

Development of An Inflammatory Response-Related Gene Signature To Predict Prognosis and Immunotherapy Response For Patients With Glioma

Zhen Zhao

Wuhan Union Hospital

Jianglin Zheng

Wuhan Union Hospital

Yi Zhang

The Third Affiliated Hospital of Zhengzhou University

Xiaobing Jiang

Wuhan Union Hospital

Chuansheng Nie

Wuhan Union Hospital

Hongyang Zhao (✉ hyzunion@163.com)

Wuhan Union Hospital <https://orcid.org/0000-0003-3895-614X>

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Abstract

Inflammatory response plays a crucial role in the development and progression of gliomas. However, the prognostic value of inflammatory response-related genes has never been comprehensively investigated for glioma. In this study, we identified 39 differentially expressed genes (DEGs) between glioma and normal brain tissue samples, of which 31 inflammatory response-related genes are related to the prognosis of glioma. The 8 optimal inflammatory response-related genes were selected to construct prognostic inflammatory response-related gene signature (IRGS) through the least absolute shrinkage and selection operator (LASSO) penalized Cox regression analysis. The effectiveness of the IRGS was verified in the training (TCGA) and validation (CGGA-693 CGGA-325 and Rembrandt) cohorts. The Kaplan-Meier curve revealed a significant difference in the OS between the high- and low-risk groups. The receiver operating characteristic curve (ROC) shows the powerful predictive ability of IRGS. Meanwhile, a nomogram with better accuracy was established to predict overall survival (OS) based on the independent prognostic factors (IRGS, age, WHO grade, and 1p19q codeletion). In addition, patients in the high-risk group had higher immune, stroma, and ESTIMATE scores, lower tumor purity, higher infiltration of immunosuppressive cells, higher expression of immune checkpoints, higher expression of TIDE and Exclusion, and lower expression of MSI Expe Sig. Thus, the patients in the low-risk group had significantly higher respond rate of immune checkpoint inhibitors (ICIs). A novel prognostic signature incorporated 8 inflammatory response-related genes was associated with the prognosis, immune landscape and the immunotherapy response in patients with gliomas. Thus, the signature can be suitable for future clinical application to predict the prognosis of patients with glioma.

1. Introduction

Glioma is the most common type of malignant tumor in the central nervous system, accounting for 40–50% of brain tumors, with an estimated annual incidence of 3 to 8 per 100,000 individuals[1, 2]. Despite progress in comprehensive treatments, such as maximal tumor resection, adjuvant chemotherapy, adjuvant radiotherapy, and immunotherapy, the median survival time of glioma patients is still very short due to the aggressiveness of the glioma, high recurrence rate, and resistance to treatment[3, 4]. Glioma is classified into 4 grades by the World Health Organization (WHO) according to their prognosis and morphological characteristics[5]. Especially glioblastoma (GBM), WHO grade IV, is the most malignant glioma, with the median overall survival (OS) of only 8 months, and the 5-year survival rate of 7.2%[1, 6]. Traditionally, the WHO classification is considered an important criterion for the prognosis of gliomas. However, the greatly differences for the clinical prognosis and treatment effectiveness of patients with the same WHO grade have been found due to the heterogeneity of gliomas[7]. Therefore, to solve this limitation, the construction of new biomarkers for accurately predicting treatment response and prognosis carries great clinical importance for patients with gliomas.

The appearance of white blood cells in tumors was initially discovered by Rudolf Virchow in the 19th century, which provides a connection between inflammatory response and cancers. With the rapid development of molecular biology, the important molecular mechanisms of inflammatory response in

tumor formation have gradually been elucidated by some studies in recent year[8, 9]. Wu et al. indicated that PTPN2 could be induced by inflammatory response and oxidative stress and its deficiency depressed glioma cell growth[10]. Meanwhile, inflammatory markers, derived from the routinely available parameters in the blood and their derivatives, appear to have the relationship of diagnostic and prognostic value in many neoplastic diseases including intracranial tumors[11–13]. For instance, white blood cells are widely existed in various tumors, especially malignant tumors, which are closely related to the important biological characteristics of tumors such as proliferation, migration, immune escape and prognosis[14]. Likewise, several peripheral blood-derived biomarkers such as the neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR) have been validated as prognostic inflammatory markers in various types of cancers[15–17]. Except for the inflammatory response factors in the blood, some inflammatory response-related genes are also used as biomarkers to predict the metastatic potential and prognosis of some cancers[18, 19]. However, interestingly, the relationship between inflammatory response-related genes and the prognosis of patients with gliomas remains unclear.

Immunotherapy, emerged in recent years, is considered to be one of the most promising treatment methods. For example, a variety of therapeutic antibodies that block immune checkpoints, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD1), showed good effects in the treatment of many tumors such as liver cancer, non-small cell lung cancer, glioma and so on[20, 21]. Unfortunately, immunotherapy based on immune checkpoints is not always useful for patients due to the heterogeneity of tumors and the complex tumor microenvironment. Therefore, it is greatly crucial and urgent to find more immunotherapy targets and clarify the molecular mechanism of more detailed immunotherapy response.

With the tremendous progress of modern sequencing technology, many RNA-seq transcriptome data of cancers can be found in some public databases, such as The Cancer Genome Atlas (TCGA), Chinese Glioma Genome Atlas (CGGA), and Rembrandt databases. In this study, we systematically analyzed RNA-seq transcriptome data and clinical data of glioma patients from these databases. Based on 8 inflammatory response-related genes, a prognostic inflammatory response-related gene signature (IRGS) of glioma patients was constructed in the TCGA database. Meanwhile, a reliable predictive nomogram model incorporating IRGS and clinicopathological characteristics was established and validated in the independent database. In addition, the correlations of IRGS with immune landscape, Tumor mutation burden, and the efficacy of immunotherapy were investigated. We consider that the IRGS has potential in patient management and can serve as potential therapeutic biomarkers for glioma in the clinical practice.

2. Materials And Methods

2.1 Patient data collection

The RNA-seq transcriptome data and corresponding clinical information of patients with glioma were extracted from TCGA, CGGA (CGGA-693 and CGGA-325), and Rembrandt databases. The RNA-seq

transcriptome data of TCGA and CGGA were quantified with Fragments Per Kilobase of transcript per Million mapped reads (FPKM) standardized by using the RSEM algorithm, whereas the Rembrandt data set was normalized microarray format[6]. Patients without survival data or OS less than 30 days were excluded from analysis for which they might die of the acute complications, rather than of the glioma itself. Therefore, a total of 1896 glioma patients were included in this study. The TCGA data set (n=631) was used as the training cohort, the CGGA-693 data set (n=656), CGGA-325 data set (n=309) and Rembrandt data set (n=300) were used as the verification cohorts. The clinicopathological characteristics of the included glioma patients are shown in Table 1. Then, the RNA-seq transcriptome data of normal brain tissue samples were searched from the Genotype-Tissue Expression (GTEx) database. In addition, the 200 inflammatory response-related genes were collected from the Molecular Signatures Database V7.4 (<https://www.gsea-msigdb.org/gsea/msigdb>). Further details on these genes are summarized in the **Supplementary Table S1**. If the gene expression data is detected in less than 50% of the samples, the gene is excluded in order to facilitate the subsequent data analysis.

Table 1
 Characteristics of glioma patients in training and validation cohorts.

Clinicopathological characteristics	Training cohort	Validation cohorts		
	TCGA (631)	CGGA-693 (656)	CGGA-325 (309)	Rembrandt (300)
Age (years)				
<45	296	357	178	110
>=45	336	298	131	181
NA	0	1	0	9
Gender				
Female	243	282	115	92
Male	333	374	194	146
NA	55	0	0	62
WHO grade				
II	201	172	97	66
III	222	248	73	57
IV	153	236	135	144
NA	65	0	4	33
Histology				
Astrocytoma	154			
Oligoastrocytoma	110			
Oligodendroglioma	159			
Glioblastoma	153			
NA	55			
IDH status				
Mutant	401	332	165	NA
Wild type	221	276	143	NA
NA	9	48	1	NA
1p19q codeletion				
Codel	157	137	62	12

Clinicopathological characteristics	Training cohort	Validation cohorts		
	TCGA (631)	CGGA-693 (656)	CGGA-325 (309)	Rembrandt (300)
Non-codel	468	453	239	91
NA	6	66	8	197
MGMT promoter status				
Methylated	449	304	151	NA
Unmethylated	150	217	140	NA
NA	32	135	18	NA

2.2 Identification of differentially expressed genes and cluster analysis

The differentially expressed genes (DEGs) between glioma samples in TCGA cohort and healthy brain tissue samples in GTEx cohort were identified through the differential-expression analysis with R package “limma” (\log_2 fold-change $|\log_{FC}| > 1$ and an adjusted false-discovery rate FDR < 0.05). Furthermore, Search Tool for the Retrieval of Interacting Genes (STRING) software (<http://www.string-db.org/>) was used to analyze the interactions and evaluate the level of interactions among the above DEGs.

In order to investigate the best gene subgroups, the glioma patients of TCGA was divided into different clusters through the application of the “ConensusClusterPlus” package in R based on the expression of DEGs. The resampling procedure was used to sample 80% of the sample 50 times, the similarity distance between the samples was estimated by Euclidean distance, and the “km dist” as a clustering algorithm was used to select reliable and stable subgroup classification[22]. The “proportion of ambiguous clustering” was used to select an optimal value of clusters with the criteria is that the clusters have high consistency, and the area under the cumulative distribution function curve does not increase significantly[23].

2.3 Development and validation of a prognostic IRGS

The univariate Cox regression analysis was conducted by using the survival coxph function of the R package to explore the relationship between DEGs and prognosis value ($p < 0.05$ was used as the significance threshold). Then the least absolute shrinkage and selection operator (LASSO) penalized Cox regression analysis was performed to construct a prognostic model in order to minimize the risk of overfitting[24, 25], which was performed within the TCGA cohort by using the R package “glmnet” and “survival”. The ten-fold cross-validation was used to selecting the optimal penalty parameter λ of the prognostic model and was followed the minimum criteria (i.e. the value of λ corresponding to the lowest partial likelihood deviance). Subsequently, the risk score of each patient was calculated according to the

regression coefficient of the model and the expression of the corresponding gene. The calculation formula is shown below:

$$Riskscore = \sum_{i=1}^n (Coef_i * X_i)$$

where n represents the number of all the selected gene; i represents the serial number of each gene; X_i and $Coef_i$ refer to the expression level of each selected gene and corresponding coefficient, respectively. The median risk score was considered as the cut-off value to divide the patients into high- or low-risk group. The Kaplan-Meier survival curve analysis with log-rank test were used to evaluate the ability of predicting OS between the high- and low-risk groups by using the R packages "survival" and "Survminer". The receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC) were calculated to evaluate the accuracy of the inflammatory response-related gene through the R software package "timeROC". The above analyses were performed simultaneously in the training and validation cohorts.

2.4 Establishment and pvaluation of a nomogram

The univariate and multivariate Cox regression analyses were performed for identifying the independent prognostic factors in terms of the risk scores and clinicopathological characteristics. Next, a nomogram was established on the independent prognostic factors in the TCGA cohort by using the R packages "rms", "regplot", and "Hmisc". The calibration curves and C-index were performed in the training and validation cohorts to evaluate the availability of this nomogram[26]. In addition, The ROC curves were also plotted to assess the accuracy of the nomogram for OS prediction in the training and validation cohorts.

2.5 Functional enrichment analysis

The DEGs between the high-risk group and the low-risk group were identified with the criteria of $|\log_2FC| > 2$ and adjusted $p < 0.05$ by BH method. Then Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted to predict the function of these DEGs through the R packages "clusterProfiler", "org.Hs.eg.db", and "rich lot".

2.6 Evaluation of immune landscape of glioma microenvironment

The infiltration levels of immune cells and stromal cells in different tumor tissues were analyzed by immune score and stromal score. The immune scores and stromal scores of glioma patients were calculated using the ESTIMATE algorithm via the R package "estimate"[27]. CIBERSORT, a deconvolution algorithm with 1000 permutations, was applied to estimate the abundance of 22 immune cells[28]. Patients with CIBERSORT $p \geq 0.05$ were excluded from the subsequent analysis. These immune cells included as follows: resting memory CD4+ T cells, activated memory CD4+ T cells, Tfh cells, Tregs, $\gamma\delta$ T cells, CD8+ T cells, naive CD4+ T cells, naive B cells, memory B cells, plasma cells, resting NK cells, activated NK cells, macrophages (M0, M1 and M2), resting DCs, activated DCs, resting mast cells, activated mast cells, eosinophils, neutrophils and monocytes. We evaluated the differences between the

high- and low-risk groups in the abundance of 22 immune cells and the expression levels of immune-related molecules.

2.7 Evaluation of tumor mutation burden and immune response

Tumor mutation burden (TMB) has been defined as the total number of somatic, coding, base substitution, and indel mutations per megabase of genome examined. In this study, we selected a novel TME-scoring algorithm to explore the differences in TME between the high- and low-risk groups. Furthermore, the tumor immune dysfunction and exclusion (TIDE) algorithm was applied to evaluate the predictive efficiency of risk scores for the Immune checkpoint inhibitions (ICIs) response in glioma[29]. In addition, the distributions of risk scores between non-respond and respond groups, and the differences of response rates between high- and low- risk groups are also analyzed and compared.

2.8 Statistical analysis

All statistical analyses and graph visualization were performed using the R software (version 4.0.1, <http://www.R-project.org>). The PERL programming language (version, 5.30.2, <http://www.perl.org>) was used to process RNA-seq transcriptome data. The Chi-square test or two-sided Fisher's exact test was performed for the comparison of categorical variables between the high- and low-risk groups, similarly, correlations between the risk score and clinicopathological parameters were the same. The Student's t-test or one-way ANOVA test was utilized to compare the continuous variables with normal distribution between two groups or more than two groups. While Mann-Whitney U test was used to compare continuous variables with non-normal distribution between two groups, and Kruskal Wallis test was used to compare continuous variables with non-normal distribution in more than two groups. The survival advantage was evaluated using the Kaplan-Meier curve and compared by the log-rank test for the high- and low-risk groups. Univariate and multivariate cox proportional hazard models were applied to estimate the hazard ratios of variables and determine independent prognostic factors. The statistical significance level was set at two-tailed $p < 0.05$.

3. Result

3.1 Identification of the DEGs between glioma and healthy brain tissue samples

These data were pre-processed before identifying the DEGs, the normalization and batch effects removal from GTEx and TCGA cohorts was conducted by R package "sva". 39 DEGs were identified between in glioma samples (n = 697) and healthy brain tissue samples (n = 1157), with the absolute value of the log₂-transformed fold change (FC) > 1 and the adjusted P-value (FDR) < 0.05 as the threshold levels of significance. Among of the identified inflammatory response-related DEGs, 17 up-regulated genes and 22 down-regulated genes were identified, which indicated that inflammatory response-related genes exerted important biological functions in the tumorigenesis of glioma. The heatmap and volcano plot of the

DEGs are shown in Figure 1A and B. In addition, the network and correlation analysis of 39 inflammatory response-related genes were performed to identify their interactions (Figure 1C and D).

3.2. The clinicopathological characteristics of the two clusters of glioma patients.

According to the expression similarity of inflammatory response-related genes and the criteria for selecting the number of clusters, $k=2$ was considered as the most appropriate value, with clustering stability increasing from $k = 2$ to 10 in the TCGA datasets (Figures 2A-C). Hence the patients from TCGA cohort were divided into two clusters named cluster 1 and 2. Subsequently, the principal component analysis (PCA) was used to compare the transcriptional profile between cluster 1 and cluster 2 subgroups. The results displayed that there is a clear boundary between the two subgroups (Figure 2D). In order to compare the survival distributions of the two clusters, the Kaplan-Meier survival curves of the them performed with log-test showed the significantly shorter OS in the cluster 1 subgroup than the cluster 2 subgroup (Figure 2E, $p < 0.001$). Moreover, the clinicopathological characteristics, survival status and immune scores between different clusters were identified, and 39 inflammatory response-related gene expression values were screened out as a heat map (Figure 2F). The cluster 1 subgroup is significantly correlated with dead ($p < 0.001$), older patients ($p < 0.001$), higher grade ($p < 0.001$), glioblastoma and astrocytoma ($p < 0.0001$), IDH-Wild type ($p < 0.001$), Non-codel 1p19q codeletion ($p < 0.001$), Unmethylated MGMT status promoter ($p < 0.001$), higher Immune score ($p < 0.001$), higher Stromal score ($p < 0.001$), higher ESTIMATES score ($p < 0.001$), lower Tumor purity ($p < 0.001$). While cluster 2 subgroup is significantly correlated with the opposite results.

3.3 Construction and validation of the prognostic IRGS

Univariate Cox regression analyses were first performed for inflammatory response-related DEGs, and the results showed that 31 genes were significantly related to the OS of glioma in the Supplementary Table S2 ($p \leq 0.05$). Among these 31 genes, 22 were protective factors and 9 were risk factors for prognosis. Above-mentioned 31 inflammatory response-related genes were incorporated into the LASSO penalized Cox regression analysis in the TCGA cohort. Ultimately, 8 optimal prognostic inflammatory response-related genes were acquired and included in the risk model, including GABBR1, CALCRL, EBI3, BTG2, SEMA4D, SELL, SERPINE1, and MMP14 (Figures 3A–B). The survival analyses of these 8 inflammatory response-related genes were indicated that high expression of these genes was all significantly correlated with poor OS in **Supplementary Figure S1**. The risk score of each patient was calculated as follows: Risk score = $(-0.2807 \times \text{expression value of GABBR1}) + (-0.1803 \times \text{expression value of CALCRL}) + (-0.1065 \times \text{expression value of EBI3}) + 0.0589 \times \text{expression value of BTG2} + (-0.0381 \times \text{expression value of SEMA4D}) + (-0.0186 \times \text{expression value of SELL}) + (0.0671 \times \text{expression value of SERPINE1}) + (0.1027 \times \text{expression value of MMP14})$. There were six genes (GABBR1, CALCRL, EBI3, BTG2, SEMA4D, and SELL) related to good prognosis based on their negative coefficients, while SERPINE1 and MMP14 was the opposite, indicating a good prognosis (Figure 3C). Subsequently, the median risk score, as the cut-off value, was used to stratified the glioma patients into the high- and low-risk groups. In the TCGA cohort, the Kaplan-Meier curve demonstrated that the survival in the low-risk group was significantly better than

in the high-risk group (log-rank test $p < 0.001$; Figure 3D). The distribution plot of the risk score and survival status showed that the higher the risk score, the more deaths of glioma patients (Figure 3H). A satisfactory prediction performance of the prognostic model was confirmed by the AUC for 1-, 3-, and 5-year OS (AUC = 0.876, 0.903, and 0.842, respectively; Figure 3L).

To test the stability of the prognostic model constructed on the TCGA cohort, the same analyses were performed in the CGGA-693 cohort, CGGA-325 cohort and the Rembrandt cohort. Similarly, patients in the low-risk group had better survival outcomes than the patients in the high-risk group (Figures 3E-G, I-K). Furthermore, the AUCs for predicting the 1-, 3-, and 5-year survival were 0.762, 0.768, and 0.743 in the CGGA-693 cohort, respectively (Figure 3M), the AUCs for predicting the 1-, 3-, and 5-year survival were 0.768, 0.854, and 0.890 in the CGGA-325 cohort, respectively (Figure 3N), the AUCs for predicting the 1-, 3-, and 5-year survival were 0.689, 0.826, and 0.788 in the Rembrandt cohort, respectively (Figure 3O). Based on these results, we confirmed the accuracy and stability of predictive signature.

3.4 Correlation analysis between the prognostic model and clinicopathological characteristics

The association of the 8 selected genes, and clinicopathologic parameters between high- and low-risk groups was analyzed in the TCGA cohort, and the results showed that the genes were up-regulated in the high-risk group and the protective genes were up-regulated in the low-risk group (Figure 4A). Meanwhile, the differences of age, gender, histology, WHO grade, IDH status, 1p19q codeletion and MGMT promoter status between high- and low-risk groups were compared in all training cohort and validation cohorts and showed in the **Supplementary Tables S3-6**. In addition, the values of risk score between glioma patients stratified by clusters and various clinicopathological characteristics were also compared. In the TCGA cohort, glioma patients with the clinicopathological characteristics of the cluster 1 subgroup, age >45 years, more malignant type of histology, higher WHO grade, IDH wild type, 1p19q codeletion non-codel and MGMT promoter unmethylated showed significantly higher levels of risk score, while no differences were observed between patients satisfied by gender (Figures 4B-I). Likewise, the risk score of glioma patients in the CGGA-693, CGGA-325 and Rembrandt cohorts was identified the similar results to the TCGA cohort (**Supplementary Figures S2A-C**).

We performed subgroup survival analyses to determine whether the clinicopathological characteristics would decrease the prediction accuracy of the prognostic IRGS. The results showed that patients with high-risk score had worse survival outcomes than those with low-risk score in all subgroups, except for the grade IV subgroup in the TCGA cohort (**Supplementary Figures S3A-M**). The other cohorts showed the same results but IDH-Wild subgroup in the CGGA-693 cohort and the 1p19q-Noncodel subgroup in the Rembrandt cohort (**Supplementary Figures S4A-C**).

3.5 Establishment and evaluation of a nomogram

The univariate Cox regression and multivariate Cox regression were analysed subsequently for identifying the OS-related factors in the TCGA, CGGA-693, CGGA-325, and Rembrandt cohorts (Figures 5A-D). And the

risk score based on 8 selected genes was confirmed as an independent prognostic factor without being affected by other clinicopathological characteristics (Figures 5A-D). Then, the independent prognostic factors in the TCGA cohort (Age, WHO grade, 1p19q and Risk score) were used to establish the nomogram for predicting the 1-, 3-, and 5-year prognoses of the glioma patients (Figure 6A). The internal evaluation was initially performed. The C-index was 0.857 (95%CI: 0.812-0.896) and the calibration curves indicated an excellent match between the actual and nomogram-predicted probability of 1-, 3-, and 5-year OS (Figure 6B). The ROC curve analysis showed that the nomogram had highest predictive accuracy of 5-year OS than risk score or clinicopathological features (Figure 6F). Likewise, the external evaluation of this nomogram was conducted in the CGGA-693, CGGA-325 and Rembrandt cohorts. The C-indices were 0.794 (95%CI: 0.757-0.831), 0.833 (95%CI: 0.781-0.885), 0.849 (95%CI: 0.730-0.968) in the CGGA-693, CGGA-325 and Rembrandt cohorts, respectively. The calibration curves all illustrated a satisfactory match between the actual and nomogram-predicted probability of 1-, 3-, and 5-year OS in these three cohorts (Figures 6C-E). Furthermore, the ROC curve analysis showed that the nomogram had highest predictive accuracy of 5-year OS than risk score or clinicopathological features in three validation cohorts (Figures 6G-I). Meanwhile, The ROC curve analysis also exhibited that the nomograms had the highest accuracy in predicting 1-, and 3-year OS in comparison to other independent prognostic factors (**Supplementary Figure S5**). Thus, the nomogram has the potential as a quantitative method to predict the prognosis of patients with glioma.

3.6 Functional enrichment analysis

The GO function and KEGG pathway enrichment analyses were conducted to characterize the biological functions of DEGs between the high- and low-risk groups. As a result, the GO function analyses revealed significant enrichment of inflammatory response-related functions, including response to molecule of bacterial origin, response to lipopolysaccharide, and positive regulation of cytokine production (Figure 7A). Meanwhile, the DEGs were also significantly enriched in several immune-related biological processes, for instances T cell activation and regulation of immune effector process (Figure 7A). Besides, the KEGG pathway analyses identified that some KEGG pathways were correlative with immune-related pathways including the Cytokine-cytokine receptor interaction, Chemokine signaling pathway and Toll-like receptor signaling pathway (Figure 7B).

3.7 Correlation of the prognostic IGRS with the immune landscape of glioma microenvironment and immunotherapy

The correlation of the prognostic IGRS with the immune landscape of glioma microenvironment was further investigated for the immune-related functions enriched by DEGs between high- and low-risk groups. Firstly, the immune system scores were compared between high- and low-risk groups, and the result indicated that the high-risk group showed significantly higher immune, stroma and ESTIMATE scores and lower tumor purity than the low-risk group (Figures 7C-F). Then, we analyzed the different extent of immune cell infiltrations between the low- and high-risk groups for all the samples. The differences were observed in the high-risk group with lower abundance of naive B cells, activated NK cells, activated mast cells, and eosinophils, but higher abundance of memory B cells, plasma cells, CD8+ T

cells, CD4⁺ memory activated T cells, regulatory T cells, gamma delta T cells, M0-type macrophages, M1-type macrophages, resting mast cells and neutrophils (Figure 7G).

The expressions of immune checkpoint proteins were all up-regulated in the high-risk group (Figure 8A). We next determined whether a correlation existed between the immune checkpoint protein and prognostic inflammatory response-related genes. The correlation matrix of and was demonstrated that the immune checkpoint protein had a significant negative correlation with the inflammatory response-related genes such as GABBR1, CALCRL, EBI3, BTG2, SEMA4D, and SELL, except for SERPINE1 and MMP14 (Figure 8B). Moreover, a comparative analysis of tumor mutation burden in the two subgroups showed the high-risk group had significantly higher tumor mutation burden (Figure 8C).

Recently, more and more study has reported the ICIs targeting immune-checkpoints such as PD-1 and PD-L1 could improve the efficiency in treatment of tumors. We found that the expression of TIDE and Exclusion was significantly higher in the high-risk group, while the expression of MSI Expe Sig was significantly lower, with the comparison to the low-risk group (Figure 8D). However, there was no statistically difference about the expression of Dysfunction between the two groups (Figure 8D). Then, the distributions of risk scores between non-respond and respond groups was compared and indicated the non-respond group with significantly higher risk scores, which inferred that patients with no respond to chemotherapy had the poor prognosis (Figure 8E). The TIDE algorithm, which was conducted to predict the immunotherapy responders through transcriptomic data, was used to explore whether prognostic genes could predict immunotherapeutic benefit in glioma. The ROC curve showed the superior predictive performance with AUC value of 0.833 in predict immunotherapeutic benefit (Figure 8F). Meanwhile, the result revealed that the response rates to ICI treatment were significantly higher in low-risk patients (92%) compared with high-risk patients (44%) ($p < 0.001$, Figure 8G). Thus, the more survival benefit was likely to be obtained from immunotherapy for patients in the low-risk group.

4. Discussion

Glioma, the most common malignant brain tumor, is notorious for its characteristics of low cure rate and high recurrence rate[30]. Currently, the problem faced by most neurosurgeons is that the prognosis of most patients with glioma has not been significantly improved by through the existing universal treatment plan. The high heterogeneity of glioma is the main limitation, which leads to inconsistent treatment response and prognosis. Therefore, it is very important and urgent to develop personalized treatment plans for glioma patients. The rapid development of bioinformatics and sequencing technology provides a new perspective to solve this problem. Previous studies indicated that hypoxia-related gene signature[31], m6A-related gene signature[32], ferroptosis-related gene signature[33], immune-related gene signature[34], and energy metabolism-related gene signature[35] are used as prognostic biomarkers to predict the prognosis of glioma. No matter prognosis of 1-year OS, 3-year OS or 5-year OS, they all exhibited superior predictive performance, which makes researchers confident in individually predicting the prognosis of patient by genomics. Inflammatory response, just like the above biological processes, is also an important factor in the occurrence, development and treatment of various

tumor cells[36]. Lin et al.[19] constructed prognostic signature based on 8 inflammatory response genes to predict the prognosis of liver cancer, and the signature showed the OS of 1,3,5-year with AUC is 0.685, 0.626, 0.605, respectively. Nevertheless, the inflammatory response-related gene signature as prognostic marker for glioma is still unclear.

In this study, 39 DEGs between glioma and normal brain tissues were differentiated from the 200 inflammatory response-related genes. Then 31 prognostic DEGs were identified after univariate Cox analysis, 8 of them were selected to construct prognostic IRGS. Whether in the training cohort (TCGA) or the validation cohorts (CGGA-693, CGGA-325 and Rembrandt), IRGS has been confirmed the strong capability in predicting survival outcomes of glioma patients. Subsequently, the prognostic IRGS was incorporated with other independent prognostic factors (age, WHO grade, and 1p19q codeletion) to construct a nomogram with superior OS prediction ability. The functional enrichment analysis revealed the potential difference in inflammatory response sensitivity between the high-risk group and the low-risk group. Meanwhile, immune-related biological processes and pathways were also observed through functional enrichment analysis. Thus, we further revealed the differential immune landscape between the two risk subgroups by comparing the immune, stromal and ESTIMATES scores, tumor purity, abundance of immune cells, and expression levels of immunoregulatory molecules. In addition, the assessment of tumor mutation burden, immune checkpoints, and immunotherapy response are also analyzed in detail based on IRGS risk stratification. To our knowledge, this is the first study to predict the prognosis of glioma with IRGS.

The prognostic IRGS constructed in our study incorporated 8 inflammatory response-related gene such as GABBR1, CALCRL, EBI3, BTG2, SEMA4D, SELL, SERPINE1, and MMP14. Except for GABBR1 and SEMA4D, other genes are up-regulated in glioma tissues. Among these genes, SERPINE1 and MMP14 are associated with poor prognosis, whereas the remaining 6 genes with good prognosis. GABBR1 is a metabotropic G protein-coupled receptor that mediates the inhibitory effect of γ -aminobutyric acid, Yang et al. demonstrated that 5 miRNAs promote the proliferation and invasion of colorectal cancer cells by inhibiting GABBR1[37]. As a G protein-coupled seven transmembrane domain receptor, CALCRL has been identified as a potential tumor suppressor for lung adenocarcinoma by Lu et al.[38]. A β -subunit of IL-12 family member (EBI3) was confirmed to promote T- and B-cell differentiation through the gp130-STAT3 signal pathway, and thereby playing a crucial role in inhibiting tumor cell proliferation and invasion[39]. In the non-small-cell lung cancer models, overexpression of BTG2 prevented the lung cancer cell metastasis by inhibiting cell invasion[40]. SEMA4D is an important member of the semaphorin subfamily and plays an important role in the nervous and immune systems. Chen et al.[41] firstly reported that highly expressed SEMA4D plays an important role in osteolytic bone metastasis of lung cancer by inhibiting osteoblast differentiation, thereby providing a potential strategy for targeting osteoblasts to treat bone metastasis. However, some studies declared to promote tumor cell invasion and metastasis by tumor angiogenesis in some cancers such as prostate cancer and colon cancer[42]. SELL, encoding the selectin L protein (CD62L), mediates the adhesion of cells to the vascular endothelium[43]. While the role of SELL in tumorigenesis and development has not been reported yet. Conversely, SERPINE1, as a member of the superfamily encoding serine protease inhibitors, works by inhibiting proteolytic activity and promoting

angiogenesis. Previous studies demonstrated that the overexpression of SERPINE1 may lead to the spread and metastasis of colon cancer, and is a poor prognostic indicator for malignant tumors such as breast cancer and gastric adenocarcinoma[44, 45]. MMP14 is also named as membrane type 1 matrix metalloproteinase, which participates in the degradation of basement membrane and extracellular matrix, and thus promotes tumor invasion and metastasis[46]. It was of great significance that these genes closely correlated to the origin and metastasis of some cancers, while the detailed action mechanism remains to further investigate.

These inflammatory response genes are involved in the biological processes of many cancers and have predominant predictive performance as a prognostic signature. However, whether these genes affect the prognosis of glioma patients through inflammatory response remains to be clarified. The KEGG analysis revealed that DEGs between different risk subgroups were significantly enriched in pathways related to inflammatory response and cancer, which further confirmed that the inflammatory response has a closely connection with tumor progression. Furthermore, the DEGs between different risk subgroups were also enriched in many immune-related biological processes such as T cell activation and regulation of immune effector process and signal pathways, which remind us that inflammatory response-related genes to may be related to immunity. Thus, we subsequently analyzed the immune scores and immune cell infiltration between the two risk subgroups. Further analyses found that the high-risk group exhibited higher immune scores, higher abundance of immunosuppressive cells (Treg, CD8+ cell)[47], and higher expression levels of immune checkpoints and macrophage associated molecules. In contrast, tumor killer cells (activated NK cells) showed a higher abundance in the low-risk group. To some extent, these results illustrated that inflammatory response-related genes is related to the immune landscape of the glioma microenvironment. From the above results, it can be concluded that the antitumor immunity of high-risk group is significantly weakened, and thus we speculated that this may be an important reason for their poor prognosis. However, the potential molecular mechanism between inflammatory response-related genes and glioma immunity has not been elucidated, and further research is needed to investigate.

With the continuous improvement of gene sequencing technology in the last few decades, the knowledge of researchers for molecular structure has continued to strengthen. Cancer treatment has shifted from chemotherapy and radiotherapy targeting tumors broadly to antibody-based immunotherapies that modulate immune responses against tumors more precisely[48]. ICIs, as the first generation of immunotherapy, play a pivotal role by blocking receptor and/or ligand interactions of molecules, such as PD-1/ PD-L1 pathway and CTLA-4[49]. Some prior studies have been described the therapeutic antibodies targeted therapy for immune checkpoints, which indicated robust and durable responses in patients with many cancers[50], including glioma[51]. In the current study, the high-risk group had a significantly higher immune checkpoints than the low-risk group, and the immune checkpoints was positively correlated with the expression of genes related to poor prognosis, and negatively correlated with genes related to good prognosis. In addition, the response rate to ICIs in the high-risk group was significantly lower than the low-risk group. Therefore, our prognostic model showed superior capacity in predicting the expression level of immune checkpoints, and had the potential to guide immunotherapy.

Certainly, there are some limitations warranted mention in this study. Firstly, it is a retrospective study, thus the results should be further validated its clinical utility in prospective studies. Secondly, these four cohorts have various degrees of deficiencies in clinical information, especially, the Rembrandt cohort is more serious, leading to insufficient verification of some research results. In addition, we were not able to validate in clinical practice owing to the lack of glioma samples. Thirdly, GO and KEGG enrichment analysis and subsequent analysis of immune microenvironment and immune checkpoints are only evaluated in the TCGA cohort, not verified in other cohorts. Lastly, the potential molecular mechanism between inflammatory response-related gene and immunotherapy has not yet been elucidated, and further experiments are needed to explore it.

5. Conclusion

In conclusion, we performed a comprehensive analysis of the inflammatory response-related gene in the four independent cohorts and established a new prognostic signature incorporating 8 inflammation-related genes for glioma patients. The signature exhibited robust capacity in predicting survival outcomes of glioma patients, and was correlated with functional analysis, immune landscape of glioma microenvironment, and immunotherapy. Accordingly, our study provides insight in inflammatory response-related gene for predicting the prognosis of glioma, which is crucial for the development of personalized glioma treatment therapies.

Declarations

Ethics approval and consent to participate: The study received full approval from the ethics committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology and informed consent was waived due to the retrospective nature of the study.

Consent for publication: Not applicable.

Availability of data and materials: Publicly available datasets were analyzed in this study. This data can be found here: The data analyzed in this study can be acquired in the TCGA (<https://portal.gdc.cancer.gov/>), CGGA (<http://www.cgga.org.cn/>) and Rembrandt (<http://gliovis.bioinfo.cnio.es/>) websites.

Competing interests: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors Contributions: Zhen Zhao and Jianglin Zheng constructed this study. Zhen Zhao, Jianglin Zheng, Yi Zhang, and Chuansheng Nie performed the data analysis, figures plotted, and writing. Xiangbing Jiang, Hongyang Zhao were responsible for the critical reading of the manuscript. All authors contributed to the article and approved the submitted version.

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References

1. Ostrom QT, Patil N, Cioffi G, Waite K, Kruchko C, Barnholtz-Sloan JS: **CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2013-2017.** *Neuro Oncol* 2020, **22**(12 Suppl 2):v1-v96.
2. Gusyatiner O, Hegi ME: **Glioma epigenetics: From subclassification to novel treatment options.** *SEMIN CANCER BIOL* 2018, **51**:50–58.
3. Cheng M, Zhang ZW, Ji XH, Xu Y, Bian E, Zhao B: **Super-enhancers: A new frontier for glioma treatment.** *Biochim Biophys Acta Rev Cancer* 2020, **1873**(2):188353.
4. Guan X, Zhang C, Zhao J, Sun G, Song Q, Jia W: **CMTM6 overexpression is associated with molecular and clinical characteristics of malignancy and predicts poor prognosis in gliomas.** *EBIOMEDICINE* 2018, **35**:233-243.
5. Chen R, Smith-Cohn M, Cohen AL, Colman H: **Glioma Subclassifications and Their Clinical Significance.** *NEUROTHERAPEUTICS* 2017, **14**(2):284–297.
6. Zheng J, Zhou Z, Qiu Y, Wang M, Yu H, Wu Z, Wang X, Jiang X: **A Prognostic Ferroptosis-Related lncRNAs Signature Associated With Immune Landscape and Radiotherapy Response in Glioma.** *Front Cell Dev Biol* 2021, **9**:675555.
7. Santangelo A, Rossato M, Lombardi G, Benfatto S, Lavezzari D, De Salvo GL, Indraccolo S, Dehecchi MC, Prandini P, Gambari R *et al.*: **A molecular signature associated with prolonged survival in glioblastoma patients treated with regorafenib.** *Neuro Oncol* 2021, **23**(2):264–276.
8. Mantovani A, Allavena P, Sica A, Balkwill F: **Cancer-related inflammation.** *NATURE* 2008, **454**(7203):436–444.
9. Ikwegbue PC, Masamba P, Mbatha LS, Oyinloye BE, Kappo AP: **Interplay between heat shock proteins, inflammation and cancer: a potential cancer therapeutic target.** *AM J CANCER RES* 2019, **9**(2):242–249.
10. Wu L, Wang F, Xu J, Chen Z: **PTPN2 induced by inflammatory response and oxidative stress contributed to glioma progression.** *J CELL BIOCHEM* 2019, **120**(11):19044–19051.
11. Chen M, Zheng SH, Yang M, Chen ZH, Li ST: **The diagnostic value of preoperative inflammatory markers in craniopharyngioma: a multicenter cohort study.** *J Neurooncol* 2018, **138**(1):113–122.
12. Marinari E, Allard M, Gustave R, Widmer V, Philippin G, Merkler D, Tsantoulis P, Dutoit V, Dietrich PY: **Inflammation and lymphocyte infiltration are associated with shorter survival in patients with high-grade glioma.** *ONCOIMMUNOLOGY* 2020, **9**(1):1779990.
13. Zhao Z, Xiao D, Nie C, Zhang H, Jiang X, Jecha AR, Yan P, Zhao H: **Development of a Nomogram Based on Preoperative Bi-Parametric MRI and Blood Indices for the Differentiation Between Cystic-Solid Pituitary Adenoma and Craniopharyngioma.** *FRONT ONCOL* 2021, **11**:2588.

14. McMillan DC: **Systemic inflammation, nutritional status and survival in patients with cancer.** *Curr Opin Clin Nutr Metab Care* 2009, **12**(3):223–226.
15. Yang T, Mao P, Chen X, Niu X, Xu G, Bai X, Xie W: **Inflammatory biomarkers in prognostic analysis for patients with glioma and the establishment of a nomogram.** *ONCOL LETT* 2019, **17**(2):2516–2522.
16. Yang HB, Xing M, Ma LN, Feng LX, Yu Z: **Prognostic significance of neutrophil-lymphocyteratio/platelet-lymphocyteratioin lung cancers: a meta-analysis.** *Oncotarget* 2016, **7**(47):76769-76778.
17. Bao Y, Yang M, Jin C, Hou S, Shi B, Shi J, Lin N: **Preoperative Hematologic Inflammatory Markers as Prognostic Factors in Patients with Glioma.** *WORLD NEUROSURG* 2018, **119**:e710-e716.
18. Budhu A, Forgues M, Ye QH, Jia HL, He P, Zanetti KA, Kammula US, Chen Y, Qin LX, Tang ZY *et al*: **Prediction of venous metastases, recurrence, and prognosis in hepatocellular carcinoma based on a unique immune response signature of the liver microenvironment.** *CANCER CELL* 2006, **10**(2):99–111.
19. Lin Z, Xu Q, Miao D, Yu F: **An Inflammatory Response-Related Gene Signature Can Impact the Immune Status and Predict the Prognosis of Hepatocellular Carcinoma.** *FRONT ONCOL* 2021, **11**:644416.
20. Johnston MP, Khakoo SI: **Immunotherapy for hepatocellular carcinoma: Current and future.** *World J Gastroenterol* 2019, **25**(24):2977–2989.
21. Xu S, Tang L, Li X, Fan F, Liu Z: **Immunotherapy for glioma: Current management and future application.** *CANCER LETT* 2020, **476**:1–12.
22. Ghosh A, Barman S: **Application of Euclidean distance measurement and principal component analysis for gene identification.** *GENE* 2016, **583**(2):112–120.
23. Zheng Z, Mao S, Guo Y, Zhang W, Liu J, Li C, Yao X: **N6methyladenosine RNA methylation regulators participate in malignant progression and have prognostic value in clear cell renal cell carcinoma.** *ONCOL REP* 2020, **43**(5):1591–1605.
24. Sauerbrei W, Royston P, Binder H: **Selection of important variables and determination of functional form for continuous predictors in multivariable model building.** *STAT MED* 2007, **26**(30):5512–5528.
25. Simon N, Friedman J, Hastie T, Tibshirani R: **Regularization Paths for Cox's Proportional Hazards Model via Coordinate Descent.** *J STAT SOFTW* 2011, **39**(5):1–13.
26. Harrell FJ, Lee KL, Mark DB: **Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors.** *STAT MED* 1996, **15**(4):361–387.
27. Yoshihara K, Shahmoradgoli M, Martinez E, Vegesna R, Kim H, Torres-Garcia W, Trevino V, Shen H, Laird PW, Levine DA *et al*: **Inferring tumour purity and stromal and immune cell admixture from expression data.** *NAT COMMUN* 2013, **4**:2612.
28. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA: **Profiling Tumor Infiltrating Immune Cells with CIBERSORT.** *Methods Mol Biol* 2018, **1711**:243–259.

29. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, Li Z, Traugh N, Bu X, Li B *et al*: **Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response.** *NAT MED* 2018, **24**(10):1550–1558.
30. Xu S, Tang L, Dai G, Luo C, Liu Z: **Expression of m6A Regulators Correlated With Immune Microenvironment Predicts Therapeutic Efficacy and Prognosis in Gliomas.** *Front Cell Dev Biol* 2020, **8**:594112.
31. Lin W, Wu S, Chen X, Ye Y, Weng Y, Pan Y, Chen Z, Chen L, Qiu X, Qiu S: **Characterization of Hypoxia Signature to Evaluate the Tumor Immune Microenvironment and Predict Prognosis in Glioma Groups.** *FRONT ONCOL* 2020, **10**:796.
32. Chai RC, Wu F, Wang QX, Zhang S, Zhang KN, Liu YQ, Zhao Z, Jiang T, Wang YZ, Kang CS: **m(6)A RNA methylation regulators contribute to malignant progression and have clinical prognostic impact in gliomas.** *Aging (Albany NY)* 2019, **11**(4):1204–1225.
33. Zhuo S, Chen Z, Yang Y, Zhang J, Tang J, Yang K: **Clinical and Biological Significances of a Ferroptosis-Related Gene Signature in Glioma.** *FRONT ONCOL* 2020, **10**:590861.
34. Zhang M, Wang X, Chen X, Zhang Q, Hong J: **Novel Immune-Related Gene Signature for Risk Stratification and Prognosis of Survival in Lower-Grade Glioma.** *FRONT GENET* 2020, **11**:363.
35. Jiang Z, Liu Z, Li M, Chen C, Wang X: **Increased glycolysis correlates with elevated immune activity in tumor immune microenvironment.** *EBIOMEDICINE* 2019, **42**:431-442.
36. Chen W, Xia T, Wang D, Huang B, Zhao P, Wang J, Qu X, Li X: **Human astrocytes secrete IL-6 to promote glioma migration and invasion through upregulation of cytomembrane MMP14.** *Oncotarget* 2016, **7**(38):62425-62438.
37. Longqiu Y, Pengcheng L, Xuejie F, Peng Z: **A miRNAs panel promotes the proliferation and invasion of colorectal cancer cells by targeting GABBR1.** *Cancer Med* 2016, **5**(8):2022-2031.
38. Lu M, Fan X, Liao W, Li Y, Ma L, Yuan M, Gu R, Wei Z, Wang C, Zhang H: **Identification of significant genes as prognostic markers and potential tumor suppressors in lung adenocarcinoma via bioinformatical analysis.** *BMC CANCER* 2021, **21**(1):616.
39. Ma N, Fang Y, Xu R, Zhai B, Hou C, Wang X, Jiang Z, Wang L, Liu Q, Han G *et al*: **Ebi3 promotes T- and B-cell division and differentiation via STAT3.** *MOL IMMUNOL* 2019, **107**:61-70.
40. Chen Z, Chen X, Lu B, Gu Y, Chen Q, Lei T, Nie F, Gu J, Huang J, Wei C *et al*: **Up-regulated LINC01234 promotes non-small-cell lung cancer cell metastasis by activating VAV3 and repressing BTG2 expression.** *J HEMATOL ONCOL* 2020, **13**(1):7.
41. Chen WG, Sun J, Shen WW, Yang SZ, Zhang Y, Hu X, Qiu H, Xu SC, Chu TW: **Sema4D expression and secretion are increased by HIF-1alpha and inhibit osteogenesis in bone metastases of lung cancer.** *Clin Exp Metastasis* 2019, **36**(1):39–56.
42. Li H, Wang JS, Mu LJ, Shan KS, Li LP, Zhou YB: **Promotion of Sema4D expression by tumor-associated macrophages: Significance in gastric carcinoma.** *World J Gastroenterol* 2018, **24**(5):593–601.

43. Dashti N, Mahmoudi M, Gharibdoost F, Kavosi H, Rezaei R, Imeni V, Jamshidi A, Aslani S, Mostafaei S, Vodjgani M: **Evaluation of ITGB2 (CD18) and SELL (CD62L) genes expression and methylation of ITGB2 promoter region in patients with systemic sclerosis.** *RHEUMATOL INT* 2018, **38**(3):489–498.
44. Mazzocchi G, Paziienza V, Panza A, Valvano MR, Benegiamo G, Vinciguerra M, Andriulli A, Piepoli A: **ARNTL2 and SERPINE1: potential biomarkers for tumor aggressiveness in colorectal cancer.** *J Cancer Res Clin Oncol* 2012, **138**(3):501–511.
45. Li L, Zhu Z, Zhao Y, Zhang Q, Wu X, Miao B, Cao J, Fei S: **FN1, SPARC, and SERPINE1 are highly expressed and significantly related to a poor prognosis of gastric adenocarcinoma revealed by microarray and bioinformatics.** *Sci Rep* 2019, **9**(1):7827.
46. Jin Y, Liang ZY, Zhou WX, Zhou L: **High MMP14 expression is predictive of poor prognosis in resectable hepatocellular carcinoma.** *PATHOLOGY* 2020, **52**(3):359–365.
47. Zhou SL, Zhou ZJ, Hu ZQ, Huang XW, Wang Z, Chen EB, Fan J, Cao Y, Dai Z, Zhou J: **Tumor-Associated Neutrophils Recruit Macrophages and T-Regulatory Cells to Promote Progression of Hepatocellular Carcinoma and Resistance to Sorafenib.** *GASTROENTEROLOGY* 2016, **150**(7):1646–1658.
48. Cao R, Yuan L, Ma B, Wang G, Tian Y: **Tumour microenvironment (TME) characterization identified prognosis and immunotherapy response in muscle-invasive bladder cancer (MIBC).** *Cancer Immunol Immunother* 2021, **70**(1):1–18.
49. Topalian SL, Drake CG, Pardoll DM: **Immune checkpoint blockade: a common denominator approach to cancer therapy.** *CANCER CELL* 2015, **27**(4):450–461.
50. Huang J, Xu J, Chen Y, Zhuang W, Zhang Y, Chen Z, Chen J, Zhang H, Niu Z, Fan Q *et al*: **Camrelizumab versus investigator's choice of chemotherapy as second-line therapy for advanced or metastatic oesophageal squamous cell carcinoma (ESCORT): a multicentre, randomised, open-label, phase 3 study.** *LANCET ONCOL* 2020, **21**(6):832–842.
51. Yang T, Kong Z, Ma W: **PD-1/PD-L1 immune checkpoint inhibitors in glioblastoma: clinical studies, challenges and potential.** *Hum Vaccin Immunother* 2021, **17**(2):546–553.

Figures

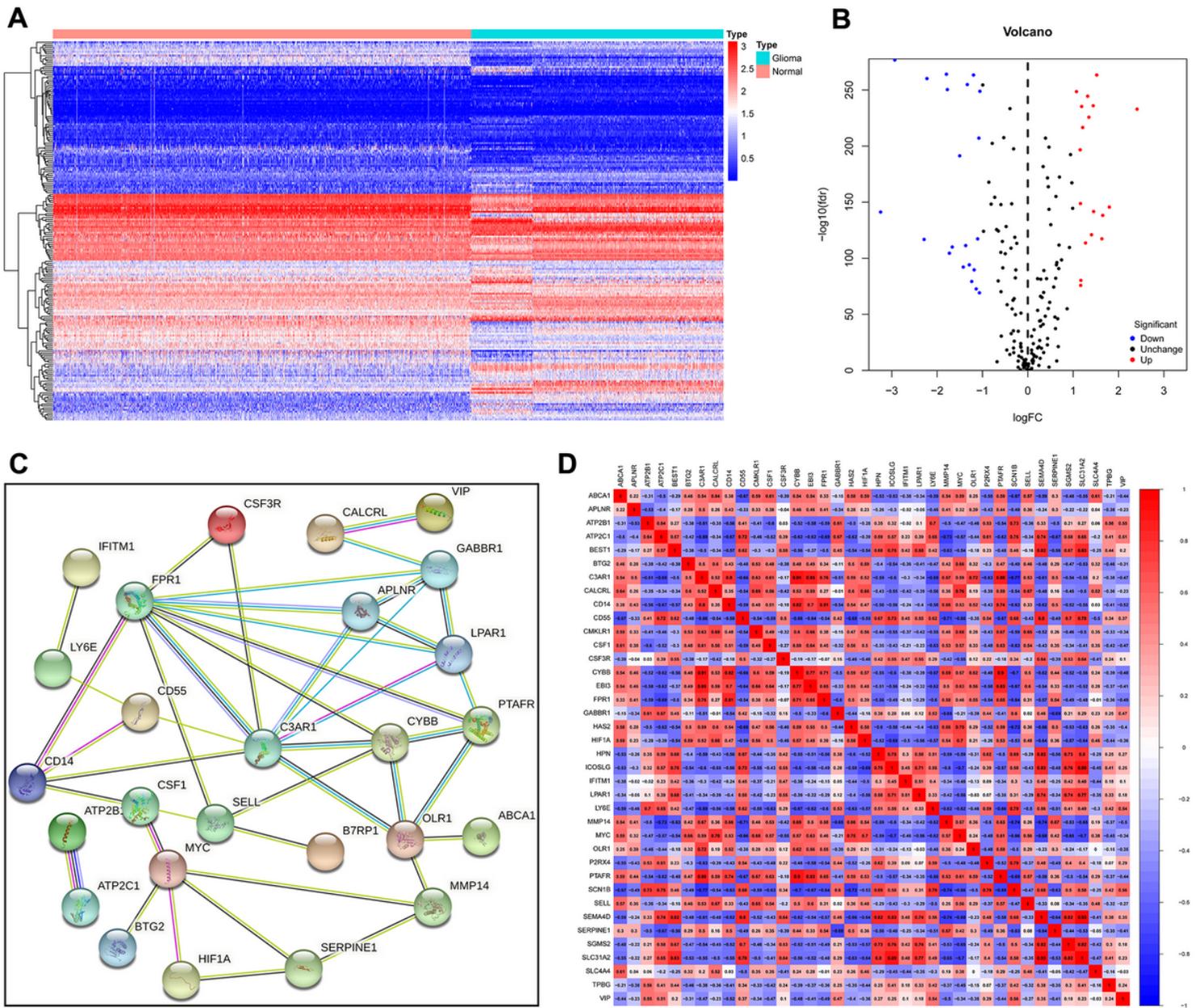


Figure 1

Identification and analysis of differentially expressed genes (DEGs). (A) A heatmap and (B) volcano plot showing the differentially expressed genes between glioma and healthy brain tissue samples. The red, green, and black dots represent the upregulated genes, downregulated genes, and no difference, respectively. (C) Network and (D) correlations among 39 inflammatory response-related genes.

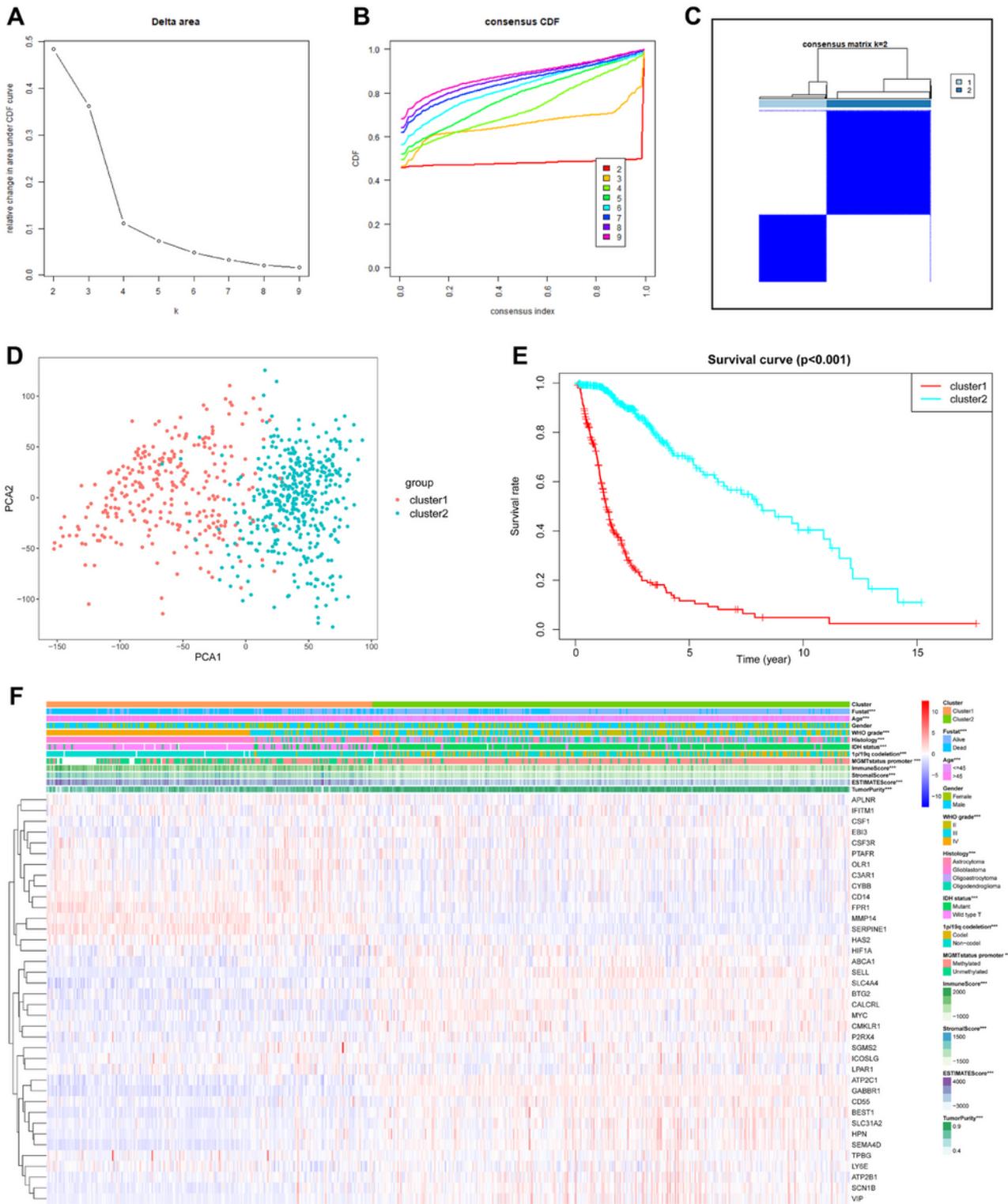


Figure 2

Differential clinicopathological features and overall survival of gliomas in the cluster 1 and cluster 2 subgroups. (A) Relative change in area under CDF curve for k = 2 to 10. (B) Consensus clustering cumulative distribution function (CDF) for k = 2 to 10. (C) Consensus clustering matrix for k=2. (D) principal component analysis (PCA) between cluster1 and cluster2 subgroups. (E) Kaplan–Meier overall

survival (OS) curves for 309 CGGA glioma patients. (F) Heatmap and clinicopathologic features of the two clusters defined by the inflammatory response-related genes consensus expression.

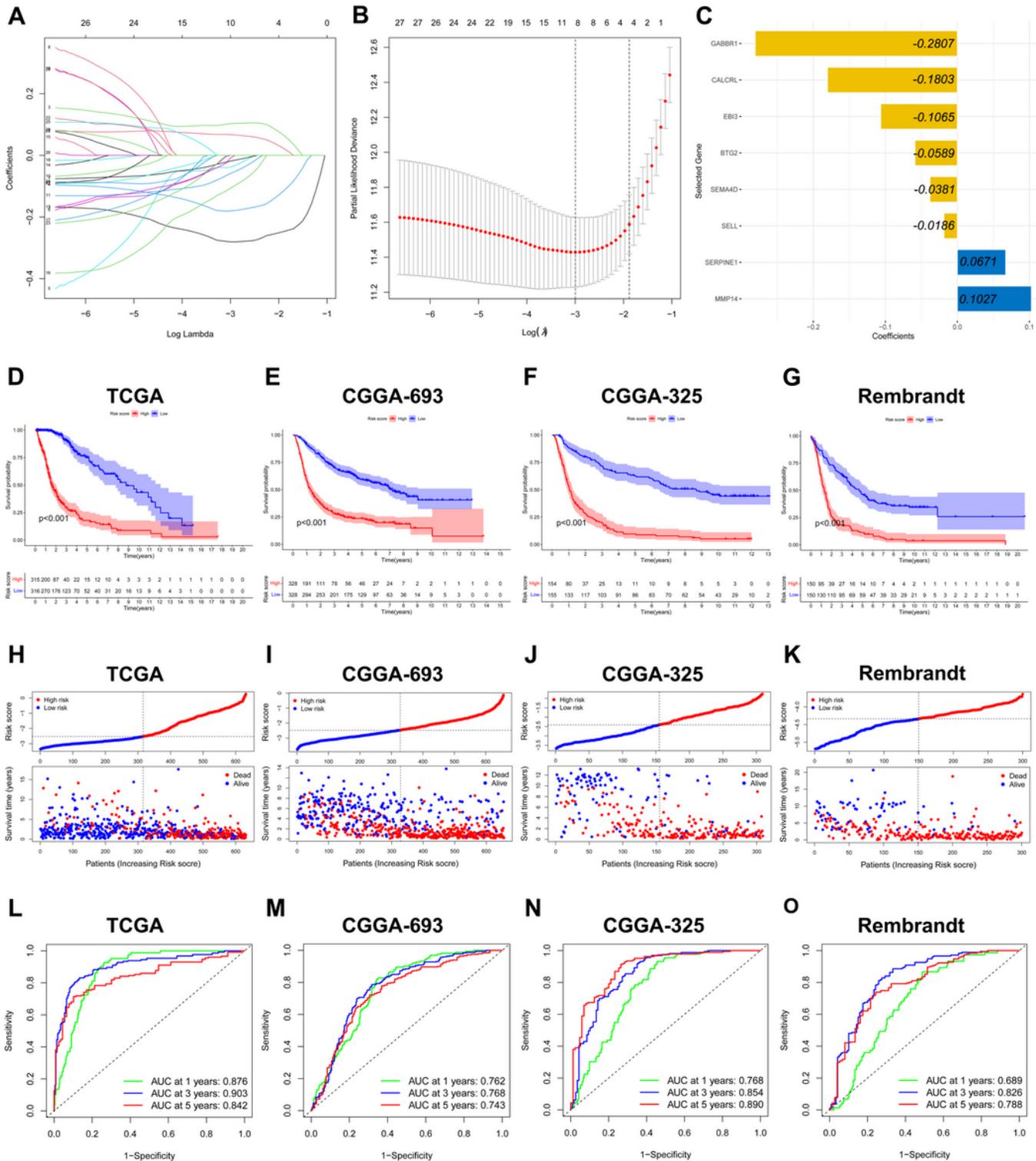


Figure 3

Construction and validation of the prognostic inflammatory response-related genes signature (IRGS). (A, B) The least absolute shrinkage and selection operator (LASSO) regression was performed with the minimum criteria. (C) LASSO coefficients of 8 selected inflammatory response-related genes. (D–G) The

Kaplan-Meier curves for survival in the TCGA, CGGA-693, CGGA-325 and Rembrandt cohorts, respectively. (H–K) The distribution plots of the risk score and survival status in the TCGA, CGGA-693, CGGA-325 and Rembrandt cohorts, respectively. (L–O) The receiver operating characteristic (ROC) curve analyses of the prognostic IRGS in predicting 1-, 3-, and 5-year overall survival (OS) in the TCGA, CGGA-693, CGGA-325 and Rembrandt cohorts, respectively.

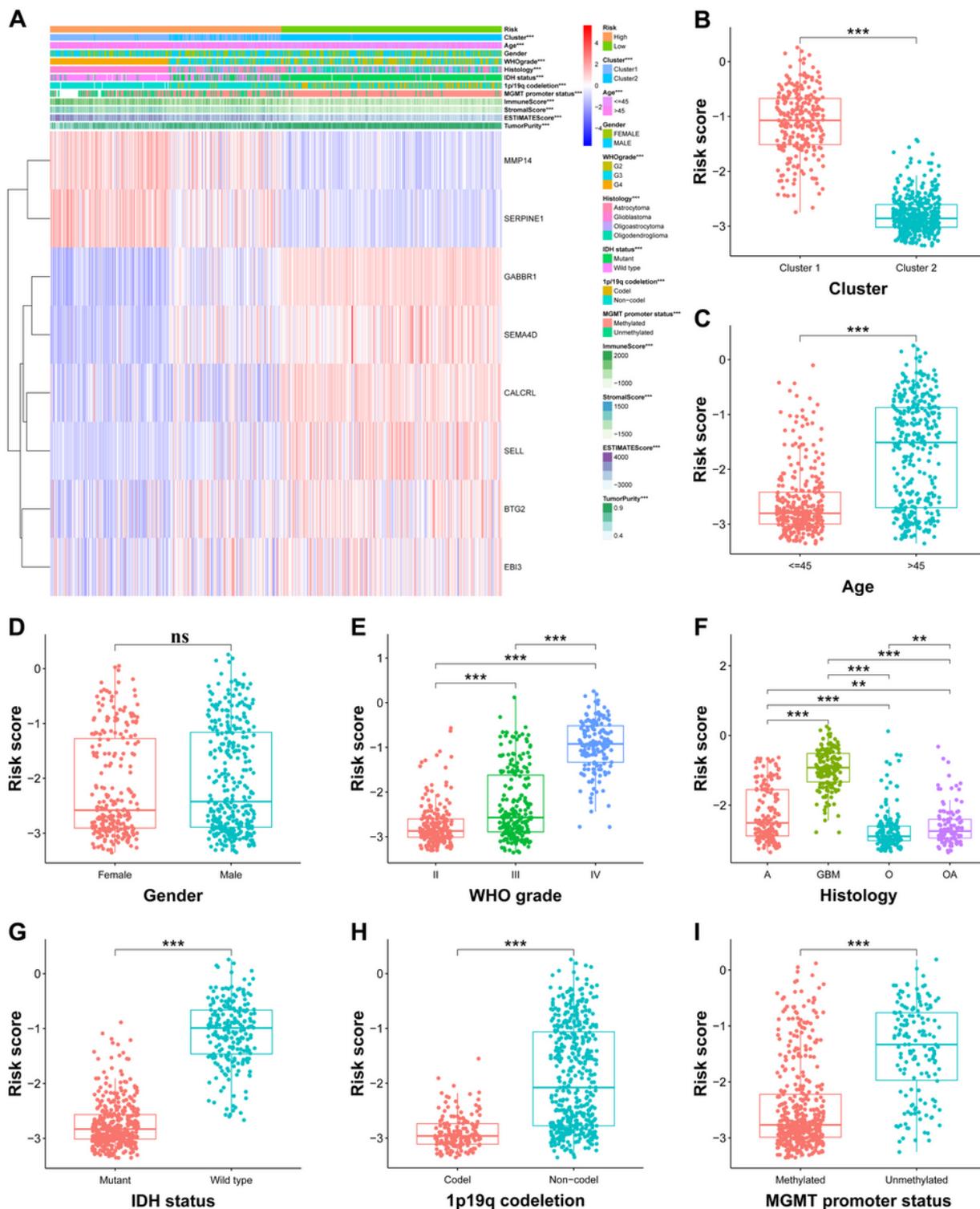


Figure 4

Correlation analysis between the prognostic IRGS and clinicopathological characteristics in the TCGA cohort. (A) A heatmap showing the distribution of clinicopathological characteristics and expression levels of 8 selected inflammatory response-related genes in the high- and low-risk groups. (B–I) Different levels of risk scores in glioma patients stratified by cluster, age, gender, WHO grade, histology, IDH status, 1p19q codeletion and MGMT promoter status. A, astrocytoma; O, oligodendroglioma; OA, oligoastrocytoma; GBM, glioblastoma. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and ns No significance.

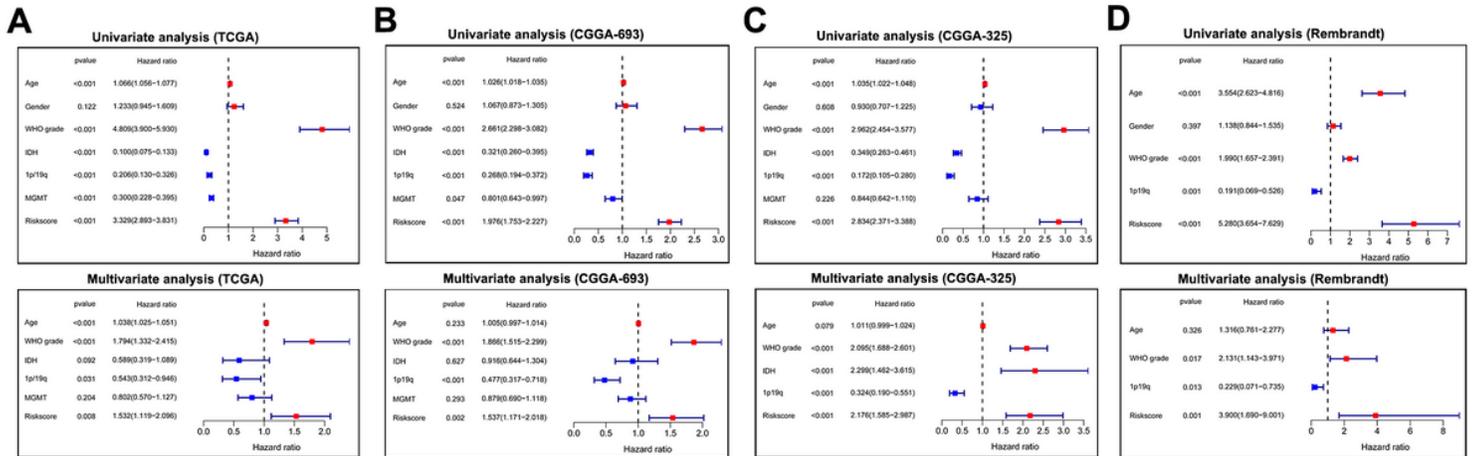


Figure 5

The univariate Cox regression and multivariate Cox regression were performed on four variables including age, gender, WHO grade, IDH status, 1p19q codeletion and MGMT promoter status. (A-D) The univariate Cox regression and multivariate Cox regression for identifying the OS-related factors in the TCGA, CGGA-693, CGGA-325, and Rembrandt cohorts, respectively.

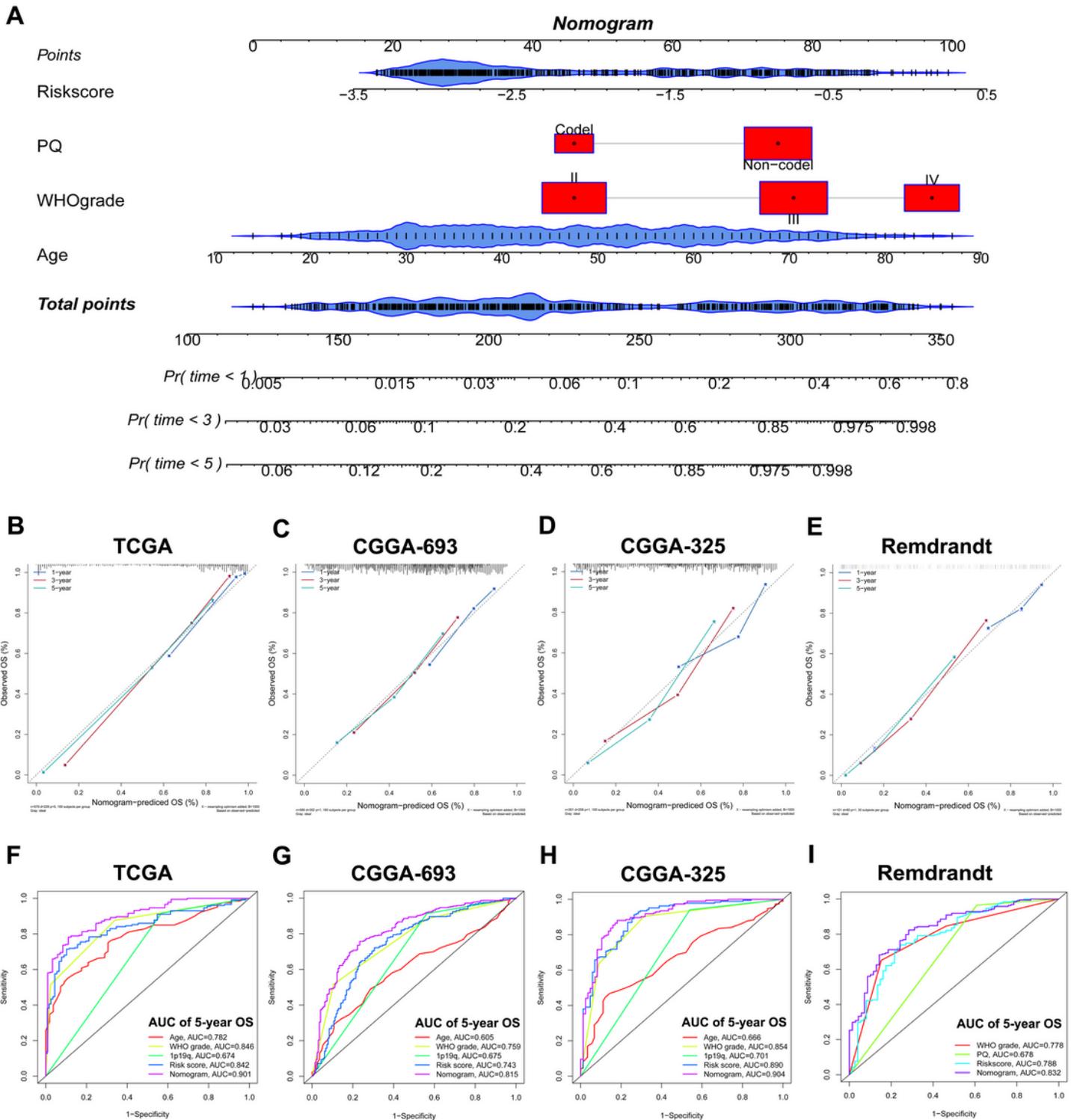


Figure 6

Establishment and evaluation of a nomogram in the TCGA cohort. (A) Nomogram based on IRGS, age, WHO grade and 1p19q codeletion. (B–E) Calibration curves showing the concordance between predicted and observed 1-, 3-, and 5-year overall survival (OS) in the TCGA, CGGA-693, CGGA-325 and Rembrandt cohorts, respectively. (F–I) The receiver operating characteristic (ROC) curve analyses of the nomogram in predicting 5-year OS in the TCGA, CGGA-693, CGGA-325 and Rembrandt cohorts, respectively.

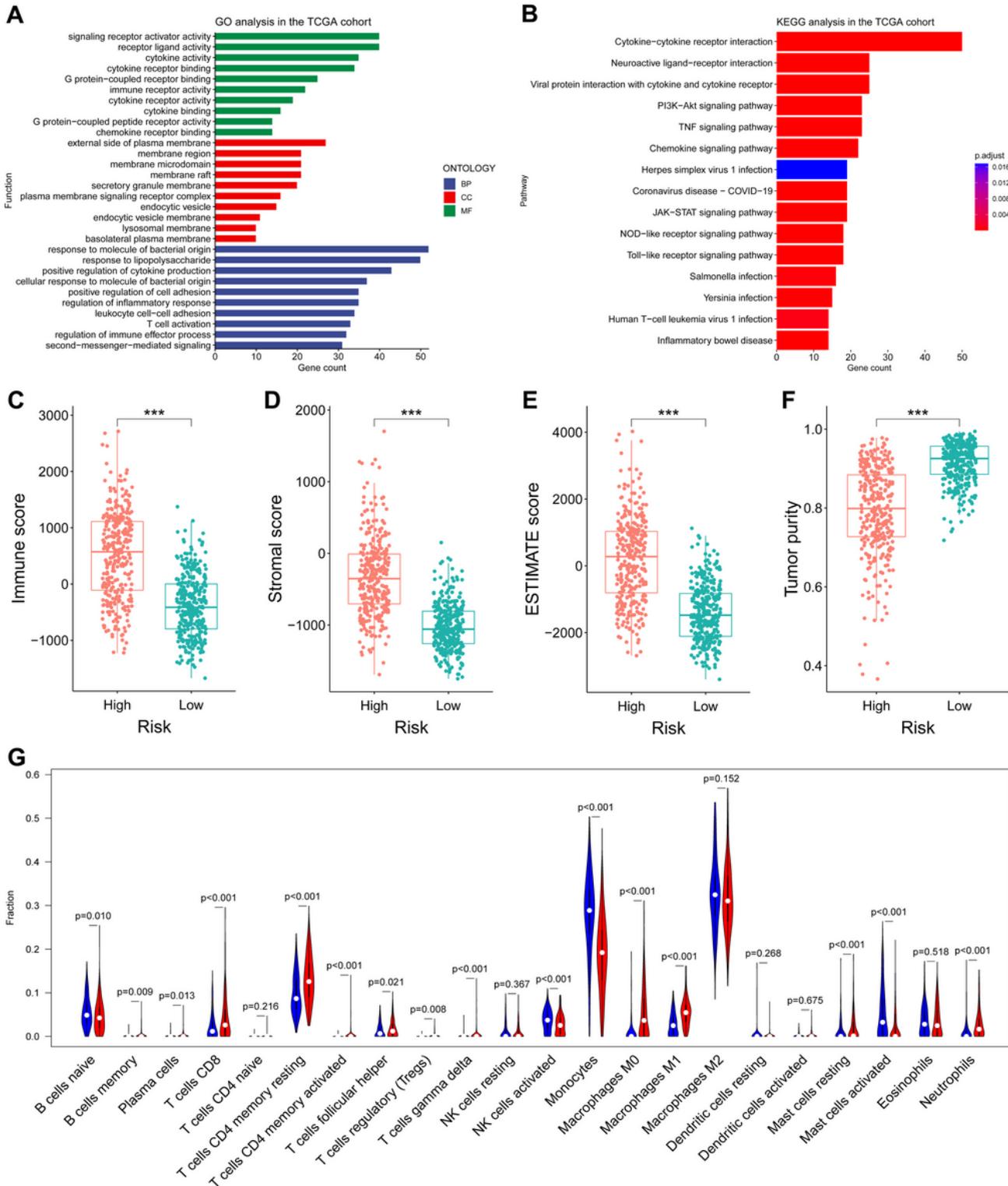


Figure 7

Functional enrichment analysis and immune landscape of glioma microenvironmental in the TCGA cohort. (A, B) Go analysis and KEGG analysis in the TCGA cohort. (C-F) Comparison of immune scores, stromal scores, ESTIMATE scores and tumor purity between the high- and low-risk groups. (E) The abundance of 22 immune cells in the high- and low-risk groups.

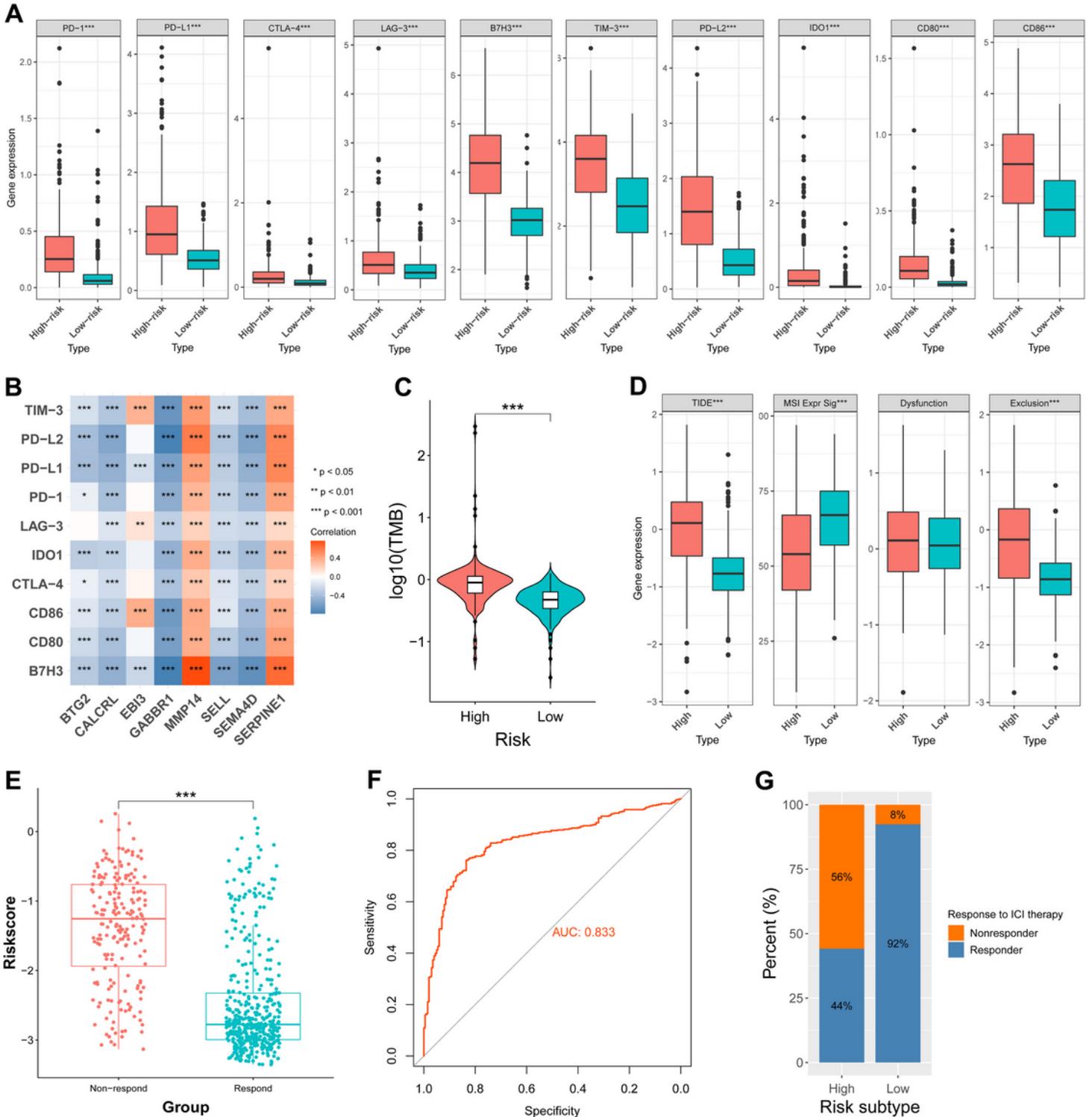


Figure 8

Evaluation of immune checkpoints and immunotherapy responsiveness in the TCGA cohort. (A) The expression levels of immune checkpoints and macrophage associated molecules in the high- and low-risk groups. *** $p < 0.001$. (B) Correlation analysis between the prognostic IRGS and immune checkpoints. (C) Comparative analysis of tumor mutation burden in the high- and low-risk groups. (D) The expression of TIDE, Exclusion, MSI Expe Sig and Dysfunction between high- and low-risk groups. (E) The distributions

of risk scores between non-respond and respond groups. (F) The ROC curve of predicting immunotherapeutic benefit. (G) Comparative analysis of the response rates to ICI treatment in the high- and low-risk groups.

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