



Title	Prognostic role of H3K27M mutation, histone H3K27 methylation status, and EZH2 expression in diffuse spinal cord gliomas
Author(s)	Ishi, Yukitomo; Takamiya, Soichiro; Seki, Toshitaka et al.
Citation	Brain Tumor Pathology, 37(3), 81-88 https://doi.org/10.1007/s10014-020-00369-9
Issue Date	2021-07
Doc URL	https://hdl.handle.net/2115/82148
Rights	This is a post-peer-review, pre-copyedit version of an article published in Brain Tumor Pathology. The final authenticated version is available online at: http://dx.doi.org/10.1007/s10014-020-00369-9
Type	journal article
File Information	Brain Tumor Pathol_37_81.pdf



Prognostic Role of H3K27M Mutation, Histone H3K27 Methylation Status, and EZH2 Expression in Diffuse Spinal Cord Gliomas

Yukitomo Ishi, MD¹; ishi-y@huhp.hokudai.ac.jp

Soichiro Takamiya, MD¹; soichiro.tkmy@gmail.com

Toshitaka Seki, MD¹; tseki@med.hokudai.ac.jp

Kazuyoshi Yamazaki, MD¹; mt.kazu71@gmail.com

Kazutoshi Hida, MD²; kazuhida@ab.inbox.ne.jp

Kanako C. Hatanaka MD³; kyanack@huhp.hokudai.ac.jp

Yusuke Ishida, MD⁴; yuishida@abox9.so-net.ne.jp

Yoshitaka Oda, MD⁴; yoshitaka_oda_0622@yahoo.co.jp

Shinya Tanaka, MD⁴; tanaka@med.hokudai.ac.jp

Shigeru Yamaguchi, MD¹; yama-shu@med.hokudai.ac.jp

¹Department of Neurosurgery, Hokkaido University School of Medicine, North 15 West 7, Kita-ku, Sapporo 060-8638, Japan

²Department of Neurosurgery, Sapporo Azabu Neurosurgical Hospital, Sapporo, Japan

³Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan

⁴Department of Cancer Pathology, Hokkaido University School of Medicine, Sapporo, Japan

Corresponding Author:

Shigeru Yamaguchi, MD, PhD

Department of Neurosurgery

Hokkaido University Graduate School of Medicine
North 15 West 7, Kita-ku, Sapporo 060-8638, Japan
Phone: (+81)11-706-5987
Fax: (+81)11-708-7737
E-mail: yama-shu@med.hokudai.ac.jp

Abstract

OBJECTIVE: To clarify clinical significance of the *H3F3A K27M* mutation (H3K27M) and analyze the correlation between H3K27M, H3K27me3 status, and EZH2 expression and prognosis in spinal cord gliomas.

METHODS: Patients with spinal cord diffuse glioma regardless of World Health Organization (WHO) grade underwent genetic analysis for *H3F3A*, *HIST1H3B*, *TERT* promoter, *IDH1/2*, and *BRAF*. H3K27me3 status and EZH2 expression were analyzed through immunohistochemistry. Thereafter, the association between H3K27M, H3K27me3 status, and EZH2 expression and prognosis was retrospectively analyzed using the log-rank test.

RESULTS: A total of 26 cases, 5 with WHO grade 4, 9 with grade 3, and 12 with grade 2 glioma, were analyzed. Although WHO grade 2 cases tended to present favorable overall survival, the difference was not statistically significant. H3K27M, which was detected in four grade 4 cases (80%) and three grade 3 cases (33%), was not associated with prognosis among grade 3 and 4 cases. Among WHO grade 2–4 cases, the combination of retained H3K27me3 and negative EZH2 expression was correlated with favorable overall survival ($p = 0.03$).

CONCLUSION: The combination of H3K27me3 status and EZH2 expression was considered as a potential prognostic marker in WHO grade 2–4 diffuse spinal cord gliomas.

(197 words)

KEYWORDS:

spinal cord, glioma, H3F3A, H3K27me3, EZH2

SHORT TITLE:

H3K27M, H3K27me3 AND EZH2 IN SPINAL CORD GLIOMAS

ABBREVIATIONS; DMG = diffuse midline glioma; FFPE = formalin-fixed paraffin-embedded; GBM = glioblastoma; H3K27M = histone H3K27M mutation; HE = hematoxylin and eosin; 1/2; IHC = immunohistochemical staining; KDM6A = lysine demethylase 6A; MGMT = methylguanine methyltransferase; MRI = magnetic

resonance imaging; OS = overall survival; PRC2 = polycomb recessive complex 2; TMZ = temozolomide; WHO = World Health Organization

Introduction

A somatic missense mutation at amino acid position 27 of the *H3F3A* or *HIST1H3B* gene (*H3K27M*), which changes a lysine residue into a methionine residue, has been frequently detected in pediatric glioblastomas (GBMs) [1], diffuse intrinsic pontine gliomas [2], thalamic gliomas in young adults [3], and spinal cord gliomas [4, 5]. *H3F3A* and *HIST1H3B* encode histone variants H3.3 and H3.1 and regulate gene expression [1, 2]. Due to unfavorable prognosis in gliomas harboring the H3K27M mutation, “diffuse midline glioma with H3K27M mutation (DMG-H3K27M)” has been newly established in the 2016 World Health Organization (WHO) Classification of Central Nervous System Tumors [6].

Spinal cord gliomas have been more infrequent compared to intracranial gliomas, which account for 6%–8% of primary spinal cord tumors [7]. Although DMG-H3K27M frequently occurs in the spinal cord [8], little has been known regarding the clinical characteristics of DMG-H3K27M in the spinal cord due to its rarity.

Loss of trimethylation in residual H3K27 (H3K27me3), which generates global transcriptional dysregulation, has been a hallmark in gliomas harboring H3K27M [9, 10]. Polycomb repressive complex 2 (PRC2) consists of enhancer of zeste homolog 2 (EZH2) and several proteins that act as methyltransferases for H3K27 [11]. Inactivation of PRC2 due to mutant H3K27M protein has been considered to cause H3K27me3 loss [12]. H3K27me3 loss has been observed in several types of malignant tumors of the central nervous system or peripheral nerves, such as malignant peripheral nerve sheath tumor caused by an inactivating mutation in genes of the PRC2 component [13, 14] or posterior fossa type A ependymomas classified by DNA methylation status [15]. Although H3K27me3 loss has been frequently observed in DMG-H3K27M, the prognostic role of H3K27me3 status in spinal cord gliomas has remained unclear.

High EZH2 expression has been reported as a marker of poor prognosis in DMG-H3K27M [16]. In addition, EZH2 expression is required for the maintenance of cancer stem cells in cerebral GBM [17]. Therefore, EZH2 has been associated with patient prognosis in gliomas other than DMG-H3K27M. Nonetheless, the association between EZH2 expression and prognosis in spinal cord gliomas has remained unknown to date.

The present study thus aimed to clarify the clinical significance of H3K27M mutation in spinal cord gliomas, as well as determine the correlation between

H3K27me3 status and EZH2 expression and prognosis in spinal cord gliomas.

Methods

Patient population

This retrospective study included patients with spinal cord diffuse glioma. Pathological diagnosis was provided by institutional pathologists based on the WHO classification at diagnosis. All WHO grades of cases were analyzed in this study. Patient's clinical data, including age, gender, tumor location, type of operation, postoperative treatment, and clinical course, were obtained from their medical record and retrospectively analyzed. Overall survival (OS) was defined as the duration between initial surgery and censoring or death. Approval from the institutional review board was obtained prior to study initiation; as this study was retrospective, the requirement for informed consent was waived.

Genetic analysis

DNA was extracted from frozen tumor tissue using an AllPrep DNA/RNA Mini Kit (Qiagen, Tokyo, Japan) or from a formalin-fixed paraffin-embedded (FFPE) block using ReliaPrep™ FFPE gDNA Miniprep System (Promega, Madison, USA). Mutation hotspots at codons 27 and 34 of *H3F3A*, codon 27 of *HIST1H3B*, the *TERT* promoter (C228T, C250T), codon 132 of *IDH1*, codon 172 of *IDH2*, and codon 600 of *BRAF* were screened using Sanger sequencing (Supplementary Table). Genomic DNA was amplified through polymerase chain reaction (PCR) using Amplitaq Gold Quick Taq® HS DyeMix (TOYOBO, Osaka, Japan). The oligonucleotide primers used for PCR are summarized in Supplementary Table. Cycle sequencing was conducted using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with forward and reverse PCR primers as sequencing primers. Sequencing data were obtained using the Genetic Analyzer 3130 Avant (Applied Biosystems, Foster City, CA, USA).

Immunohistochemical staining

A FFPE block was cut into 4-μm sections. Antigen-retrieval was performed by pressure-cooking for 3 min in 10 mM sodium citrate buffer (pH 6.0). Endogenous peroxidase was blocked using peroxidase blocking solution (Dako, Carpinteria, CA,

USA). Antibodies against H3K27me3 (1:200, No. ab6002, Abcam, Cambridge, UK), EZH2 (1:50, No. 5246S, Cell Signaling Technology, Danvers, USA) and methylguanine methyltransferase (MGMT) (1:25, No. bsm-51234M, Bioss, Boston, USA) diluted with Can Get Signal Immunostain SolutionA (TOYOBO, Osaka, Japan) were added followed by overnight incubation at 4 °C. Antibodies were detected using the EnVision Dual Link system-HRP (Dako, Carpinteria, CA, USA) followed by the addition of Liquid DAB+ Substrate Chromagen System (Dako, Carpinteria, CA, USA) for visualization. Slides were counterstained with Meyer's hematoxylin (Sakura Finetek Japan, Tokyo, Japan) and dehydrated in 70%, 80%, and 100% ethanol and xylene. Mean positive rate of H3K27me3 and EZH2 staining were calculated by two pathologists independently and more than 70% of the cells positive for H3K27me3 were considered retained H3K27me3 [18], while none of the cells negative for EZH2 were considered negative [19].

Statistical analysis

Statistical analysis was performed using EZR (Saitama Medical Centre, Jichi Medical University, <http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html>; Kanda, 2012), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0). A *p* value less than 0.05 derived using the log-rank test and Mann-Whitney U test was considered statistically significant.

Results

Patient demographics and genetic landscape of spinal cord glioma

A total of 26 cases, 5 with WHO grade 4, 9 with grade 3, and 12 with grade 2 disease, were analyzed herein (Table 1). Chemotherapy was performed in 10 cases including 5 cases with temozolomide (TMZ), and radiotherapy was performed in 17 cases. H3K27M mutation was detected in four grade 4 cases (80%) and three grade 3 cases (33.3%) but not in grade 2 cases (Fig. 1). All cases with an H3K27M mutation harbored a H3F3A mutation. H3K27M mutation and other recurrent genetic alternations were presented in a mutually exclusive fashion. One case with H3K27M

had been initially diagnosed as WHO grade 2 following biopsy but was subsequently diagnosed as grade 3 after additional resection, which was considered as the final diagnosis (Supplementary Fig. 1). Sixteen patients including 7 cases with H3K27M presented positive MGMT staining on immunohistochemical staining (IHC), while expression of MGMT was not associated with OS (Supplementary Fig. 2).

Clinical features of H3K27M and wild type H3K27 cases in WHO grade 3 and 4 spinal cord gliomas

Given that H3K27M mutations were detected exclusively within WHO grade 3 and 4 cases, a comparison between H3K27M and wild type H3K27 cases was conducted among these high-grade cases. Age at onset did not significantly differ between H3K27M and wild type H3K27 cases, while H3K27M cases presented bimodal peaks among teenagers and those in their fifties (Supplementary Fig. 3A). Both H3K27M and wild type H3K27 cases had few lower thoracic cord tumors with a median involvement of 4 levels in both of these groups (Supplementary Fig. 3B).

Although WHO grade 2 cases tended to present favorable OS compared to grade 3 and 4 cases, the difference was not statistically significant (Fig. 2A), suggesting the necessity of novel prognostic markers other than pathological diagnosis. Among WHO grade 3 and 4 cases, OS in H3K27M and wild type H3K27-cases did not significantly differ (Fig. 2B).

Analysis of H3K27me3 status and EZH2 expression in WHO Grade 2–4 spinal cord gliomas

Considering that WHO grade 2 cases included those with poor (i.e., died within several years) and good prognosis (i.e., long-term survival over 10 years) (Supplementary Fig. 4), we attempted to identify novel prognostic markers in WHO grade 2–4 in diffuse spinal cord gliomas. Given previous reports showing that H3K27M-mutated gliomas present reduced H3K27me3, we analyzed H3K27me3 status and expression of EZH2, a component of PRC2 that acts as a methyltransferase for H3K27, through immunohistochemistry (Fig. 3, Fig. 4A). All cases with H3K27M exhibited loss of H3K27me3 that was consistent with previous reports [9, 10], while a subset of cases without H3K27M also presented reduced H3K27me3. Notably, four grade 2 cases who died several years after initial surgery presented reduced

H3K27me3. Moreover, EZH2 expression was positive mainly in WHO grade 3 and 4 cases.

Prognostic role of H3K27me3 status and EZH2 expression

Although the correlation between OS and H3K27me3 status and EZH2 expression was not statistically significant, cases with retained H3K27me3 and negative EZH2 expression tended to present long-term survival (Supplementary Fig. 5). Among WHO grade 2–4 cases, those with retained H3K27me3 and negative EZH2 expression presented extremely favorable outcomes compared to the other types ($p = 0.03$; Fig. 4B). Majority of the H3K27me3(+)/EZH2(–) cases had been diagnosed as WHO grade 2, with only one grade 3 case presenting long-term survival belonging to this group (Fig. 4A).

Discussion

Prognostic role of H3K27M mutation in diffuse midline gliomas

Although DMG-H3K27M has currently been classified as WHO grade 4, recent studies on spinal cord high-grade gliomas have reported slightly favorable outcomes among H3K27M-mutated cases than among wild type H3K27 cases [20, 21]. Similarly, although this study included small number of cases, our results showed no significant difference in prognostic outcomes between H3K27M and wild type H3K27 cases. However, another study had reported that H3K27M-mutated spinal cord gliomas had worse prognosis compared to wild type H3K27 cases [22].

Aside from spinal cord cases, previous reports on brainstem tumors have indicated that H3K27M-mutated cases had worse outcomes compared to wild type H3K27 cases [23, 24]. Moreover, one study showed that cases with a K27M mutation in the *H3F3A* gene presented poorer outcomes than those with the same mutation in the *HIST1H3B* gene [25]. Studies involving thalamic cases have shown that H3K27M mutation cases had unfavorable or similar outcomes compared to wild type H3K27 cases [22, 26].

Previous reports indicated the association of MGMT promoter methylation and H3K27M-mutant gliomas, which induces resistance for TMZ in laboratory investigation [27]. Because this study included only 5 cases with patients who underwent TMZ therapy for initial treatment, efficacy of TMZ administration was not

clear. However, recent clinical study suggested non-efficacy of TMZ for grade 4 cases mostly consisted with H3K27M-mutant and MGMT promoter unmethylated cases [28].

Clinical significance of H3K27me3 status and EZH2 expression

Although H3K27M-mutated cases are classified as WHO grade 4, diffuse midline glioma of the pons with wild type H3K27 has been proposed based on unfavorable outcomes regardless of histological grade or H3K27 mutational status [29]. However, the present study showed that WHO grade 2 spinal cord gliomas included various cases with long-term survival, as well as those with poor prognosis. Therefore, concepts involved in diffuse midline glioma with wild type H3K27 should not be applied to spinal cord cases.

Our results suggest that the combination of H3K27me3 status and EZH2 expression could be a novel and precise prognostic marker for detecting cases with favorable outcome. In addition to pathological diagnosis and molecular analysis, studies on H3K27me3 and EZH2 would provide supportive information for clinical practice involving spinal cord diffuse gliomas.

As surgical procedures for spinal cord gliomas usually provide only a limited amount of tumor tissue, and some it would not be rare that the surgical specimen is not sufficient amount for genetic testing [20]. Our result suggest that cases of spinal cord gliomas with good prognosis by IHC of H3K27me3 and EZH2 that does not require much amount of tumor tissue. We consider that prognostic markers that assessed by IHC that can be performed with small amount of tissue would be efficient method for spinal cord gliomas.

Biological mechanisms in retained H3K27me3 and negative EZH2 cases

Because EZH2 is a exclusive methyltransferase of H3K27[30], cases with retained H3K27me3 without EZH2 expression in this study seems to be strange phenomenon. We consider 3 possible mechanisms that explain the presence of such cases. Firstly, modification of H3K27 is also regulated by demethylases of H3K27 such as lysine demethylase 6A (KDM6A) [30, 31]. As previous report show that low expression of KDM6A increases H3K27me3 level in bladder cancer [31], some EZH2-negative cases in this study might obtain retained H3K27me3 by extremely low expression of such demethylases of H3K27. Secondly, compensatory function of enhancer of zeste homolog

1 (EZH1) and EZH2 to obtain retained H3K27me3 has been reported in malignant lymphomas [32]. Although EZH1 expression is not investigated in this study, some enzymes other than EZH2 might act as methyltransferase of H3K27 and induce retained H3K27me3 without EZH2 expression. Thirdly, minute but effective expression might be not detected by IHC using specific antibody.

Previous pathological study of pediatric gliomas by IHC presented that the majority of low and lower gliomas presented higher positive H2K27me3 staining and no EZH2 staining, and EZH2 expression was higher in GBM regardless to H3K27M mutation or wild type [10]. Although the mechanism of tumorigenesis in spinal cord gliomas with retained H3K27me3 and EZH2 expression in our study is still unclear, we consider that they would have biological characteristics similar to these low or lower gliomas in the research by Venneti et al [10]. Because there have been no reports that describe H3K27me3 status and EZH2 expression, to our knowledge, it is unclear whether our hypothesis is applicable in entire diffuse midline gliomas or solely in spinal cord gliomas.

Role of genetic analysis for spinal cord gliomas

Various studies comparing the prognosis between H3K27M and wild type H3K27 spinal cord gliomas [20-22] have shown that the prognostic role of H3K27M still remains unclear. However, a case presented in Supplementary Figure 1 suggests that detection of H3K27M could be useful in preventing the underestimation of pathological diagnosis. In addition, several high-grade wild type H3K27 cases in the present study have exhibited long-term survival. Despite the lack of statistical significance for the prognostic role of H3K27M, presence of cases harboring wild type H3K27 who exhibited favorable outcomes should be recognized, even with high-grade pathology.

Although DMG-H3K27M carries unfavorable outcomes, recent studies have identified potential treatments by targeting the H3K27M mutation [11, 33-35]. Further clarification of the molecular background in spinal cord gliomas would result in novel treatment for wild type H3K27 cases as well.

Limitations of this study

This study includes a limited number of cases with 26 patients in a single institution. Moreover, lack of clinical variables including extent of surgical resection and postoperative chemoradiotherapy is a limitation of this study. Owing to the limited number of study population, we were unable to perform sufficient statistical analysis with clinical variables. Validation cohort study with independent data set including much number of cases and analysis with clinical variables would be required to confirm the confidence of prognostic role of H3K27me3 status and EZH2 expression in spinal cord gliomas. Because most of studies with genetic analysis of spinal cord glioma consist with limited number of cases[4, 5, 20, 21], multi-center study would be desirable for such rare disease.

Conclusion

Although spinal cord gliomas harboring H3K27M presented unfavorable outcomes, differences between H3K27M and wild type H3K27 cases were not statistically significant. Moreover, our results suggest that the combination of H3K27me3 status and EZH2 expression could be novel prognostic marker among diffuse spinal cord gliomas, including WHO grade 2–4 cases.

Acknowledgments

The authors would like to thank Enago (www.enago.jp) for the English language review.

Conflict of Interest

The authors declare that they have no conflict of interest.

Figure legends

FIG. 1. Genetic landscape of spinal cord gliomas included in this study

FIG. 2. Kaplan–Meier survival curve for spinal cord gliomas included in this study

A: No significantly difference in overall survival (OS) was observed between each WHO grade.

B: No significantly difference in OS was observed between H3K27M-mutant and wild type H3K27 cases with WHO grades 3 and 4.

FIG. 3. Illustrative findings of immunohistochemistry with H3K27me3 and EZH2 antibodies for spinal cord diffuse gliomas

A and B: Immunohistochemical staining (IHC) illustrating diffuse astrocytoma presenting retained H3K27me3 (A) and negative EZH2 expression (B).

C and D: Immunohistochemical staining (IHC) illustrating glioblastoma harboring an H3K27M mutation presenting loss of H3K27me3 (C) and positive EZH2 expression (D)

FIG. 4. Association between overall survival and WHO grade, H3F3A K27 status, H3K27me3 status, and EZH2 expression in grade 2–4 cases.

A: The upper graph indicates overall survival of each case (white, alive; black, dead). The lower chart indicates WHO grade, *H3F3A* K27 status, H3K27me3 status, and EZH2 expression in each case.

B: Kaplan–Meier survival curve showing significantly longer survival among cases with retained H3K27me3 and negative EZH2 ($p = 0.03$).

References

1. Schwartzenuber J, Korshunov A, Liu XY et al. (2012) Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* 482:226-231
2. Wu G, Broniscer A, McEachron TA et al. (2012) Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nature genetics* 44:251-253
3. Aihara K, Mukasa A, Gotoh K et al. (2014) H3F3A K27M mutations in thalamic gliomas from young adult patients. *Neuro-oncology* 16:140-146
4. Gessi M, Gielen GH, Dreschmann V et al. (2015) High frequency of H3F3A (K27M) mutations characterizes pediatric and adult high-grade gliomas of the spinal cord. *Acta Neuropathol* 130:435-437
5. Shankar GM, Lelic N, Gill CM et al. (2016) BRAF alteration status and the histone H3F3A gene K27M mutation segregate spinal cord astrocytoma histology. *Acta Neuropathol* 131:147-150
6. Hawkins C, Ellison DW, Sturm D (2016) Diffuse midline glioma, H3 K27M-mutant. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) WHO Classification of Tumours of the Central Nervous System. International Agency for Research on Cancer, Lyon, pp 57 - 59
7. Ryu SJ, Kim JY, Kim KH et al. (2016) A retrospective observational study on the treatment outcomes of 26 patients with spinal cord astrocytoma including two cases of malignant transformation. *Eur Spine J* 25:4067-4079
8. Solomon DA, Wood MD, Tihan T et al. (2015) Diffuse Midline Gliomas with Histone H3-K27M Mutation: A Series of 47 Cases Assessing the Spectrum of Morphologic Variation and Associated Genetic Alterations. *Brain pathology*. doi:10.1111/bpa.12336
9. Bender S, Tang Y, Lindroth AM et al. (2013) Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. *Cancer Cell* 24:660-672
10. Venneti S, Garimella MT, Sullivan LM et al. (2013) Evaluation of histone 3 lysine 27 trimethylation (H3K27me3) and enhancer of Zest 2 (EZH2) in pediatric glial and glioneuronal tumors shows decreased H3K27me3 in H3F3A K27M mutant glioblastomas. *Brain pathology* 23:558-564
11. Mohammad F, Weissmann S, Leblanc B et al. (2017) EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas. *Nat Med* 23:483-492
12. Lewis PW, Muller MM, Koletsky MS et al. (2013) Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science* 340:857-861
13. Lee W, Teckie S, Wiesner T et al. (2014) PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. *Nature genetics* 46:1227-1232
14. Cleven AH, Sannaa GA, Briaire-de Bruijn I et al. (2016) Loss of H3K27 trimethylation is a diagnostic marker for malignant peripheral nerve sheath tumors and an indicator for an inferior survival. *Mod Pathol* 29:582-590
15. Panwalkar P, Clark J, Ramaswamy V et al. (2017) Immunohistochemical analysis of H3K27me3 demonstrates global reduction in group-A childhood posterior fossa ependymoma and is a powerful predictor of outcome. *Acta neuropathologica* 134:705-714
16. Karlowee V, Amatya VJ, Takayasu T et al. (2019) Immunostaining of Increased

- Expression of Enhancer of Zeste Homolog 2 (EZH2) in Diffuse Midline Glioma H3K27M-Mutant Patients with Poor Survival. *Pathobiology* 86:152-161
17. Suva ML, Riggi N, Janiszewska M et al. (2009) EZH2 is essential for glioblastoma cancer stem cell maintenance. *Cancer research* 69:9211-9218
 18. Otsuka H, Kohashi K, Yoshimoto M et al. (2018) Immunohistochemical evaluation of H3K27 trimethylation in malignant peripheral nerve sheath tumors. *Pathol Res Pract* 214:417-425
 19. Wu Z, Wang Q, Wang L et al. (2013) Combined aberrant expression of Bmi1 and EZH2 is predictive of poor prognosis in glioma patients. *J Neurol Sci* 335:191-196
 20. Alvi MA, Ida CM, Paolini MA et al. (2019) Spinal cord high-grade infiltrating gliomas in adults: clinico-pathological and molecular evaluation. *Mod Pathol*. doi:10.1038/s41379-019-0271-3
 21. Yi S, Choi S, Shin DA et al. (2019) Impact of H3.3 K27M Mutation on Prognosis and Survival of Grade IV Spinal Cord Glioma on the Basis of New 2016 World Health Organization Classification of the Central Nervous System. *Neurosurgery* 84:1072-1081
 22. Wang L, Li Z, Zhang M et al. (2018) H3 K27M-mutant diffuse midline gliomas in different anatomical locations. *Hum Pathol* 78:89-96
 23. Khuong-Quang DA, Buczkowicz P, Rakopoulos P et al. (2012) K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol* 124:439-447
 24. Buczkowicz P, Bartels U, Bouffet E et al. (2014) Histopathological spectrum of paediatric diffuse intrinsic pontine glioma: diagnostic and therapeutic implications. *Acta Neuropathol* 128:573-581
 25. Castel D, Philippe C, Calmon R et al. (2015) Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol* 130:815-827
 26. Liu Y, Zhang Y, Hua W et al. (2019) Clinical and Molecular Characteristics of Thalamic Gliomas: Retrospective Report of 26 Cases. *World Neurosurg*. doi:10.1016/j.wneu.2019.03.061
 27. Abe H, Natsumeda M, Okada M et al. (2019) MGMT Expression Contributes to Temozolomide Resistance in H3K27M-Mutant Diffuse Midline Gliomas. *Front Oncol* 9:1568
 28. Chai RC, Zhang YW, Liu YQ et al. (2020) The molecular characteristics of spinal cord gliomas with or without H3 K27M mutation. *Acta Neuropathol Commun* 8:40
 29. von Bueren AO, Karremann M, Gielen GH et al. (2018) A suggestion to introduce the diagnosis of "diffuse midline glioma of the pons, H3 K27 wildtype (WHO grade IV)". *Acta Neuropathol* 136:171-173
 30. Huynh JL, Casaccia P (2013) Epigenetic mechanisms in multiple sclerosis: implications for pathogenesis and treatment. *Lancet Neurol* 12:195-206
 31. Ler LD, Ghosh S, Chai X et al. (2017) Loss of tumor suppressor KDM6A amplifies PRC2-regulated transcriptional repression in bladder cancer and can be targeted through inhibition of EZH2. *Sci Transl Med* 9
 32. Yamagishi M, Hori M, Fujikawa D et al. (2019) Targeting Excessive EZH1 and EZH2 Activities for Abnormal Histone Methylation and Transcription Network in Malignant Lymphomas. *Cell Rep* 29:2321-2337 e2327
 33. Hashizume R, Andor N, Ihara Y et al. (2014) Pharmacologic inhibition of

- histone demethylation as a therapy for pediatric brainstem glioma. *Nat Med* 20:1394-1396
34. Piunti A, Hashizume R, Morgan MA et al. (2017) Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat Med* 23:493-500
 35. Mount CW, Majzner RG, Sundaresh S et al. (2018) Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M(+) diffuse midline gliomas. *Nat Med* 24:572-579

Table 1. Summary of cases with spinal cord gliomas analyzed in this study.

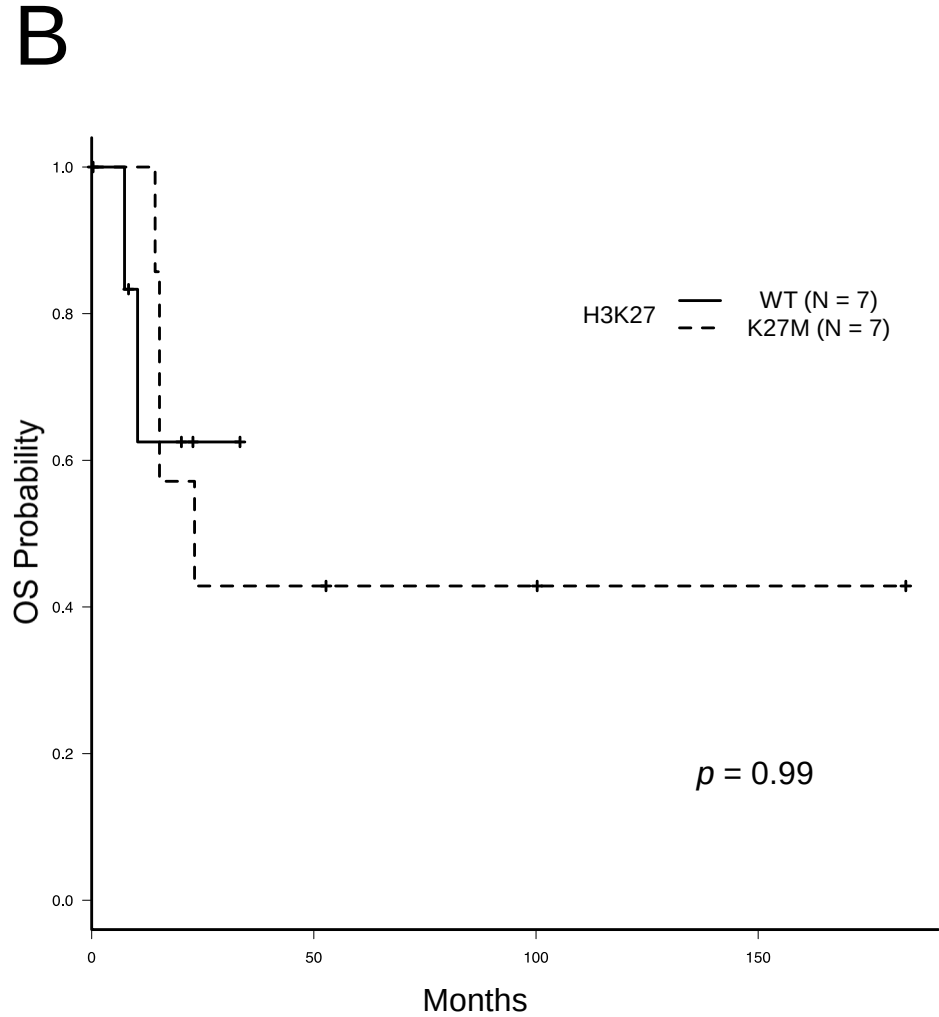
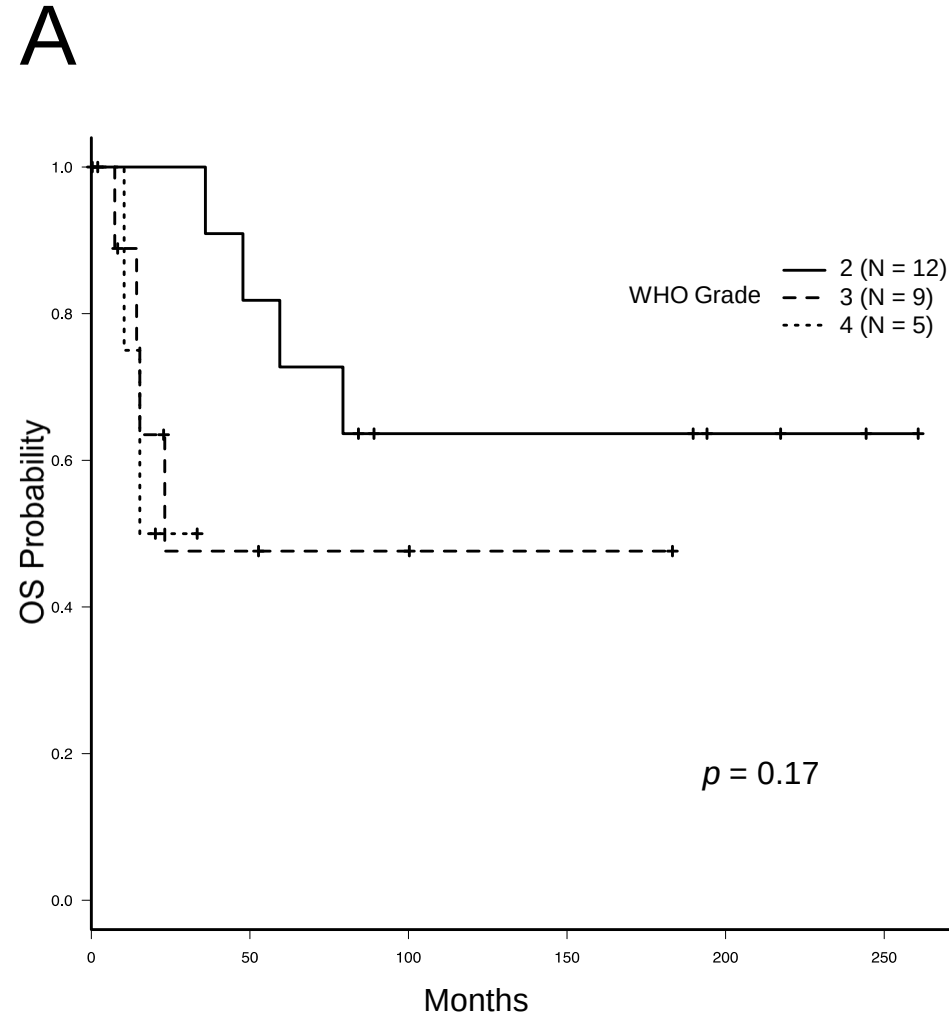
	All cases	World Health Organization Grade		
		4	3	2
Number of cases	26	5	9	12
Age	35.5	37	31	42
[median (range)]	(2–75)	(13–55)	(12–75)	(10–73)
Gender	16/18	4/1	3/5	6/6
(male/female)				
H3K27M	7	4	3	0
(%)	(18.9)	(80)	(33.3)	(0)
Chemotherapy	10	3	6	1
(temozolomide)	(5)	(1)	(4)	(0)
Radiotherapy	17	4	9	4

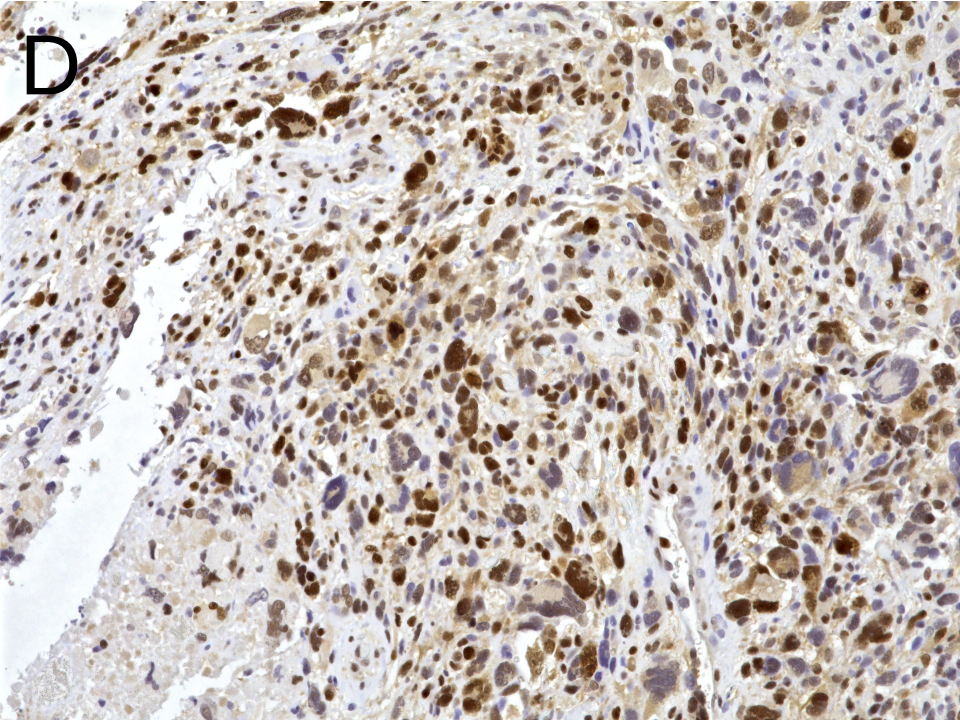
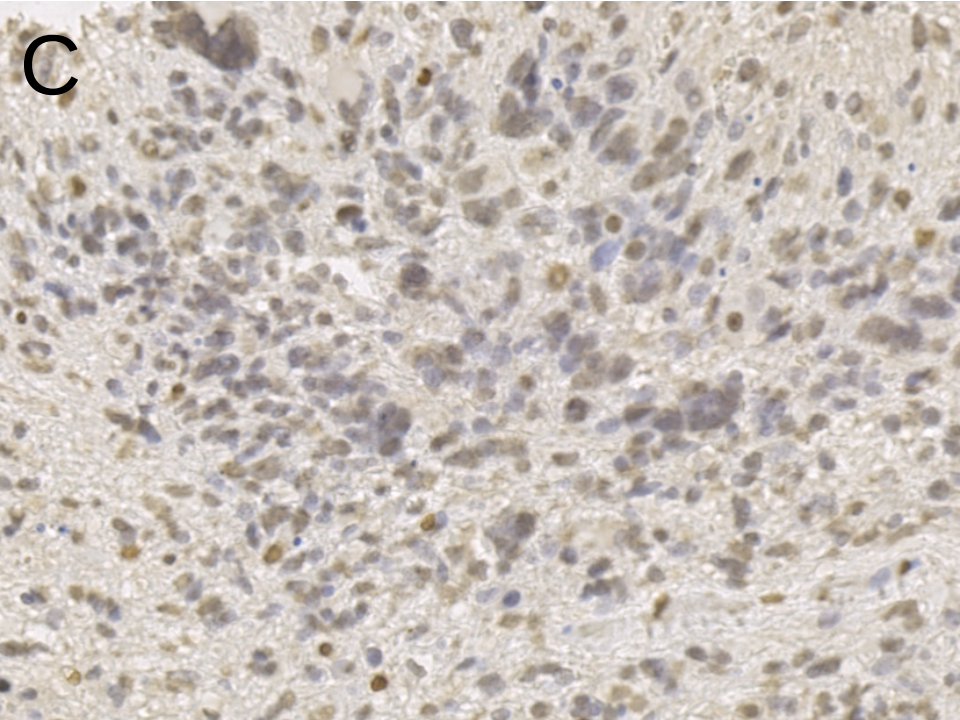
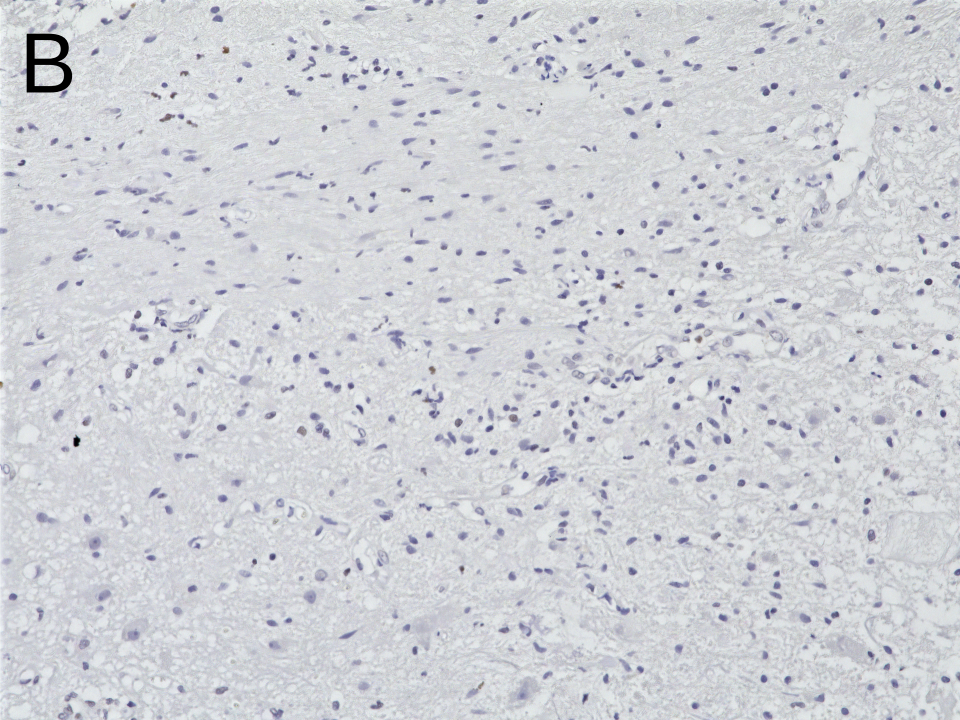
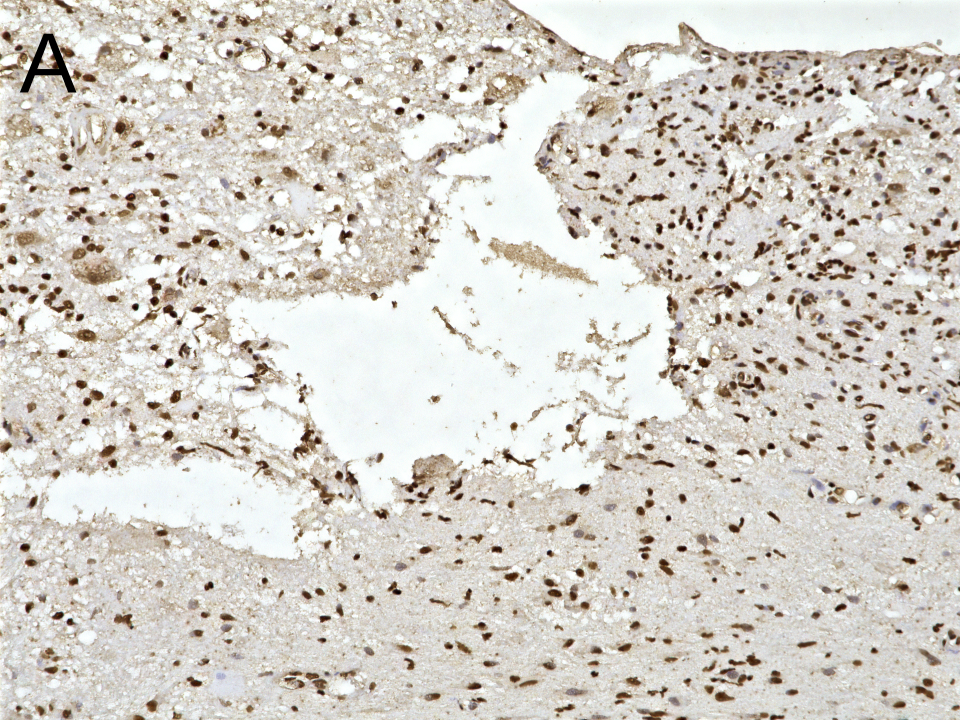
N = 26

WHO Grade	4				3								2													
<i>H3F3A</i>																										
<i>HIST1H3B</i>																										
<i>TERT</i>																										
<i>IDH1/2</i>																										
<i>BRAF</i>																										

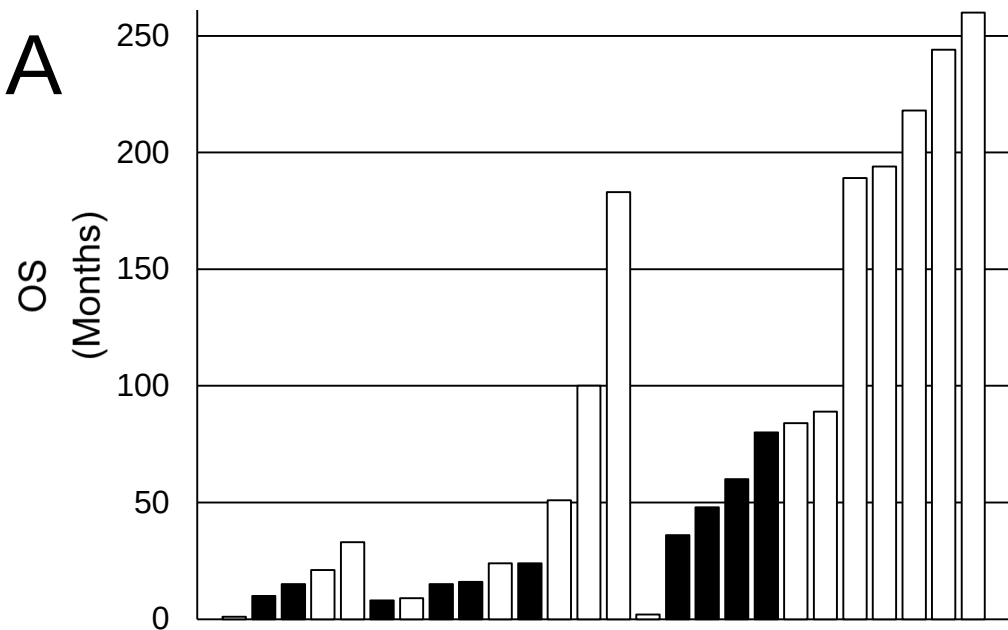
Genetic status

	Mutant
	Wild type





A



WHO Grade

4

3

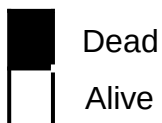
2

H3F3A

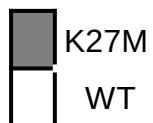
H3K27me3

EZH2

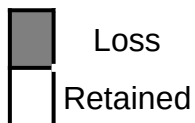
OS



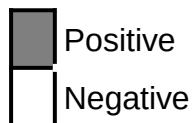
H3F3A



H3K27me3



EZH2



B

