Summary. In recent years, frequent isocitrate dehydrogenase 1/2 (IDH1/IDH2) gene mutations were found in a variety of tumors, which specifically alter arginine residues of catalytic active site in IDH1/IDH2 and confer new enzymatic function of directly catalyzing alpha-ketoglutarate (α-KG) to R-2-hydroxyglutarate (2-HG). 2-HG could competitively inhibit α-KG–dependent enzymes and might therefore contribute to tumorigenesis. In addition, mutation status of IDH1/IDH2 is closely related to the progress and prognosis of certain tumors. Thus IDH1/IDH2 is considered to be a promising biomarker for early diagnosis and prognosis and targeted therapy. In this study, the current research on IDH1/IDH2 mutation, especially the mechanisms and clinical characteristics related to tumor, are reviewed.

Key words: Isocitrate dehydrogenase 1/2 (IDH1/IDH2), Mutation, Methylation, Metabolism, Tumorigenesis

Introduction

Metabolic abnormality has been considered to play an important role in tumorigenesis and tumor progression, since the discovery by Otto Warburg in 1924 that tumor cells prefer the glycolytic pathway to obtain energy even under conditions of adequate oxygen supply. However, the mechanisms involved in this metabolic change are not fully understood. In recent years, gene mutations of three metabolizing enzymes including fumaric acid dehydrogenase, succinate dehydrogenase, and isocitrate dehydrogenase (IDH) have been found in cancer cells, which further confirm that cell metabolism and development of tumor are closely related. IDH, a key enzyme in the Krebs cycle, catalyzes isocitrate into alpha-ketoglutarate (α-KG) and thus plays an important role in biological material and energy metabolism. IDH1/IDH2 mutations are the most common metabolic enzyme gene mutations that are usually found in glioma and some subtypes of acute myeloid leukemia (AML); sometimes these mutations are also detected in chondrosarcoma, cholangiocarcinoma, paraganglioma, colorectal cancer, prostate cancer, and lung cancer (Losman et al., 2013; Ye et al., 2013). Mutations alter the active site of the enzyme and confer new catalytic activity to IDH1/IDH2, i.e. reducing α-KG to high levels of R-2-hydroxyglutarate (2-HG) affects a variety of signaling pathways related to cellular proliferation and differentiation.

Biological characteristics of the wild-type IDH

IDH is a key enzyme in the Krebs cycle. It catalyzes the oxidative decarboxylation of isocitrate into α-KG, and at the same time reduces NAD+/NADP+ into nicotinamide adenine dinucleotide (NADH)/nicotinamide adenine dinucleotide phosphate (NADPH). Therefore, IDH plays an important role in life activities. Three subtypes of IDH (IDH1, IDH2, and IDH3) are present in mammals, where IDH1 and IDH2 are NADP+ dependent. Human IDH1 and IDH2 genes are located on chromosomes 2q33.3 and 15q26.1, respectively. IDH1 and IDH2 are present in the cytosol, peroxisome, and mitochondria, catalyzing NADP+ and isocitrate into α-KG and NADPH, and this reaction is reversible (Kim et al., 2012). IDH1 and IDH2 are homodimeric enzymes; two subunits of the homodimer combine to form the active site of the enzyme. A number of conserved amino acid sequences in the active site (such as IDH1<sup>R132</sup> and IDH2<sup>R172</sup>) determine the binding specificity of coenzyme and substrate affinity (Zhao et al., 2009; Kim
Isocitrate dehydrogenase in human tumors

and Liau, 2012).

IDH3 is an NAD+-dependent IDH present in the mitochondria. Yang et al. (2014) reported that IDH3 upregulated aflatoxin B1-induced liver injury, suggesting that it is involved in the phosphatidylinositol 3'-kinase/Akt pathway-mediated oxidative stress.

**IDH1 mutations and their functions in human tumors**

IDH1/IDH2 mutations were first discovered in adult glioblastoma multiforme tumors (GBMs) (Parsons et al., 2008), and they were also observed in AML, chondrosarcoma, bile duct cancer, paraganglioma, colorectal cancer, prostate cancer, lung cancer, and other tumors (Losman et al., 2013). All IDH mutations are heterozygous mutations located in the conserved regions of the catalytic active site of the enzyme, where IDH1R132H and IDH2R172H are the most common mutation sites.

Initially, it was considered that IDH mutations lead to the loss of enzymatic activity. But Dang et al. (2010) analyzed the levels of metabolite in wild IDH1 and IDH1R132H cells and found markedly elevated levels of 2-HG in IDH1R132H cells. The 2-HG was transformed from isotopically labeled α-KG, indicating that the mutant IDH1 obtained new catalytic activity, i.e., an NADPH-dependent reduction of α-KG to generate 2-HG (Fig. 1). Later, these findings were also confirmed in a series of animal models and cellular models delivered with mutant IDH1/IDH2 (Sasaki et al., 2012a,b; Zhang et al., 2013).

**Mechanisms of IDH mutation-induced tumorigenesis**

α-KG not only plays a role in metabolism, but also is involved in regulating a variety of important cellular signaling pathways. At least 60 α-KG-dependent dioxygenases are present in eukaryotic cells. These dioxygenases are involved in collagen synthesis, fatty acid metabolism, DNA damage repair, DNA/RNA/chromatin modification, hypoxic stress response, and other important cellular activities, which have a very wide range of biological functions (Losman and Kaelin, 2013). These enzymes require Fe2+ and α-KG to fulfill enzymatic activity. Because 2-HG and α-KG show very similar molecular structure, presumably 2-HG plays a role in tumor promotion by competitively inhibiting α-KG-dependent dioxygenases (Losman and Kaelin, 2013).

Sasaki et al. (2012) found that the serum level of 2-HG was significantly elevated in IDH1R132H mouse model, and older mice showed leukemia-like phenotypes including increased number of early hematopoietic progenitor cells, splenomegaly, anemia, and extramedullary hematopoiesis. Additionally, the methylation pattern of DNA CpG islands was altered in >80% of the bone marrow cells. Lu et al. (2012) expressed IDH2R172K and IDH2R140Q mutations in 3T3-L1 adipocytes. They found differentiation arrest and significantly increased levels of histone methylation markers in those cells. These findings suggest that IDH1/IDH2 gene mutations and their products such as 2-HG may promote tumorigenesis by affecting cell differentiation and signaling pathways. Therefore, inhibition of α-KG-dependent dioxygenase activity by 2-HG is considered to be the main mechanism of carcinogenicity caused by IDH mutations (Fig. 2).

**2-HG inhibits prolyl hydroxylase**

Prolyl hydroxylase (PHD) is one of the α-KG-dependent dioxygenase families, which can regulate the level of expression of hypoxia-inducible factor-1α (HIF-1α). The latter is an important transcription regulator involved in the regulation of key signaling pathways related to apoptosis, metabolism, and angiogenesis during tumorigenesis and tumor progression (Zhao et al., 2009; Xu et al., 2011; Lu et al., 2012; Sasaki et al., 2012a,b; Zhang et al., 2013).

Zhao et al. (2009) found that the cytoplasmic levels of HIF-1α were significantly increased in U-87MG and human embryonic kidney 293T cells transfected with IDH1R132H mutant; at the same time, HIF-1α target genes glucose transporter-1 (Glut1), vascular endothelial growth factor (VEGF), and phosphoglycerate kinase 1 (PKG1) were also upregulated. Results of immunohistochemistry from 26 cases of glioma revealed that HIF-1α and VEGF were higher in glioma samples with IDH mutations than those without the mutation (P<0.001). Sasaki et al. (2012) delivered IDH1R132H mutation into mouse embryos and found that the levels of expression of HIF-1α and its target gene VEGF were upregulated in embryonic rat brain cells. Chowdhury et al. (2011) further verified that 2-HG could inhibit the
activity of PHDs in vitro, resulting in elevated levels of HIF-1α. The evidence above supports the hypothesis that the product of mutated IDH1/IDH2 2-HG competitively inhibits the binding of α-KG to PHDs and thereby affects its regulatory function, leading to intracellular accumulation of HIF-1α and initiation of expression of downstream target genes to promote tumorigenesis. In contrast, Koivunen et al. (2012) reported that 2-HG could enhance the activity of PHDs both in vitro and in vivo. Williams et al. (2011) analyzed 120 cases of glioma with IDH1R132H mutation and found that only certain subtypes of gliomas showed upregulated HIF-1α. These data suggest that upregulated HIF-1α is not entirely due to the inhibitory effect of 2-HG on PHDs and that there may be other mechanisms such as hypoxia- or growth factor-dependent transcriptional regulation. It was also reported that the proliferation of AML cell lines in hypoxia or overexpression of HIF-1α occurred with slower proliferation, terminal differentiation, and apoptosis (He et al., 2013). Therefore, this hypothesis about IDH mutations and HIF-1α remains to be explored.

2-HG inhibits DNA demethylase

Ten-eleven translocation (TET) family enzymes are the main enzymes that regulate DNA demethylation, and TET family enzymes catalyze 5-methylcytosine to 5-hydroxymethyl-cytosine (5-hmC) (Losman and Kaelin, 2013). The TET family has three members, and TET2 is the most important regulatory enzyme of DNA demethylation. Clinical observation uncovered that IDH and TET2 mutations resulted in similar DNA methylation profiles in AML, and these two types of mutations were mutually exclusive, suggesting that these two mutations may share common oncogenic pathways (Figueroa et al., 2010).

Noushmehr et al. (2010) profiled the cancer genome of 207 GBM specimens and found that IDH1 mutations were highly correlated with the glioma-CpG island methylator phenotype (G-CIMP) of glioma-specific promoters: 18 of 23 (78%) G-CIMP-positive GBM cases harbored IDH1 mutation, but all 184 G-CIMP-negative tumors were of IDH1 wild type. The G-CIMP and IDH mutations were also tightly correlated in different subgroups of gliomas. To further verify the relationship between IDH1 mutations and G-CIMP, Turcan et al. (Turcan et al., 2012) used the same genotype immortalized human astrocytes to construct cell models expressing IDH1R132H and wild-type IDH1, respectively. They found that a large number of genes in the astrocytes with IDH1R132H occurred with hypermethylation, the level of 5-hmC was significantly reduced, and methylation markers (H3K9, H3K27, and H3K36) were significantly increased, while no significant changes were observed in cells with wild-type IDH1. In the studies by Xu et al. (2011) and Sasaki et al. (2012a,b), similar changes in DNA methylation were also observed in animal models and cells. These findings suggest that the mutant IDH1R132H and its product 2-HG inhibit the 5mC hydroxylation catalyzed by TET2, resulting in abnormal DNA methylation and tumorigenesis.

2-HG inhibits histone demethylase

Histone methylation is an important epigenetic modification, and it is closely related to the formation of heterochromatin, gene expression, inactivation, and DNA damage repair. JmjC domain-containing histone

![Fig. 2. A proposed model for R-2-HG in tumorigenesis (modified from Ye et al., 2013) R-2-HG produced by the mutant IDH1/IDH2 promotes tumorigenesis by inhibiting 5mC hydroxylase (TET2) and lysine demethylases (KDM), leading to the demethylation of DNA and histone, respectively. The epigenetic alterations associated with IDH1/IDH2 mutations result in changes in gene expression and tumorigenesis. R-2-HG does not inhibit, but rather stimulates Egln1, which promotes the degradation of HIF-1α by hydroxylation. HIF-1α might suppress leukemogenesis, but this may not apply to other cancer types with IDH1/IDH2 mutations.](image-url)
demethylase (JHDM) family is an important histidine demethylase, which catalyzes the demethylation of histone H3K4, H3K9, H3K27, H3K36, and H4K20. Abnormal histone demethylation is associated with the occurrence and progression of nervous diseases and a variety of human cancers (Kooistra and Helin, 2012). Xu et al. (2011) reported that 2-HG suppressed the demethylation activity of JHDMs by competitively inhibiting α-KG in U-87MG cells with IDH1R132H; this mechanism was further confirmed in vivo in 10 glioma samples combined with IDH1R132H mutation and 10 cases with wild-type IDH1. Lu et al. (2012) found that introduction of either IDH1R132H or cell-permeable 2-HG mutation into cell models caused upregulation of a variety of histone methylation markers. Moreover, histone methylation preceded a rise in DNA methylation as cells were passaged in culture, suggesting that histone methylation may induce DNA methylation. In addition, in both cell models and animal models, there was coexistence of histone methylation and DNA methylation (Xu et al., 2011; Sasaki et al., 2012a,b). It is known that histone modifications and DNA methylation can interplay, but the relationship between histone methylation and target genes is poorly understood and requires extensive research to reveal the factors.

Other possible mechanisms of IDH mutation-induced tumorigenesis

Other family members of α-KG-dependent dioxygenases can also be inhibited by 2-HG. Collagen hydroxylase family member P4HA1 has been shown to be inhibited by 2-HG in vitro (Koivunen et al., 2012; Sasaki et al., 2012a,b). In addition, other α-KG-dependent dioxygenases such as FIH1, which regulates HIF-1α transcriptional activity, DNA damage repair-related ABH family, and RNA demethylation-associated FTO, etc, have also been inhibited by 2-HG (Losman and Kaelin, 2013).

Specific inhibitors (AGI-5198 and AGI-6780) for IDH1/IDH2 mutants can promote the differentiation of leukemia and glioma cells and the growth of xenograft tumors, but they cannot reverse the methylation status of DNA and histones (Rohle et al., 2013; Wang et al., 2013). Among the IDH1/IDH2 mutants, only IDH1R132, IDH1R100, IDH2R172, and IDH2R140 can produce 2-HG (Table 1) (Ward et al., 2012). IDH mutation is one of the early genetic changes in the progression of glioma, which occurs earlier than TP53 mutation and 1p/19q deletions (Watanabe et al., 2009; Yan et al., 2009). It suggests that in addition to competitive inhibition of α-KG-dependent dioxygenases by their product 2-HG, IDH mutations may affect multiple signaling pathways in tumors.

In fact, metabolic changes induced by IDH mutations per se are sufficient to produce a number of direct impacts: NADPH is an important electron donor of glutathione, thioredoxin, and some transcription factors including NF-κB and AP1, and it is important for the regulation of intracellular redox state. IDH mutations lead to increased consumption of NADPH in cells and are more susceptible to reactive oxygen species damage.

<table>
<thead>
<tr>
<th>IDH Mutation</th>
<th>Occurs In</th>
<th>WT activity</th>
<th>Neomorphic</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1 R132_</td>
<td>glioma</td>
<td>NO</td>
<td>YES</td>
<td>R(-)-2HG production</td>
</tr>
<tr>
<td></td>
<td>leukemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>chondrosarcoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDH1 R100_</td>
<td>glioma</td>
<td>NO</td>
<td>YES</td>
<td>R(-)-2HG production</td>
</tr>
<tr>
<td>IDH1 G97D</td>
<td>colon CA cell lines</td>
<td>NO</td>
<td>YES</td>
<td>R(-)-2HG production</td>
</tr>
<tr>
<td>IDH1 Y139D</td>
<td>predicted</td>
<td>NO</td>
<td>YES</td>
<td>R(-)-2HG production</td>
</tr>
<tr>
<td>IDH2 R172_</td>
<td>glioma</td>
<td>NO</td>
<td>YES</td>
<td>R(-)-2HG production</td>
</tr>
<tr>
<td></td>
<td>leukemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>chondrosarcoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDH2 R140_</td>
<td>leukemia</td>
<td>R(-)-2HG aciduria</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>IDH1 V71I</td>
<td>SNP</td>
<td>YES</td>
<td>NO</td>
<td>WT activity</td>
</tr>
<tr>
<td>IDH1 V178I</td>
<td>SNP</td>
<td>YES</td>
<td>NO</td>
<td>WT activity</td>
</tr>
<tr>
<td>IDH1 I99M</td>
<td>leukemia (one case)</td>
<td>YES</td>
<td>NO</td>
<td>WT activity</td>
</tr>
<tr>
<td>IDH1 G123R</td>
<td>thyroid CA (one case)</td>
<td>YES</td>
<td>NO</td>
<td>WT activity</td>
</tr>
<tr>
<td>IDH1 I130M</td>
<td>thyroid CA (one case)</td>
<td>YES</td>
<td>NO</td>
<td>WT activity</td>
</tr>
<tr>
<td>IDH1 H133Q</td>
<td>thyroid CA (one case)</td>
<td>YES</td>
<td>NO</td>
<td>WT activity</td>
</tr>
<tr>
<td>IDH2 V294M</td>
<td>melanoma (one case)</td>
<td>YES</td>
<td>NO</td>
<td>WT activity</td>
</tr>
<tr>
<td>IDH1 G70D</td>
<td>thyroid CA (six cases)</td>
<td>NO</td>
<td>NO</td>
<td>loss of function</td>
</tr>
<tr>
<td>IDH1 A134D</td>
<td>thyroid CA (two cases)</td>
<td>NO</td>
<td>NO</td>
<td>loss of function</td>
</tr>
<tr>
<td>IDH1 R49C</td>
<td>pediatric glioblastoma (one case)</td>
<td>NO</td>
<td>NO</td>
<td>loss of function</td>
</tr>
<tr>
<td>IDH2 F394_</td>
<td>T-cell angioimmunoblastic lymphoma (two cases)</td>
<td>NO</td>
<td>NO</td>
<td>loss of function</td>
</tr>
</tbody>
</table>

Modified from: Ward et al., 2012.
leading to cell membrane damage, changes in enzymatic activity, and oxidative damage to DNA and RNA, thereby activating relevant oncogenic signaling pathways and resulting in tumor development and progression.

**Value of IDH mutations in clinical diagnosis and treatment**

**IDH mutations contribute to tumor classification**

Conventional cancer diagnostic grading is based on histopathologic analysis, but there are many limitations in the histopathologic method when the samples are difficult to access or the samples resemble each other in morphology. In recent years with the development of molecular biology, cancer diagnostic grading has evolved from the cellular level to the molecular level, and is no longer limited to traditional histopathologic analysis.

**IDH** mutation is closely correlated with tumor histological classification, and **IDH** mutation is an early event in the progression of glioma (Watanabe et al., 2009; Yan et al., 2009). Therefore, it can be recognized as one of the diagnostic markers of glioma, which will not only lead to more timely and comprehensive diagnosis, but also contribute to further study of tumor pathogenesis and biological characteristics. Moreover, abnormally elevated levels of 2-HG in the peripheral blood is the important biochemical indicator for **IDH**/**IDH** mutation (Dang et al., 2010; Ward et al., 2012). 2-HG can serve as a sensitive and specific predictor if physiological 2-HG range is determined.

**IDH mutation analysis helps to predict prognosis and develop targeted therapy**

The patients with glioma with **IDH** mutations tend to have a better prognosis (Houillier et al., 2010; Songtao et al., 2012), while the expression of mutant **IDH**R132H** decelerated proliferation and migration capabilities of glioma cells, and mice transplanted with **IDH**R132H gliomas also exhibited longer survival (Bralten et al., 2011). Given the important role of mutant **IDH**/**IDH** and their products in cancer progression, it is feasible to develop cancer therapy targeting the mutant enzymes and their products. Rohle et al. (2013) and Wang et al. (2013) synthesized small molecules AGI-5198 and AGI-6780 to specifically inhibit the activity of mutant **IDH**/**IDH**. These compounds inhibited the proliferation of glioma cells and leukemic cells, restored the expression of cytokines related to differentiation, and induced differentiation of tumor cells, which confirmed the feasibility of targeted therapy for these tumors with mutant **IDH**/**IDH**. Recently, the Food and Drug Administration has approved the clinical trials of AG-120 and AG-221, which are targeted inhibitors for mutant **IDH** and **IDH**. In April 2014, Agios reported the results of their clinical trial of a drug designed to inhibit the mutant **IDH** enzyme. Of the seven patients with advanced AML as well as **IDH** mutations, tumor cells were undetectable in the peripheral blood of five patients after five cycles of drug administration (Ledford, 2014). This provides a new therapeutic strategy for such tumors.

**Perspective**

Mutations of **IDH**/**IDH** are closely related to tumor progression and prognosis, and the detection of **IDH**/**IDH** mutations and their product 2-HG has potential clinical applications. In-depth study of **IDH**/**IDH** mutations will not only contribute to the diagnosis of cancer using **IDH**/**IDH** mutation and 2-HG as biomarkers, but also help in the development of targeted therapeutics for these mutations and their metabolites, which has positive implications for the prevention of cancer and individualized treatment.

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