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Emerging Roles of Wild-type and Mutant IDH1 in Growth, Metabolism and Therapeutics of Glioma

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Abstract: Glioblastoma is one of the most devastating human malignancies and is categorized into primary and secondary glioblastoma subtypes that develop through different genetic pathways. Isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) are key enzymes linking cellular metabolism to epigenetic regulation and redox states. Hot spot mutations of *IDH1* is early and frequent genetic alterations in secondary glioblastoma as well as in grade II and III glioma and represent a major biomarker with diagnostic, prognostic, and predictive implications. Mutant IDH proteins acquire neomorphic enzymatic activity to produce D-2-hydroxyglutarate, a putative oncometabolite that could induce epigenetic changes

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at DNA and RNA levels. On the other hand, recent studies show that primary glioblastoma increases expression of wild-type IDH1, which confers therapeutic resistance. In this chapter, we introduce the current understanding of the biological roles of wild-type and mutant IDH enzymes in glioblastoma. We discuss the challenges hampering the development of IDH targeted therapeutics and the current status of IDH1 mutant inhibitor development.

Keywords: glioblastoma; glioma; IDH1; metabolism; therapeutics

INTRODUCTION

Gliomas are the most common primary malignant brain tumor in adults. Many WHO grade I gliomas are well-circumscribed, surgically curable tumors and have different molecular drivers than those seen in grade II, III, and IV gliomas. Even though grade II glioma is categorized as a low-grade glioma, it is incurable due to its diffusely infiltrative nature and that it almost inevitably progress to high-grade III glioma and grade IV secondary glioblastoma over time (1). Remarkably, over 70% of grade II gliomas and secondary glioblastoma possess heterozygous missense mutations in the gene encoding cytosolic enzyme *IDH1* (2–4), which confer a neomorphic enzyme activity that converts α-ketoglutarate (αKG) to D-2-hydroxyglutarate (D-2HG) (Figure 1) (5). Mutations in mitochondrial isozyme IDH2 have also been identified in gliomas, but they are much less common and mutually exclusive with mutations in *IDH1* (3, 6, 7). Though naturally existing D-2HG is at negligible levels, the intracellular concentrations of D-2HG reach 10–30 mM in the glioma with the *IDH1* mutation (5). D-2HG appears to be a major intracellular effector of *IDH1* mutated glioma and is considered as an oncometabolite, altering epigenetics and setting the cellular state permissive to malignant transformation (8–10).

There are three distinct groups of gliomas with different molecular drivers, mutations, epigenetic signatures, and clinical behavior: (i) *IDH* wild-type gliomas (primary glioblastoma); (ii) *IDH* mutant with a 1p/19q deletion; and (iii) *IDH* mutant with a p53 mutation (11). This distinction between the three groups of

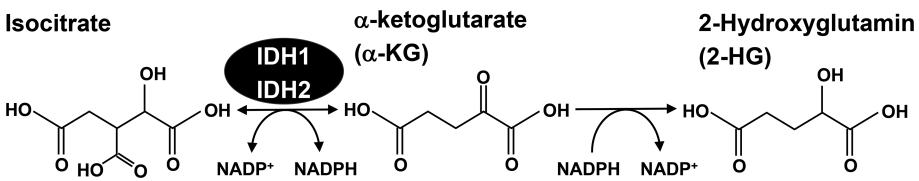


Figure 1. Wild-type IDH converts isocitrate and α-ketoglutarate to each other, and mutant IDH converts α-ketoglutarate to 2-hydroxyglutarate. Isocitrate dehydrogenase (IDH) interconverts isocitrate and α-ketoglutarate. Three subtypes of human IDH are known: IDH1 (cytosolic, $NADP^+$ dependent) and IDH2 (mitochondrial, $NADP^+$ dependent) and IDH3 (mitochondrial, NAD^+ dependent). IDH1 and IDH2 mutations have been reported in a variety of cancers such as, glioma, acute myeloid leukemia and bile duct cancer. Mutated IDH converts α-ketoglutarate to 2-hydroxyglutarate, which worsens the prognosis of gliomas. IHD1 and IDH2 mutations produce D-2-hydroxyglutarate, which has been considered "oncometabolite."

gliomas is currently driving clinical management as well as placing greater emphasis on the molecular and genetic differences of *IDH* mutant and *IDH* wild-type gliomas. This chapter discusses the role of wild-type and mutant IDH1 enzymes in the progression of glioma, and emerging therapy targeting the glioma with wild-type or mutant IDH1.

METABOLIC CHARACTERIZATION OF WILD-TYPE IDH1 GLIOMA

There are three isocitrate dehydrogenase isozymes—IDH1, IDH2, and IDH3—that are expressed in mammalian cells. IDH1 is a cytosolic enzyme, while IDH2 and IDH3 are mitochondrial enzymes. Both IDH1 and IDH2 use $NADP^+$ as an electron acceptor to convert isocitrate to αKG, co-producing an NADPH per reaction. IDH3 uses NAD^+ as an electron acceptor. Notably, the ratios of NADPH/$NADP^+$ determine the intracellular redox potential, affecting the thermodynamic driving force of many reactions, in particular providing electrons for lipids and deoxyribonucleotide and reducing oxidized precursors to maintain a reduced intracellular condition and ameliorate oxidative damage (Figure 2). The IDH1 and IDH2-dependent reaction is reversible, while IDH3-dependent reaction is

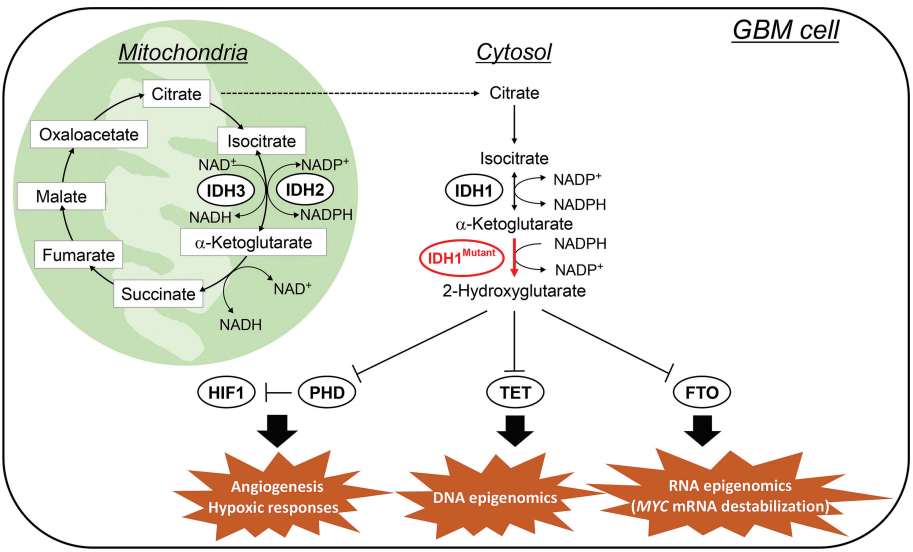


Figure 2. D-2HG generated by *IDH1* mutation interferes various pathways resulting in glioblastoma exacerbation. Citrate, the mitochondrial metabolite, flows out to cytosol. Isocitrate, synthesized by cytosolic citrate, is used as a substrate for the IDH1-mediated catabolism. The mutant IDH1 produces D-2-hydroxyglutarate (D-2HG) as an oncometabolite. In glioblastoma, accumulated D-2HG causes (i) angiogenesis and hypoxic responses through depression of HIF1 by PHD inhibition; (ii) reprograming of DNA epigenomics through TET inhibition; and (iii) reprograming of RNA epigenomics including destabilization of *MYC* mRNA through FTO inhibition. D-2HG, 2-hydroxyglutarate; FTO, fat mass and obesity-associated protein; HIF1, hypoxia inducible factor 1; IDH1, isocitrate dehydrogenase 1; PHD, prolyl hydroxylase domain -containing protein (PHD); TET, ten-eleven translocation enzyme.

irreversible (12–14). The reversible nature of IDH1 and IDH2 reaction plays an important role in reductive carboxylation, which enables cells lipogenesis under the conditions that decrease the TCA cycle coupled-oxidative phosphorylation (for example, hypoxia, VHL mutation) (15, 16) (Figures 1–3).

Wild-type IDH1 is overexpressed in many primary glioblastoma

Although wild-type *IDH1* has had much less attention compared to the research on glioma with *IDH1* mutation, several studies have revealed that wild-type IDH1 is overexpressed in several types of cancers, including non-small cell lung carcinoma (NSCLC) (17, 18), pancreatic adenocarcinoma (PDAC) (19), and primary glioblastoma (20, 21). Importantly, these studies show that IDH1 is overexpressed in over 60% of primary glioblastoma patients and is correlated with poor overall survival. Wahl et al. and Calvert et al. independently demonstrated that knocking down endogenous *IDH1* by shRNA, or pharmacological inactivation of IDH1 by the IDH1 inhibitor GSK864, decreases glioblastoma growth *in vitro* and extends survival of mice harboring intracranial glioblastoma, while overexpression of wild-type IDH1 shortened the survival of the glioblastoma mouse (20, 21).

Targeting the wild-type IDH to increase the therapeutic efficacy of radiation and chemotherapies

With regard to the primary glioblastoma, even with aggressive multimodal radiation and chemotherapy after surgery, only marginal improvements on survival are made (average of 2 months), with a median survival of just 14.6 months (22, 23). The use of tumor treating fields (TTFs) with the standard of care therapy in glioblastoma (IR plus TMZ) in a randomized open-label trial of 695 glioblastoma patients, reporting that median progression-free survival was 6.7 months in the TTF plus standard of care group versus 4 months in the standard of care group alone (24). Recent studies suggest that the IDH1 enzyme is a potential clinical target for glioblastoma therapy (25). The rationale is that IDH1 activity is considered to increase cytoplasmic NADPH/$NADP^+$ ratios, which promotes lipid biosynthesis and increases cellular defense against oxidative stress. Suppression of IDH1 activity could alter cellular metabolism, potentially lowering the ratio of NADPH/$NADP^+$, which sensitizes cells to oxidative stresses (Figure 3). Given that radiotherapy induces cell death through induction of reactive oxygen species (ROS) and DNA lesions (26, 27), targeting the IDH1 enzyme in glioma with wild-type *IDH1* allele (for example, primary glioblastoma) has exciting therapeutic potential.

Wahl *et al.* further demonstrated that knocking down wild-type *IDH1* in primary glioblastoma cell lines (wild-type IDH1) decreases the ratio of NADPH/$NADP^+$, as well as levels of deoxynucleotides and reduced glutathione (GSH) and increases the efficacy of radiation. This radiosensitization effect of IDH1 knockdown is reversed by treatment of anti-oxidant N-acetyl cysteine and/or nucleotide precursors, pointing that IDH1-dependent NADPH production is critical for glioblastoma radioresistance (20, 28). Likewise, suppression of mitochondrial IDH2 also increases radiosensitivity of primary glioblastoma cells (29). Furthermore, IDH1 inhibition decreases GSH and NADPH levels in the glioblastoma initiating

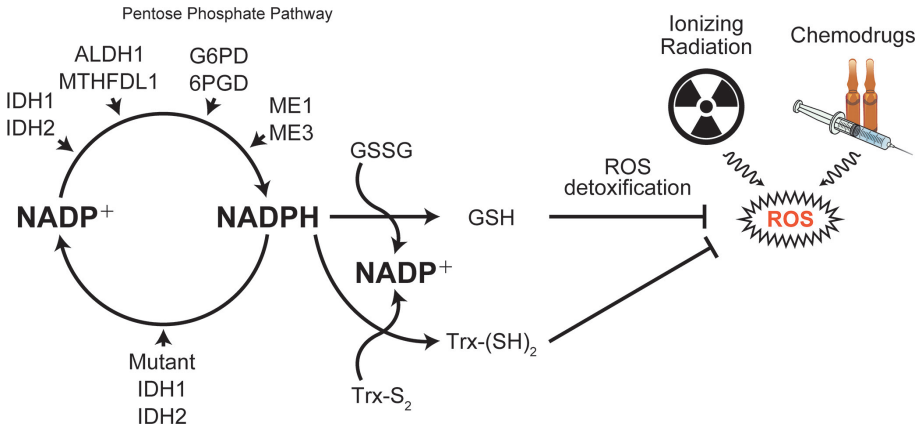


Figure 3. Effects of wild-type and mutant IDH enzymes on redox status and therapeutic efficacy. IDHs mutation may increase therapeutic efficacy of radiation and chemotherapies. IDH1, 2, and the other enzymes reduce $NADP^+$ to NADPH. NADPH also reduces GSSG or Trx-S_2 to GSH or Trx-$(SH)_2$, respectively. GSH and Trx-$(SH)_2$ detoxify ROS and decrease the effect of the radiation or chemotherapy treatment for glioblastoma patients. The decrease of NADPH/$NADP^+$ ratio by the inhibition of IDH enzymes has potential to increase the efficacy of the current treatment for glioblastoma. 6PGD, 6-Phosphogluconate dehydrogenase; ALDH1, aldehyde dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; GSSG, glutathione disulfide; IDH, isocitrate dehydrogenase; ME, malic enzyme; $NADP^+$ /NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; Trx, thioredoxin.

cells/glioblastoma stem cells carrying EGFR-amplification, making them more susceptible to EGFR inhibitor treatment (30). Serum-free culturing techniques have revealed a sub-population of "glioblastoma initiating cells" that may have increased radiation resistance and lead to recurrence after radiation treatment (31–34). The possibility of better targeting these cells via IDH1 inhibition may lead to better radiation response and delayed recurrence (22–24).

BIOLOGICAL IMPACT OF D-2HG ON GLIOBLASTOMA

A point mutation in the *IDH1* gene was initially identified through exome sequencing of colon tumor and glioblastoma samples (2, 35). Mutations in *IDH1* mostly occur at Arg-132 residue (R132) located within the catalytic domain, which is the binding site for isocitrate. R132H is the most common alteration, comprising >80% of all *IDH1* mutations in gliomas. Surprisingly, a study undertaking metabolomics analysis shows that the mutation of IDH enzymes bestows a new enzymatic function of reducing alpha-ketoglutarate (αKG) to D-2-hydroxyglutarate (D-2HG, or R-2HG) using NADPH as an electron donor (5). In the presence of the *IDH1* mutation, the D-2HG molecule, which is normally found at minute levels, can increase to millimolar amounts (5). D-2HG generated by *IDH1* mutation interferes various pathways resulting in glioblastoma exacerbation (Figure 2). Understandably, there has been considerable interest in what role this potential new "oncometabolite" might have on cells. Following the discovery of the *IDH1* mutation, many investigators sought to determine what new malignant traits this

mutation would bestow upon a cell. Unexpectedly, it appeared that in general, the addition of the *IDH1* mutation led to slower growth in most brain tumor models (36). This was a perplexing result and was hypothesized to be the reason why *IDH1* mutant tumors had a better prognosis compared to glioblastomas without the *IDH1* mutation. Extensive studies have shown the complexity of the IDH1 mutational effects and important issues affecting the interpretation of past research. The impact of D-2HG on transcriptional landscape, with particular emphasis on the recent discovery of the new effect of D-2HG on RNA epigenomics, is discussed below.

D-2HG induces epigenetic alternations by increased DNA and histone methylation

Even though mechanistic understanding of *IDH* mutations and D-2HG effects on gliomagenesis remain to be clarified, compelling evidence from Turcan *et al*. shows that the glioma-associated *IDH* mutation promotes hypermethylation of histone and DNA through its accumulated product D-2HG (37) (Figure 2). Mechanistically, because of its structural similarity to αKG, it has been considered that supra-physiologically elevated D-2HG levels inhibit enzymes, such as DNA demethylase ten-eleven translocation enzymes (TETs) and histone lysine demethylases (KDMs) that utilize αKG as a co-substrate (Figure 2). Consequently, *IDH1* mutation provokes epigenetic reprogramming of the transcriptional landscape of glioma (8, 38–41).

D-2HG inhibits RNA demethylase, FTO, leading to aberrant RNA methylation and growth suppression

While the dynamic covalent modifications (for example, methylation) to DNA and histones play critical roles in regulating gene transcriptions, an emerging research area is epigenetic regulation of RNA. Over 160 different chemical modifications in RNA have been identified (42). Among them, N^6-methyladenosine (m^6A) has been considered the most prevalent modification of RNA Pol II transcripts (43–45). In general, m^6A modification is enriched near the 5' untranslated terminal region (UTR) as well as the stop codon and 3′ UTR, which regulates mRNA transcription (46), splicing (47), export (48), stability (49), and translation (50, 51). Like methylation on DNA and histones, m^6A is a reversible modification, and fat mass and obesity-associated protein (FTO) is the first RNA demethylase identified for the removal of the methyl group of m^6A in mRNA with some extent to m^1A in specific tRNAs using αKG as a co-substrate (52, 53).

Accumulating evidence shows that m^6A mRNA modification is critical for glioblastoma stem cells self-renewal and tumorigenesis. Though there is some apparent discrepancy in terms of the role of methyltransferase responsible for m^6A in glioblastoma (48–50), the consensus is that inhibition of FTO significantly suppresses glioblastoma stem cells on culture and intracranial growth of glioblastoma stem cells in a mouse xenograft model (54–56). Importantly, our recent study has uncovered that D-2HG is a potent inhibitor for the FTO activity *in vitro* and *in vivo*, leading to the aberrant accumulation of m^6A mRNA in leukemia and glioma cells expressing *IDH1* mutant (57). In leukemic cells, FTO inhibition by D-2HG

decreases stability and thus, expression levels of *MYC*, one of the master regulators of hyper-anabolism and cell proliferation (57) (Figure 2), though whether this mechanism can be extended to glioma remains to be clarified. The results of our study revealed a surprising functional link between FTO and *IDH* mutations that could potentially explain why *IDH* mutated tumors bear proliferative disadvantage.

POTENTIAL IMPACTS OF *IDH1* MUTATION ON CELLULAR METABOLISM

The fact that *IDH1* mutant tumors carry a better prognosis than those without *IDH1* mutations has led to the hypothesis that this new enzyme may have deleterious effects on cellular metabolism. In accordance with this hypothesis, our study has shown that intracellular metabolism in *IDH*-mutated glioblastoma is significantly different from that in *IDH* wild-type glioblastoma, in particular, prominent in nucleotide metabolism pathway (58). To investigate this hypothesis, many investigators have overexpressed the *IDH1* mutant gene and then performed mass spectroscopy to examine the differences between parental and transformed lines. However, the results so far are mixed and need further studies to interpret the results. Here, we briefly summarize the experimental results, which appear controversial in some cases, and discuss the technical challenge of faithfully recapitulating the *IDH1* mutated glioma cellular status.

All studies to date have found that the addition of the *IDH1* mutant enzyme comes with a metabolic burden that makes the cell less fit and resilient. The first studies focused on the fact that the *IDH1* mutant enzyme was converting large amounts of αKG into D-2HG, making the assumption that αKG was derived largely from glutamine. Taking these facts together, it was hypothesized that the *IDH1* mutation led to cells becoming glutamine deficient. Seltzer et al. confirmed this by showing that the addition of the *IDH1* mutant enzyme made cells more vulnerable to glutaminase inhibition (59). Another set of studies focused on the fact that the *IDH1* mutant enzyme consumes one molecule of NADPH and produces a molecule of $NADP^+$ and, therefore, might have an effect on the level of ROS. Results on this topic have been mixed and sometimes appear controversial. Attempts to knock-in the *IDH1* mutation under a Nestin neural stem cell driver were embryonic lethal, but the salvaged cells had lower levels of ROS (60). In contrast, overexpressing the *IDH1* mutant enzyme in U87MG cells increased ROS levels and made the cells more vulnerable to radiation (61).

More recently, it was discovered that *IDH1* has an important role in reductive carboxylation, which is the ability of the cell to convert glutamine to citrate without going through the TCA cycle. This allows the cell to participate in lipogenesis and membrane synthesis in a hypoxic environment. The presumption would be that with a mutation in the *IDH1* enzyme, the native function of the enzyme would be diminished. Again, results have been mixed, with Grassian et al. reporting that overexpression of the *IDH1* mutant gene inhibited the ability of cells to perform reductive carboxylation under hypoxia (62). On the other hand, Reitman et al. using the same cell line found that the *IDH1* mutation actually facilitated the

ability of the cell to convert glutamine into fatty acids (palmitate) under hypoxic conditions (63).

A potential caveat and technical challenge to investigate the roles of IDH1 mutation on cellular metabolism

All of these studies suffer from two methodological problems. The first is that the metabolic effect of the *IDH1* mutant enzyme depends on the baseline metabolic background of the host cell. There is increasing evidence that the *IDH1* mutation is likely one of the first mutations to occur in gliomagenesis (64) and thus over many rounds of cell growth and selection, the cells have time to adjust and adapt to whatever metabolic effects the *IDH1* mutation may have. If the *IDH1* mutation were as detrimental to cellular function as studies suggest, the *IDH1* mutation would be deleted along with the other genes that impede accelerated cellular growth, for example, *PTEN, p53,* and *NF1*. The second is that these studies fail to answer the metabolic effect of blocking the *IDH1* mutant enzyme in a glioma cell that already has it.

In order to address these shortcomings, our group performed genetic and metabolic profiling on a panel of patient-derived *IDH1* wild-type and *IDH1* mutant glioblastoma cultures and compared these results with *IDH1* mutant overexpression models to determine the accuracy and differences of these models (58). We found that *IDH1* wild-type glioblastoma cells had a high genetic expression of *de novo* nucleotide synthesis genes and disproportionately shunted glucose through the pentose phosphate pathway for *de novo* nucleotide synthesis. In contrast, *IDH1* mutant glioblastoma cells were enriched for DNA repair response genes. Consistent with these predictions, *IDH1* wild-type glioblastomas were more vulnerable to *de novo* nucleotide synthesis inhibitors, and *IDH1* mutant glioblastomas were better able to repair DNA after radiation (58), which is also supported by our other studies (65–67). Initially, we assumed that the observed changes in transcriptome and metabolism were due to the direct metabolic effect of *IDH* mutation. However, surprisingly and importantly, there was no difference seen in nucleotide synthesis when the *IDH1* mutant enzyme was overexpressed on an *IDH1* wild-type background or when D-2HG production was blocked by an *IDH1* mutant inhibitor (58).

Similar to previous studies, overexpression of the *IDH1* mutant enzyme depleted TCA cycle intermediates and led to much slower growth (58). In contrast, inhibiting D-2HG formation in endogenous *IDH1* mutant cells had no effect on either growth or the level of TCA cycle intermediates. Furthermore, the baseline levels of TCA cycle intermediates were roughly equal between the *IDH1* mutant and *IDH1* wild-type cultures (58). Taken together, these results suggest that the *IDH1* mutation has different effects on different cellular backgrounds and is largely well tolerated in endogenous *IDH1* mutant glioma cells. Although *IDH1* mutant and *IDH1* wild-type gliomas have different metabolic vulnerabilities, these may be largely due to their differences in growth speed and genetic contexts after the long-term gliomagenesis. Further dedicated studies are needed to clarify whether the *IDH1* mutation by itself is sufficient to induce the metabolic complexity and heterogeneity of *IDH* mutated glioma.

THREE POSSIBLE MODELS EXPLAIN THE APPARENTLY PARADOXICAL OUTCOMES OF *IDH1* MUTATION

Why would a mutation that slows growth be selected over neighboring cells without that mutation and a presumably faster rate of growth? We raise three working models.

Model 1. Increased stress resilience by IDH1 mutation

One possibility is that the *IDH1* mutation enables cells to resist death or anti-growth signals in their microenvironment. This theory is supported by the discovery that the D-2HG molecule could inhibit the function of alpha-ketoglutarate-dependent enzymes by outcompeting alpha-ketoglutarate (23). his led to the hypothesis that the *IDH1* mutation might give a cell the ability to resist environmental influences and prevent differentiation from a progenitor cell to a more differentiated and less prolific cell type. In several cellular contexts, notably fat cells (8), chondrocytes (68) and liver cells (61), overexpressing the *IDH1* mutation in precursor/stem cells prevented those cells from differentiating. In each of these previous studies, there was a key mediator gene that was essential for differentiation. During differentiation, this gene was activated by the demethylation of a key histone mark in the promoter or enhancer region. In the presence of the *IDH1* mutation or high levels of 2-HG, this histone demethylation was prevented, and the cell failed to differentiate and instead maintained its proliferative potential. However, trying to show that the *IDH1* mutation has the ability to block differentiation in neural cells has been more elusive. Overexpressing the *IDH1* mutation in a mouse sub-ventricular zone (SVZ) stem cell culture changed the default differentiation from a GFAP-positive astrocyte to a TUJ1 positive neuron; however, it did not prevent differentiation or lead to increased growth (8).

Model 2. Chronic malignant evolution via epigenomic repression

A second possibility is that the effect of the *IDH1* mutation is slow but over time can gradually convert the epigenetic state of a cell to a more malignant phenotype. The most popular model of this theory is that the *IDH1* mutant enzyme impairs the ability of the TET enzymes to demethylate DNA. In this case, the activity of the DNA methyltransferase (DNMT) enzyme family is unopposed and leads to a gradual increase of methylation throughout the genome. The methylation of CpG islands, particularly in key regulatory regions, tends to decrease the expression of those genes. Over time more and more tumor suppressor genes would become repressed until the cell becomes tumorigenic. The strongest evidence for this theory comes from a study using overexpression of the *IDH1* mutant enzyme in an astrocyte line. This led to the gradual induction of Nestin expression and a small increase in growth over many passages. This long-term *IDH1* mutant expression was also associated with increased DNA and histone methylation (37).

Model 3. IDH1 mutation acts as a mutator

A third possibility is that the *IDH1* mutation predisposes to further mutations. When studying patients with *IDH1* mutant tumors, it was noted that following resection, when the tumor eventually grew back, the tumors had often acquired a new set of mutations. Mutations that were present in the first tumor were not present in the second tumor. The only mutations that were always present were *IDH1* and p53 (64). This result implies that the *IDH1* mutation is likely the initial mutation in gliomagenesis and is sufficient to generate enough mutations for tumorigenesis multiple times throughout a patient's life. However, first, p53 must be rendered non-functional. It is not clear how the *IDH1* mutation leads to further mutations. One possibility is the methylation and down-regulation of DNA repair machinery, in particular the gene encoding O-6-Methylguanine-DNA Methyltransferase (*MGMT),* a DNA repair enzyme removing the guanine-alkyl group induced by alkylating agents such as temozolomide. *IDH1* mutation and *MGMT* methylation are correlated, although there are *IDH1* mutant tumors with unmethylated MGMT (69, 70). As additional evidence, while MGMT methylation is an independent predictor of a positive response to temozolomide in *IDH1* wild-type glioma cells, it is not a predictor of chemotherapy response in *IDH1* mutant cells, implying that either MGMT itself or the MGMT pathway may be non-functional in *IDH1* mutant cells (71). Another possibility is that the *IDH1* mutation may lead to higher levels of endogenous ROS, predisposing to DNA damage. While presumably the *IDH1* mutant enzyme would lead to an alteration in the NADPH/$NADP^+$ equilibrium, it is not obvious *a priori* what effect this would have on total endogenous ROS levels. Consequently, the question of whether the addition of the *IDH1* mutation to cells causes an increase or a decrease in ROS levels is still a matter of debate, with different studies showing conflicting results (60, 61).

THERAPEUTIC SENSITIVITY AND RESISTANCE OF *IDH* MUTANT GLIOMAS

Standard therapy for a newly diagnosed glioblastoma involves maximal safe surgical resection, temozolomide, and fractionated radiation. This protocol has been validated by randomized controlled trials (22, 23). However, these trials were based on a mixed cohort of *IDH1* mutant and *IDH1* wild-type patients. Presumably, given demographics and prevalence, the majority of these patients were *IDH1* wild-type. This means that the results of these trials may not necessarily translate to *IDH1* mutant gliomas. *IDH1* mutant gliomas are associated with longer survival, and some have assumed that this is due to a better response to adjuvant therapy (temozolomide and radiation) (72). However, arguing against this assumption is the observation that *IDH1* wild-type gliomas show increasing therapeutic response and increased survival to higher doses of temozolomide, whereas *IDH1* mutant gliomas do not show any improvement with higher doses of temozolomide (73). Due to this concern, additional chemotherapy regimens were tried, and recent trials have shown that CCNU is effective in combination with radiation in *IDH1* mutant low-grade gliomas (74). These clinical trials present

multiple logistical difficulties related to the low incidence of the disease and the relatively long and variable survival.

As many such questions remain unanswered, the most relevant and perhaps most controversial is the question of whether *IDH1* mutant gliomas are more or less sensitive to radiation than their *IDH1* wild-type counterparts. Multiple studies have found conflicting results that seem to depend on the cell line model used and even the culture conditions. Studies using serum culturing conditions found that the *IDH1* mutation is associated with increased radiation sensitivity in both overexpression (61, 75) and endogenous (76) *in vitro* studies. In contrast, overexpression (75) and endogenous models (58) grown in serum-free conditions show radiation resistance. There is a shortage of mouse models of *IDH1* mutant gliomas; however, in one of the few studies to utilize a mouse model, the *IDH1* mutation was associated with radiation resistance via upregulation of DNA damage response genes (77). The general clinical consensus is that radiation is effective against *IDH1* mutant gliomas, and no randomized clinical trial is to test this assertion is forthcoming.

Small molecule *IDH1* mutant inhibitor

Following the discovery of the *IDH1* mutation, there was a great deal of interest in developing mutant IDH targeted therapy, leading to a series of potent small molecule inhibitors against mutant IDH1 (for example, AGI-5198) and IDH2 enzyme (e.g., AGI-6780) (Figure 4) (78). In the case of the leukemia model TF-1, mutant IDH2 specific inhibitor AGI-6780 prevented the changes seen following IDH2 mutant expression and induced differentiation of the *IDH2* mutated leukemic cells (79). Consistent with these findings, early clinical trials with similar inhibitors in acute myeloid leukemia are also promising (80). After taking the *IDH1* mutant inhibitor, patients with *IDH1* mutant acute myeloid leukemia show a progressive decrease in the number of immature tumor-type myeloid cells with a corresponding increase in mature differentiated cells. Most encouragingly, unlike traditional chemotherapy, there is no myelosuppression seen across the other myeloid lineages.

However, in the case of *IDH1* mutant glioma models, the results were more mixed. The first attempt to treat an *IDH1* mutant glioma with the inhibitor was met with some success. Treatment of mutant IDH1 inhibitor AGI-5198, the first prototype inhibitor (78, 81), decreased glioma size and increased expression of GFAP, suggesting differentiation (82). However, later attempts to repeat this data have failed. In one of the more thorough studies, Tateishi et al. treated *IDH1* mutant cells with AGI-5198 for over a year and found no difference in either DNA methylation or histone modification, and there was a slight increase in growth with the addition of AGI-5198 (83). These variable results from the preclinical studies are, in part, likely due to the poor metabolic stability and low blood-brain barrier penetrance of the compound (78).

One difficulty with mutant IDH1 inhibitor to treat brain tumor models is the issue of time. While Turcan et al. was able to demonstrate an increase in methylation with the addition of the *IDH1* mutant enzyme, the effect required the cells to undergo 40 passages and presumably several hundred cell divisions (37). Even then, the majority of the newly methylated sites were only partially methylated.

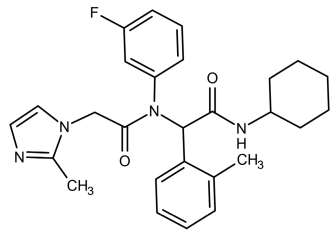
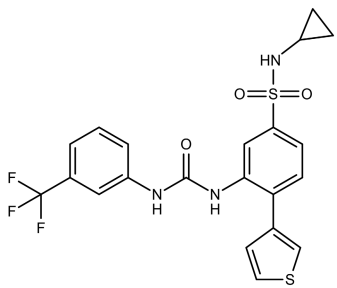
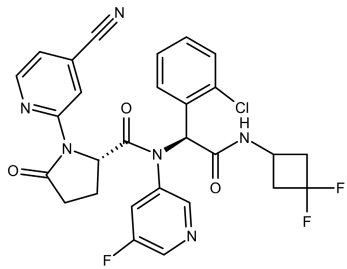
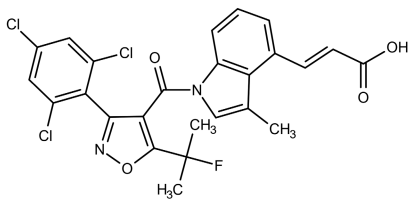
	Target	Generation	Chemical structure
AGI-5198	IDH1-R132H/C	1st	
AGI-6780	IDH2-R140Q	1st	
AG-120	IDH1-R132H/C	2nd	
DS-1001b	IDH1-R132H/C	2nd	

Figure 4. Potential molecules for treatment of IDH mutated cancers. The chemical structure of inhibitors of IDH mutants are shown. AGI-5198, AG-120, and DS-1001b target the IDH1 mutants (R132H and R132C), and AGI-6780 targets an IDH2 mutant (R140Q). AG-120 and DS-1001b are expected as the next-generation therapeutics for curing glioblastoma.

Any study that fails to find an effect of adding the *IDH1* mutant enzyme can be criticized for not giving the cells enough time no matter how much time was given. One possibility for the disagreement between the two studies is that while the *IDH1* mutation is able to induce methylation, once the methylation is induced, it is irreversible. It is also difficult to translate these results into a clinical context where the vast majority of cells in the brain are post-mitotic.

Nevertheless, given the potential for targeted efficacy with limited off-target toxicity, many IDH1 mutant inhibitors have entered clinical trials. AG-120/Ivosidenib is showing a good safety profile and a trend for tumor stability in non-enhancing tumors (84) (Clinical Trials: NCT02073994; NCT03343197). Furthermore, clinical trials with another next-generation compound with greater blood-brain barrier penetration, DS-1001b (85), are currently enrolling for glioma patients with an *IDH1* mutation (NCT03030066; NCT04458272) (Figure 4).

CONCLUSION

In this chapter, we introduced the impact of wild-type and mutant *IDH1* on glioblastoma metabolism, growth, and current therapeutic approach. Since its discovery nearly a decade ago, the *IDH1* mutation has fast become one of the most well-known and complicated metabolic mutations found in cancer. Convincing evidence exists that it is the initial mutation that begins the process of tumorigenesis. Despite the difficulty of modeling its behavior *in vitro*, significant strides have been made to link the derangement in metabolic function to its deregulation of epigenetics and, finally, its effect on growth. Overexpression models of *IDH1* mutant function likely over-estimate the negative effects of the mutant enzyme on growth and metabolic function. In several studies, inhibiting the *IDH1* mutant enzyme in endogenous cultures seems to have minimal effects on either growth or the metabolic state of the cell. Our study also demonstrates the tumor-suppressive effect of the accumulated D-2HG by FTO inhibition. However, all studies demonstrate *IDH1* mutant and *IDH1* wild-type gliomas have different metabolic properties, pointing that they may have distinctive vulnerabilities allowing for the possibility of personalized therapy. Collectively, these results suggest that further and broader investigation of the mechanistic role of these enzymes in *IDH1*-wild-type and mutant glioma is warranted.

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