INTRODUCTION

Glioma is the most common form of malignant primary brain tumor. WHO grading from 2016 classifies gliomas into four grades (I–IV) according to their histopathological signatures with astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas being recognized as lower-grade gliomas (LGGs).1,2 Importantly, the survival of LGG patients ranges widely when subgrouped by histological signature. The most frequent treatment approaches for LGGs are surgical resection combined with chemoradiotherapy.3 However, tumor...
recurrence and malignant progression inevitably occur following treatment, largely owing to the high invasiveness of residual tumor cells and the surgically unapproachable location. Moreover, in some cases, LGGs may rapidly progress to high-grade glioblastoma multiforme brain (GBM) tumors, significantly affecting quality of life for these patients.

The histopathological classifications of LGGs are widely recognized; however, this feature alone does not satisfactorily predict patient survival. On this basis, clinical decisions are frequently made using genetic and molecular classifications. For example, IDH1/2, EGFR, and ATRX are well-known indicators for predicting OS in patients. Further evidence also shows that codeletion of 1p/19q with hypermethylation of the MGMT promoter is critical prognostic factor in LGGs. Current WHO classifications delineate LGGs into three distinct subgroups on the basis of 1p/19q codeletion and IDH mutation. However, the predictive role of these factors in evaluating patient survival outcomes is inadequate since LGG patients presenting with the same signature often have different outcomes. Thus, more comprehensive studies are warranted to identify better predictive models to improve patient management and outcomes.

Epigenetic alterations are now widely recognized as a cancer hallmark. The enzymes involved in cancer epigenomic deregulation can be classified into four functional categories: writers, readers, editors, and erasers. Many epigenetic regulators with oncogenic properties have been intensively investigated in past studies, for example, IDH1 mutations in glioma have been linked with a hypermethylation phenotype which induces global gene expression alterations. Moreover, accumulating evidence shows that epigenetic modifications are tightly linked with the elevated adaptiveness of cancer cells to the harsh tumor microenvironment, together with their increased invasiveness, therapeutic resistance, and recurrence. Instructively, studies have also revealed that the cellular plasticity of glioma cells is highly dependent on epigenetic modifications and their intercellular crosstalk within the microenvironment. However, the predictive efficiency of individual gene modifications remains inadequate, thus limiting the use of this approach in clinical assessments. To improve clinical applications, further investigation of epigenetic enzyme genes is required to construct a more effective integrated predictive model.

The current study focused on deriving an epigenetic gene signature that can predict LGG patient outcomes. Transcriptomic comparisons undertaken to identify deregulated genes were distilled down to a 13-gene epigenetic signature with verification across three data sets, including the Kamoun, Gravendeel, and TCGA cohorts. The high-risk signature was strongly linked with worse clinical outcomes for LGG patients. In addition, multiple oncogenic pathways were found to be activated in the high-risk group with an interestingly association uncovered between the high risk score and immune suppression pathways. Adding to the bioinformatic analyses, we confirmed the oncogenic role of one of the identified genes, SMYD2, using in vitro and in vivo assays.

### 2 MATERIALS AND METHODS

#### 2.1 Ethics

In this study, the usage of cell line and experimental animals (SCID mice) was approved by the Scientific Ethics Committee of Xi’an Jiaotong University, Xi’an, China.

#### 2.1.1 Patients and data sets

A total of 711 lower-grade gliomas (LGGs) were investigated in this study. mRNA expression data of public data sets were obtained from “Gliovis” (http://gliovis.bioinfo.cnio.es/). Clinical and molecular information from TCGA data set (n = 481), Kamoun data set (n = 126), and Gravendeel data set (n = 104) was also collected. In this study, the TCGA data set was used as the discovery set. The other 2 data sets were applied as validation sets. Patient selection criteria: LGG patients with survival less than 1 month, were excluded from this study. We have summarized the clinicopathological characteristics for all patients in Table 1 and Table S1.

#### TABLE 1 Clinicopathological Characteristics and Genetic Alterations of LGG Patients in the TCGA Cohort.

| Variable          | Subgroup | TCGA (N = 481) | N | %  |
|-------------------|----------|----------------|---|----|
| Age               | <=40     | 204            | 42.4% |
|                   | >40      | 221            | 45.9% |
|                   | NA       | 56             | 11.6% |
| Gender            | male     | 235            | 48.9% |
|                   | female   | 190            | 39.5% |
|                   | NA       | 56             | 11.6% |
| Grade             | II       | 211            | 43.9% |
|                   | III      | 227            | 47.2% |
|                   | NA       | 43             | 8.9%  |
| IDH status        | WT       | 85             | 17.7% |
|                   | mutant   | 393            | 81.7% |
|                   | NA       | 3              | 0.6%  |
| Chr 1p19q status  | non-codel| 347            | 72.1% |
|                   | codel    | 169            | 35.1% |
|                   | NA       | 0              | 0.0%  |
| MGMT promoter     | unmethylated| 83            | 17.3% |
|                   | methylated| 398            | 82.7% |
|                   | NA       | 0              | 0.0%  |
| TERT status       | WT       | 146            | 30.4% |
|                   | mutant   | 120            | 24.9% |
|                   | NA       | 215            | 44.7% |
| ATRX status       | WT       | 301            | 62.6% |
|                   | mutant   | 177            | 36.8% |
|                   | NA       | 3              | 0.6%  |
2.1.2 | Data processing and risk score construction

Transcriptomic data from the TCGA, Kamoun, and Gravendeel data sets were systematically analyzed with the aim to identify a gene signature that captures epigenetic modifications of LGG tumor cells, hereafter referred to as deregulated epigenetic enzyme gene (DEEG) signature.

For the discovery phase, differentially expressed genes (DEGs) were derived from the TCGA data set using the “Gene Expression Profiling Interactive Analysis (GEPIA)” (http://geopia.cancer-pku.cn/). 207 normal brain specimens from the GTEx Portal were used as controls. TCGA and GTEx samples were re-analyzed (re-aligned to hg38 genome and expressions are called using RSEM and Kallisto methods) by the same RNA-seq pipeline. Gene transcripts per million (TPM) data were used for analysis. Genes of |log2FC| >1 and FDR-adjusted P value <0.01 were identified as differentially expressed genes. A total of 5745 DEGs were identified and applied against the next screening step of 212 epigenetics enzyme genes to identify a total of 57 deregulated epigenetic enzyme genes (DEEGs). Further analysis using LASSO Cox regression revealed 13 of the 57 selected DEEGs were found to be powerful prognostic LGG biomarkers. The coefficients of 13 DEEGs were used to construct a risk score (RS) model and to assess the predictive accuracy of the risk score; time-dependent ROC curve analyses were performed.

2.2 | Bioinformatic analysis

GO (Gene ontology) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were carried out by using a well-known online software (https://david-d.ncifcrf.gov/). To visualize the potential links between the DEEGs, we constructed the protein-protein network using the STRING database. MCODE method in Cytoscape was used to identify clusters among the DEEGs. Gene set enrichment analysis (GSEA) was performed using a well-known online tool (http://software.broadinstitute.org/gsea/index.jsp). During the analysis, risk score was regarded as a phenotype.

2.3 | Analyses of immune signature

The 782 immune metagenes were obtained from previous study. The metagenes contain 28 cell subpopulations. The single-sample gene set enrichment analysis (ssGSEA) was performed to determine the enrichment scores for all the cases. Analysis for the link of gene expression with immune infiltration was based on an online tool “TIMER (Tumor Immune Estimation Resource, https://cistrome.shinyapps.io/timer/)”.

2.4 | Statistics

The statistical analyses were carried out using the R software (version 4.0.0), SPSS (version 22.0), or Prism 6 (GraphPad Software).
SUZ12, SMYD2, and ZBTB33 significantly contributed to better outcomes (Figure 1C and S2D). Subsequently, based on these 13 biomarkers, we constructed an epigenetic risk score model. The formula for the risk score is presented in Figure S2E. Using the mRNA expression and regression coefficient of each gene, we calculated the risk score for each case in the TCGA data set. The distribution of the risk scores, OS, and OS status in the TCGA cohort is presented in Figure 1D and 1E.

### 3.2 The risk score model harbors robust prediction value for LGG patients

We next evaluated the prognostic value of the epigenetic score by Kaplan-Meier (K-M) analyses of patient overall survival using the TCGA data set (Table 1) and then verified the results against the validation data sets (Table S1). Based on the median value of risk scores, the tumor samples were assigned into low- or high-risk
As anticipated, patients in the high-risk group demonstrated significantly poorer outcomes compared with those in the low-risk group (Figure 2A). Consistently, K-M survival curves showed that high-risk patients in the validation data sets had shorter overall survival than low-risk classified patients (Figure 2B and 2C). Additionally, we evaluated the predictive ability of the risk score using ROC analysis against overall survival at 1, 2, and 3 years (Figure 2D-F). ROC analyses comparing the performance of the risk signature against of IDH and 1p19q status (Figure S3A-C) showed that the risk score produced higher AUC values (0.874, 0.85, and 0.866 for 1, 2, and 3 years, respectively). Further analyses of validation data sets confirmed the predictive value of the risk score with AUC values being 0.865, 0.825, and 0.799 (validation data set 1), and 0.81, 0.757, and 0.774 (validation data set 2), respectively. Collectively these analyses highlight the superior performance of the epigenetic signature in predicting patient outcomes.

We further considered our findings in relation to the WHO classification of three different LGG subgroups according to the IDH mutation and 1p/19q codeletion status. K-M analyses were performed on patients with high- and low-risk LGGs based on their molecular subtypes. Notably, even within different subgroups we observed that the 13-gene epigenetic signature was still effective in the prediction of patient outcomes (Figure 2G). Moreover, in addition to the molecular subtypes, we applied K-M analyses against other clinical factors including MGMT promoter status, sex, age, grade, and histology diagnosis. These results showed that the risk score still harbors robust stratification ability for LGG patients (Figure S4A-K).

**Figure 2** The risk score model harbors robust prediction value for LGG patients. (A-C) Kaplan-Meier curve comparing overall survival of LGG patients subgrouped by the risk score in the TCGA data set (A), Kamoun data set (B), and Gravendeel data set (C). (D-F) Time-dependent ROC analysis (1, 2, and 3 years) according to the risk score in the TCGA data set (D), Kamoun data set (E), and Gravendeel data set (F). (G) Kaplan-Meier curve comparing overall survival of LGG patients with high and low risk. The patients with IDH mutation, wild-type IDH, 1p19q codeletion, and without 1p19q codeletion were analyzed separately.
3.3 | The epigenetic gene signature robustly identifies poor molecular signatures in LGGs

We further investigated the link between the risk score and the aforementioned clinical factors in LGGs. Clinicopathological and genetic alterations, including age, sex, grade, IDH mutation status, MGMT promoter status, Chr. 1p19q status, TERT mutation status, and ATRX mutation status, are summarized in Table 1. As shown in Figure 3A, higher risk scores demonstrated stronger associative trends with older age, higher tumor grade, wild-type (WT) IDH, 1p19q non-codeletion (non-codel), and non-methylation of the MGMT promoter. We subgroups the LGGs based on these signatures and compared the risk score value between each group. The result further validated our observations (Figure 3B). In addition, analyses using Pearson’s chi-squared test (Table 2) demonstrated that higher risk score was significantly linked to higher tumor grades (grade III, \( p < 0.001 \)), WT IDH (\( p < 0.001 \)), non-codel status of 1p19q (\( p < 0.001 \)), non-methylation status of the MGMT promoter (\( p < 0.001 \)), and WT status of TERT (\( p < 0.001 \)). However, no significant differences were identified with sex or age.

Next, we performed univariate and multivariate Cox regression analyses to determine if the prediction ability of the epigenetic signature was independent of other factors. The univariate analysis showed that age, grade (II vs. III), IDH mutation status (mutant vs. WT), 1p19q status (codel vs. non-codel), MGMT promoter methylation status (methylated vs. unmethylated), and the risk score all derived significant hazard ratios (HR) in the TCGA data set (Table S3). Factors giving significant results were applied in a multivariate analysis where the risk score was found to be prognostic independently of other clinical factors for patient overall survival (HR =2.50, 95% CI =1.658–3.77) (Figure 3C).

3.4 | Nomogram model construction based on the epigenetic signature

To further assess the clinical application of the epigenetic model, we constructed a nomogram, incorporating risk score and clinicopathological parameters such as age and grade (Figure 3D). The nomogram generated to predict the 1-year, 3-year, and 5-year overall survival in the TCGA data set demonstrated good calibration and discrimination ability for predicting LGG patient outcomes (Figure 3E). Indeed, the high C-index (0.86) indicated that our model exhibited very good performance in this assessment.

3.5 | Pathway enrichment analysis of the epigenetic gene signature

We next sought to explore the molecular basis of the epigenetic signature using gene set enrichment analysis (GSEA) to better identify the underlying oncogenic pathways involved. Here we employed the TCGA data set using the risk score as a phenotype label (high risk vs. low risk) (Table S4). This analysis showed that the interferon alpha/gamma response, the epithelial-mesenchymal transition (EMT), the G2/M checkpoint, angiogenesis, the TNF-α signaling via NFκB, and the E2F targets were significantly activated in the high-risk group (Figure 4A and S5). The same pathway enrichments were observed in the validation data sets (Figure 4B, 4C and S5), collectively indicating that critical pathways linked with tumor invasion and infiltration such as epithelial-mesenchymal transition (EMT) and angiogenesis are activated in high-risk LGGs. Moreover, the therapeutic response-associated pathways were found to be highly enriched in the high-risk group, including G2/M checkpoint and E2F target pathways. Interestingly, we also found that the pathways strongly associated with immune response were significantly activated in the high-risk group (interferon response, inflammation response, and TNF-α signaling, respectively). The latter finding suggests that epigenetic status could contribute to intra-tumoral immune heterogeneity, thereby contributing a critical role in the cellular interactions between tumor cells and immune cells.

3.6 | The risk score model is closely associated with immune suppression in LGGs

Given the above findings, we sought to explore the link between the risk score and immune status in LGGs. We first performed ssGSEA to assess the infiltration scores of different immune cell subpopulations (Figure 5A). Interestingly among the 28 cell subpopulations, myeloid-derived suppressor cells (MDSCs), natural killer (NK) T cells, and active dendritic cells showed significantly higher correlation with the high risk score (Figure 5B and 5C). Previous studies have shown that the majority of infiltrating immune cells in glioma are microglia, blood-derived macrophages, and MDSCs. In addition, MDSCs is a well-known cell population tightly linked with immune suppression in glioma, inducing T-cell apoptosis, Treg cell activation, and functional impairment of NK cells and active dendritic cells. Thus, we performed further focused investigations on MDSCs.

FIGURE 3 The epigenetic gene signature robustly identifies poor molecular signatures in LGGs. (A) Heat map of the risk score and 13 identified genes in the TCGA data set. Grade, IDH mutation status, 1p/19q codeletion status, TERT promoter status, BRAF, V600E status, and MGMT promoter status are annotated in the upper panel. (B) Dot plot of the risk score in LGG patients subgrouped by the status of IDH, 1p19q, MGMT promoter, grade, age, gender, TERT status, and ATRX status. (C) Forest plot showing the multivariate Cox regression analysis with risk score, age, grade, IDH mutation status, MGMT promoter status, and 1p19q status. RS, risk score. ** \( p < 0.001 \), ns, not significant. (D) The nomogram for predicting the 1-year, 3-year, and 5-year OS in LGG patients. RS, risk score. (E) The calibration plots showed that the nomogram model harbors robust ability in predicting patient outcome.
Risk score and clinical characteristics

Multivariate Cox for Risk score and clinical factors

(B)

(D)

(E)

Nomogram-Predicted Probability of 1 year OS
Nomogram-Predicted Probability of 3 year OS
Nomogram-Predicted Probability of 5 year OS
We observed a significantly higher meta-score for MDSCs in the high-risk group (Figure 5D). Moreover, correlation analyses between risk score and the meta-score further confirmed this observation (Figure 5E). Similar correlation result was observed in the validation data sets (Figure S6A and S6B). Infiltration correlation analysis using the “TIMER” online tool showed that PCNA was the main contributor to MDSC enrichment in LGGs (Figure 5F). In addition to the infiltration of immune suppressive cells in LGGs, we also investigated the relationship between the risk score and the expression of immune suppressive biomarkers. Notably, the results revealed that the epigenetic signature was tightly linked with mRNA expression levels of PD-L1, PD-1, CTLA-4, TGFβ1/2/3, CCL5, IL-10, CSF-1, and CSF-1R, highlighting an elevated immune suppressive microenvironment in these tumor samples (Figure 5G and S6C). In addition, correlation analyses identified that the epigenetic score was positively associated with these immune suppression biomarkers (Figure 5H and S6D). Collectively, these data propose a strong link between the risk score model and tumor immune suppression.

### 3.7 Functional validation of oncogenic role of SMYD2 in glioma

We next investigated the oncogenic role of SMYD2, one of the 13 identified biomarkers where analysis of the TCGA data set showed significantly elevated expression of in the high-risk group (Figure S7A).

To directly investigate the functional role of SMYD2, we infected U373 glioma cells with lentiviruses expressing either non-targeting control (shNT) or two independent shRNAs targeting SMYD2 (SMYD2#1 and #2, respectively). The silencing efficacy was evaluated by qRT-PCR, indicating significant reductions in SMYD2 mRNA expression with a relatively higher silencing efficacy of shSMYD2#1 compared with shSMYD2#2 (Figure 6A). Assessment of the in vitro growth of the U373 glioma cells demonstrated that their proliferative capacity was attenuated after SMYD2 silencing in comparison to the shNT-treated cells (Figure 6B). As further in vivo validation, shNT or shSMYD2-bearing U373 glioma tumor cells were intracranially injected into the brains of SCID mice. The outcome revealed a significantly prolonged survival time in the shSMYD2 group (Figure 6C) with serial bioluminescent imaging (Figure 6D and 6E) confirming that SMYD2 silencing attenuated the tumor forming ability and progression of xenografted glioma cells. As independent confirmation of these data, experiments utilizing the SMYD2 inhibitor, LLY-507, demonstrated that targeting SMYD2 was effective in inhibiting the proliferation of U373 glioma cells (Figure 6F). Remarkably, shSMYD2 treatment significantly reduced the expression of three other epigenetic regulators in the 13-gene risk set, namely HDAC1, PCNA, and SUZ12, implying that SMYD2 expression contributes to other elements of the epigenetic risk score (Figure S7B). Collectively these data indicate that SMYD2 is essential to glioma tumor cell proliferation in vitro and tumor formation in vivo.

Finally, given the importance of temozolomide (TMZ) in the therapeutic management of glioma following surgical resection, we investigated whether SMYD2 confers TMZ resistance to glioma. Glioma cells were treated with TMZ alone and in combination with shRNA and inhibitor approaches to target SMYD2. Instructively, both shRNA silencing of SMYD2 (Figure 6G) and LLY-507 inhibitor treatment (Figure 6H) potentiated the effects of TMZ of cell growth.

### TABLE 2 Clinicopathological Correlations of the Epigenetic Enzyme Gene Signature in the TCGA Cohort

| Variable                  | N     | High risk score | Low risk score | p value |
|---------------------------|-------|-----------------|----------------|---------|
| Age                       | <=40  | 96              | 108            | 0.456   |
|                           | >40   | 112             | 109            |         |
| Gender                    | male  | 112             | 123            | 0.557   |
|                           | female| 96              | 94             |         |
| Grade                     | II    | 86              | 125            | 0.001   |
|                           | III   | 127             | 100            |         |
| IDH status                | WT    | 80              | 5              | <0.001  |
|                           | mutant| 160             | 233            |         |
| Chr 1p19q status          | non-codel | 220       | 103            | <0.001  |
|                           | codel | 21              | 137            |         |
| MGMT promoter             | unmethylated | 70         | 13             | <0.001  |
|                           | methylated | 171       | 227            |         |
| TERT status               | WT    | 81              | 65             | 0.035   |
|                           | mutant| 51              | 69             |         |
| ATRX status               | WT    | 134             | 167            | 0.001   |
|                           | mutant| 106             | 71             |         |
when compared with control group. Together these data suggest that SMYD2 influences the resistance of glioma cells to TMZ with implications for the chemoradiotherapy of glioma.

4 | DISCUSSION

Several studies have already described significant links between the distinct molecular subtypes and poor patient prognosis in glioma. Nevertheless, the clinical application of these factors, especially for individual patients, has thus far not been satisfactory. Recent evidence shows that the epigenetic modifications in tumor cells play a critical role in the progression of many cancers including gliomas. Thus, in this study, we focused on the exploration and construction of an epigenetic signature to provide an alternative assessment model that could be used clinically.

We used the resources of the TCGA as our discovery cohort and screened for known epigenetic enzyme genes that are aberrantly expressed in LGGs. Consequently, we identified a 13 deregulated epigenetic enzyme gene (DEEG) signature which after validation showed high predictive value for patient outcomes. Moreover, the high risk score was tightly linked with malignant LGG features including wild-type IDH status, unmethylated MGMT promoter, and non-codeletion status of 1p19q. Additionally, pathway enrichment analysis showed that the epigenetic signature was strongly associated with a variety of oncogenic pathways including immunity as further described below.

Collectively, our risk score model defined a variety of oncogenic genes that are involved in multiple epigenetic modification processes, depicting a general map of epigenetic alternations for LGGs and enabling higher prediction value for patient outcome. Of the 13 selected biomarkers, higher expression of IDH1, HDAC1, PCNA, SUZ12, PHF8, SMYD2, and ZBTB33 was significantly correlated with worse outcome in LGG patients. Many of these enzymes are strongly implicated in LGG tumorigenesis. For instance, the mutation status of IDH1 and less commonly IDH2 has been well recognized as crucial factors for classification and prognostic prediction in LGGs. The mutation of IDH1 or IDH2 leads to the elevated production of the oncometabolite 2-hydroxyglutarate, which is thought to induce significant epigenetic alterations and promote the initiation and progression of tumor cells. For HDAC1, a well-known histone deacetylase, a number of studies
YU et al. have validated its crucial role in tumor progression. One recent study also found that PCNA is strongly associated with the stemness and radio-resistance in glioma tumor cells. SUZ12, a critical factor in the PRC2 complex, has also been found to promote therapeutic resistance and intra-tumoral heterogeneity in GBM. Another study found that ZBTB33 is highly expressed in gliomas and tightly linked with proliferation, invasion, and EMT phenotype. For PHF8 and SYMD2, even though reports have confirmed their oncogenic roles in other cancer types, little is known about the function of these biomarkers in glioma.

**FIGURE 5** The risk score model is closely associated with immune suppression in LGGs. (A) Heat map of different immune cell populations in LGGs from TCGA data set. The ssGSEA scores was applied. (B) The correlation matrix showing the correlation between 28 immune cell subpopulations and the risk score. (C) Radar map showing the immune infiltration difference between high- and low-risk subgroups. (D) Dot plot showing the meta_score for MDSCs in high- or low-risk groups for TCGA data set. (E) Correlation analysis of the risk score with meta_score of MDSCs in TCGA data set. (F) Infiltration correlation analysis of PCNA with MDSC, using “Timer” web tool. (G) Dot plot showing the expression of PD-L1, CTLA-4, TGFβ2, CCL5, and CSF-1R in high- or low-risk groups. (H) Correlation analysis of the risk score with mRNA expression of PD-L1, CTLA-4, TGFβ2, CCL5, and CSF-1R.***p < 0.001

**FIGURE 6** Functional validation of SMYD2 oncogenic role in glioma. (A) qRT-PCR analysis of SMYD2 mRNA expression in U373 glioma cells treated with NT-shRNA or SMYD2-shRNA. GAPDH served as control. (B) In vitro cell viability assay of U373 glioma cells treated with either NT-shRNA or SMYD2-shRNA. (C) Kaplan-Meier curve comparing overall survival of mice intracranially injected with U373 glioma cells pre-treated with either NT-shRNA or SMYD2-shRNA. Log-rank test. (D) Representative bioluminescence images of mice injected with glioma cells pre-infected with either NT-shRNA or SMYD2-shRNA on day 25. (E) Signal quantification for Figure 6D. (F) In vitro cell viability assay of U373 glioma cells treated with either DMSO or LLY-507 (SMYD2 inhibitor) on day 3. (G) In vitro cell viability assay of U373 glioma cells treated with/without TMZ after pre-treatment with either NT-shRNA or SMYD2-shRNA. (H) In vitro cell viability assay of U373 glioma cells treated with/without TMZ after pre-treatment with either DMSO or LLY-507 (SMYD2 inhibitor). **p < 0.01, ***p < 0.001
On this basis, we chose to further explore the oncogenic role of SMYD2 in glioma. SMYD2 functions as a lysine methyltransferase and has been previously shown to promote proliferation, epithelial-mesenchymal transition, and invasion of a variety of cancer cell types.\textsuperscript{38-40} In addition, one study found that positive expression of SMYD2 was associated with poor prognosis in patients with hepatocellular carcinoma.\textsuperscript{41} The interaction between SMYD2 and EZH2,\textsuperscript{39} a critical factor in glioma progression, further highlights its potential oncogenic role in LGGs. Here we showed through shRNA silencing and inhibitor approaches that SMYD2 is essential for cell proliferation, tumor formation, and TMZ resistance in glioma tumor cells. However, further mechanistic investigations are warranted for a deeper understanding of SMYD2 functions in this context.

One highly interesting observation concerned the strong enrichment of a variety of immune-associated pathways in the high-risk group, indicating a potential link between our risk score model and the immune microenvironment in LGGs. To verify our hypothesis, the ssGSEA analysis and expression correlation analysis were further carried out to explore the link of the epigenetic gene signature with immune status in LGGs. The analyses confirmed the link between higher infiltration of MDSCs and the high risk score. Accumulating evidence from the literature reveals a critical role of MDSCs in immune suppression, as well as their prominent role in tumor invasion, and therapeutic resistance.\textsuperscript{42} Thus, the samples with a high risk score are likely to be more immune suppressive. Furthermore, the high risk scores were also closely associated with elevated mRNA expression for immune suppression biomarkers (TGFβ1/2/3, etc.) and the immune checkpoint biomarkers (PD-1, PD-L1, and CTLA-4). Previous studies have reported that TGFβ is capable of slowing down the anti-tumor activation of T cells and lead to a more immune-suppressive microenvironment.\textsuperscript{27,43} In addition, the binding of PD-L1 (tumor cells) with PD-1 (T cells) can lead to exhaustion of activated T cells.\textsuperscript{44,45} Therefore, tumor cells create an immunosuppressive environment via this interaction to escape from immune system attack.\textsuperscript{45} Recent evidence has also shown that PD-L1 engagement can also inhibit phagocytosis of tumor-associated macrophages.\textsuperscript{46} Additionally, cytotoxic T lymphocyte–associated protein-4 (CTLA-4), another crucial immune checkpoint marker expressed on T cells, is capable of decreasing the activation of T helper and effector cells while stimulating Tregs.\textsuperscript{26,47} Thus, it is likely that the higher infiltration of multiple immune cells in high-risk group promotes malignant LGG progression through a variety of mechanisms including the increased involvement of MDSCs and the effects of immune checkpoint and suppression markers which disable the activation of active immune cells.

The limitations of this study should be noted in particular, the reliance on data derived from bioinformatic analyses. While we did perform preliminary investigations on the role of SMYD2, many key elements of the study need further verification. For example, the elevated infiltration of immune cells such as MDSCs needs to be further verified. Our mouse model experiments used SCID mice which lack an immune component, so other approaches are needed to better define this aspect. In addition, the clinical application of the risk score model in immune therapy assessment may need further verification. Previous studies (Tumor Immune Dysfunction and Exclusion, TIDE)\textsuperscript{48} have established an online data set for immune response prediction; however, its application in glioma is limited due to the lack of appropriate clinical samples from glioma patients undergoing immunotherapy. A comprehensive data set of glioma patients with detailed immune therapy response record is therefore necessary for this verification.

Additionally, the risk score may also guide the current standard post-surgical treatments, including chemotherapy and irradiation treatment. High correlations between the risk score and unmethylated MGMT promoter indicate that patients with high risk scores may display an unfavorable response to temozolomide (TMZ), the most frequently used chemotherapy drug for glioma.\textsuperscript{49} Similarly, the enrichment of multiple oncogenic pathways, including G2/M checkpoint,\textsuperscript{21} E2F targets,\textsuperscript{22} and EMT,\textsuperscript{19} is strong indicator for an irradiation-resistant phenotype. These findings therefore imply utility for the risk score model in therapeutic response assessment. However, further analyses of patients with detailed therapeutic records are necessary for the validation of our hypothesis.

In conclusion, we focused on understanding of the epigenetic enzyme deregulation in LGGs. Through a comprehensive analysis, a novel, 13-gene epigenetic signature has been identified and validated. This signature harbors robust risk stratification ability and facilitates a better prediction of overall outcome for LGG patients compared with existing criteria. Our findings suggest that epigenetic deregulations are tightly linked to oncogenic process alterations, and the signature can be applied as a prediction tool in clinical assessment. Furthermore, the risk score may help guide and assess immunotherapy.

ACKNOWLEDGEMENTS
We thank all the members that contribute to this work. The authors would like to express their gratitude to EditSprings (https://www.editsprings.com/) for the expert linguistic services provided.

CONFLICTS OF INTEREST
We claim that no conflicts of interest exist in the submission and the manuscript has been approved by all authors.

DATA AVAILABILITY STATEMENT
The data used to support the findings of this study are available from the corresponding author upon request.

ORCID
Hai Yu \(\text{https://orcid.org/0000-0001-9404-8037}\)

REFERENCES
1. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol. 2016;131(6):803-820.
2. Brat DJ, Verhaak RG, Aldape KD, et al. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N Engl J Med*. 2015;372(26):2481-2498.

3. Weller M, van den Bent M, Tonn JC, et al. European Association for Neuro-Oncology (EANO) guideline on the diagnosis and treatment of adult astrocytic and oligodendroglial gliomas. *Lancet Oncol*. 2017;18(6):e315-e329.

4. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med*. 2009;360(8):765-773.

5. Sun Y, Zhang W, Chen D, et al. A glioma classification scheme based on coexpression modules of EGFR and PDGFRα. *Proc Natl Acad Sci USA*. 2014;111(9):3538-3543.

6. Liu X-Y, Gerges N, Korshunov A, et al. Frequent ATRX mutations and loss of expression in adult diffuse astrocytic tumors carrying IDH1/IDH2 and TP53 mutations. *Acta Neuropathol*. 2012;124(5):615-625.

7. Weller M, Stupp R, Hegi ME, et al. Personalized care in neuro-oncology coming of age: why we need MGMT and 1p/19q testing for malignant glioma patients in clinical practice. *Neuro-oncology*. 2012;14(Suppl 4):iv100-iv108.

8. Timp W, Feinberg AP. Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. *Nat Rev Cancer*. 2013;13(7):497-510.

9. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674.

10. Yang Z, Jones A, Widschwendter M, Tschendorff AE. An integrative pan-cancer-wide analysis of epigenetic enzymes reveals universal patterns of epigenetic deregulation in cancer. *Genome Biol*. 2015;16(1):140.

11. Noushmehr H, Weisenberger DJ, Diefes K, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of human solid tumors. *Nat Genet*. 2012;44(5):615-625.

12. Bowman RL, Wang Q, Carro A, Verhaak RG, Squatrito M. GlioVis combined BMI1 and EZH2 inhibition. *Nat Med*. 2017;23(11):1352-1361.

13. Ni T, Li X-Y, Lu NA, et al. Snail1-dependent p53 repression regulates expansion and activity of tumour-initiating cells in breast cancer. *Nat Cell Biol*. 2016;18(11):1221-1232.

14. Laugesen A, Helin K. Chromatin repressive complexes in stem cells, development, and cancer. *Cell Stem Cell*. 2014;14(6):735-751.

15. Song Y, Jiang Y, Tao D, et al. NFIAT2-HDAC1 signaling contributes to the malignant phenotype of glioblastoma. *Neuro-oncology*. 2020;22(1):46-57.

16. Ong DST, Hu B, Ho YW, et al. PAF promotes stemness and radioresistance of glioma stem cells. *Proc Natl Acad Sci USA*. 2017;114(43):E90 86-E9095.

17. Bao S, Wu Q, McLendon RE, et al. Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep*. 2017;18(1):248-262.

18. Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Can Res*. 2017;77(21):e108-e110.

19. Wendt MK, Balanis N, Carlin CR, Schiemann WP. STAT3 and epithelial-mesenchymal transition in carcinomas. *JAK-STAT*. 2014;3(2):e28975.

20. Swanson KR, Rockne RC, Claridge J, Chaplain MA, Alvord EC Jr, Anderson AR. Quantifying the role of angiogenesis in malignant progression of gliomas: in silico modeling integrates imaging and histology. *Can Res*. 2011;71(24):7366-7375.

21. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756-760.

22. Yu H, Li Z, Wang M. Expression and prognostic role of E2F transcription factors in high-grade glioma. *CNS Neurosci Ther*. 2020;26(7):741-753.

23. Raychaudhuri B, Rayman P, Huang P, et al. Myeloid derived suppressor cell infiltration of murine and human gliomas is associated with reduction of tumor infiltrating lymphocytes. *J Neurooncol*. 2015;122(2):393-401.

24. Bruni D, HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer*. 2020;20(11):662-680.

25. Perring P, Lim M. Immunosuppressive mechanisms of malignant gliomas: parallels at Non-CNS sites. *Front Oncol*. 2015;5:153.

26. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell*. 2015;27(4):450-461.

27. Derynck R, Turley SJ, Akhurst RJ. TGFβ biology in cancer progression and immunotherapy. *Nat Rev Clin Oncol*. 2020.

28. Aldiniucci D, Borghese C, Casagrande N. The CCL5/CCR5 axis in cancer progression. *Cancers*. 2020;12(7):1765.

29. Bloch O, Crane CA, Kaur R, Safaee M, Rutkowski MJ, Parsa AT. Gliomas promote immunosuppression through induction of B7–H1 expression in tumor-associated macrophages. *Clin Cancer Res: Off J Am Assoc Cancer Res*. 2013;19(12):3165-3175.

30. Pyonteck SM, Akkari L, Schuhmacher AJ, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med*. 2013;19(10):1264-1272.

31. Jin X, Kim LJY, Wu Q, et al. Targeting glioma stem cells through combined BMI1 and EZH2 inhibition. *Nat Med*. 2017;23(11):1352-1361.

32. Nt T, Li X-Y, Lu NA, et al. Snail1-dependent p53 repression regulates expansion and activity of tumour-initiating cells in breast cancer. *Nat Cell Biol*. 2016;18(11):1221-1232.

33. Laugesen A, Helin K. Chromatin repressive complexes in stem cells, development, and cancer. *Cell Stem Cell*. 2014;14(6):735-751.

34. Song Y, Jiang Y, Tao D, et al. NFIAT2-HDAC1 signaling contributes to the malignant phenotype of glioblastoma. *Neuro-oncology*. 2020;22(1):46-57.

35. Ong DST, Hu B, Ho YW, et al. PAF promotes stemness and radioresistance of glioma stem cells. *Proc Natl Acad Sci USA*. 2017;114(43):E90 86-E9095.

36. Lee SJ, Kim YS, Lee KH, et al. Small molecule inhibitor of NFIAT2 promotes breast cancer cell growth through inhibition of NFIAT2-HDAC1 signaling. *Cell Death Dis*. 2018;9(3):326.

37. Meng F, Liu X, Lin C, et al. SMYD2 suppresses APC2 expression to activate the Wnt/β-catenin pathway and promotes epithelial-mesenchymal transition in colorectal cancer. *Am J Cancer Res*. 2020;10(3):997-1011.

38. Li X, Zhou JX, Calvet JP, Godwin AK, Jensen RA, Li X. Lysine methylation and demethylation promote breast cancer cell growth through regulation of BMI1 and EZH2 in breast cancer cells. *Cancer Cell*. 2017;23(11):1244-1255.

39. Zuo S-R, Zuo X-C, He Y, et al. Positive expression of SMYD2 is associated with poor prognosis in patients with primary hepatocellular carcinoma. *J Cancer*. 2018;9(2):321-330.

40. Kumar V, Patel S, Tcyganov E, Gabriolivich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. *Trends Immunol*. 2016;37(3):208-220.

41. Akhurst RJ, Hata A. Targeting the TGFβ signalling pathway in disease. *Nat Rev Drug Discovery*. 2012;11(10):790-811.

42. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immune checkpoint receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*. 2000;192(7):1027-1034.
45. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. Sci Trans Med. 2016;8(328):328rv4.
46. Gordon SR, Maute RL, Dulken BW, et al. PD-1 expression by tumor-associated macrophages inhibits phagocytosis and tumour immunity. Nature. 2017;545(7655):495-499.
47. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015;161(2):205-214.
48. Fu J, Li K, Zhang W, et al. Large-scale public data reuse to model immunotherapy response and resistance. Genome Med. 2020;12(1):21.
49. Hegi ME, Genbrugge E, Gorlia T, et al. MGMT promoter methylation cutoff with safety margin for selecting glioblastoma patients into trials omitting temozolomide: a pooled analysis of four clinical trials. Clin Cancer Res: Off J Am Associat Cancer Res. 2019;25(6):1809-1816.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Yu H, Zhang D, Lian M. Identification of an epigenetic prognostic signature for patients with lower-grade gliomas. CNS Neurosci Ther. 2021;27:470–483. https://doi.org/10.1111/cns.13587