Original Article

Autophagy related DNA methylation signature predict clinical prognosis and immune microenvironment in low-grade glioma

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Background: Low-grade glioma (LGG) is a common malignant tumor of the central nervous system. The clinical prognosis of different patients varies greatly, so exploring appropriate markers that affect the prognosis and treatment of LGG is important. The purpose of this study was to identify the potential effect of autophagy-related DNA methylation on the prognosis and immune microenvironment in LGG.

Methods: The methylation profile, transcription data and corresponding clinical information of 451 patients with LGG were obtained from The Cancer Genome Atlas (TCGA). Another methylation data and clinical information of 110 patients with LGG from Chinese Glioma Genome Atlas (CGGA) were used as the validation set. Through univariate and multivariate COX regression analysis, we identified the autophagy-related genes (ARGs) associated with methylation levels and prognosis, and established a risk assessment signature. The receiver operating characteristic (ROC) and Kaplan-Meier (KM) survival curve were used to verify the model's effectiveness in predicting prognosis. Patients were divided into low- and high-risk groups based on risk scores. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were used to explore the differences in biological functions between the two groups. ESTIMATE and CIBERSORT algorithms were used to explore differences in immune infiltration and immunotherapy sites. Pearson correlation analysis was used to analyze the relative relationship between methylated cg sites and corresponding genes.

Results: A total of 6 ARGs (*ARSB*, *CFLAR*, *WIPI2*, *RB1*, *ERN1*, *RAB24*) were selected that were associated with methylation levels and prognosis. The area under the curve (AUC) =0.96, and the KM survival curve P<0.0001, which proves that the risk assessment model has a good effect in predicting the prognosis of LGG. GO and KEGG enrichment analysis showed that the model mainly involved major histocompatibility complex (MHC) II receptors, antigen processing and presentation, and immune cell differentiation. In addition, we also found differences in immune infiltration and immune checkpoints between high- and low-risk groups.

Conclusions: The methylation levels of these 6 ARGs have a strong predictive potential for LGG, and the methylation regulation of ARGs has an important impact on the immune microenvironment of LGGs.

Keywords: Low-grade glioma (LGG); autophagy; DNA methylation; prognosis; immune microenvironment

Submitted Feb 10, 2022. Accepted for publication May 17, 2022.
doi: 10.21037/tcr-22-310
View this article at: https://dx.doi.org/10.21037/tcr-22-310

Introduction

Glioma is the most usual primary malignant tumor of the central nervous system with a worse clinical prognosis. According to the 2016 World Health Organization (WHO) Classification of Tumors of the Central Nervous System, it is divided into grades I–IV, of which grades II–III are named diffuse low-grade gliomas (LGG) (1,2). The prognosis of LGGs is significantly different, some patients relapse into recurrent high-grade gliomas, while other patients have longer progression-free survival after treatment (3). LGG appears diffuse and infiltrative, often closely associated with important functional areas of the brain. Complete surgical resection is difficult, and despite the continuous advances in postoperative radiotherapy and chemotherapy in the past few decades, the clinical prognosis has not improved significantly (4). Novel treatment and potential targets need to be further detected.

Epigenetic modification refers to changing the expression of key genes in the development of tumors without transforming the DNA sequence, including the loss of tumor suppressor genes and the abnormal activation of proto-oncogenes (5). Various epigenetic mechanisms, including DNA methylation, histone modifications, and noncoding RNAs, were proved to regulate tumorigenesis, progression, and treatment in multiple tumors (6). DNA methylation is the most widely studied epigenetic modification, and abnormal DNA methylation can be found in a variety of tumors, including gliomas (7,8). DNA methylation can affect the changes in various biological behaviors and signaling pathways in immune cells, normal cells and tumor cells, which has a faster regulation speed compared with changes in genome sequences. It is an important target for effective tumor therapy.

Autophagy is a highly conserved cellular dynamic self-digestion process, which mainly includes the formation of autophagosomes, the fusion and degradation of autophagosomes and lysosomes (9). Autophagy is beneficial to regulate the energy metabolism of cells, eliminate damaged organelles and misfolded proteins, can maintain the stability of the intracellular environment (10). The occurrence of excessive autophagy can cause type II programmed cell death (11). Autophagy plays a two-sided role in the tumor field, not only regulating the death of tumor cells, but also maintaining the homeostasis of tumor cells to face various stress. Multiple autophagy-related genes (ARGs) in glioma have been shown to be closely related to the proliferation, invasion, metastasis and apoptosis of glioma cells (12,13). However, the detailed regulatory mechanism of autophagy in glioma and the effect of ARGs on the prognosis of LGG patients in actual clinical practice are still unclear. ARGs are often regulated by epigenetic modifications at both the transcriptional and translational levels, and DNA methylation of autophagy regulatory elements plays an important role in LGG. For example, methylation silencing of the ARG *ULK2* is critical for astrocyte transformation and tumor growth (14). Epigenetic modification of autophagy is an important direction in cancer and cancer therapeutics (15).

The heterogeneity of the tumor microenvironment (TME) in LGGs is currently a major obstacle to clinical treatment (16). Changes in the autophagy pathway of immune cells and tumor cells in the microenvironment affect tumor progression and therapy (17). Tumor cells can escape from immunity by suppressing immune responses and reducing immunogenicity (18,19). Zhan *et al.* reported that the autophagy pathway mediates tumor progression by regulating immune responses including immune cell function and cytokine production (20). Therefore, targeting autophagy to modulate changes in the glioma immune microenvironment has great potential to combat LGGs and predict prognosis. At present, there are few studies on the effect of changes in the methylation level of ARGs on the tumor immune microenvironment, and the definite mechanism remains unexplored. This study preliminarily explored the effect of DNA methylation on ARGs, screened ARGs methylation levels related to prognosis, constructed a risk prognosis model, and analyzed the impact of the model on the tumor immune microenvironment. This study helps to decipher the possible mechanism of the epigenetic modification of autophagy on prognosis and treatment sensitivity, and provides a new research target for the prognosis prediction and treatment of LGG. We present the following article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-310/rc).

Methods

Data of LGG collecting from TCGA and pretreatment

The DNA methylation data (Illumina HumanMethylation450 BeadChip), gene expression profile and corresponding clinical information of LGG patients were originated from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/) and MEXPRESS (21,22). The workflow of

this article was depicted in *Figure 1*. The collection criteria for LGG were still in accordance with the 2016 WHO classification of central nervous system tumors. By matching DNA methylation data and expression profile, and removing samples with incomplete clinical information, we finally collected 451 LGG samples. The average β value of multiple cg sites of one gene were used as the methylation level of the gene. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Screening of ARGs whose methylation levels associating with prognosis [prognosis-related differentially methylated ARGs (DMARGs)]

Differentially methylated genes (DMGs) of normal and LGG patients were obtained from DiseaseMeth version, which were screened by 'limma' package with the standard of $|\log_2$fold change (FC)$|$ >0.2 and P<0.05. The datasets of ARGs were downloaded from Human Autophagy Database (HADb; http://autophagy.lu/clustering/index.html). Selected DMARGs associated with prognosis by COX regression analysis (23). The intersections of these results were showed by Venn diagram.

Construction and validation of the prognostic risk model

The prognostic risk score model was constructed by least absolute shrinkage and selection operator (LASSO) coefficient and the corresponding relative methylation level by formula: risk score = sum (the relative methylation level × corresponding coefficient) (24). The risk score of each patient was calculated and divided into high- and low-risk groups by median risk score. The Kaplan-Meier (KM) survival curve was used to contrast the difference in prognosis between the two groups. The ROC curve and the Chinese Glioma Genome Atlas (CGGA) database data was introduced as a validation set to assess the performance of this prognostic signature.

Functional enrichment in high- and low-risk groups

R packages 'org.Hs.eg.db, clusterProfiler' were applied to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis on the differentially expressed genes of high- and low-risk groups, the standard is P<0.05. Gene set enrichment analysis (GSEA) software (version 4.1.0) was used to analyze enrichment results on two groups based on hallmark gene sets (H). Normalized enrichment score |NES| >1.5 and nominal (NOM) P<0.05 was considered statistical significance.

Distinctions in the immune microenvironment analysis between two groups

To explore the potential differences of immune microenvironment in two groups, the ESTIMATE algorithm was used to calculate the immune scores, stromal scores of the high- and low-risk groups (25). The Cibersort algorithm which calculated enrichment of different immune cells by deconvolution algorithm, was applied to examine distribution of 22 types of immune infiltrating cells (26). We also examined gene expression of inhibitory immune checkpoints to assess the effect of immunotherapy.

Analysis of DNA methylation cg sites

To further explore the impact of methylation sites on gene expression and prognosis, the heatmap showed the relationship between different cg sites of 6 DMARGs and clinical features. The correlation between DNA methylation cg sites and the relative expression of the corresponding genes were examined by Pearson correlation analysis.

Statistical analysis

Statistical analysis and graphing were performed using R language (version 4.1), GraphPad Prism (version 8.0.1) and Adobe Illustrator 2019. Differences between the two groups were tested by *t*-test, analysis of variance (ANOVA) was used to test the significance of the difference of more than two samples, and log-rank test was used for survival curves. P<0.05 was regarded as statistically significant.

Results

Identification of DMARGs

The ARG set was downloaded from HADb, and 35 differential methylation of ARGs (28 down-regulated, 7 up-regulated) were extracted between the normal tissue and LGG patients ($|\log_2$FC$|$>0.1, P<0.05, from DiseaseMeth). The results were displayed in the heatmap and volcano plot (*Figure 2A*,*2B*). STRING network showed the association of 35 DMARGs (*Figure 2C*) and 21 prognosis related DMARGs (*Figure 2D*). Through univariate and multivariate

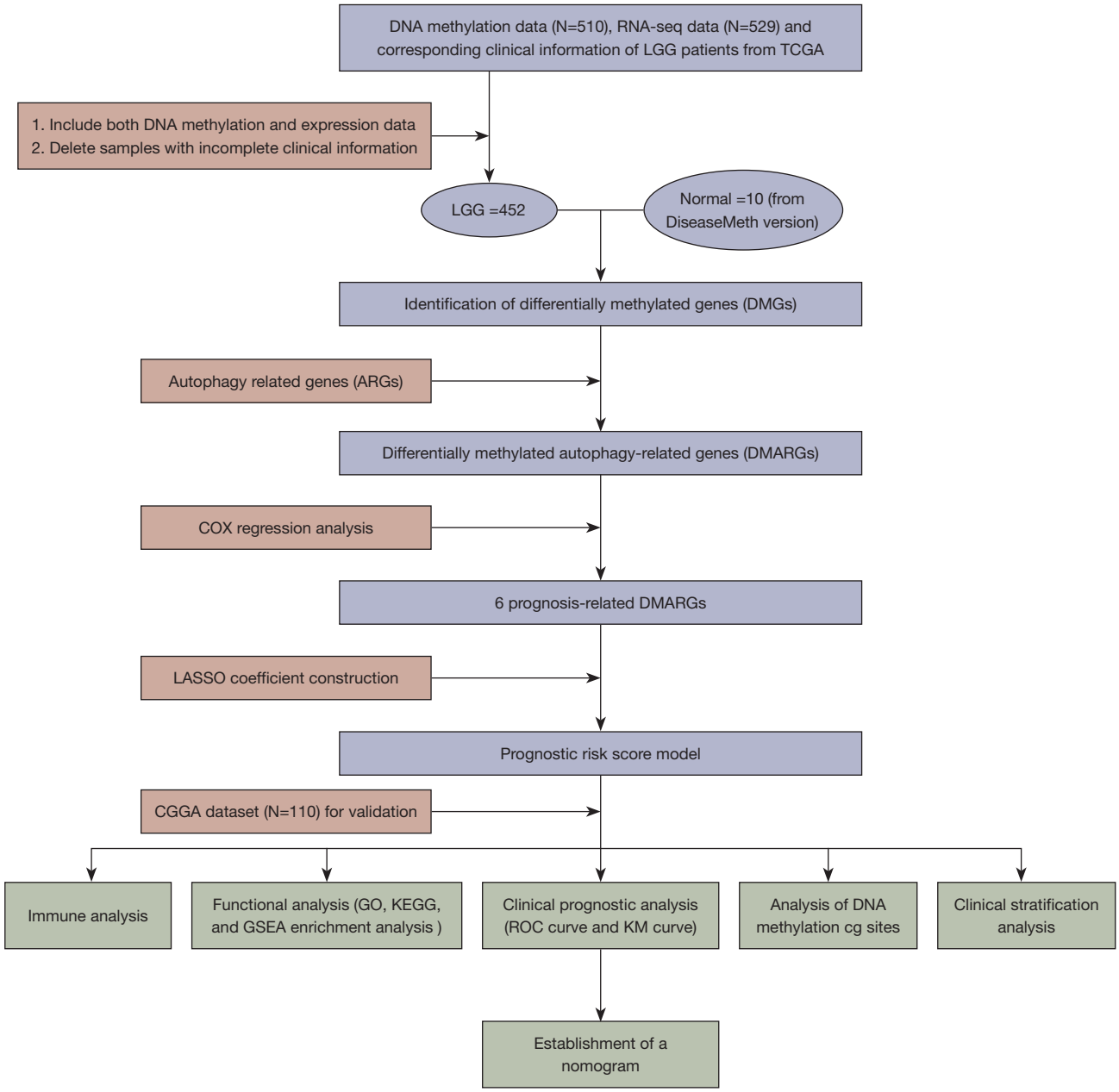
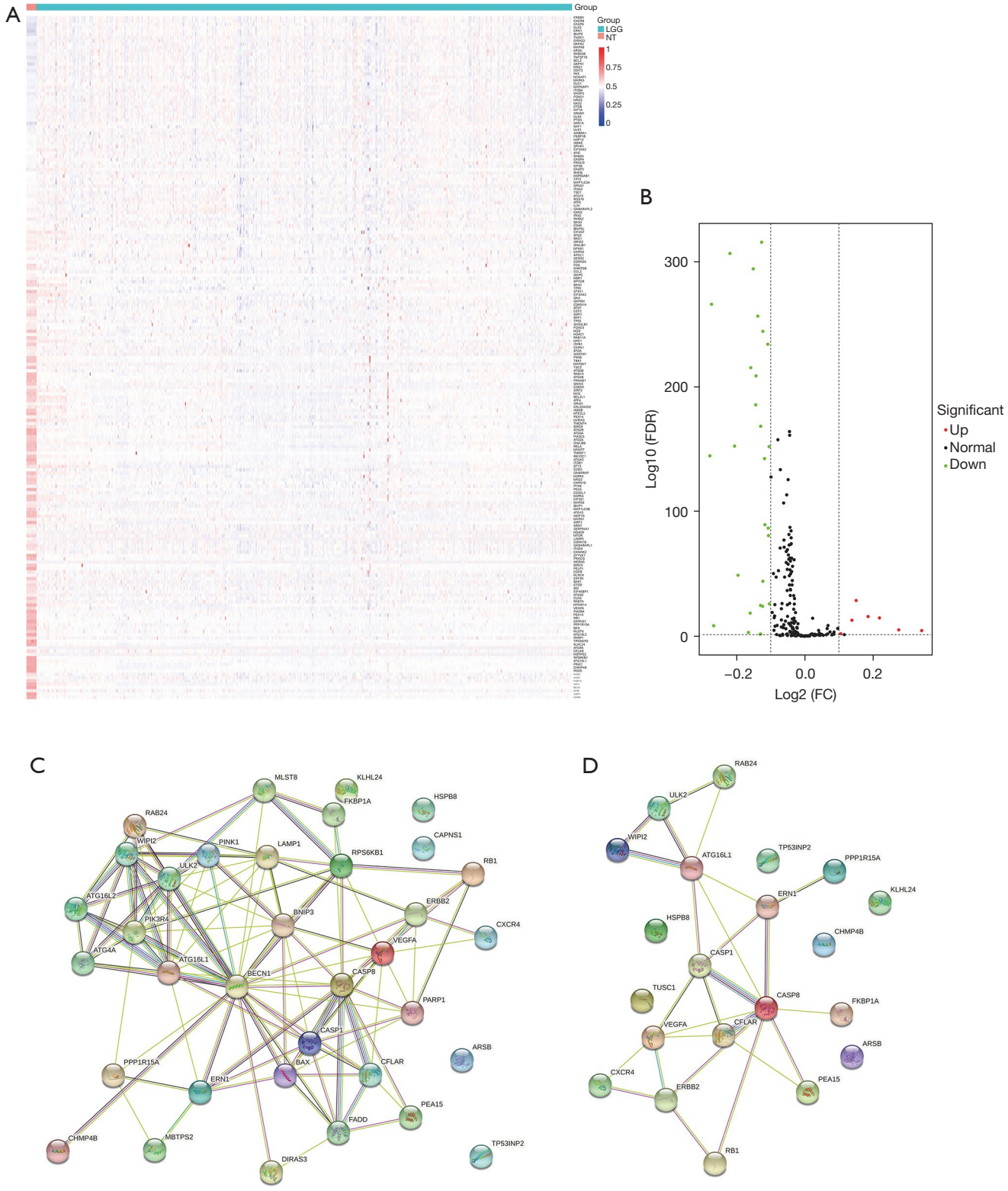


Figure 1 The workflow chart of this research. LGG, low-grade glioma; LASSO, least absolute shrinkage and selection operator; CGGA, Chinese Glioma Genome Atlas; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, gene set enrichment analysis; ROC, receiver operating characteristic; KM, Kaplan-Meier.

COX regression analysis, we finally screened out 6 DMARGs (*ARSB*, *CFLAR*, *WIPI2*, *RB1*, *ERN1*, *RAB24*) for constructing prognostic signature (*Figure 2E*). Venn diagrams revealed that the intersections were used for further analysis (*Figure 2F*).

Establishment and validation of survival prognostic model with DMARGs in LGG cohort

The regression coefficient of 6 DMARGs was determined by LASSO COX regression analyses. According to coefficient



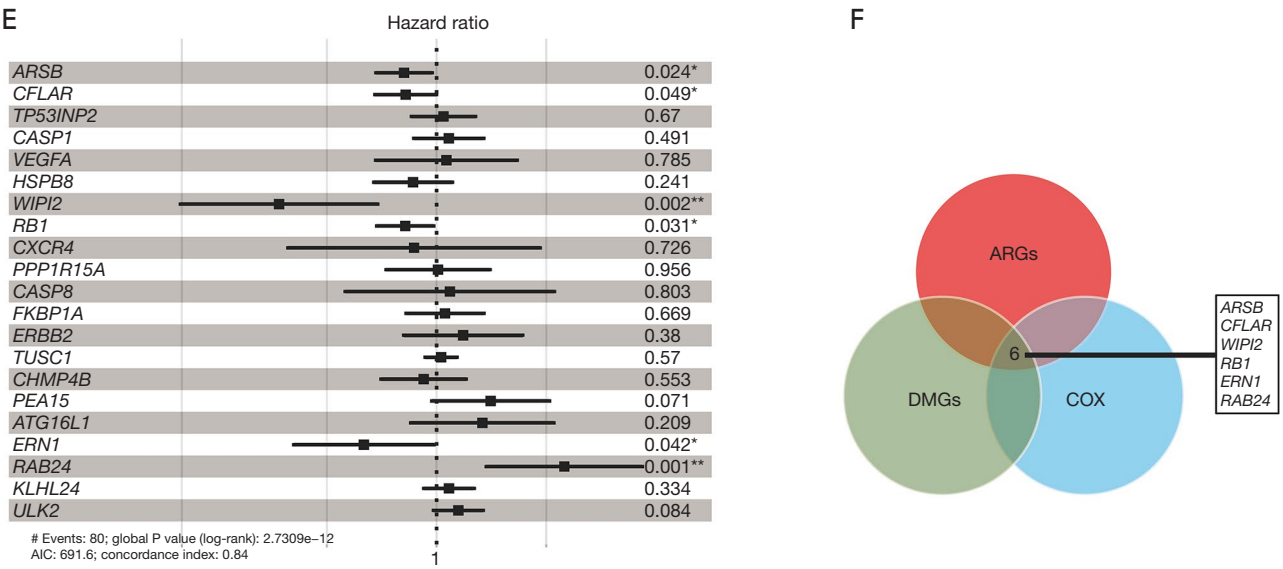


Figure 2 Identification of DMARGs in LGG. (A) Heatmap of methylation level of all ARGs between normal samples and LGG tissues (from DiseaseMeth); (B) volcano plot of DMARGs in tumor tissues and normal samples, the green spots represent down-regulation, and red spots represent up-regulation; (C) STRING network of association of 35 DMARGs; (D) STRING network of association of 21 prognosis related DMARGs; (E) multivariate COX regression analysis of prognosis related DMARGs; (F) Venn diagram showing the 6 DMARGs (the intersection of the DMGs, ARGs and COX regression analysis). *, P<0.05; **, P<0.01. LGG, low-grade glioma; NT, normal tissue; FDR, false discovery rate; FC, fold change; AIC, Akaike Information Criterion; ARGs, autophagy related genes; DMGs, differentially methylated genes; DMARGs, differentially methylated autophagy-related genes.

values and corresponding methylation values of 6 DMARGs obtained by multivariate COX regression analysis, we calculated the risk score of LGG patients. The risk score = (5.7092 × methylation level of *RAB24*) + (−1.1684 × methylation level of *ARSB*) + (−4.3193 × methylation level of *WIPI2*) + (−1.4733 × methylation level of *RB1*) + (−3.2461 × methylation level of *ERN1*) + (−1.4838 × methylation level of *CFLAR*). Patients were divided into high- and low-risk groups according to the median of risk score (*Figure 3A*). The survival status of each patient was shown in *Figure 3B*, and the number of patients who died increased with the risk score increasing. The heatmap (*Figure 3C*) showed methylation levels of 6 DMARGs between high- and low-risk groups. KM survival analysis (*Figure 3D*) showed that the survival prognosis of patients in the high-risk group was significantly worse (log-rank P=3E-10). The prediction accuracy of the model was evaluated using the receiver operating characteristic (ROC) curve, and the results showed that the model had good prediction accuracy (*Figure 3E*), with the best effect at 30 months [area under the curve $(AUC)_{max}$ =0.96]. The analysis of CGGA methylation data as a validation dataset showed similar results, proving that the prognostic signature has a good effect on the prognosis assessment of LGG (*Figure 3F,3G*). The nomogram based on risk score and other clinical information predicted 1-, 2-, and 3-year prognosis of LGG patients (*Figure 3H*).

The relationship between risk scores of prognostic model and clinical features

The heatmap combined the clinical information of the patients [age, tumor stage, isocitrate dehydrogenase (IDH) mutation status, 1p/19q co-deletion status] to display the methylation levels and mRNA expression of the 6 DMARGs (*Figure 4A,4B*). *ARSB*, *CFLAR*, *WIPI2*, *RB1*, and *ERN1* were hypermethylated in the low-risk group, which were protective factors, and *RAB24* was hypomethylated in the low-risk group, which was a risk factor for the prognosis of LGG. The 6 DMARGs were divided into hypermethylated and hypomethylated groups according to their methylation levels, and the KM survival curves showed prognostic differences (*Figure 4C*). Pearson correlation analysis showed the relationship between the overall methylation levels of the 6 DMARGs and the

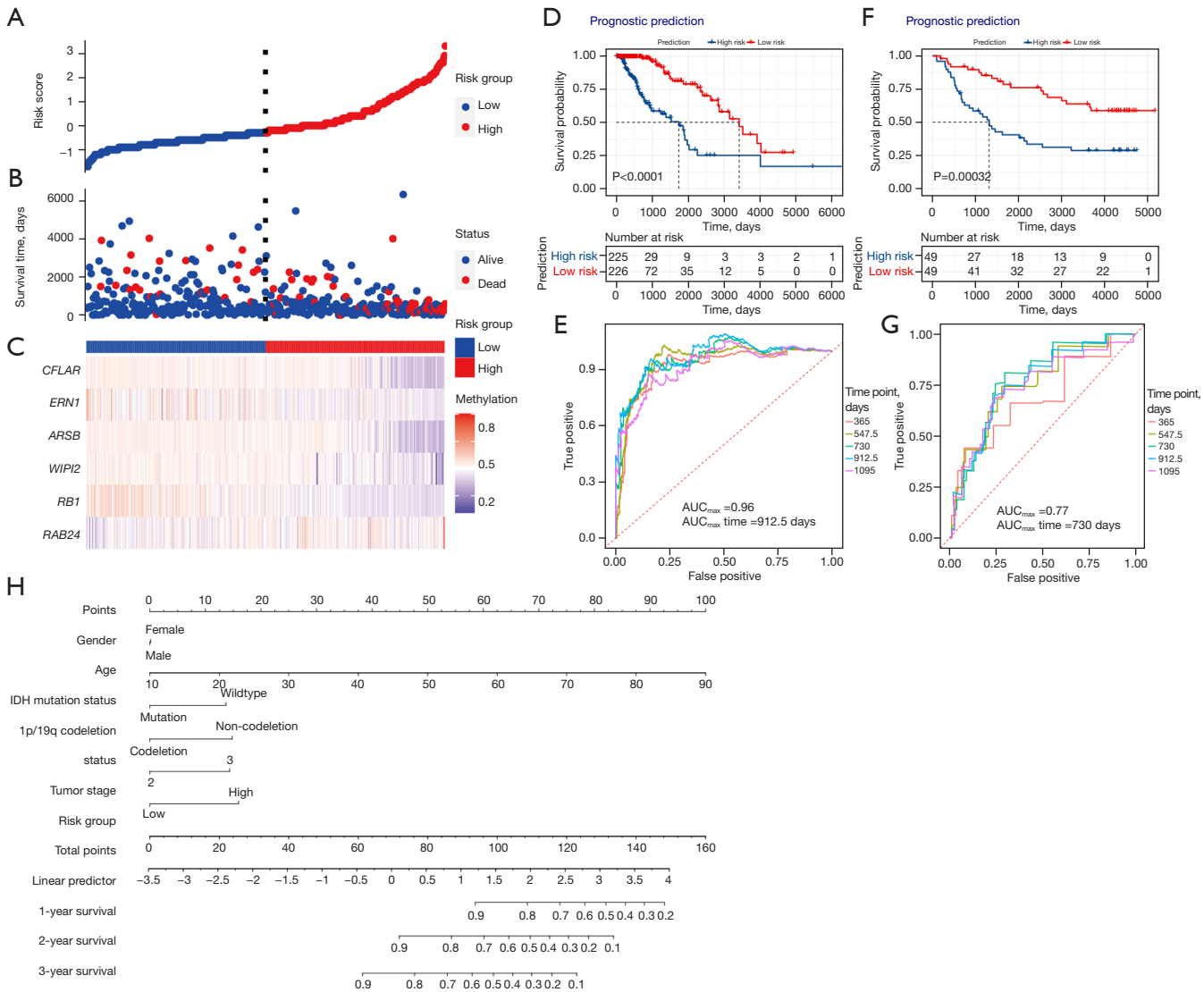


Figure 3 Development of risk score signature and validation based on the 6 DMARGs. (A) Distribution of the risk score for the DMARGs signature in LGG; (B) the survival status of each patient in risk signature; (C) heatmap showing the methylation level of 6 DMARGs in LGG; (D) the KM curve for OS of the two risk groups in LGG patients with TCGA dataset; (E) time-dependent ROC curve analysis of the risk score in LGG patient with TCGA dataset (AUC_{max} =0.96); KM curve (F) and ROC curve (G) of CGGA methylation data as a validation dataset; (H) a nomogram based on risk score and other clinical information for predicting 1-, 2-, and 3-year prognosis of LGG patients in TCGA database. AUC, area under the curve; IDH, isocitrate dehydrogenase; DMARGs, differentially methylated autophagy-related genes; LGG, low-grade glioma; KM, Kaplan-Meier; OS, overall survival; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic; CGGA, Chinese Glioma Genome Atlas.

corresponding mRNA expression (*Figure 4D*).

Function enrichment analysis between low- and high-risk groups

To analyze the biological functions of DEGs in the low- and high-risk groups, we performed functional enrichment analysis. GO enrichment analysis revealed that the down-regulated DEGs of high-risk group were significantly enriched in terms of skeletal system development, antigen processing and presentation via major histocompatibility complex (MHC) class II, extracellular matrix organization

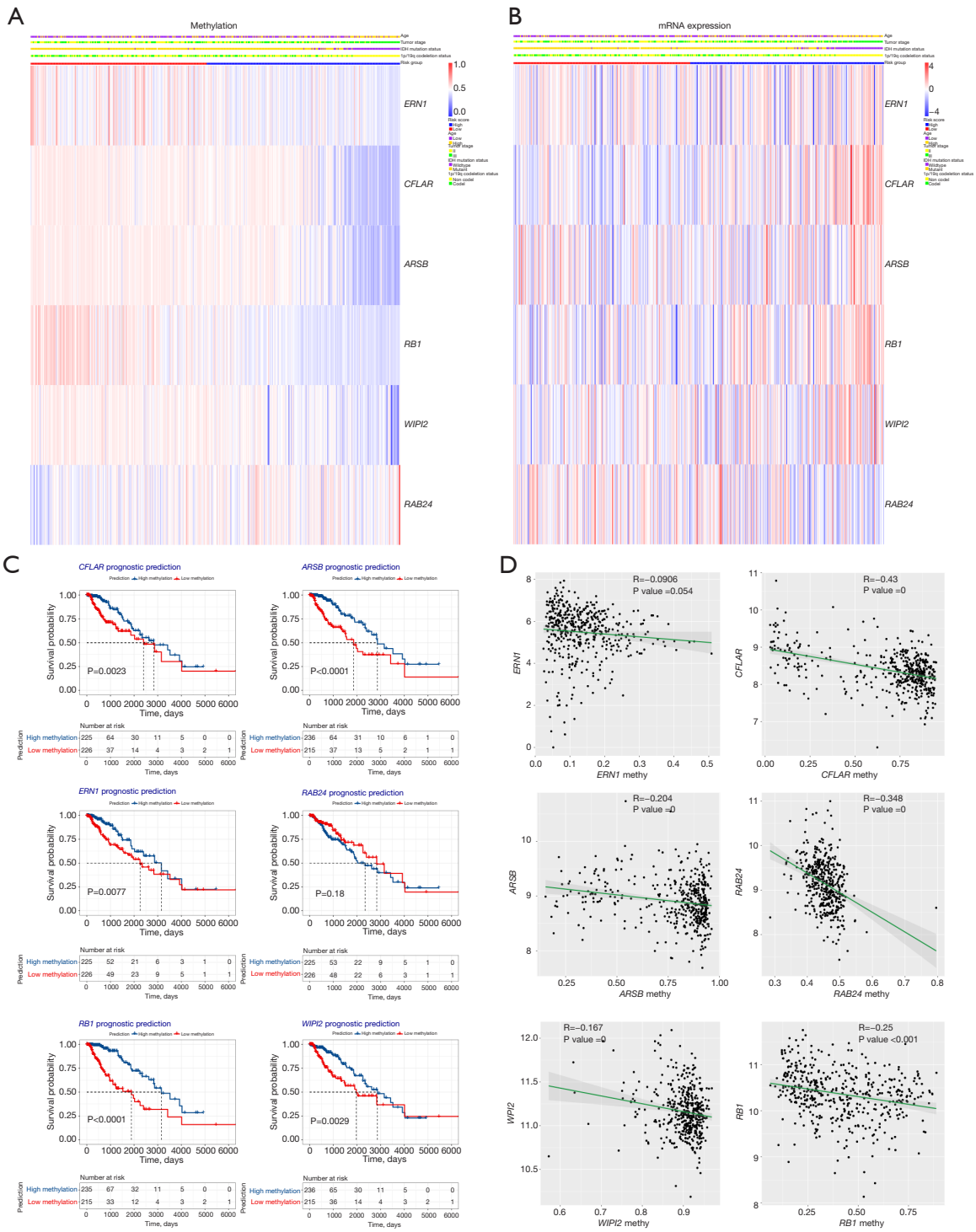


Figure 4 Relationship between methylation level of 6 DMARGs in risk signature with clinical factors and prognosis. (A) The heatmap showed the relationship between methylation level of 6 DMARGs and the clinical features (age, grade, IDH status, 1p/19q codeletion status); (B) the heatmap showed the relationship between relative expression of 6 DMARGs and the clinical features (age, grade, IDH status, 1p/19q codeletion status); (C) KM curves showed prognostic values of 6 DMARGs in risk signature respectively ; (D) Pearson correlation analysis showed the relationship between methylation levels and the corresponding mRNA expression of the 6 DMARGs. IDH, isocitrate dehydrogenase; DMARGs, differentially methylated autophagy-related genes; KM, Kaplan-Meier.

in biological process (BP), MHC class II protein complex, collagen-containing extracellular matrix, MHC protein complex in cellular component (CC) and MHC class II receptor activity, extracellular matrix structural constituent, immune receptor activity in molecular function (MF) (*Figure 5A*). The KEGG enrichment analysis showed that DEGs mainly involved in infection and immune diseases, immune cell differentiation and antigen processing and presentation (*Figure 5B*). GSEA enrichment analysis showed that DNA repair, *G2M* checkpoint, *E2F* targets, *P53* pathway, and glycolysis were enriched in high-risk group in hallmark gene sets (*Figure 5C*).

Differences in the immune microenvironment of two groups

The enrichment analysis results demonstrated that the risk assessment model constructed by DMARGs was closely related to the tumor immune microenvironment. Immunotherapy targeting immune checkpoints is an important direction for current tumor treatment. Therefore, we detected the expression of 7 common immune checkpoints (*PD-1*, *PD-L1*, *PD-L2*, *LAG3*, *TIM3*, *B7H3*, *CTLA4*) between the high- and low-risk groups. The results showed that the expression of immune checkpoints in the high-risk group was generally increased, which was more conducive to the immune escape of tumors (*Figure 6A*). The ESTIMATE algorithm revealed that the immune, stromal, and ESTIMATE scores of the high-risk group were significantly higher than those of the low-risk group (*Figure 6B*). In addition, we used the CIBERSORT algorithm to calculate the abundance of 22 types of immune cells in LGG patients, as well as the differences in the degree of immune cell infiltration between high- and low-risk groups. The results showed that compared with the low-risk group, resting memory CD4 T cells, regulatory T cells (Tregs), resting NK cells, M2 macrophages, resting mast cells significantly increased, Plasma cells, naive CD4 T cells, and activated mast cells reduced in the high-risk group (*Figure 6C*,*6D*).

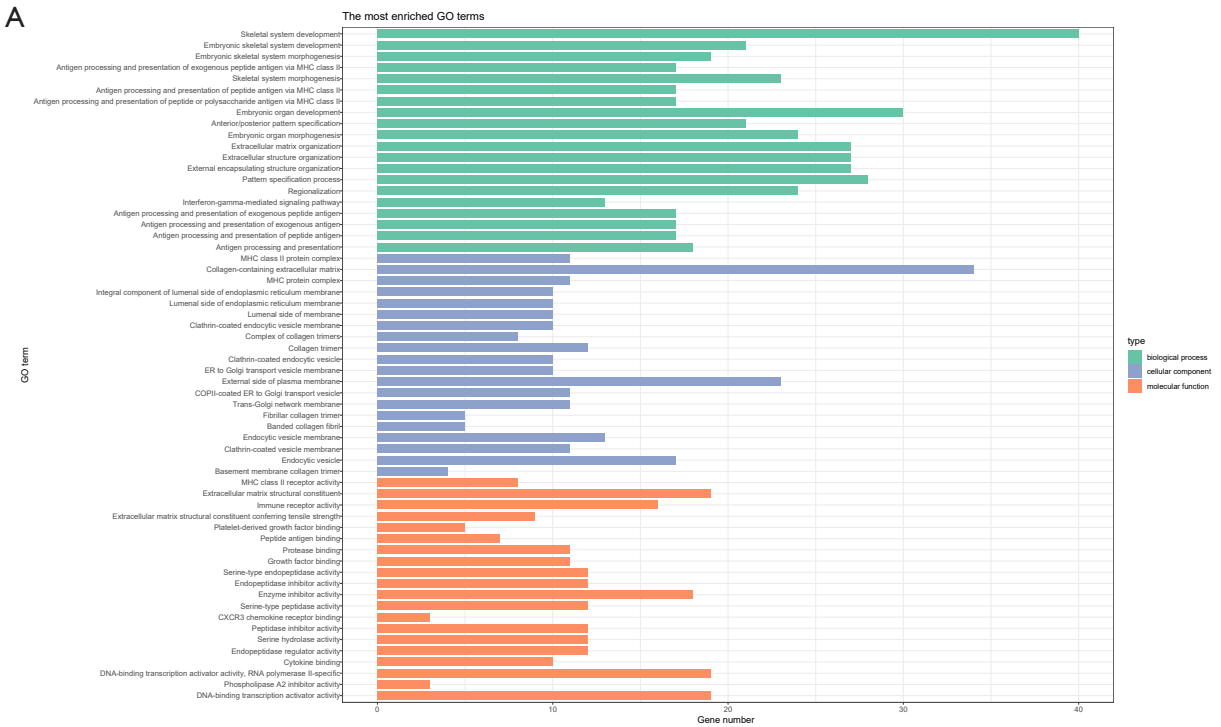
Associations between cg sites of methylations and expressions of the 6 DMARGs

To further explore the role of each cg site in DMARGs, as shown in *Figure 7A*, the heatmap showed the methylation levels of each cg site in 6 DMARGs between high- and low-risk groups. Pearson correlation analysis showed the relationship between each cg sites and the relative expression of the corresponding gene, clinical information (gender, histological type, tumor location) (*Figure 7B*).

Discussion

LGGs were prone to relapse to higher pathological grades of gliomas. Although a variety of therapeutic measures had been used in clinical practice in the past years, the survival expectations of patients with LGGs were still not optimistic (3,27). The clinical prognosis and therapeutic effects of gliomas are quite different, and the current histopathological classification could not meet the needs. Classification based on genomic changes and epigenetic differences will help us to identify carcinogenic factors, prognostic markers and knowledge of treatments in LGGs (28,29). IDH mutation, 1p/19q co-deletion, TERT promoter mutation and MGMT methylation have been certified to be prognostic biomarkers (30,31). Therefore, screening new biomarkers that can predict prognosis and serve as therapeutic targets is an important direction for LGG research (32).

The factors mediating tumor progression were complex, and epigenetic modification was considered to be an important part of tumor development and treatment (33). Epigenetics referred to heritable changes in gene expression that are not accompanied by changes in DNA sequence, including DNA methylation, chromatin remodeling, and noncoding RNAs. Many studies had shown that DNA methylation was significantly different in normal cells and different subtypes of gliomas, and extensive hypermethylation and hypomethylation of different CpG islands are characteristic of glioma (34). For example, Wang *et al.* demonstrated that hypermethylation of the miR-338-5p promoter regulated the aberrant expression of *ETS-1*, which was a key regulator of tumor cell proliferation and invasion, and was associated with prognosis of astrocytoma patients (35). There was also a public database-based bioinformatics analysis showing that *PODNL1* methylation acted as a prognostic biomarker and correlates with immune infiltration and immune checkpoints in LGG (36). da Costa Rosa *et al.* evaluated the effect of a DNA demethylating agent combined with all-trans retinoic acid in *IDH1* mutant gliomas, and the combination of 5-Aza and atRA reduced the growth of *IDH1 R132H* glioma cells and increased differentiation and apoptosis (37). Therefore, the methylation level of various genes in glioma cells is a standard biomarker for predicting tumor prognosis (38).



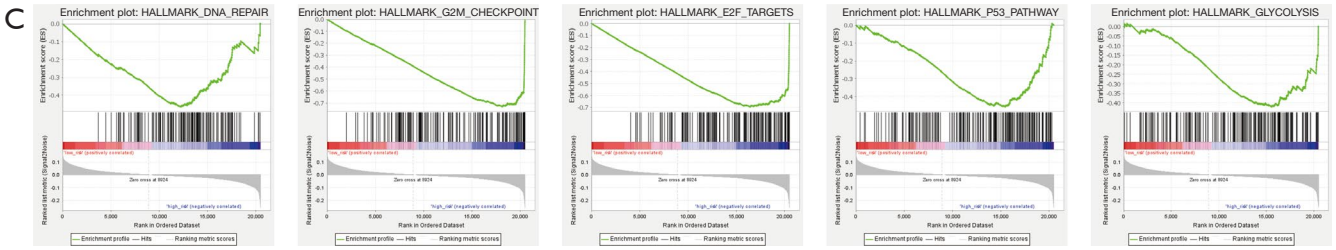


Figure 5 Functional annotation and pathway enrichment analysis between high- and low-risk groups. (A) GO enrichment analysis including BP, CC, and MF in the DEGs between high- and low-risk groups; (B) KEGG pathways enrichment in DEGs between high- and low-risk groups; (C) GSEA of hallmark gene sets in high- and low-risk groups. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function; DEGs, differentially expressed genes; GSEA, gene set enrichment analysis.

On the other hand, the role of ARG methylation in LGG was still not fully understood. Autophagy played a dual role in LGG with different subtypes, stages and gene expression, could either promote tumor cell survival or cause tumor cell death (39). The epigenetic regulation of autophagy was very complex and regarded as a critical role in tumor progression. For example, the generation of cancer stem cells depends on autophagy, which helps to improve the viability of tumor cells, leading to drug resistance and recurrence of tumors (40). Epigenetic modifications of autophagy were also involved in multiple tumor-related signaling pathways. Some modifications can reduce tumorigenicity by inhibiting proliferation and metastasis, promoting apoptosis and lethal autophagy, improving therapeutic response and achieving a better prognosis. One study had confirmed that the down-regulation of *HIF1A* gene in medulloblastoma cells leads to a significant increase in the methylation of the *ATG16L1* promoter and a significant decrease in the methylation of the *EPAS1* promoter, which decrease cell proliferation (41). In addition, the development and drug resistance of tumors are also related to the epigenetic modification of autophagy. Silencing of *MAP1LC3Av1* methylation might attenuate autophagy and promote the occurrence of gastric cancer (42). MicroRNA-20a-5p inhibited autophagy and reduced cisplatin resistance by regulating *DNMT3B*-mediated *RBP1* DNA methylation in ovarian cancer (43). Long non-coding RNA *H19* induced autophagy activation by reducing Beclin1 promoter methylation through the *SAHH/DNMT3B* axis, leading to tamoxifen resistance in breast cancer (44). Therefore, abnormal epigenetic modification of autophagy regulators was a key factor in regulating tumor progression, epigenetic modification is reversible, and abnormal epigenetic modification state could be restored to normal state by treatment (45). The epigenetic regulation of autophagy was also related to the glioma immune microenvironment (46).

Tumor cells had a variety of immune evasion mechanisms. Clinical immunotherapy was limited by the difference in the tumor immune microenvironment. Various signaling pathways affected the changes in the autophagy of immune cells and tumor cells in the TME (47). Autophagy could affect the proliferation, antigen presentation and function of immune cells, in addition, tumor cells could reduce the immune response by producing abnormal immune checkpoint signals, resulting in immune escape (48,49). PD-1 was an important T cell suppressor, and its binding to PD-L1 of tumor cells could significantly inhibit the proliferation and activity of cytotoxic T cells, resulting in immune escape of tumor cells. Study had shown that autophagy enables pancreatic cancer cells to perform immune evasion by degrading MHC I, and autophagy inhibition reverses immune evasion in animal models of pancreatic cancer (50). Yang *et al.* demonstrated that knockdown of m6A mRNA demethylase FTO increased m6A methylation in PD-1 (*PDCD1*), *CXCR4* and *SOX10* in melanoma cells, regulating melanoma tumorigenicity and sensitive to anti-PD-1 therapy (51). A study proved that highly selective *HDAC6* inhibitors promote *HDAC6* degradation in glioblastoma and induce antitumor immune responses by inhibiting autophagy (52). Therefore, utilizing the immune microenvironment was an important way to treat tumors including gliomas. Autophagy could regulate the immune microenvironment of tumors, and regulating autophagy might improve the effectiveness of immunotherapy.

In this study, LGG patients were collected from the

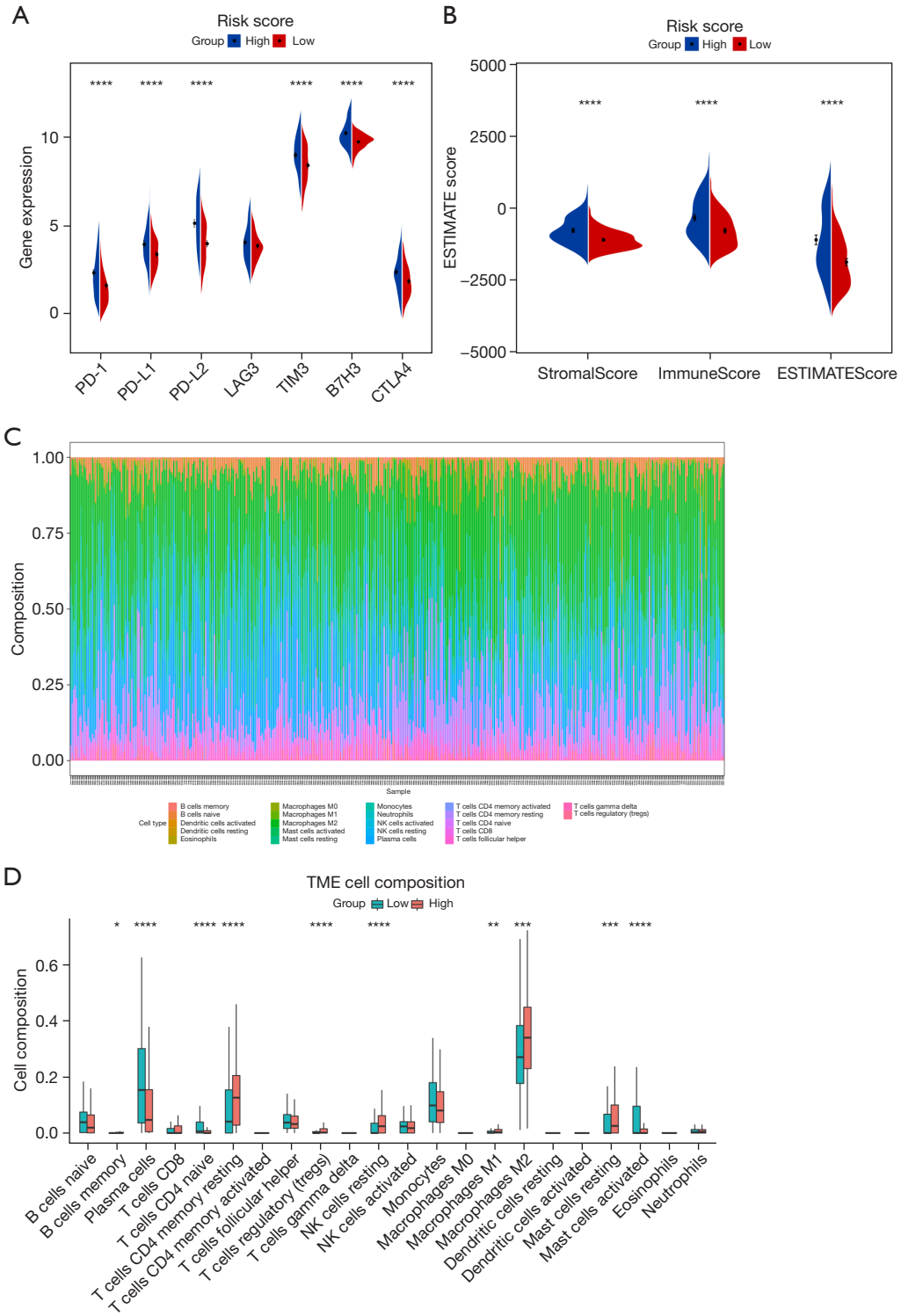
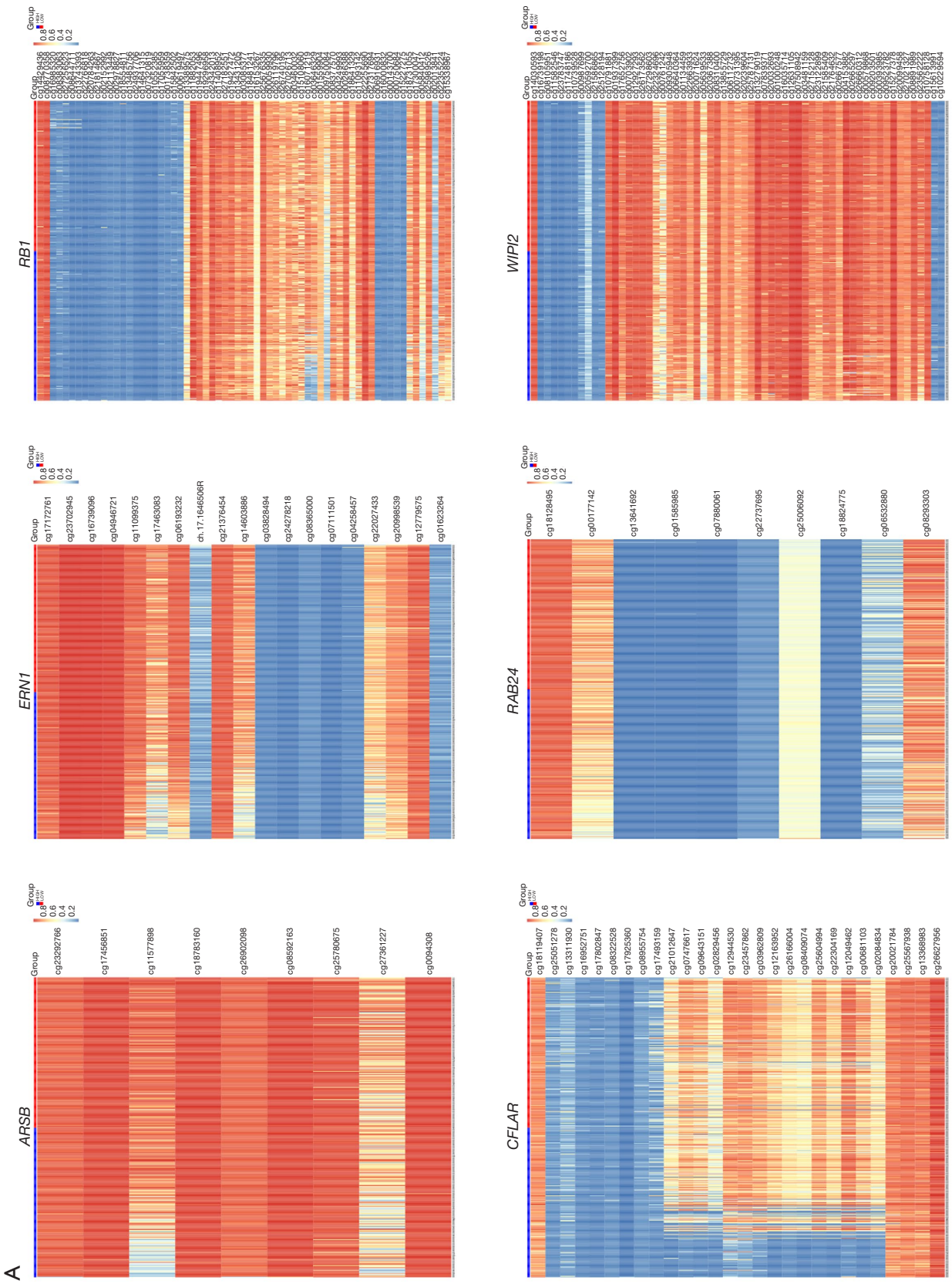


Figure 6 Immune microenvironment differences between high- and low-risk groups. (A) Differences in immune checkpoint gene expression between high- and low-risk groups; (B) the violin plot of immune, stromal and ESTIMATE scores between high- and low-risk groups; (C) the abundance of 22 types of immune cells in LGG patients with TCGA dataset; (D) the box plot showed the 22 different immune cell infiltration between high- and low-risk groups. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. TME, tumor microenvironment; LGG, low-grade glioma; TCGA, The Cancer Genome Atlas.



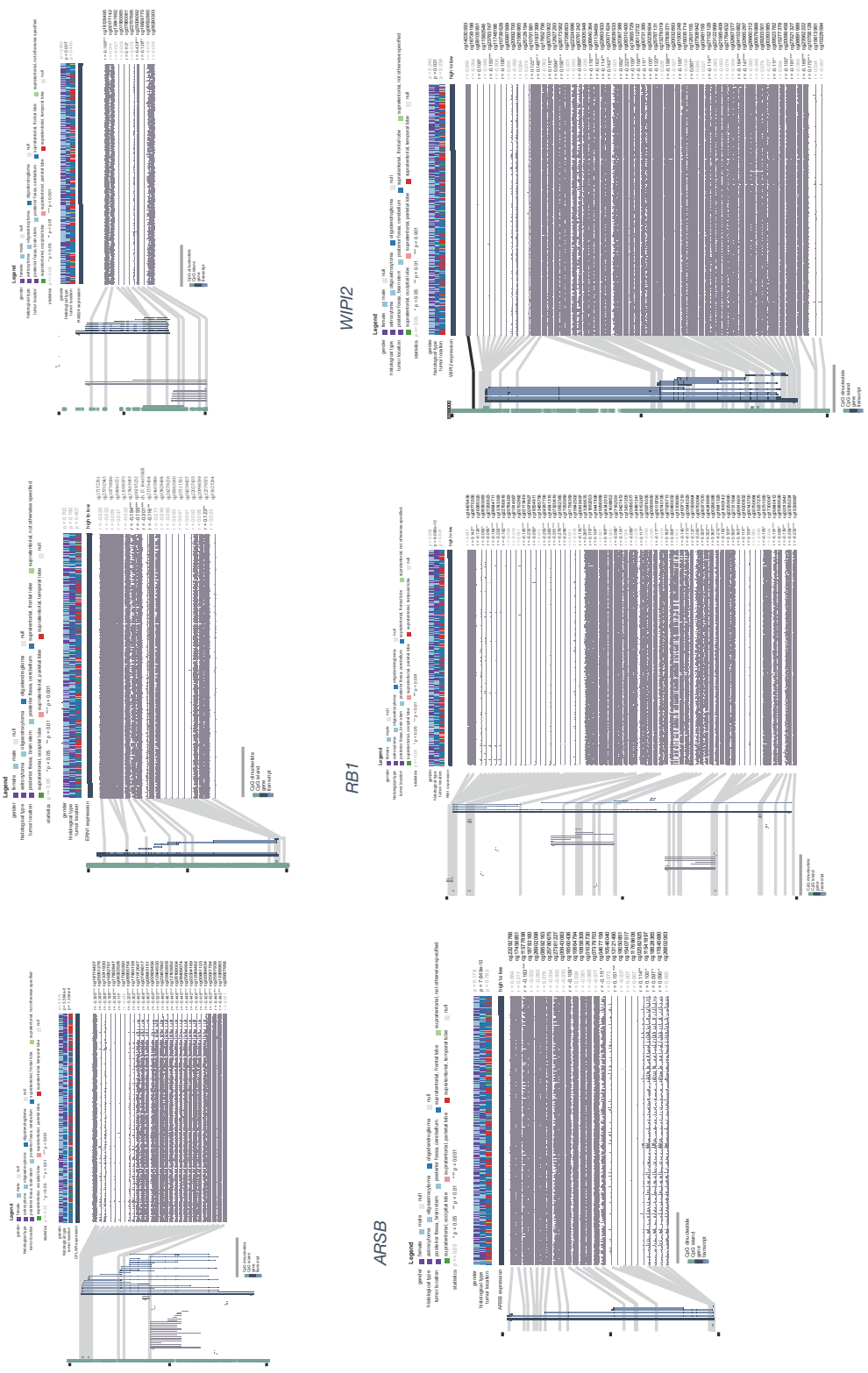


Figure 7 cg sites of the 6 DMARGs. (A) The methylation level of cg sites in 6 DMARGs between high- and low-risk groups; (B) the association between the gene relative expression and methylation level of cg sites (from MEXPRESS). DMARGs, differentially methylated autophagy-related genes.

public database, and DMARGs between normal tissues and LGGs were screened. COX regression analysis was performed on the DMARG set, and 6 DMARGs (*ARSB*, *CFLAR*, *WIPI2*, *RB1*, *ERN1*, *RAB24*) associated with prognosis were further screened to construct a risk assessment model. The median of the risk score was used as the cut-off point to divide into high- and low-risk group, and the KM survival curve proved that the survival prognosis of the high-risk group was poorer. At the same time, the ROC curve (AUC =0.96) proved the excellent prediction effect of the model. In addition, we demonstrated that risk score is an important factor affecting the prognosis of LGGs through a nomogram prediction model. The results of GO and KEGG enrichment analysis suggested differences in the immune microenvironment between high- and low-risk groups. We found that the expression of inhibitory immune checkpoints was significantly elevated in the high-risk group, which was beneficial for tumor immune escape. The immune infiltration scores and immune cell infiltration were also significantly different between high- and low-risk groups, providing a theoretical basis for the clinical application of immunotherapy. However, LGG sometimes were considered to be immunologically cold tumors, calculation of immune cell infiltration using CIBERSORT might be biased. The true differences between the two groups still needs further verification. Finally, we analyzed the relationship between gene methylation cg sites and the relative expression of corresponding genes, and further explored the methylation sites of ARGs that affect the prognosis of LGG patients.

Autophagy was an important factor in regulating the tumor immune microenvironment, the 6 ARGs in this model had also been reported to be related to the immune response of tumors. The innate immune response was regulated by toll-like receptors (TLRs), and dysregulation of TLRs' function could occur in multiple malignant tumors. West *et al.* found that the expression of *TLR2* was significantly increased in gastric cancer, and the DNA expression matrix showed that the growth response of human gastric cancer cells induced by *TLR2* was associated with the up-regulation of the anti-apoptotic gene *CFLAR* (53). Meanwhile, Plaza-Sirvent *et al.* showed that the low expression of *CFLAR* would contribute to high apoptosis rate of Treg, which was important for regulating immune responses and might be a target for tumor immunotherapy (54). A study had shown that dysregulation of *RAB24* is closely related to oncogenic genetic alterations of *MXD3*, which mediate immune cell dysfunction in a variety of tumors (55). Guo *et al.* demonstrated cocaine-induced autophagy could reduce microglia activation and the release of inflammatory factors, while the activation of *ERN1*-dependent endoplasmic reticulum stress pathway was involved in this induction of autophagy (56). Liao *et al.* reported *rVP1* increased the formation of LC3-associated autophagosomes via *WIPI1* and *WIPI2* and promotes the migration of macrophages, which was important for regulating immune responses and antitumor activity. Knockdown of *WIPI1* and *WIPI2* could inhibited *rVP1*-mediated autophagy and reduce *MAPK1/3* phosphorylation and activation of *MMP9* (57). A recent study showed that *SNX5* initiated autophagy during viral infection via recruitment of *WIPI2* to regulate intracellular immune and inflammatory responses (58). Regrettably, the impact of these 6 ARGs on the immune microenvironment remained to be studied in LGG because there were few related studies in LGG.

However, risk assessment models based on bioinformatics still had certain limitations. The genes used to build prognostic models from public databases had only statistically significant associations, and their interactions cannot be confirmed for the time being. In this study, the biological functions and signaling pathways of genes still need to be verified *in vivo* and *in vitro* experiments, and their clinical significance also needs to be verified in a large-scale multi-center clinical cohort.

Conclusions

In this study, a total of 6 DMARG associated with prognosis were screened. The methylation level of these genes could be used to predict prognosis of LGG patients and have a close relationship with immune microenvironment of tumor. This study further demonstrated the link between clinical prognosis, TME and methylation of ARG, providing a theoretical basis for the study of epigenetic regulation of autophagy. The results should be verified by more experiments.

Acknowledgments

Funding: This work was supported by Natural Science Foundation of China (Grant No. 81771270 to QP).

Footnote

Reporting Checklist: The authors have completed the

TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-310/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-310/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-310/coif). QP reports that this work was supported by the National Natural Science Foundation of China (81771270). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Cite this article as: Qiao Q, Wang Y, Zhang R, Pang Q. Autophagy related DNA methylation signature predict clinical prognosis and immune microenvironment in low-grade glioma. Transl Cancer Res 2022;11(7):2157-2174. doi: 10.21037/tcr-22-310

