

Isocitrate dehydrogenase mutation as a therapeutic target in gliomas

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Abstract: Isocitrate dehydrogenases (IDH) are important enzymes that catalyze the oxidative decarboxylation of isocitrate to α -ketoglutarate (α -KG), producing NADPH in the process. More than 80% of low-grade gliomas and secondary glioblastoma (GBM) harbor an IDH mutation. IDH mutations involve the catalytic pocket of the enzyme and lead to a neomorphic ability to produce 2-hydroxyglutarate (2HG) while oxidizing NADPH to NADP⁺. 2HG is considered as an ‘oncometabolite’ which is thought to be responsible for many, if not all, biologic effects of IDH mutations. 2HG accumulation competitively inhibits α -KG-dependent dioxygenases, including histone lysine demethylases and DNA demethylases, resulting in a hypermethylation phenotype with alterations in cellular epigenetic status as well as a block in cellular differentiation. IDH mutations have been suggested as an important early event in tumorigenesis, however it remains unclear whether IDH mutation by itself causes cancer or if it requires other oncogenic events to initiate tumorigenesis. Significant efforts have been made to better understand the mechanisms of IDH mutations in tumor initiation and progression, and to develop targeted therapies for IDH-mutated tumors. This review provides an overview of the function of mutant IDH, and the current understanding of the role IDH mutations play in gliomagenesis. In addition, several potential therapeutic strategies for IDH-mutant gliomas, including mutant IDH inhibitors which have entered clinical evaluation in glioma patients, will be discussed.

Keywords: Isocitrate dehydrogenases (IDH); IDH1; IDH2; glioma; targeted therapies; 2-hydroxyglutarate (2HG)

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Introduction

There have been major advances in the understanding of isocitrate dehydrogenase (IDH) mutations in various types of cancers since their discovery in 2008 by Parsons *et al.* (1). In their integrated genomic analysis of human glioblastoma (GBM), IDH mutation was observed in 12% of GBM samples and the majority of these were in tumors that had progressed from lower grade gliomas (secondary GBM).

Subsequently >70% of WHO grade II/III gliomas were found to harbor an IDH mutation (2). IDH mutations also occur in acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN), cholangiocarcinoma, enchondroma and chondrosarcoma, and other solid cancers. Significant efforts have been made since its discovery to improve our knowledge about the role IDH mutations play in tumorigenesis and progression. It has become clear that IDH mutations are associated with

many epigenetic and metabolic changes in these tumors. In this review we provide an overview of the function of mutant IDH and the current understanding of the role IDH mutations play in tumorigenesis. In addition, we discuss several potential therapeutic strategies for IDH-mutant gliomas based on emerging preclinical and clinical data, including therapies targeting mutant IDH and other IDH mutation-associated pathways.

Functions of normal IDH

There are three isozymes in the IDH family: IDH1 is located in the cytosol and peroxisome and IDH2 and IDH3 are located in the mitochondria. Normal IDH catalyzes the NADP⁺-dependent oxidative decarboxylation of isocitrate to α -ketoglutarate (α -KG), producing CO₂ and NADPH in the process (3). It, therefore, plays an important role in biosynthesis of central metabolites in the tricarboxylic acid (TCA) cycle as well as in the generation of cellular NADPH. IDH1 is particularly important in the brain as this is the main source of NADPH (4).

Functions of mutant IDH

The majority of IDH mutations involve the catalytic pocket of the enzyme. IDH1 mutations predominantly occur at arginine 132 resulting in substitutions, including R132H (most common, 88%), R132C, R132L, R132S, and R132G (2). IDH2 mutations typically occur at R140 or R172. Of IDH2 mutations, R172K is most common. IDH1 and IDH2 mutations are mutually exclusive.

A landmark study of a large-scale metabolomics screen in IDH-mutant GBM cells found that IDH1^{R132H} mutation leads to a neomorphic ability to produce (R)-2-hydroxyglutarate (2HG) while oxidizing NADPH to NADP⁺ (5). This process results in 2HG accumulation with levels up to 100-fold higher in IDH mutant versus IDH wild type cells (6). 2HG is converted directly from α -KG by the mutant IDH, therefore relies on the presence of a wild-type IDH which converts isocitrate to α -KG in the TCA cycle (7). This may explain the rareness of loss of heterozygosity of IDH.

2HG is considered as an 'oncometabolite' which is thought to be responsible for many, if not all, biologic effects of IDH mutations. In normal cells without IDH mutation, 2HG is present at trace levels and has no known metabolic function (8). It is rapidly converted back to α -KG by 2HG dehydrogenases. In IDH-mutant cells, the

production of 2HG is far greater than its clearance, leading to high concentrations of 2HG in these cells.

Role of IDH mutations in tumor formation and progression

IDH mutations have been suggested as an important early event in tumorigenesis. Large scale genomic analyses of IDH-mutant gliomas found that IDH1 mutation occurred before acquisition of other mutations such as TP53 mutations or 1p/19q co-deletion (9,10), and IDH1^{R132H} was the only mutation that was consistently present in both the initial and recurrent glioma samples (11). IDH mutation was also maintained in 95% of recurrent secondary GBMs (12). Further supporting evidence of IDH mutation being an early genetic event comes from studies of hematopoietic disorders where IDH1/2 mutations have been found in premalignant diseases such as early MDS, MDS/MPN (13-15). IDH mutations have also been identified in other premalignant solid tumors, such as enchondromas, cartilaginous tumors that can progress to malignant chondrosarcomas.

It remains unclear whether IDH mutation by itself causes cancer or if it requires other oncogenic events to initiate tumorigenesis. IDH-mutant tumors typically harbor other cancer-associated genetic alterations. IDH mutation is associated with ATRX, TP53 and TERT mutations in diffuse astrocytoma, and co-deletion of chromosome arms 1p and 19q in oligodendroglioma (16). In mouse IDH-mutant AML models, there was a long latency and incomplete penetrance of tumors, suggesting IDH mutation is not sufficient to fully drive tumorigenesis by itself (17-19). Similarly, IDH1^{R132H} expression in several cell types of the murine central nervous system failed to form tumor on its own (20). However, once established, at least in IDH1-mutant glioma xenograft models in mice, ongoing expression of IDH1 mutation appears to be necessary for tumor maintenance as pharmacological inhibition of mutant IDH1 results in tumor growth delay and induction of cell differentiation (21).

Epigenetic modifications by IDH mutations via 2HG accumulation

2HG is structurally similar to α -KG. At high concentrations, 2HG competitively inhibits α -KG-dependent dioxygenases including the Jumonji domain containing (JmjC) family of histone lysine demethylases (KDMs) and the ten-

eleven translocation (TET) family of DNA hydroxylases that catalyze the sequential oxidation of 5-methylcytosine (5mC) to eventual DNA demethylation (22-27). These inhibitory effects on histone and DNA demethylases result in a hypermethylation phenotype with alterations in cellular epigenetic status. IDH-mutant tumors have been shown to display a CpG island methylator phenotype (CIMP) (28,29). In glioma, this DNA hypermethylation signature is known as glioma CIMP (G-CIMP). The notion that IDH mutation is the molecular basis of G-CIMP has been supported by preclinical data showing that introduction of mutant IDH1 into primary human astrocytes induces extensive DNA hypermethylation and reshapes the methylome in a fashion that mirrors the changes observed in G-CIMP (30).

IDH mutations and subsequent accumulation of 2HG have been shown to prevent the histone demethylation that is required for lineage-specific progenitor cells to differentiate (31). This suggests that IDH mutations can promote tumorigenesis by blocking cellular differentiation via hypermethylation of genes involved in differentiation.

CTCF insulator protein organizes the genome into topological domains by creating chromatin loops and boundaries (32,33). The hypermethylator phenotype of IDH-mutant gliomas has been shown to disrupt CTCF binding and domain boundaries, allowing a potent constitutive enhancer to interact with the normally quiescent PDGFRA oncogene promoter (34). This provides an epigenetic mechanistic link between IDH mutation and activation of PDGFRA. Furthermore, a demethylating agent was able to restore the insulator function, resulting in reduction of PDGFRA expression in IDH-mutant astrocytoma model. Widespread CTCF and domain boundary disruption is likely and this could lead to other oncogene activations in IDH-mutant tumors.

Potential therapeutic targets in IDH-mutant gliomas

Patients with WHO grade II and III gliomas that harbor an IDH mutation have a better response to treatment and a prolonged survival compared to those with IDH wild-type gliomas (35,36). In addition, these lower grade gliomas respond better to chemo-radiotherapy than to radiotherapy alone (37,38). IDH mutant gliomas maintain the mutant IDH allele even after acquiring further oncogenic driver mutations (11,39). This may indicate that IDH mutant gliomas, even at progression or after malignant transformation to higher grade glioma, may remain

vulnerable to the targeted therapies developed specifically for IDH mutations. The therapeutic effects may be further enhanced by combining different targeted therapies or with traditional chemotherapeutics or radiation. In this section, various potential therapeutic targets in IDH-mutant gliomas are discussed based on emerging preclinical and clinical data.

Mutant IDH enzymes

The notion that these neomorphic mutations of IDH1 and IDH2 are the mechanistic basis for malignant transformation led to the development of drugs targeting the mutant IDH1/2 enzymes. Moreover, IDH mutations are tumor-specific and expressed uniformly in all tumor cells (10). In a preclinical study using a fully transformed cells with endogenous IDH1 mutation, a targeted inhibitor of mutant IDH1 induced demethylation of histone H3K9me3 and expression of genes associated with gliogenic differentiation via reduction of 2HG production (21). Mutant IDH1 inhibition also delayed the growth of IDH1-mutant, but not IDH1 wildtype, glioma cells. In another study, a mutant IDH2 inhibitor was able to induce cellular differentiation of primary human AML cells harboring an IDH2 mutation (40). Mechanistic studies in an IDH2 mutant erythroleukemia model showed that inhibition of IDH2 by a small molecule inhibitor reversed both histone and DNA methylation changes within days and weeks, respectively (41). Methylation of histone and DNA is closely linked to mRNA expression and differentiation, therefore these preclinical study results provide proof-of-concept that targeting mutant IDH1/2 is a potential therapeutic strategy for IDH-mutant tumors.

Whether inhibition of mutant IDH and subsequent reduction in 2HG production are sufficient to halt tumor growth in IDH-mutant gliomas and other solid tumors remains unclear. There are studies that have shown no or minimal effects on CIMP or tumor growth despite near complete abrogation of 2HG production by pharmacologic inhibition alone (42-45). These conflicting observations between tumor types and cell lines may be due to potentially differing effector pathways of mutant IDH and 2HG in cellular-, (epi)genetic-, and organ-level context.

Multiple IDH inhibitors have been developed and several of them are currently undergoing clinical evaluation in phase I/II trials in patients with IDH mutant tumors. AG-221, a selective small molecule inhibitor of IDH2 was the first to enter clinical trial in late 2013. Preliminary

Table 1 Targeted therapies for IDH-mutant gliomas currently undergoing clinical evaluation

Drug	Mechanism of action	Indication	Trial phase	Comments	Clinicaltrials.gov identifier
Mutant IDH inhibitors					
AG-881	Mutant IDH1 and IDH2 inhibitor	Advanced solid tumors, including gliomas, with an IDH1/2 mutation	Phase I	Recruiting, penetrates blood-brain barrier	NCT02481154
AG-120	Mutant IDH1 inhibitor	Advanced solid tumors, including gliomas, with an IDH1 mutation	Phase I	Active, not recruiting	NCT02073994
AG-221	Mutant IDH2 inhibitor	Advanced solid tumors, including gliomas, with an IDH2 mutation	Phase I/II	Completed, results awaited	NCT02273739
BAY1436032	Mutant IDH1 inhibitor	IDH1 mutant advanced solid tumors	Phase I	Recruiting	NCT02746081
DS-1001b	Mutant IDH1 inhibitor	IDH1 ^{R132} mutant glioma	Phase I	Recruiting	NCT03030066
IDH305	Mutant IDH1 inhibitor	Advanced malignancies, including gliomas, with an IDH1 ^{R132} mutation	Phase I	Suspended recruitment	NCT02381886
Metabolic pathway					
Metformin and chloroquine	Antidiabetic and antimalarial (to exploit metabolic vulnerability exhibited by IDH mutant tumors)	IDH1/2 mutant glioma, cholangiocarcinoma, cholangiosarcoma	Phase Ib	Recruiting	NCT02496741
Immunotherapy					
Avelumab (with hypofractionated radiation)	Programmed death-ligand 1 (PD-L1) inhibitor	Progressive or recurrent IDH1/2 mutant glioblastoma	Phase II	Recruiting	NCT02968940
IDH1 ^{R132H} -dendritic cell vaccine	Immunotherapy	IDH1 ^{R132} mutant glioma	Phase I	Recruiting	NCT02771301
IDH1 ^{R132H} peptide vaccine	Immunotherapy	IDH1 ^{R132} mutant glioma	Phase I	Recruiting	NCT02454634
PEPIDH1M vaccine	Immunotherapy	Progressive or recurrent grade II glioma with IDH1 mutation	Phase I	Recruiting	NCT02193347
CB-839	Glutaminase inhibitor	Advanced and/or treatment refractory solid tumors, including IDH1/2 mutant gliomas	Phase I	Recruiting	NCT02071862

IDH, isocitrate dehydrogenase.

data from phase I trial of this agent in patients with IDH2-mutant relapsed or refractory AML demonstrated clinical responses in about 40% of the patients (46). Preliminary data of the dose escalation phase I trial of AG-120, an IDH1 inhibitor, in solid tumors reported a clinical benefit rate (defined as progression free at 6 months) of 37% among all patients and 25% in glioma patients (47). AG-881, a pan-inhibitor of the mutant IDH1 and IDH 2 enzymes, has been shown to fully penetrate the brain-blood barrier

in preclinical studies, therefore it is of particular interest whether this agent has better clinical efficacy in glioma patients. At the time of writing, there are six IDH inhibitor clinical trials for gliomas (*Table 1*).

DNA hypermethylation

Given the evidence that IDH mutations cause epigenetic alterations and a cellular differentiation block by inhibiting

histone and DNA demethylases and the implication that these epigenetic alterations lead to malignant transformation, histone deacetylase inhibitors and DNA demethylating agents have been investigated in preclinical models of IDH-mutant tumors (42,45,48). Decitabine and azacytidine are DNA demethylating agents, approved by Food and Drug Administration (FDA) for MSD, and they inhibit DNA (cytosine-5-)-methyltransferase 1 (DNMT1). A preclinical study of decitabine in patient-derived IDH1 mutant glioma cells demonstrated that decitabine can effectively reverse the pathologic DNA hypermethylation induced by mutant IDH, and induce cellular differentiation in these cells (45). In this study, decitabine also decreased growth of these cells *in vitro* and IDH1-mutant xenografts in mice. Similarly, another study showed that long-term administration of low dose azacytidine results in reduction of DNA methylation of promoter loci, induction of glial differentiation, reduction of cell proliferation and a significant reduction in tumor growth in an endogenous IDH1 anaplastic astrocytoma model (42). The effects of both decitabine and azacytidine were durable even after the treatment was stopped.

These demethylating agents may be useful not only in the recurrent setting but also as a maintenance therapy following surgical resection of IDH-mutant gliomas. Due to the infiltrative growth pattern of gliomas it is almost impossible to remove all of the tumor cells by resection, therefore tumor recurrence is virtually inevitable over time despite gross total resection and adjuvant chemoradiotherapy, and often these tumors recur as higher grade gliomas. Long-term low dose treatment with a DNA demethylating agent following initial treatment may induce cell differentiation in the remaining IDH-mutant glioma cells, thereby delaying or even preventing recurrence. Targeting the pathologic DNA hypermethylation in IDH-mutant cells with DNA demethylating agents may represent a potential therapeutic strategy that warrant further clinical evaluation in patients with IDH-mutant gliomas.

Vulnerability of IDH-mutant cells to NAD⁺ depletion

IDH mutations have also been shown to affect canonical metabolic pathways including NAD⁺ production, glutamine catabolism and the TCA cycle (3,43,44,49-51). Tateishi *et al.* reported intriguing data showing that depletion of 2HG via direct inhibition of mutant IDH1 was insufficient to halt the formation or growth of progressive IDH1-mutant gliomas *in vitro* and *in vivo* in mice (44). This

finding suggests that other genetic and/or epigenetic factors may modulate the effect of direct mutant IDH1 inhibition. The authors then discovered that IDH-mutant cells were more sensitive, compared to IDH wild-type cells, to depletion of the coenzyme NAD⁺, an essential component of intracellular signaling pathways implicated in cancer cell growth (52). Depletion of NAD⁺ using small molecule inhibitors of NAMPT, a salvage NAD⁺ synthesis enzyme, resulted in selective cytotoxicity in IDH-mutant glioma cells, and in mice harboring intracerebral IDH-mutant glioma xenografts, treatment with NAMPT inhibitors resulted in a marked survival extension (44). They also found that IDH1 mutation led to reduced expression of Naprt1, a rate-limiting enzyme in the salvage NAD⁺ pathway, via 2HG-mediated hypermethylation of the NAPRT1 promoter CpG island. This explains the lower basal levels of NAD⁺ found in IDH-mutant cells which may render them less able to compensate for further depletion of NAD⁺ induced by NAMPT inhibition. A metabolic study of IDH-mutant glioma cell lines treated with NAMPT inhibitors showed that NAD⁺ depletion disrupts TCA cycle metabolism and induces autophagy by activating AMP-activated protein kinase (AMPK), a key regulator of the metabolic checkpoint which responds to nutrient- and energy-poor conditions (44). Collectively, these data suggest that NAMPT inhibition represents a promising synthetic lethal therapeutic approach that effectively inhibits both NAD⁺ salvage pathways (NAMPT and Naprt1), leading to a metabolic crisis that activates AMPK and initiates autophagy in IDH-mutant gliomas.

IDH mutation-associated genome instability

Mutant IDH uses NADPH as a cofactor in the conversion of α -KG to 2HG, resulting in alterations in the NADPH/NADP⁺ ratio. A significantly reduced NADPH production has been reported in IDH-mutant glioma samples (4). NADPH is an essential reducing factor required for cellular defense mechanism against reactive oxygen species through regeneration of reduced glutathione (GSH) from oxidized glutathione (GSSG) by glutathione reductase. In IDH-mutant cell lines, IDH mutation resulted in increased DNA double-strand breaks after ionizing radiation via reduction of IDH-mediated NADPH production (53). This observation suggests that IDH mutations lead to DNA damage and genome instability, which in turn render these IDH-mutant cells more sensitive to radiation. Mutant IDH inhibitor restored IDH-mediated NADPH production

with a subsequent radioresistance of IDH-mutant cells, suggesting that treatment with mutant IDH inhibitor during radiation therapy may abolish the beneficial effects on survival of IDH-mutant glioma patients.

Alkylating agents, such as temozolomide, procarbazine and lomustine, are commonly used chemotherapeutics in glioma patients. Chemotherapy-induced DNA lesions are repaired by DNA repair enzymes such as O⁶-methylguanine-DNA methyltransferase (MGMT) and alkB homolog (ALKBH) proteins (54-56). The G-CIMP status correlates well with MGMT promoter methylation in gliomas (30), suggesting a link between IDH mutation and MGMT methylation. ALKBHs are α -KG dependent, therefore, 2HG accumulation in IDH-mutant cells inhibit the function of these DNA repair proteins, leading to increased DNA double-strand breaks after alkylating treatment (56). As a result, IDH mutations sensitize IDH-mutant tumor cells to alkylating agents compared to IDH wildtype.

Recently IDH1/2 mutations have been shown to cause a homologous recombination (HR) defect via 2HG-induced inhibition of α -KG-dependent dioxygenases, particularly KDM4A and KDM4B (57). This “BRCAness” phenotype was demonstrated in a range of clinically relevant models, including patient-derived IDH-mutant glioma cell lines, and these cells showed a selective sensitivity to poly(adenosine 5'-diphosphate-ribose) polymerase (PARP) inhibition via synthetic lethal interactions. Treatment with a mutant IDH inhibitor reversed this HR defect and eliminated the associated PARP inhibitor sensitivity. Olaparib and veliparib, PARP inhibitors, have been shown to penetrate the blood-brain barrier (58-60). PARP inhibition using these drugs as a potential treatment for IDH-mutant gliomas warrant further investigation.

Collectively, these studies provide evidence that IDH mutations are associated with increased genome instability, thereby greater vulnerability to therapies causing further DNA damage or interfering with DNA repair. This may explain, at least partly, the more favorable therapeutic responses and better prognosis observed in IDH-mutant gliomas (37,61).

Dependence of IDH-mutant cells on oxidative mitochondrial metabolism

IDH1 mutations have been shown to compromise cellular metabolism under hypoxic conditions in IDH1-mutant cell lines, resulting in increased dependence on

oxidative mitochondrial metabolism and inability to induce reductive glutamine metabolism (43). This suggests that IDH-mutant cells may be more sensitive to inhibitors that perturb mitochondrial metabolism. Interestingly, inhibition of mutant IDH1 was unable to reverse the metabolic phenotype, a defect in reductive carboxylation activity, suggesting that the metabolic alterations may be independent of 2HG production (43).

Further evidence that IDH-mutant cells are vulnerable to mitochondrial metabolism inhibition was provided by a preclinical metabolomics study of IDH1^{R132H}-mutant cells (62). Metabolic reprogramming of IDH1-mutant cells resulted in an inability of these cells to generate energy from various carbon substrate fuels. Treatment with metformin, a mitochondrial biguanide poison, decreased cell growth and survival of IDH1-mutant cells by altering the oxidative metabolism of glutamine and glucose via the TCA cycle. In addition, this study also showed that the metabolic vulnerability of IDH1-mutant cells to metformin appeared independent of 2HG production.

The importance of mitochondria on the growth of IDH-mutant tumors was also suggested by another study in which IDH1 mutant glioma samples and IDH1^{R132H}-expressing cancer cells were found to be hypersuccinylated, preferentially in the mitochondria (63). 2HG was shown to inhibit the mitochondrial TCA cycle enzyme succinate dehydrogenase (SDH) resulting in mitochondrial respiration impairment via succinyl-CoA accumulation and hypersuccinylation. This mitochondrial dysfunction induced anti-apoptotic protein BCL-2 accumulation at the mitochondria membrane, leading to apoptosis resistance in these cells. Overexpression of a desuccinylase SIRT5 was shown to reverse these processes and inhibit growth of hypersuccinylated tumors.

There is currently one phase I trial exploring the combination of metformin and chloroquine in IDH-mutant tumors including gliomas (*Table 1*). Further investigations exploring the combination of mutant IDH inhibition and oxidative mitochondrial metabolism inhibition as a potential therapeutic strategy for IDH-mutant tumors are warranted.

α -KG homeostasis via glutaminase inhibition

2HG has been shown to primarily drive from glutamine, where glutamine is hydrolyzed by glutaminase to produce glutamate which is converted to α -KG (5). α -KG is then converted to 2HG by mutant IDH. A small molecule glutaminase inhibitor, BPTES, has been shown to reduce

glutaminase activity with subsequent reduction in glutamate and α -KG levels in both IDH-mutant and IDH-wildtype GBM cells, but interestingly, only the growth of IDH mutant cells was slowed (64). The growth reduction seen in this study was modest (about 20% reduction) although the reduction of glutaminase activity was significant, indicating that cellular metabolism is able to compensate for changes made by glutaminase inhibition. Glutaminase inhibition alone, therefore, is unlikely to be an effective therapy, however, it may exert synergistic effects on tumor growth when combined with other therapeutic strategies. Another potent glutaminase inhibitor, CB-839, is currently undergoing phase I evaluation in various solid tumors including IDH mutant tumors (Table 1).

IDH mutation-associated hypoxia-inducible factor-1 α (HIF-1 α) pathway

α -KG is essential for prolyl hydroxylases (PHD) which promotes HIF-1 α degradation therefore a reduction in α -KG, as in IDH mutant cells, abrogate degradation of HIF-1 α . In glioma cell lines, IDH1 knockdown induced the expression of HIF-1 α target genes, including glucose transporter 1 (Glut1), vascular endothelial growth factor (VEGF), and phosphoglycerate kinase 1 (PGK1), that are implicated in glucose metabolism, angiogenesis, and other signaling pathways that are critical to tumor growth (65). Consistent with this, IDH1 mutations correlated with elevated HIF-1 α and VEGF levels in human glioma samples. Similarly, another preclinical study also found increased HIF-1 α and cell proliferation in IDH-mutant glioma cell lines (66). In addition, this study showed that IDH mutation activated nuclear factor- κ B (NF- κ B) in a HIF-1 α dependent manner, which induced high expression levels of oncogenes, cyclins D1 and E and c-Myc in these cells. These results suggest that IDH mutations contribute to tumor angiogenesis and proliferation by promoting the HIF-1 α pathway. In cultured cells expressing IDH1 mutation, it has been shown that an α -KG derivative reversed the induction of HIF-1 α levels (65). Drugs targeting HIF-1 α or drugs mimicking α -KG may thus warrant further investigation as a potential therapy for IDH-mutant gliomas.

Mammalian target of rapamycin (mTOR) signaling pathway

KDM4 is a subfamily of the Jumanji family of histone

demethylases and is inhibited by 2HG via α -KG inhibition (25). 2HG-induced KDM4 inhibition was recently shown to promote oncogenic activation of mTOR independently of the PI3K/AKT/TSC1–2 pathway by reducing the levels of DEPTOR (67), a negative regulator of both mTORC1 and mTORC2 which are associated with cell growth and autophagy (mTORC1), and cell survival and cytoskeleton organization (mTORC2) (68). This mTOR activation via the mutant IDH/2HG/KDM4/DEPTOR pathway may provide an additional molecular mechanism to explain the oncogenic activity of IDH1/2 mutant gliomas and represents a potential therapeutic strategy.

Immunotherapy

Given the evidence that IDH mutation is an early event in tumorigenesis and is present homogeneously in all glioma tumor cells at specific codons, these mutations are ideal immunotherapy targets, especially as a maintenance therapy for preventing recurrence of these diffuse infiltrating tumors. Schumacher *et al.* demonstrated that IDH1^{R132H} contained an immunogenic epitope suitable for mutation-specific vaccination (69). Immunization with mutated IDH1 peptides resulted in a significant prolongation of survival in an intracranial glioma mouse model (70). Immunotherapy using peptide and dendritic cell vaccinations are currently being investigated in patients with IDH-mutant gliomas (Table 1).

Natural killer (NK) cells are critical to the innate immune system and respond to tumor formation. Lysis of tumor cells by NK cells is mediated by interaction between NK group 2D (NKG2D) receptor on NK cells and stress-inducible ligands on target cells, such as NKG2D ligand (NKG2D-L) (71). Correlation between NKG2D-L expression and improved clinical outcomes have been shown in human cancers (72). Zhang *et al.* discovered that IDH-mutant glioma cells were resistant to NK-mediated lysis due to transcriptional silencing of NKG2D-L by 2HG-induced genomic hypermethylation (71). Decitabine upregulated the expression of NKG2D-L and sensitized the cells to NK cell-mediated cytotoxicity.

In the IDH1-vaccine study by Schumacher *et al.*, only 4 of 25 patients with IDH-mutant gliomas exhibited evidence of spontaneous T-cell response to the IDH1 epitope (69). This may be explained by the impaired innate immune activity shown by Zhang *et al.*, as innate immune activity is required for induction and maintenance of T-cell mediated immunity. Demethylating agents may therefore facilitate

potent antitumor T-cell mediated immune responses of vaccine therapy for IDH-mutant gliomas. Further investigations in clinical trials are warranted for this combined approach.

Conclusions

An IDH mutation is found in the vast majority of low grade gliomas and secondary GBM and it appears to be an early event. Significant efforts have been made to better understand the role IDH mutations play in tumor initiation, maintenance and progression. A wide range of epigenetic and metabolic alterations caused by mutant IDH-mediated 2HG accumulation via inhibition of α -KG-dependent dioxygenases have been implicated, and many different potential therapeutic avenues are being explored. Specific aberrant changes, that drive tumor formation and growth, may differ in different tumor types, further complicating identification of therapeutic targets in these tumors. Drug combinations that exert synergistic effects on more than one target simultaneously may be more effective. Finally, although a matter of controversy IDH mutations represent a “hit and run” genetic event in gliomas potentially explaining the general lack of efficacy of first-generation IDH1 inhibitors and suggesting that other synthetic lethal or immune-targeting strategies may be more effective.

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