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An Immunogenic Cell Death-Related Classification Predicts Prognosis and Response to Immunotherapy in Glioblastoma

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Abstract

To investigate the immunogenic Cell Death gene's potential mechanism and prognostic value in glioblastoma. Information on GBM samples from The Cancer Genome Atlas database was downloaded, ICD genes were obtained, genotyping, integrated bioinformatics to verify the prognostic value of genotyping, and finally, prognostic model construction. Two subtypes associated with the ICD gene were obtained by consensus clustering, and the high ICD subtype (risk) group was associated with poor prognosis, high mutations in the PTEN gene, high stromal score, and high immune score. We also constructed a new classification system for GBM based on ICD characteristics. This study is the first to use immunogenic cell death genes for genotyping and successfully build a prognostic model.

Keywords

Immunogenic Cell Death, Prognostic Value, Classification, Immunotherapy, Glioblastoma

1. Introduction

Glioblastoma (GBM) is the most common and lethal primary tumor in the human brain [1]. It is also the glioma with the worst prognosis, with a median survival of approximately 12 - 15 months [2]. The primary treatment modalities currently available are surgery, chemotherapy, anti-angiogenic therapy, radiation therapy, immunotherapy, and tumor electric field therapy [2]. Despite advances in the treatment of GBM, exciting outcomes are usually not observed, and patients diagnosed with GBM typically have a poor prognosis and poor

quality of life as the disease progresses. It has been found to have a heterogeneous population of genetically unstable and highly infiltrative cells, ultimately leading to drug resistance development [2] [3] [4] [5]. Statistical reports based on the U.S. Central Brain Tumor Registry for 2021 and 2020 found an increase in the incidence of 0.07 from 2014-2018 compared to 2013-2017 [6] [7]. With the incidence rate increasing year by year, the underlying pathogenic mechanism of GBM is unclear. Therefore, research to explore the underlying mechanisms is urgent and has significant practical implications for improving patient prognosis, improving the quality of patient survival, and reducing the socioeconomic burden.

Immunogenic cell death (ICD) is a specific variant of Regulated cell death (RCD) driven by stress and can induce adaptive immunity against dead cell antigens. Immunogenic cell death is not a recently proposed mode of death; it was presented as early as 2005 by Noelia *et al.* and in 2013 by Kroemer *et al.*, who hypothesized that ICD and its destruction by pathogens also play an essential role in antiviral immune responses and further discussed the characteristics of ICD, immune effect-related ICD pathways [8] [9]. Three months ago, Kroemer *et al.* [10] found that when cells exhibit sufficient antigenicity, their death in the presence of infected malignant cells can peak the adaptive immune response, which is executed by cytotoxic T lymphocytes and triggers immune memory, suggesting a crucial role of ICD in immune surveillance [10]. The discovery of ICD has facilitated the development of new therapeutic agents, personalized treatment, and therapeutic strategies.

In this study, we explored the potential mechanism and typing of immunogenic cell death genes in glioblastoma and the relationship with tumor mutation burden, tumor microenvironment, immune checkpoints, HLA genes, and copper death genes by integrating bioinformatics approach, and prognostic model construction by finding prognosis-related genes, which ultimately mediates sound immunotherapeutic effects.

2. Materials and Methods

2.1. Data Download and ICD Gene Acquisition, Differential Analysis, and Genotyping

Download Counts data, clinical information, and tumor mutation data of glioblastoma from the TCGA database, then perform gene ID conversion and tumor mutation burden calculation. Thirty-four ICD genes were identified by Garg *et al.* [11], and gene expression matrices were also obtained, followed by differential analysis with the screening criteria of $|\log FC| \geq 0.5$ and P-value < 0.05. Protein-protein interaction (PPI) network construction was also performed using the STRING (https://string-db.org/, version: 11.5) online web tool, followed by genotyping using the "ConsensusClusterPlus" package and heat map for gene classification, which was finally used to classify them into two groups of high and low risk [12].

2.2. Genotyping for Survival Analysis, Differential Analysis

Matrix data were combined with survival information for survival analysis of genotypes, and genotypes were analyzed for differences with |logFC| ≥ 2 and FDR < 0.05. The study used the "wilcoxTest" function in R. GO, and KEGG was screened for relevant enriched pathways with FDR < 0.05.

2.3. Fractal Difference Analysis of Tumor Mutation Burden and Immune Infiltration, Immune Cell Assessment

Tumor mutation burden was explored by plotting waterfall plots using the "maftools" package. Immune infiltration was assessed using the "estimate" expression matrix. Differences in immune infiltration were also compared between different analyses of the ICD (using the "stat_compare_means" function in R The immune cell assessment was performed using the CIBERSORT (https://cibersortx.stanford.edu/) online website, followed by correlation analysis and difference analysis of immune cells [13] [14].

2.4. Genotyping and Correlation Analysis of HLA, Immune Checkpoint, and Copper Death Genes

Twenty-four HLA genes, 48 immune checkpoint genes, and 13 copper death genes were obtained from the literature and subsequently analyzed for differences using the "stat_compare_means" function in R [15] [16].

2.5. Genotyping and Correlation Analysis of HLA, Immune Checkpoint, and Copper Death Genes

First, the matrix data of the CGGA database mRNAseq-325 was downloaded, and then the primary glioblastoma in it was screened for ICD-related genes. Secondly, the ICD gene matrix in the TCGA database and the ICD gene matrix in the CGGA database were intersected to obtain the ICD genes common to both. Again, genes with significant prognostic effects were screened using one-way COX analysis; finally, predictive models were constructed using LASSO regression.

2.6. Integrated Bioinformatics Analysis of Prognostic Models

For prognostic model genes survival analysis, risk mapping, independent prognostic factor analysis, drug prediction (using the "pRRophetic" package), immune cell correlation analysis (based on TIMER), immune function difference analysis, immunotherapy using TIDE (Tumor Immune Dysfunction and E exclusion, http://tide.dfci.harvard.edu/) online web tool, and prognostic value of the model using ROC curves.

3. Results

3.1. Data Collation and Differential Analysis of ICD Genes, Genotyping

A total of 174 GBM samples (5 normal and 169 tumor samples) were downloaded from the TCGA database, and an additional 12,696 mutated genes were

obtained. Differential analysis of 34 ICD genes yielded 20 differentially expressed ICD genes, and the heat map showed their differential expression in tumor and normal tissues was significant (Figure 1A). PPI showed a potential association between differential ICD genes (Figure 1C). Genotyping showed they were most appropriate when typed as 2 (Figure 1B). After that, the heat map visualization of gene typing showed the association between 34 ICD genes and types 1 and 2 (Figure 1D).

3.2. Genotyping for Survival Analysis, Differential Analysis, and Enrichment Analysis

Survival analysis of typing showed that the prognosis of the high-risk group of ICD was significantly lower than that of the low-risk group of ICD ($P < 0.05$) (Figure 2A). A total of 258 differential genes (including 232 up-regulated genes and 26 down-regulated genes) were obtained by differential analysis after genotyping (Figure 2B & Figure 2C). The GO terminology analysis found that the differential genes were mainly enriched in immune-related pathways (Figure 3A). KEGG enrichment analysis showed that they were enriched in multiple tumor-related pathways. E.g., PI3K-Akt signaling pathway, TNF signaling pathway, NF–kappa B signaling pathway, and JAK-STAT signaling pathway (Figure 3B).

3.3. Genotyping for Tumor Mutation Burden and Immune Infiltration Assessment

Tumor mutation burden in the high and low-risk groups of ICD showed higher mutation rates in the top 5 highly mutated genes, PTEN in the high-risk group and TP53, EGFR, TTN, and MUC16 in the low-risk group (Figure 3D & Figure 3E). The Estimate assessment found higher immune scores and stromal scores but lower tumor purity in the high risk ($P < 0.05$) (Figure 3C). The CIBERSORT immune cell assessment found a negative correlation between Monocytes and Macrophages M0 and a positive correlation between T cells gamma delta and Plasma cells (Figure 4B). Differential expression of B cells naïve, B cells memory, T cells CD4 memory activated, T cells gamma delta, and Dendritic cells resting was found in high and low-risk groups (Figure 4C).

3.4. Relationship between Genotyping and HLA Genes, Immune Checkpoint, and Copper Death Genes

A total of 22 HLA genes were found to be different ($P < 0.05$) (Figure 5A), 30 immune checkpoint genes were different ($P < 0.05$) (Figure 5B), and eight copper death genes were different ($P < 0.05$) in the high and low-risk groups (Figure 5C).

3.5. Construction of Prognostic Models

A total of 30 ICD-related genes were obtained by taking the intersection of the two data sets, seven prognosis-related genes were obtained by one-way Cox

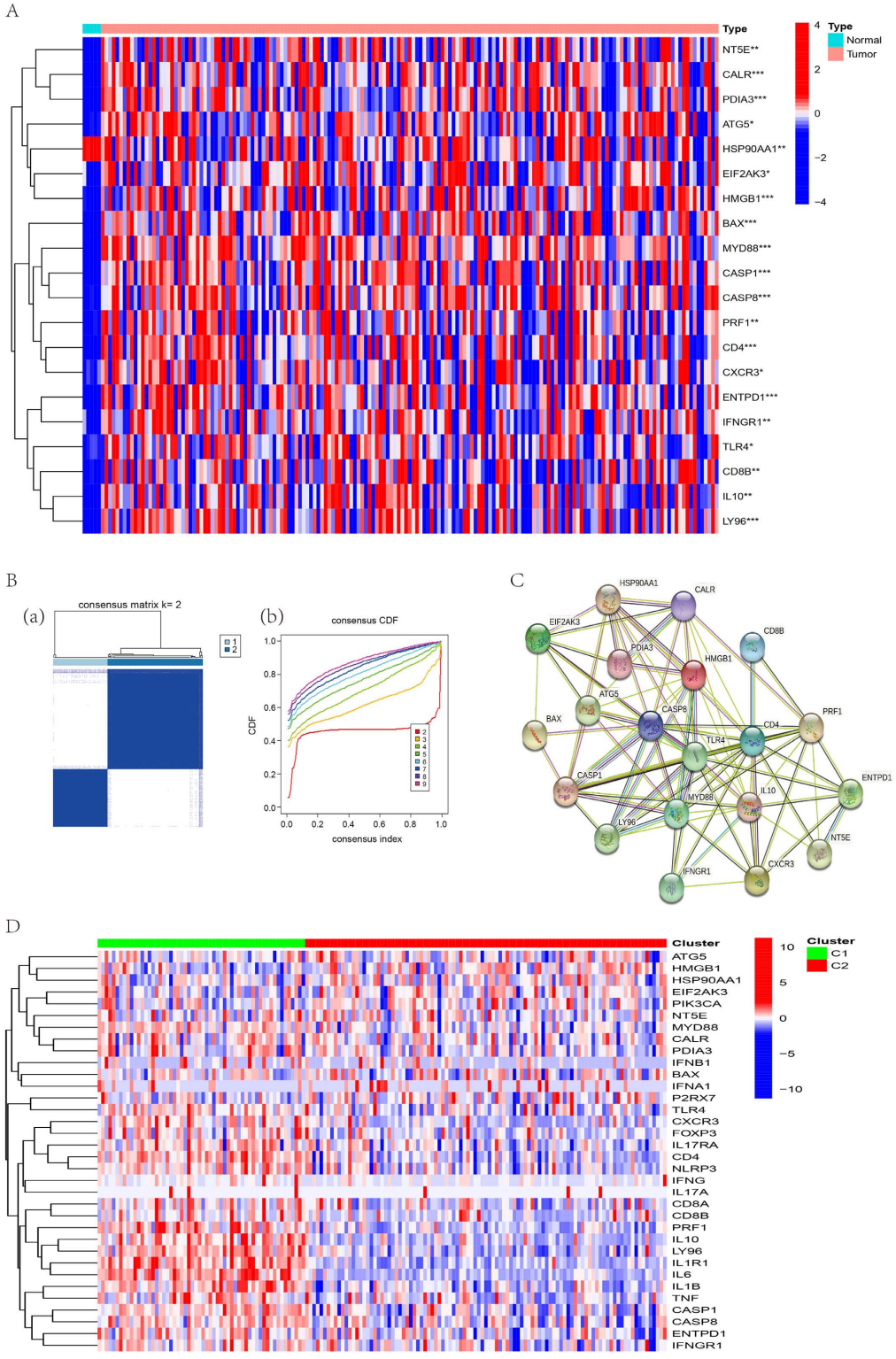

Figure 1. Screening process of ICD genes. A. Heat map of difference analysis between TCGA tumor group and normal values; blue represents low expression, and red represents high expression. B. Genotyping results based on consensus clustering. (a) Representative typing of 2 with approximately less typing crossover, representing that its classification as type 2 is highly desirable; (b) Consensus clustering shows the relative change in the area under the cumulative distribution function (CDF) curve for k = 2 to 10. C. PPI results for differential ICD genes. D. Heat map of differences when genes are classified as type 2. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

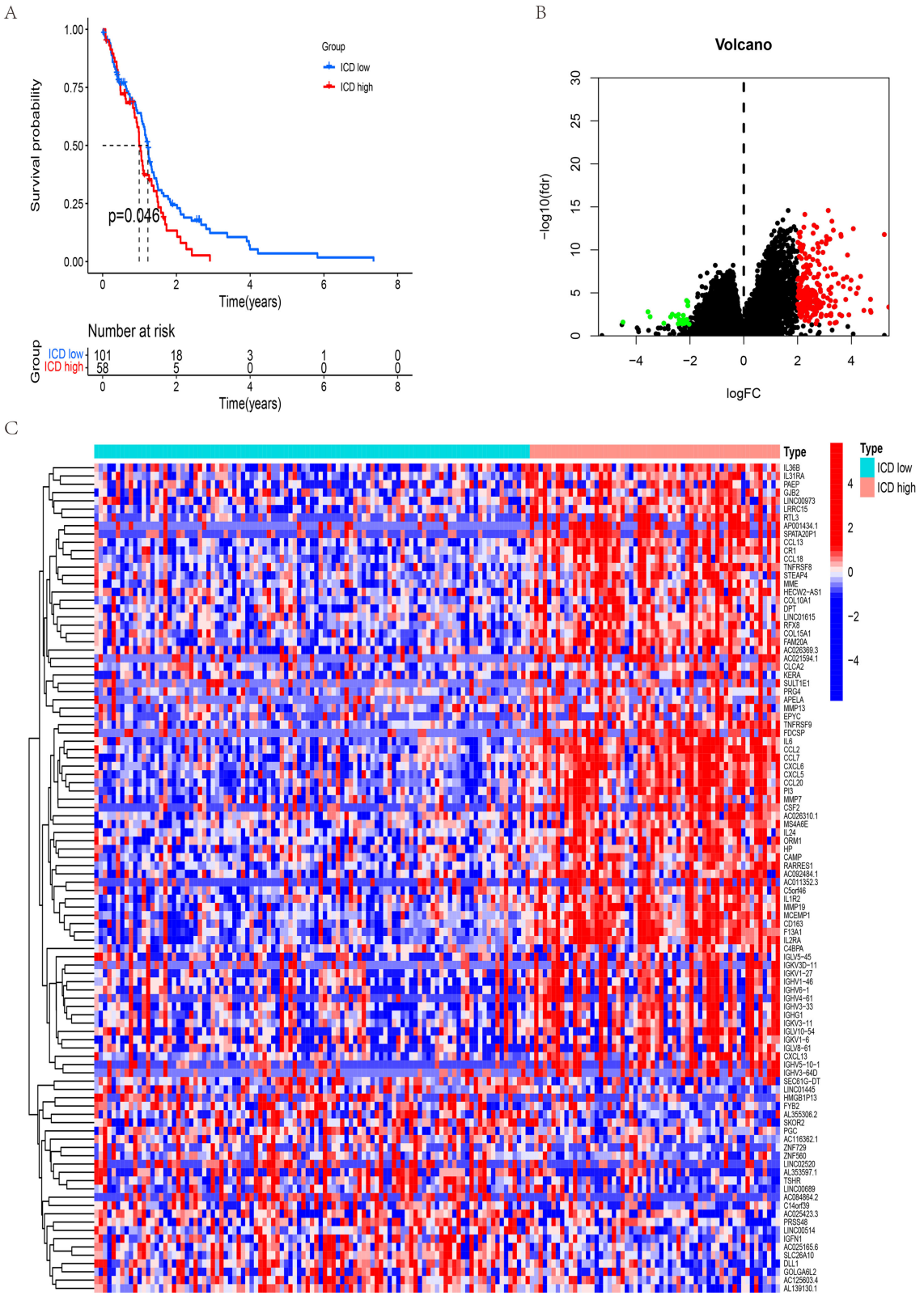

Figure 2. Survival analysis of genotyping, differential analysis results. A. Differential analysis of genotyping, red represents ICD high-risk group, blue represents ICD low-risk group, X-axis represents survival time, Y-axis represents survival rate; B. Volcano plot of differential analysis, red represents upregulated genes, green represents downregulated genes, and black represents stable expression genes; C. Differential analysis between ICD high and low-risk groups.

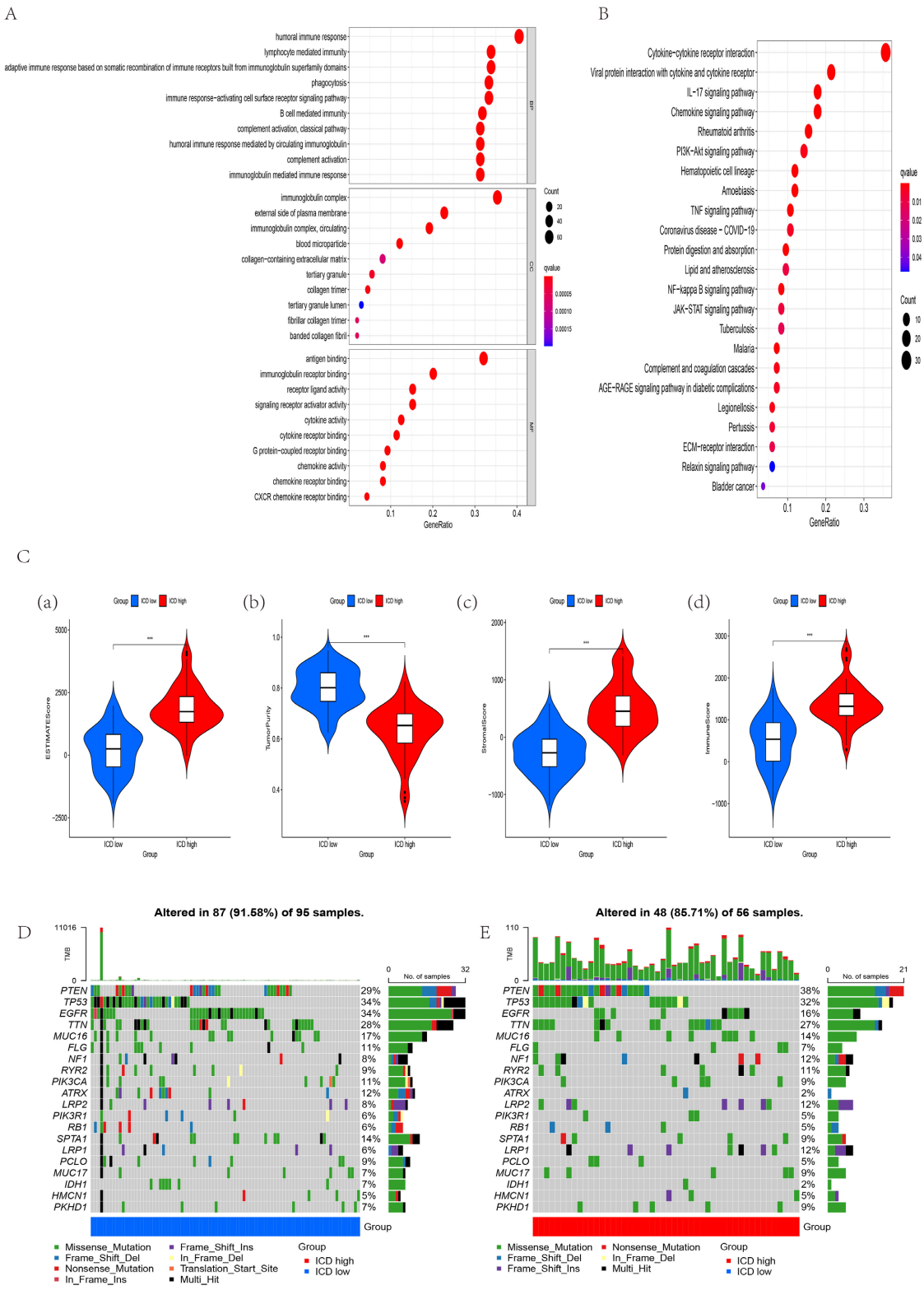

Figure 3. Enrichment analysis, immune infiltration analysis, and tumor mutation burden analysis between high and low-risk groups of ICD. A. GO analysis of high and low-risk groups. Bp, cc, and mf represent different terms. Different colors represent different significant levels; B. KEGG analysis of high and low-risk groups; C. Immune infiltration analysis of high and low-risk groups. Red means the high-risk group, and blue represents the low-risk group. D. Tumor mutation burden in high- and low-risk groups.

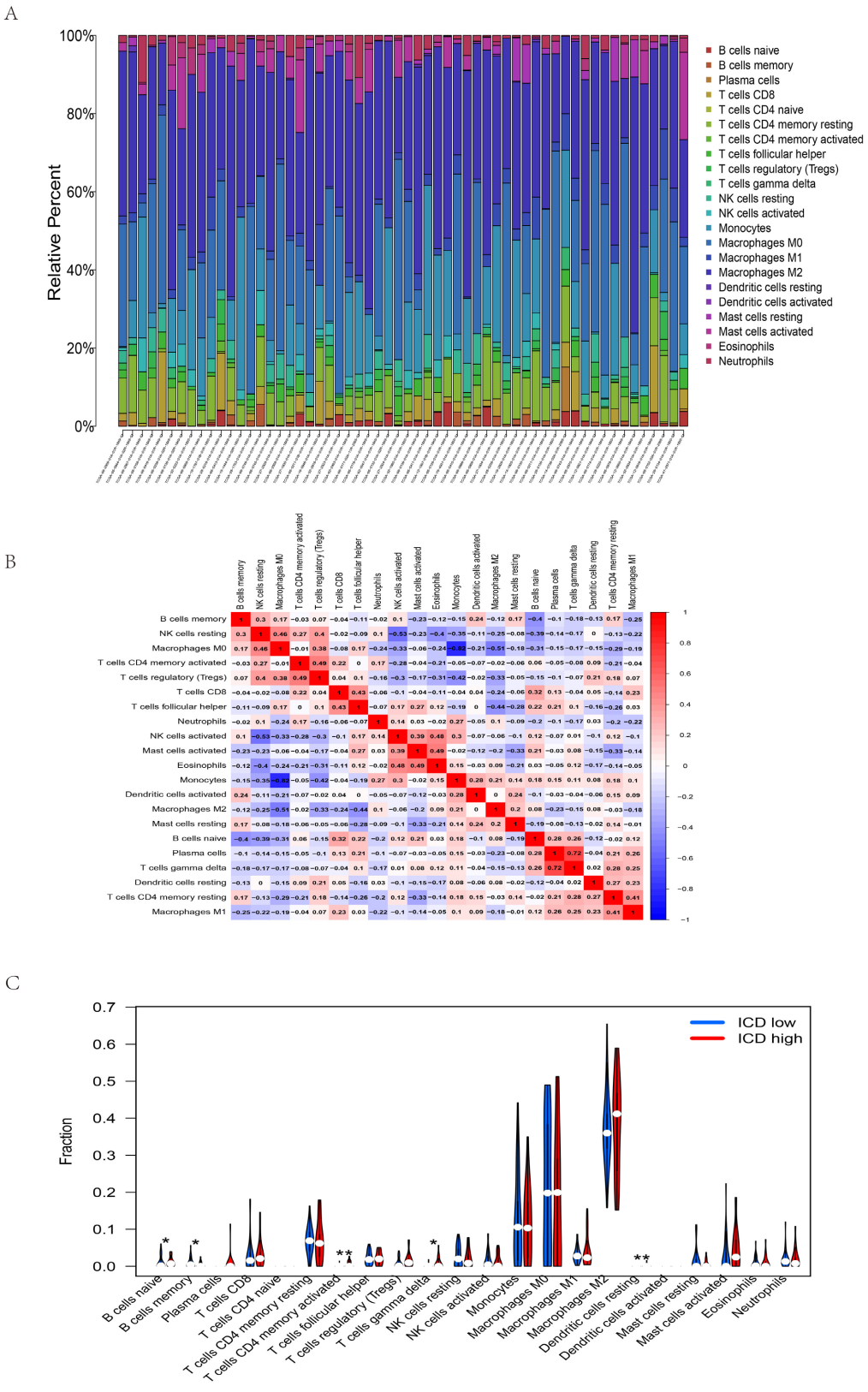

Figure 4. Analysis of immune cell infiltration levels in high and low-risk groups. A. Presentation of immune cell content in each sample; B. Analysis of immune cell and immune cell correlation, C. Analysis of differences in immune cells between high and low-risk groups.

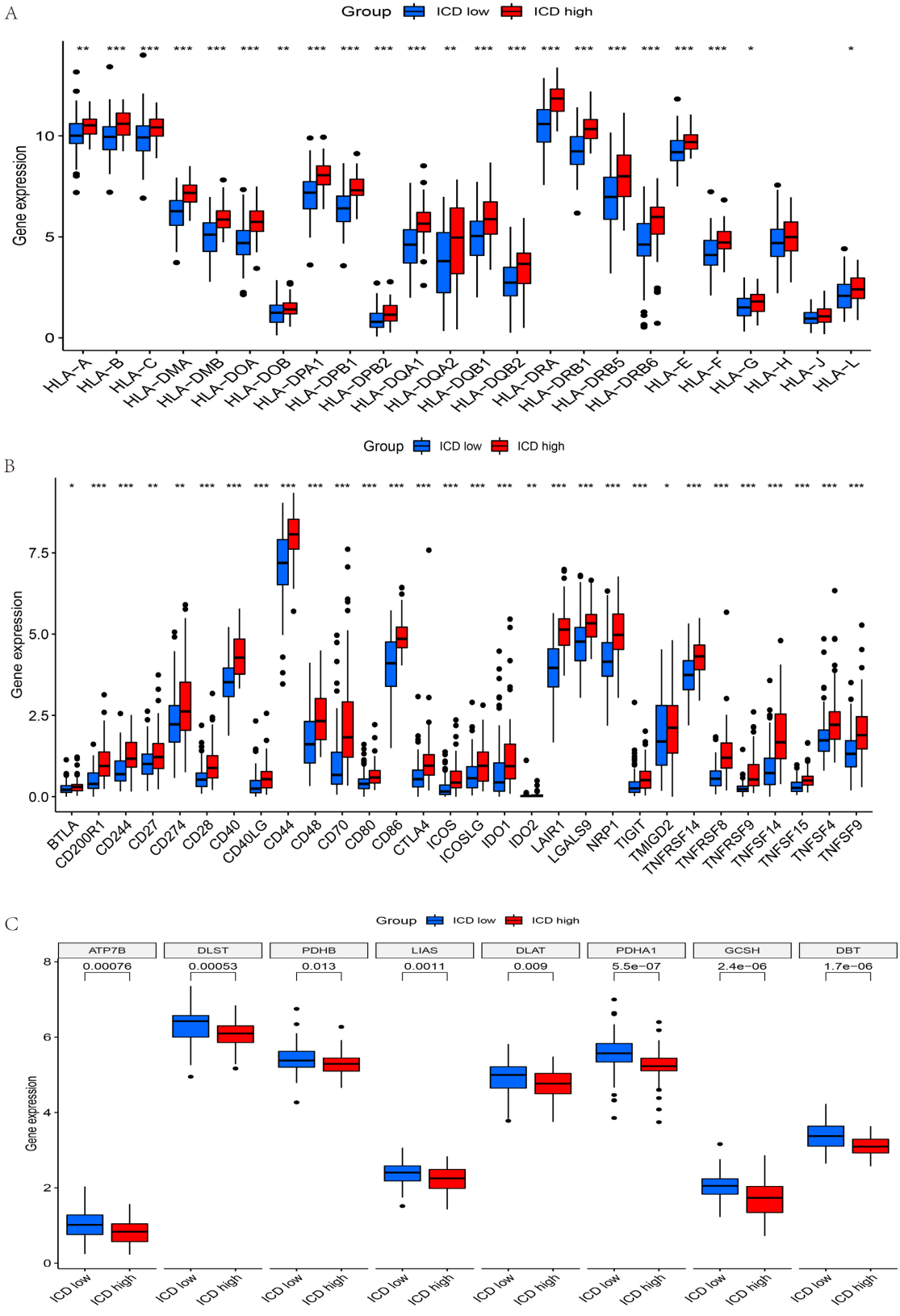

Figure 5. Differential analysis of HLA genes, immune checkpoint genes, and copper death genes between high and low-risk groups. A. Differential analysis of HLA genes between high and low-risk groups; B. Differential analysis of immune checkpoint genes between high and low-risk groups; C. Differential analysis of copper death genes between high and low-risk groups.

analysis (Figure 6A), and five genes were further analyzed by lasso regression for model construction (Figure 6B & Figure 6C), and the risk score was calculated as risk score = FOXP3 exp * 0.35 + IL6 exp * 0.048 + LY96 exp * 0.038 + MYD88 exp * 0.193 + PDIA3 exp * 0.126, survival analysis showed that the prognosis of the high-risk group was significantly lower than that of the low-risk group ($P < 0.05$) (Figure 6D & Figure 6E), The results of the CGGA cohort verified that it was consistent with the TCGA cohort. Independent prognostic factor analysis showed that the risk score could be used as an independent prognostic factor (Figure 6G & Figure 6H); drug prediction showed that the high-risk group was more sensitive to 32 drugs ($P < 0.05$), 13 drugs without laboratory evidence, 11 sensitive drugs in the high-risk group, and two sensitive drugs in the low-risk group. Treatment of GBM ($P < 0.05$) (Figure 8). The TIDE analysis revealed that the TIDE score was significantly lower in the low-risk group than in the high-risk group ($P < 0.05$) (Figure 7D), reflecting that immunotherapy may benefit patients with low-risk scores. The correlation analysis showed a negative correlation between the risk score and the TCGA cohort for Eosinophil, NK cells activated negatively and T cells regulatory (Tregs) positively (Figure 7A), Monocytes, NK cells activated negatively and Neutrophils, T cells CD4 memory triggered completely in the CGGA cohort (Figure 7B). The TCGA cohort was analyzed for immune cell correlation, and the results showed differences in multi-platform immune cells ($P < 0.05$) (Figure 7E), and further analysis of their immune functions revealed differences in all immune pathways ($P < 0.05$) (Figure 7C). In addition, ROC curves showed good prognostic value of the predictive model (Figure 8N & Figure 8O).

4. Discussion

Glioblastoma is a primary brain tumor in the brain, and its treatment is diverse, but its prognosis has not been significantly improved, which requires further research. A related study found that the PI3K-Akt signaling pathway was associated with glioblastoma signaling pathway is associated with drug resistance in glioblastoma, tumor proliferation, and invasion, and tumor cell senescence and apoptosis [17] [18] [19]. Rhoj can promote angiogenesis in glioblastoma through the TNF signaling pathway [20]. The NF-kB pathway is associated with radiation resistance in glioblastoma [21] [22] [23]. The JAK-STAT signaling pathway is a potential therapeutic associated with tumor proliferation, anti-apoptosis, angiogenesis, stem cell maintenance, and immunosuppression [24].

Tumor mutation burden analysis revealed a higher mutation rate of the PTEN gene in the high-risk group and the rest of the genes in the low-risk group, which was found to be a common mutation in glioblastoma and its association with glioblastoma drug resistance, and the rest of genes were expressed higher in the low-risk group, which was linked to the subsequent TIDE treatment of GBM [25]. We could find that the tumor mutation burden may be relevant to their TIDE treatment. In addition, immune infiltration was higher in the high-risk group, and multiple immune cells were differentially expressed.

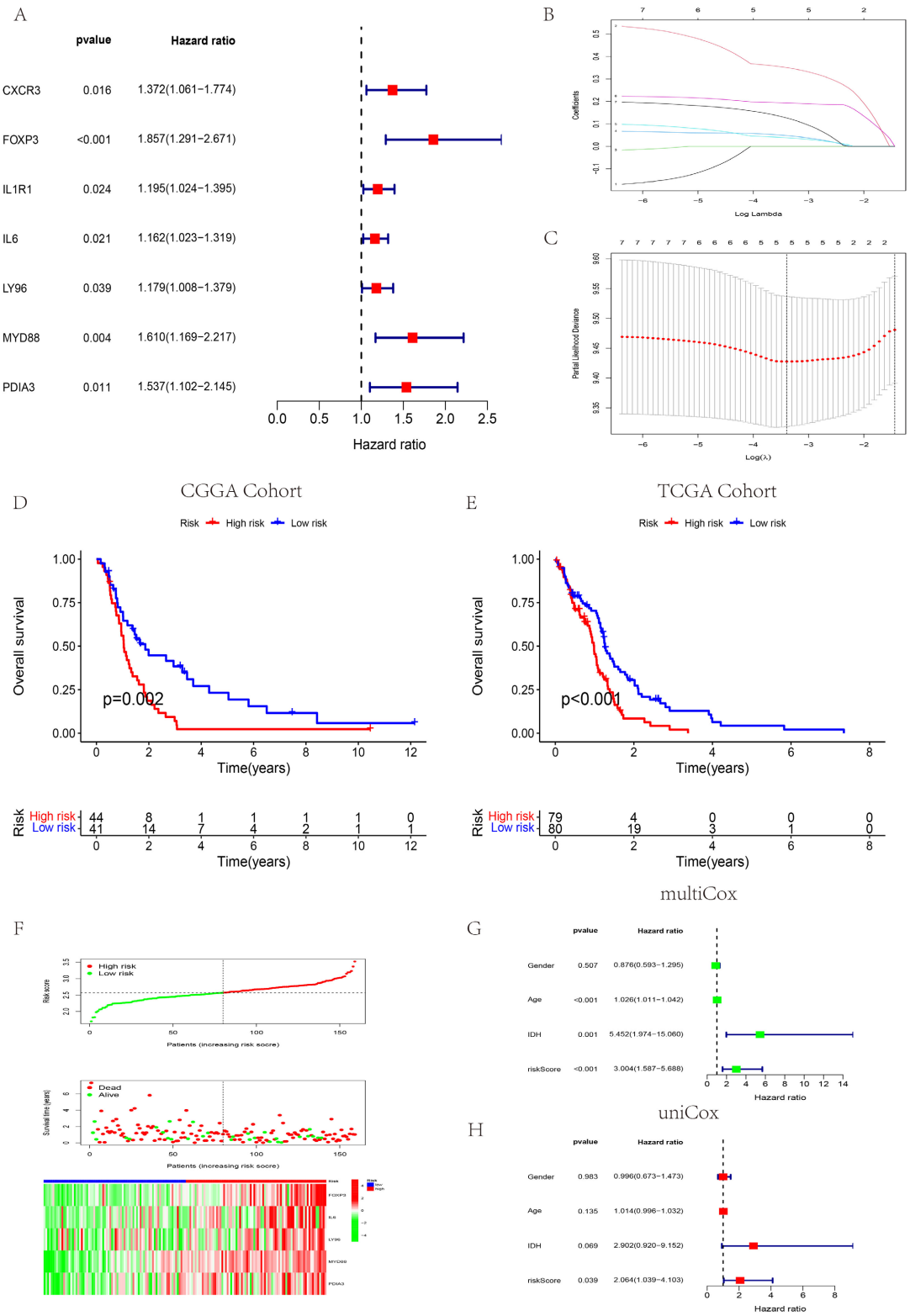

Figure 6. Construction of prognostic model for ICD genes. A. Forest plot of univariate analysis of ICD genes. B&C. Lasso Cox analysis to obtain the five genes most associated with prognosis for ICD genes for model construction. D. Survival analysis of prognostic model based on CGGA data. E. Survival analysis of prognostic model based on TCGA data. F. Risk plot of prognostic model. G. Risk plot of prognostic model. Forest plot of univariate analysis of the prognostic model. H. Forest plot of multivariate analysis of the prognostic model.

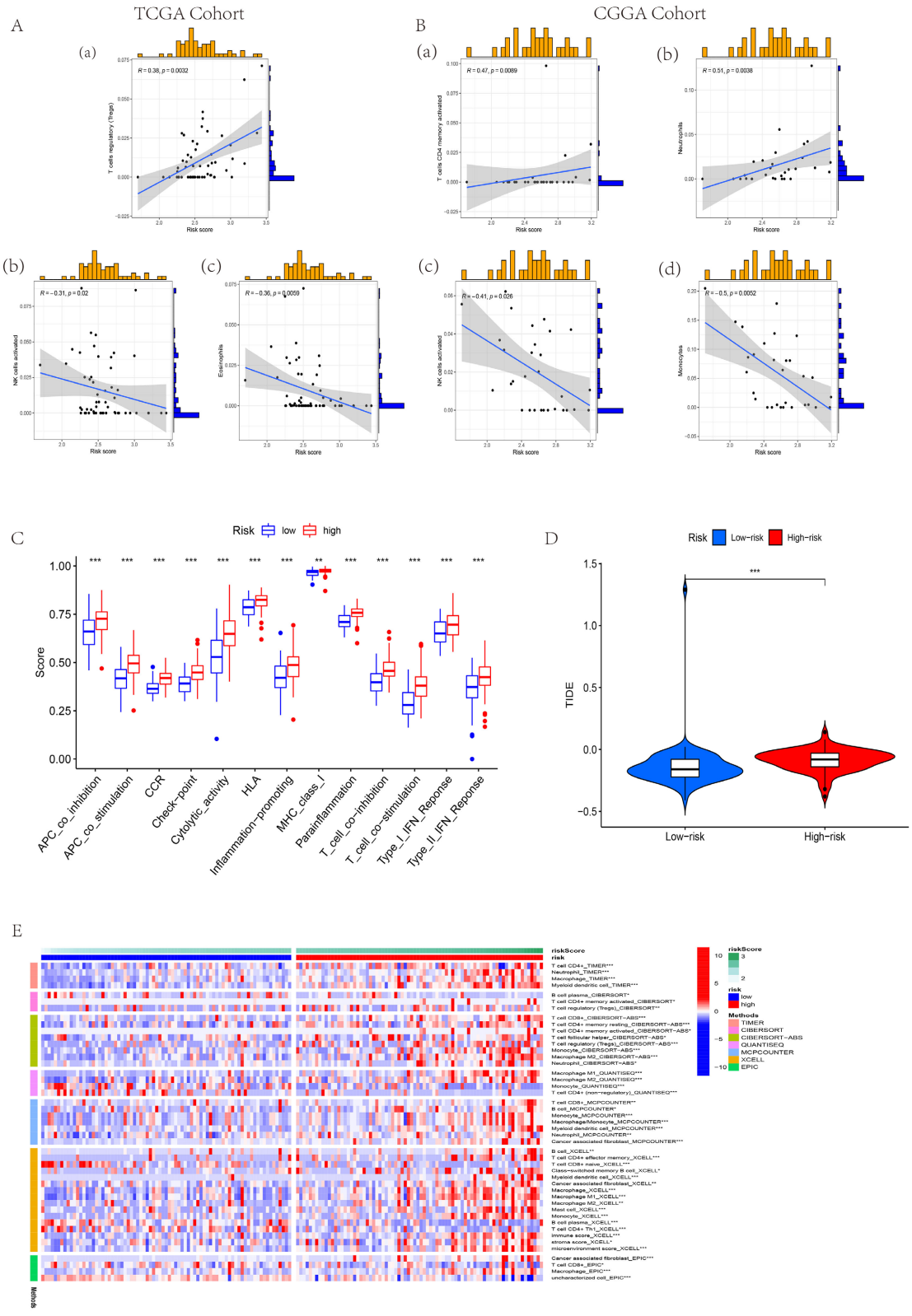

Figure 7. Immune cells, immune function, and TIDE analysis of the ICD genetic prognostic model. A. Correlation analysis of risk scores and immune cells. B. for validation results based on the CGGA database. C. Difference analysis of immune function between high and low-risk groups of the prognostic model. D. Difference analysis of TCDE in high and low-risk groups. E. Difference analysis of immune cells based on TIMER. *P < 0.05, **P < 0.01, ***P < 0.001, & ****P < 0.0001.

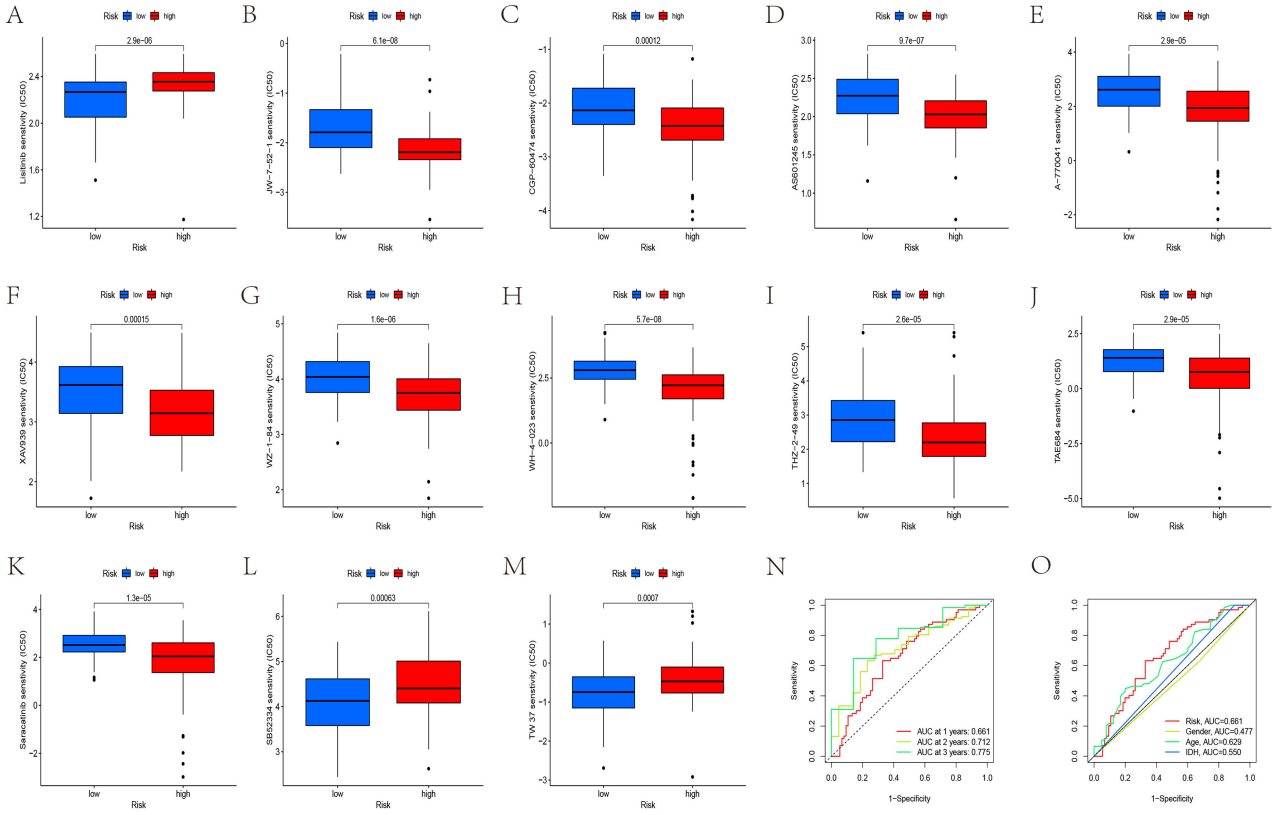

Figure 8. Drug prediction and ROC curve analysis of the prognostic model. A-M. Drug prediction results of the prognostic model. N&O. The ROC curve prediction analysis of the prognostic model.

In contrast, it was differentially expressed in 91.7% of HLA genes and 62.5% of immune checkpoint genes in the high- and low-risk groups and was more highly expressed in the high-risk group than in the low-risk group but was more highly expressed in the low-risk group than in the high-risk group. In addition, we further constructed ICD-related prognostic models in which several genes and GBM were correlated, among which FOXP3 plays an essential role in the immunosuppressive microenvironment of glioma through regulatory T cells (Treg), and FOXP3 overexpression was associated with GBM cell resistance to TMZ [26] [27]. il6 plays an essential role in the immunosuppressive microenvironment of glioma through phosphorylation of (p)-STAT3-MIR155-3p pathway to cause glioblastoma autophagy [28]. In 2018, Wang *et al.* found that targeting IL-6 produced by endothelial cells may be a potential therapeutic strategy for GBM [29]. Nakamura *et al.* studied CNS lymphoma and GBM samples and found that recurrent mutations in CD79B and MYD88 are a hallmark of primary CNS lymphoma [30]. Chiavari *et al.* found that reduced expression of the PDIA3 gene restricts the pro-tumor polarization process of microglia toward the M2 phenotype [31]. In addition, the novel cyclic RNACircRFX3 as a sponge of MicroRNA-587 could regulate the PDIA3 gene, thereby promoting glioblastoma progression [32]. ly96 gene is currently not found to correlate with GBM. A total of 32 potential drugs were predicted in drug prediction (**Table 1**), and 19 drugs

Table 1. Drug prediction results.

Drug name	Drug sensitivity	Evidence
17-AAG	High-risk group	Sauvageot et al. [33]
AG-014699 (Rucaparib)	High-risk group	Zhang *et al.* [34]
Bortezomib	High-risk group	Su *et al.* [35]
Dasatinib	High-risk group	Alhalabi *et al.* [36]
Lapatinib	High-risk group	Yu *et al.* [37]
LY317615	High-risk group	Graff *et al.* [38]
MG-132	High-risk group	Li *et al.* [39]
OSI-930	High-risk group	Yang *et al.* [40]
Paclitaxel	High-risk group	Yang *et al.* [41]
Pazopanib	High-risk group	Haraldsdottir *et al.* [42]
PD-0332991	High-risk group	Michaud *et al.* [43]
Rapamycin	High-risk group	Le *et al.* [44]
Ruxolitinib	High-risk group	Delen *et al.* [45]
Temsirolimus	High-risk group	Kaley *et al.* [46]
TGX221	High-risk group	Yang *et al.* [47]
Z-LLNle-CHO	High-risk group	Monticone *et al.* [48]
BMS-754807	Low-risk group	Fuentes-Baile *et al.* [49]
FK866	Low-risk group	Feng *et al.* [50]
Salubrinal	Low-risk group	He *et al.* [51]

have been confirmed by relevant evidence [33]-[51]. In addition, 11 sensitive drugs in the high-risk group and two sensitive drugs in the low-risk group were not found to treat GBM, so our study can provide a reference for clinical drug development.

In this study, the ICD gene was typed by a complete bioinformatics method, and integrated bioinformatics analysis and prognostic model construction were performed after typing, which is the first application of the ICD gene in glioblastoma. Secondly, the sample size is still tiny and may be biased; finally, there is a lack of relevant laboratory validation tools.

5. Conclusion

In this study, we uncovered the relationship between ICD genotyping based on ICD genotyping and GBM immune infiltration and tumor microenvironment, and our findings will be helpful for the immunotherapy of GBM patients. In addition, we developed an ICD gene-related prognostic model, integrated bioinformatics to verify its good predictive value, and obtained potential therapeutic agents.

Author Contributions

XL designed the study. XL performed the bioinformatics analysis and interpreta-

tion of the data. XL wrote the manuscript. XL revised the manuscript and gave final approval for publishing the version.

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Data Availability Statement

The datasets TCGA for this study can be found in The Cancer Genome Atlas [https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga].

The datasets CGGA for this study can be found in Chinese Glioma Genome Atlas [http://www.cgga.org.cn/].

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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