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 2.²,⁵,⁸,¹¹,¹² 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 mutations.
Role of Mutant IDHs in 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, whereas IDH2 is encoded at 15q26.1. IDH3 is a heterotrimer formed by three gene products: IDH3A (15q25.1-2), IDH3B (20p13), and IDH3G (Xq28). IDH1 and IDH2 change NAD+ to NADH, whereas IDH3 change NAD+ to NADPH. Only IDH3 is involved in the tricarboxylic acid cycle.

IDH: isocitrate dehydrogenase.

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

| Types of IDH                  | Author       | IDH1/2 | IDH1/2 | IDH1/2 | IDH1/2 | IDH1 | IDH1 | IDH1 |
|------------------------------|--------------|--------|--------|--------|--------|------|------|------|
| Author                       | Hartmann     | 0%     | 0%     | 0%     | 0%     | 10%  |      |      |
| et al.5)                     | Mukasa       |        |        |        |        |      |      |      |
| et al.7)                     | Sonoda       |        |        |        |        |      |      |      |
| et al.9)                     | Yan et al.11 |        |        |        |        |      |      |      |
| et al.6)                     | Ichimura     |        |        |        |        |      |      |      |
| et al.8)                     | Sanson       |        |        |        |        |      |      |      |
| et al.10)                    | Watanabe     |        |        |        |        |      |      |      |

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% Oligodendrogloma 87% 76% 67% 84% 68% 76% 79% Anaplastic oligodendrogloma 75% 67% 50% 94% 60% 49% 75% Oligoastrocytoma 83% 57% 100% 50% 76% 94% Anaplastic oligoastrocytoma 72% 80% 75% 100% 78% 63% 71%

Table 2  Frequency of specific isocitrate dehydrogenase mutations in gliomas

| Gene | Amino acid change | Author     | Hartmann et al.5 | Yan et al.11 | Sanson et al.8 | Pusch et al.12 |
|------|------------------|------------|------------------|--------------|----------------|----------------|
| 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. 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. 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). In the current review, we focused on the role of IDH1 and IDH2 mutations on gliomagenesis.

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Role of IDHs

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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. The α-KG has many important roles via α-KG-dependent enzymes.

α-KG-dependent enzymes

The dioxygenases incorporate both atoms of molecular oxygen (O2) 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 O2 are subsequently converted to succinate and CO2, and one oxygen atom is attached to a hydroxyl group in the substrate. More than 60 α-KG-dependent dioxygenases are established in...
humans. 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.

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. 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. However, IDH1 mutation has been revealed to have a neomorphic function which converts α-KG to 2-hydroxyglutarate (2-HG) (Fig. 4). The glioma cells with overexpressed R132H IDH1 showed higher levels of 2-HG than the same cells with overexpressed wild-type IDH1. 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. IDH2 mutations on either Arg172 or Arg 140 also gain this new ability.

In leukemia, Losman et al. reported that 2-HG was sufficient to promote leukemogenesis. In gliomas, several reports have shown that the addition of 2-HG exhibited similar patterns of changes as those by overexpressed mutant IDHs. 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-κB, which induced the expression of cyclin D1 and E and c-myc, which were involved in cell proliferation. 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.

With respect to cellular transformation, an in vitro study using immortalized human astrocytes showed that 2-HG levels in IDH1R132H overexpressed cells...
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-CIMP: 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. \(^{42}\)
Verhaak et al. classified GBMs into proneural, neural, classical, and mesenchymal subtypes based on the gene expressions, and gliomas with G-CIMP exhibited gene expression of the proneural type. 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.

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.

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.

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 gliomagenesis (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 1α, alteration of cell microenvironment. α-KG: α-ketoglutarate, 2-HG: 2-hydroxyglutarate.
Role of Mutant IDHs in Gliomagenesis

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. 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. 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. Similarly, ectopically expressed IDH1 mutants increased HIF-1α in U87 cells. 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. 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.

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.

In gliomas containing IDH1 mutations, the levels of NADPH decrease. 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).

Treatment Targeting for the IDH Mutant

Recently, high throughput compound screen identified AGI-5198 as a potent inhibitor of R132H mutated IDH1. 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. 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.

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.

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