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MINI-SYMPOSIUM: CANCER METABOLISM IN BRAIN TUMORS

2-Hydoxyglutarate: D/Riving Pathology in gLiomaS

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Abstract

Common pathways and mechanisms can be found in both cancers and inborn errors of metabolism. 2-Hydroxyglutarate (2-HG) acidurias and isocitrate dehydrogenase (IDH) 1/2 mutant tumors are examples of this phenomenon. 2-HG can exist in two chiral forms, D(R)-2-HG and L(S)-2-HG, which are elevated in D- and L-acidurias, respectively. D-2-HG was subsequently discovered to be synthesized in IDH 1/2 mutant tumors including ~70% of intermediate-grade gliomas and secondary glioblastomas (GBM). Recent studies have revealed that L-2-HG is generated in hypoxia in IDH wild-type tumors. Both 2-HG enantiomers have similar structures as α-ketoglutarate (α-KG) and can competitively inhibit α-KG-dependent enzymes. This inhibition modulates numerous cellular processes, including histone and DNA methylation, and can ultimately impact oncogenesis. D-2-HG can be detected *in vivo* in glioma patients and animal models using advanced imaging modalities. Finally, pharmacologic inhibitors of mutant IDH 1/2 attenuate the production of D-2-HG and show great promise as therapeutic agents.

IDH 1/2 MUTATIONS IN GLIOMAS

IDH 1 mutations were first discovered by next-generation sequencing studies in 12% of glioblastomas (GBM) (71). This intriguing finding prompted a more comprehensive analysis of a variety of gliomas including World Health Organization (WHO) grade II and grade III tumors, leading to the surprising detection of both IDH 1/2 mutations in ~70% of grade II and grade III gliomas and GBM that arise from these intermediate-grade lesions (termed secondary GBM) (7, 101). Subsequently, IDH 1/2 mutations have been found in other cancers, including acute myeloid leukemia, chondrosarcomas, Ollier and Maffucci syndromes (characterized by multiple central cartilaginous tumors), cholangiocarcinomas, angioimmunoblastic T-cell lymphoma and rare cases of melanoma, colon, adult medulloblastoma and thyroid cancers (2, 3, 9, 10, 58, 69, 81, 95, 96, 102). IDH 1/2 mutant gliomas occur more frequently in young adult patients and are very rare in children (37, 49, 59, 61, 98, 101).

The discovery of IDH 1/2 mutations in gliomas has significantly impacted our clinical approach to glioma patients. IDH 1/2 mutations in high- and low-grade gliomas confer a favorable prognosis relative to wild-type tumors. Further, IDH 1/2 mutations in grade II and grade III gliomas have a better outcome regardless of WHO grade, while wild-type tumors tend to behave like GBM (11, 29, 35, 39, 50, 75, 87). Thus, the IDH mutational status is paramount in intermediate-grade gliomas (31). From a biological perspective, the mechanisms by which IDH 1/2 mutations confer a better prognosis remain to be elucidated.

IDH 1/2 mutations are invariably missense and monoallelic. In gliomas, more than 90% of mutations involve IDH1 at the arginine residue at the enzymatic active site (R132H) (7, 12, 37, 101). This observation is in contrast to other tumors such as AML where IDH1 and IDH2 mutations occur at equal frequency. This finding suggests an important role for wild-type IDH1 in glial biology. The high frequency of IDH1 R132H mutations can be leveraged in diagnostic neuropathology using an antibody that specifically detects this mutation (14). Immunohistochemistry with this antibody has been proven very useful in readily detecting the mutation as a molecular surrogate and in histopathologic diagnostic challenges such as identifying single infiltrating tumor cells in limited biopsy material, differentiating recurrent tumor from reactive atypia and separating gangliogliomas from infiltrating astrocytomas (12, 13, 38). Other IDH1 mutations are rare but still involve the R132 residue where the arginine residue is replaced by other amino acids (such as C, G, S, L, V) (25). While IDH2 mutations are rare in gliomas, when they occur they also affect the catalytic arginine sites of the enzyme: R172 and R140 (25).

2-HG IS A METABOLITE PRODUCED IN IDH 1/2 MUTANT TUMORS AND 2-HG ACIDURIA

Wild-type IDH proteins (IDH1–3) are enzymes closely related to the tricarboxylic acid (TCA) cycle and catalyze the conversion of isocitrate to α-ketoglutarate (α-KG, also referred to as 2-oxoglutarate). IDH2 and IDH3 are mitochondrial enzymes, while IDH1 is mainly cytosolic. All three isoforms of IDH are critical for the generation of reducing equivalents. IDH3 is a heterotetrameric complex (formed by two α, one β and one γ subunits) that uses NAD^+ as a cofactor to generate NADH and this mitochondrial reaction is irreversible (103). On the other hand, both IDH2 and IDH1 are homodimers and use $NADP^+$ as a cofactor to produce NADPH (97). Monoallelic IDH1 mutations were initially hypothesized to promote oncogenesis through loss-of-function in enzymatic capacity to generate α-KG from isocitrate by the mutant allele along with dominant negative

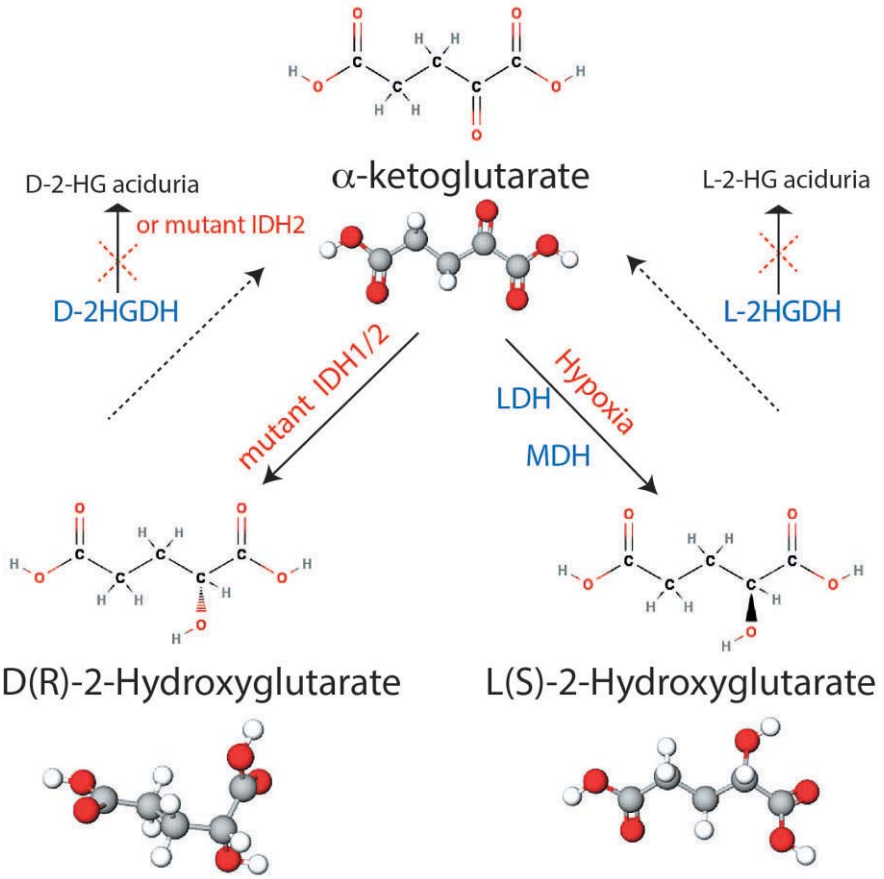

Figure 1. Enantiomers of 2-hydroxyglutarate (2-HG). In normal cells, D(R)-2-HG is produced at very low levels and is metabolized back to α-ketoglutarate (α-KG) by D-2-HG dehydrogenase (D-2-HGDH). D-2-HGDH loss-of-function mutations and IDH2 gain-of-function mutations result in D-2-HG aciduria. In cancers, D(R)-2-HG is generated in high concentrations by the activity of mutant IDH 1/2. L(S)-2-HG is synthesized at very low levels in normal cells and is metabolized back to α-KG by L-2-HG dehydrogenase (L-2-HGDH). L-2-HGDH loss-of-function mutations result in L-2-HG aciduria. In tumor hypoxic environments, both lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) can convert α-KG to L-2-HG.

activity against the wild-type allele (104). Subsequent studies have revealed a gain-of-function activity of the mutant enzyme that uses α-KG as a substrate along with NADPH consumption to generate the metabolite (D)-2-HG in millimolar concentrations (26, 36, 95). In proliferating cells *in vitro*, α-KG and subsequently (D)-2-HG is primarily derived from the amino acid glutamine, with smaller contributions from glucose carbons (26, 93, 97).

2-HG is a metabolite that has been studied in the context of inborn metabolic disorders termed 2-HG acidurias described initially in 1980 (15, 28). 2-HG is made in low concentrations in normal cells and can exist in two enantiomeric isoforms: D (also termed R)-2-HG and L (also termed S)-2-HG (inspiring the title of this article) (46) (Figure 1). These chiral forms of 2-HG are metabolized by dehydrogenases termed D-2-hydroxyglutarate dehydrogenase (D-2-HGDH) and L-2-hydroxyglutarate dehydrogenase (L-2-HGDH) (Figure 1). Germline loss-of-function mutations of either of these enzymes cause impaired 2-HG metabolism. This leads to accumulation of the metabolite resulting in D- or L-2-HG aciduria, respectively (83, 85). Combined D-2- and L-2-hydroxyglutaric aciduria is a rare disorder and is characterized by mutations in SLC25A1, the mitochondrial citrate transporter (65).

Intriguingly, D-2-HG aciduria patients do not show brain tumors (with the caveat that many of these patients have relatively shorter lifespan) but can exhibit developmental delays, seizures, enlarged ventricles, benign CNS (central nervous system) cysts and multifocal cerebral white matter abnormalities (46, 91). D-2-HGDH loss-of-function heterozygous mutations are also noted in a small subset of diffuse large B-cell lymphoma, resulting in modest elevations on D-2-HG (51). In addition to D-2-HGDH loss-of-function mutations, IDH2 gain-of-function mutations, similar to those seen in AML, involving the R140 residue are also noted in a fraction of D-2-HG aciduria patients and can be modeled in mice (1, 45). The absence of an association of brains tumors in humans and mouse models of D-2-HG aciduria suggests that the pathogenesis of tumors bearing IDH 1/2 mutations may be multifactorial.

IDH 1/2 MUTATIONS AND D-2-HG IN GLIOMA ONCOGENESIS

IDH 1/2 mutations and D-2-HG impact oncogenesis by impairing cell differentiation (Figure 2). Immortalized astrocytes, neuronal stem cells, erythroleukemia cell lines, liver progenitor cells and chondrosarcoma cell culture models expressing mutant forms of IDH show arrested cell differentiation (44, 54, 56, 57, 76, 90). Further, cell permeable forms of D-2-HG negatively impact differentiation in glioma and leukemia cell models (54, 56). This suppression of differentiation, when coupled with a second genetic hit, is hypothesized to drive glioma pathogenesis (Figure 2). The second hit hypothesis is strengthened by the observation that the IDH1 R132H mutation alone is insufficient to form gliomas in mouse models *in vivo* (77). It is speculated that IDH 1/2 mutations suppress the ability of early glial precursors to differentiate into

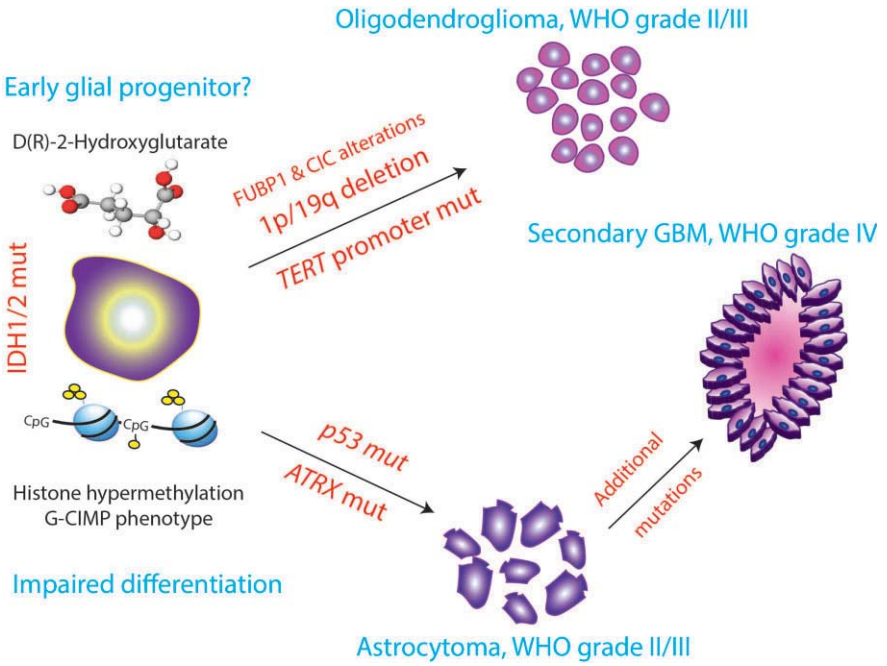

Figure 2. Isocitrate dehydrogenase (IDH) 1/2 mutation is an early event in glioma genesis. IDH 1/2 mutations are thought to occur as an early event in glioma biology in early glial progenitors. D-2-hydroxyglutarate (D-2-HG) is thought to impair cell differentiation in these cells as a result of histone and DNA hypermethylation (G-CIMP). When these cells acquire 1p/19q co-deletions (corresponding to FUBP1 and CIC alterations) and TERT promoter mutations, they give rise to oligodendrogliomas. When these cells accumulate p53 and ATRX mutations, they are thought to develop into astrocytomas and secondary glioblastomas (GBM) on acquiring additional mutations.

mature oligodendrocytes or astrocytes. When this cell additionally accumulates p53 and ATRX mutations, it is thought to give rise to astrocytic tumors (Figure 2). On the other hand, when this IDH 1/2 mutant cell acquires 1p/19q deletions (corresponding to CIC and FUBP1 alterations) along with TERT promoter mutations, it is thought to give rise to oligodendrogliomas (Figure 2). Interestingly, mutant CIC cooperates with IDH1 mutations to increase levels of D-2-HG (20). Finally, the association of IDH 1/2 mutations with astrocytomas, oligodendrogliomas and oligoastrocytomas supports this hypothesis (37, 49, 98). This model has led to the suggestion that IDH 1/2 mutations occur as an early event in gliomagenesis.

IDH 1/2 mutations are thought to impair cell differentiation by directly reprogramming the epigenetic state of the cell by influencing DNA and histone methylation (Figures 2 and 3). Prior analyses of DNA methylation in GBM revealed a subset of tumors that displayed widespread DNA hypermethylation across the genome. These tumors are referred to as the glioma-associated-CpG island hypermethylator phenotype (G-CIMP) (66). Similar to IDH mutant tumors, these gliomas have better prognostic outcomes than non-G-CIMP tumors (66). Moreover, G-CIMP is strongly associated with IDH 1/2 mutations in both GBM and intermediate-grade gliomas (23, 27, 48, 66, 90). Expression of IDH1 R132H mutation in immortalized astrocytes and other cell lines results in DNA hypermethylation similar to that seen in G-CIMP (27, 57, 90).

The mechanism by which IDH 1/2 mutations reprogram DNA methylation is directly linked to the structure of 2-HG. Both D- and L-isoforms of 2-HG are similar in structure to α-KG (Figure 3). The metabolite α-KG along with oxygen and iron are critical cofactors for reactions driven by a family of enzymes termed α-KG-dependent dioxygenases. These enzymes regulate a number of cellular reactions, including DNA methylation, histone

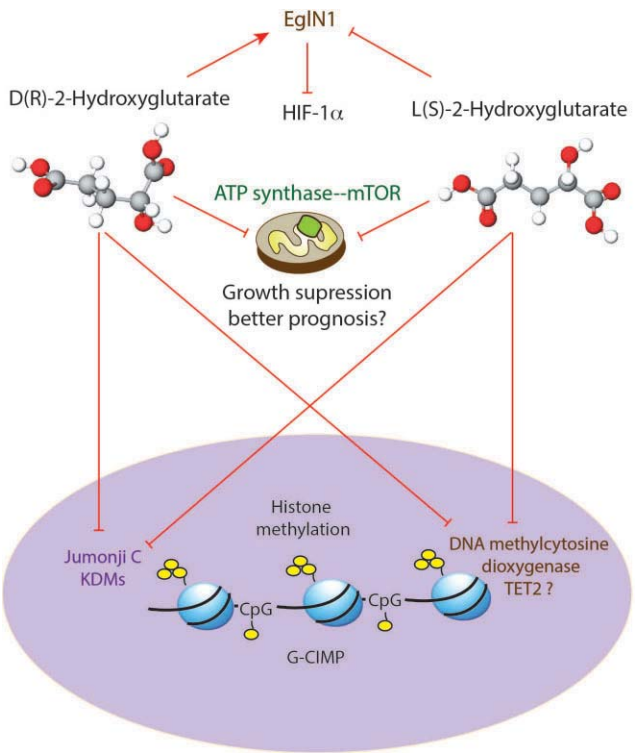

Figure 3. D-2-hydroxyglutarate (D-2-HG) and L-2-hydroxyglutarate (L-2-HG) have similar and differential effects. D-2-HG is thought to potentiate eglin1, while L-2-HG antagonizes eglin1, leading to HIF-1α stabilization. Both D- and L-2-HG can inhibit DNA and histone demethylases, resulting in histone and DNA hypermethylation (G-CIMP phenotype). Both enantiomers also inhibit ATP synthase and mammalian target of rapamycin (mTOR) inhibit growth of glioma cells and may contribute to better prognosis seen in IDH1/2 gliomas.

methylation, carnitine synthesis, hypoxia sensing and collagen modifications (52). The structural similarity of D- and L-2-HG to α-KG is thought to result in a competitive inhibition of α-KG-dependent enzymatic reactions (22, 100). Interestingly, D-2-HG is made at very low levels under normal conditions and is considered a by-product of cellular metabolism (generated by the enzyme hydroxyacid–oxoacid transhydrogenase) (86). D-2-HG accumulation is prevented by its conversion to α-KG by D-2-HGDH (Figure 2) (46). Activity of D-2-HGDH can increase α-KG levels directly and indirectly by enhancing IDH2 function in an unknown manner (51). Elevated α-KG concentrations drive demethylation of histones and DNA by potentiating the function of α-KG-dependent demethylases (51). In IDH mutant tumors, the concentration of D-2-HG produced from the activity of mutant IDH 1/2 is so high that it overwhelms the activity of D-2-HGDH, resulting in accumulation of D-2-HG and inhibiting the function of these α-KG-dependent enzymes resulting in DNA and histone hypermethylation (22, 55, 100).

The strongest support for this hypothesis in relation to DNA methylation comes from the leukemias where IDH 1/2 mutations and loss-of-function mutations in the DNA methylating enzyme TET2 are mutually exclusive (32). TET2 is an α-KG-dependent DNA methylating enzyme (methyl cytosine dioxygenase) that hydroxylates 5-methylcytosine to 5-hydroxylmethycytosine (88). Further, TET2 knockdown in leukemia cells blocks differentiation in a fashion similar to IDH2 mutations (54). While TET2 promoter methylation is seen in a small fraction of IDH 1/2 wild-type low-grade gliomas, no mutations in the TET family of proteins are reported in gliomas (43). Further, no significant relationship was observed between IDH 1/2 mutations and decreased 5-hydroxylmethycytosine in glioma samples, suggesting that this effect is not driven by TET2 inhibition (41, 63, 68). The mechanisms of how IDH 1/2 mutations and D-2-HG reprogram DNA methylation in gliomas and cause the G-CIMP phenotype require further elucidation.

Similar to its effects on inhibiting DNA methylating enzymes, D-2-HG can also inhibit histone-demethylating enzymes resulting in histone hypermethylation (22, 55, 100) (Figure 3). The jumonji C family of histone methyltransferases depend on α-KG for their activity (22). Cell permeable forms of D-2-HG can increase histone methylation marks in adipocytes (55). Similarly, IDH mutations expressed in various cell lines and IDH 1/2 mutant oligodendrogliomas induce histone hypermethylation as evidenced by increased trimethylation of histone marks such as H3K9, H3K27 and H3K36 (27, 55, 90, 92, 100). Together, DNA and histone hypermethylation affect the expression of many genes and are thought to contribute to suppressing cell differentiation.

D-2-HG also impacts the function of other α-KG-dependent dioxygenases. While conditional knock-in of IDH1 R132H in mice does not induce tumor formation, it does alter collagen maturation by blocking prolyl-hydroxylation. This abnormal maturation leads to defective vasculature and cerebral hemorrhage in the brain (77). Interestingly, effects on prolyl-hydroxylation may be context-dependent. EglN1 is an α-KG and oxygen-dependent prolyl hydroxylase that enables HIF1-α degradation by the VHL complex. In hypoxia, EglN1 enzymatic activity is decreased, resulting in HIF1-α stabilization. Instead of blocking EGln1 activity, D-2-HG is thought to potentiate EglN1 activity, resulting in enhanced proliferation of immortalized human astrocytes in soft agar in an undefined manner (Figure 3) (44). Along similar lines, both α-KG and 2-HG (both D- and L-isoforms) can extend the lifespan of *Caenorhabditis elegans* by inhibiting ATP synthase. Inhibition of ATP synthase is thought to lower mitochondrial respiration and attenuate mammalian target of rapamycin (mTOR) signaling, resulting in lowered tumor cell growth and viability (Figure 3) (19, 33). These findings could potentially relate to the overall better survival outcomes in IDH 1/2 mutant glioma patients compared with wild-type tumors. In addition to influencing α-KG-dependent cellular functions, D-2-HG can also inhibit the activity of cytochrome *c* oxidase in the mitochondrial electron transport chain. This inhibition causes increased apoptosis in IDH2 mutant leukemic cell lines and is of potential therapeutic use (16). Thus, D-2-HG can affect distinct cellular phenomena, many of which need to be further defined in IDH 1/2 mutant gliomas.

TUMOR MICROENVIRONMENT, L-2-HG AND ITS ROLE IN ONCOGENESIS

L-2-HG acidurias are characterized by loss-of-function mutations in L-2-HGDH and subsequent L-2-HG accumulation. Patients with this disorder show delayed development and seizures similar to D-2-HG acidurias. Additionally, cerebellar dysfunction, dystonia and spasticity, tremors, and white matter changes in the cerebrum, cerebellum and basal ganglia can be observed (45). In contrast to D-2-HG-acidurias, astrocytomas, bone tumors, gliomatosis cerebri and Wilm's tumors have been described as rare features of L-2-HG aciduria patients (45, 53). Further, renal cancers with VHL mutations show decreased expression of L-2-HGDH, resulting in elevated levels of L-2-HG (80). Together, these observations suggest that L-2-HG may play a role in oncogenesis. D-2-HG is produced by IDH 1/2 mutations, but how is L-2-HG synthesized and how does this affect tumor pathogenesis? Recent studies suggest that hypoxic tumor microenvironments result in increased L-2-HG production from α-KG accompanied by the consumption of NADH (40, 67). This phenomenon was not dependent on the activity of wild-type IDH 1/2 enzymes but was mediated in a context-dependent manner to different degrees by the enzymes malate dehydrogenase and lactate dehydrogenase (Figure 3) (40, 67).

As discussed previously, decreased EglN1 (α-KG-dependent prolyl hydroxylase) enzymatic activity results in HIF1-α stabilization. While D-2-HG potentiates EglN1 activity, L-2-HG inhibits EglN *in vitro* and promotes HIF-1α accumulation (Figure 3) (44, 54). This model would suggest a reciprocal feedback mechanism in which hypoxic generation of L-2-HG stabilizes HIF-1α, further promoting hypoxia. However, L-2-HG synthesis was independent of the effects of HIF-1α, suggesting that L-2-HG production was driven by other mechanisms (67). Hypoxia results in increased cytosolic and mitochondrial NADH production caused by redirection of glucose metabolism to anaerobic glycolysis (78). Generation of L-2-HG is thought to be a cellular adaptive response to consume these excessive reducing equivalents. L-2-HG production was also directly related to α-KG substrate availability. In hypoxia, glutamine metabolism is reprogrammed such that glutamine becomes the main TCA cycle substrate to generate α-KG (60, 62, 99). In glioblastoma cell lines, glutamine-generated α-KG was the main substrate for L-2-HG synthesis (40), although glucose-derived α-KG also contributed to L-2-HG production in other cell

lines (67). Further, L-2-HG accumulation in hypoxia reduced glycolytic flux through a negative feedback loop, perhaps to limit additional NADH synthesis via glycolysis (67). These data suggest that L-2-HG production may be an adaptive response to maintain redox balance in hypoxic tumor cells.

While the effects of D-2-HG and L-2-HG on EglN1 are thought to be opposed, both metabolites inhibit α-KG-dependent dioxygenases including DNA and histone demethylases (Figure 3). Intriguingly, L-2-HG is thought to be a more potent inhibitor of enzymes like TET2 (44, 100). In glioma cells, L-2-HG in hypoxia caused increased H3K9me3, depending on inhibition of the activity of the histone demethylase KDM4C (40). This effect corresponded to increased H3K9me3 in hypoxic niches in IDH 1/2 wild-type GBM tumor samples. Similarly, L-2-HG accumulation in VHL-mutated renal cancer causes increased DNA and histone methylation (80). Overall, these data suggest that L-2-HG mediates epigenetic changes in hypoxic tumor niches in IDH wild-type gliomas resulting in tumor heterogeneity.

IMAGING OF 2-HG *IN VIVO*

The accumulation of intracellular D-2-HG to millimolar concentrations in IDH1/2-mutated tumors can be leveraged to image these tumors *in vivo* and thus non-invasively determine the IDH mutational status. Several imaging modalities including conventional magnetic resonance spectroscopy (MRS) and hyperpolarized ^{13}C MRS can detect 2-HG *in vivo*. Because IDH wild-type tumors and normal tissues do not accumulate appreciable amounts of D- or L-2-HG under normal conditions, spectroscopic detection has the possibility to spatially discriminate tumor from normal tissue based on altered biochemistry. In the past several years, interest in D-2-HG imaging has increased dramatically with a focus on its potential as a biomarker for IDH 1/2 mutations, a potential early marker for treatment response and as a tool for distinguishing tumor progression from pseudoprogression in IDH 1/2 mutated gliomas.

Conventional MRS was introduced in the 1970s as a method of defining the biochemical environment of tissues (82). A technical description of MRS theory and methodology is beyond the scope of this review, but in brief, the technique provides a proton spectrum for individual voxels that allows for the detection and quantification of major neurometabolites, including N-acetylaspartate (NAA), choline (Cho), creatine (Cr), alanine (Ala) and lactate. While many nuclei can be monitored by spectroscopy, proton MRS is used clinically because of the high abundance of protons, their favorable gyromagnetic ratio and the ability to perform proton MRS without additional hardware beyond what is typically used in a clinical scanner (82). Most brain tumors have elevated Cho and decreased NAA compared with normal brain and therefore a common clinical use of MRS is to assess the NAA/Cho ratio to distinguish glioma from normal brain or treatment effects (82).

The initial discovery that L-2-HG could be monitored by MRS was made in the early 2000s when patients with congenital L-2-HG aciduria were found to have an abnormal singlet spectral peak between 2.25 and 2.7 ppm in all voxels of the brain analyzed (34, 79). Subsequent studies have shown that the MRS spectra of both D- and L-enantiomers are identical (84). Therefore, once the oncogenic IDH1/2 mutations were shown to produce D-2-HG, there was tremendous interest to apply MRS techniques to image IDH1/2-mutated tumors. Initial publications demonstrating MRS detection of D-2-HG in IDH1-mutated brain tumors came in 2012 from the University of Texas Southwestern (UTSW) (21) and from the Massachusetts General Hospital (MGH) (4). These groups both noted that one of the main difficulties in the MRS detection of D-2-HG was its spectral proximity to nearby glutamine/glutamate and NAA resonance peaks (8). The MGH group overcame this difficulty by using two-dimensional correlation spectroscopy, while the UTSW group optimized echo time and used J-difference spectroscopy to minimize the signal from overlapping metabolites. Both groups demonstrated that MRS detection of D-2-HG occurred only in patients with IDH1-mutated gliomas and found concentrations of 1.7–8.9 mM of D-2-HG. The 100% specificity associated with these advanced MRS techniques is a major improvement over standard MRS techniques for D-2-HG detection, which had false-positive rates near 20% (72).

The clinical use of MRS to detect D-2-HG is in its infancy, but possible opportunities abound. The Memorial Sloan Kettering Cancer Center group has recently presented their results of incorporating D-2-HG MRS into standard imaging for glioma patients (24). D-2-HG MRS was performed on 107 consecutive grade II–IV glioma patients, 75% of which contained mutated IDH 1/2. 2-HG MRS was negative in all patients with wild-type IDH and all those with mutant IDH 1/2 tumors with volume less than 8 cm^3. Approximately 35% of patients with mutant IDH 1/2 had positive D-2-HG MRS scans. Intriguingly, results from a subset of patients who received multiple scans while undergoing active treatment suggested that a drop in D-2-HG levels is associated with a treatment response. While the specificity of D-2-HG MRS is excellent, increased sensitivity is needed, especially for smaller tumors. Furthermore, applying this technique as a biomarker for treatment response or as a marker for early progression will require much additional study.

An alternative means of imaging D-2-HG is by using hyperpolarized carbon 13 (^{13}C) MRS rather than proton spectroscopy. Because MRS visualizes only paramagnetic atoms, this technique detects only ^{13}C rather than the more abundant ^{12}C. Typically, ^{13}C is difficult to image by spectroscopy because of its low natural abundance (approximately 1%) and unfavorable gyromagnetic ratio, both of which result in a nearly insurmountably low signal-to-noise ratio (47). An increasingly utilized technology to overcome these limitations is hyperpolarized ^{13}C MRS. In this technique, a ^{13}C-enriched metabolic tracer is obtained to interrogate the metabolic pathway of interest (eg, pyruvate to investigate lactate dehydrogenase or α-ketoglutarate to investigate the TCA cycle). Immediately prior to tracer administration and scanning, the ^{13}C tracer is "hyperpolarized" by exposing it to microwaves and unpaired electron donors while at extremely low temperatures in a technique known as dynamic nuclear polarization (DNP) (6). This process alters the Boltzmann distribution of ^{13}C and can increase sensitivity by more than 10 000-fold. Because ^{13}C only remains hyperpolarized for a very short time, the tracer is then immediately infused into the patient where it is detected by MRS. More importantly, the conversion of the administered metabolite into its downstream products can be measured by simultaneously measuring the disappearance of ^{13}C in the administered metabolite and the appearance of ^{13}C into potential products. Therefore, unlike conventional MRS, hyperpolarized ^{13}C MRS allows for the detection of dynamic metabolic fluxes in real time (64).

The mutant IDH 1/2-catalyzed conversion of α-KG to D-2-HG has recently been imaged using hyperpolarized ^{13}C MRS (18). In this study, α-KG tracer was synthesized with ^{13}C at the first carbon position ([1-^{13}C] α-KG). After hyperpolarization, this tracer was administered to rats bearing either IDH1 WT or mutant GBM xenografts and conversion of [1-^{13}C] α-KG into [1-^{13}C] D-2-HG was seen only in the IDH1 mutant tumors (18). Interestingly, the conversion of α-KG into glutamate is catalyzed by branched-chain amino acid transaminase 1 (BCAT1) and the activity of BCAT1 is severely diminished in IDH 1-mutated gliomas (89). Therefore, combined analysis of the formation of hyperpolarized [1-^{13}C] D-2-HG and hyperpolarized [1-^{13}C] glutamate from a single tracer ([1-^{13}C] α-KG) could give more robust information about the IDH 1/2 mutational status of a tumor (89). While these observations are yet to be translated to the clinic, potential applications abound. Changes in the flux through individual metabolic pathways can indicate a response to chemotherapy in as little as 24 h after treatment initiation (70). Therefore, early changes in the observed conversion of α-KG to D-2-HG could reveal which patients with IDH1/2-mutated gliomas are receiving a benefit from chemotherapy.

INHIBITION OF D-2-HG AS A THERAPEUTIC TARGET IN IDH 1/2 MUTANT GLIOMAS

Pharmacologic inhibitors of mutant IDH1 and IDH2 have been recently synthesized and show great promise in cell lines, IDH 1/2 tumor animal models and AML patients (17, 42, 54, 73, 74, 94). Treatment of oligodendroglioma cell lines bearing 1p/19q co-deletions and IDH1 R132H mutations with IDH1 mutant specific inhibitors showed significant decreases in D-2-HG production. This was accompanied by modest growth inhibition in subcutaneous glioma models *in vivo*. Importantly, the inhibitor resulted in demethylation of histone H3K9me3 and promoted the expression of genes associated with glial differentiation, suggesting that its therapeutic effects arose from reversing D-2-HG-mediated hypermethylation. Intriguingly, DNA methylation did not appreciably decrease after treatment (74). Treatment of patient-derived AML cells with a mutant IDH2 inhibitor induced differentiation *in vitro* with early reversal of increased histone methylation, but in contrast to glioma, models showed a slower reversal of DNA hypermethylation (42, 94). Early data from a clinical trial in IDH mutant human subjects with AML show promising results (5). Other approaches target the metabolic pathway of D-2-HG generation. As D-2-HG is mainly derived from glutamine, inhibition of glutaminase, which metabolizes glutamine to glutamate, reduces D-2-HG production, cell growth and histone hypermethylation of IDH1-mutant cells (30). While these approaches show great promise, blood–brain barrier permeability issues poses a challenge that needs to be overcome for effective treatment in IDH 1/2 mutant gliomas.

SUMMARY

2-HG can exist as two chiral isoforms: D-2-HG and L-2-HG. Both molecules are produced in negligible quantities in normal cells as by-products of metabolism and are degraded by enantiomer-specific dehydrogenases. D-2-HG accumulates in D-2-HG aciduria as a result of the loss-of-function mutations in D-2-HGDH and gain-of-function IDH2 mutations. L-2-HG is elevated in L-2-HG aciduria as a result of the loss-of-function mutations in L-2-HGDH. In cancers, D-2-HG is generated from α-KG by gain-of-function IDH 1/2 mutations. IDH 1/2 wild-type tumors synthesize L-2-HG from α-KG by the enzymes MDH and LDH in hypoxia, albeit at lower concentrations. Both isoforms of 2-HG are similar in structure to α-KG, resulting in inhibition of enzymes like ATP synthase and α-KG-dependent dioxygenases such as histone and DNA demethylases (L-2-HG is thought to be more potent than D-2-HG). They can also differ in function; while D-2-HG potentiates the activity of Egln1 (a prolyl hydroxylase enzyme that destabilizes HIF-1α), L-2-HG is thought to inhibit it. The production of L-2-HG is thought to be an adaptive response to help adapt cells to hypoxic redox changes. Thus, both isoforms of 2-HG are implicated in cancers and can impact the epigenetic state of the tumors. Because of its high concentrations, D-2-HG can be detected *in vivo* in IDH 1/2 mutant gliomas using MRS and hyperpolarized ^{13}C MRS imaging techniques. Finally, lowering D-2-HG using IDH 1/2 mutation-specific small-molecule inhibitors shows great promise in treating these patients.

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