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Original Article

Diffuse midline glioma with *H3* K27M mutation in the spinal cord: A series of 33 cases

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We investigated the risk factors for diffuse midline gliomas of the spinal cord (DMGSCs). Seventy patients with spinal cord gliomas in two hospitals were analyzed retrospectively. Sixty-nine patients that underwent surgery achieved partial or gross total removal. The patients were subdivided into some groups, based on age, WHO grade, tumor location within the cord, tumor size, and molecular profile: immunohistochemical expression of p53 and ATRX, and mutational status of *Histone 3* (*H3*), and *BRAF*. Thirty-three patients had an *H3* K27M mutation (47%). Some clinical characteristics were significantly different between *H3* K27M mutant and *H3* wild-type tumors. The main risk factors for DMGSCs were male sex, glioblastomas, and ≤ 2 spinal cord segments. The median survival period of patients with *H3* K27M mutant tumors was significantly shorter than those with *H3* wild-type tumors (17.0 ± 3.7 months *vs* censored, $P < 0.0001$). In the DMGSC subgroup, patients with thoracic cord tumors had a significantly better prognosis than those with cervical cord tumors (31.0 ± 6.0 *vs* 10.0 ± 4.8 months). Patients > 45 years of age survived significantly longer than patients < 19 years ($P = 0.001$). In conclusion, *H3* K27M mutation significantly predicts a worse outcome of spinal cord gliomas. Anatomical location and age are the main risk factors for DMGSCs.

Key words: diffuse midline glioma, H3 K27M mutation, overall survival, spinal cord, tumor location.

INTRODUCTION

Primary spinal cord tumors are relatively rare, representing approximately 2–16% of primary central nervous system tumors.[1–3] Approximately 2.4–4% of primary spinal cord tumors are diffuse astrocytomas (WHO grades III–IV).[1] The novel entity of "diffuse midline gliomas" in the 2016 WHO guidelines further divided rare spinal cord gliomas into *H3* wild-type and *H3* K27M mutant subtypes. A limited number of cases of diffuse midline gliomas of the spinal cords (DMGSCs) have been reported in the literature; however, the prognosis is contradictory.[4–7] For this study, 70 spinal cord glioma cases were collected from two hospitals in China. The associated clinical and pathological data were analyzed, and the cases were divided into two groups based on *H3* aberrations. The differences between *H3* mutant gliomas and *H3* wild-type gliomas were explored to determine the risk factors affecting prognosis.

MATERIALS AND METHODS

Patient population and clinical data

Of the examined 70 patients with diffuse gliomas of the spinal cord, 35 originated from Beijing Tsinghua Changgung Hospital, obtained at the period from November 2014 to December 2016, and the rest 35 were from Xuanwu Hospital, Capital Medical University (from January 2009 to January 2017) were retrospectively investigated.[8] Except for one biopsy, all patients (69, 98.6%) underwent partial to gross total resection. The extent of resection was defined based on intraoperative fluorescence (sodium fluorescein), gross total resection (resection of all), subtotal resection (≥ 80%), and partial resection (< 20%).[9] Because the 2016 WHO guidelines do not clarify whether histological grade is a prognostic factor for DMGSCs, we investigated the relationship between histological grade and K27M mutation in the histone 3 (H3) gene (*H3*). While conventional magnetic resonance imaging has a limited effect on the grading of spinal cord gliomas,[10] histological grade was used as the only criterion for tumor grading in this study. Hematoxylin and eosin (HE)-stained sections from these cases were reviewed, and

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histological diagnosis and grading were determined by two trained pathologists based on the WHO Classification of Tumours of the Central Nervous System (2007 edition) for astrocytomas.[11] The clinical data were collected for all patients, including age at diagnosis, sex, tumor location and length, immunohistochemical features, and genetic status of *H3* and *BRAF*. Patients were subdivided into some groups, based on different factors: age (< 19, 19–45, > 45), sex, grade (WHO grades II, III, and IV), location (cervical, thoracic, and lumbar), length (≤ 2 segments, 3–4 segments, and ≥ 5 segments), and molecular aberration. Most of the patients did not live in Beijing. They came to our hospitals with prepared radiological data and returned to their local hospitals for adjuvant therapy after surgery. Therefore, the radiological data and details of adjuvant therapy for these patients were not obtained.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded (FFPE) tissue sections. The primary antibodies used were against H3 K27M mutant (rabbit polyconal, Cat. No. SAB4500352, 1:100; Sigma-Aldrich, St Louis, MO, USA), isocitrate dehydrogenase 1 (IDH1) R132H mutant (mouse monoclonal, clone H09, 1:500; Dianova, Hamburg, Germany), α-thalassemia mental retardation X-linked (ATRX) (rabbit polyclonal, Cat. No. HPA001906, 1:100; Sigma-Aldrich), p53 (mouse monoclonal, clone DO-7, 1:100; Dako, Glostrup, Denmark), oligodendrocyte transcription factor 2 (Olig2) (rabbit polyclonal, Cat. No. AB9610, 1:250; Millipore, Billerica, MA, USA), glial fibrillary acidic protein (GFAP) (rabbit polyclonal, Cta. No. Z0334, 1:1000; Dako), and Ki-67 (mouse monoclonal, clone MIB-1, 1:50; Labvision, Fremont, CA, USA). Immunoreaction was detected using Leica Bond automated staining processors. For H3 K27M immunohistochemistry, astrocytoma cell nuclei were used as a positive reaction control, and endothelial cells were used as a negative reaction control. For ATRX immunohistochemistry, complete loss of nuclear staining in tumor cells with retained expression in the nontumor cells was considered to be a loss of nuclear protein expression. For p53 immunohistochemistry, positivity was considered when more than 10% of the tumor cell nuclei were strongly immunoreactive.[8,12]

Table 1 Clinical and pathological characteristics of 70 patients in the spinal cord

Character	Total	*H3* K27M mutant	*H3* wild-type (%)	*P*-value
Number (%)	70	33 (47.1)	37 (52.9)	
Mean age (years)	30.7 ± 15.6	29.4 ± 12.3	32.0 ± 17.9	0.456
Age at diagnosis (years), *n* (%)				0.341
< 19	18 (25.7)	8 (24.2)	10 (27.0)	
19–45	39 (55.7)	21 (63.6)	18 (48.6)	
> 45	13 (18.6)	4 (12.1)	9 (24.3)	
Sex, *n* (%)				**0.017**
Male	45 (64.3)	26 (78.8)	19 (51.4)	
Female	25 (35.7)	7 (21.2)	17 (48.6)	
Histologic grade, *n* (%)				**< 0.0001**
WHO grade II	29 (41.4)	5 (15.2)	24 (64.9)	
WHO grade III	17 (24.3)	9 (27.3)	8 (21.6)	
WHO grade IV	24 (34.3)	19 (57.6)	5 (13.5)	
Location, *n* (%)				0.459
Cervical	26 (39.4)	11 (34.4)	15 (44.1)	
Thoracic	36 (54.6)	18 (56.3)	18 (52.9)	
Lumbar	4 (6)	3 (9.4)	1 (2.9)	
Mean length of tumor (segment)	4.3 ± 2.4	4.6 ± 3.3	4.5 ± 2.9	
Length of tumor, *n* (%)				**0.006**
≤ 2 segments	21 (21.4)	15 (48.4)	6 (16.2)	
3–4 segments	25 (34.3)	11 (35.5)	14 (37.8)	
≥ 5 segments	22 (31.4)	5 (16.1)	17 (45.9)	
Molecular alteration, *n* (%)				
p53-positive	33 (47.1)	20 (60.6)	13 (36.1)	**0.042**
p53-negative	36 (51.4)	13 (39.4)	23 (63.9)	
ATRX-lost	20 (33.3)	10 (31.3)	10 (35.7)	0.714
ATRX-retained	40 (66.7)	22 (68.8)	18 (64.3)	
Median overall survival (months)	32.3 ± 2.8	17.0 ± 3.7	—	**< 0.0001**

Boldface type indicates statistical significance ($P < 0.05$, using χ^2-test).

Gene sequencing

Total DNA was extracted using a Simplex OUP FFPE DNA Kit (Triplex International Biosciences, Beijing, China) according to the manufacturer's protocol for FFPE tissue samples. *BRAF* V600E mutation was detected by amplification refractory mutation system polymerase chain reaction (ARMS-PCR) using a *BRAF* V600E Mutation Detection Kit (Amoy Diagnostics, Xiamen, China) according to the manufacturer's instructions. Mutational analysis of the histone H3.3/H3.1 gene, including *H3F3A* and *HISTIH3B/C*,[7] was performed by Sanger sequencing after amplification by PCR. The PCR conditions and primers were the same as previously reported.[8] Sanger sequencing was performed on an ABI 3500 DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Statistics

SPSS 22.0 software was used for statistical analysis. Values were expressed as mean ± SEM. Continuous variables were summarized using the mean and standard deviations or the median and interquartile ranges. Categorical variables were summarized using frequencies and proportions and compared between patients with and without a mutation by Pearson $\chi 2$-test or Fisher's exact test. The survival periods between different subgroups were displayed using Kaplan–Meier curves and were examined by the log-rank test. Cox proportional hazards regression analysis was used to determine which factors were multivariately related to survival.

RESULTS

Clinical data and epidemiological features

Seventy cases of primary intramedullary spinal cord gliomas from patients of all ages in the two hospitals were analyzed. The patients' age at diagnosis was 30.7 ± 15.6 years (range 2–66). The male to female ratio (M:F) was 1.8:1. The 70 cases included 29 WHO grade II, 17 WHO grade III, and 24 WHO grade IV gliomas. The thoracic spinal cord was the most common site (54.6%) of the tumors. The average length of span by tumors was 4.5 segments.

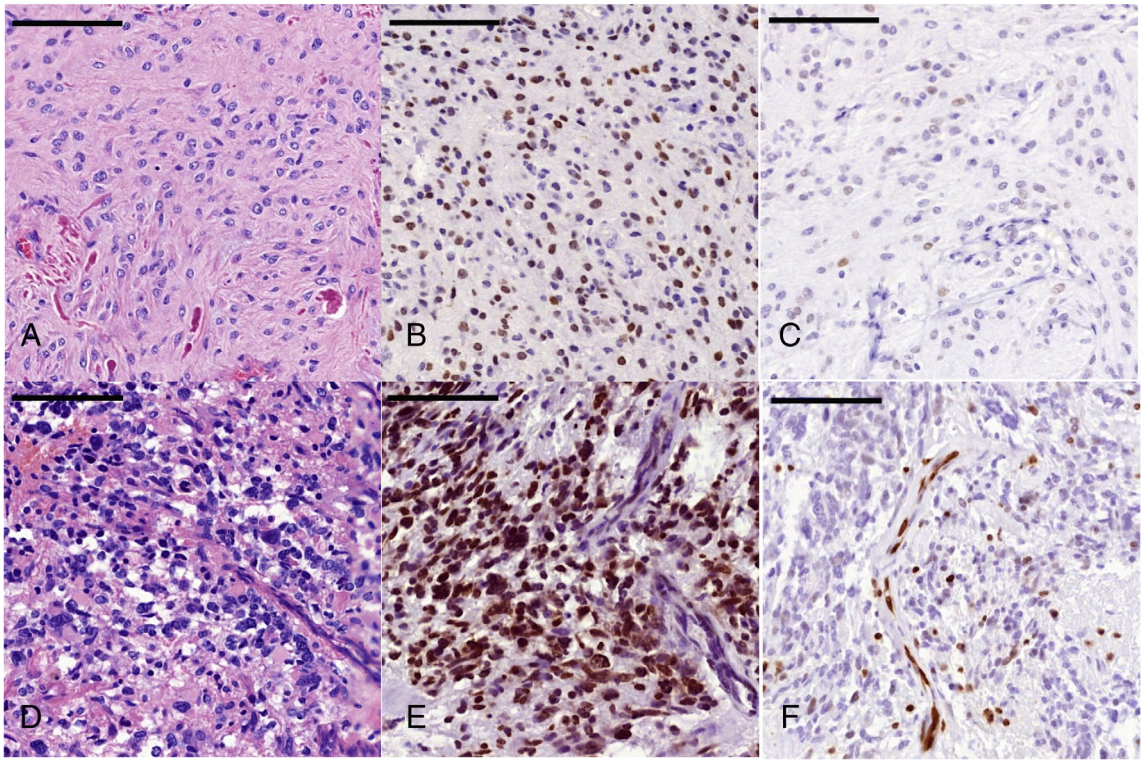

Fig 1 Histological findings in case 32 diagnosed as having a WHO grade II glioma with *H3* K27M mutant (A–C) and in case 24 diagnosed as having a WHO grade IV glioma with *H3* K27M mutant (D–F). (A) Tumor cells show mild to moderate dysplasia and no mitosis on an HE-stained section. (B) The tumor cell nuclei are strongly positive for *H3* K27M on immunohistochemistry. (C) Some of the tumor cell nuclei are weakly positive for p53 (< 10%) on immunohistochemistry; the result is interpreted as negative. (D) Tumor cells show moderate to severe dysplasia, with microvascular hyperplasia on an HE-stained section. (E) The tumor cell nuclei are positive for *H3* K27M on immunohistochemistry. (F) The tumor cell nuclei are negative for ATRX on immunohistochemistry. Scale bars: 100 μm (A–F).

Table 2 The overall survival of 33 DMGSCs and univariate and multivariate Cox analyses

Variety	Survival period Mean ± SEM (months)	Univariate		Multivariate	
		HR (95%CI)	*P*-value	HR (95% CI)	*P*-value
Age at diagnosis (years)					
< 19 (*vs* > 45)	13.5 ± 9.6	2.060 (0.429–9.894)	0.367	1.253 (0.170–9.242)	0.825
19–45 (*vs* > 45)	15.2 ± 4.3	1.170 (0.333–4.112)	0.806	0.709 (0.171–2.943)	0.636
> 45	25.0 ± 4.1	-	-	-	-
Sex				NA	NA
Male	15.2 ± 2.8	-	-	-	-
Female	19.0 ± 8.6	1.144 (0.44–2.976)	0.782	-	-
Histologic grade				NA	NA
WHO grade II	25.0 ± 0.0	-	-		
WHO grade III (*vs* WHO grade II)	15.2 ± 5.7	0.671 (0.140–3.212)	0.671		
WHO grade IV (*vs* WHO grade II)	17.0 ± 4.3	0.906 (0.259–3.175)	0.877		
Location					
Cervical	10.0 ± 4.8	-	-	-	-
Thoracic (*vs* cervical)	31.0 ± 6.0	0.225 (0.095–0.686)	**0.007**	0.261 (0.097–0.703)	**0.008**
Lumbar (*vs* cervical)	9.9	0.937 (0.198–4.439)	0.935	0.936 (0.198–4.434)	0.934
Length of tumor					
≤ 2 segments (*vs* ≥ 5 segments)	15.2 ± 6.0	1.320 (0.345–5.047)	0.685	1.767 (0.388–8.049)	0.462
3–4 segments (*vs* ≥ 5 segments)	13.0 ± 2.8	2.220 (0.583–8.462)	0.243	1.185 (0.264–5.311)	0.825
≥ 5 segments	25.0 ± 8.8	-	-		
Molecular alteration					
p53-positive	13.5 ± 2.3	1.243 (0.504–3.067)	0.636	NA	NA
p53-negative	20.0 ± 3.8	-	-	-	-
ATRX-lost	25.0 ± 9.7	0.752 (0.283–1.997)	0.567	NA	NA
ATRX-retained	17.0 ± 4.0	-	-	-	-

Boldface type indicates statistical significance ($P < 0.05$, using Cox proportional hazards regression analysis). CI, confidence interval; DMGSC, diffuse midline gliomas of the spinal cord; HR, hazard ratio; NA, not applicable.

A total of 33 *H3* K27M mutant tumors were identified, accounting for 47.1%. Compared with *H3* wild-type tumors, *H3* K27M mutant tumors appeared to occur in younger male patients (age, 29.4 ± 12.3 *vs* 32.0 ± 17.9, $P = 0.456$; M:F, 26:7 *vs* 19:18, $P = 0.017$, respectively) and were of smaller size (χ^2-test, $P = 0.006$) (Table 1).

The three pathological grades of glioma were unevenly distributed in two molecular subgroups (χ^2-test, $P = 0.000079$). High-grade glioma was more common in the *H3* K27M mutant group.

Findings in immunohistochemical and gene sequencing analyses

Our sequencing results for *H3F3A* and *HISTIH3B/C*[7] were consistent with immunohistochemical observations of H3 K27M (Fig. 1). The mutation frequency in high-grade gliomas was 68.3% (28/41). The *H3.3* K27M mutation was the only aberration (NM_002107.4: c.80A>T [p.K27M]); no other variants of *H3* mutation were detected. No cases had the *BRAF* V600E mutation. Immunohistochemically, among the examined gliomas, 47.8% (33/69) were p53-positive and 3.3% (20/60) were ATRX-negative. The frequency of p53 positivity was significantly different between the two subgroups (χ^2-test, $P = 0.042$) and was more frequent in *H3* K27M mutant tumors (Table 1). All the examined gliomas were negative for IDH1 R312H.

Overall survival and results of univariate and multivariate analyses

The mutational status of *H3* K27M and survival data were available for 64 patients. The survival periods of 33 patients with DMGSCs were 17.0 ± 3.7 months. This was significantly shorter than that of those with *H3* wild-type tumors (log-rank test, $P = 0.000011$) but similar to that of those with WHO grade IV tumors (17.0 ± 4.2 months). The survival period for DMGSCs increased with age (Table 2). Pairwise comparisons of the log-rank test revealed that patients > 45 years of age survival period significantly longer than those < 19 years ($P = 0.001$) (Fig. 2); however, this could not be proven by Cox analysis. The log-rank test and Cox regression revealed that tumor location was a risk factor for DMGSCs. The median survival period of patients with thoracic spinal cord tumors was significantly longer than that of those with cervical spinal cord tumors (31.0 *vs* 10.0 months) ($P = 0.008$, hazard ratio [HR] = 0.261, 95% confidence interval [CI] = 0.097–0.703). Male sex, high grade, p53 positivity, and ATRX expression were associated with

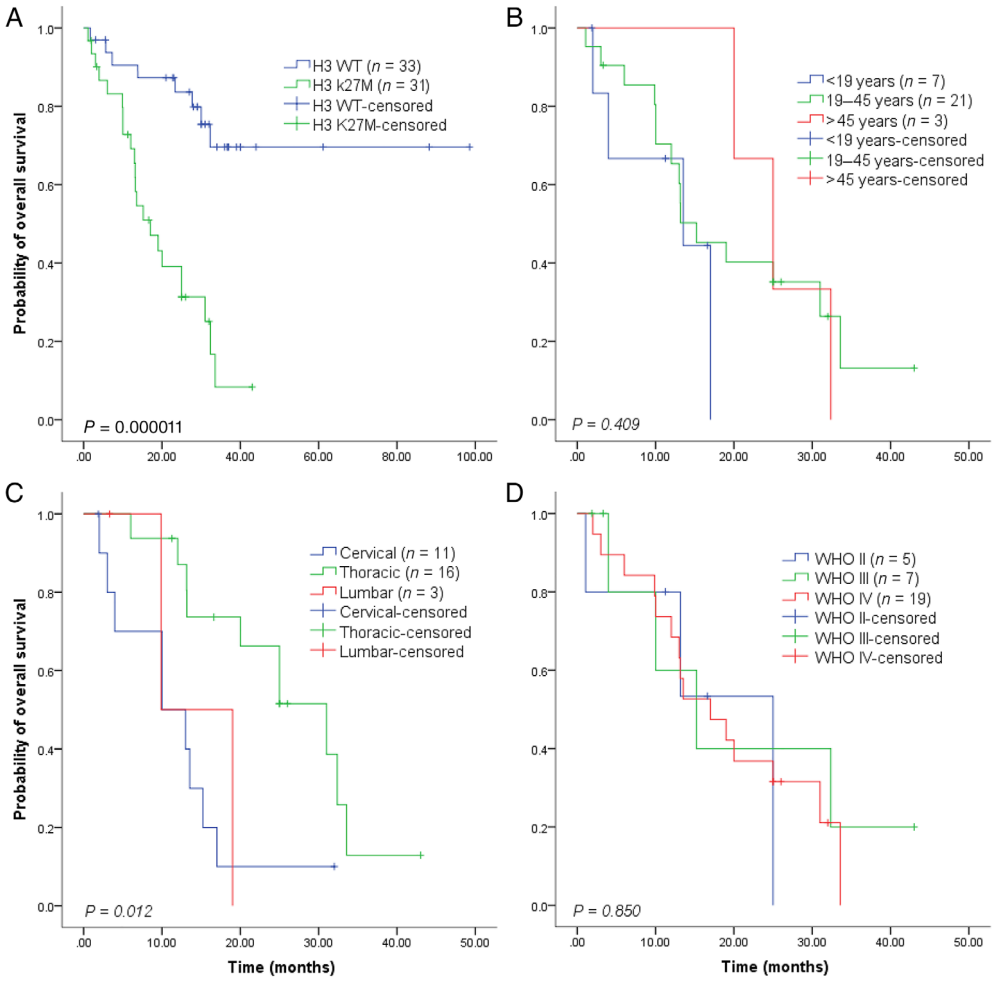

Fig 2 Kaplan–Meier survival curves and log-rank tests for *H3* K27M and associated risk factors for DMGSCs. (A) Patients with *H3* K27M-mutant tumors have a significantly shorter overall survival than those with *H3* wild-type (WT) tumors ($P = 0.000011$). (B–D) In different risk factors for *H3* K27M mutant tumors, there is no statistical difference among the three age subgroups. However, using pairwise comparisons of log-rank test, patients > 45 years have a more favorable prognosis than those < 19 years ($P = 0.001$) (B). Patients with thoracic cord tumors live longer than those with cervical tumors ($P = 0.012$) (C). There is no significant difference in overall survial between different histological grade subgroups ($P = 0.850$).

shorter survival, although there was no statistical significance.

DISCUSSION

Since 2016, the World Health Organization has proposed "diffuse midline glioma, *H3* K27M mutant" as an entity, and recommended that all diffuse midline gliomas should be regarded as WHO grade IV gliomas.[13] These guidelines mainly came from the study of diffuse intrinsic pontine gliomas (DIPGs). A limited number of histopathological tumor entities have been identified in the spinal cord, identical to DMGSCs. The risk factors for DMGSCs remain unclear. The present study searched for several risk factors and molecular aberrations for DMGSCs and attempted to determine their correlations with survival period. In the cohort, the *H3* K27M mutation was detected in 33/70 patients (47.1%), and the frequency of this mutation in high-grade gliomas was 68.3% (28/41). After reviewing the literature, it was found that the frequency of *H3* K27M mutation in spinal cord gliomas is 40–53%, which is slightly lower than that in thalamic (54.2–69%) and pontine (58.5–94%) gliomas.[5,6,8] The only type of mutation in our cohort was *H3.3* K27M, but Meyronet, *et al.* detected one glioma with *H3.1* K27M mutation in the spinal cord.[14]

Although five WHO grade II gliomas and nine WHO grade III gliomas were included, the overall survival period of 33 patients with DMGSCs was 17.0 ± 3.7 months; it is similar to that of those with glioblastomas (GBMs) in our cohort (17.0 ± 4.2 months) but profoundly shorter than that of those with *H3* wild-type gliomas (right censored) and that of those with WHO grade III gliomas (30.0 ± 14.9 months). The present study reviewed the literature and analyzed their data (Table 3). The survival period of patients with DMGSCs ranged from 1.5 to 132 months, and the median overall survival ranged from 4.8 to 40.7 months.\[4–6,8,14–20] There is a considerable gap between them. According to the above analysis results, the present study suggests that *H3* K27M mutation is a critical risk factor for spinal cord gliomas.

Affected by the H3K27 mutation, the prognosis of patients with low-grade DMGSCs is slightly better than

Table 3 Summary of recent studies on DMGSCs

Authors and public year	Number of mutant cases (%*)	Male/female	Median age (range) years	Overall survival (range) months	Number of LGG	Number of HGG
Hochart *et al.* (2015)[14]	1	0/1	7	132	1	0
Gessi *et al.* (2015)[4]	17 (56.7)	13/4	14 (9–62)	ND	1	16
Solomon *et al.* (2016)[5]	9 (53.0)	5/4	25 (4–41)	ND	3	6
Shankar *et al.* (2016)[15]	4	3/1	13 (9–25)	ND	0	4
Meyronet *et al.* (2017)[13]	6	4/2	33 (19–52)	6.0 (1.5–37)	2	4
Pagès *et al.* (2018)[17]	1	0/1	14	ND	1	0
Kleinschmidt-DeMasters and Mulcahy Levy (2018)[16]	6	0/6	30 (6–72)	9.7 (3.2–19.6)	2	4
Wang *et al.* (2018)[8]	16 (45.7)	13/33	24	13.2	4	12
Yi *et al.* (2018)[18]	20 (80.0)	14/6	ND	40.7	4	16
Karremann *et al.* (2018)[6]	6 (54.6)	4/2	ND	4.8	0	6
Alvi *et al.* (2019)[19]	6 (46.2)	5/1	35.1 (18–56)	ND	0	6

*The proportion of *H3* K27M mutant gliomas in the cohort. HGG, high-grade gliomas; LGG, low-grade gliomas; ND, no data.

that of patients with high-grade DMGSCs, but it is not statistically significant (25.0 *vs* 17.0 months, $P = 0.877$). To further confirm this result, we used the keywords "H3K27M or H3 or spinal cord glioma or diffuse midline glioma" to search the literature in PubMed. In all studies mentioning *H3* K27M-mutant low-grade gliomas (WHO I-II, 2007 edition), the survival period of patients ranges from six to 132 months, and the overall survival is 37.0 ± 13.1 months,[14,15,17] which is significantly shorter than that of patients with WHO grade II astrocytomas in the spinal cord (70–91 months),[21,22] and similar to that of patients with primary spinal anaplastic astrocytomas (14–37.4 months).[23–26] Hence, these data further confirmed that WHO grade II astrocytomas with *H3* K27M mutation should be regarded as high-grade gliomas.

DMGSCs are more common in young adults. The reported average age at diagnosis ranges from 25 to 39.1 years,[4,5,19] which is similar to our result (29.4 years). In this study, Kaplan–Meier curves showed that the survival period gradually increased with age: 13.5 ± 9.6 months (< 19 years), 15.2 ± 4.3 months (19–45 years), and 25.0 ± 4.1 months (> 45 years). Pairwise comparisons of the log-rank test revealed that patients > 45 years of age had a significantly better outcome than patients < 19 years ($P = 0.001$), but Cox regression did not prove this result. Therefore, age may be one of the prognostic factors for DMGSCs, but more data are required to verify this issue.

Due to the limited number of cases in the study, patients were divided into three groups based on tumor location: cervical, thoracic, and lumbar segments. The thoracic spinal cord segment was the most common location for DMGSCs and *H3* wild-type tumors. This is consistent with the results of previous studies.[4,19] In addition, univariate and multivariate analyses indicated that the location was a risk factor for DMGSCs. Patients with thoracic cord tumors had a significantly better prognosis than those with cervical cord tumors ($P = 0.008$, HR = 0.261, 95% CI = 0.097–0.703), suggesting that patients with tumors in the thoracic region have a 0.261 times higher risk of death than those in the cervical segment. Similar results were reported in other studies.[27–29] This finding can probably be explained by the assumption that cervical tumors can infiltrate or affect the brainstem, thus causing central respiratory failure. The cervicomedullary junction has internal fibers, such as pyramidal decussation, and tend to direct tumor growth.[30] The 11-month survival period of DIPGs proves that the prognosis of brainstem tumors is worse than that of DMGSCs.[31] Another possible reason for this is more radical resection and radiotherapy for gliomas in the lower spinal cord segments.[29]

The frequency of p53 positivity was significantly different between the two groups ($P = 0.042$), and it was more frequent in the mutant group (60.6%). According to the reviewed literature, p53 positivity is common in DMGSCs (20–50%)[5,16] and WHO III–IV spinal cord astrocytomas (60–67%).[32] The co-occurrence of the *H3* K27M and *TP53* mutations in gliomas had a trend of poorer prognosis (13.5 ± 2.3 months) compared to that of gliomas without *TP53* mutation (20.0 ± 3.85 months) but had no significant effect on survival period. Some authors postulate that the *H3* K27M mutation is a key driver mutation, and that the tumor suppressor gene product p53 positivity, indicative of a loss-of function mutation, plays an essential role in tumor development in both pediatric and adult spinal cord high-grade gliomas.[15,33] Thirteen DMGSCs did not show any p53 positivity in this cohort; eight of them were high-grade tumors. The present study suggests that the *TP53* mutation might be an accelerating factor for the progression of DMGSCs.

To summarize, spinal cord gliomas in males and young adults and high-grade gliomas located in the thoracic

region were prone to DMGSCs. High grade and *H3* K27M mutation were both poor prognosis factors for spinal cord gliomas. Tumor location was a prognostic factor for DMGSCs. Patients who had tumors in the thoracic region lived longer than those in the cervical region. In addition, age is a potential risk factor.

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DISCLOSURE

The authors declare that they have no conflicts of interest in association with the present study.

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