

Weighted Gene Co-Expression Network Analysis Identifies an Immunogenic Cell Death Signature to Predict Therapeutic Responses and Prognosis of Glioblastoma

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Submitted: 27 February 2024 Revised: 29 March 2024 Accepted: 12 April 2024 Published: 1 June 2024

Background: Induction of immunogenic cell death (ICD) breaks down the immunosuppressive tumor microenvironment (TME) and controls tumor progression, but the correlation between glioblastoma (GBM) and ICD is unclear. Therefore, this study aims to investigate the potential prognostic value of ICD-associated genes in GBM.

Methods: We collected 34 ICD-related genes from various sources. Utilizing public databases, we extracted relevant GBM data and delineated prognosis-related ICD gene modules using weighted gene co-expression network analysis (WGCNA). Least absolute shrinkage and selection operator (LASSO) algorithm was employed to develop a risk model, whose accuracy was confirmed by including an independent Gene Expression Omnibus (GEO) dataset. The biological functions and pathways associated with these signals were analyzed by performing enrichment analysis, and the tumor immune infiltration capacity was evaluated. The R package oncoPredict was used to infer the drug sensitivity of patients in different risk groups using data from the Genomics of Drug Sensitivity in Cancer 2 (GDSC2) database with expression profiling.

Results: Thirty-four ICD-associated genes were differentially expressed in GBM samples and two gene modules significantly associated with prognosis were identified. Based on these gene modules, vitamin D receptor (*VDR*) and cell death-inducing DFF45-like effector B (*CIDEB*) were identified as two signature genes for the prognostic prediction of GBM. Subsequently, multivariate Cox analysis confirmed the validity of this signature as an independent factor for evaluating overall survival in GBM. Receiver operating characteristic (ROC) curves also supported an effective prediction of the signature (1-year area under the ROC curve (AUC): 0.667; 3-year AUC: 0.727; 5-year AUC: 0.762). We observed that the high-risk group had higher immune cell infiltration and sensitivity to some drugs.

Conclusions: This work developed a novel ICD-related prognostic model for GBM patients. Our findings highlight the potential of using ICD as a promising prognosis indicator in GBM, contributing to the current understanding of the intricate interplay between ICD and tumor microenvironment.

Keywords: glioblastoma; immunogenic cell death; tumor microenvironment; immunotherapy; prognosis

Introduction

Cancer treatment for primary malignant brain and central nervous system tumors remains a challenge and is characterized by a dismal prognosis. Five-year survival chance for patients diagnosed with these tumors is around 35.8% [1–3]. Glioma, also known as neuroepithelial tissue tumors, represents about 30% of all brain and central nervous system malignancies. Despite its relatively low incidence rate, the impact is profound, leading to significant disability and mortality upon diagnosis. Notably, glioblastoma (GBM) is the most common subtype among all gliomas, accounting for approximately 57.3% of all gliomas and 48.3% of malignant central nervous system tumors [4]. Recent

study mainly focused on the anti-tumor immunotherapy in glioma [5]. Biomarker-guided treatments and immunotherapy inhibitors exhibited great potential for treating GBM [6]. Nonetheless, drug resistance in tumors remains a significant problem in cancer therapy [7]. Hence, the discovery of reliable biomarkers and risk models is essential for better prediction of the clinical outcome of GBM patients. Such discoveries are also imperative to evaluate the efficacy of anti-tumor therapies.

Immunogenic cell death (ICD) is a distinct form of regulated cell death (RCD) which stimulates immune responses against tumor cells. ICD was realized through the release of damage-associated molecular patterns (DAMPs), including secretion of adenosine triphosphate (ATP), re-

lease of high mobility group box-1 (HMGB1), and calreticulin exposure on cell surface, from dying tumor cells. These DAMPs activate dendritic cells and recruit immune cells, thereby triggering antitumor immune responses [8–10]. ICD has been regarded as a new modality in cancer immunotherapy because of its ability to enhance T cell-mediated tumor killing [11]. Ruan *et al.* [12] suggested that ICD can break down the immunologically heterogeneous tumor microenvironment, which in turn initiates T cell-mediated adaptive immunity and might have the potential to be used in long-term tumor control. In addition, antitumor drug-induced ICD has been increasingly explored. For instance, Tukaramrao *et al.* [13] found that chemotherapeutic drug TPH104 alone can induce ICD in breast cancer by promoting the stimulatory capacity of dendritic cells (DCs). Hence, a rational and safe utilization of ICD inducers in tumor therapy holds the potential to activate antitumor immunity and achieve long-term antitumor effects. However, the mechanism of cellular ICD and the specific prognostic features in GBM are still unknown.

This research calculated the immune score for all the samples in The Cancer Genome Atlas-glioblastoma (TCGA-GBM) using Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data (ESTIMATE) algorithm. Subsequently, immune-related modules were sectioned by weighted gene co-expression network analysis (WGCNA). Through stability selection and least absolute shrinkage and selection operator (LASSO)-Cox regression analysis, we developed a 2-gene prognostic model. The model validity was assessed using independent validation sets and compared with previous models. Additionally, the analysis of infiltrating immune cells and pathway enrichment demonstrated a significant association between our prognostic model and components of innate immunity. In summary, this study successfully developed a reliable ICD-related prognostic model for GBM based on transcriptomics data.

Materials and Methods

Data Acquisition and Preprocessing

We utilized the University of California Santa Cruz (UCSC)-Xena website (<https://xenabrowser.net/datapages/>) to collect data of tumor and normal control samples from the TCGA (<https://portal.gdc.cancer.gov/>), Genotype-Tissue Expression (GTEx, <https://www.gtexportal.org/home/>), and Therapeutically Applicable Research To Generate Effective Treatments (TARGET) (<https://ocg.cancer.gov/programs/target>) databases. Tumor samples from the TCGA-GBM dataset were selected for analysis and the brain samples from the GTEx cohort served as control. By excluding samples without complete survival information or with survival time less than 1 day (d), 141 GBM samples and 152 control samples were included in the analysis.

For validation purpose, expression profiles of patients in the GSE83300 cohort were analyzed on the GPL6480 platform and selected as the validation cohort. Similar to the previous step, samples in this dataset without complete survival information or with survival time shorter than 1 d were excluded. In this way, a total of 50 GBM samples were included in the analysis. Expression profile data and clinical data were collected from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo>). The GEO data were processed as follows: (1) Removing unloaded probes; (2) Deleting probes matching to several genes; (3) The final expression value was calculated as the median expression value for genes with several probes.

WGCNA and Modules Related to Immune

Using the WGCNA, we captured gene patterns across various samples, facilitating the identification of highly correlated gene sets and the exploration of potential therapeutic targets or biomarker genes in terms of their connectivity within gene set and their association with various phenotypes [14]. To obtain the ICD-related hub genes, we first used the single-sample gene set enrichment analysis (ssGSEA) method, which took ICD-free ICD-related genes as ICD-associated pathways and then calculated the ICD pathway scores for each GBM sample. Subsequently, the candidate genes were subjected to WGCNA analysis. Co-expression network was constructed by the WGCNA algorithm in the R package WGCNA to further investigate the association between specific features of ICD-related genes and modules.

Scale independence and average connectivity analysis on modules with different power values were used to determine software threshold parameters. Accordingly, co-expression network was created by mapping all the relevant genes. To represent gene expression similarity, the adjacency matrix was then transformed into a topological overlap matrix (TOM). Under a cut height of 0.25 and a minimum module size of 30, dynamic tree-cutting algorithm was employed to categorize the genes into different modules. The analysis included 13,937 genes, with a threshold mad value of 0.01.

To assess the association between ICD-related genes and gene modules, the enrichment score of the 34 ICD-related genes for each sample in the GBM cohort was calculated by ssGSEA method. The correlation analysis identified modules with significant positive and negative correlations with ICD-related genes under the criteria of gene significance (GS) >0.6 and module membership (MM) >0.7. Subsequently, the hub genes were obtained and used to further select prognostic factors with $p < 0.05$ by univariate Cox regression. To select signature genes for creating a prognostic model, LASSO analysis was performed. Based on the expression levels of these signature genes and their corresponding regression coefficients, each patient was assigned with a risk score:

$$\text{Score} = \sum_{i=1}^n \text{Coefficient}_i \times \text{Expression}_i$$

Coefficient referred to weight coefficient of a gene, while expression was the amount of expression of the gene.

High score and low score groups were divided according to the median score. The “survminer” R package (version 0.4.9, Statistics Section, Department of Economy Finance and Statistics, University of Perugia, Perugia, Italy) was utilized to analyze the distinct prognoses between the two groups [15]. The prognostic prediction capacity was tested using the training and validation sets and reflected in the time-dependent receiver operating characteristic (ROC) curves plotted by the “timeROC” R package (version 0.4, University of Bordeaux, Bordeaux, France) [16].

Genetic Variation Analysis of the ICD-Related Genes

From the TCGA database, we obtained genetic variation data of colorectal cancer, including copy number variations (CNVs) and single nucleotide polymorphisms (SNPs). The “maftools” software package (version 2.8.05, National University of Singapore, Kent Hill, Singapore) [17] was employed to analyze and visualize the types and frequencies of gene mutations in R21. To enhance the visualization of chromosomal alterations, copy number alteration (CNA) summary plots were generated using the “ggplot2” package in R (version 3.4.4, NIHR Great Ormond Street Hospital Biomedical Research Centre, London, UK), and the distribution of ICD-related genes was visualized using the “RCircos” package in R22 (Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA).

Function and Pathway Enrichment of the Hub Genes

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis on the hub gene sets were performed utilizing the R package “clusterProfiler” (version 0.4.6, Baylor College of Medicine, Houston, TX, USA) [18].

Immune Cell Infiltration in the TME

To measure the relative abundance of different cell infiltrates in the tumor microenvironment (TME) of breast cancer patients, the ssGSEA algorithm was employed. The gene set from Charoentong’s study [19] that encompassed a diverse range of human immune cell subtypes including macrophages, regulatory T cells, activated dendritic cells, natural killer T cells, and activated cluster of the differentiation 8 (CD8⁺) T cells was used to identify the types of immune cells infiltrating the tumor immune microenvironment (TIME).

Protein Interaction Network to Study Synergistic Biological Functions

The interaction between target genes and proteins was extracted from the STRING database (<https://cn.string-db.org/>). Isolated points in the network were removed, and Cytoscape software (version 3.9.1, Institute for Systems Biology, Seattle, WA, USA) [20] was employed for analyzing and visualizing the resulting network.

Statistical Analysis

All the statistical analyses were performed in R software (version 4.1.2, Columbia University Irving Medical Center, New York, NY, USA; <https://www.R-project.org>). The tidyverse package in R (version 2.0.0, Department of Biostatistics and Informatics, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO, USA) was employed for data integration and visualization. Kaplan-Meier (K-M) curves and log-rank test were performed to assess significant differences in overall survival (OS) between the groups. Wilcoxon and Kruskal–Wallis tests were applied to compare continuous and ordered categorical variables, respectively. Unordered categorical variables such as the distribution of immunotherapy patients across different groups were compared with Fisher’s exact test. Heatmaps were generated using the ComplexHeatmap package in R (version 3.20, Division of Theoretical Bioinformatics Heidelberg Center for Personalized Oncology, German Cancer Research Center, Heidelberg, Germany). Univariate and multivariate Cox regression analyses were conducted, and the statistics table containing sample information was generated using the gtsummary package in R (version 1.7.2, <https://github.com/dsjoberg/gtsummary>). In all analyses, *p* value below 0.05 was considered as statistically significant. Statistical significance levels were indicated as follows: * for *p* < 0.05, ** for *p* < 0.01, *** for *p* < 0.001, and **** for *p* < 0.0001.

Results

Variation Landscape of ICD-Related Genes in GBM Patients

A total of 34 ICD-related genes were collected, as identified in the study of Garg *et al.* [21]. Initially, a differential analysis of the 34 ICD-correlated genes between 141 GBM samples and 152 control samples revealed significant changes in the expressions of 30 ICD-correlated genes among GBM patients. Interferon-alpha1 (*IFNA1*), heat shock protein 90 alpha family class A member 1 (*HSP90AA1*), forkhead box protein 3 (*FOXP3*), and cluster of the differentiation 8 alpha (*CD8A*) were significantly downregulated in GBM patients compared with the control group, while the remaining 26 genes were significantly upregulated (*p* < 0.05, Fig. 1A). Analysis of CNV showed frequent alterations in ICD-related genes. Notably, phosphatidylinositol-4, 5-bisphosphate 3-kinase cat-

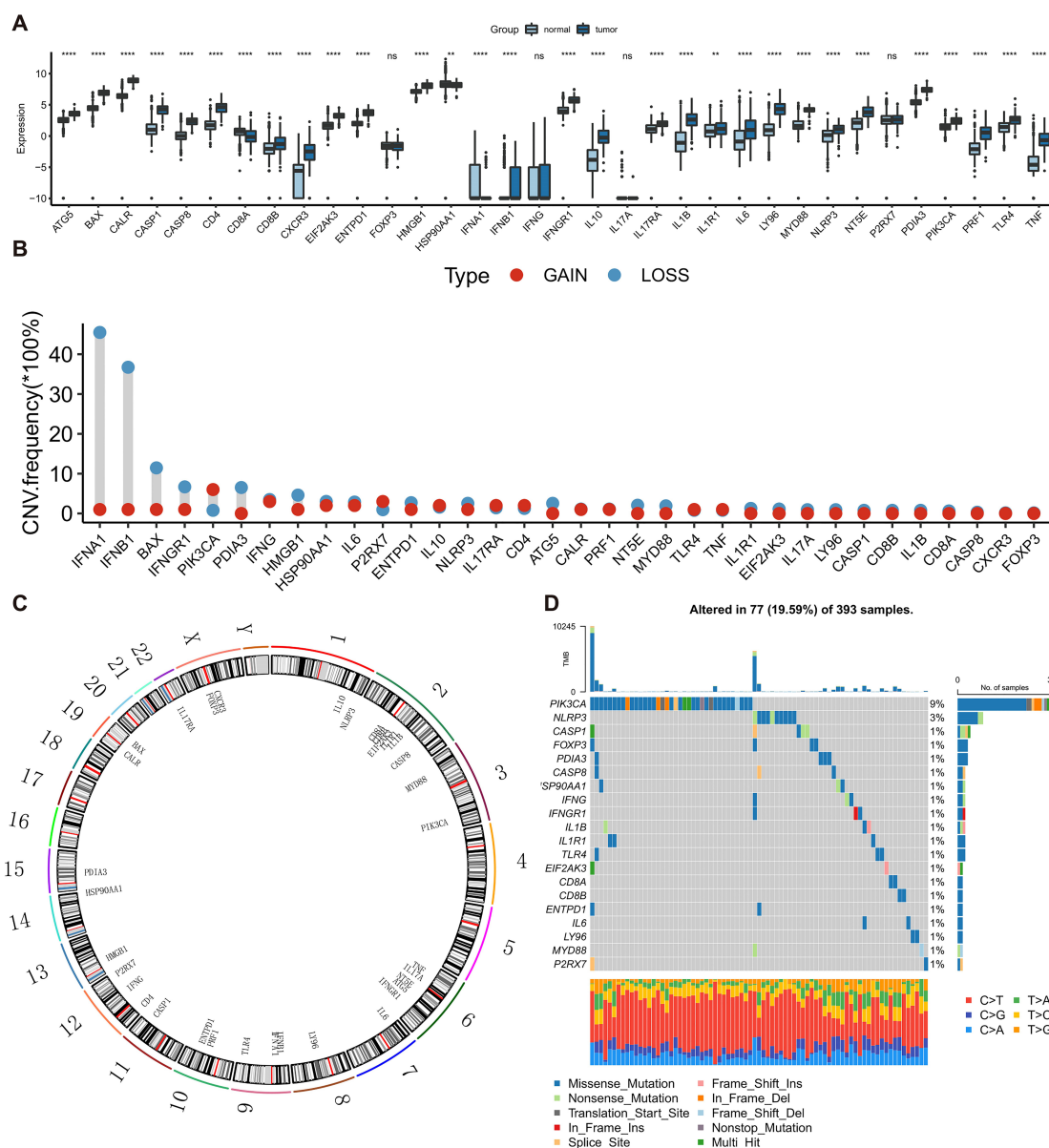


Fig. 1. Analysis of immunogenic cell death (ICD)-correlated genes in the glioblastoma (GBM) landscape. (A) Differential expressions of genes correlated with ICD in primary cancer samples and normal brain tissue. (B) Copy number variation (CNV) in genes related to ICD. (C) Chromosomal location of ICD-related genes and PPI network interactions. (D) Analysis of single nucleotide variation (SNV) alterations in ICD-correlated genes in the The Cancer Genome Atlas-glioblastoma (TCGA-GBM) cohort. ns $p > 0.05$, ** represents $p < 0.01$, and **** represents $p < 0.0001$. *HMGB1*, high mobility group box-1; *IFNB1*, interferon beta1; *FOXP3*, forkhead box protein 3; *IFNA1*, interferon-alpha1; *CD8A*, cluster of the differentiation 8 alpha; *NLRP3*, NLR-family pyrin domain-containing protein 3; *PIK3CA*, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha; *IFNGR1*, interferon gamma receptor 1; *HSP90AA1*, heat shock protein 90 alpha family class A member 1; *ATG5*, autophagy-related gene 5; *CALR*, calreticulin; *CXCR3*, C-X-C motif chemokine receptor 3; *EIF2AK3*, eukaryotic translation initiation factor 2 alpha kinase 3; *CASP1*, caspase-1; *CD4*, cluster of the differentiation 4; *P2RX7*, purinergic receptor P2X, ligand-gated ion channel 7; *TLR4*, toll-like receptor 4; *PDI3*, protein disulfide isomerase family A member 3; *NT5E*, ecto-5'-nucleotidase; *IL*, interleukin; *MYD88*, myeloid differentiation factor-88; *PRF1*, perforin; *ENTPD1*, ectonucleoside triphosphate diphosphohydrolase 1.

alytic subunit alpha (*PIK3CA*) had significant copy number gain, while *IFNA1*, interferon beta1 (*IFNB1*), *Bax*, and interferon gamma receptor 1 (*IFNGR1*) had extensive copy number loss (Fig. 1B). The chromosomal locations of all

ICD-related genes are shown in Fig. 1C. Subsequently, we analyzed variants in ICD-related genes among GBM patients. The findings revealed that approximately 19.59% (77/393) of TNBC patients had mutations in these genes.

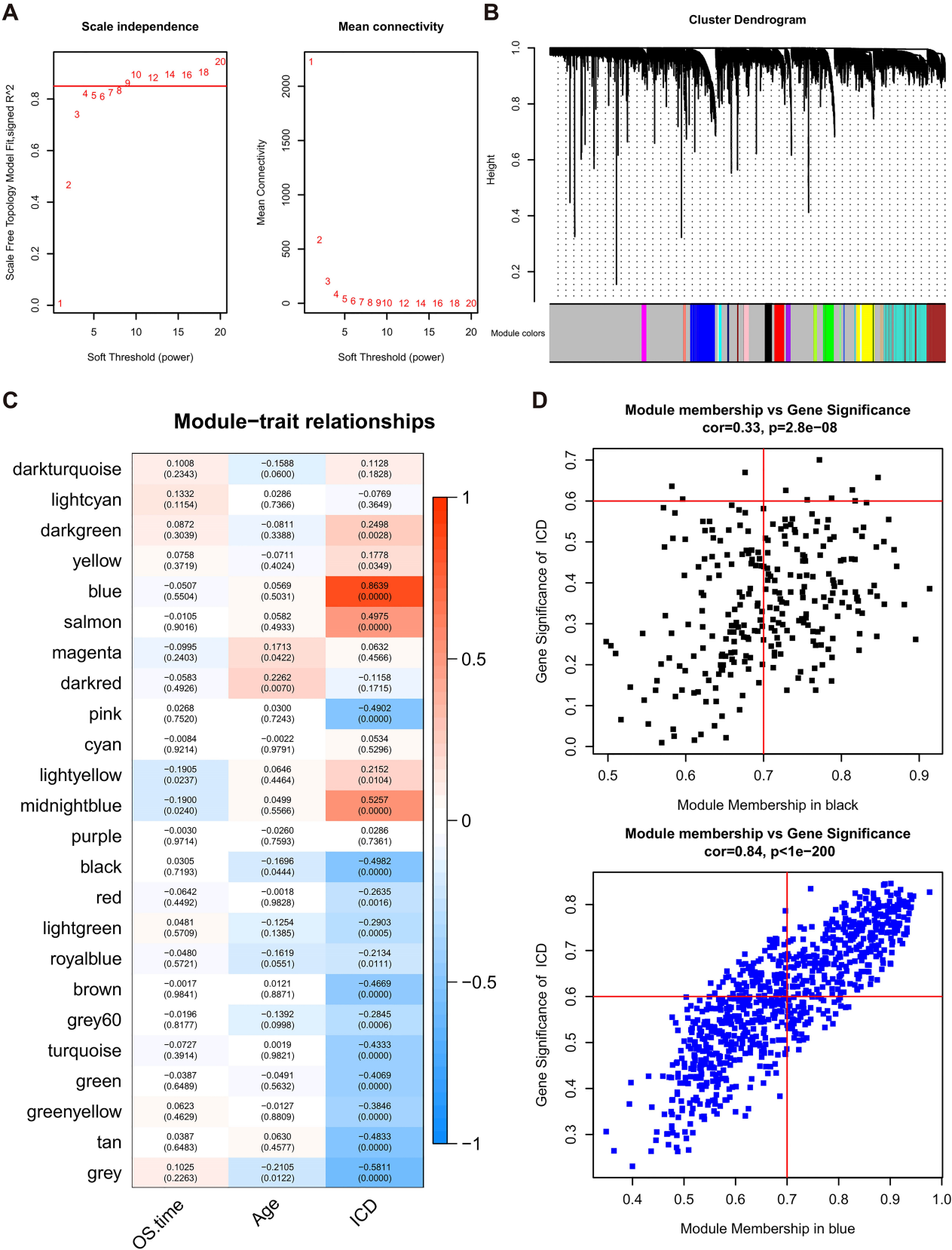


Fig. 2. Differences in the enrichment of ICD-related pathways. (A) Results under soft threshold screening. (B) Gene clustering and weighted gene co-expression network analysis (WGCNA) analysis were used to section different co-expressed modules. (C) Correlation between sample characteristics and 24 identified co-expression modules. (D) Results of core gene screening.

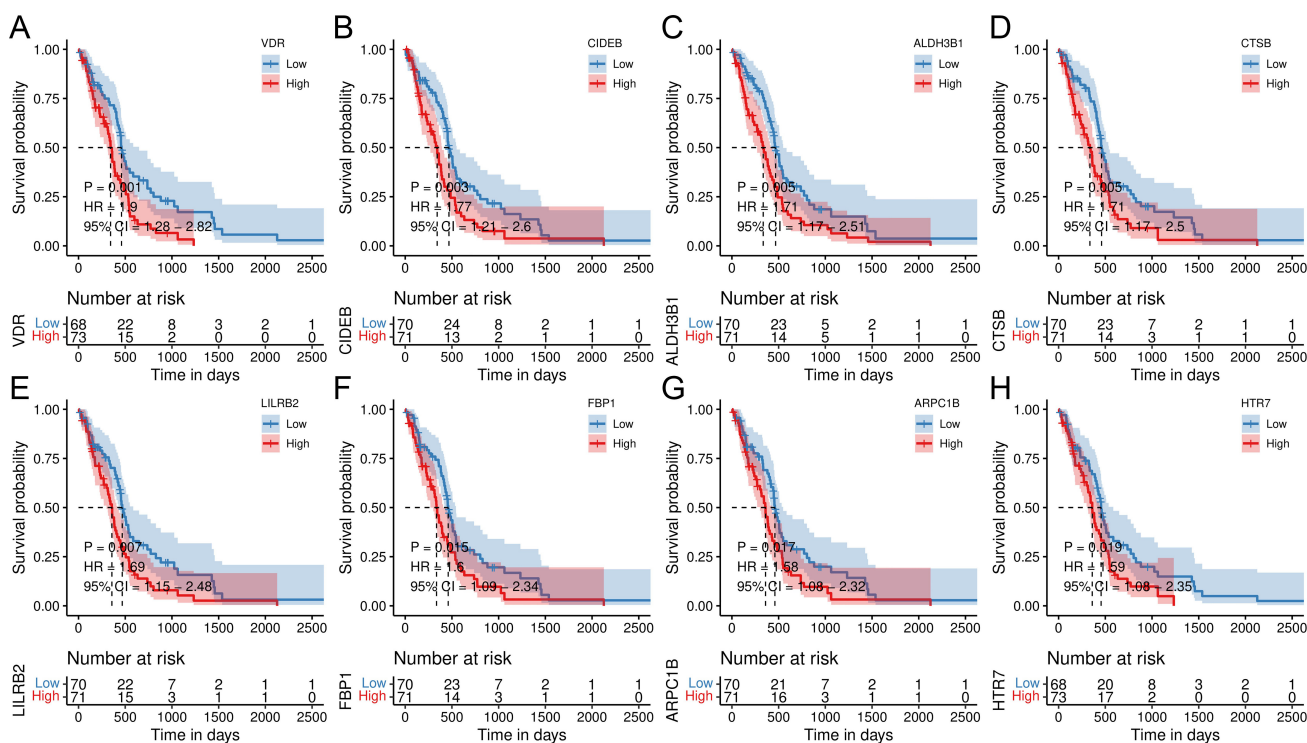


Fig. 3. Kaplan-Meier (K-M) analysis shows the survival curves of eight genes. (A) K-M curve of vitamin D receptor (*VDR*). (B) K-M curve of cell death-inducing DFF45-like effector B (*CIDEB*). (C) K-M curve of aldehyde dehydrogenase 3B1 (*ALDH3B1*). (D) K-M curve of Cathepsin B (*CTSB*). (E) K-M curve of lymphocyte immunoglobulin-like receptor B2 (*LILRB2*). (F) K-M curve of fructose 1,6-bisphosphatase 1 (*FBP1*). (G) K-M curve of actin related protein 2/3 complex subunit 1B (*ARPC1B*). (H) K-M curve of serotonin receptor 7 gene (*HTR7*). HR, hazard ratio; CI, confidence interval.

Among the top 20 mutations in ICD-related genes, *PIK3CA* displayed the highest mutation frequency (9%), followed by NLR-family pyrin domain-containing protein 3 (*NLRP3*) with a mutation frequency of approximately 3% (Fig. 1D). These findings indicated that ICD-correlated genes played a pivotal role in the development of GBM.

Screening ICD-Related Core Modules Based on WGCNA

The ICD-related pathway scores for each GBM sample were calculated by the ssGSEA method and subsequently included as sample characteristics in further analysis. WGCNA was performed on the candidate genes using the TCGA-GBM cohort as the reference. According to the scale-free nature of the network, the optimal soft threshold power (denoted as $r(\beta)$) was estimated to be 9 (Fig. 2A). The cluster diagram in Fig. 2B displayed the co-expressed genes identified from the modules. Based on the Pearson correlation coefficient between each module, the sample characteristics showed that the “black” and “blue” modules were the most negatively and positively related to ICD in tumors, respectively (Fig. 2C). The hub genes in these two modules with $GS > 0.6$ and $MM > 0.7$ were selected for further analysis (Fig. 2D), and this step yielded a total of 319 genes.

Subsequently, the genes obtained in above step were subjected to GO and KEGG enrichment analyses (Supplementary Fig. 1). The KEGG analysis showed that enriched pathways were related to body immunity, such as *Staphylococcus aureus* infection and leishmaniasis. The GO analysis demonstrated enrichment in immune-related functions, including myeloid leukocyte activation and leukocyte-mediated immunity.

Development of an ICD-Correlated Prognosis Signature for GBM Patients

To screen prognostic factors by performing univariate Cox regression analysis, we utilized the 319 core genes obtained from the previous step. Under the criterion of $p < 0.05$, 23 significant prognostic factors were filtered. Using these 23 prognostic factors, survival analysis was performed in the TCGA-GBM cohort. We observed significant survival differences in patients grouped by the expression levels of these factors ($p < 0.05$). Among these factors, there were eight prognostic factors exhibiting the most pronounced survival differences between different groups (Fig. 3).

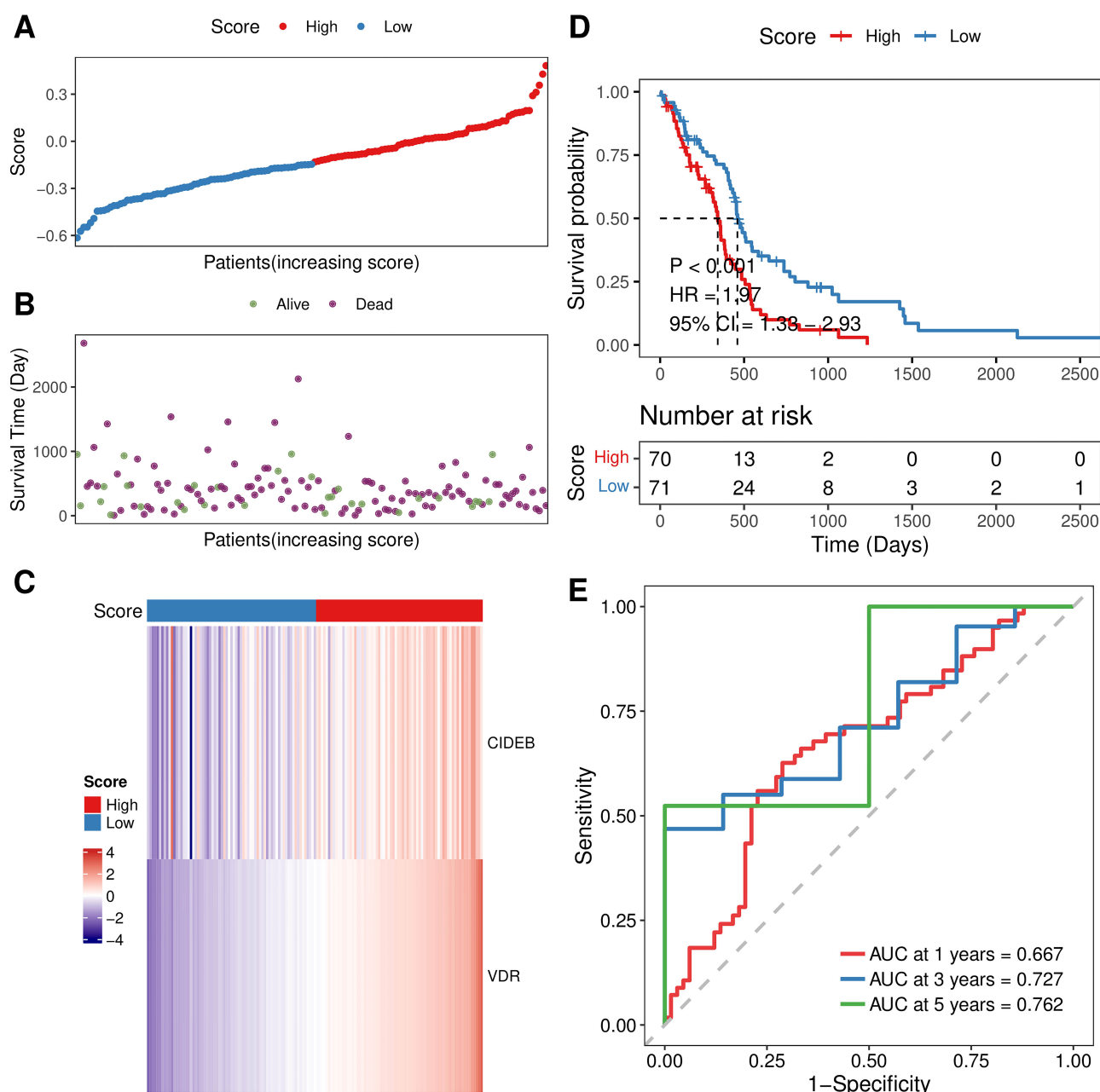


Fig. 4. Construction of ICD-related prognosis risk model based on the TCGA-GBM cohort. (A) Score distribution of each sample in the training set cohort. (B) Sample survival in the training set cohort. (C) Expression heat map in high and low score groups of signature. (D) Survival of high and low score groups. (E) Time-dependent receiver operating characteristic (ROC) curve shows the 1-, 3-, and 5-year area under the ROC curve (AUC).

Evaluation and Verification of the ICD-Related Prognostic Risk Model

Subsequent LASSO-COX analysis of these 23 prognostic factors identified vitamin D receptor (*VDR*) and cell death-inducing DFF45-like effector B (*CIDEB*) as two independent prognostic factors for GBM patients (Supplementary Fig. 2). The regression coefficients for *VDR* and *CIDEB* were 0.022 and 0.127, respectively. Using the formula (score = *CIDEB* × 0.022 + *VDR* × 0.127), each patient was assigned with a risk score. According to the me-

dian score, GBM patients were divided into high score and low score groups. K-M curve analysis demonstrated that high-score patients had a poor prognosis. Time-dependent ROC analysis revealed area under the ROC curve (AUC) values of 0.667 at 1 year, 0.727 at 3 years, and 0.762 at 5 years, which demonstrated an accurate prediction of the model (Fig. 4A-E).

In the independent validation dataset, GBM patient samples were divided into high and low risk score groups by the median risk score. Consistent with the results of the

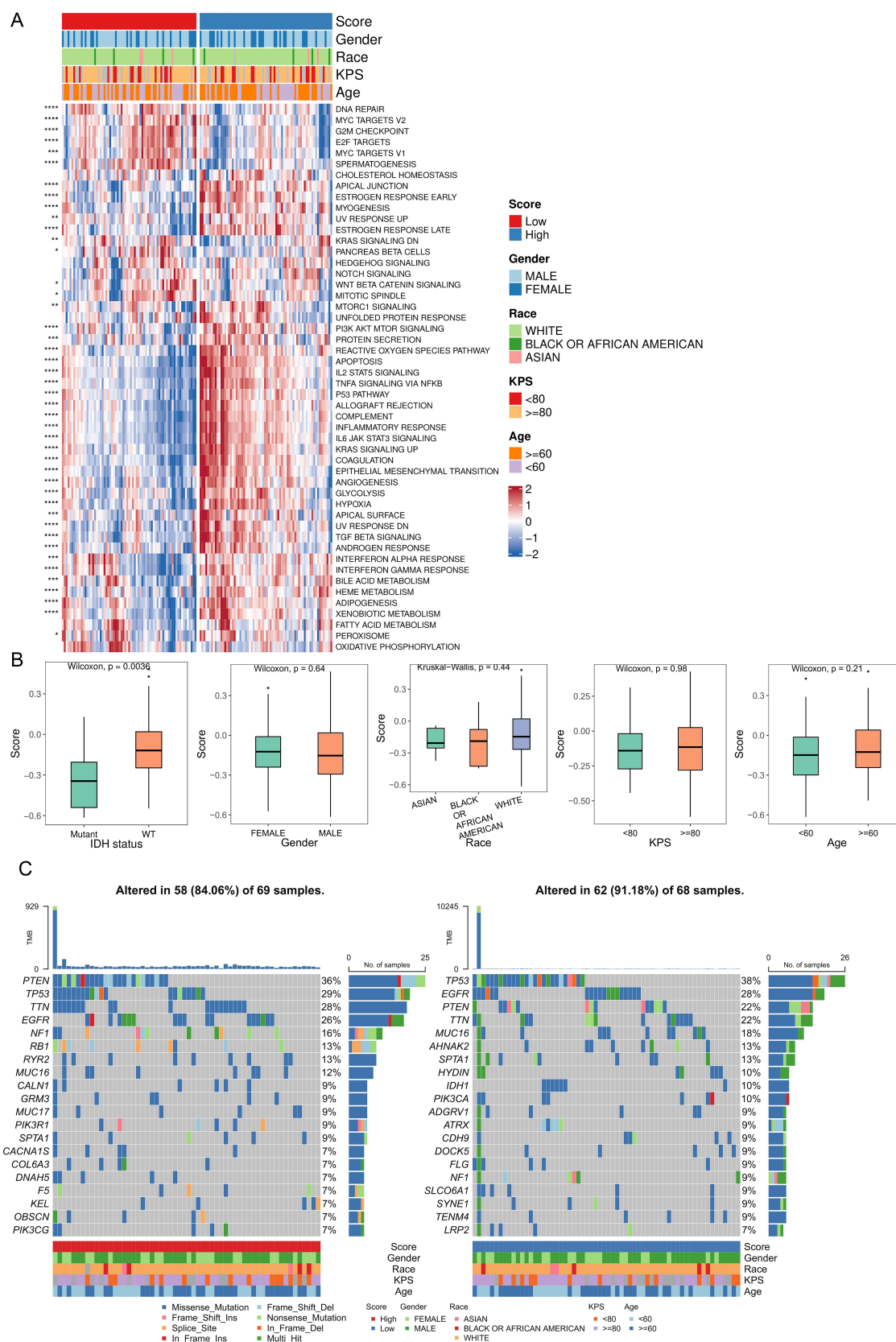


Fig. 5. Molecular features of the ICD-related prognostic risk model. (A) HALLMARK pathway enrichment score between groups. (B) Risk score of different clinical features in the training set. (C) Top 20 somatic mutant genes with the most variants in the high- and low-risk groups (the high score group at left, the low score group at right). KPS, Karnofsky performance status. * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$, and **** represents $p < 0.0001$.

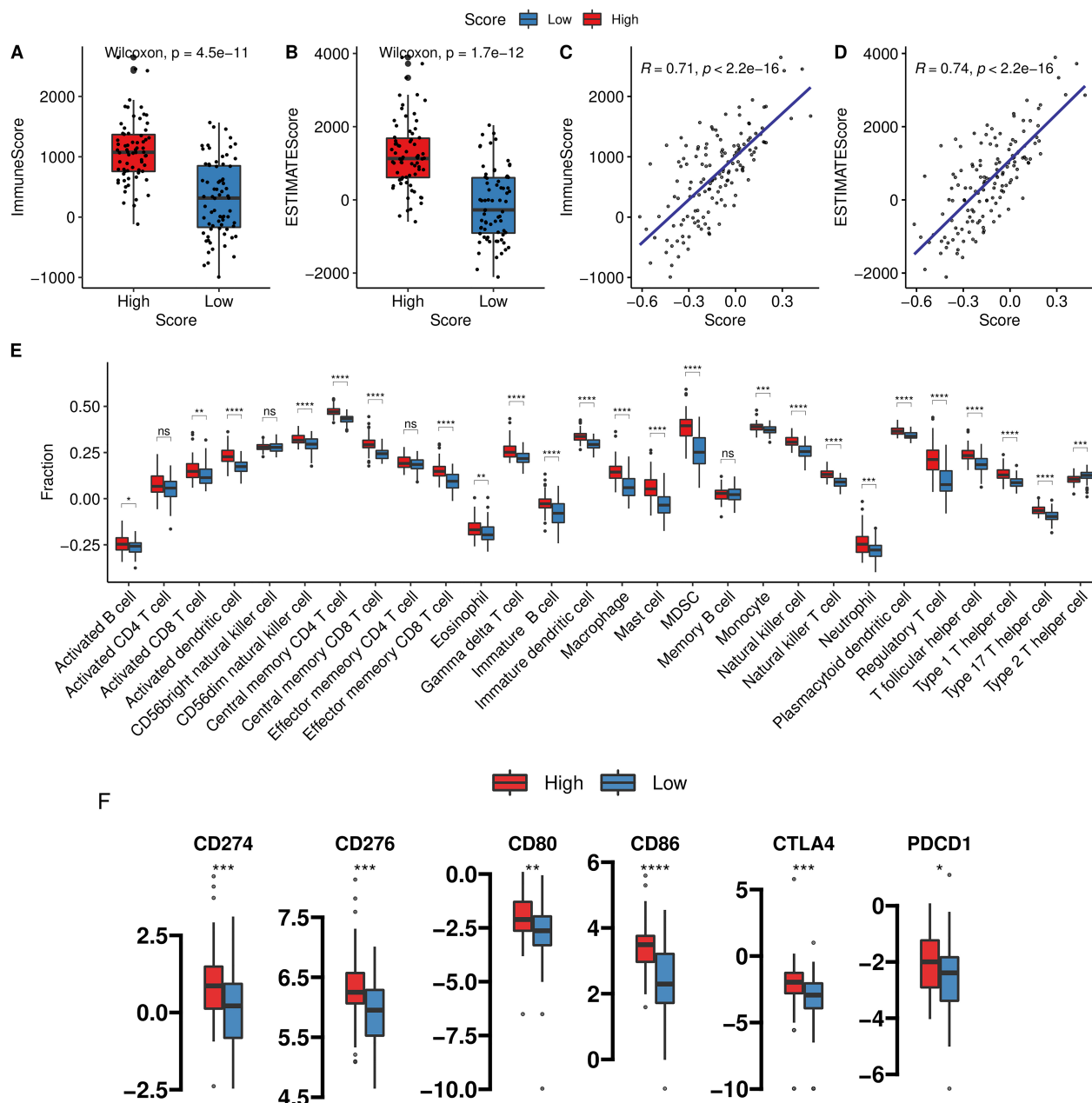


Fig. 6. ICD risk score characterized differential tumor immune microenvironment features. (A) Immune scores of patients classified by high and low scores. (B) ESTIMATE scores of patients classified by high and low scores. (C) Spearman correlation between immunity score and ICD risk score. (D) Spearman correlation between ESTIMATE score and ICD risk score. (E) Comparison of immune infiltration in high-score and low-score patients. (F) Analysis on the expression differences of immune checkpoints of the two risk groups. * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$, **** represents $p < 0.0001$, and ns represents no significant. R, correlation; CD, cluster of the differentiation.

training cohort, high-risk patients exhibited shorter survival time than low-score patients ($p < 0.05$, **Supplementary Fig. 3**). The AUC values in this cohort were 0.675 at 1 year and 0.836 at 3 years, further confirming the predictive capability of our model.

Multivariate Cox analysis was performed to assess whether the score was an independent prognostic factor for the OS of GBM. The univariate Cox analysis revealed that

the score in the training set data showed a significant correlation with OS. After adjustment, the score was confirmed by the multivariate Cox analysis as an independent predictor of OS (**Supplementary Table 1**). These results were further supported using an independent validation cohort. The results of the univariate and multivariate Cox analysis are shown in **Supplementary Table 2**.

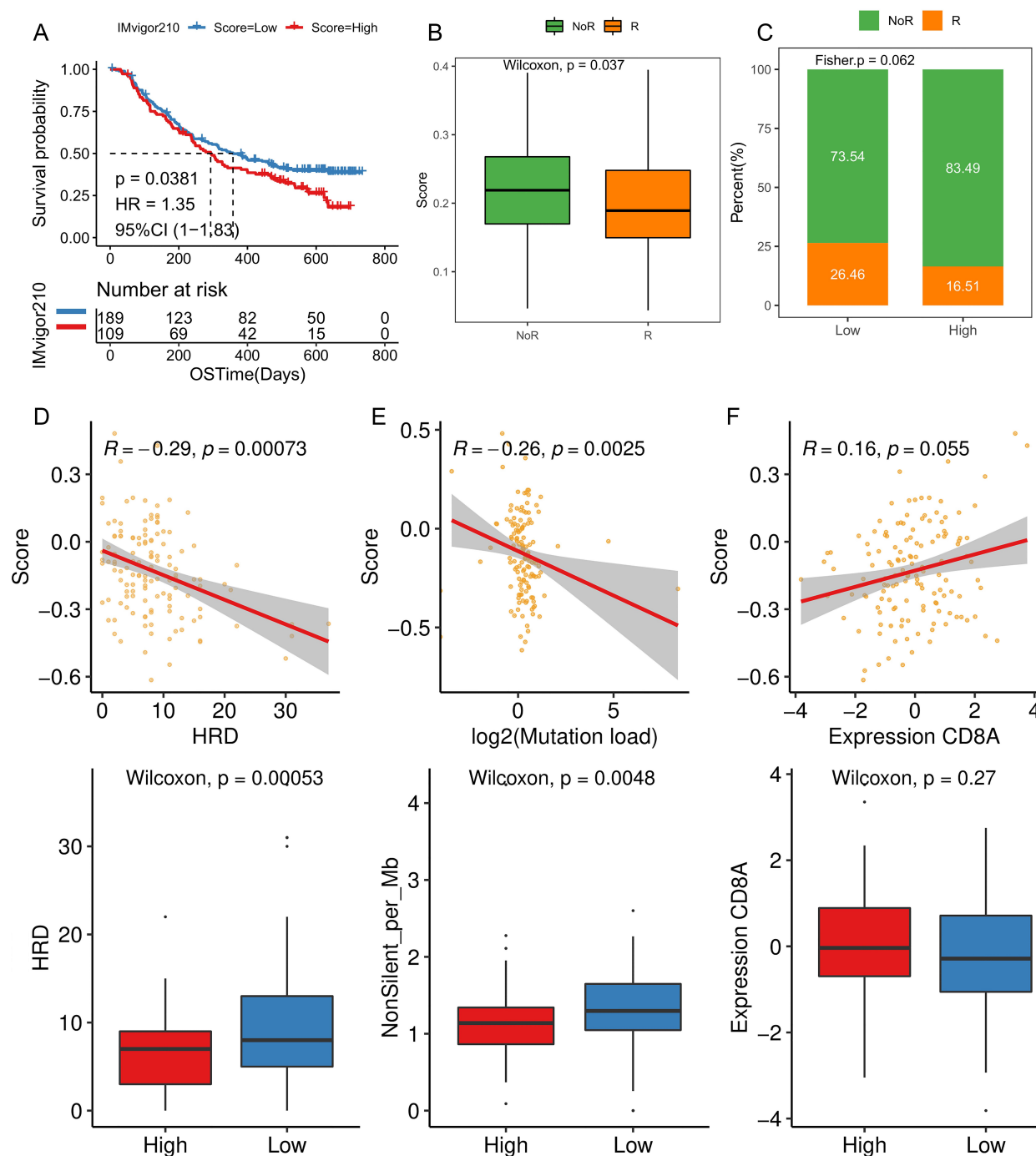


Fig. 7. Efficacy of ICD-related prognostic risk score in immunotherapy. (A) Survival by high and low groups based on the immunotherapy cohort IMvigor210. (B) Risk scores for patients with different immune tolerance. (C) Immune tolerance of patients in the two groups. (D) Correlation between ICD-related risk score and HRD. (E) Correlation between ICD-related risk score and mutational load. (F) Correlation between ICD-related risk score and cluster of the differentiation 8 alpha (CD8A). HRD, homologous recombination deficiency.

Molecular Characteristics Related to the ICD-Related Prognostic Risk Model

The hallmark pathway scores for patients in the training set cohort were computed by ssGSEA (Fig. 5A). The analysis revealed significant enrichment differences across

multiple pathways between different risk groups, indicating their close association with immune activities within the body ($p < 0.05$). Furthermore, the correlation study between the clinical features of GBM and model score showed higher risk scores of patients with wild-type Isocitrate de-

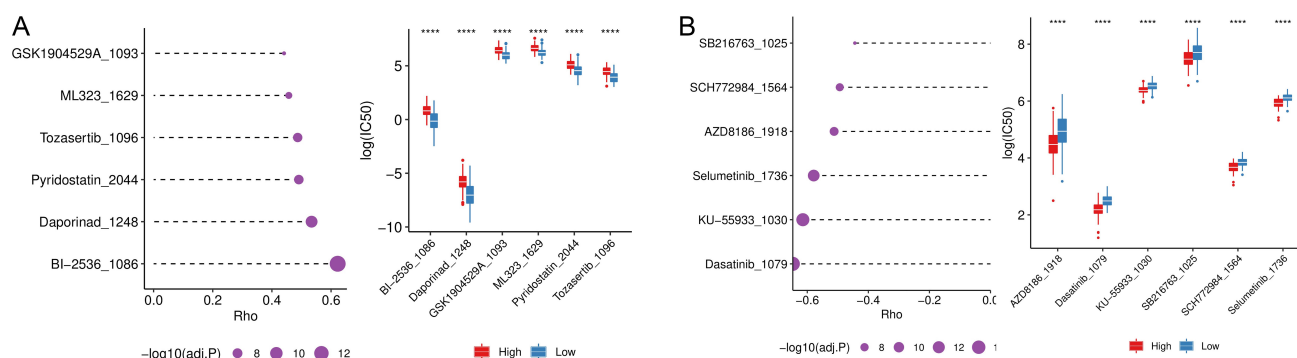


Fig. 8. Prediction of drug sensitivity based on ICD-related prognostic risk scores. (A) The top 6 drugs showing the strongest positive relation with the risk score and drug sensitivity differences among patients in different risk groups. (B) The top 6 drugs showing the strongest negative relation with risk scores and drug sensitivity differences among patients in different risk groups. **** represents $p < 0.0001$.

hydrogenase (IDH) (Fig. 5B). Such a finding was consistent with previous studies that GBM patients without IDH mutations tended to have a poorer prognosis. Additionally, we conducted a comparison of somatic mutations between the two risk groups, and the results are presented in Fig. 5C.

Dissection of Tumor Microenvironment Heterogeneity Using the ICD-Related Prognostic Risk Score

Furthermore, Estimation of STromal and Immune cells in Malignant Tumor tissues using Expression data (ESTIMATE) results showed that high-score patients in the GBM cohort exhibited significantly higher immune scores than low-score patients ($p < 0.05$; Fig. 6A,C). Importantly, patients' ESTIMATE scores were significantly positively correlated with the previously determined score ($p < 0.05$; Fig. 6B,D). These findings demonstrated that high-score patients tended to have “hot” tumors characterized by increased immune activity. Additionally, immune cell infiltration in the two risk groups was quantified (Fig. 6E). Substantial differences in the number of activated CD8⁺ T cells, activated B cells, and other cell types were observed between the two groups, with the high-risk group having greater immune infiltration. Analysis on the expression differences of immune checkpoints of the two risk groups ($p < 0.05$; Fig. 6F) showed higher expression of immune checkpoints in the high-score group, indicating that this group could benefit more from taking molecular targeted therapy or immune checkpoint inhibitor therapy.

Accuracy of the ICD-Related Prognostic Risk Model for Evaluating the Immunotherapy Response

Utilizing the IMvigor210 data of immunotherapy cohort, significant prognostic differences between the high- and low- score groups were observed ($p < 0.05$). Patients in the high score group exhibited an unfavorable prognosis and less active response to immunotherapy (Fig. 7A–C). These findings strongly indicated the presence of immune

tolerance in the high-score patients, which may potentially limit the effectiveness of immunotherapeutic interventions.

Furthermore, the relationship between two crucial factors (homologous recombination deficiency (HRD) and mutation load) and the risk score was explored. Our analysis indicated that both HRD and mutation load were relatively low in the high risk score group, presenting a negative correlation with the model score (Fig. 7D–F). These findings suggested an association between the model score and genomic stability, indicating the potential involvement of immune-related pathways in modulating HRD and mutation load in GBM patients.

The ICD-Related Prognostic Risk Score Guides Chemotherapy Strategy Selection

To predict patients' sensitivity to drugs stored in the Genomics of Drug Sensitivity in Cancer 2 (GDSC2) database, we employed the R package oncoPredict with expression profiling. The Spearman correlation coefficient was computed to determine the relationship between the log half-maximal inhibitory concentration (IC₅₀) and the score for each drug. Subsequently, we selected the top 6 drugs based on their positive and negative correlations with the risk score, according to descending p -value. The distribution differences and correlation coefficients of the log IC₅₀ values for each drug between the high- and low-score groups are shown in Fig. 8.

Discussion

GBM is a malignant primary brain tumor characterized by aggressive behaviors, infiltrative growth, and limited treatment options [22]. Despite considerable efforts to advance the clinical treatments for GBM, the treatment for this cancer remains a challenge in the field of oncology. ICD stimulates immune response against tumor cells, therefore exploring the potential of ICD might be an effective approach for developing novel cancer immunotherapies to

promote immune recognition and eradicate cancer cells [23,24]. However, the correlation between ICD and GBM is still unclear. Using the TCGA-GBM cohort, WGCNA identified a set of 23 ICD-related genes. Subsequently, LASSO regression was performed and screened two important prognostic signatures to construct a risk model for GBM. Notably, this risk model demonstrated a great significance in OS prediction and had the potential to be used as an independent prognosis indicator, specifically for GBM patients.

We constructed an ICD-correlated prognostic signature whose top five genes were *VDR*, *CIDEB*, aldehyde dehydrogenase 3B1 (*ALDH3B1*), Cathepsin B (*CTSB*), and lymphocyte immunoglobulin-like receptor B2 (*LILRB2*). *VDR* has been extensively studied in GBM [25–27]. Recent research found that it may regulate GBM through the VDR/B-cell Lymphoma 6 (*BCL6*)/protein 53 (p53) axis [28]. *ALDH3B1*, by inducing cell cycle arrest and epithelial-mesenchymal transition, can affect GBM cell proliferation and migration [29]. *CTSB* could regulate the expression of tumor sphere formation and stemness markers in GBM, which might be achieved by the targeted negative regulation of *CTSB* via miR-140 [30]. *LILRB2* promotes GBM tumor progression by facilitating the formation and expansion of myeloid-derived suppressor cells [31]. *CIDEB* is mainly involved in the biogenesis of very low-density lipoprotein transport vesicles [32], but its role in GBM has not been explored.

In recent years, infiltrating immune cells have gained significant attention in tumor researches [33,34]. In our study, we observed significant changes in the immune microenvironment between the low- and high-score groups. In particular, the infiltration ratio of central memory cluster of the differentiation 4 (CD4⁺) T cells, central memory CD8⁺ T cells, CD56dim natural killer cells, activated dendritic cells, and other cells was significantly lower in the high score group. Conversely, the infiltration ratio of type 2 T helper cells was significantly higher. Among the nontumor populations in the GBM tumor microenvironment, microglia/macrophages were the most abundant, but their proportion was significantly reduced in the high score group, suggesting the need for further investigation of the underlying mechanism.

Conclusions

In this study, an ICD-correlated prognostic signature has been confirmed as a significant predictor of the prognosis of GBM patients. Furthermore, we observed distinctively different immune states between the low-risk and high-risk groups. The current findings highlight the potential of using the model as an effective biomarker for GBM prognosis in future clinical applications.

Availability of Data and Materials

The datasets supporting the findings of this study are available in the GEO (<https://www.ncbi.nlm.nih.gov/geo/>). For the discovery cohort database, further inquiries can be directed to the corresponding author.

Author Contributions

LC: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Software, Validation, Visualization, Writing-original draft. RZ: Formal analysis, Visualization, Writing-review & editing. QJ: Formal analysis, Writing-original draft. XW: Formal analysis, Funding acquisition, Writing-original draft. BZ: Data curation, Formal analysis, Writing-original draft. XF: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Project administration, Writing-review & editing. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

We would like to acknowledge the support of the Natural Science Foundation of Tianjin and The National Natural Science Foundation of China.

Funding

This research was supported by the Natural Science Foundation of Tianjin (21JCQNJC00130) and the National Natural Science Foundation of China (82072212).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.23812/j.biol.regul.homeost.agents.20243806.419>.

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