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Biological Significance of Mutant Isocitrate Dehydrogenase 1 and 2 in Gliomagenesis

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Abstract

Mutations of the isocitrate dehydrogenase (IDH) genes are considered an important event that occurs at an early stage during gliomagenesis. The mutations often occur in grade 2 or 3 gliomas and secondary glioblastomas. Most IDH mutations are associated with codon 132 and 172 in *IDH1* and *IDH2* in gliomas, respectively. While IDH1 and IDH2 catalyze the oxidative decarboxylation of isocitrate to form α-ketoglutarate (α-KG), IDH1 and IDH2 mutations convert α-KG to 2-hydroxyglutarate (2-HG). The accumulation of oncometabolite 2-HG is believed to lead progenitor cells into gliomas, inhibiting several α-KG-dependent enzymes, including ten-eleven translocation enzymes, histone demethylases, and prolyl hydroxylases, although the mechanisms have not been fully revealed. Herein, we review the contribution of *IDH1* and *IDH2* mutations to gliomagenesis.

Key words: α-KG-dependent enzymes, glioma, gliomagenesis, 2-hydroxyglutarate, *IDH* mutations

Introduction

The classification of gliomas has changed. In the current edition of the World Health Organization (WHO) classification of the central nervous system published in 2007, gliomas were divided mainly on the basis of histological findings.[1)] In the next WHO classification, it was suggested that molecular information should be combined to generate a new integrated diagnosis, as recommended by the International Society of Neuropathology-Haarlem Consensus Guidelines.[2,3)] It is undisputed that many types of molecular pathogenesis have contributed to the revision of the classification, and one of the most important factors to affect the revision is the discovery of the mutation of the isocitrate dehydrogenase (IDH) 1 gene.

The first report regarding *IDH1* mutation in gliomas was published in 2008 by Parsons et al. In the study, recurrent mutations in the active site of *IDH1* were found in 12% of glioblastoma (GBM) patients. They reported that mutations in *IDH1* occurred in younger patients and in most patients with secondary GBMs and were associated with an increased overall survival relative to the patients with wild-type *IDH1*.[4)]

IDH1 and *IDH2* mutations are mainly found in grade 2 and 3 gliomas and secondary GBMs. The frequencies of *IDH1* and *IDH2* mutations in different types of gliomas reported in past studies have been variable and are summarized in Table 1.[5–11)]

The frequency of each mutated location is summarized in Table 2.[5,8,11,12)] Interestingly, nearly all *IDH* mutations involve a single amino acid substitution: the arginine residue at codon 132 in *IDH1* and the arginine residue at codon 172 or codon 140 in *IDH2*. However, R140 mutations are not found in gliomas.[13)] The most common alteration is R132H (c.395G > A), which accounts for approximately 90% of all *IDH* mutations. R132C, R132S, R132G, and R132L occur in less than 5% of all *IDH* mutations. Some cases showed *IDH2* mutation, which occurred at Arg172. R172K is the main amino acid change occurring during *IDH2* mutation. Although *IDH1* and *IDH2* mutations occur exclusively in most cases, only rare cases exhibit both mutations. Only 4 out of 743 reported cases with *IDH1* or *IDH2* mutation showed both *IDH1* and *IDH2* mutations.[5)] There has been no report to show the relationship of *IDH3* mutation and gliomas.[2)]

Many studies have revealed that IDH mutant gliomas are more likely to contain mutations in *TP53* or a loss of chromosome 1p or 19q and are less likely to contain alterations in PTEN, EGFR, CDKN2A, or CDKN2B.[11,14)] Watanabe et al. demonstrated several cases in which *TP53* mutation or 1p/19q loss occurred after the acquisition of *IDH1*

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Table 1 Frequency of isocitrate dehydrogenase mutations in different types of gliomas

Types of IDH	IDH1/2	IDH1/2	IDH1/2	IDH1/2	IDH1	IDH1	IDH1
Author	Hartmann et al.[5]	Mukasa et al.[7]	Sonoda et al.[9]	Yan et al.[11]	Ichimura et al.[6]	Sanson et al.[8]	Watanabe et al.[10]
Pilocytic astrocytoma		0%		0%	0%		10%
Diffuse astrocytoma	74%	59%	0%	90%	59%	83%	88%
Anaplastic astrocytoma	65%	28%	62%	73%	52%	50%	78%
Secondary glioblastoma		46%	67%	85%	50%		82%
Primary glioblastoma		6%	5%	5%	3%		5%
Oligodendroglioma	87%	76%	67%	84%	68%	76%	79%
Anaplastic oligodendroglioma	75%	67%	50%	94%	60%	49%	75%
Oligoastrocytoma	83%	57%		100%	50%	76%	94%
Anaplastic oligoastrocytoma	72%	80%	75%	100%	78%	63%	71%

IDH: isocitrate dehydrogenase.

Table 2 Frequency of specific isocitrate dehydrogenase mutations in gliomas

	Author	Hartmann et al.[5]	Yan et al.[11]	Sanson et al.[8]	Pusch et al.[12]
Gene	Amino acid change				
IDH1	R132H	89.4%	83.5%	89%	91.5%
	R132C	3.9%	4.1%	3.2%	4.3%
	R132S	1.5%	2.4%	1.9%	1.6%
	R132G	1.3%	0.6%	4.5%	1.9%
	R132L	0.3%	4.1%	1.3%	0.6%
IDH2	R172K	2.7%	2.4%		
	R172M	0.8%	1.8%		
	R172W	0.7%			
	R172G		1.2%		

IDH: isocitrate dehydrogenase.

mutation; however, there was no case in which an *IDH1* mutation occurred after the acquisition of a *TP53* mutation or the loss of 1p/19q.[10] An additional report showed that *IDH1* mutations were detected in 36/45 cases of low-grade astrocytomas that became malignant and were consistent in all consecutive high-grade gliomas.[15] These facts suggested that *IDH* mutations occur at the early stage during gliomagenesis and occur in a progenitor cell that can give rise to both cell types (astrocytic and oligodendrocytic) (Fig. 1).[10,14,15] In the current review, we focused on the role of *IDH1* and *IDH2* mutations on gliomagenesis.

Role of IDHs

In human cells, there are three types of IDHs: IDH1, IDH2, and IDH3. IDH1 catalyzes the oxidative decarboxylation of isocitrate to form α-ketoglutarate (α-KG) using nicotinamide adenine dinucleotide phosphate (NADP+) as a cofactor to generate NADPH. Although these three enzymes show the same enzymatic reaction, there are few differences among them. IDH1 is located in the cytosol and the peroxisomes, whereas IDH2 and IDH3 are located in the mitochondria. IDH1 and IDH2 are homodimeric enzymes. *IDH1* is encoded at 2q33,[16] whereas *IDH2* is encoded at 15q26.[17] IDH3 is a heteroctamer formed by three gene products: *IDH3A* (15q25.1-2), *IDH3B* (20p13), and *IDH3G* (Xq28).[18–21] IDH1 and IDH2 convert NADP+ to NADPH, whereas IDH3 change NAD+ to NADH. Only IDH3 is involved in the tricarboxylic acid cycle (Fig. 2).[2]

IDH1 and IDH2 produce NADPH and α-KG. NADPH is reported to be involved in many cellular processes, including the defense against oxidative stress, glucose metabolism, and lipid metabolism.[22,23] The α-KG has many important roles via α-KG-dependent enzymes.

α-KG-dependent enzymes

The dioxygenases incorporate both atoms of molecular oxygen (O_2) into their substrates. The dioxygenases whose activities require α-KG as cofactors are often termed α-KG-dependent dioxygenases. When they function, α-KG and O_2 are subsequently converted to succinate and CO_2, and one oxygen atom is attached to a hydroxyl group in the substrate.[22] More than 60 α-KG-dependent dioxygenases are established in

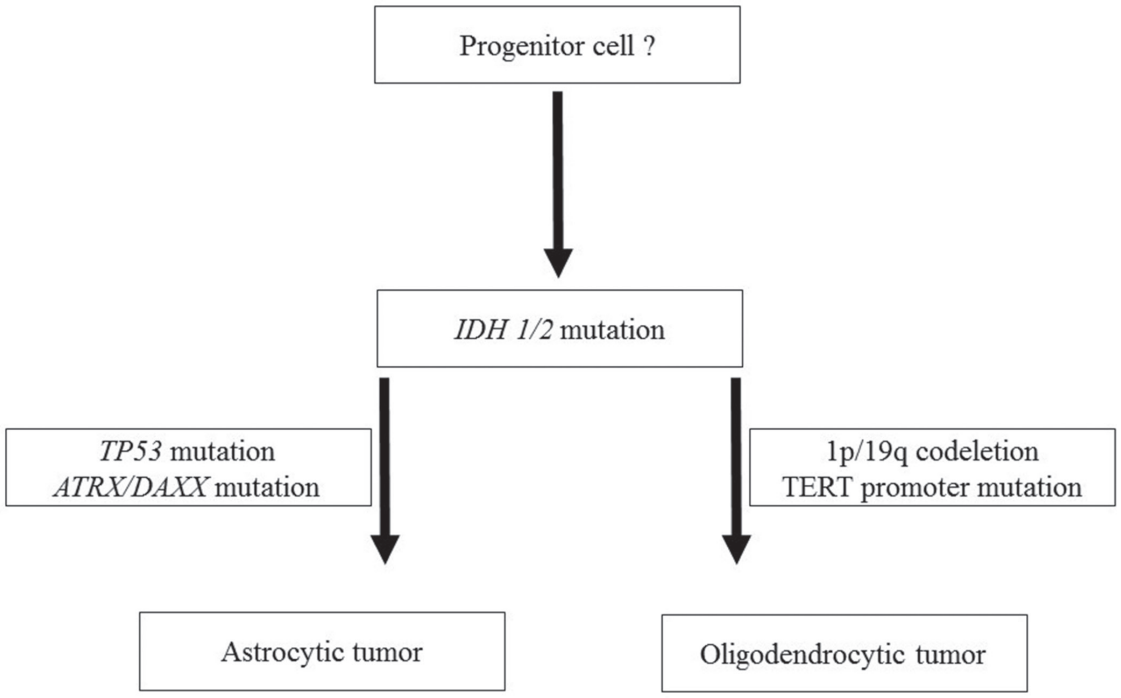

Fig. 1 ***IDH*** **mutation in gliomagenesis.** ***IDH*** **mutations have been considered to occur at an early stage during gliomagenesis. Additional genetic aberration induces both cell types (astrocytic and oligodendrocytic). IDH: isocitrate dehydrogenase.**

humans.[2,24] They are associated with various pathways, including collagen, histone, and transcription factors, alkylated deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), lipids, antibiotics, 5-methylcytosine (5mC) of genomic DNA, and 6-methyladenine of RNA (Fig. 3A). Therefore, the changes of α-KG-dependent dioxygenases by mutant IDHs are considered to affect multiple cellular pathways.[22]

Role of Mutant IDHs

The mutations of *IDH1* occur at a single amino acid residue of the *IDH1* active site. Initially, the mutation of IDH1 was considered to be a dominant negative inhibitor and to suppress the function of normal IDH1 to convert isocitrate to α-KG.[25] Zhao et al. reported that IDH1 mutations decrease the affinity of the enzyme for its substrate and dominantly inhibit the activity of wild-type IDH1 by forming catalytically inactive heterodimers.[25] However, IDH1 mutation has been revealed to have a neomorphic function which converts α-KG to 2-hydroxyglutarate (2-HG) (Fig. 4).[26] The glioma cells with overexpressed R132H IDH1 showed higher levels of 2-HG than the same cells with overexpressed wild-type IDH1.[26] Interestingly, tumor samples containing *IDH1* mutations showed 2-HG levels as high as 100-fold greater than tumor samples without *IDH1* mutations; however, there was no difference in the levels of α-KG or isocitrate between them.[26] *IDH2* mutations on either Arg172 or Arg 140 also gain this new ability.[27,28]

In leukemia, Losman et al. reported that 2-HG was sufficient to promote leukemogenesis.[29] In gliomas, several reports have shown that the addition of 2-HG exhibited similar patterns of changes as those by overexpressed mutant IDHs.[30,31] Therefore, 2-HG appears to be the main contributor to gliomagenesis in gliomas with *IDH1* mutation.

Mechanism of Gliomagenesis by Mutant IDHs

There have been inconsistent reports regarding the effect of *IDH1* mutation on glioma cell proliferation. Wang et al. reported that the overexpression of mutant IDH1 promoted cell proliferation. Overexpressed IDH1 mutant activated NF-kB, which induced the expression of cyclin D1 and E and c-myc, which were involved in cell proliferation.[32] On the other hand, Bralten et al. reported that the overexpression of mutant IDH1 reduced the proliferation due to a reduced cell cycle activity in glioma cell lines.[33]

With respect to cellular transformation, an *in vitro* study using immortalized human astrocytes showed that 2-HG levels in IDH1R132H overexpressed cells

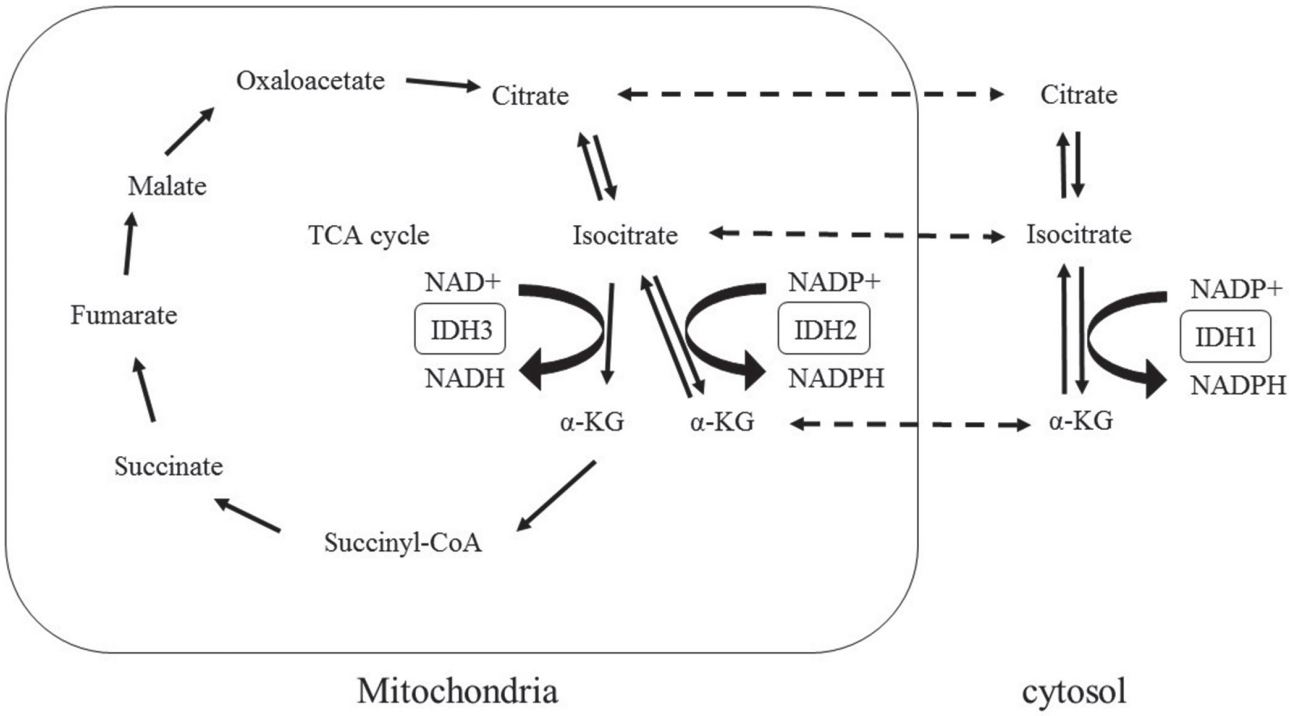

Fig. 2 The role of IDHs. IDH1 is located in the cytosol and the peroxisomes, whereas IDH2 and IDH3 are located in the mitochondria. IDH1 and IDH2 catalyze the oxidative decarboxylation of isocitrate to form α-ketoglutarate using NADP+ as a cofactor to generate NADPH, whereas IDH3 converts NAD+ to NADH. IDH: isocitrate dehydrogenase, NAD+: nicotinamide adenine dinucleotide, NADP+: nicotinamide adenine dinucleotide phosphate.

increased compared with wild-type IDH1 overexpressed cells and that IDH1R132H-overexpressed immortalized human astrocytes obtained the ability to form colonies in soft agar.[34,35]

Although accurate descriptions of the mechanisms of gliomagenesis have not yet been achieved, an important phenomenon is that the accumulation of 2-HG competes with α-KG because of their similar constructs and subsequently inhibits many α-KG-dependent enzymes.[27,31] Many studies have suggested the contribution of the inhibition of α-KG-dependent enzymes by 2-HG to gliomagenesis via various mechanisms, including modified histone methylation, altered DNA methylation, and regulated hypoxia-inducible factor 1 α (HIF-1α) (Fig. 3B).[21,25,30,34,36]

Although 2-HG inhibits α-KG-dependent enzymes, it does not inhibit several α-KG-dependent dioxygenases to an equal degree. Chowdhury et al. reported that the concentrations of 2-HG varied from approximately 25 μM for the inhibition of histone demethylase JMJD2A to more than 5 mM for the inhibition of HIF prolyl hydroxylase,[37] which may provide clues of what pathways most contribute to gliomagenesis. The leading hypothesis is that mutant IDHs affects DNA and histone methylation, which induces gene expressions that subsequently lead to gliomagenesis.

Inhibition of DNA Methylation

Ten-eleven Translocation (TET): The TET family of DNA hydroxylases catalyzes the conversion of 5mC to 5-hydroxymethylcytosine (5hmC) during DNA demethylation.[38] *IDH1* mutation was reported to inhibit the activity of the TET family proteins, and 2-HG was additionally reported to inhibit the activity of TET hydroxylases.[31] The 2-HG in gliomas inhibits the TET family, which may contribute to global DNA methylation in G-CIMP. Supporting this hypothesis, an acute myeloid leukemia (AML) containing an inactivating mutation of *TET2* showed a hypermethylation phenotype compatible with G-CIMP.[39] Additionally, it was observed that *IDH* and *TET* mutations were mutually exclusive in the AML samples.[39]

On the other hand, several studies showed inconsistent results. Ko et al. showed that TET2 loss-of-function was predominantly associated with decreased methylation at CpG sites.[40] Patients with wild-type *TET2* showed a higher degree of hypermethylation than those with *TET2* mutations in chronic myelomonocytic leukemia.[41]

G-CMP: The genome-wide methylation profile analysis showed that gliomas with *IDH1* mutations had a unique CpG island methylation at a larger number of loci than gliomas without *IDH1* mutations.[42]

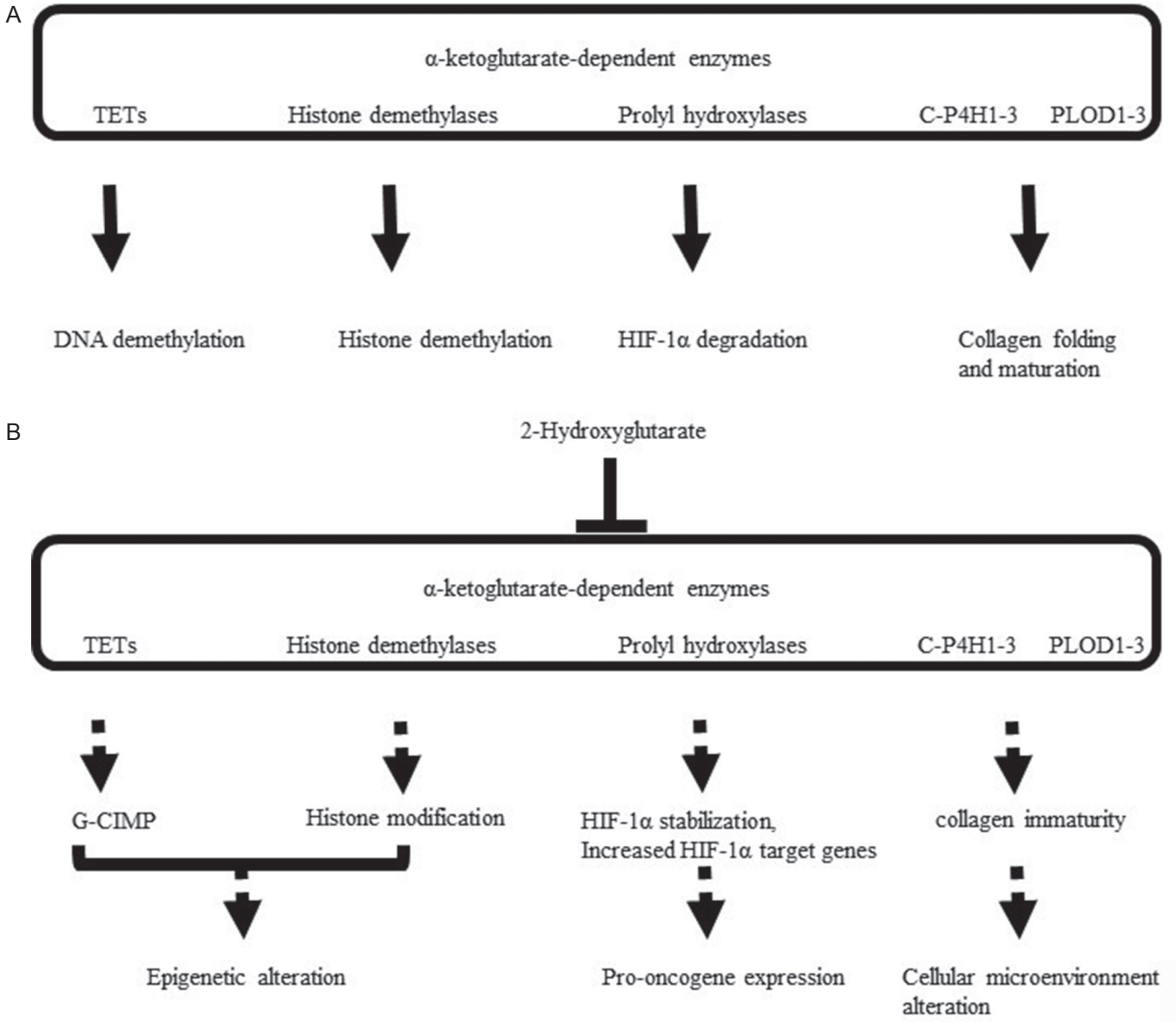

Fig. 3 Enzymatic function of α-KG-dependent enzymes (A) and supposed mechanism in which inhibition of α-KG-dependent enzymes by 2-HG contribute to gliomageneis (B). The accumulation of 2-HG competes with α-KG and subsequently inhibits many α-KG-dependent enzymes. The inhibition of α-KG-dependent enzymes by 2-HG contributes to gliomagenesis via various mechanisms, including epigenetic alteration, stabilization of hypoxia-inducible factor 1 α, alteration of cell microenvironment. α-KG: α-ketoglutarate, 2-HG: 2-hydroxyglutarate.

Verhaak et al. classified GBMs into proneural, neural, classical, and mesenchymal subtypes based on the gene expressions,[43] and gliomas with G-CMP exhibited gene expression of the proneural type.[42] Turcan et al. examined the methylation data from grade 2 and 3 gliomas and found that a distinct G-CIMP phenotype was dependent on the presence of *IDH* mutation.[36]

The introduction of mutant IDH1 into immortalized astrocytoma resulted in their development of G-CIMP after long passages. The gene expression programs that occurred in astrocytes expressing mutant IDH1 were similar to those in low-grade gliomas harboring *IDH1* mutation.[36]

Hill et al. demonstrated that in 80% of cases, the hypermethylator status that is associated with *IDH1* mutation in secondary GBM was retained in both the early and late tumor of the same patient, suggesting limited alterations to genome-wide methylation during gliomagenesis, and that the CIMP phenotype occurred at an early stage of glioma progression.[44]

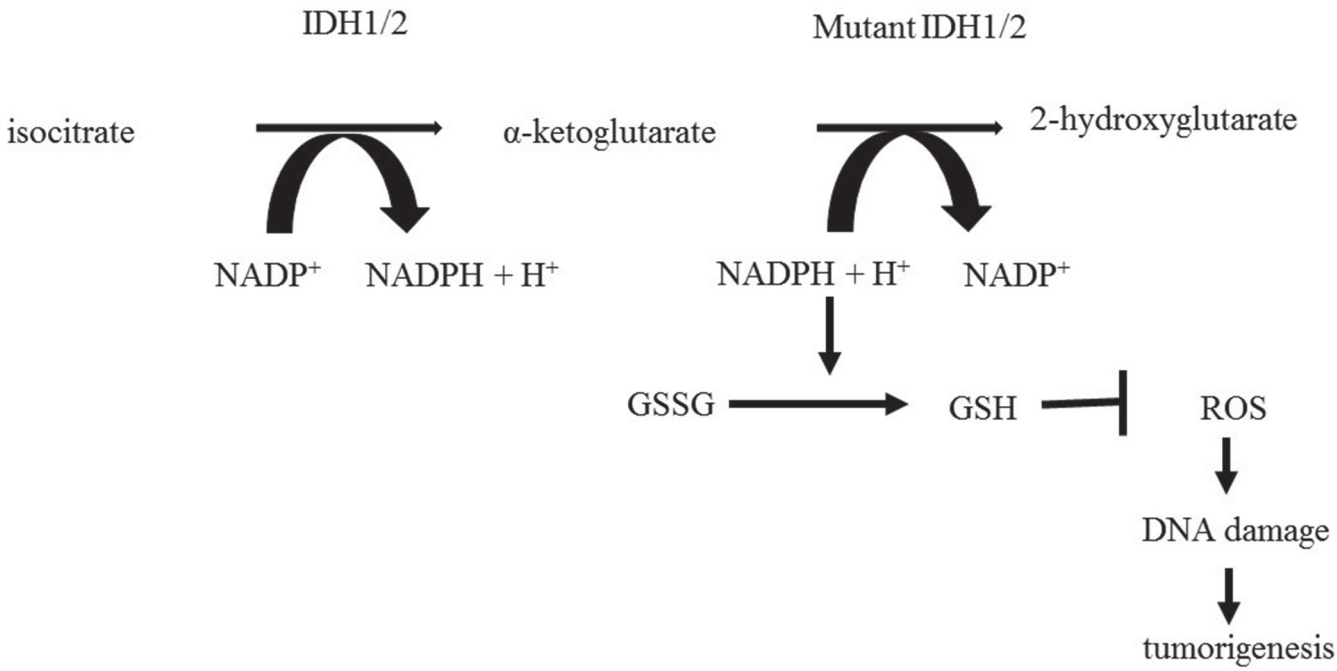

Fig. 4 Enzymatic function of wild-type and mutant IDHs and supposed mechanism in which mutant IDHs contribute to gliomageneis. Wild-type IDH1 and IDH2 convert isocitrate to α-ketoglutarate (α-KG) using NADP+ as a cofactor to generate NADPH. Mutant IDHs convert α-KG to 2-hydroxyglutarate (2-HG). Decreased NADPH results in a decrease in the level of GSH, which induces increased ROS in gliomas containing the *IDH* mutations. ROS induces deoxyribonucleic acid damage, which may lead to tumorigenesis. IDH: isocitrate dehydrogenase, NADP+: nicotinamide adenine dinucleotide phosphate, ROS: reactive oxygen species.

Inhibition of Histone Demethylase

The 2-HG competes with α-KG and inhibits histone demethylase. The addition of 2-HG increased the dimethylation of H3K9 and H3K79 by 5- and 10-fold, respectively. Ectopic expression of R132H IDH1 in U87 induced H3K4 monomethylation, H3K27 dimethylation, H3K4 trimethylation, H3K9 dimethylation, and H3K79 dimethylation. Increased H3K79 dimethylation levels were found in glioma samples with *IDH1* mutation compared to glioma samples without *IDH1* mutation.[31]

Similarly, Lu et al. reported that late-passage immortalized astrocytes that were the transfection of mutant IDH1 showed an accumulation of histone methylation marks. H3K9me3 levels were significantly elevated earlier by passage 12 after cells were infected with mutant IDH, whereas the elevation of H3K27me3 levels occurred after later passages. The elevations of H3K79me2 were lower, and H3K4me3 levels were not changed by 27 passages. Increases in DNA methylation were not found before passage 17, which was later than the occurrence of increased H3K9me3.[30] How histone methylations contribute to gliomagenesis is not yet clear; however, the status of histone demethylation is considered to be associated with cell differentiation and tumorigenesis.

Inhibition of Differentiation

The IDH mutations result in a block in cell differentiation and the promotion of cell proliferation.[30] The introduction of mutant IDH2 or the addition of 2-HG to 3T3-L1 cells was associated with the repression of the inducible expression of lineage-specific differentiation genes and blocked the adipocyte differentiation. The introduction of mutant *IDH1* in immortalized astrocytes induced these cells into a stem cell-like phenotype, decreasing the expression of the astrocyte marker glial fibrillary acidic protein (GFAP) and increasing the expression of the neural marker nestin. The differentiation was blocked by the inhibition of histone demethylation.[30] Kernytsky et al. demonstrated that IDH2 mutant inhibition reversed histone methylation and promoted differentiation in TF-1 with overexpressed mutant IDH2.[45]

Inhibition of HIF-1α

With respect to the association of mutant IDHs with HIF-1α, there are two opposite hypotheses. The accumulation of 2-HG has been shown to inhibit prolyl-hydroxylase (PHD) enzymes, which are one of the α-KG-dependent enzymes. PHD regulates the stability and activation of HIF-1α. Under normal

oxygen conditions, PHD hydroxylates HIF-1α at specific proline residues, creating a binding site for the von Hippel-Lindau protein ubiquitin-ligase protein complex, which subsequently ubiquitinates HIF-1α for proteasomal degradation.[23,27] Under hypoxic conditions, oxygen-dependent hydroxylation does not occur, which results in the accumulation of HIF-1α and its subsequent translocation into the nucleus and induction of HIF-1α downstream gene targets.[23] Therefore, mutant IDHs inhibit PHD, stabilize the expression of HIF-1α, and subsequently result in an increase of the expression of HIF-1α target genes, such as *VEGF*, *GLUT1*, and *PGK1*, which might promote tumor cell growth, invasion, angiogenesis, and metastasis. Supporting this hypothesis, the rise in HIF1-α levels was reported to be reversible by α-KG derivative.[25] Similarly, ectopically expressed IDH1 mutants increased HIF-1α in U87 cells.[31] On the other hand, Koivunen et al. reported that the accumulation of 2-HG enhanced EGLN activity and that it lead to decrease the expression of HIF-1α in immortalized astrocytes with the introduction of mutant IDH1. In their report, decreased HIF-1α and increased EGLN1 contributed to cellular transformation.[34] Intriguingly, none of the expression levels of HIF-1α, GLUT1, PGK1, and VEGF are significantly different between samples with and without IDH mutant in an AML.[46]

Other Possibilities

The 2-HG has been reported to inhibit PLOD1-3 and C-P4H1-3 to impair collagen maturation, which leads to basement membrane aberrations that might play a part in the progression of glioma.[23,47]

In gliomas containing *IDH1* mutations, the levels of NADPH are decreased. NADPH is reported to maintain cellular redox balance and regulate reactive oxygen species (ROS). NADPH is required for the conversion of glutathione disulfide to GSH, which is a major antioxidant against oxidative stress by ROS. Therefore, a decrease of NADPH+ results in a decrease in the level of GSH, which induces increased ROS in gliomas containing the *IDH1* mutation. ROS induces DNA damage, which may promote the incidence of malignancy (Fig. 4).[23]

Treatment Targeting for the IDH Mutant

Recently, high throughput compound screen identified AGI-5198 as a potent inhibitor of R132H mutated IDH1.[48] AGI-5198 showed high selectivity; IC50 of AGI-5198 for mutant IDH1 was 70 nM, whereas IC50 for WT IDH1 was more than 100 uM. AGI-5198 reduced the levels of 2-HG in a dose-dependent manner in R132H mutated glioma cells, and suppressed the efficacy of colonogenicity. AGI-5198 treatment markedly increased the GFAP-positive astrocytes and reduced the nestin-positive neural progenitor cells. Oral administration of the drug in mice reduced the growth of the xenografted subcutaneous R132H IDH1 glioma.[48] AG-120 and AG-221 were developed from AGI-5198, targeting for IDH1 and IDH2 mutations, respectively. The clinical trials for these drugs have been starting.[27]

Conclusion

From the information described above, it is reasonable to consider that IDH mutations induce oncometabolite 2-HG, which inhibits several α-KG-dependent enzymes, such as TET2, PHD, and histone demethylases, and subsequently directs progenitor cells into gliomas by altering epigenetics and blocking normal differentiation processes. However, the details of many of these pathways of gliomagenesis remain unclear. Moreover, several contradictory results with respect to the hypothesized pathways have been reported. Further studies are required to reveal the true pathways of gliomagenesis.

Conflicts of Interest Disclosure

All authors have no conflicts of interest.

References

1) Louis DN, Ohgaki H, Wiestler OD, Cavenee WK: *WHO Classification of Tumors of the Central Nervous System, ed 4*. International agency for research on cancer, Lyon
2) Ichimura K, Narita Y, Hawkins CE: Diffusely infiltrating astrocytomas: pathology, molecular mechanisms and markers. *Acta Neuropathol* 129: 789–808, 2015
3) Louis DN, Perry A, Burger P, Ellison DW, Reifenberger G, von Deimling A, Aldape K, Brat D, Collins VP, Eberhart C, Figarella-Branger D, Fuller GN, Giangaspero F, Giannini C, Hawkins C, Kleihues P, Korshunov A, Kros JM, Beatriz Lopes M, Ng HK, Ohgaki H, Paulus W, Pietsch T, Rosenblum M, Rushing E, Soylemezoglu F, Wiestler O, Wesseling P; International Society Of Neuropathology—Haarlem: International Society Of Neuropathology—Haarlem consensus guidelines for nervous system tumor classification and grading. *Brain Pathol* 24: 429–435, 2014
4) Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW: An integrated genomic analysis of

human glioblastoma multiforme. *Science* 321: 1807–1812, 2008
5) Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W, Weller M, Herold-Mende C, Unterberg A, Jeuken JW, Wesseling P, Reifenberger G, von Deimling A: Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. *Acta Neuropathol* 118: 469–474, 2009
6) Ichimura K, Pearson DM, Kocialkowski S, Bäcklund LM, Chan R, Jones DT, Collins VP: IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. *Neuro-oncology* 11: 341–347, 2009
7) Mukasa A, Takayanagi S, Saito K, Shibahara J, Tabei Y, Furuya K, Ide T, Narita Y, Nishikawa R, Ueki K, Saito N: Significance of IDH mutations varies with tumor histology, grade, and genetics in Japanese glioma patients. *Cancer Sci* 103: 587–592, 2012
8) Sanson M, Marie Y, Paris S, Idbaih A, Laffaire J, Ducray F, El Hallani S, Boisselier B, Mokhtari K, Hoang-Xuan K, Delattre JY: Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. *J Clin Oncol* 27: 4150–4154, 2009
9) Sonoda Y, Kumabe T, Nakamura T, Saito R, Kanamori M, Yamashita Y, Suzuki H, Tominaga T: Analysis of IDH1 and IDH2 mutations in Japanese glioma patients. *Cancer Sci* 100: 1996–1998, 2009
10) Watanabe T, Nobusawa S, Kleihues P, Ohgaki H: IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am J Pathol* 174: 1149–1153, 2009
11) Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD: IDH1 and IDH2 mutations in gliomas. *N Engl J Med* 360: 765–773, 2009
12) Pusch S, Schweizer L, Beck AC, Lehmler JM, Weissert S, Balss J, Miller AK, von Deimling A: D-2-Hydroxyglutarate producing neo-enzymatic activity inversely correlates with frequency of the type of isocitrate dehydrogenase 1 mutations found in glioma. *Acta Neuropathol Commun* 2: 19, 2014
13) Arita H, Narita Y, Yoshida A, Hashimoto N, Yoshimine T, Ichimura K: IDH1/2 mutation detection in gliomas. *Brain Tumor Pathol* 32: 79–89, 2015
14) Agnihotri S, Aldape KD, Zadeh G: Isocitrate dehydrogenase status and molecular subclasses of glioma and glioblastoma. *Neurosurg Focus* 37: E13, 2014
15) Juratli TA, Kirsch M, Robel K, Soucek S, Geiger K, von Kummer R, Schackert G, Krex D: IDH mutations as an early and consistent marker in low-grade astrocytomas WHO grade II and their consecutive secondary high-grade gliomas. *J Neurooncol* 108: 403–410, 2012
16) Narahara K, Kimura S, Kikkawa K, Takahashi Y, Wakita Y, Kasai R, Nagai S, Nishibayashi Y, Kimoto H: Probable assignment of soluble isocitrate dehydrogenase (IDH1) to 2q33.3. *Hum Genet* 71: 37–40, 1985
17) Oh IU, Inazawa J, Kim YO, Song BJ, Huh TL: Assignment of the human mitochondrial NADP(+)-specific isocitrate dehydrogenase (IDH2) gene to 15q26.1 by in situ hybridization. *Genomics* 38: 104–106, 1996
18) Brenner V, Nyakatura G, Rosenthal A, Platzer M: Genomic organization of two novel genes on human Xq28: compact head to head arrangement of IDH gamma and TRAP delta is conserved in rat and mouse. *Genomics* 44: 8–14, 1997
19) Huh TL, Kim YO, Oh IU, Song BJ, Inazawa J: Assignment of the human mitochondrial NAD+ -specific isocitrate dehydrogenase alpha subunit (IDH3A) gene to 15q25.1–>q25.2 by in situ hybridization. *Genomics* 32: 295–296, 1996
20) Kim YO, Park SH, Kang YJ, Koh HJ, Kim SH, Park SY, Sohn U, Huh TL: Assignment of mitochondrial NAD(+)-specific isocitrate dehydrogenase beta subunit gene (IDH3B) to human chromosome band 20p13 by in situ hybridization and radiation hybrid mapping. *Cytogenet Cell Genet* 86: 240–241, 1999
21) Waitkus MS, Diplas BH, Yan H: Isocitrate dehydrogenase mutations in gliomas. Neuro Oncol 18: 16–26, 2016
22) Yang H, Ye D, Guan KL, Xiong Y: IDH1 and IDH2 mutations in tumorigenesis: mechanistic insights and clinical perspectives. *Clin Cancer Res* 18: 5562–5571, 2012
23) Zhang C, Moore LM, Li X, Yung WK, Zhang W: IDH1/2 mutations target a key hallmark of cancer by deregulating cellular metabolism in glioma. *Neuro-oncology* 15: 1114–1126, 2013
24) Rose NR, McDonough MA, King ON, Kawamura A, Schofield CJ: Inhibition of 2-oxoglutarate dependent oxygenases. *Chem Soc Rev* 40: 4364–4397, 2011
25) Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, Yu W, Li Z, Gong L, Peng Y, Ding J, Lei Q, Guan KL, Xiong Y: Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. *Science* 324: 261–265, 2009
26) Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, Su SM: Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 462: 739–744, 2009
27) Dimitrov L, Hong CS, Yang C, Zhuang Z, Heiss JD: New developments in the pathogenesis and therapeutic targeting of the IDH1 mutation in glioma. *Int J Med Sci* 12: 201–213, 2015
28) Ward PS, Cross JR, Lu C, Weigert O, Abel-Wahab O, Levine RL, Weinstock DM, Sharp KA, Thompson CB: Identification of additional IDH mutations associated with oncometabolite R(-)-2-hydroxyglutarate production. *Oncogene* 31: 2491–2498, 2012
29) Losman JA, Looper RE, Koivunen P, Lee S, Schneider RK, McMahon C, Cowley GS, Root DE, Ebert BL,

Kaelin WG Jr: (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science* 339: 1621–1625, 2013
30) Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, Edwards CR, Khanin R, Figueroa ME, Melnick A, Wellen KE, O'Rourke DM, Berger SL, Chan TA, Levine RL, Mellinghoff IK, Thompson CB: IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature* 483: 474–478, 2012
31) Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT, Liu LX, Jiang WQ, Liu J, Zhang JY, Wang B, Frye S, Zhang Y, Xu YH, Lei QY, Guan KL, Zhao SM, Xiong Y: Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell* 19: 17–30, 2011
32) Wang G, Sai K, Gong F, Yang Q, Chen F, Lin J: Mutation of isocitrate dehydrogenase 1 induces glioma cell proliferation via nuclear factor-κB activation in a hypoxia-inducible factor 1-α dependent manner. *Mol Med Rep* 9: 1799–1805, 2014
33) Bralten LB, Kloosterhof NK, Balvers R, Sacchetti A, Lapre L, Lamfers M, Leenstra S, de Jonge H, Kros JM, Jansen EE, Struys EA, Jakobs C, Salomons GS, Diks SH, Peppelenbosch M, Kremer A, Hoogenraad CC, Smitt PA, French PJ: IDH1 R132H decreases proliferation of glioma cell lines in vitro and in vivo. *Ann Neurol* 69: 455–463, 2011
34) Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S, Losman JA, Joensuu P, Bergmann U, Gross S, Travins J, Weiss S, Looper R, Ligon KL, Verhaak RG, Yan H, Kaelin WG Jr: Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. *Nature* 483: 484–488, 2012
35) Ohba S, Mukherjee J, See WL, Pieper RO: Mutant IDH1-driven cellular transformation increases RAD51-mediated homologous recombination and temozolomide resistance. *Cancer Res* 74: 4836–4844, 2014
36) Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AW, Lu C, Ward PS, Thompson CB, Kaufman A, Guryanova O, Levine R, Heguy A, Viale A, Morris LG, Huse JT, Mellinghoff IK, Chan TA: IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 483: 479–483, 2012
37) Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagg EA, Rose NR, Leung IK, Li XS, Woon EC, Yang M, McDonough MA, King ON, Clifton IJ, Klose RJ, Claridge TD, Ratcliffe PJ, Schofield CJ, Kawamura A: The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep* 12: 463–469, 2011
38) Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A: Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 324: 930–935, 2009
39) Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanthakumar A, Fernandez HF, Tallman MS, Sun Z, Wolniak K, Peeters JK, Liu W, Choe SE, Fantin VR, Paietta E, Löwenberg B, Licht JD, Godley LA, Delwel R, Valk PJ, Thompson CB, Levine RL, Melnick A: Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* 18: 553–567, 2010
40) Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, An J, Lamperti ED, Koh KP, Ganetzky R, Liu XS, Aravind L, Agarwal S, Maciejewski JP, Rao A: Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature* 468: 839–843, 2010
41) Pérez C, Martínez-Calle N, Martín-Subero JI, Segura V, Delabesse E, Fernandez-Mercado M, Garate L, Alvarez S, Rifon J, Varea S, Boultwood J, Wainscoat JS, Cruz Cigudosa J, Calasanz MJ, Cross NC, Prósper F, Agirre X: TET2 mutations are associated with specific 5-methylcytosine and 5-hydroxymethylcytosine profiles in patients with chronic myelomonocytic leukemia. *PLoS One* 7:e31605, 2012
42) Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape K; Cancer Genome Atlas Research Network: Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* 17: 510–522, 2010
43) Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN; Cancer Genome Atlas Research Network: Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 17: 98–110, 2010
44) Hill VK, Shinawi T, Ricketts CJ, Krex D, Schackert G, Bauer J, Wei W, Cruickshank G, Maher ER, Latif F: Stability of the CpG island methylator phenotype during glioma progression and identification of methylated loci in secondary glioblastomas. *BMC Cancer* 14: 506, 2014
45) Kernytsky A, Wang F, Hansen E, Schalm S, Straley K, Gliser C, Yang H, Travins J, Murray S, Dorsch M, Agresta S, Schenkein DP, Biller SA, Su SM, Liu W, Yen KE: IDH2 mutation-induced histone and DNA hypermethylation is progressively reversed by small-molecule inhibition. *Blood* 125:296–303, 2015
46) Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, Koboldt DC, Fulton RS, Delehaunty KD, McGrath SD, Fulton LA, Locke DP, Magrini VJ, Abbott RM, Vickery TL, Reed JS, Robinson JS, Wylie T, Smith SM, Carmichael L, Eldred JM, Harris CC,

Walker J, Peck JB, Du F, Dukes AF, Sanderson GE, Brummett AM, Clark E, McMichael JF, Meyer RJ, Schindler JK, Pohl CS, Wallis JW, Shi X, Lin L, Schmidt H, Tang Y, Haipek C, Wiechert ME, Ivy JV, Kalicki J, Elliott G, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson MA, Baty J, Heath S, Shannon WD, Nagarajan R, Link DC, Walter MJ, Graubert TA, DiPersio JF, Wilson RK, Ley TJ: Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med* 361: 1058–1066, 2009

47) Sasaki M, Knobbe CB, Itsumi M, Elia AJ, Harris IS, Chio II, Cairns RA, McCracken S, Wakeham A, Haight J, Ten AY, Snow B, Ueda T, Inoue S, Yamamoto K, Ko M, Rao A, Yen KE, Su SM, Mak TW: D-2-hydroxyglutarate produced by mutant IDH1 perturbs collagen maturation and basement membrane function. *Genes Dev* 26: 2038–2049, 2012

48) Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, Tsoi J, Clark O, Oldrini B, Komisopoulou E, Kunii K, Pedraza A, Schalm S, Silverman L, Miller A, Wang F, Yang H, Chen Y, Kernytsky A, Rosenblum MK, Liu W, Biller SA, Su SM, Brennan CW, Chan TA, Graeber TG, Yen KE, Mellinghoff IK: An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* 340: 626–630, 2013

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