The Biological Behavior of High-Grade Glioma in H3K27M Mutant Circumscribed Midline Gliomas

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Abstract

Purpose Due to the prognosis of circumscribed middle gliomas (CMGs) with H3K27M mutation is still unclear. This study explored the prognostic stratification of CMGs with H3K27M mutation.

Methods: One hundred and sixty middle gliomas (MGs) were identified over 10 years. Immunohistochemistry was done for H3K27M, ATRX, IDH1, and P53, and Sanger sequencing performed for IDH and H3 genes. The clinicopathological characteristics were reviewed and survival analysis performed.

Results:

1. H3K27M mutation was associated with a poor prognosis ($p = 0.00017$) for brainstem gliomas, but not for thalamic gliomas ($p = 0.3$). In the elder adults (≥ 40 years), there was no correlation between H3K27M mutation and the prognosis for MGs ($p = 0.49$).
2. For H3K27M mutant MGs, there was no difference in prognosis between diffuse midline gliomas (DMGs) and CMGs ($p = 0.211$), also between the CNS WHO grades.
3. The prognosis of H3K27M mutant CMGs of CNS WHO grade 1 was worse than that of H3K27M wild-type CMGs ($p = 0.0024$), even worse than of DMGs without H3K27M mutation of CNS WHO grade 2 ($p = 0.0066$). There was no significant difference in prognosis from DMGs with histological grades CNS WHO grade 3 and 4 ($p = 0.46$).

Conclusions:

The weight value of H3K27M affecting prognosis is affected by location and age. CMGs with H3K27M mutation have biological behavior similar to high-grade gliomas. It is recommended that Treatment management was referenced to high-grade glioma for CMGs with H3K27 mutation.

1. Background

The novel tumor type 'H3K27M mutant diffuse midline glioma (DMG-H3m)' added in CNS WHO 2016 edition corresponds to CNS WHO grade 4 regardless of its histological grade. This classification arrangement explains and solves some clinical puzzles. It is worth noting that CMGs such as pilocytic astrocytoma, ganglioglioma and other circumscribed midline glioma (CMG) can also be accompanied by H3K27M mutation. Some of these tumors can survive for a long time[1; 2], and some have a poor prognosis[3]. There are many disputes on classification for these tumors. For clarification, in March 2018, the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy– Not Official (cIMPACT-NOW) strictly defined DMG-H3m and explicitly excluded H3K27M mutant CMGs. It is considered that the biological behavior of H3K27M mutant CMG needs to be further studied[4]. The fifth edition of CNS WHO CNS tumor classification in 2021 does not independently describe CMGs with H3K27M mutation [5]. The current pathological integrated diagnosis for midline gliomas (MG) fails to

explain these phenomena clearly. It confuses neurooncologists in their treatment decisions. This study will evaluate the prognostic factors of MGs and explore the optimal treatment management for MGs.

2. Materials And Methods

2.1 Research Object

This study collected 160 paraffin-embedded specimens of midline gliomas obtained through surgery or biopsy at Guangdong Sanjiu Brain Hospital from September 2012 to December 2019. The tumor sites of all included cases were reviewed by neuroimaging experts and neurooncologists, including brainstem, thalamus, hypothalamus, suprasellar and basal ganglia. The degree of resection was observed by MRI postoperative re-examination, including total resection (100%), subtotal resection (91% - 99%), partial resection (10% - 90%), and biopsy (less than 10%). All included subjects were primary neuroepithelial tumors of the central nervous system located in the midline. The histological classification and grade were carried out according to the 2007 WHO CNS tumor classification (not Integration of molecular genetics characteristics). The histopathological diagnosis were determined by two or more senior neuropathologists. The total survival time (OS) was calculated for all patients with the operation time as the starting time. The study was approved by the ethics committees of Guangdong Sanjiu Brain Hospital. Informed consent was obtained from all patients

2.2 experimental methods and steps

The H3 K27M mutation was identified with immunohistochemistry (IHC) using H3 K27M specific antibodies. IDH1R132H, p53, and ATRX status were also evaluated by IHC. The used primary antibodies were as follows: H3 K27M mutation-specific antibody (ABE419; EMD Millipore, Billerica, MA; 1:1000 dilution), IDH1 R132H mutation-specific antibody (clone H09; Dia- Nova, Hamburg, Germany; 1:500 dilution), ATRX (HPA001906; Sigma, St Louis, MO; 1:100 dilution), p53 (clone DO-7; Dako, Glostrup, Denmark; 1:00 dilution), Ki-67 (MIB-1; Labvision, Fremont, CA; 1:50 dilution). All stainings were performed using Roche Benchmark XT automated staining processors. The standards for judging H3 K27M, ATRX, and P53 staining were the same as in previous studies[6; 7; 8]. Genomic DNA was extracted using the TIANamp FFPE DNA Kit (DP331; Tiangen Biotech, Beijing, China) according to the manufacturer's protocol for formalin-fixed, paraffin-embedded samples. Mutational analysis of coding exons of the histone H3.3/H3.1 gene was performed using polymerase chain reaction (PCR) and Sanger sequencing of formalin-fixed, paraffin-embedded material only for 60 cases because of limited tissue samples. IDH1 and IDH2 were sequenced by Sanger sequencing after amplification by PCR only for 90 cases. The PCR conditions and primers were the same as reported before[9; 10]. Sanger sequencing was performed using 3730xl DNA Analyzer Technology (Applied Biosystems, Foster City, CA), according to the manufacturer's protocol.

2.3 Result Analysis and Statistical Methods

The incidence of the various tumor was described by the number of cases and the constituent ratio. Median survival time was used to describe survival data. The survival time was measured from the date of surgery to the date of last follow-up or death. Survival rates and curves were estimated using the Kaplan-Meier method, and group comparisons were assessed using the log-rank test. Variables that were found to be prognostic according to the univariate analysis were subsequently evaluated using proportional Cox regression for multivariate analysis. When bilateral p value < 0.05, the difference was considered statistically significant. All statistical analyses were performed in R (version R 3.6.3).

3 Results

3.1 Clinical and Imaging Features

Among 160 patients with midline glioma, the clinical symptoms depend on the location of the disease, including headache, dizziness, weakness, visual impairment, nausea and vomiting, memory loss, language disorder, seizures, swallowing dysfunction, abnormal gait, disturbance of consciousness and facial numbness, etc. The male to female ratio is 103:57, aged from 1 to 68 years old, with a median age of 30 years. Patients with tumors in the brain stem are younger, with a median age of 19 years. There were 54 patients (33.8%) ≤ 18 years old, 55 patients (34.5%) 18-39 years old, and 51 patients ≥ 40 years old (31.7%). There were 69 cases (43.1%) involved the brain stem, 63 cases (39.4%) involved the thalamus, 3 cases (1.9%) located in the hypothalamus and 25 cases (15.6%) located in other midline parts (sellar region, suprasellar and basal ganglia). MRI showed that irregular masses or swelling growth masses with clear boundaries, involving the brain stem and thalamus or Multiple parts of midline. According to the MR signal characteristics, the real part shows a low slightly or low signal on T1WI and an apparent high signal on T2WI (Figure 1). The tumor is enhanced and edematous in varying degrees, and occasionally hydrocephalus occurred.

3.2 Histological Performance

In 160 cases of midline glioma, the histological grades can be CNS WHO grade 1-4. there were 30 cases of grade 1, of which 13 cases of H3K27M mutant and 17 cases of H3K27M wild type; there were 43 cases of grade 2, 39 cases of grade 3, and 48 cases of grade 4. There were 124 cases of histological manifestations of DMG, accounting for 68.9%, including 42 cases of CNS WHO grade 2 diffuse glioma, 34 cases of grade 3 anaplastic glioma and 48 cases of grade 4 glioblastoma (including 1 case of small blue round cell embryonic tumor-like morphology). 36 cases (31.1%) showed histological features of circumscribed glioma, including 17 cases of pilocytic astrocytoma, 15 cases of glioneuronal, and 4 cases of other types of CMG. H3K27M mutant midline glioma has various morphological manifestations (Figure 2a-h).

3.3 Immunohistochemical Phenotype and Molecular Test Results

In all 160 cases of MG underwent H3K27M and H3K27Me3 immunohistochemical detection, 75 cases of H3K27M were positive, and the corresponding H3K27me3 expression was absent. 97.3% (73/75) of the cases were diffusely positive for H3K27M and negative for H3K27me3; two cases showed a "mosaic" appearance positive. Among 136 cases, IDH1R132H immunohistochemical test shows positive in 4 cases (2.9%, 4/132), and 1 case accompanied with H3K27M mutation(Figure 2i-l). Among 103 cases that underwent ATRX immunohistochemical detection, there were 27 cases of ATRX expression loss (23.9%, 27/113). Among 52 cases of 75 H3K27M mutation tumors that underwent ATRX detection, there were 19 cases of expression loss (36.5%, 19/52). Among 128 cases that underwent P53 immunohistochemical detection, 46 cases were overexpressed (35.9%, 46/128); among 75 cases of H3K27M mutation tumors, 47 cases underwent P53 detection 23 cases overexpression were performed (48.9%, 23/47) Deletion of ATRX expression and TP53 overexpression were correlated with H3K27 mutation ($P=0.004$; $P=0.02$). The Ki67 proliferation index with H3K27M mutation is more often expressed as high expression.

Among the 60 specimens, H3.1 and H3.3 gene were detected by Sanger sequencing. There were 24 cases of H3.3 K27M mutation and 1 case of H3.1 K27M mutation. Sanger sequencing results are entirely consistent with immunohistochemistry results. The specificity and sensitivity of immunohistochemistry detection are both 100%. Sanger detected 90 cases for IDH1/IDH2. There were also 8 cases of gene mutations (8.9%, 8/90), of which 4 cases were IDH1 R132H (CGT>CAT) mutations, and 2 cases were IDH1 R132C (CGT>TGT) mutations. 1 case of IDH1 R132G (CGT>GGT), 1 case of IDH2 R172M (AGG>ATG) mutation. The clinicopathological characteristics of 160 cases of MG are shown in Table 1 and Figure 3.

Table 1
Clinical pathological findings of 160 cases of MG

Clinical case characteristics	Total No(%)	<i>H3K27M</i> Wild Type (n=85)	<i>H3K27M</i> Mutant (n=75)	<i>p</i> -value
Age				
≤18	54 (33.8)	21 (24.7%)	33 (44.0%)	<0.001
19~39	55(34.3)	25(29.4%)	30(40.0%)	
≥40	51(31.9)	39 (45.9%)	12 (56.0%)	
Gender				
Male	103 (64.4)	58 (68.2%)	45 (60.0%)	0.278
Female	57 (35.6)	27 (31.8%)	30 (40.0%)	
Location				
Brain stem	69 (43.1)	29 (34.1%)	40 (53.3%)	0.014
others	91 (56.9)	56 (65.9%)	35 (46.7%)	
Histological classification				
DMG/PA	124 (77.5)	65 (76.5%)	59 (78.7%)	0.143
PA	17(10.6)	6(7.1%)	11(14.7%)	
GG	15(9.4)	11(12.9%)	4(5.3%)	
Other	4(2.5)	3(3.5%)	1 (1.3%%)	
Histological grade				
1	30 (18.8)	17 (20.0%)	13 (17.3%)	0.95
2	43 (26.9)	23 (27.1%)	20 (26.7%)	
3	39 (24.4)	21 (24.7%)	18 (24.0%)	
4	48 (30.0)	24 (28.2%)	24 (32.0%)	
IDH1 R132H(IHC)				
Negative	132 (82.5)	72(84.7%)	60 (80.0%)	0.201
Positive	4 (2.5)	3 (3.5%)	1 (1.3%)	
NA	24 (15.0)	10 (11.8%)	14 (18.7%)	
IDH1/IDH (Sequencing)				
Negative	82(51.3)	52(61.2)	30(40.0)	1.000

Clinical case characteristics	Total No(%)	<i>H3K27M</i> Wild Type (n=85)	<i>H3K27M</i> Mutant (n=75)	<i>p</i> -value
Positive	8(5.0)	5(5.9)	3(4.0)	
NA	70(43.7)	28(32.9)	42(56.0)	
Ki67 Expression				
Low/medium expression	63 (39.4)	46 (54.1%)	17 (22.7%)	<0.001
High expression	70 (43.7)	31 (36.5%)	39 (52.0%)	
NA	27 (16.9)	8 (9.4%)	19 (25.3%)	
P53 Expression				
Weak Positive	82 (51.2)	58 (68.2%)	24 (32.0%)	0.020
Strong and hight Positive	46 (28.8)	23 (27.1%)	23 (30.7%)	
NA	32 (20.0)	4 (4.7%)	28 (37.3%)	
loss of ATRX Expression				
No loss	86 (53.7)	53 (62.4%)	33 (44.0%)	0.004
Loss of expression	27 (16.9)	8 (9.4%)	19 (25.3%)	
NA	47 (29.4)	24 (28.2%)	23(30.7%)	

3.4 Prognostic Analysis Data And Follow-Up Data

1. In 153 cases of midline glioma (Seven cases of lost follow-up were excluded), the median survival time was 15.6 months. The median survival time of H3K27M mutant MGs was 12.1 months, while that of H3K27M wild-type MGs was 21.0 months. H3K27M mutation was associated with a poor prognosis ($p=0.00042$). In 67 cases of brainstem glioma, the median survival time was 13.6 months, and that of H3K27M mutant brainstem glioma was 11.4 months. H3K27M mutation was associated with a poor prognosis ($p=0.00017$). In 61 cases of thalamic glioma, the median survival time was 15.27 months, and that of H3K27M mutant thalamic glioma was 17.6 months, while that of H3K27M wild-type thalamic glioma was 14.9 months. There was no correlation between H3K27M mutation and prognosis ($p=0.3$) (Figure 4a-b).

2. The median survival time of 75 H3K27M MGs was 12.1 months, and the prognosis of children (≤ 18 years) was worse than that of adults ($p=0.02$). The prognosis of patients ≤ 18 years old, 19-39 years old, and ≥ 40 years old was significantly different ($p=0.05$). The prognosis of patients ≥ 40 years old was relatively good, with a median survival time of 25.0 months. For patients more aged than 40 years, the

median survival time of H3K27M wild-type midline glioma was 11.0 months, and the prognosis was not significantly different from that of H3K27M mutant midline glioma ($p=0.49$) (Figure 4c). The overall survival time of patients with H3K27M mutation can benefit from radiotherapy and chemotherapy. There is no significant difference in prognosis with tumor location, histological findings, and histological grades (CNS WHO grade 1 vs. grade 2, CNS WHO grade 1 vs. grade 2, 3, 4). The prognosis of the tumor is not related to the expression of Ki67, the expression of P53, and whether the expression of ATRX is lost, and is not related to the scope of surgical resection (Table 2).

3. For the 30 cases assessed as CNS WHO grade 1 by histopathology, the median survival time of H3K27M mutant patients was 15.6 months, which had a worse prognosis than H3K27M wild type ($p=0.0024$). The prognosis of the midline glioma with H3K27M mutation of CNS WHO grade 1 by histopathology was even worse than that of H3K27M wild-type glioma in grade 2 by histopathology ($p=0.0066$) and the prognosis of which was not significantly different from that of H3K27M wild-type midline glioma with grade 3 and 4 by histology ($p=0.46$). (Figure 4d-f).

Table 2
Evaluation of Prognostic Factors of H3K27M Mutant Glioma (Kaplan-Meire method and Cox regression)

Feature	HR(95%CI).x	P value	HR(95%CI).y	mupvalue
Age(≤18 vs. >18)	0.543(0.322~0.917)	0.0207	0.659(0.365~1.19)	0.168
Gender(M vs. F)	0.609(0.358~1.04)	0.0649	0.367(0.186~0.723)	0.00374
brain stem vs. other midline)	0.697(0.413~1.18)	0.174	NA	NA
Location (brain stem vs. thalamus, hypothalamus)	0.678(0.395~1.16)	0.155	NA	NA
Histological Classification (circumscribed vs. Diffuse)	1.46(0.806~2.64)	0.211	NA	NA
Histological grade (grade 1/2 vs. grade 3/4)	1.62(0.954~2.73)	0.0716	NA	NA
Histological grade (grade 1 vs. grade 2)	1.08(0.498~2.34)	0.846	NA	NA
Histological grade (grade 1 vs. grade 2/3/4)	1.45(0.756~2.77)	0.262	NA	NA
Ki67 expression (low/medium expression vs. high expression)	1.31(0.664~2.57)	0.437	NA	NA
P53 expression level (high expression vs. low expression)	1.06(0.545~2.05)	0.868	NA	NA
ATRX expression (loss vs. complete)	1.45(0.727~2.87)	0.291	NA	NA
Surgical scope (biopsy/partial resection vs. total resection/subtotal resection)	0.597(0.278~1.28)	0.182	NA	NA
Radiotherapy (yes / not)	0.289(0.151~0.554)	6.86E-05	0.357(0.14~0.91)	0.031
Chemotherapy (yes / not)	0.408(0.226~0.736)	0.00209	0.711(0.3~1.69)	0.439

5. Discussion

It was challenge in diagnosis and treatment for MG because of the incomplete matching between the CNS WHO grade and prognosis. In 2012, Sturm D found high frequency of H3K27M mutations in pediatric DMG, which was significantly associated with shorter patient survival " [11], which attracted extensive attention. DMG with H3-K27M mutation" was recognized as a novel type 2016 edition of the WHO Classification of Tumors of the Central Nervous System(CNS), which was corresponded to CNS WHO grade 4 regardless of its histological grades[12]. However, H3K27M mutant MG can occur at any age and any type of neuroepithelial tumors. Notably, the histopathological classification a, location of tumors and the age have an essential impact on the biological behavior. However, it is unclear about the prognosis of non-diffuse MG with H3K27M mutation. Therefore, for CMGs such as pilocytic astrocytoma or ganglioglioma with H3K27M mutation, it was explicitly indicated that it was not suitable for CNS WHO grade 4 and was not classified as H3K27M mutant DMG[4]. The arrangement of H3 mutant CMGs have not been determined until the fifth WHO classification of tumors of CNS[5]. Actually, H3K27M mutant CMGs is not uncommon, and our data are similar to the results of recent studies[1; 3; 13]. Previous studies on the prognosis of H3K27M mutant CMG were inconsistent [1; 2; 3; 14]. At present, although there are a number of reports that show the prognosis of H3K27M mutant CMG better than DMG, the overall prognosis is still poor compared with CMGs without H3K27M mutation[15].

This cohort showed that there was no significant difference in the prognosis between H3K27M mutant CMGs of CNS WHO grade 1 and DMGs of CNS grade 2-4. The prognosis of CNS WHO grade 1 CMSs with H3K27M mutation is worse than that of without H3K27M mutation. This shows that H3K27M mutation does has a negative impact on the prognosis of CMSs. What has attracted our attention is that the prognosis of CNS WHO grade 1 CMSs with H3K27M mutation is worse than that of CNS WHO grade 2 DMGs without H3K27M mutation ($p = 0.0024$). And there was no difference in prognosis between CNS WHO grade 1 CMSs with H3K27M mutation and high-grade (such as CNS WHO grade 3 or 4) DMGs without H3K27M mutation. This further suggests that H3K27M mutation does affect the biological behavior of CNS WHO grade 1 CMGs, prognosis of which was similar with high grade glioma. Therefore, we suggest that H3K27M mutant MG be managed as high-grade glioma even if it is just a CNS WHO grade 1 CMG.

In recent years, the prognostic stratification of H3K27M mutant MG, including the affection to prognosis by age, location, or molecular genetic characteristics, has attracted much attention. In this study, the age of H3K27M mutant MG is related to the prognosis of the tumor, and the children group (age ≤ 18 years) is connected to poor prognosis, with similar results as recently reported [16; 17; 18]. The prognosis of elder adults (≥ 40 patients) was significantly better. More interestingly, there was no difference in prognosis between H3K27M mutation and H3K27M wild-type MG in patients who are ≥ 40 years old. Thus, age is an important prognostic factor in patients with H3K27M mutant MG. In addition, for MG at different midline positions, the weighted value of H3K27M mutation on prognosis is different. Our cohort found that in brainstem MG, H3K27M mutation is related to poor prognosis. Still, for thalamic MG, H3K27M mutation has no correlation with tumor prognosis, which has similar results as reported in

recent years[19]. There is no significant difference in the prognosis between the brainstem and other midline tumors in our cohort, which is inconsistent with the conclusion of recent studies that "the prognosis of adult H3K27M mutant brainstem gliomas is worse than thalamic gliomas"[18; 19; 20]. Whether the classification of H3K27M mutant MG in the thalamus and brainstem should be treated differently needs further study. Also, it should be noted that non-midline gliomas can be accompanied by H3K27M mutation, and their prognosis is better than that of H3K27M mutant DMG[19; 21]. The H3K27M mutant gliomas located in the cerebral hemisphere should be treated separately[19; 20;22].

H3 gene mutations in midline gliomas include H3.3 and h3.1 variants, about 80% - 90% of which are mutated into H3.3 coding gene H3F3A, and the other 10% -20% have H3.1 coding gene HIST1H3B. There is also the rare coding gene HIST1H3C[23; 24]. The H3K27M immunohistochemical antibody can detect k27m mutations of h3.1 and H3.3 genes simultaneously, and H3K27me3 is expressed as a lack of expression [24; 25]. This study was similar to the results in recent years, H3K27M immunohistochemical staining has high sensitivity and specificity, while H3K27me3 has insufficient specificity[8; 19; 24; 26]. Some rare mutations of H3K27, such as HK27I mutation, cannot be detected by immunohistochemistry H3K27M, but H3K27Me3 can show a deletion[24]. Therefore, H3K27me3 helps to find rare mutations in daily diagnosis. In addition, the loss of H3K27me3 expression can also be caused by EZHIP overexpression caused by other reasons (such as EGFR gene mutation). It should be noted that these cases H3K27Me3 expression loss have a poor prognosis[27]. Therefore, the 2021 edition of WHO CNS revised "diffuse midline glioma, H3K27M mutant" to "diffuse midline glioma, H3K27 altered[5] to broaden the molecular connotation of diffuse midline glioma. The vast majority of the immunohistochemical expression in our cases is a diffuse expression. Still, it can be "mosaic" expression, suggesting that H3 histone mutation may be the initiating factor for tumor development, otherwise, it may also be a subclonal mutation [28]. Current studies have shown that H3K27M mutation is related to some molecular changes. Schwartzenuber[29] and other studies have shown that the mutation rate of ATRX-DAXX and TP53 in H3K27M mutant gliomas accounts for about 31% and 54%, respectively. Although ATRX and p53 genes are associated with H3K27M mutation, they do not correlate with tumor prognosis[19] Similar results are also found in this study. In addition, H3K27M mutation and EGFR gene amplification, TERT mutation, and MGMT promoter methylation are mutually exclusive [26; 30; 31], BRAF-V600E mutation and IDH gene mutation are occasionally accompanied by H3K27M mutation[13; 32]. It is particularly noteworthy that the hotspot mutation of the midline glioma IDH gene is not IDH1 R132H[32]. The incidence rate of IDH1 R132H mutation is similar to this study. The biological behavior of the midline glioma with IDH mutation and H3K27 mutation needs further study due to few. Recent studies have found that 30% of cases have PDGFRA gene amplification, 20% have ACVR1 gene mutation, and they have a poorer prognosis [33]. and H3K27M mutation with FGFR1 mutation is associated with a good prognosis[34]. Studies have also found that MCL1 gene amplification is associated with poor prognosis of midline gliomas Independent of H3K27M mutation [30]. Otherwise, MG can benefit from radiotherapy or chemotherapy regardless of age, location, grade, tumor type and molecular genetic characteristics. And the extent of surgical resection was not associated with prognosis, whether biopsy, partial resection, majority resection, or total resection

In conclusion, H3K27M mutant MG has unique clinicopathological and molecular genetic characteristics, and there are more challenges in diagnosis and treatment. The management of patients needs integration such as age, location, histological types and grades. In particular, the H3K27M mutant CMG should arouse our attention. Although some studies have confirmed that the prognosis of H3K27M mutant DMG is relatively good, it often presents as a high-grade glioma with aggressive behavior. Therefore, it is suggested that these patients should be managed according to high-grade gliomas, which can benefit from radiotherapy and chemotherapy.

Declarations

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

L. C. And Z. L. are co-corresponding authors.

H. L., J. T., M. L.: contributed equally.

H. L., J. T., M. L.: analyzed the data, performed the experiments and wrote the manuscript.

Z.Z, D. D.: provided essential material, analyzed the imaging features and clinical data.

W. C., M. L., B. C., and C. F.: performed the experiments and reviewed the pathologic diagnosis

L. C. And Z. L. supervised the study and critically reviewed the manuscript.

DATA AVAILABILITY

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Guangdong Sanjiu Brain Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

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Figures

Figure 1

MRI features of diffuse midline glioma with H3K27M mutation

a-c: showed the pons was diffuse swelling, hypo-intensity on the axial T1WI (a), hyper-intensity on the axial T2WI (b), no enhancement on the axial enhanced T1WI (c). d-f: showed the right thalamic tumor was inhomogeneity hypo-intensity on the axial T1WI(d), hyper-intensity on the axial T2WI (e), and obvious inhomogeneity enhancement on the axial enhanced T1WI (f). The third ventricle was obviously compressed, and the bilateral lateral ventricles showed obstructive hydrocephalus. g-i: showed the hypothalamic tumor was inhomogeneity hypo-intensity on the coronal T1WI(g) hyper-intensity on the sagittal T2WI(1h), and obvious enhancement on the sagittal enhanced T1WI(1i). j-l showed multiple, inhomogeneity hypo-intensity on the axial T1WI (1j), hyper-intensity on the axial T2WI(1k). On the axial

enhanced T1WI(1l),the lesion in the right optic nerve inner orbital was inhomogeneity obvious enhancement, and the other lesions were no enhancement. mSagittal enhancement of the whole spine (m) showed the periphery of the whole spinal cord, brainstem, and cauda equina were extensive multiple line-like and nodular enhanced lesions.

Figure 2

Histopathologic and immunohistochemical features of midline glioma with H3K27M mutation: Histopathologic features of cases diagnosed as diffuse astrocytoma(A, HE×400) oligodendroglioma(B, HE×200), Anaplastic astrocytoma(C, HE×400) glioblastoma(D, HE×100) Pleomorphic xanthoastrocytoma(E, HE×200), embryonal tumours-like with diffuse small blue cells(F, HE×200), pilocytic astrocytoma(G, HE×200). ganglioglioma(H, HE×200), H3 K27M was diffuse and strongly positive in the nuclei of tumor cells(I, IHC×200), The gliomas were loss of expression of H3 K27me3(J, IHC×200), H3 K27M showed a "mosaic" appearance positive in some cases (K, HE×200), H3 K27M was strongly positive in the nuclei of gangliocellular tumor cells in ganglioglioma(L, HE×200).

Figure 3

The clinicopathological features of midline glioma. H3K27M mutation is common in midline gliomas, which occurs in any age, predominately in children and young adults, with the most common locations being brain stem, thalamus. H3K27M mutations are common in both DMGs and CMGs, and the histological grades presents CNS WHO grade 1-4. H3K27M mutations are usually mutually exclusive with IDH mutations, but in rare cases occur simultaneously. H3K27M mutation is associated with high expression of P53 and ATRX loss.

Figure 4

Kaplan-Meier survival curves and log-rank tests for MGs: A, The H3 K27M mutation was associated with poorer OS in the brainstem(A), but not in the thalamus (B). For the elder adults (≥ 40 years old), there was no association in prognosis with H3K27M mutation(C). The prognosis of H3 K27M mutant CMGs of CNS WHO grade 1 was poorer than H3 K27M wild type CMG of CNS WHO grade 1(D), even than H3 K27M wild type DMG of CNS WHO grade 2(E) and was no difference from H3 K27M wild type DMG of CNS WHO grade 3 or 4(F).