Original Paper

Integrative Analysis of DNA Methylation and Gene Expression Identify a Three-Gene Signature for Predicting Prognosis in Lower-Grade Gliomas

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Key Words

Lower-grade glioma • Promoter methylation • Prognosis • Signature

Abstract

Background/Aims: In the current study, we performed an integrated analysis of genome-wide methylation and gene expression data to find novel prognostic genes for lower-grade gliomas (LGGs). **Methods:** First, TCGA methylation data were used to identify prognostic genes associated with promoter methylation. Second, candidate genes that were stably regulated by promoter methylation were explored. Third, Cox proportional hazards regression analysis was used to generate a prognostic signature, and the signature genes were used to construct a survival risk score system. **Results:** Three genes (*EMP3*, *GSX2* and *EMILIN3*) were selected as signature genes. These three signature genes were used to construct a survival risk score system. The high-risk group exhibited significantly worse overall survival (OS) and relapse-free survival (RFS) as compared to the low-risk group in the TCGA dataset. The association of the three-gene prognostic signature with patient' survival was then validated using the CGGA dataset. Moreover, Kaplan-Meier plots showed that the three-gene prognostic signature risk remarkably stratified grade II and grade III patients in terms of both OS and RFS in the TCGA cohort. There was also a significant difference between the low- and high-risk groups in *IDH* wild-type glioma patients, indicating that the three-gene signature may be able to help in

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predicting prognosis for patients with *IDH* wild-type gliomas. **Conclusion:** We identified and validated a three-gene (*EMP3*, *GSX2* and *EMILIN3*) prognostic signature in LGGs by integrating multidimensional genomic data from the TCGA and CGGA datasets, which may help in fine-tuning the current histology-based tumors classification system and providing better stratification for future clinical trials.

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Introduction

Gliomas are the most common primary malignancies in the central nervous system and show great heterogeneity in terms of their histopathology and clinical outcomes. According to the 2016 World Health Organization (WHO) classification system, gliomas include astrocytoma, oligoastrocytoma, oligodendroglioma and glioblastoma (GBM), and ranged in grades I to IV [1, 2]. Lower-grade gliomas (LGGs), which include WHO grades II and III astrocytoma, anaplastic astrocytoma, oligodendroglioma and anaplastic oligodendroglioma, exhibit an infiltrative nature and an intrinsic tendency to recur or progress to glioblastoma, a WHO grade IV glioma [1, 3]. Because of their highly invasive nature, complete neurosurgical resection is impossible for LGGs. The presence of residual tumor usually results in recurrence and malignant progression, albeit at highly variable intervals [4]. The survival of LGGs ranges widely, from 1 to 15 years, and some LGGs patients show impressive therapeutic sensitivity [5]. Though molecular diagnosis has been adopted in the classification of LGGs to create a more objective and precise tumor classification system [6, 7], the known molecular markers can only partly explain LGG prognosis at present. Thus, continued efforts to improve prediction accuracy and facilitate therapeutic strategies for LGGs are warranted.

DNA methylation is a vital form of epigenetic modification that is involved in the pathogenesis of cancer [8]. Promoter methylation of the gene encoding O6-methylguanine-DNA methyltransferase (MGMT) is closely related with its activity and can effectively predict gliomas' responsiveness to alkylating agents [9, 10]. Increased methylation in 5' upstream regulatory sites is negatively correlated with the expression of some tumor-suppression genes [11], which suggests that alterations in DNA methylation may have potential uses in the functional characterization and diagnosis of gliomas.

Transcriptomic data are typically used to explore the underlying characteristics of cancer and design new drug targets. Verhaak et al. classified GBM into Proneural, Neural, Classical, and Mesenchymal subtypes by using gene expression data of GBM [12]. Weller et al. have also identified eight transcriptionally different groups (five *IDH1/2* mutant and three *IDH1/2* wild types) of glioma by analyzing transcriptome-wide data derived from primary tumor samples [13]. Recent studies have confirmed that *IDH* mutation can impair histone demethylation [14] and predict better survival for glioma patients [15, 16]. However, current molecular classification cannot ensure the precise diagnosis and personalized medicine for LGG patients.

In this study, LGG DNA methylation data from The Cancer Genome Atlas (TCGA) and transcriptomic data from TCGA and the Chinese Glioma Genome Atlas (CGGA) were used to identify novel prognostic genes that are potentially regulated by promoter methylation. The relationships between DNA promoter methylation and gene expression as well as disease survival were analyzed for LGG patients. We believe that the findings will help to further improve molecular diagnosis and individualized therapy for LGGs.

Materials and Methods

Lower-grade glioma datasets

TCGA LGG datasets, including DNA methylation and gene expression data obtained from the same patient' population, were downloaded from the University of California, Santa Cruz, cancer browser: <https://genome-cancer.ucsc.edu/> (Version: 2015-02-24). The DNA methylation data were generated by

the Illumina Infinium Human Methylation 450 platform. The gene promoter methylation status was obtained by calculating the average of all CpG sites in a given promoter region. According to the Illumina Infinium Human Methylation450 platform annotation file, the promoter regions included TSS1500, TSS200, and the 5' untranslated region (5'UTR) [17]. Gene expression data from the TCGA LGG dataset were obtained from the Illumina HiSeq 2000 RNA Sequencing platform. In total, 473 samples (225 grade II and 248 grade III LGGs) with clinical information were eligible for the prognosis analysis and the correlation analysis between DNA methylation and gene expression levels. The CGGA microarray dataset, which was generated by Agilent Whole Human Genome Array (<http://cgga.org.cn/>), was also downloaded for use as a validation dataset. From this dataset, 131 samples (97 grade II and 34 grade III) were included, and the clinical data were also downloaded for prognostic analysis. The clinical characteristics of LGG patients in the TCGA and CGGA datasets were shown in Table 1.

Prognosis and correlation analysis

Overall survival (OS) and relapse-free survival (RFS) were used to indicate prognosis in our analysis. OS is defined as the time interval between resection and the date of death or the last follow-up. RFS is the period from resection to the first radiological evidence of tumor recurrence. To evaluate the prognostic value of DNA promoter methylation in TCGA LGG patients, promoter methylation status was stratified using four different cut-off values, which included the mean, the median (=the second quartile), quartile (the first quartile versus the third quartile) and the beta value=0.5. The beta value was used to estimate the methylation level of the probes. Methylation was considered to have prognosis significance when an adjusted $P < 0.05$ occurred for all four cut-off values. The correlation between promoter methylation level and the mRNA expression of the corresponding gene was investigated by using Spearman's correlation analysis [18]. A Spearman $|r| \geq 0.6$ with an adjusted $P < 0.05$ indicated a strong and significant correlation [19]. Cox proportional hazard regression analysis was performed to analyze the influence of gene expression on disease prognosis. The prognostic genes generated from above TCGA LGG dataset were then validated using the CGGA microarray dataset to further determine the genes' prognostic significance in LGG patients. The analysis flow chart was shown in Fig. 1.

Table 1. Clinical characteristics of the lower-grade glioma datasets. Notes: TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas; KPS: karnofsky performance score; IDH: isocitrate dehydrogenase; MGMT: O-6-methylguanine-DNA methyltransferase; TERT: Telomerase reverse transcriptase; ATRX: Alpha thalassemia/mental retardation syndrome X-linked; NR=not recorded

	TCGA cohort	CGGA cohort
No. of patients	473	131
Age, mean (year)	43	39.9
Sex, No. (%)		
male	260 (54.9)	72 (55.0)
female	212 (44.8)	59 (45.0)
WHO Grade, No. (%)		
II grade	225 (47.6)	97 (74.0)
III grade	248 (52.4)	34 (26.0)
KPS, No. (%)		
KPS $\geq$ 70	266 (56.2)	113 (86.2)
KPS $\leq$ 70	18 (3.8)	18 (13.8)
unknown	189 (40.0)	0 (0)
IDH status, No. (%)		
IDH mutant	383 (81.0)	84 (64.1)
IDH wild type	88 (18.6)	45 (34.4)
unknown	2 (0.4)	2 (1.5)
1p/19q codeletion, No. (%)		
1p/19q non-codeletion	316 (66.8)	-
1p/19q codeletion	157 (33.2)	-
MGMT promoter methylation, No. (%)		
unmethylated	81 (17.1)	-
methylated	392 (82.9)	-
TERT promoter status, No. (%)		
TERT mutant	132 (27.9)	-
TERT wild type	166 (35.1)	-
unknown	175 (37.0)	-
ATRX status, No. (%)		
ATRX mutant	174 (36.8)	-
ATRX wild type	297 (62.8)	-
unknown	2 (0.4)	-
Radiotherapy, No. (%)		
Yes	274 (57.9)	NR
No	166 (35.1)	NR
unknown	33 (7.0)	NR
Temozolomide (TMZ), No. (%)		
Yes	74 (15.6)	NR
No	88 (18.6)	NR
unknown	311 (65.8)	NR

Fig. 1. Flow chart of procedure for building the three-gene signature based on TCGA and CGGA datasets.

Statistical analysis

Statistical analyses were performed with R software (Version 3.3.1), SPSS 13.0 (SPSS Inc., Chicago, IL, USA), and GraphPad Prism 5.0 (Graphpad Inc., San Diego, CA, USA). Comparisons between groups were performed using Student's *t*-tests. Kaplan-Meier survival analysis was performed to assess the survival profiles, and log-rank test was performed to determine the significance of the differences between groups. Univariate Cox proportional hazards regression analysis was carried out to assess the association between gene expression and overall survival. For the multivariable analysis, a Cox proportional hazards model was established for OS and RFS with a limited forward-LR procedure, which was adjusted for potential confounding covariates. Hazard ratio (HRs) and 95% confidence intervals (CIs) were used to describe the relative risk. Statistical significance was accepted when $P < 0.05$.

Results

Whole-genome analysis of gene promoter methylation's effect on LGGs prognosis based on TCGA dataset

We first extracted data regarding gene promoter CpG sites from the Illumina Infinium Human Methylation 450 platform. A total of 129,225 promoter CpG sites in 18,963 mapping Refseq-annotated genes were selected. The methylation status for each gene promoter in each sample was obtained by calculating the average of all CpG sites in the same promoter region. A total of 2526 promoter methylation showed significant association with LGG patients' overall survival at four cut-off values (adjusted $P < 0.05$, (for all online suppl. material, see www.karger.com/doi/10.1159/000489954) Suppl. Table 1).

Identification of gene promoter methylation relevant gene expression changes in LGGs

To investigate the correlation between promoter methylation and gene expression changes, both the DNA methylation and the gene expression data for 473 samples were downloaded and Spearman's nonparametric correlation analysis was carried out. We

Fig. 2. The association of *EMP3*, *GSX2* and *EMILIN3* promoter methylation with its gene expression, as well as patients' survival. (A) Heatmap of Methylation Probes in *EMP3*, *GSX2* and *EMILIN3* promoter regions. (B) Kaplan-Meier plot for overall survival (OS) between patients with hyper-methylation and hypo-methylation of *EMP3*, *GSX2* and *EMILIN3* promoter in TCGA dataset. (C) Kaplan-Meier plot for relapse-free survival (RFS) between patients with hyper-methylation and hypo-methylation of *EMP3*, *GSX2* and *EMILIN3* promoter in TCGA dataset. (D) The correlation between three genes promoter methylation status and gene expression levels was assessed using Spearman's correlation analysis. (E) The mRNA expression of *EMP3*, *GSX2* and *EMILIN3* in hyper-methylation and hypo-methylation promoter groups. The results are mean $\pm$ SD, *** $P < 0.001$.

observed that mean promoter methylation levels were strongly negatively correlated with corresponding gene expression for 100 genes (Spearman $r < -0.6$ and adjusted $P < 0.05$, see online suppl. material, Suppl. Table 2). To further investigate whether the promoter methylation of the 100 negatively correlated genes affected stable gene expression, the samples were divided into two groups using a methylation beta value = 0.5 as the cut-off value. The inclusion criteria were a >1.5-fold change in gene expression and an adjusted $P < 0.05$ between the hypo- and hyper-methylation groups. We also investigated the association of gene expression with overall survival using univariate Cox proportional hazard regression analysis ($P < 0.05$). Of the 100 genes included, eleven genes (*LOC100130776*, *NCRNA000092*, *RBP1*, *TSTD1*, *TOM1L1*, *DDIT4L*, *EMILIN3*, *PRICKLE3*, *EMP3*, *GSX2* and *NSUN7*) met the above criteria (adjusted $P < 0.05$ between the hypo- and hyper-methylation groups; gene expression level associated with OS; see online suppl. material, Suppl. Table 3). The eleven candidate genes were then used to construct a prognostic signature using a multivariable Cox proportional hazards model. Finally, three genes, *EMP3*, *GSX2* and *EMILIN3*, were selected as signature genes, and correlations between promoter methylation and genes expression for *EMP3*, *GSX2* and *EMILIN3* were shown in Fig. 2.

Generation and validation of three-gene prognostic signature

We further constructed a survival risk score system using the regression coefficient from the multivariable Cox proportional hazards model. The risk score was calculated as follows: risk score = (0.597* expression level of *EMP3* + 0.825* expression level of *GSX2* + 0.942* expression level of *EMILIN3*). Patients were divided into low-risk and high-risk groups according to their risk scores, and the cut-off value was set at 75th percentile [20] (Fig. 3A). As shown in Fig. 3B, the three signature genes were remarkably overexpressed in the high-risk

Fig. 3. Identification of the three-gene prognostic signature with TCGA LGGs cohort. (A) The distribution of three-gene expression and risk score in TCGA cohort, the expression of three genes were transformed by z-score, with red indicating higher expression and blue indicating lower expression. The risk scores for all patients in TCGA LGGs cohort are plotted in ascending order and marked as low-risk (blue) or high-risk (red), as divided by the threshold (vertical black line). (B) The *EMP3*, *GSX2* and *EMILIN3* mRNA was differentially expressed between high and low-risk groups. The results are mean $\pm$ SD, *** $P < 0.001$. Kaplan-Meier plot for OS (C) and RFS (D) in TCGA LGGs cohort stratified by three-gene prognostic signature in high and low-risk.

Table 2. Cox proportional hazards models of the three-gene signature risk scores in TCGA LGGs cohort. Notes: HR: hazard ratio; CI: confidence interval; KPS: karnofsky performance score

Overall survival	Univariate		Multivariate	
	P-value	HR[95%CI]	P-value	HR[95%CI]
Age (year): >40 vs ≤ 40	3.37E-15	1.070 [1.052-1.091]	9.38E-15	1.058 [1.053 - 1.071]
Gender: male vs female	0.4686	1.165 [0.770-1.763]	ns	-
KPS: ≤ 70 vs > 70	0.0113	2.129 [1.187-3.820]	ns	-
Tumor grade: Grade III vs II	5.0E-06	2.851 [1.821-4.465]	0.023	1.745 [1.080 - 2.819]
Risk score: high vs low	1.19E-18	6.374 [4.223-9.662]	5.15E-13	5.813 [3.605 - 9.372]

Fig. 4. Validation of the three-gene prognostic signature with CGGA LGGs cohort. (A) The distribution of three-gene expression and risk score in CGGA LGGs cohort, with red indicating higher expression and light blue indicating lower expression. The risk scores for all patients in CGGA cohort are plotted in ascending order and marked as low-risk (blue) or high-risk (red), as divided by the threshold (vertical black line). (B) The *EMP3*, *GSX2* and *EMILIN3* mRNA was differentially expressed between high and low-risk groups. The results are mean $\pm$ SD, *** P <0.001. (C) Kaplan-Meier plot for OS between high and low-risk groups in CGGA LGGs cohort.

Fig. 5. Three-gene signature performance in grade II and III and IDH1 wild-type subsets in the TCGA LGGs cohort. (A) Comparison of the three-gene signature with the molecular subtypes and the cluster from Ceccarelli et al. for 471 LGG samples from the TCGA database. The three-gene signature is indicated in the first row. (B and D) Kaplan-Meier survival plots for OS and RFS between grade II and grade III patients in the TCGA LGGs cohort. (C) Kaplan-Meier plot for OS between grade II or grade III patients with high-risk and low-risk in TCGA lower-grade glioma patients. (E) Kaplan-Meier plot for RFS between grade II or grade III patients with high-risk and low-risk in TCGA lower-grade glioma patients. (G) Kaplan-Meier survival plots for OS between *IDH* mutant patients and wild type patients in the TCGA LGGs cohort. (H) Kaplan-Meier survival plots for OS between *IDH* mutant patients and wild-type patients with high-risk and low-risk in the TCGA LGGs cohort.

group. In addition, the high-risk group exhibited significantly worse OS (HR=5.45, 95%CI=3.10-9.59, $P=1.55E-20$) and RFS (HR=3.55, 95%CI=2.10-5.99, $P=1.02E-10$) as compared with the low-risk group after adjusting for age, Karnofsky performance score (KPS) and tumor grade (Fig. 3C and D, Table 2).

The CGGA dataset was used to validate the three-gene prognostic signature obtained from the TCGA LGGs dataset. Based on the same risk score cut-off value as the TCGA LGGs dataset, CGGA LGG patients were grouped into low-risk and high-risk groups. Significant differences in the expression levels of all three genes were observed between the two groups (Fig. 4A and B). Kaplan-Meier plot indicated that the high-risk group exhibited significantly worse OS as compared with the low-risk group (HR=3.61, 95%CI=1.37-9.53, $P=0.0007$, Fig. 4C).

Clinical and molecular features of low- and high-risk LGG patients

Comparison of the three-gene signature with the molecular subtypes and the cluster from Ceccarelli et al. for 473 LGG samples from the TCGA database were shown in Fig. 5A. To explore the three-gene signature with respect to prognosis among glioma patients within WHO grades II and III, we further investigated the association between risk score and survival in patient data from the TCGA cohort. As shown in Fig. 5B and D, patients with grade III glioma exhibited significantly worse OS (HR=2.79, 95%CI=1.85-4.21, $P=1.69E-06$) and RFS (HR=1.63, 95%CI=1.12-2.38, $P=0.0092$) than those with grade II glioma. Moreover, the three-gene prognostic signature risk stratified grade II and grade III patients remarkably well in terms of both OS (grade II + high-risk vs grade II + low-risk: HR=3.92, 95% CI=1.28-12.06, $P=0.0001$; grade III + high-risk vs grade III + low-risk: HR=3.61, 95%CI=1.87-6.94, $P=1.86E-08$, Fig. 5C) and RFS (grade II + high-risk vs grade II + low-risk: HR=2.15, 95%CI=0.94-4.92, $P=0.0178$; grade III + high-risk vs grade III + low-risk: HR=3.48, 95%CI=1.72-7.06, $P=2.95E-07$, Fig. 5E). When both WHO grade and the risk score were considered concomitantly, the grade II patients in the low-risk group showed the best outcomes, while the patients with grade III glioma in the high-risk group showed the worst outcome (Fig. 5C and E).

IDH mutation is a widely acknowledged molecular marker that predicts better prognosis for LGG patients [12, 14, 21]. IDH mutation status was known for 471 patients in the TCGA LGGs cohort. We observed that IDH mutant patients showed significantly better OS as compared with IDH wild-type patients (HR=5.48, 95%CI=2.899-10.36, $P=5.30E-21$, Fig. 5F). To clarify whether the three-gene signature acted independently of the IDH mutation as a prognostic indicator for LGGs, we evaluated the association between risk-score and prognosis in LGG patients stratified by IDH mutation status. As shown in Fig. 5G, a significant difference in OS was observed between the high- and low-risk groups in the IDH wild-type patients (HR=3.545, 95%CI=1.42-35.75, $P=0.0336$), and this association was also observed among IDH mutant patients (HR=3.44, 95%CI=1.103-11.40). When both risk score and

Fig. 6. Three-gene signature performance in grade II and III subsets and IDH mutation and wild type subsets in the CGGA LGGs cohort. (A) Kaplan-Meier survival plots for OS and between grades II and III patients in the CGGA LGGs cohort. (B) Kaplan-Meier plot for OS between grade II or grade III patients with high-risk and low-risk in CGGA lower-grade glioma patients. (C) Kaplan-Meier survival plots for OS between IDH mutant patients and wild type patients in the CGGA LGGs cohort. (D) Kaplan-Meier survival plots for OS between IDH mutant patients and wild type patients with high-risk and low-risk in the CGGA LGGs cohort.

IDH mutation status were considered, we observed that *IDH* wild-type patients with high-risk scores showed the worst prognosis. We further validated the findings using the CGGA dataset (Fig. 6). These data indicated that the three-gene signature may act as a prognostic factor for patients with *IDH* wild-type gliomas.

Discussion

Despite recent progress in neurosurgery, radiotherapy and chemotherapy, the survival of LGG patients ranges widely. To improve prediction accuracy and facilitate therapeutic strategies for LGGs, the genetic and epigenetic landscapes of LGGs have been extensively studied [3, 14, 16, 22, 23]. In the case of LGGs, *IDH*, *ATRX*, *TP53*, *TERT* and 1p/19q co-deletion are well-established genetic markers. In particular, *IDH* mutation and 1p/19q deletion were adopted as biomarkers for classifying gliomas in the 2016 WHO classification of central nervous system tumors [1]. DNA methylation, an epigenetic modification via the methylation of cytosin in carbon 5, has been widely reported in human gliomas [24-26]. For example, about 40% of gliomas present with *MGMT* promoter methylation, which acts as a responsiveness biomarker for alkylating agents [9, 10]. However, the role of epigenetic alterations in LGGs and the potential role of DNA methylation as a prognostic biomarker remain largely unclear.

In this study, we first utilized the TCGA methylation data to identify prognostic genes associated with promoter methylation. Next, we screened for genes that were stably regulated by promoter methylation. Then, the Cox proportional hazards regression analysis was carried out to generate a prognostic signature. We observed that this three-gene (*EMP3*, *GSX2* and *EMILIN3*) signature can be used as a prognostic indicator for LGGs patients from both the TCGA and the CGGA datasets. *EMP3* is a member of the PMP22/*EMP*/*MP20* gene family that plays a role in cell proliferation and cell-cell interactions [27]. Previous studies reported that CpG island hypermethylation in the *EMP3* promoter region is frequent in LGGs [28-30]. Moreover, *EMP3* promoter hypermethylation is associated with a favorable prognostic significance in OS in patients with oligodendrogial and glioblastoma [29, 31, 32]. In line with previous studies, we found that promoter methylation in *EMP3* correlated negatively with its expression and lower *EMP3* expression predicted better prognosis in LGGs patients. Our findings further support an oncogenic role on the part of *EMP3* in glioma. Of course, the exact role of *EMP3* in the development of glioma requires further investigation.

GSX2 (*GS homeobox 2*, also known as *GSH2*) is a brain-specific class II homeobox gene that is involved in brain development and neuronal differentiation. The role of *GSX2* in tumor development is contradictory. In pancreatic cancer, *GSX2* has been identified as a novel methylation-sensitive tumor suppressor gene and associated with tumor nose metastasis stage [33]. However, studies in acute myeloid leukemia have suggested *GSX2* as a putative oncogene [34-36]. The influence of *GSX2* promoter methylation on LGGs prognosis remains largely unknown. In this study, we found that hypermethylation of the *GSX2* promoter was correlated negatively with its mRNA expression and that *GSX2* hypomethylation and higher *GSX2* mRNA expression were associated with worse OS and RFS in LGG patients.

EMILIN3 (elastin microfibril interfacier 3), a glycoprotein of the extracellular matrix, belongs to the elastin microfibril interface-located protein (MEILIN)/multimerin family. Previous study using whole genome copy number variation (CNV) analysis revealed that the *EMILIN3* gene was significantly increased in copy number in colorectal cancer samples as compared to non-cancerous samples [37]. However, the role of *EMILIN3* in gliomas remains unclear. In this study, we found that higher *EMILIN3* expression was associated with worse OS and RFS in LGG patients and that promoter methylation in *EMILIN3* was negatively correlated with gene expression. According to previous studies, the mechanism via which *EMILIN3* affects glioma development may be explained by its interaction with heparin and heparin sulfate proteoglycans (HSPG) on the extracellular matrix [38-40]. Further studies are needed to explore the exact mechanisms of *EMILIN3* in the tumor microenvironment

and its pro-tumorigenic function in gliomas.

The survival differences between WHO grade II and WHO grade III gliomas were highly significant, and some LGGs patients showed impressive therapeutic sensitivity [3, 5]. Our results indicated that the three-gene prognostic signature risk score could precisely predict clinical outcomes in LGG patients, and that low-risk score patients with grade II tumors showed the best outcomes, while patients with high-risk score and grade III gliomas showed the worst outcomes. In addition to tumor grades II and III, risk score can also be used to stratify *IDH* wild-type glioma patients. The majority of LGGs belongs to *IDH* mutation category, while *IDH* wild-type patients account for only 20-30% [41]. Moreover, *IDH* mutation status is a widely acknowledged molecular marker for LGGs, and *IDH* wild-type patients are characterized by significantly worse survival outcomes than *IDH* mutant patients [12, 14, 21]. *IDH* wild-type LGGs more frequently show molecular genetic alterations similar to GBM, such as *TP53* mutation, no 1p/19q co-deletion, *TERT* promoter mutation, *CDKN2A*, *EGFR* amplification, and *PTEN* deletion [12, 21, 41]. In addition, the DNA methylation analysis performed by Cecarelli et al. has revealed subtypes of *IDH* mutants and *IDH* wild-type gliomas, and that *IDH* wild-type subtypes labeled Pilocytic Astrocytoma-like is associated with favorable survival [16]. In this study, a significant difference in OS between the high- and low-risk groups was observed among the *IDH* wild-type patients, and that *IDH* wild-type patients with high-risk score showed the worst prognosis. These data indicated that three-gene signature may act as a prognostic factor for *IDH* wild-type gliomas.

In summary, we identified and validated a three-gene prognostic signature that can act as a prognostic factor for LGGs using public database. We also found that the expression of all three genes was regulated by promoter methylation. Our findings support the hypothesis that genes that are tightly controlled by promoter methylation level are more likely to be associated with cancer outcomes. Importantly, the three-gene prognostic signature risk performed well in the stratification of grade II and grade III patients and *IDH* wild-type cohorts, which may help to make the current histology-based tumor classification system more precise and provide better stratification for future clinical trials. In the future, clinical test kits can be developed to detect the expression of three genes in glioma samples, which may help to apply this classification to clinical settings. Regarding the three genes identified in our study, for the first time, high expression of *EMILIN3* was shown to predict poor survival in LGGs. Further experiments are needed to explore the precise role of these genes in glioma progression, and their potential application for glioma prognosis.

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Disclosure Statement

No conflict of interests exists.

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