomas [108], and in diffuse gliomas where biopsy samples are very small [110]. Moreover, IHC detection of \textit{IDH1 R132H} mutations has been demonstrated to be reliable and consistent across different laboratories, whereas \textit{IDH} sequencing procedures have been demonstrated to give inconsistent results from one laboratory to another [114].

In order for \textit{IDH} mutation status to be used to guide the clinical management of patients in the future, it is important to ensure that reliable methods are available for this purpose, and guidelines have now been established to advise on the use of IHC and DNA sequencing for diagnostic \textit{IDH} testing [115].

Measurement of 2HG levels can also be used for diagnostic purposes and may also be used in certain circumstances to monitor response to therapy. 2HG can be detected noninvasively in patients with \textit{IDH} mutant glioma using magnetic resonance spectroscopy imaging of the brain [116,117], and although the clinical relevance of this technology is not yet defined, it may in the future enable preoperative prediction of tumor histology, intraoperative visualization of tumor margins, monitoring of treatment and early detection of disease recurrence. Serum measurements of 2HG levels in glioma patients do not appear to correlate with \textit{IDH} mutation status or tumor size and, therefore, serum 2HG assessment may not be useful in the diagnosis or monitoring of treatment in glioma [118]. However, 2HG can be detected in the serum and urine of patients with \textit{IDH} mutant AML [49] and its levels decrease with response to treatment [49] suggesting that 2HG may serve as a noninvasive biomarker of disease activity in this setting. Furthermore, post-treatment serum 2HG levels <200 ng/ml correlate with longer overall survival in \textit{IDH} mutant AML suggesting that serum 2HG measurement may also provide prognostic information in these patients [48].

\textit{IDH} mutations could serve as therapeutic targets, and recently two compounds specifically targeting mutant \textit{IDH1 R132H} (AGI 5198) [119] and \textit{IDH2 R140Q} (AGI 6780) [120] have been developed. Although the potential clinical benefit of such inhibitors is not yet clear, their emergence raises new hope for the treatment of \textit{IDH}-mutated tumors. Treatment of erythroleukemic cells expressing \textit{IDH1 R132H} [63] or \textit{IDH2 R140Q} [120] with the corresponding inhibitors blocked D-2HG production, reverting growth factor-independent proliferation and restoring the ability of these cells to differentiate. Moreover, \textit{ex vivo} treatment of primary \textit{IDH2 R140 AML} cells with AGI-6780, decreased the levels of 2-HG, relieving the block of differentiation consequent to the expression of mutant \textit{IDH2} [120]. Other studies additionally demonstrated that the targeted inactivation of \textit{IDH1 R132H} by the inhibitor AGI-5198 impaired growth of \textit{IDH1}-mutant glioma cells and mouse xenografts, and promoted gliogenic differentiation [121].
In addition to inhibiting the activity of mutant IDH, it may also be possible to directly target IDH mutations with antisense oligonucleotides, or to pharmacologically revert the epigenetic changes associated with such mutations using HDAC inhibitors or DNA methyltransferase inhibitors [122]. Early clinical studies have demonstrated the HDAC inhibitor vorinostat to be active in GBM [123] and to be well tolerated as a single agent [124], and when combined with temozolomide [125]. Furthermore, valproic acid, which has HDAC-inhibitory activity, has been demonstrated to prolong survival in some glioma patients [125] and to increase the sensitivity of glioma cells to radiation [126]. HDAC inhibitors have also shown promise in early clinical trials in AML [127,128]. It is important to note, however, that these studies were not stratified on the basis of IDH mutational status so direct conclusions as to the efficacy of HDAC inhibitors in IDH mutant versus IDH wild-type disease cannot be drawn.

Directly targeting 2HG production by inhibiting the conversion of glutamine to α-KG with siRNA and the small molecule inhibitor bis-2-(5-phenyl-acetamido1,2,4-thiadiazol-2-yl) ethyl sulfide, slows the growth rate of IDH mutant glioma cells, suggesting that ‘starving’ mutant IDH cells of α-KG may have therapeutic benefit [92]. However, it is also possible that treatment with exogenous α-KG may be beneficial, due to a reduction in the competitive inhibitory effect of 2HG on α-KG-dependent enzymes. Targeting other aspects of the glutamate system may also hold promise. Talampanel, an antagonist of the AMPA glutamate receptor was used in a Phase II trial in combination with radiotherapy and temozolomide in patients with newly diagnosed GBM. A 5.7-month improvement in median survival was observed when compared with historical controls that had received radiotherapy and temozolomide alone [129]. Although, again, this study did not stratify patients based on IDH mutation status, and therefore conclusions specific to IDH mutant disease cannot be drawn. It should also be noted that in a Phase II trial of single-agent talampanel in patients with recurrent GBM and anaplastic glioma, no significant antitumor response was observed and the trial was terminated early due to treatment futility [130].

**Conclusion & future perspective**

The fact that abnormal histone and DNA methylation patterns are a common feature of IDH mutant tumors suggests that the inhibition of JHDMs and TET by 2HG are probably responsible for tumorigenesis, rather than TCA cycle dysregulation *per se*. The development of a pseudohypoxic state resulting from the stabilization of HIF1α is also likely to play a role. The absolute effects of these phenomena, however, remain unknown and further research, perhaps using genetically engineered mouse models, is required to characterize the ‘downstream’ genetic effects and pathways that give IDH mutant cells their protumorigenic potential. The first of such models was developed using a human IDH1 R132H mutant glioma stem cell line in an orthotopic xenograph mouse. This model may be of use in the future to test IDH-targeted therapies [131]. Subsequently a hematopoietic-specific conditional *idh1* R132H knock-in mouse was developed, in which a specific leukemic methylation pattern was observed [68]. Most recently a brain-specific *idh2* R132H conditional knock-in mouse was generated, which was associated with the development of intracerebral hemorrhage *in utero* and perinatal lethality [60]. Elevated D-2HG levels were observed in the embryonic brains of these mice, as well as defective collagen protein maturation, suggesting that the inhibition of C-P4H was responsible for the resulting phenotype [60]. Moreover elevated levels of HIF1α, and upregulation of *VEGF*, *Glut-1* and *Pgk1* were observed, further demonstrating the potential of D-2HG to inhibit HIF-PHD [60]. To date, no tumor-forming genetically engineered IDH mutant model has been established, which possibly reflects the toxic effect of D-2HG during murine embryogenesis. It is hoped that the development of such *in vivo* models will enable us to better understand the relationship between IDH mutations and tumorigenesis and to develop effective therapies against IDH-mutated tumors in the future. The potential clinical application of IDH1 and IDH2 mutations as diagnostic, prognostic and predictive tools also requires further evaluation possibly through clinical trials stratified by IDH mutation status.

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Executive summary

**Background**
- Isocitrate dehydrogenases (IDHs) convert isocitrate to α-ketoglutarate (α-KG).
- IDH1 functions in the cytoplasm.
- IDH2 and IDH3 function in the mitochondria.
- Mutations in IDH1 and IDH2 have been discovered in glioma, acute myeloid leukemia and other solid tumors.

**How do IDH mutations promote tumorigenesis?**
- Mutant IDH converts α-KG to D-2HG.
- D-2-hydroxyglutarate (D-2HG) accumulates in IDH mutant tumors.
- Excess production of D-2HG or reduced α-KG production are likely to promote tumorigenesis.

**Effects of D-2HG on the activity of α-KG-dependent enzymes**
- Accumulated D-2HG may act as an oncometabolite by inhibiting α-KG-dependent enzymes.
- Inhibition of histone demethylases and TET may result in aberrant histone and DNA methylation.
- Hypermethylation is a feature of IDH mutant glioma.
- Upregulation of HIF1α may also occur as a result of HIF-α inhibition.

**IDH mutation effects on tricarboxylic acid cycle function & glucose sensing**
- Initial studies failed to demonstrate alterations in tricarboxylic acid cycle function associated with mutant IDH.
- Subsequent studies suggested that IDH mutations can result in tricarboxylic acid cycle downregulation.
- IDH mutant tumors may rely on glutamine and glutamate to maintain cellular α-KG levels, or as an alternative energy source to glucose.
- IDH mutations may also lead to aberrant glucose sensing.

**IDH mutation & cellular response to oxidative stress**
- IDH1/2 protect against reactive oxygen species.
- Knockdown of wild-type IDH1/2 enhances cellular oxidative damage.
- IDH mutations may result in increased oxidative stress and DNA damage.
- Current evidence from in vivo studies is, however, conflicting.

**Clinical relevance**
- IDH mutations could have a role as prognostic and predictive biomarkers in glioma.
- IDH mutation testing has become a standard diagnostic tool in glioma.
- Assessment of 2-hydroxyglutarate levels can be used for diagnostic purposes and to monitor response to therapy.
- IDH mutations are potential therapeutic targets.
- Specific inhibitors of IDH1/2 have been developed that can block D-2HG production and promote differentiation.
- Other potential therapies include HDAC inhibitors, drugs targeting the glutamate system and α-KG itself.

**Conclusion & future perspective**
- Inhibition of JHDMs and TET by 2-hydroxyglutarate are likely to be responsible for tumorigenesis associated with IDH mutations.
- The development of a ‘pseudohypoxic state’ is also likely to play a role.
- The absolute effects of these phenomena, however, remain unknown.
- A better understanding of the relationship between IDH mutations and tumorgenesis may enable the development of effective therapies against IDH-mutated tumors in the future.
- The development of genetically engineered mouse models may be key to this.
- The potential clinical application of IDH1/2 mutations as diagnostic, prognostic and predictive tools also requires further evaluation.

**References**
Papers of special note have been highlighted as:
- of interest

7. Metallo CM, Gameiro PA, Bell EL et al. Reductive glutamine metabolism by IDH1...
Review

Krell, Mulholland, Frampton, Krell, Stebbing & Bardella


- Initially demonstrated the association between *IDH* mutations and glioma


- Confirmed the findings by Dang et al [29] that mutant *IDH* converts α-ketoglutarate to 2-hydroxyglutarate.


- Demonstrated the neomorphic enzyme function of mutant *IDH*.


43. Haliloglu G, Jobard F, Ogue K et al. L-2-hydroxyglutaric aciduria and brain tumours in children with mutations in the


- Illustrated the potential role of D-2-hydroxyglutarate (D-2HG) as an oncometabolite by demonstrating its ability to inhibit α-ketoglutarate-dependent dioxygenases.


- Demonstrates the accumulation of D-2HG in the brains of idh1 R132H knock-in NestinCre mice.


- Demonstrates the ability of D-2HG to modify histones and block cell differentiation.


- Illustrates the association between IDH mutation and hypermethylation in glioma.


- DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer:


* Demonstrates the effects of inhibiting mutant IDH1 in glioma cells, delaying growth and reversing the block to differentiation cause by mutant IDH, and offers the hope of a promising potential therapeutic option.


