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MGMT Expression Contributes to Temozolomide Resistance in H3K27M-Mutant Diffuse Midline Gliomas and MGMT Silencing to Temozolomide Sensitivity in IDH-Mutant Gliomas

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Abstract

Histone H3 mutations are frequently found in diffuse midline gliomas (DMGs), which include diffuse intrinsic pontine gliomas and thalamic gliomas. These tumors have dismal prognoses. Recent evidence suggests that one reason for the poor prognoses is that O^6-methylguanine-DNA methyltransferase (MGMT) promoter frequently lacks methylation in DMGs. This review compares the epigenetic changes brought about by histone mutations to those by isocitrate dehydrogenase-mutant gliomas, which frequently have methylated *MGMT* promoters and are known to be sensitive to temozolomide.

Key words: MGMT, diffuse midline gliomas, Histone H3 mutation, resistance, epigenetics

Introduction

Diffuse midline gliomas (DMGs), including diffuse intrinsic pontine gliomas (DIPGs) and thalamic gliomas, have dismal prognoses: 8–11 months for DIPGs[1,2] and about 25 and 12 months for World Health Organization (WHO) grades 3 and 4 thalamic gliomas, respectively.[3,4] Possible explanations for the poor prognosis include difficulty of surgery[5] and the ineffectiveness of temozolomide.[6]

It is well known that malignant gliomas with isocitrate dehydrogenase (IDH) mutation have a good prognosis[7,8] compared to IDH-wildtype gliomas. A majority of IDH-mutant gliomas are known to have O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation and respond to temozolomide.[9]

Recent genetic studies have shown that up to 90% of DMGs have mutations in histone H3.3 H3K27M encoding the gene *H3F3A* or H3.1 H3K27M encoding *HIST1H3B*.[10–15] H3.3 H3K27M mutations are about 2.5-fold more frequent, present at an older age, have a gender predisposition toward boys, and carry a worse prognosis compared to DIPGs with H3.1 H3K27M mutations.[12] Epigenetic studies have shown that histone mutations cause DNA hypomethylation,[16,17] whereas IDH mutation causes DNA hypermethylation.[17,18] We review the increasing evidence that this epigenetic modification renders IDH-mutant gliomas sensitive to temozolomide, but not DMGs.[19]

IDH-mutant Gliomas Have Frequent *MGMT* Promoter Methylation and Are Sensitive to Temozolomide

A seminal study in glioblastomas showed that recurrent mutations in *IDH1* is seen in approximately 10% of glioblastomas.[20] Subsequent studies have shown that *IDH1* and *IDH2* mutation are frequently seen in WHO grades 2 and 3 astrocytomas and oligodendrogliomas,[8] and that IDH-mutation is a vital, early event in gliomagenesis.

IDH mutations are known to be gain-of-function mutations, which produce the oncometabolite R-2-hydroxyglutarate (2HG).[21] The 2HG is structurally similar to alpha-ketoglutarate (α-KG), which is necessary to produce the DNA demethylase TET2 and histone demethylases (JMJs). 2HG competitively inhibits DNA and histone demethylases,[22] causing diffuse deoxyribonucleic acid (DNA)

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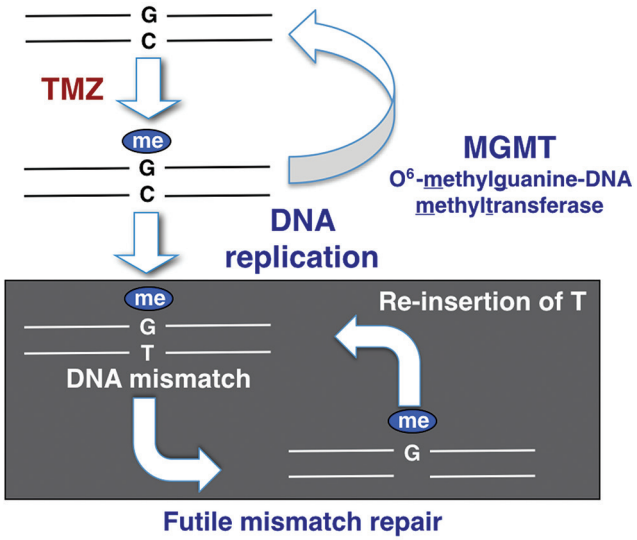

Fig. 1 A schematic drawing showing the relationship between *MGMT* promoter methylation and MGMT protein expression. When the *MGMT* promoter is methylated, transcription is repressed and thus MGMT protein is not produced.

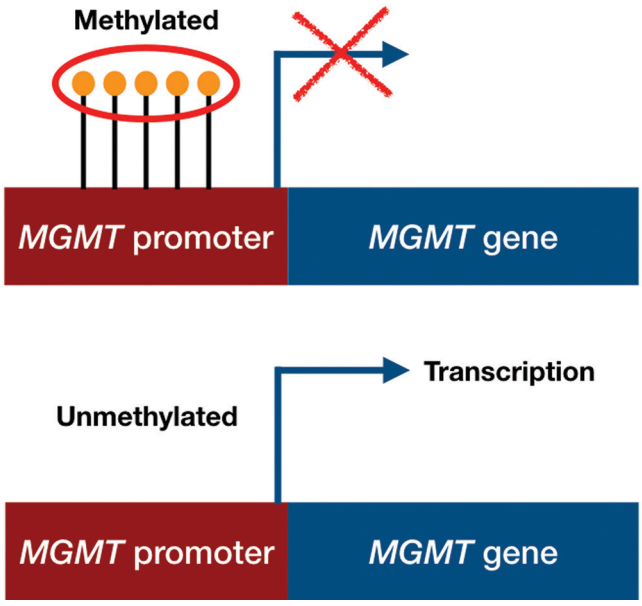

Fig. 2 The main mechanism of action of temozolomide is to add a methyl-group at the O⁶ position of guanine (G) in the DNA of glioma cells, causing a methyl-guanine (meG)-to-thymine (T) mismatch at DNA replication, instead of cytosine (C). Mismatch repair genes locate the meG-T mismatch and remove the T, only to have a T re-inserted. This insertion and removal of T, called the "futile mismatch repair", contributes to the vulnerability of tumor DNA and ultimately leads to apoptosis. MGMT, which is expressed in normal cells but lost in a percentage of brain tumors, removes the methyl group at the O⁶ position of guanine added by temozolomide, neutralizing its effect.

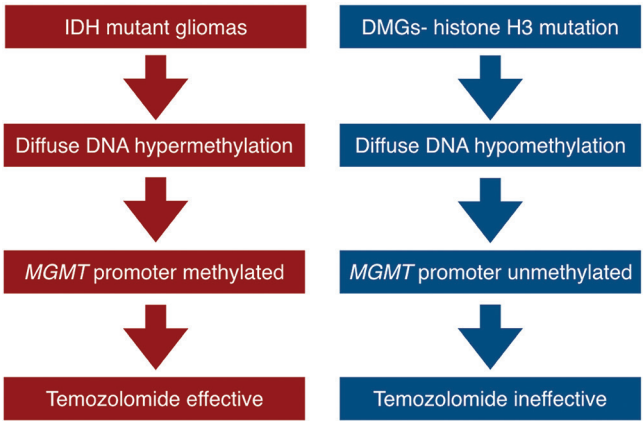

Fig. 3 A flow chart showing the relationship between epigenetic changes in DNA, *MGMT* promoter methylation and response to temozolomide in IDH-mutant gliomas (left side) and diffuse midline gliomas with histone H3K27 mutations (right side).

hypermethylation [the so-called "glioma-CpG island methylator phenotype (G-CIMP) phenotype"][18] and histone hypermethylation.[23]

A large proportion of G-CIMP cases are known to have *MGMT* promoter methylation. Data from the NOA-04 trial found that 96% of G-CIMP cases had methylated *MGMT* promoters.[24] Also, 88% of oligodendrogliomas, which are known to harbor IDH mutations, were found to have *MGMT* promoter methylation.[25]

It is well known that *MGMT* promoter methylation is a predictive factor of response to temozolomide.[9,26] The main mechanism of action of temozolomide is to add a methyl-group at the O⁶ position of guanine (G) in the DNA of glioma cells, causing a methyl-guanine (meG)-to-thymine (T) mismatch at DNA replication, instead of cytosine (C) (Fig. 1). Mismatch repair genes locate the meG-T mismatch and remove the T, only to have a T re-inserted. This insertion and removal of T, called the "futile mismatch repair", contributes to the vulnerability of tumor DNA and ultimately leads to death of the tumor cell. MGMT, which is expressed in normal cells but lost in a percentage of brain tumors, removes the methyl group at the O⁶ position of guanine added by temozolomide, neutralizing its effect (Fig. 1). MGMT expression is epigenetically regulated.[9] Thus, *MGMT* promoter methylation inhibits the transcription of MGMT leading to MGMT silencing (Fig. 2).

Taken together, we can conclude that IDH-mutant gliomas express the G-CIMP phenotype, frequently have *MGMT* promoter methylation and are sensitive to temozolomide (Fig. 3, left side). Secondary

glioblastomas harboring IDH-mutations are known to be sensitive to temozolomide therapy.[27]

Histone H3-mutant Diffuse Midline Gliomas Have Frequent Unmethylated *MGMT* Promoter and Are Resistant to Temozolomide

In contrast to IDH-mutation, in which diffuse DNA hypermethylation occurs, epigenetic studies have shown that histone mutations, including H3K27M and H3G34R/V (seen in pediatric glioblastoma of the cerebrum), cause DNA hypomethylation.[16,17,28] Recent studies suggest that MGMT is almost always expressed in DMGs. None of the 46 DMGs with confirmed *H3F3A* mutation showed *MGMT* promoter methylation in a report by Banan et al.[29] Similarly, Korshunov et al.[30] reported that *MGMT* promoter was methylated in only 3% of DIPGs with H3K27M mutations.

Furthermore, Oka et al.[31] showed that MGMT was expressed in 9 out of 11 (82%) brainstem gliomas in which immunohistochemical analysis of MGMT was feasible. From these reports, we can postulate that epigenetic changes driven by histone H3K27M mutation cause frequent lack of *MGMT* promoter methylation, thus expression of MGMT and resistance to temozolomide therapy (Fig. 3, right side).

Future Directions and Therapeutic Implications

Temozolomide is a key drug used in the treatment of glioblastomas, and is often used in the treatment of WHO grade 3 malignant gliomas as well. However, increasing evidence suggests that temozolomide is not effective in DMGs. Despite some effort for aggressive surgical intervention,[4] the clinical outlook for DMGs remain dismal. Here, we outline just some of the new preclinical and clinical efforts to eradicate this disease.

Epigenetic modification

Since global reduction of H3K27 methylation is a key epigenetic event in H3K27M mutant DMGs, pharmacologic restoration of H3K27 methylation either by enhancing H3K27 methyltransferase (PRC2) activity or by inhibiting H3K27 demethylase activity for the lysine 27 residue is a rational method to treat DMGs.[32] The latter can be achieved by using the H3K27 demethylase inhibitor GSKJ4. Decreased histone methylation at H3K27 causes increased histone acetylation in DIPG, which can also be targeted. The HDAC inhibitor panobinostat was found to be effective in DIPG cell lines through a screening of 83 drugs. Panobinostat was found to increase H3 acetylation and restore H3K27 trimethylation.[19] This data has led to the commencement of clinical trials looking at the efficacy of panobinostat in DIPGs. Two recent high-profile papers show the efficacy of BET bromodomain inhibitors, which prevent the interaction of BRD4 with acetylated histone, leading to the repression of BRD4 transcriptional targets and proliferation.[33,34]

Targeting of associated mutations

Epigenetic modification can be very toxic, as drugs will affect the epigenetic status of normal cells as well as tumor. Less toxic treatments including localized delivery and targeted treatments need to be explored. One potential avenue of treatment is targeting of mutations associated with H3K27M mutations. Mutations in activin receptor type 1 (*ACVR1*) are frequently seen in H3.1 H3K27M mutant, but not H3.3 H3K27M mutant DMGs.[12,35] *ACVR1* encodes for type I bone morphogenic protein (BMP) receptor ALK2, and mutation of this receptor leads to constitutive activation of BMP signaling pathway.[35] Targeted treatment using the ALK2 inhibitor LDN-193189 showed moderate response *in vitro*.[15]

FGFR1 mutations are seen in 4–27% of thalamic high-grade gliomas, but not DIPGs,[35] and is a potential target for thalamic gliomas.

Targeting of PARP

As stated above, the main mechanism of action for temozolomide is to add a methyl-group at the O^6-position of guanine, which is removed by MGMT (Fig. 1). However, temozolomide is also known to methylate adenine at N^3-position and guanine at the N^7-position. These do not generally induce cytotoxicity, as poly(ADP-ribose) polymerase (PARP) activation allows for base excision repair of damaged DNA. Evidence suggests that inhibition of PARP or depletion of NAD^+ which is a co-enzyme of PARP, can lead to cytotoxicity.[36] Interestingly, a study by Chornenkyy et al.[37] shows PARP1 expression in DIPG cell lines and sensitivity to the PARP inhibitor niraparib.

Inhibition of PTEN/AKT/mTOR signaling pathway

Approximately, 70% of DIPGs have either *AKT* gain or phosphatase and tensin homolog deleted on chromosome 10 (PTEN) loss,[38,39] suggesting that targeting of the PTEN/AKT/mechanistic target of rapamycin (mTOR) signaling pathway is a potential therapeutic strategy for DIPGs. Miyahara et al.[40] and others[41] reported the efficacy of dual mTOR inhibition *in vitro* and *in vivo*.

Immunotherapy

Okada and colleagues established T cell receptor-transduced T cells recognizing a peptide sequence encompassing the H3.3K27M mutation. Preclinical data shows significant suppression of glioma xenografts in mice.[42] Development of a peptide vaccine recognizing IDH1 R132H mutant glioma[43] has led to exploration of a similar peptide vaccine recognizing H3K27M.[44] Major histocompatibility complex (MHC) class 2 response, which allows proteins to be degraded into peptides and sent to the surface of the cell,[45] enables intracellular mutant proteins to be expressed at the surface of tumor cells. A Phase I clinical trial (NCT02960230) testing the safety of an H3.3K27M peptide vaccine is currently underway.

Convection-enhanced delivery and other methods of delivery

Convection-enhanced delivery (CED) of drugs to the brainstem remains a promising candidate for treatment of DIPGs. Preclinical brainstem tumor models have been treated with CED of various drugs including temozolomide.[46] In Japan, Saito et al.[47] have published a case report showing radiographical response after CED of nimustine hydrochloride (ACNU) in a patient with recurrent glioblastoma infiltrating into the brainstem. Intranasal delivery (IND) is also a promising method of delivery, as it is far less invasive than CED.[48] IND was shown to be effective in a brainstem tumor model when combined with nanoliposomal chemotherapy.[49]

Conclusion

This paper focused on what we currently known about the reason H3 mutant DMGs are not sensitive to temozolomide. Epigenetic changes brought about by H3 mutation cause DMGs to frequently lack *MGMT* promoter methylation thus express MGMT. Since radical surgery is difficult in almost all cases of DMGs, there is an urgent need for new, more effective therapies targeting DMGs. Safe, local delivery, as well as more targeted therapies are rapidly being developed and tested, but a real breakthrough remains elusive. Worldwide collaboration in research as well as clinical treatment is critical to overcome this uncommon but deadly disease.

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Conflicts of Interest Disclosure

The authors have no conflicts of interest to declare.

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