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REVIEW ARTICLE

Correlation between *IDH*, *ATRX*, and *TERT* promoter mutations in glioma

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Abstract
According to the 2016 World Health Organization (WHO) classification of central nervous system tumors, diffuse astrocytic and oligodendroglial tumors are differentiated by the presence of *isocitrate dehydrogenase 1 or 2* (*IDH1/2*) mutation and the combined loss of the short arm of chromosome 1 and the long arm of chromosome 19 (1p/19q co-deletion). *IDH*-mutant astrocytoma often has *p53* and *alpha-thalassemia/mental retardation syndrome X-linked* (*ATRX*) mutation, showing the alternative lengthening of telomeres (ALT) phenotype, while *IDH*-mutant and 1p/19q-co-deleted oligodendroglioma often have wild-type *p53* and *telomerase reverse transcriptase* (*TERT*) promoter mutation, showing telomerase activation. This study analyzed *IDH*, *ATRX*, and *TERT* promoter mutations, and the correlation between them. Immortalized cells overcome the telomere-related crisis by activating telomerase or ALT. In glioma, telomerase is mainly activated by *TERT* promoter mutation, while ALT is usually associated with *ATRX* mutation. Although the mechanism of how *ATRX* mutation induces ALT remains unclear, ATRX loss alone is believed to be insufficient to induce ALT. Treatments targeting telomere maintenance are promising.

Keywords *ATRX* · Glioma · *IDH* · *TERT*

Introduction

In the 2016 World Health Organization (WHO) classification of central nervous system (CNS) tumors, diffuse astrocytic and oligodendroglial tumors are differentiated by the presence of *isocitrate dehydrogenase 1 or 2* (*IDH*1/2) mutation. *IDH1/2*-mutant gliomas are further subdivided on the basis of the presence of a combined loss of the short arm of chromosome 1 and the long arm of chromosome 19 (1p/19q co-deletion). Diffuse or anaplastic astrocytoma does not have 1p/19q co-deletion, while oligodendroglioma or anaplastic oligodendroglioma shows 1p/19q co-deletion [1]. Astrocytoma often has *p53* and *alpha-thalassemia/mental retardation syndrome X-linked* (*ATRX*) mutation, showing the alternative lengthening of telomeres (ALT) phenotype, while oligodendroglioma often has wild-type (WT) *p53* and *telomerase reverse transcriptase* (*TERT*) promoter mutation, showing activation of telomerase [1] (Fig. 1). This study analyzed *IDH*, *ATRX*, and *TERT* promoter mutations and the correlation between them.

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IDH mutation

IDHs are a group of enzymes that catalyze the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (α-KG). The human body contains three types of IDHs: IDH1, IDH2, and IDH3 [2]. IDH1 is located in the cytosol/peroxisomes, while IDH2 and IDH3 are present in the mitochondria [3]. IDH1 and IDH2 are nicotinamide adenine dinucleotide phosphate (NADP) dependent, while IDH3 is nicotinamide adenine dinucleotide (NAD) dependent [4]. Only IDH3 is associated with the tricarboxylic acid cycle [5]. Mutant IDH1/2 converts α-KG into 2-hydroxyglutarate (2-HG) [6].

IDH1/2 mutation has been reported in not only gliomas [7] but also other tumors, such as acute myelogenous leukemia [8] and chondrosarcoma [9]. In addition, 2-HG

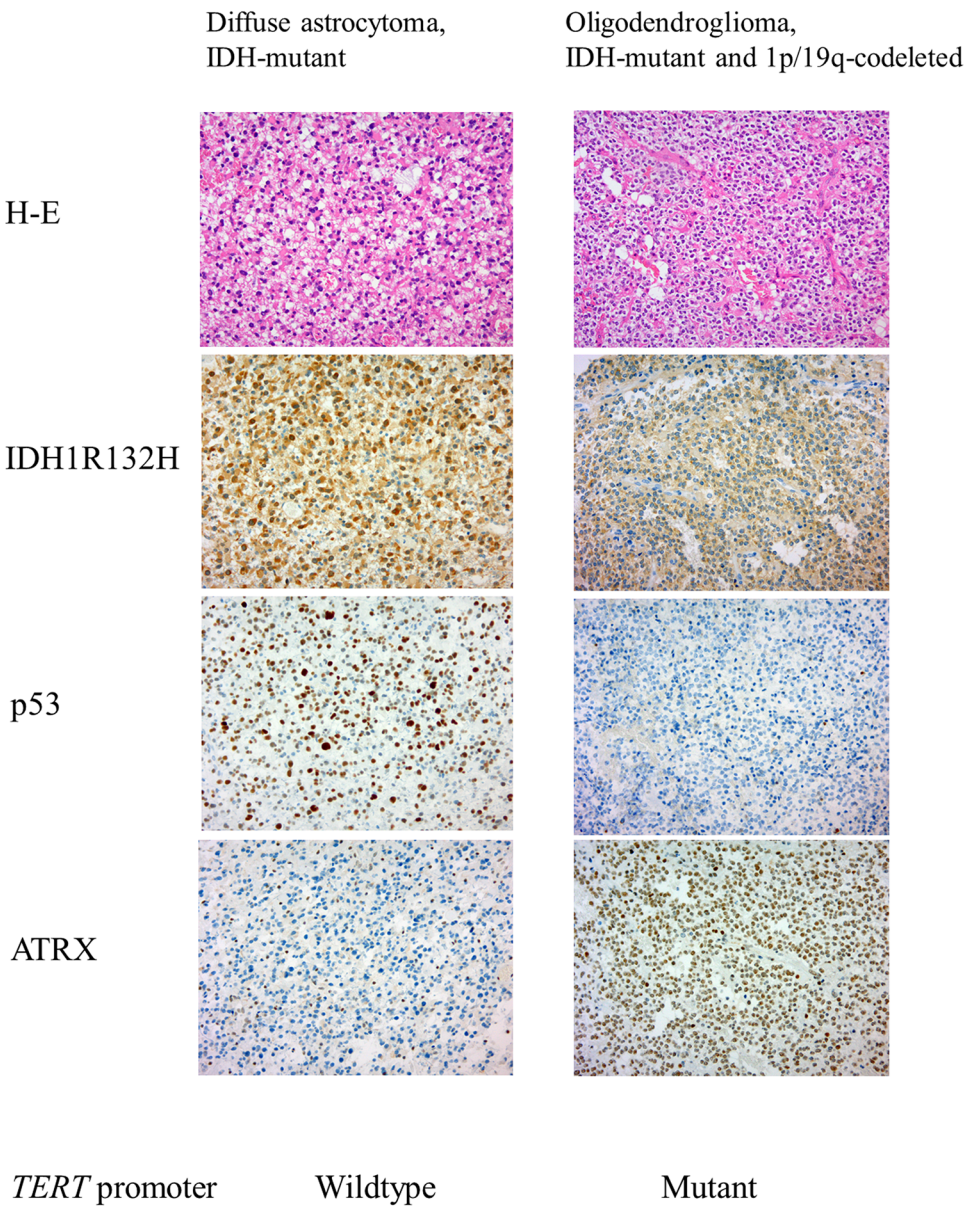

Fig. 1 The pathological characteristics of diffuse astrocytoma, IDH-mutant, and oligodendroglioma, IDH-mutant and 1p/19q-codeleted. These figures show representative pictures of diffuse astrocytoma, IDH-mutant, and oligodendroglioma, IDH-mutant and 1p/19q-codeleted. Both types of tumors are usually immunopositive for IDH1R132H. Diffuse astrocytoma, IDH-mutant is often immunopositive for p53 and immunonegative for alpha-thalassemia/mental retardation syndrome X-linked (ATRX), while oligodendroglioma, IDH-mutant and 1p/19q codeleted is often immunonegative for p53 and immunopositive for ATRX having *telomerase reverse transcriptase* promoter mutation

accumulation reportedly contributes to tumorigenesis in these cases [7].

After Parsons [10], many studies have been conducted on *IDH* mutations in glioma [11–14]. Nearly all *IDH* mutations involve a single amino acid substitution. The mutation occurs at the arginine residue at codon 132 in *IDH1* and codon 140 or 172 in *IDH2*, although R140 mutations are not found in glioma. The commonest is the R132H mutation (c.395G > A), which accounts for ~ 90% of all *IDH* mutations. *IDH1* and *IDH2* mutations usually occur exclusively [7]. *IDH1/2* mutation is reportedly detected in 80–90% of grade II and III glioma and 5% of primary glioblastoma [3]. After publication of the 2016 WHO classification of CNS tumors, a glioma needs to have an *IDH* mutation to be classified as an oligodendroglioma [1]. Since the IDH1R132H alteration occupies ~ 90% of *IDH* mutations, immunohistochemistry (IHC) evaluation with the IDH1R132H antibody can cover ~ 90% of *IDH1/2* mutation cases. In addition, direct DNA sequence analysis is performed to detect the *IDH* mutations, depending on the facilities.

IDH mutation has been detected as a common mutation between primary and recurrent tumors [15–17]. It has also been detected as a common mutation among samples from multiple regions in the same patients [16]. Therefore, *IDH* mutation is considered to occur at the early stage of tumorigenesis and as a driver mutation in *IDH*-mutant gliomas [7, 15]. The mechanism underlying gliomagenesis by mutant *IDH1/2* has not been clearly elucidated. However, several possible hypotheses are reported (Fig. 2). The structures of 2-HG and α-KG are

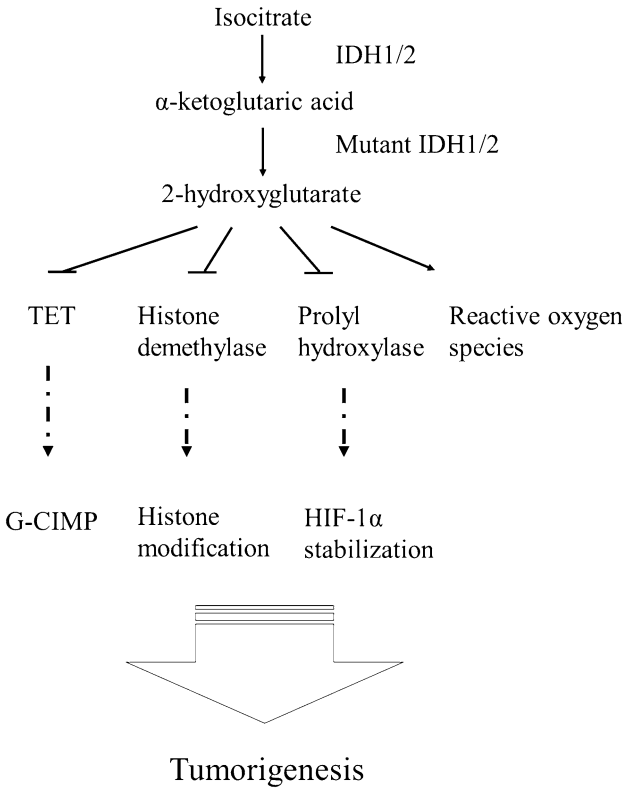

Fig. 2 The hypothesis of mutant IDH1/2-induced tumorigenesis. Mutant IDH1/2-induced 2-hydroxyglutarate (2-HG) competes with α-ketoglutaric acid (α-KG) and inhibits α-KG-dependent enzymes, such as the ten-eleven translocation (TET) enzyme family, histone demethylases, and prolyl-hydroxylase. G-CIMP induced by inhibition TET family, or histone methylation leads to gene expression. 2-HG stabilizes hypoxia-inducible factor 1-alpha (HIF-1α) and expression and increases the expression of HIF-1α target gene by inhibition of prolyl-hydroxylase. 2-HG also increases reactive oxygen species through the NAPD/NAPDH balance. These factors are considered to contribute to tumorigenesis

similar, and 2-HG competes with α-KG and inhibits α-KG-dependent enzymes, such as the ten-eleven translocation (TET) enzyme family of five methylcytosine hydroxylases and Jumonji C domain-containing histone demethylases [15, 18]. Mutant IDH-induced 2-HG in glioma inhibits the TET family, which might lead to global DNA methylation in the glioma CpG island methylation phenotype (G-CIMP) [19]. Gliomas with G-CIMP show gene expression of the proneural type classified by Verhaak et al. [20, 21]. These epigenetic changes are believed to affect gene expression, contributing to tumorigenesis. Histone demethylase inhibition by 2-HG increases histone methylation. This epigenetic status might also affect gene expression [18]. 2-HG reportedly stabilizes hypoxia-inducible factor 1-alpha (HIF-1α) expression and subsequently results in an increase in HIF-1α target gene expression by inhibition of prolyl-hydroxylase enzymes [22]. Consequently, *IDH*-mutant glioma cells escape from a hypoxic environment [4]. In addition, 2-HG increases reactive oxygen species through the NAPD/NAPDH balance, contributing to tumorigenesis [23].

IDH1R132H is a main mutation among *IDH* mutations, so IHC evaluation using IDH1R132H antibody is widely used. To check other types of mutations, direct DNA sequence analysis is also performed. IHC is reportedly 100% sensitive and specific in detecting IDH1R132H mutations [24]. The consistency rate between IHC and DNA sequence analysis is reported to be 88% in diffuse glioma patients [25]. As noninvasive methods, mutant *IDH1/2*-induced 2-HG is reported to be detectable by magnetic resonance spectroscopy [26].

IDH1/2 mutation is considered a driver mutation in *IDH*-mutant glioma, and inhibition of mutant *IDH1/2* is a promising treatment for such gliomas. Several clinical trials are ongoing [4, 27]. In experimental studies, the effects of IDH inhibitors are controversial. IDH inhibitors inhibit the proliferation of *IDH1*-mutant glioma cells [28], while inhibition of *IDH* mutation is ineffective [29] or works only in a narrow window of time [30].

Telomere length in gliomas

Telomeres are DNA–protein complexes that protect chromosome ends. These protein complexes are called Shelterin, which comprise TRF1, TRF2, POT1, TIN1, TPP1, and RAP1 [31]. Telomeres in vertebrates comprise a region of 3000–20,000 TTAGGG repeats at the ends of chromosomes [32]. The length of a telomere shortens after each cell division, and cells go to the arrest stage after limitation [33]. To overcome this issue, many types of tumor cells maintain the telomere length via telomerase activation, while some types of tumors elongate the telomere length by telomere-independent manner, which is known as ALT [32]. These oncogenic changes in glioma are generally exclusive [34]. Telomerase has two principle components, the 1132-amino acid telomerase reverse transcriptase (TERT) and an associated telomerase RNA molecule (TERC) [35]. *TERT* promoter mutation increases TERT expression and activates telomerase activity [36, 37]. Another mechanism is ALT, with which ATRX, death-associated protein 6 (DAXX), and histone H3.3 are reportedly associated [32, 38].

IDH-mutant diffuse or anaplastic astrocytoma shows the ALT phenotype, while *IDH*-mutant and 1p/19q-co-deleted oligodendroglioma or *IDH*-WT glioblastoma shows *TERT* promoter mutation [36, 39, 40]. Although both tumor types have the same *IDH* mutation, why astrocytoma selects the ALT phenotype and oligodendroglioma selects *TERT* promoter mutation to resolve telomeric dysfunction and maintain telomere length remains unknown.

Telomerase

TERT promoter mutation occurs at positions 124 and 146 bp upstream of the *TERT* ATG start site, called C228T and C250T, respectively [34]. These mutations are exclusive. Messenger RNA (mRNA) expression of *TERT* increases in gliomas with *TERT* promoter mutation compared to gliomas without *TERT* promoter mutation [36, 37]. The telomere length is shorter in glioma with *TERT* promoter mutation compared to glioma without *TERT* promoter mutation [37]. *TERT* promoter mutations generate identical 11 bp sequences that form a de novo binding site. The E26 transformation-specific transcription factor GA-binding protein selectively binds to the site and activates TERT [41]. *TERT* promoter mutation is believed to be associated with tumorigenesis in two phases: by promoting immortalization and genomic instability. In the first phase, *TERT* promoter mutation extends the cellular life span by extending the shortest telomeres but does not prevent most telomere shortening. In the second phase, the critically short telomeres inducing genome instability and telomerase are upregulated. Consequently, cells maintain growth [42]. *TERT* promoter mutation is considered the main mechanism of *TERT* transcript upregulation; however, other mechanisms have also been reported [43, 44]. For example, mutant *IDH* activates the *TERT* promoter, increasing histone lysine methylation and c-Myc/Max binding at the *TERT* promoter [44].

Several studies have reported the correlation between *TERT* promoter mutation and prognosis in glioma [39, 45–47], but few studies have compared prognoses within the same diagnosis based on the 2016 WHO classification [46]. Pekmezci et al. showed that of 291 grade II or III oligodendroglioma, IDH-mutant and 1p/19q-codeleted patients, 94% had only *TERT* promoter mutation, 0.69% had only *ATRX* mutation, 1.7% had both *TERT* promoter and *ATRX* mutation, and 4% had neither *TERT* promoter nor *ATRX* mutation [46]. The group with *TERT* promoter mutation had significant better overall survival (OS) compared to the group with *TERT* promoter WT group [46]. Of 154 *IDH*-WT astrocytoma patients, 60% showed only *TERT* promoter mutation, and 2% had both *TERT* promoter and *ATRX* mutation. The group with *TERT* promoter mutation had significantly worse OS compared to the *TERT* promoter WT group [46]. Akyerli et al. classified hemispheric diffuse glioma using the *IDH* and *TERT* promoter mutation status: both *IDH* and *TERT* promoter mutation, only *IDH* mutation, only *TERT* promoter mutation, and neither *IDH* nor *TERT* promoter mutation. Every group had a distinct demographic, anatomical, clinical, and prognostic correlation [39], and the *TERT* promoter mutation status of tumors was unchanged over time or recurred [39]. Similarly, in a meta-analysis, Vuong et al. classified WHO grade II or III glioma patients into four groups on the basis of the *IDH* and *TERT* promoter mutation status and showed the difference in OS between the groups (both *IDH* and *TERT* promoter mutation > only *IDH* mutation > neither *IDH* nor *TERT* promoter mutation > only *TERT* promoter mutation) [47].

Telomere maintenance is expected to be a new therapeutic target. Several approaches have been followed, such as vaccines, antisense oligonucleotides, and small-molecule inhibitors, to inhibit *TERT*, *TRRC*, or *TERT* promoter mutation [48–53]. However, these reagents might be ineffective in tumors with the ALT phenotype.

ALT

Although telomerase activity is the most frequent mechanism for maintaining telomere length, 10%–15% of cancers also show a telomerase-independent mechanism for elongating telomeres (ALT) [54]. Although the ALT phenotype is uncommon in tumors, it is common in certain cancer subtypes, including glioma and sarcoma [32, 54]. Among gliomas, ALT is often detected in WHO grade II or III *IDH*-mutant astrocytoma, and *IDH*-mutant glioblastoma [46]. Tumor cells with the ALT phenotype depend on the activation of a homologous recombination DNA repair mechanism to maintain telomere length [55]. ALT often begins with the loss of chromatin-remodeling proteins in telomeres, resulting in DNA damage response, recombination, and abnormal protein behavior, which reportedly initiates ALT [32, 55].

Addition of ATRX suppresses the ALT phenotype, so ATRX loss is associated with the ALT phenotype [56]. However, studies have reported that ATRX loss alone is insufficient to induce the ALT phenotype [56, 57]. In contrast, IDH1R132H overexpression in glioma cell lines downregulates *ATRX* and induces telomere lengthening consistent with ALT [58]. The authors suggested that IDH1R132H alone is sufficient to diminish ATRX expression and induces ALT. Inconsistent with their findings, neither overexpressed IDH1R132H alone nor *ATRX* knockout alone induces the ALT phenotype [44, 57]. Exogenous IDH1R132H expression combined with *ATRX* mutation induces the ALT phenotype [57]. Mutant *IDH1* downregulates a Shelterin protein, RAP1, which leads to telomere dysfunction. Mutant *IDH1* also downregulates XRCC, which leads to nonhomologous end-joining (NHEJ) inhibition. This downregulation of RAP1 and XRCC reportedly contributes to ALT in glioma cells with ATRX loss [57].

The cIMPACT Update 2 report showed that diffuse astrocytic-appearing WHO grade II or III glioma with *IDH* mutation and ATRX nuclear expression loss and/or strong, diffuse p53 immunopositivity can be diagnosed as *diffuse*

astrocytoma, IDH-mutant or *anaplastic astrocytoma, IDH*-mutant without checking the 1p/19q co-deletion status [59]. IHC analysis for ATRX, not sequence analysis for *ATRX*, was described in this report. Since *ATRX* is relatively large, it is difficult to perform the assay to detect the mutation as a routine test. Therefore, IHC analysis is usually performed as an alternative method for evaluating *ATRX* mutation. Ikemura et al. (2016) examined 193 patients and divided them into three groups: ATRX loss with staining loss in > 90% of tumor cells ($n = 43$), ATRX indeterminate with staining loss in 10%–90% of tumor cells ($n = 0$), and ATRX retained with staining loss in < 10% of tumor cells ($n = 150$) [60]. Other studies divided patients into two groups, with nuclear ATRX loss and with nuclear ATRX retention, using a cutoff value of 10% [61, 62].

ATRX mutation is associated with prognosis in glioma [63, 64]. However, in most cases, tumor diagnosis is performed on the basis of pre-2016 WHO classification. Since most *IDH*-mutant astrocytomas and *IDH*-mutant glioblastomas show ATRX loss and most *IDH*-mutant and 1p/19q-co-deleted oligodendrogliomas and *IDH*-WT glioblastomas show ATRX expression, the comparison between low-grade gliomas or between glioblastomas might be a comparison between astrocytoma and oligodendroglioma or between *IDH*-WT glioblastoma and *IDH*-mutant glioblastoma. In a comparison within the same diagnosis of 220 cases of *IDH*-mutant diffuse astrocytoma and 181 cases of *IDH*-mutant anaplastic astrocytoma, 78% had only *ATRX* mutation, 2% had both *TERT* and *ATRX* mutation, 5% had only *TERT* mutation, and 16% had neither *TERT* nor *ATRX* mutation. Neither *TERT* nor *ATRX* status was associated with survival [46]. In *IDH*-mutant glioblastoma patients, the rate of each mutation is similar to that in *IDH*-mutant WHO grade II and III astrocytoma patients. Sixty-three percentage had only *ATRX* mutation, 6% had both *TERT* and *ATRX* mutation, 12% had only *TERT* mutation, and 20% had neither *TERT* nor *ATRX* mutation. Neither *TERT* nor *ATRX* status was associated with survival [46].

Several new treatments have been recommended for targeting tumor cells with the ALT phenotype [35, 54]. CRISPR-Cas9 screening detected Wee1 as a target in *ATRX*-null tumor cells [65]. The ATR inhibitor reportedly disrupts ALT and triggers chromosome fragmentation and apoptosis in ALT cells [66]. Histone deacetylase inhibitors might suppress ALT through NuRD inhibition [67]. Although these treatments are expected to be effective in tumor cells with the ALT phenotype, treatments that are effective independent of activated telomerase or ALT are promising. G-quadruplexes are tetra-stranded DNA structures formed by guanine-rich sequences. Studies have reported a G-quadruplex stabilizer that activated the pathways of response to DNA damage and induce senescence in both telomerase-positive and ALT phenotype cells [68].

Conclusion

In this review, we discussed the three mutations of *IDH*, *ATRX*, and *TERT* promoter in glioma. It remains unclear why astrocytoma, IDH-mutant has ALT phenotype, whereas oligodendroglioma, IDH-mutant and 1p/19q-codeleted showed activated telomerase. Further studies may clarify it and new treatments targeting maintenance of telomere are promising.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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