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 O6-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 DIPGs1,2) 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 surgery5) and the ineffectiveness of temozolomide.6)

It is well known that malignant gliomas with isocitrate dehydrogenase (IDH) mutation have a good prognosis7,8) compared to IDH-wildtype gliomas. A majority of IDH-mutant gliomas are known to have O6-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)
hypermethylation [the so-called “glioma-CpG island methylator phenotype (G-CIMP) phenotype”] and histone hypermethylation.

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. Also, 88% of oligodendrogliomas, which are known to harbor IDH mutations, were found to have MGMT promoter methylation.

It is well known that MGMT promoter methylation is a predictive factor of response to temozolomide. 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 death of 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.

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,\(^{40}\) 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.\(^{42}\) The latter can be achieved by using the H3K27 demethylase inhibitor GSJ4. 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.\(^{12,13,55}\) ACVR1 encodes for type I bone morphogenetic 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\(^{-}\)position of guanine, which is removed by MGMT (Fig. 1). However, temozolomide is also known to methylate adenine at N\(^{\circ}\)position and guanine at the N\(^{\circ}\)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 Chornenky 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. Development of a peptide vaccine recognizing IDH1 R132H mutant glioma has led to exploration of a similar peptide vaccine recognizing H3K27M. Major histocompatibility complex (MHC) class 2 response, which allows proteins to be degraded into peptides and sent to the surface of the cell, 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. In Japan, Saito et al. 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. IND was shown to be effective in a brainstem tumor model when combined with nanoliposomal chemotherapy.

**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.

**References**

1. Hargrave D, Bartels U, Bouffet E: Diffuse brainstem glioma in children: critical review of clinical trials. *Lancet Oncol* 7: 241–248, 2006
2. Buczkowicz P, Bartels U, Bouffet E, et al.: Histopathological spectrum of paediatric diffuse intrinsic pontine glioma: diagnostic and therapeutic implications. *Acta Neuropathol* 128: 573–581, 2014
3. Eskenazi Y, Moussazadeh N, Link TW, et al.: Thalamic glioblastoma: clinical presentation, management strategies, and outcomes. *Neurosurgery* 2017
4. Saito R, Kumabe T, Kanamori M, Sonoda Y, Tominaga T: Distant recurrences limit the survival of patients with thalamic high-grade gliomas after successful resection. *Neurosurg Rev* 40: 469–477, 2017
5. Kelly PJ: Stereotactic biopsy and resection of thalamic astrocytomas. *Neurosurgery* 25: 185–195, 1989
6. Chassot A, Canale S, Varlet P, et al.: Radiotherapy with concurrent and adjuvant temozolomide in children with newly diagnosed diffuse intrinsic pontine glioma. *J Neurooncol* 106: 399–407, 2012
7. Ogura R, Tsukamoto Y, Natsumeda M, et al.: Immunohistochemical profiles of IDH1, MGMT and P53: practical significance for prognostication of patients with diffuse gliomas. *Neuropathology* 35: 324–335, 2015
8. Yan H, Parsons DW, Jin G, et al.: IDH 1 and IDH 2 mutations in gliomas. *N Engl J Med* 360: 765–773, 2009
9. Hegi ME, Diserens AC, Gorlia T, et al.: MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 352: 997–1003, 2005
10. Wu G, Diaz AK, Faugh BS, et al.: The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nat Genet* 46: 444–450, 2014
11. Aihara K, Mukasa A, Gotoh K, et al.: H3F3A K27M mutations in thalamic gliomas from young adult patients. *Neuro-oncology* 16: 140–146, 2014
12. Castel D, Philippe C, Calmon R, et al.: Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol* 130: 815–827, 2015
13. Khuong-Quang DA, Buczkowicz P, Rakopoulos P, et al.: K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol* 124: 439–447, 2012
14. Mackay A, Burford A, Carvalho D, et al.: Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. *Cancer Cell* 32: 520–537.e5, 2017
15) Taylor KR, Mackay A, Truffaux N, et al.: Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. *Nat Genet* 46: 457–461, 2014

16) Bender S, Tang Y, Lindroth AM, et al.: Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. *Cancer Cell* 24: 660–672, 2013

17) Sturm D, Witt H, Hovestadt V, et al.: Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell* 22: 425–437, 2012

18) Turcan S, Rohde D, Goenka A, et al.: IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 483: 479–483, 2012

19) Grasso CS, Tang Y, Truffaux N, et al.: Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nat Med* 21: 555–559, 2015

20) Parsons DW, Jones S, Zhang X, et al.: An integrated genomic analysis of human glioblastoma multiforme. *Science* 321: 1807–1812, 2008

21) Dang L, White DW, Gross S, et al.: Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 462: 739–744, 2009

22) Xu W, Yang H, Liu Y, et al.: Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell* 19: 17–30, 2011

23) Lu C, Ward PS, Kapoor GS, et al.: IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature* 483: 474–478, 2012

24) Wiestler B, Capper D, Hovestadt V, et al.: Assessing CpG island methylator phenotype, 1p/19q codeletion, and MGMT promoter methylation from epigenome-wide data in the biomarker cohort of the NOA-04 trial. *Neuro-oncology* 16: 1630–1638, 2014

25) Möllmann M, Wolter M, Felsberg J, Collins VP, Reifenberger G: Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendrogial tumors. *Int J Cancer* 113: 379–385, 2005

26) Jacinto FV, Esteller M: MGMT hypermethylation: a prognostic foe, a predictive friend. *DNA Repair (Amst)* 6: 1155–1160, 2007

27) SongTao Q, Lei Y, Si G, et al.: IDH mutations predict longer survival and response to temozolomide in secondary glioblastoma. *Cancer Sci* 103: 269–273, 2012

28) Ahsan S, Raabe EH, Haffner MC, et al.: Increased 5-hydroxymethylcytosine and decreased 5-methylcytosine are indicators of global epigenetic dysregulation in diffuse pontine glioma. *Acta Neuropathol Commun* 2: 59, 2014

29) Banan R, Christians A, Bartels S, Lehmann U, Hartmann C: Absence of MGMT promoter methylation in diffuse midline glioma, H3 K27M-mutant. *Acta Neuropathol Commun* 5: 98, 2017

30) Korshunov A, Ryzhova M, Hovestadt V, et al.: Integrated analysis of pediatric glioblastoma reveals a subset of biologically favorable tumors with associated molecular prognostic markers. *Acta Neuropathol* 129: 669–678, 2015

31) Oka H, Utsuki S, Tanizaki Y, et al.: Clinicopathological features of human brainstem gliomas. *Brain Tumor Pathol* 30: 1–7, 2013

32) Hashizume R: Epigenetic targeted therapy for diffuse intrinsic pontine glioma. *Neuro Med Chir (Tokyo)* 57: 331–342, 2017

33) Piunti A, Hashizume R, Morgan MA, et al.: Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat Med* 23: 493–500, 2017

34) Nagaraja S, Vitanza NA, Woo PJ, et al.: Transcriptional dependencies in diffuse intrinsic pontine glioma. *Cancer Cell* 31: 635–652.e6, 2017

35) Fontebasso AM, Papillon-Cavanagh S, Schwartzentruber J, et al.: Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nat Genet* 46: 462–466, 2014

36) Tateishi K, Higuchi F, Miller JJ, et al.: The alkylating chemotherapy temozolomide induces metabolic stress in IDH1-mutant cancers and potentiates NAD+ depletion–mediated cytotoxicity. *Cancer Res* 77: 4102–4115, 2017

37) Chornenky Y, Agnihotri S, Yu M, et al.: Poly-ADP-ribose polymerase as a therapeutic target in pediatric diffuse intrinsic pontine glioma and pediatric high-grade astrocytoma. *Mol Can Ther* 14: 2560–2568, 2015

38) Zarhoooni M, Bartels U, Lee E, et al.: Whole-genome profiling of pediatric diffuse intrinsic pontine gliomas highlights platelet-derived growth factor receptor alpha and poly (ADP-ribose) polymerase as potential therapeutic targets. *J Clin Oncol* 28: 1337–1344, 2010

39) Warren KE, Killian K, Suuriniemi M, Wang Y, Quezado M, Meltzer PS: Genomic aberrations in pediatric diffuse intrinsic pontine gliomas. *Neuro-oncology* 14: 326–332, 2012

40) Miyahara H, Yadavilli S, Natsumeda M, et al.: The dual mTOR kinase inhibitor TAK228 inhibits tumorigenicity and enhances radiosensitization in diffuse intrinsic pontine glioma. *Cancer Lett* 400: 110–116, 2017

41) Flannery PC, DeSisto JA, Amani V, et al.: Preclinical analysis of MTOR complex 1/2 inhibition in diffuse intrinsic pontine glioma. *Onco Rep* 39: 455–464, 2018

42) Chheda ZS, Kohanbash G, Okada K, et al.: Novel and shared neoantigen derived from histone 3 variant H3.3K27M mutation for glioma T cell therapy. *J Exp Med* 215: 141–157, 2018

43) Schumacher T, Bunse L, Pusch S, et al.: A vaccine targeting mutant IDH1 induces antitumour immunity. *Nature* 512: 324–327, 2014

44) Ochs K, Ott M, Bunse T, et al.: K27M-mutant histone-3 as a novel target for glioma immunotherapy. *Oncoimmunology* 6: e1328340, 2017

45) Coulie PG, van den Eynde BJ, van der Bruggen P, Boon T: Tumour antigens recognized by T lymphocytes:
at the core of cancer immunotherapy. *Nat Rev Cancer* 14: 135–146, 2014

46) Yoshimura J, Siu IM, Thomale UW, Jallo GI: The effects of temozolomide delivered by prolonged intracerebral microinfusion against the rat brainstem GBM allograft model. *Childs Nerv Syst* 28: 707–713, 2012

47) Saito R, Sonoda Y, Kumabe T, Nagamatsu K, Watanabe M, Tominaga T: Regression of recurrent glioblastoma infiltrating the brainstem after convection-enhanced delivery of nimustine hydrochloride. *J Neurosurg Pediatr* 7: 522–526, 2011

48) Hashizume R, Ozawa T, Gryaznov SM, et al.: New therapeutic approach for brain tumors: intranasal delivery of telomerase inhibitor GRN163. *Neuro-oncology* 10: 112–120, 2008

49) Louis N, Liu S, He X, et al.: New therapeutic approaches for brainstem tumors: a comparison of delivery routes using nanoliposomal irinotecan in an animal model. *J Neurooncol* 136: 475–484, 2018

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