Cancer Cell International

RESEARCH Open Access

Decoding the immune microenvironment: unveiling CD8+T cell-related biomarkers and developing a prognostic signature for personalized glioma treatment

Xiaofang Lin[1], Jianqiang Liu[2], Ni Zhang[3*], Dexiang Zhou[2*] and Yakang Liu[3*]

Abstract

Background Gliomas are aggressive brain tumors with poor prognosis. Understanding the tumor immune microenvironment (TIME) in gliomas is essential for developing effective immunotherapies. This study aimed to identify TIME-related biomarkers in glioma using bioinformatic analysis of RNA-seq data.

Methods In this study, we employed weighted gene co-expression network analysis (WGCNA) on bulk RNA-seq data to identify TIME-related genes. To identify prognostic genes, we performed univariate Cox regression and least absolute shrinkage and selection operator (LASSO) regression analyses. Based on these genes, we constructed a prognostic signature and delineated risk groups. To validate the prognostic signature, external validation was conducted.

Results CD8+T cell infiltration was strongly correlated with glioma patient prognosis. We identified 115 CD8+T cell-related genes through integrative analysis of bulk-seq data. CDCA5, KIF11, and KIF4A were found to be significant immune-related genes (IRGs) associated with overall survival in glioma patients and served as independent prognostic factors. We developed a prognostic nomogram that incorporated these genes, age, gender, and grade, providing a reliable tool for clinicians to predict patient survival probabilities. The nomogram's predictions were supported by calibration plots, further validating its accuracy.

Conclusion In conclusion, our study identifies CD8+T cell infiltration as a strong predictor of glioma patient outcomes and highlights the prognostic value of genes. The developed prognostic nomogram, incorporating these genes along with clinical factors, provides a reliable tool for predicting patient survival probabilities and has important implications for personalized treatment decisions in glioma.

Keywords Gliomas, CD8+T cells, Tumor immune microenvironment, Prognosis, Bioinformatic analysis

*Correspondence:
Ni Zhang
674561824@qq.com
Dexiang Zhou
stzdx808@163.com
Yakang Liu
liuyakang@gdph.org.cn
[1]Laboratory Department of Zengcheng Branch, Nanfang Hospital, Southern Medical University, Guangzhou, China
[2]Department of Neurosurgery, Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University, 106 Zhongshan 2nd Road, Guangzhou 510080, China
[3]Department of Physical Medicine and Rehabilitation, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Southern Medical University, 106 Zhongshan 2nd Road, Guangzhou 510080, China

Introduction

Gliomas, the most prevalent type of brain tumor originating from glial cells, pose a significant burden on morbidity and mortality rates [1]. Among these tumors, glioblastoma (GBM) stands out as the most common primary malignant brain tumor, accounting for approximately 80% of all cases. Unfortunately, GBM is associated with a disappointing prognosis and poor quality of life, with a median overall survival (OS) of less than one year [2, 3]. Despite recent advancements in our understanding of glioma biology and the development of targeted therapies through preclinical and clinical trials, the incidence of glioma recurrence, cognitive impairment, disability, and mortality remains unacceptably high [4]. Most therapies that have shown a significant survival benefit in gliomas, such as radiation and chemotherapy, rely on non-specific targeting of proliferating cells. However, their curative effects are often compromised by glioma cell invasion and immune evasion [4–6]. Therefore, it is crucial to uncover unknown factors and further investigate the known factors that contribute to the formation of the tumor microenvironment (TME). Understanding these factors helps promote glioma progression, identify malignant transformation mechanisms, and develop strategies to overcome drug resistance. Ultimately, this knowledge will enhance the diagnosis and enable personalized treatment approaches for gliomas.

TME plays a crucial role in the development and progression of cancer [7, 8]. It encompasses various components, including the extracellular matrix, non-transformed cells like fibroblasts and immune cells, as well as the vascular network recruited from neighboring tissues [9]. Within the TME, immune-related genes (IRGs) are known to exert a significant influence on tumor development, invasion, metastasis, and drug resistance [10–12]. This complex interplay is mediated by factors such as matrix, cytokines, and growth factors, ultimately impacting the efficacy of tumor treatment [13, 14]. The extracellular matrix provides structural support to the tumor and facilitates cell signaling, migration, and invasion [15, 16]. It acts as a reservoir for growth factors and cytokines, which can promote tumor cell proliferation and survival [17, 18]. Additionally, fibroblasts within the TME can undergo activation, leading to the secretion of various factors that contribute to tumor growth and invasion [19, 20]. The immune infiltrates present in the TME, including immune cells like T cells, B cells, and macrophages, have a dual role [21, 22]. On the one hand, they can mount an antitumor immune response, aiming to eliminate cancer cells [23]. On the other hand, tumor cells can manipulate the immune response to their advantage, leading to immune evasion and progression of the disease [24]. This intricate interplay between tumor cells and immune cells within the TME is a key determinant of tumor behavior and response to therapy. Furthermore, the TME is characterized by an altered vascular network, which is crucial for tumor growth and metastasis [25, 26]. Tumor cells can induce angiogenesis, the formation of new blood vessels, to ensure a sufficient supply of nutrients and oxygen for their survival and proliferation [27, 28]. However, this abnormal vasculature can also contribute to increased interstitial pressure within the tumor, limiting drug delivery and efficacy [29]. Understanding the complexity of the TME and its influence on tumor development and treatment response is essential for the development of effective therapeutic strategies. Targeting specific components or signaling pathways within the TME holds promise for improving treatment outcomes and overcoming drug resistance in cancer patients.

Compared with normal brain tissue, which typically exhibits a low-to-medium immune state, the tumor immune microenvironment (TIME) plays a crucial role in glioma progression and treatment resistance [30, 31]. A suppressive TIME can significantly impair the effectiveness of GBM treatment [32]. However, recent studies have shown that remodeling TIME using a brain-targeted liposomal honokiol and disulfiram/copper codelivery system can trigger tumor cell autophagy and promote immunogenic cell death [32]. This is accompanied by the activation of tumor-infiltrating macrophages and dendritic cells, as well as primed T and NK cells, leading to the formation of antitumor immunity and tumor regression [32]. Furthermore, a group of IRGs has been identified, which can be classified into low-risk and high-risk groups [33–35]. This suggests that IRGs play differential and complex roles in the development, progression, and metastasis of gliomas. Understanding the function of these genes within the context of TIME is critical for developing effective treatment strategies. Thus, the identification and characterization of IRGs within the TIME can provide valuable insights into the development of effective treatment strategies for gliomas.

In this study, we conducted a comprehensive analysis of the TIME in glioma patients and identified 7 IRGs that play a crucial role in glioma development and progression. Among these IRGs, three hub genes, namely, CDCA5, KIF11, and KIF4A, were identified as potential independent clinical indicators for glioma patients. Our integrated analysis of these IRGs provides valuable insights into the development of more effective antitumor immunotherapies for gliomas. These findings contribute to a deeper understanding of the complex interplay between the immune system and glioma progression and may pave the way for the development and implementation of novel treatment strategies targeting these specific IRGs.

Materials and methods

Data collection and procession

The research design is illustrated in the flowchart (Fig. 1). The process of collecting and organizing the data was a crucial aspect of this study. We accessed the required data from publicly available sources, specifically the TCGA database (http://cancergenome.nih.gov/abouttcga) and GlioVis database (http://gliovis.bioinfo.cnio.es/). These databases provided us with level 3 RNA-seq data, which had been normalized using TPM (transcripts per million) and included expression matrices. Additionally, the database contained clinical and follow-up information of patients with gliomas. Our focus was on a subset of the TCGA- low-grade glioma (LGG)/GBM samples, totaling 663 samples. These samples were utilized for constructing prognostic risk signature models and analyzing prognostic gene expression signatures. To validate the reliability of our risk signature model, we also utilized two separate datasets, namely, the REMBRANDT dataset (consisting of 444 samples) and the GRAVENDEEL dataset (consisting of 276 samples), both obtained from the GlioVis database.

Tumor immune microenvironment landscape

To further investigate the immune landscape in gliomas, we employed the TIMER algorithm [36]. This algorithm allowed us to quantify the composition of six immune cell types (including CD4+T cells, CD8+T cells, neutrophil cells, myeloid dendritic cells, macrophages and B cells) in the glioma samples, providing detailed information about the immune cell infiltrates. We utilized the estimation of STromal and immune cells in MAlignant Tumor tissues using Expression data (ESTIMATE) algorithm, implemented through the R package "ESTIMATE" [37], to assess the infiltration of stromal and immune cells as well as tumor purity based on the gene expression profile in the TCGA cohorts. The ESTIMATE algorithm calculates several scores that provide insights into the immune microenvironment. These scores include the immune score, which reflects the abundance of tumor-infiltrating immune cells, and the stromal score, which reflects the abundance of stromal cells. Additionally, the estimated score indicates tumor purity, while the tumor purity score shows its level. These scores help us understand the composition and characteristics of the tumor microenvironment. In addition to assessing the immune cell composition, we collected 25 immune cell death (ICD) modulators, such as ANXA1 and IFNB1, and 47 immune checkpoints (ICPs), including CTLA4 and PD-L1, from a previous study [38]. We examined the association of these ICD modulators and ICPs with risk scores, providing insights into their potential roles in glioma development and progression. By utilizing these algorithms and collecting additional immune-related factors, we were able to comprehensively analyze the immune landscape and investigate the potential implications of immune cell

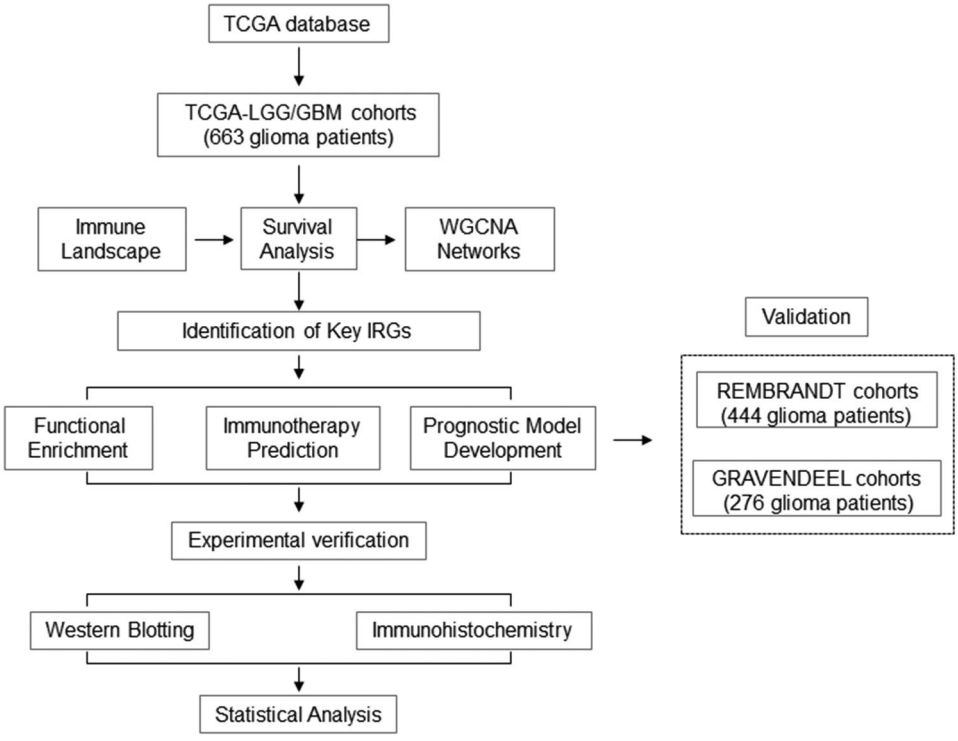

Fig. 1 Flowchart illustrating the study design

infiltrates, ICD modulators, and ICPs in gliomas. This information enhances our understanding of the immune response in gliomas and may have implications for the development of immune-based therapies.

Kaplan-Meier survival analysis

In this study, we utilized the R packages "survival" and "survminer" (https://www.bioconductor.org/) to perform Kaplan-Meier survival analyses with log-rank tests. Our aim was to investigate the overall survival (OS) of patients with gliomas based on immune cells and identified genes. We generated Kaplan-Meier curves, p-values, and hazard ratios (HR) with 95% confidence intervals (CI) through log-rank test and univariate Cox proportional hazards regression. Additionally, we utilized the R package "forestplot" (https://www.bioconductor.org/) to create a forest plot, which displayed the p values and HR with 95% CI of the identified genes. These statistical analyses allowed us to assess the prognostic value of the identified genes, providing valuable insights into the survival outcomes of patients with gliomas.

Identification of immune-related genes

To identify IRGs, we employed the R package "WGCNA" [39] to construct co-expression networks of genes and identify co-expressed gene modules that are closely associated with TIMER scores. To construct the co-expression network, we performed Pearson's correlation analysis to establish a gene similarity matrix. To enhance this matrix into a scale-free co-expression network, we applied an appropriate soft threshold power (β). This power value strengthens the connections between genes in the network. Next, we converted the gene similarity matrix into a topological overlap matrix (TOM). The TOM allows us to measure the connectivity between genes in the network. A higher TOM value indicates a stronger connectivity between genes. Finally, we assigned genes with strong correlations to the same module. We utilized the R package "Limma" to perform differential expression analysis to obtain differentially expressed genes (DEGs) and identified the intersections between these genes and strong immune-correlated gene modules, identified as IRGs [40]. Through this approach, we were able to identify genes that are closely associated with the immune response and have potential implications in the context of immune-related diseases or therapies. This information enhances our understanding of the immune system and provides valuable insights into the molecular mechanisms underlying immune-related processes.

Functional enrichment analysis

To gain a deeper understanding of the functional implications of the genes identified in our study, we employed the R package "clusterProfiler" [41]. This powerful tool allowed us to conduct Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. The GO term analysis provided us with valuable information about the biological processes, molecular functions, and cellular components associated with the identified genes. This allowed us to gain insights into the specific roles and functions of these genes in glioma biology. Meanwhile, the KEGG pathway analysis helped us understand the potential involvement of these genes in various signaling and biological pathways, providing a more comprehensive understanding of their functional implications. To ensure the significance of our enrichment results, we employed a strict cutoff criterion of FDR (false discovery rate) < 0.05. This enabled us to identify only the most relevant and statistically significant GO terms and KEGG pathways associated with the identified genes. By performing these enrichment analyses, we were able to uncover the functional implications and potential biological roles of the identified genes in gliomas.

Development and validation of prognostic risk signature model

Based on STRING database (STRING: functional protein association networks (string-db.org)) and Cytoscape software V3.9.1, five algorithms (Degree, MNC, DMNC, EPC, and MCC) from the cytoHubba plug-in were utilized in Cytoscape software to identify hub IRGs. The Absolute Shrinkage and Selection Operator (LASSO) is a regularization and dimension reduction method commonly used in biomarker screening for survival analysis, particularly when combined with the Cox regression model [42]. In our study, we employed the R package "Glmnet" [43] to perform LASSO-Cox regression analysis, utilizing 10-fold cross-validation and 1000 bootstrap samples to mitigate overfitting. The goal of this analysis was to identify Immune-Related Genes (IRGs) that are associated with prognosis and establish a prognostic risk signature model. Through feature selection, we removed potential overfitting and selected the most relevant IRGs for the model. The medium risk score was used as a criterion to classify patients into high-risk and low-risk groups. To assess the survival difference between these groups, we performed Kaplan-Meier survival analysis with a log-rank test. To evaluate the predictive accuracy of the risk score, we conducted time-dependent receiver operating characteristic (ROC) analysis using the R package "survivalROC" (https://www.bioconductor.org/). Additionally, we aimed to identify and validate independent clinical prognostic factors. To achieve this, we performed univariate and multivariate Cox regression analysis. We also constructed a nomogram for prognosis prediction using the "rms" R package (https://www.

bioconductor.org/). Age, sex, and tumor grade were included as variables in the nomogram. Furthermore, we validated the efficacy of the prognostic risk signature model by calculating the risk score for patients with gliomas from the REMBRANDT dataset and GRAVENDEEL dataset, following the same methodology as described above. By employing these analytical approaches and validation steps, we aimed to establish a robust prognostic risk signature model that can accurately predict patient outcomes in gliomas. This model incorporates relevant clinical factors and IRGs, providing valuable insights for personalized treatment strategies and clinical decision-making.

Prediction of immunotherapy responsiveness

We utilized the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm [44] to predict the potential response to immune checkpoint blockade (ICB) therapy. This algorithm leverages the tumor transcriptomic profile from the TCGA cohort to assess the likelihood of a favorable response to ICB. The TIDE algorithm is based on the integration of expression signatures related to T cell dysfunction and T cell exclusion. By incorporating these signatures, it can effectively model tumor immune evasion and evaluate the interaction with the level of cytotoxic T lymphocyte infiltration. This enables the algorithm to predict patient survival and response to immunotherapy. The TIDE score serves as an indicator of the efficacy of ICB treatment. A higher TIDE score suggests a poorer response to ICB, leading to shorter survival times for patients. Essentially, the TIDE algorithm provides valuable insights into the potential curative effect of ICB therapy and helps identify patients who may benefit the most from this treatment approach. By employing the TIDE algorithm in our study, we were able to assess the likelihood of a positive response to ICB therapy based on the tumor's transcriptomic profile. This information can guide clinical decision-making and contribute to personalized treatment strategies for patients with gliomas.

Sample collection

In this study, glioma samples comprising various grades (WHO grades II-IV) were collected from tumor patients, whereas nontumor specimens were collected from patients undergoing decompressive craniectomy for traumatic intracerebral hemorrhage. Both were approved by the Ethics Committee. The samples were sourced from Guangdong Provincial Peoples' Hospital.

Each sample was divided into two portions, one for Western Blotting and the other for immunohistochemistry (IHC). Basic patient information including gender, age, tumor location, and pathological diagnosis was recorded.

Western blotting

The protein levels of CDCA5, Eg5, and KIF4A were assessed using standard Western blot analysis. The primary antibodies applied were anti-CDCA5 (1:100; Cat# 67418-1-Ig; Proteintech), anti-Eg5 (1:100; Cat# 23333-1-AP; Proteintech) and anti-KIF4A (1:100; Cat# 14344-1-AP; Proteintech). Detection was performed using the ECL chemiluminescence system, and target protein bands were analyzed using Image J software V1.53e to determine relative density.

Immunohistochemistry

Fresh glioma specimens were fixed in 10% neutral formalin and subsequently embedded in paraffin. Sections of 4–5 μm thickness were then dewaxed and rehydrated. Antigen retrieval was accomplished using a citrate buffer under high temperature and pressure. To block endogenous peroxidase activity, 3% hydrogen peroxide was applied. The paraffin-embedded sections were then incubated overnight at 4 °C with primary antibodies for CDCA5 (1:100; Cat# 67418-1-Ig; Proteintech), Eg5 (1:100; Cat# 23333-1-AP; Proteintech), and KIF4A (1:100; Cat# 14344-1-AP; Proteintech). The following day, sections were treated with HRP-conjugated secondary antibodies, developed using DAB as the chromogen, and counterstained with hematoxylin. The stained sections were examined under a microscope, photographed, and the positive cells along with staining intensity were quantified using Image J software V1.53e.

Statistical analysis

A significance level of $p<0.05$ was used to determine statistical significance. All statistical analyses were conducted using R version 4.0.2.

Results

The infiltration of CD8+T cells showed a significant association with the prognosis of glioma patients

To investigate the TIME in patients with gliomas, we employed the TIMER algorithm to accurately quantify the abundance of six distinct immune cell populations within the TCGA cohort. Our analysis revealed intriguing and distinct patterns of immune cell infiltration in LGG and GBM, as depicted in Fig. 2A-B. Intrigued by these findings, we sought to delve deeper into the relationship between immune cell infiltration and glioma prognosis. To accomplish this, we performed log-rank tests and univariate Cox proportional hazards regression analyses to evaluate the prognostic significance of the six immune cell types. The results were striking, showing a significant difference in survival outcomes between patients with high and low immune cell infiltration. Specifically, CD4+T cells, CD8+T cells, neutrophils, myeloid dendritic cells, and macrophages

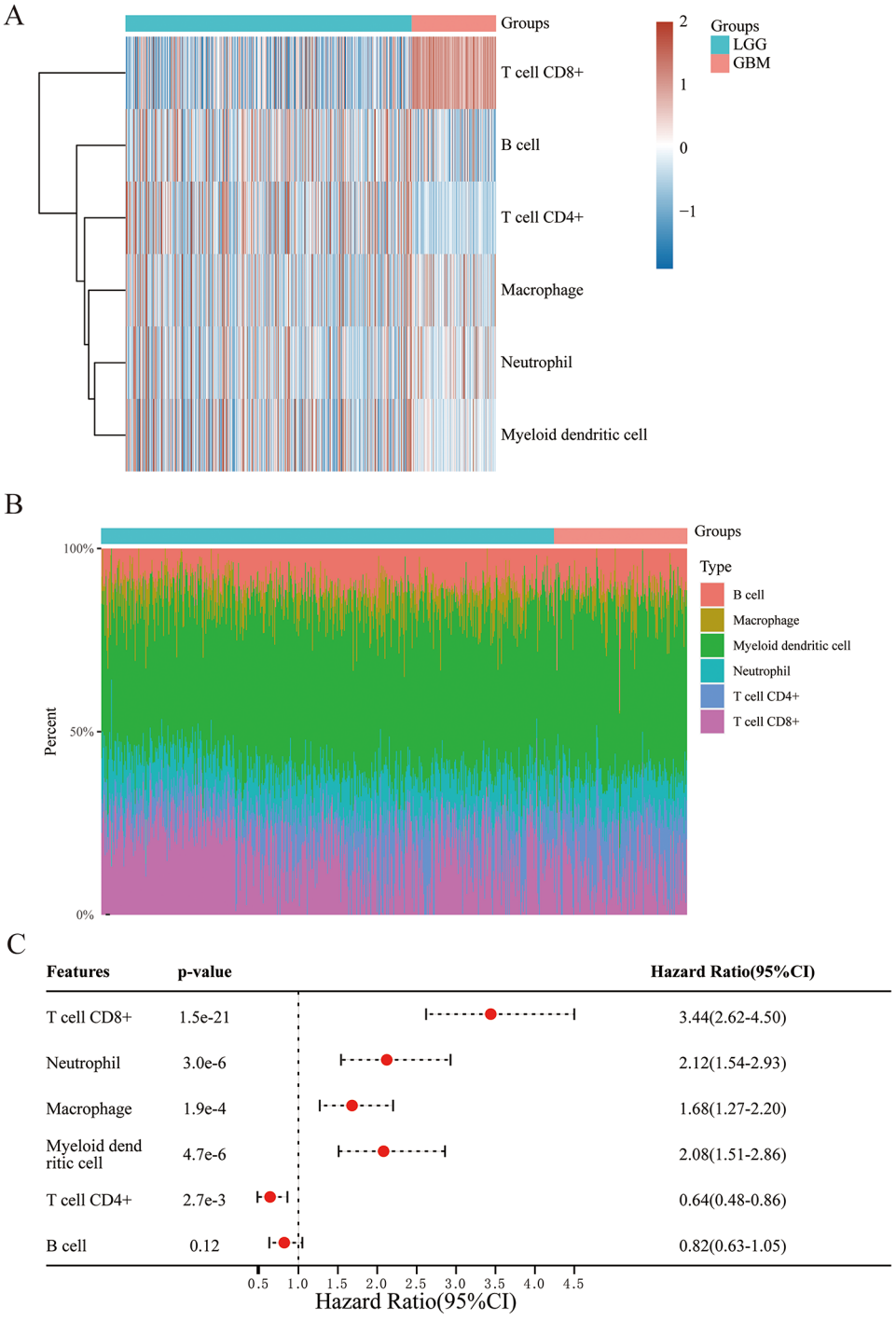

Fig. 2 The immune landscape in glioma and immune cell-related survival analysis. (**A**) Heatmap illustrating the expression scores of six immune cell types in LGG and GBM. The heatmap uses different colors to represent the expression trends observed in different samples (LGG: $n=510$, GBM: $n=153$). (**B**) Representation of the relative abundance of tumor-infiltrating immune cells in glioma, with each immune cell type depicted using distinct colors. (**C**) Survival analysis of various immune cell types using log-rank tests and univariate Cox proportional hazards regression

exhibited a strong association with glioma prognosis. It is worth noting that among the six immune cell types analyzed, CD8+T cell infiltration was found to be the most strongly correlated with glioma patient prognosis. However, interestingly, as shown in Fig. 2C, B cells did not exhibit a significant impact on patient survival rates. These findings provide valuable insights into the intricate interplay between immune cells and glioma progression, shedding light on potential therapeutic targets and prognostic indicators.

Screening for IRGs associated with CD8 + T cell infiltration in gliomas using weighted gene co-expression network analysis (WGCNA)

As illustrated in Fig. 2C, the infiltration of CD8 + T cells demonstrated a noteworthy correlation with the prognosis of glioma patients. Building upon this significant finding, a comprehensive analysis using WGCNA was performed to identify IRGs specifically associated with CD8 + T cell infiltration in gliomas. First, a thorough examination of the TCGA cohort revealed no outliers, as visually demonstrated in Fig. 3A. To determine the optimal soft threshold power, a meticulous analysis was conducted, ultimately selecting 10 as the most suitable power, as depicted in Fig. 2B and C. Subsequently, a comprehensive WGCNA was performed, leading to the identification of 15 distinct modules, as illustrated in Fig. 2D

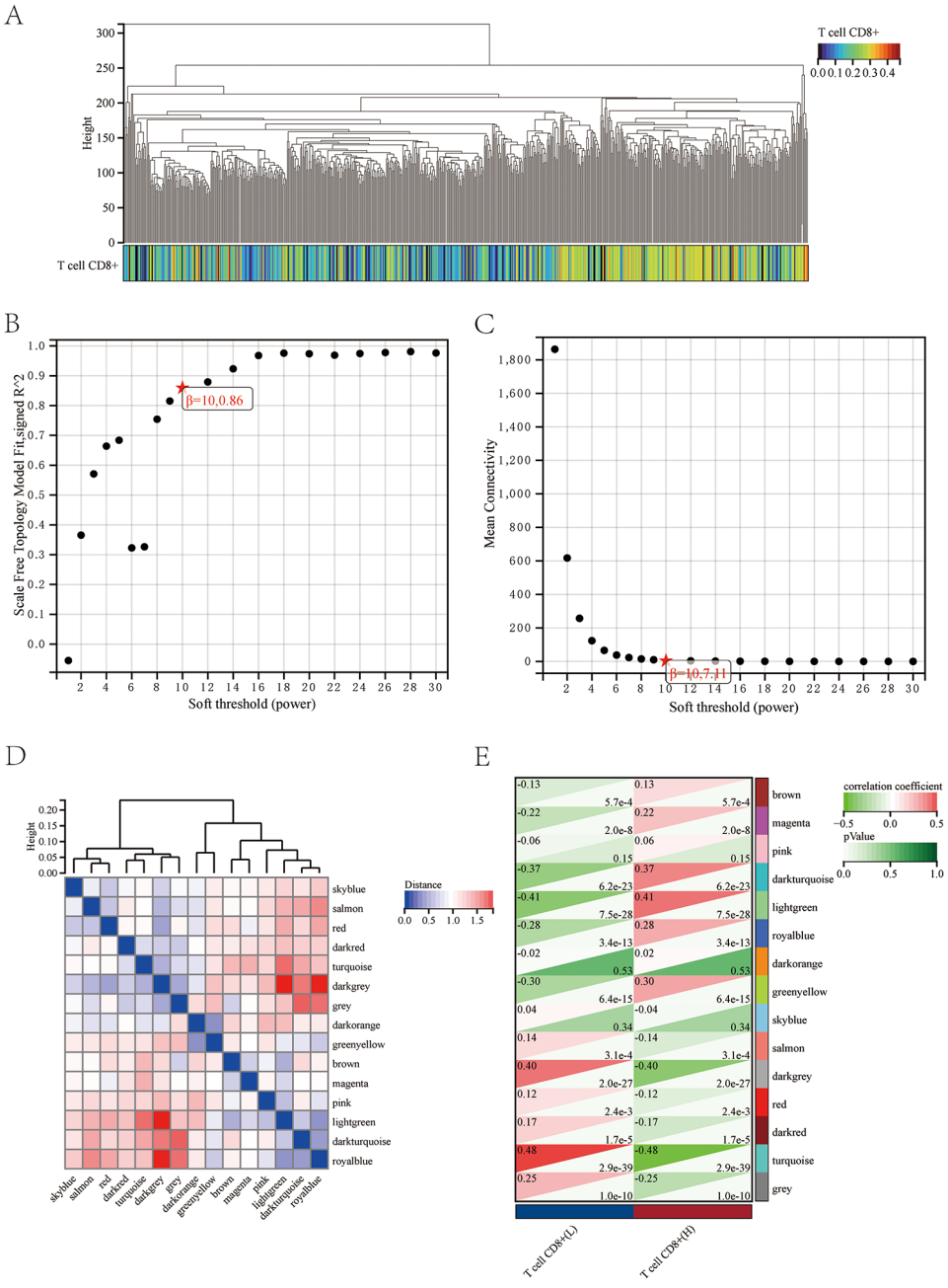

Fig. 3 Identification of the CD8 + T cell-related module using WGCNA screening. (**A**) Detection of outlier samples through sample clustering. (**B**-**C**) Evaluation of the scale-free topology fit index and mean connectivity for different soft threshold powers (β). (**D**) Heatmap displaying the correlation between different modules. (**E**) Determination of the module-trait relationship in glioma patients, highlighting the turquoise module as the most relevant module associated with CD8 + T cells

and E. To establish the association between these modules and CD8+T cell infiltration, a correlation analysis was carried out. Interestingly, the turquoise module with 807 genes exhibited the most significant correlation with high levels of infiltrating CD8+T cells, with a correlation coefficient of 0.48 and a p-value of less than 0.001, as showcased in Fig. 3E. This finding suggests that the genes within the turquoise module may play a crucial role in facilitating the infiltration of CD8+T cells in gliomas. Further investigation into these genes could provide valuable insights into the underlying mechanisms of CD8+T cell-mediated immune responses in glioma progression.

Screening of CD8+T cell-related prognostic IRGs in gliomas

To comprehensively evaluate the prognostic significance of CD8+T cell-related IRGs in gliomas, we first employed the R package "Limma" to conduct differential expression analysis. By intersecting the resulting 1273 differentially expressed genes (DEGs) with 807 CD8+T cell-related IRGs, we identified a set of 115 candidate CD8+T cell-related IRGs, as displayed in Fig. 4A. To determine the prognostic value of these candidate IRGs, we performed log-rank tests and univariate Cox proportional hazards regression analysis. Intriguingly, the expression levels

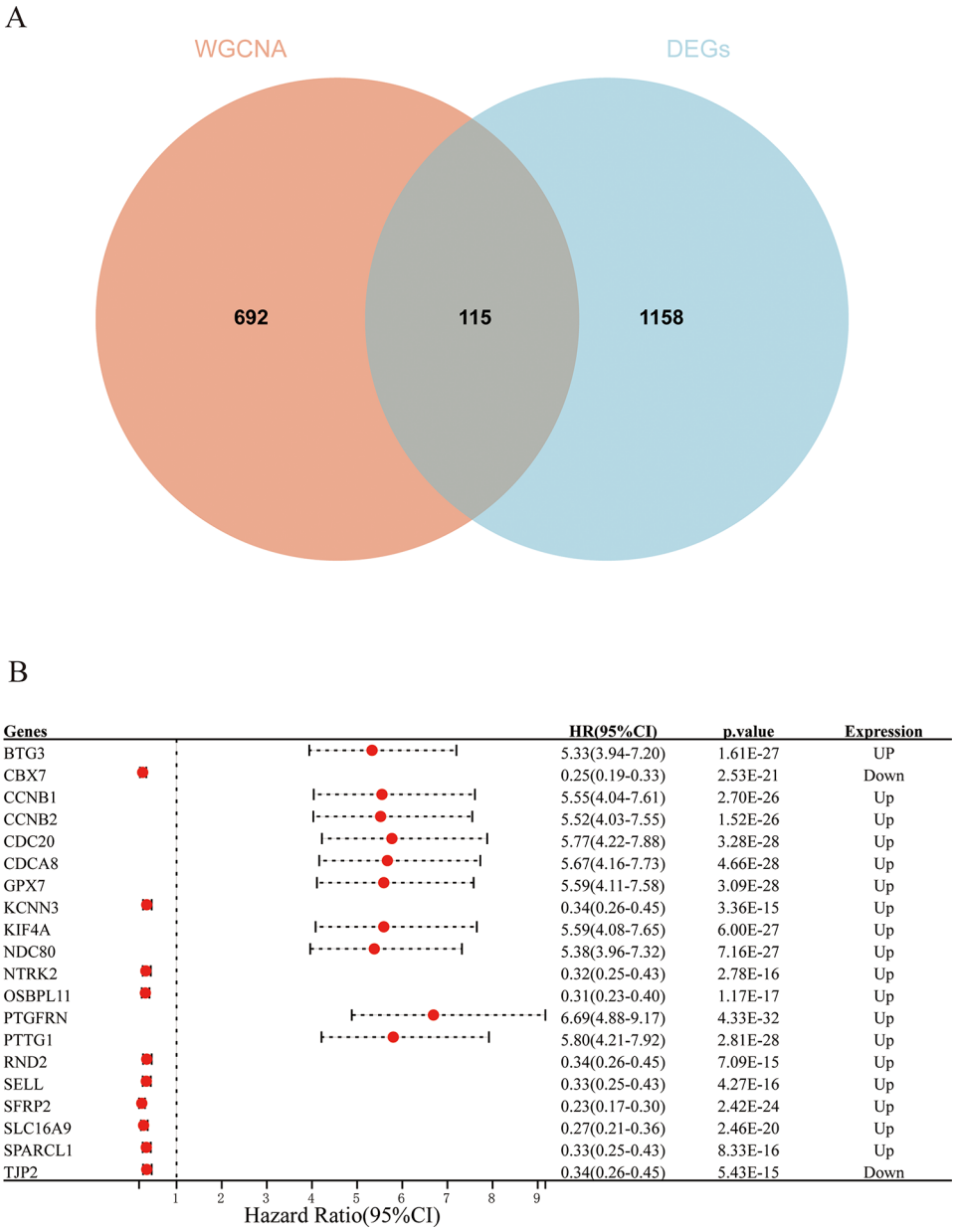

Fig. 4 Identification of immune-related genes (IRGs) associated with CD8+T cells. (**A**) Screening for candidate genes associated with CD8+T cells. (**B**) Evaluation of the prognostic significance of candidate CD8+T cell-related IRGs using log-rank tests and univariate Cox proportional hazards regression

of 110 out of the 115 candidate IRGs were found to be significantly correlated with survival prognosis. The forest plot further illustrated the top 20 CD8+T cell-related prognostic IRGs in gliomas, as shown in Fig. 4B. These findings suggest that the identified CD8+T cell-related IRGs may serve as potential prognostic biomarkers and therapeutic targets for glioma patients.

Functional enrichment analysis of candidate IRGs in gliomas

To understand the biological roles of the 110 candidate CD8+T cell-related prognostic IRGs, we performed KEGG pathway enrichment analysis and examined three GO term categories: biological process, cellular component, and molecular function. The KEGG analysis identified several significantly enriched pathways. KEGG pathway analysis identified significant enrichment in pathways such as the cell cycle, oocyte meiosis, p53 signaling, and cellular senescence (Figure S1A). A chord diagram illustrated the correlation between IRGs and these pathways (Figure S1B), while a lollipop plot depicted the KEGG pathway class distribution (Figure S1C).

In the GO term analysis, we identified several significantly enriched terms. In the BP category, key terms included cell cycle, cell division, and nuclear division, highlighting these IRGs' roles in essential cellular processes (Figure S2A). In the CC category, enriched terms like chromosome, microtubule, cytoskeleton, and kinetochore indicated the localization and interaction of these IRGs within specific cellular structures (Figure S2B). Lastly, the MF category showed that carbohydrate derivative binding, nucleotide binding, and nucleoside phosphate binding were the most enriched terms, emphasizing the IRGs' roles in molecular interactions and binding (Figure S2C).

These pathway and GO term analyses offer insights into the biological roles of CD8+T cell-related prognostic IRGs in gliomas, indicating their involvement in key cellular processes, specific cellular structures, and molecular interactions.

Construction and validation of a CD8+T cell-related prognostic risk signature model

By utilizing the STRING database, we extracted protein-protein interaction information for the 110 candidate IRGs. To identify hub IRGs, we employed five algorithms (Degree, MNC, DMNC, EPC, and MCC) from the cytoHubba plug-in in Cytoscape software, which determined 13 hub CD8+T cell-related IRGs, as depicted in Fig. 5A. To further investigate the prognostic relevance of these 13 IRGs in glioma patients, we employed LASSO-Cox regression with tenfold cross-validation. This approach allowed us to determine the optimal lambda value, which was obtained from the minimum partial likelihood deviance (Fig. 5B-C). Subsequently, we explored the 7-gene signature model (including KIF11, RRM2, KIF20A, CDC20, CDCA5, PBK, and KIF4A) in three different cohorts: TCGA, REMBRANDT, and GRAVENDEEL (Fig. 6D-F, G-I, and J-L, respectively). Kaplan-Meier survival analysis of the 7-IRG signature revealed a significant association between higher risk scores and worse survival in all three cohorts: TCGA, REMBRANDT, and GRAVENDEEL (Fig. 6E, H, and K, respectively). To evaluate the predictive efficiency of the risk score, we performed ROC curve analysis using data from the TCGA cohort (Fig. 5F). Additionally, we validated the predictive effect in the REMBRANDT and GRAVENDEEL cohorts (Fig. 6I and L, respectively). The results demonstrated that the AUC ranged from 0.86 to 0.94 at the 1-year stage, 0.74 to 0.89 at the 3-year stage, and 0.86 to 0.95 at the 5-year stage in the TCGA cohort, indicating the reliable predictive ability of the 7-IRG risk score for glioma prognosis (Fig. 5F). Similarly, the REMBRANDT and GRAVENDEEL cohorts also confirmed the efficacy of the 7-IRG risk score as an indicator for glioma prognosis (Fig. 6I and L).

Utilizing the risk score derived from our prognostic signature, along with other clinicopathological indicators of patients, we developed a nomogram that provides a comprehensive prediction of patient survival at 1-year, 3-year, and 5-year intervals (Fig. 6A). This nomogram serves as a valuable tool for clinicians to assess the likelihood of patient survival based on multiple factors. To assess the performance of the nomogram, we conducted a calibration curve analysis. The results demonstrated the reliable performance of our nomogram in predicting patient survival (Fig. 6B). The calibration curve provides a visual representation of the agreement between the predicted survival probabilities and the actual observed survival rates. The close alignment between the predicted and observed outcomes further validates the accuracy and reliability of our nomogram. The incorporation of the risk score derived from our prognostic signature, along with other clinicopathological indicators, into the nomogram enhances its predictive power. By considering multiple factors simultaneously, clinicians can obtain a more comprehensive understanding of patient prognosis.

These findings highlight the potential of the 7-IRG risk signature as a prognostic indicator for gliomas. The robust association between higher risk scores and worse survival suggests the clinical relevance of these IRGs in predicting patient outcomes. The consistent results across multiple cohorts further validate the reliability and generalizability of the risk score. This information could potentially aid in personalized treatment strategies and improve patient management in glioma cases.

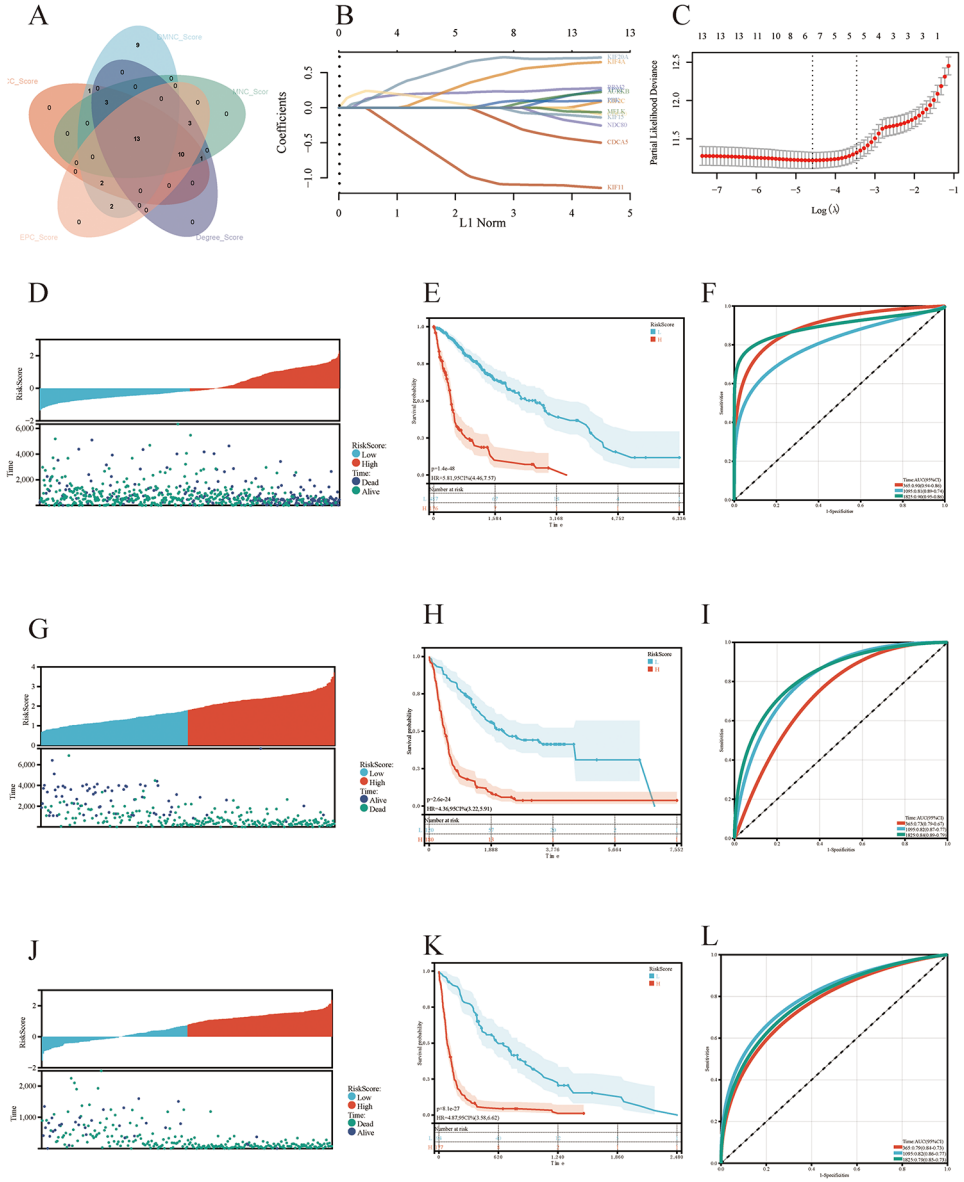

Fig. 5 Identification of hub IRGs and development of a survival predictor model based on these hub IRGs. (**A**) Five algorithms (Degree, MNC, DMNC, EPC, and MCC) from the cytoHubba plug-in were utilized in Cytoscape software to identify hub IRGs. (**B**-**C**) absolute shrinkage and selection operator (LASSO) analysis performed on 13 hub IRGs. This analysis involved determining the log (Lambda) value for each of the 13 hub IRGs in the LASSO model and identifying the most appropriate log (Lambda) value. (**D**-**F**) Prognostic analysis of the 7-gene signature in the TCGA cohort. This analysis included the distribution of risk scores (**D**), Kaplan-Meier survival curve for overall survival (OS) in the high-risk and low-risk groups (**E**), and the 1, 3, and 5-year area under the curve (AUC) values obtained from time-dependent receiver operating characteristic (ROC) curve analysis to evaluate the prognostic performance of the risk score for OS (**F**). (**G**-**I**) Prognostic validation of the 7-gene signature in the REMBRANDT cohort. (**J**-**L**) Prognostic validation of the 7-gene signature in the GRAVENDEEL cohort

The risk signature identified has important implications for immune cells, immune function, and the immunotherapeutic landscape

To evaluate the immune infiltration in different risk groups, we employed the ESTIMATE algorithm to assess the presence of stromal and immune cells, as well as tumor purity. The results of our analysis revealed that the high-risk group exhibited significantly higher immune scores, stromal scores, and estimated scores compared with the low-risk group (Fig. 7A). Conversely, the high-risk group had lower tumor purity compared to the low-risk group (Fig. 7B). These findings suggest that the high-risk group is characterized by increased immune cell infiltration and stromal components, indicating a more active and dynamic immune microenvironment. Furthermore, our analysis included correlation studies between the risk signature and immune cell infiltration. The results revealed a positive correlation between

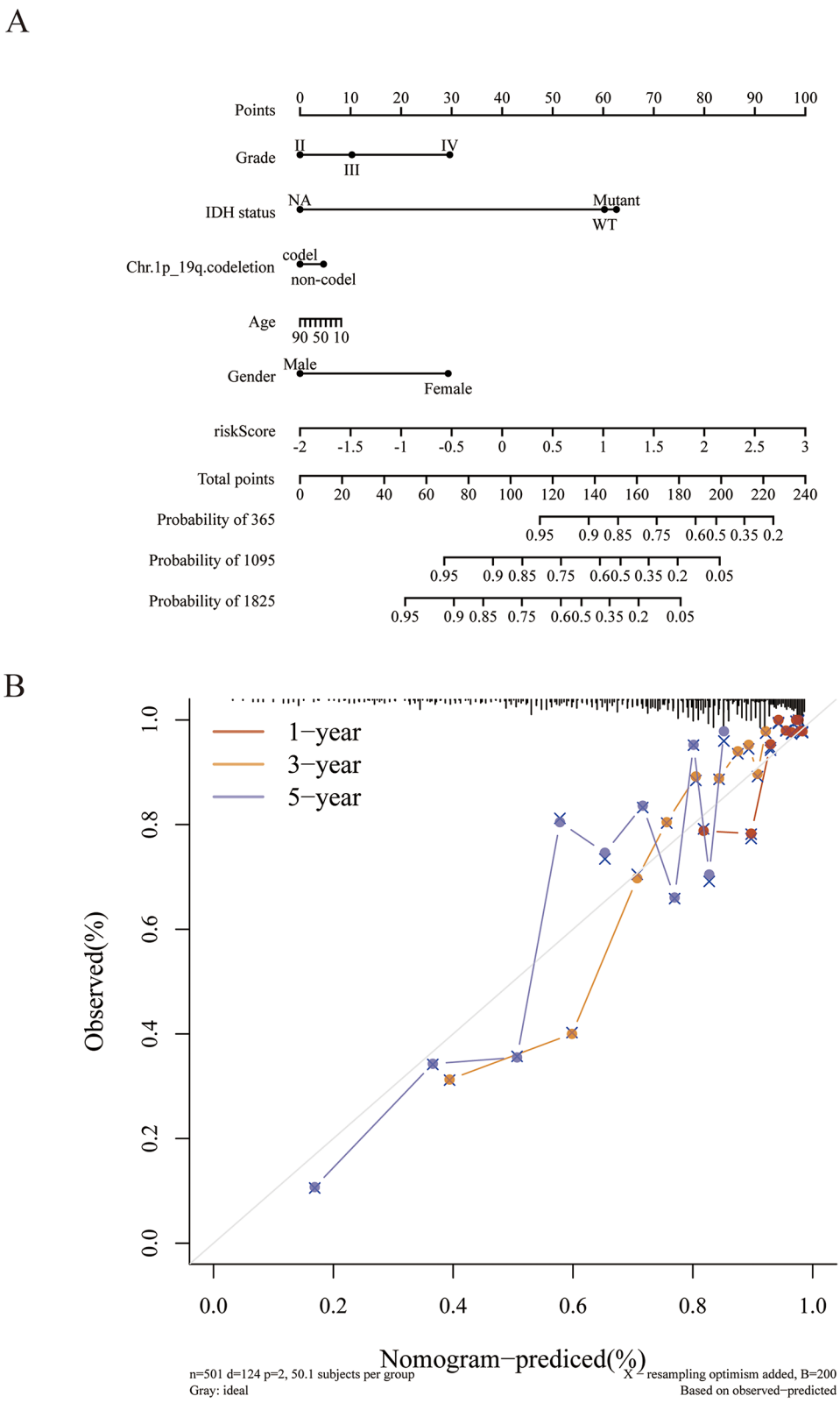

Fig. 6 Assessment of the independent prognostic value of the risk signature. (**A**) Nomograms developed for predicting the 1-year, 3-year, and 5-year survival probabilities of patient mortality based on the risk score and clinical variables, including age, gender, and grade. (**B**) The calibration curve analysis yielded results that confirmed the reliable performance of the nomogram

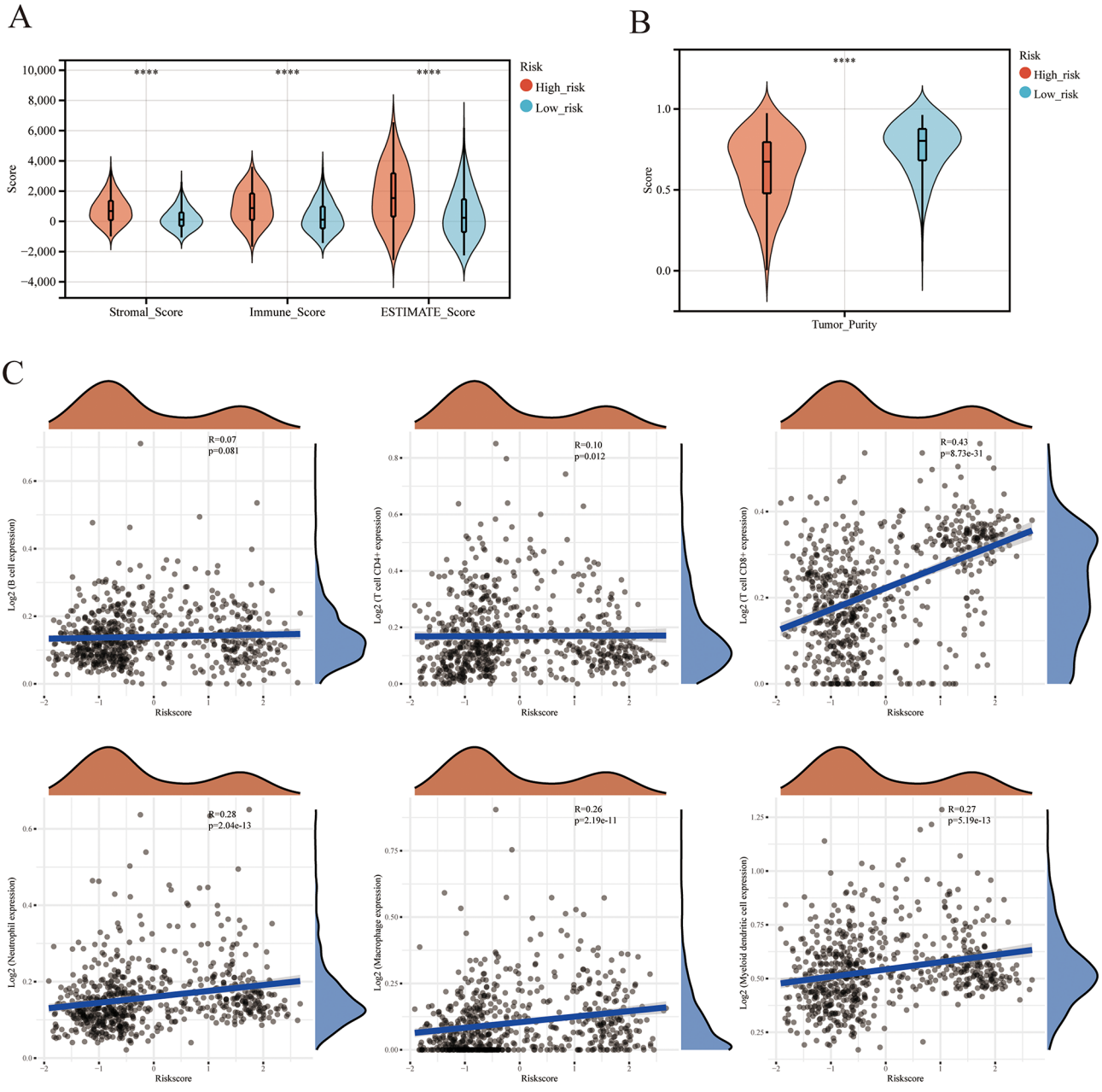

Fig. 7 Immune landscapes associated with the risk signature. (**A**-**B**) Comparison of the immune, stromal, and ESTIMATE scores (**A**) and tumor purity (**B**) between the high-risk and low-risk groups. (**C**) Spearman correlation analysis of the six immune cell scores with the risk score

the risk score and the presence of CD4+T cells, CD8+T cells, neutrophils, myeloid dendritic cells, and macrophages (Fig. 7C). However, there was no significant relationship observed between B cells and the risk score. These findings indicate that the risk signature is associated with increased infiltration of specific immune cell types, highlighting their potential role in the underlying mechanisms of disease progression and response to treatment.

Considering the crucial role of ICD modulators and ICPs in cancer immunity and the effectiveness of mRNA vaccines, we proceeded to examine the expression levels of 25 ICD modulators (Fig. 8A) and 47 ICPs (Fig. 8B) across different risk groups. Our analysis revealed significant differences in gene expression between the two groups. Notably, many well-documented genes, such as CXCL10, IFNAR2, and TLR3 (ICD modulators), as well as CD160, CD44, and PDCD1 (ICPs), exhibited differential expression. These findings hold promise for the discovery of novel targets for immunotherapy, potentially expanding the repertoire of therapeutic options available. Expanding our investigation into immunotherapy-related

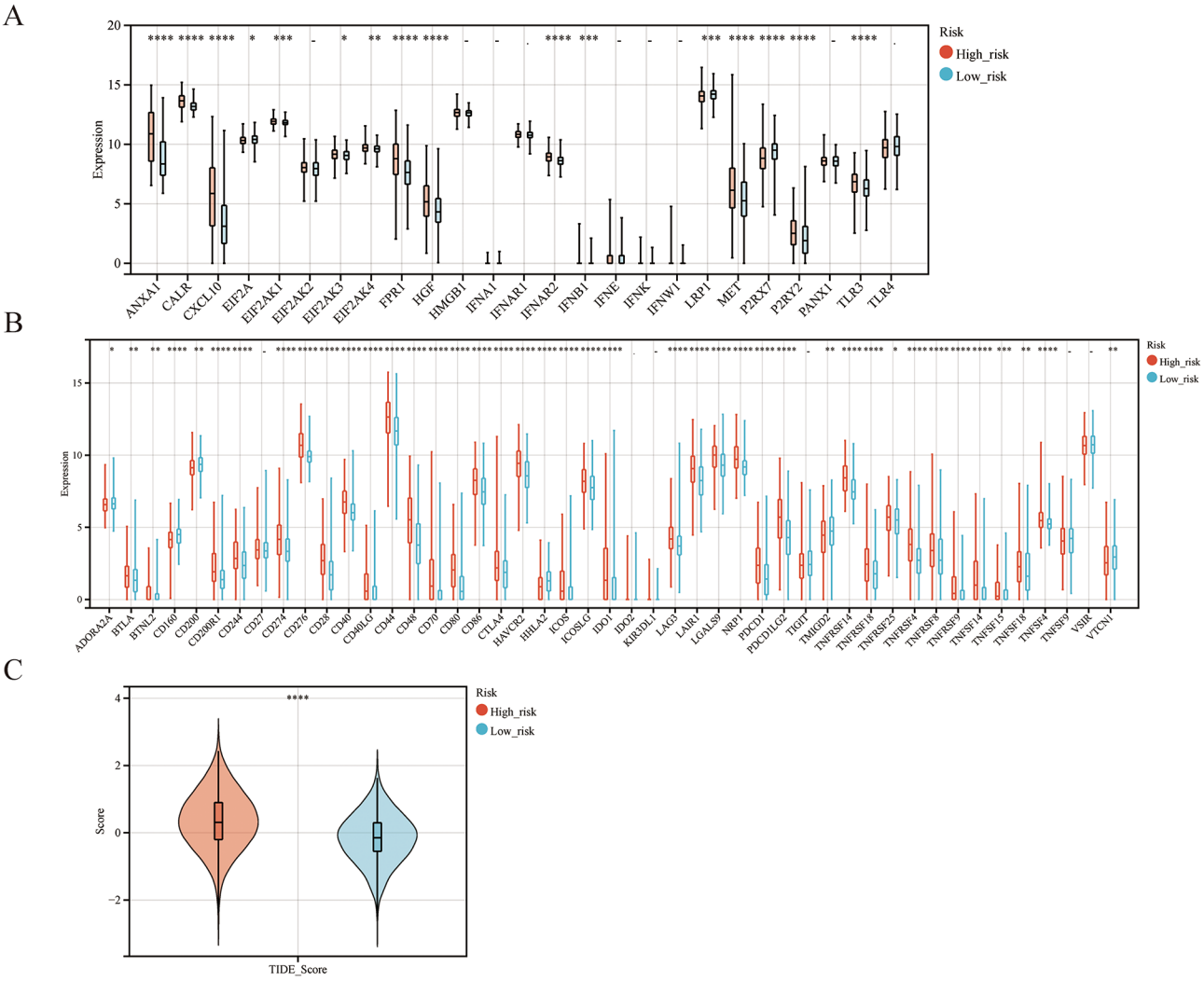

Fig. 8 Comparison analysis of the risk signature of immunogenic cell death (ICD) modulators, immune checkpoint inhibitors (ICPs), and immune checkpoint blockade (ICB) responses. (**A**, **B**) Comparison of ICD modulators (**A**) and ICPs (**B**) between the high-risk and low-risk groups. (**C**) TIDE scores of the high-risk and low-risk groups. Statistical significance was determined as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns (no significance). TIDE stands for Tumor Immune Dysfunction and Exclusion

aspects, we assessed the TIDE scores in the high-risk and low-risk groups (Fig. 8C). Interestingly, we observed higher TIDE scores in the high-risk group compared to the low-risk group. This suggests that patients in the high-risk group may be more susceptible to immune evasion mechanisms, potentially leading to reduced efficacy of immunotherapy. These findings underscore the importance of considering risk stratification when designing immunotherapeutic strategies and highlight the need for further research to enhance the effectiveness of immunotherapy in high-risk patients.

Prognostic value of 7 IRGs in gliomas

Following the identification of CD8 + T cell-related candidate IRGs and the construction of a prognostic signature, we conducted further analysis to examine the expression patterns of these signature genes in glioma. Our findings revealed that the RNA expression levels of KIF11, RRM2, KIF20A, CDC20, CDCA5, PBK, and KIF4A were significantly upregulated in both tumor samples (Fig. 9A) and the high-risk group (Fig. 9B). This suggests that these genes may play a crucial role in the progression of glioma, potentially serving as key regulators in the disease pathway.

To explore the relationship between the expression of the seven hub IRGs and OS in patients with gliomas from the TCGA cohort, we performed univariate and multivariate Cox regression analyses. These analyses considered the prognostic value of age, gender, and grade as covariates (Fig. 9C-D). The results demonstrated that the expression levels of three candidate IRGs, CDCA5, KIF11, and KIF4A, were significantly associated with

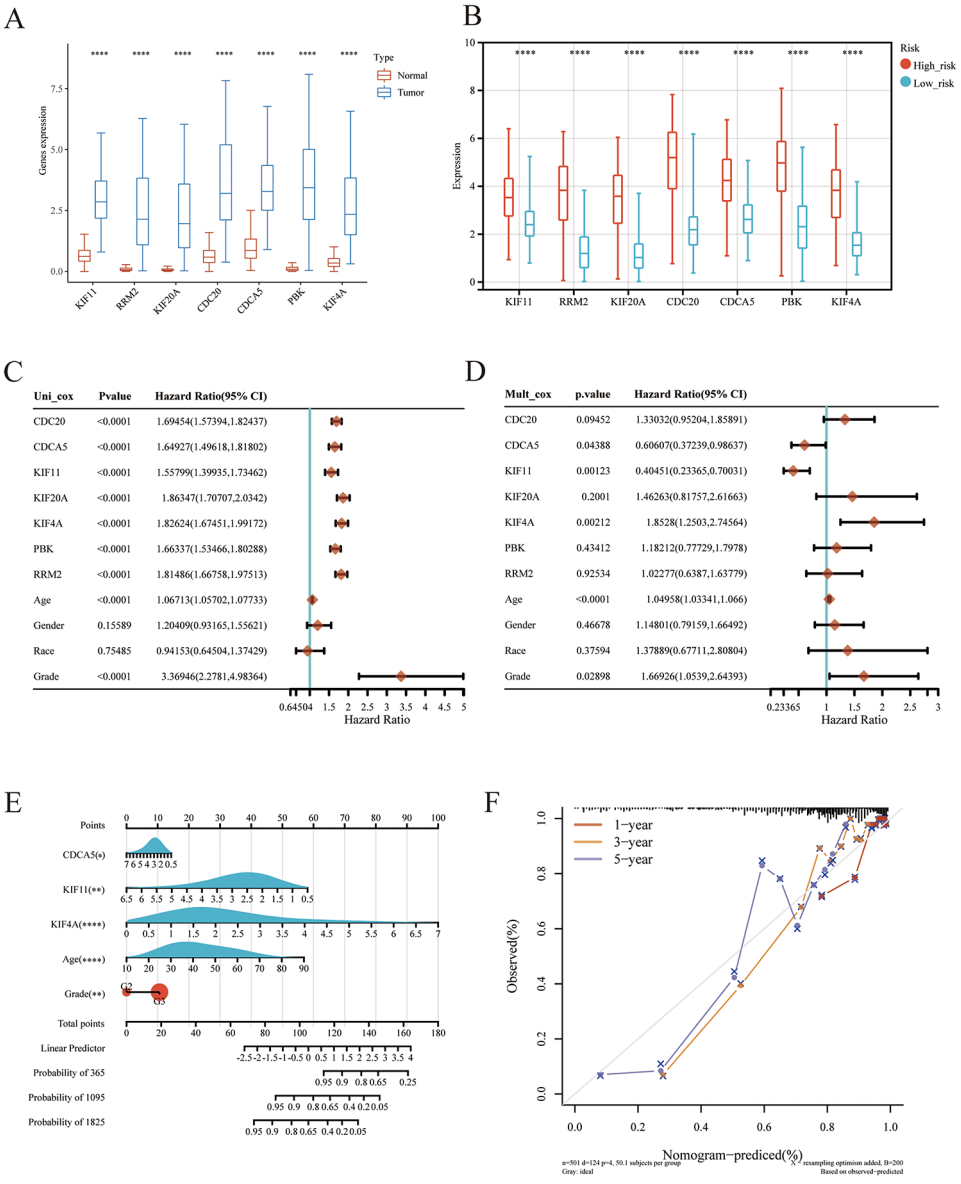

Fig. 9 Prognostic significance of hub IRGs in gliomas. (**A**-**B**) Expression analysis of hub IRGs in normal and tumor tissues (**A**), and between high-risk and low-risk groups (**B**). (**C**-**D**) Univariate (**C**) and multivariate (**D**) Cox regression analyses of the seven hub IRGs in gliomas. (**E**) Nomograms developed for predicting the 1-year, 3-year, and 5-year survival probabilities of patient mortality based on the results of multivariate Cox regression and clinical variables. (**F**) Plots depicting the calibration of the nomograms based on hub IRGs in terms of the agreement between predicted and observed 1-year, 3-year, and 5-year outcomes

OS in glioma patients from the TCGA cohort (Fig. 9E). This suggests that CDCA5, KIF11, and KIF4A can serve as independent prognostic factors in gliomas, providing valuable insights into their potential as predictive biomarkers for patient outcomes. To establish a clinically dependable predictive method for evaluating the survival probability of patient mortality at different time points, we developed a prognostic nomogram. This nomogram integrated the expression signature of CDCA5, KIF11, and KIF4A, along with age, gender, and grade (Fig. 9E). To assess the accuracy of the nomogram predictions, we established calibration plots based on the expression levels of CDCA5, KIF11, and KIF4A. These plots evaluated the agreement between the predicted survival probabilities and the observed 1-year, 3-year, and 5-year outcomes (Fig. 9F). The calibration plots demonstrated good agreement, further validating the reliability and accuracy of the nomogram predictions.

Expression of CDCA5, KIF11 and KIF4A expression using Western blotting and IHC

To further validate this hypothesis, we evaluated the protein expression levels of CDCA5, KIF11(also known as Eg5), and KIF4A in human glioma tissues as well as non-tumor brain tissues. Western blot analysis indicated significantly elevated expression of CDCA5, Eg5, and KIF4A in the glioma group compared to the non-tumor group, with P-values less than 0.01, 0.05, and 0.01, respectively (Fig. 10A). Additionally, immunohistochemistry was conducted to assess the expression of these proteins, revealing higher positive areas for both CDCA5

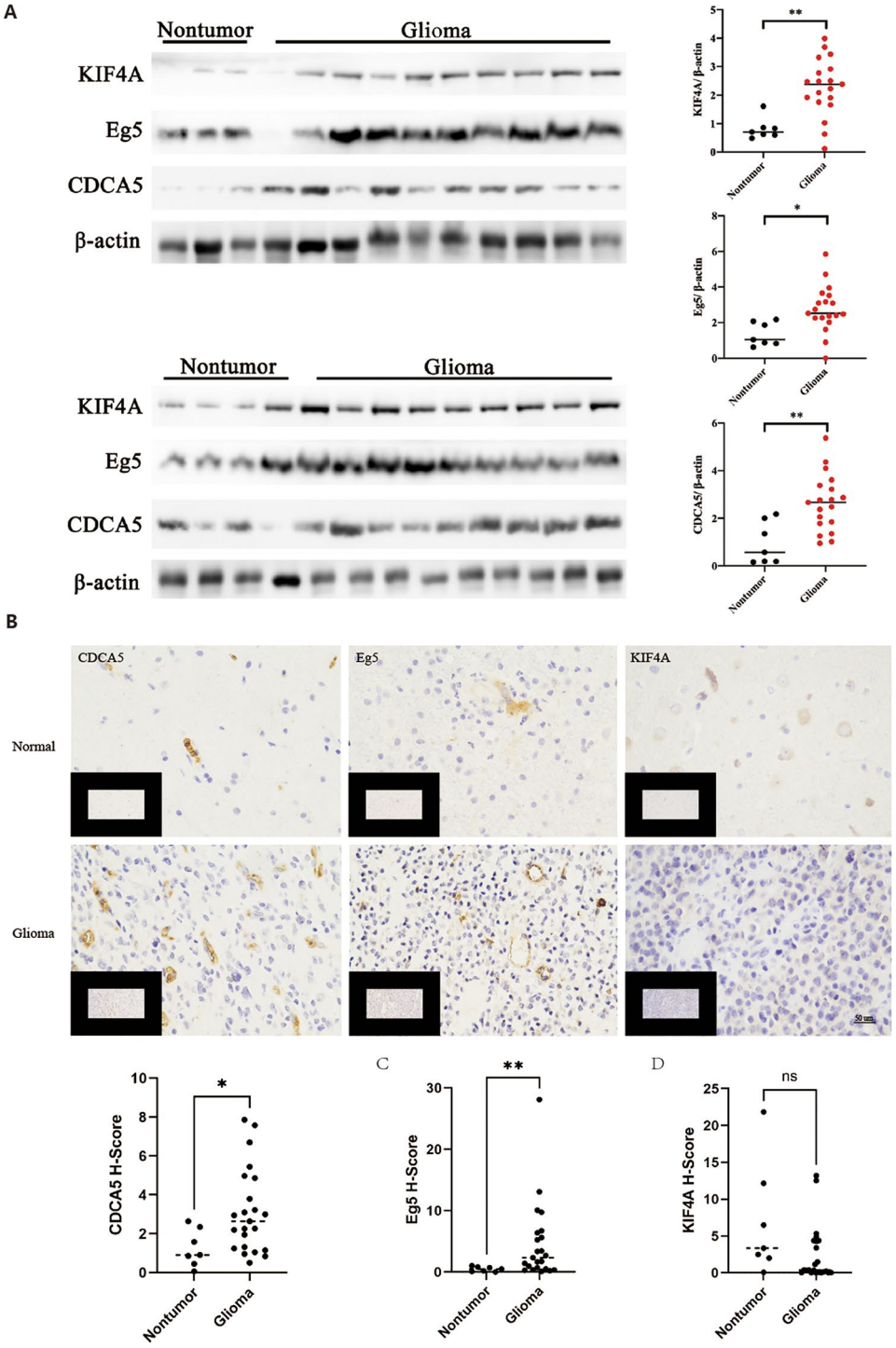

Fig. 10 Verifying the expression of CDCA5, Eg5 and KIF4A in nontumor tissue and glioma using western blotting (**A**) and IHC staining (**B**). Statistical significance was determined as follows: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns (no significance)

and Eg5 ($P<0.05$ and 0.01) in glioma samples than that in non-tumor samples. However, the expression of KIF4A shows no difference in either glioma samples nor that in non-tumor samples (Fig. 10B). These findings highlight the differential expression of CDCA5, Eg5 in glioma tissue, suggesting their potential as prognostic biomarkers for immunotherapy.

In conclusion, our study revealed the upregulation of several signature genes in glioma, suggesting their involvement in disease progression. The identification of CDCA5, KIF11, and KIF4A as independent prognostic factors highlights their potential as predictive biomarkers in gliomas. The development of a prognostic nomogram incorporating these genes, along with age, gender, and grade, provides a clinically dependable method for predicting patient survival probabilities. The calibration plots further support the accuracy of the nomogram predictions, emphasizing its potential utility in clinical practice. These findings contribute to a better understanding of glioma prognosis and offer a valuable tool for personalized treatment decision-making and patient management.

Discussion

Glioma, a highly aggressive primary tumor of the central nervous system that originates from neuroglia, poses significant challenges due to its poor prognosis and lack of effective treatment options [1, 45]. Despite these challenges, immunotherapy has emerged as a promising approach in the treatment of various types of tumors. The characterization of the TIME in gliomas is of utmost importance as it provides valuable insights into the tumor immune response and the factors that influence treatment outcomes. By understanding the intricate interactions between tumor cells and immune cells within the TIME, we can identify potential targets for immunotherapy and develop personalized treatment strategies. However, developing immunotherapy specifically targeted at gliomas is particularly challenging due to the complex nature of the TIME. Understanding the characteristics of the TIME is crucial for designing successful antitumor immunotherapies. In this study, we conducted an exploratory analysis to comprehensively characterize the TIME in gliomas. Our aim was to establish a clinically dependable predictive method for evaluating the survival probability of patients with gliomas. To achieve this, we analyzed different glioma cohorts and validated our findings to ensure their plausibility and reliability. Our findings not only deepened our understanding of the characteristics of TIME in gliomas but also provided implications for personalized immunotherapy in patients with gliomas. By establishing a clinically dependable predictive method for evaluating patient survival probability, we can better inform treatment decisions and improve patient outcomes.

Our study aimed to investigate the impact of immune cell infiltration on the survival outcomes of patients with glioma. We observed a clear distinction in survival outcomes between patients with high and low levels of immune cell infiltration. Among the six immune cell types analyzed, we found that CD8+T cell infiltration exhibited the strongest correlation with glioma patient prognosis. The presence of CD8+T cells within the tumor microenvironment has been associated with a favorable prognosis in various types of cancer. These immune cells play a crucial role in recognizing and eliminating tumor cells, thereby exerting an antitumor effect. In the context of cancer, CD8+T cells have been shown to infiltrate the tumor tissue and exert their cytotoxic activity against cancer cells. A recent study has identified immunological biomarkers that are clinically relevant and capable of distinguishing between different hypofunctional states of tumor-associated CD8+T cells [46]. The study utilized multiomics analysis to identify tumor niche-dependent exhausted and other types of hypofunctional CD8+T cell states in multiple patient cohorts and tumor types. The findings revealed that CD8+T cells in "supportive" niches, such as melanoma or lung cancer, exhibited features of tumor reactivity-driven exhaustion, while "nonsupportive" niches like glioblastoma were enriched for features of hypofunctionality that differed from canonical exhaustion. The study also highlighted the prevalence of dysfunctional CD4+: CD8+T cell interactions in glioblastoma and demonstrated that antiprogrammed cell death protein 1 (PD-1) immunotherapy facilitated glioblastoma's tolerogenic disparities, while DC vaccine partly corrected them. Overall, the study provides an atlas for evaluating different CD8+T cell hypofunctional states in immunogenic versus nonimmunogenic cancers. Our findings provide further evidence supporting the importance of immune cell infiltration, particularly CD8+T cell infiltration, in determining the survival outcomes of glioma patients. This suggests that enhancing CD8+T cell infiltration within the tumor microenvironment could potentially improve patient prognosis. These results have significant clinical implications, as they highlight the potential of immunotherapeutic strategies aimed at boosting CD8+T cell responses in glioma patients.

By intersecting the resulting 1273 DEGs with 807 CD8+T cell-related IRGs, we identified 115 candidate CD8+T cell-related IRGs. Upon further investigation, we discovered that the expression levels of 110 out of the 115 candidate IRGs were significantly correlated with survival prognosis. To gain more insight into the functional roles of these 110 candidate CD8+T cell-related prognostic IRGs, we conducted a functional

enrichment analysis. The results showed that several KEGG pathways were highly enriched, including the cell cycle, oocyte meiosis, p53 signaling pathway, and cellular senescence. In the BP category, terms such as cell cycle, cell division, and nuclear division were the most prominent. In the CC category, terms like chromosome, microtubule, cytoskeleton, and kinetochore were highly enriched. In the MF category, carbohydrate derivative binding, nucleotide binding, and nucleoside phosphate binding were among the most enriched terms. Among the enriched pathways and terms identified in our analysis, it is worth noting that some, such as the p53 signaling pathway [47–49], have been extensively studied and established as important players in glioma. The involvement of these well-known pathways further supports the validity of our findings. On the other hand, there are pathways and terms, like oocyte meiosis, that have not been extensively explored in the context of glioma. These novel findings provide intriguing insights into potential mechanisms that may contribute to glioma development and progression. Further investigation into these less-studied pathways could uncover new therapeutic targets or biomarkers for glioma. Overall, our findings provide valuable insights into the molecular mechanisms underlying gliomas. The identification of these 110 candidate CD8+T cell-related prognostic IRGs highlights their crucial roles in various cellular processes and pathways that are essential for cell proliferation, division, and survival. By understanding the involvement of these IRGs, we may gain a deeper understanding of the underlying molecular mechanisms driving gliomas. This knowledge could potentially pave the way for the development of novel therapeutic strategies aimed at targeting these specific pathways and improving the prognosis for patients with gliomas.

Following LASSO-Cox regression analysis, we identified 7 prognosis-related genes (KIF11, RRM2, KIF20A, CDC20, CDCA5, PBK, and KIF4A) for the construction of a prognostic signature in glioma. Among these genes, some have been extensively studied in the context of glioma, while others have not received much attention. For instance, KIF11 has been reported to play a crucial role in promoting tumor stemness and drug resistance in TP53 mutant glioma [50]. Studies have shown that upregulation of KIF11 contributes to the aggressive behavior of glioma cells. Similarly, RRM2 has been implicated in glioma progression, as its knockdown resulted in the upregulation of genes involved in apoptosis, proliferation, cell adhesion, and negative regulation of signaling pathways [51]. In the case of KIF20A, inhibition of this gene has been shown to induce significant apoptosis in glioma cells, suggesting its potential as a therapeutic target [52]. CDC20 overexpression has been linked to temozolomide-resistant glioma cells with epithelial-mesenchymal transition, highlighting its involvement in drug resistance mechanisms [53]. The role of PBK/TOPK in glioma has been investigated, demonstrating that targeting this gene can decrease the growth and survival of glioma initiating cells in vitro and attenuate tumor growth in vivo [54]. Additionally, KIF4A has been found to drive glioma growth by transcriptionally repressing Rac1/Cdc42, leading to cytoskeletal remodeling in glioma cells [55]. However, limited research has been conducted on the involvement of CDCA5 in glioma progression. To the best of our knowledge, no reports have specifically addressed the role of CDCA5 in glioma. This highlights the need for further investigation into the potential contribution of CDCA5 to glioma development and progression. In summary, our findings highlight the significance of these prognosis-related genes in glioma. While some genes have been extensively studied and their roles in glioma well established, others represent novel targets for future research.

Based 7-IRGs signature model, we assessed the predictive efficiency of the risk score and calculated the risk scores of patients based on our prognostic signature. We then divided the patients into high- and low-risk groups using the best cutoff values. Our results showed that patients in the high-risk group had a significantly worse prognosis than those in the low-risk group. The C-index, univariate analyses, multivariate analyses, and ROC curves all demonstrated that our signature could independently predict the prognosis of glioma patients and had a promising performance in the training set. We also validated the prognostic signature in the REMBRANDT and GRAVENDEEL cohorts, demonstrating its general applicability and validity. Based on the signature-related risk scores and clinicopathological indicators of patients, we constructed a nomogram to provide a comprehensive evaluation of patient prognosis from multiple aspects. In general, the high mortality rate and unfavorable prognosis of glioma in cancer significantly impact both family and public health systems. In response to this challenge, researchers have increasingly focused on developing diverse prognostic signatures specifically tailored for glioma patients. Zhao et al. identified a neuregulin-related TIME that can be used to predict the prognosis of gliomas. Specifically, they found that neuregulin 3 was a potential independent biomarker for predicting prognosis in LGG, while neuregulin 1 showed promise as an independent biomarker for GBM [56]. Lin et al. investigated the prognostic and immunotherapy-related biomarkers, as well as the TIME characteristics, in LGG based on mutational profiling. Through univariate and multivariate Cox regression analyses, the CIC gene was identified as a potential biomarker, and a nomogram model was established to assess the prognostic value of CIC in LGG [57]. Zhang et al. identified

fibroblast-related genes with prognostic significance and developed a novel risk signature that can assess the prognosis of glioma patients [58]. Zi et al. conducted a comprehensive bioinformatic analysis based on the gasdermin family, revealing the significant role of gasdermin family members in glioma. They constructed a prognostic algorithm comprising four genes and identified the lncRNA/miR-296-5p/GSDMD regulatory axis as a key player in glioma progression [59]. These studies have made significant advancements in refining the prediction of prognosis for patients with glioma by considering various perspectives. The models developed in these studies have demonstrated high efficacy in accurately predicting patient outcomes.

In addition to effectively predicting the prognosis of glioma patients, our prognostic signature also revealed associations between risk grouping and the immune landscape and response to immunotherapy. Our analysis showed that the high-risk group had significantly higher immune scores, stromal scores, and estimated scores compared with the low-risk group, while the high-risk group had lower tumor purity. We also observed a positive correlation between the risk score and the presence of CD4+T cells, CD8+T cells, neutrophils, myeloid dendritic cells, and macrophages, but no significant relationship with B cells. Furthermore, we observed differential expression of ICD modulators and ICPs in the two groups, and higher TIDE scores in the high-risk group compared to the low-risk group. These findings suggest that our prognostic signature may be useful in predicting the response to immunotherapy in glioma patients.

This study identified elevated expression levels of CDCA5, KIF11, and KIF4A in glioma samples from Chinese patients. However, there are several limitations that must be considered. One primary limitation is the lack of elucidation regarding the regulatory mechanisms that govern the overexpression of these genes, which constrains our understanding of their roles in glioma pathogenesis. Additionally, we failed to build up a prognostic model based on the IRGs due to our insufficient capacity and limited time. Furthermore, the study did not investigate the functional implications of the overexpression of these genes, leaving a gap in understanding their exact contributions to glioma development and progression.

Future research should aim to address these limitations through several approaches. First, detailed investigations into the regulatory mechanisms that lead to the overexpression of CDCA5, KIF11, and KIF4A are needed. This could involve exploring transcriptional, post-transcriptional, and epigenetic factors that influence gene expression in gliomas. Second, build up a prognostics model according to the expression levels of identified IRGs. Third, functional studies are essential to determine the specific roles of these genes in glioma progression. This could include experiments such as gene knockdown or overexpression studies in glioma cell lines or animal models to observe the resultant phenotypic changes. Finally, exploring potential therapeutic interventions that target these genes could provide valuable insights into new treatment strategies for glioma, improving outcomes for patients across diverse populations.

In conclusion, our study shed light on the complex nature of the TIME in gliomas and provided valuable insights for the development of personalized immunotherapy approaches. By further understanding the characteristics of the TIME, we can optimize treatment strategies and improve the prognosis of patients with gliomas.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12935-024-03517-9.

Supplementary Material 1

Supplementary Material 2

Acknowledgements
We thank the TCGA database and GlioVis database for providing free and public data sets.

Author contributions
Data curation, Jianqiang Liu and Ni Zhang; Formal analysis, Ni Zhang and Dexiang Zhou; Writing – original draft, Xiaofang Lin; Writing – review & editing, Yakang Liu and Dexiang Zhou. All authors reviewed and approved the final manuscript.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

Received: 7 July 2024 / Accepted: 25 September 2024
Published online: 01 October 2024

References

1. Ostrom QT, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C, et al. CBTRUS Statistical Report: primary brain and other Central Nervous System tumors diagnosed in the United States in 2012–2016. Neurooncology. 2019;21(Suppl 5):v1–100.
2. Bleeker FE, Molenaar RJ, Leenstra S. Recent advances in the molecular understanding of glioblastoma. J Neurooncol. 2012;108(1):11–27.
3. Ostrom QT, Gittleman H, Xu J, Kromer C, Wolinsky Y, Kruchko C, et al. CBTRUS Statistical Report: primary brain and other Central Nervous System tumors diagnosed in the United States in 2009–2013. Neurooncology. 2016;18(suppl5):v1–75.
4. Chen R, Smith-Cohn M, Cohen AL, Colman H. Glioma subclassifications and their clinical significance. Neurotherapeutics: J Am Soc Experimental Neurother. 2017;14(2):284–97.
5. Xu S, Tang L, Li X, Fan F, Liu Z. Immunotherapy for glioma: current management and future applications. Cancer Lett. 2020;476:1–12.

6. Goldbrunner R, Ruge M, Kocher M, Lucas CW, Galldiks N, Grau S. The treatment of Gliomas in Adulthood. Deutsches Arzteblatt Int. 2018;115(20–21):356–64.
7. Katsuta E, Rashid OM, Takabe K. Clinical relevance of tumor microenvironment: immune cells, vessels, and mouse models. Hum Cell. 2020;33(4):930–7.
8. Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y, et al. Role of tumor microenvironment in tumorigenesis. J Cancer. 2017;8(5):761–73.
9. Lv B, Wang Y, Ma D, Cheng W, Liu J, Yong T, et al. Immunotherapy: reshape the Tumor Immune Microenvironment. Front Immunol. 2022;13:844142.
10. Chen Y, Feng Y, Yan F, Zhao Y, Zhao H, Guo Y. A Novel Immune-related gene signature to identify the Tumor Microenvironment and Prognose Disease among patients with oral squamous cell carcinoma patients using ssGSEA: a Bioinformatics and Biological Validation Study. Front Immunol. 2022;13:922195.
11. Sun S, Guo W, Wang Z, Wang X, Zhang G, Zhang H, et al. Development and validation of an immune-related prognostic signature in lung adenocarcinoma. Cancer Med. 2020;9(16):5960–75.
12. Dai Y, Qiang W, Lin K, Gui Y, Lan X, Wang D. An immune-related gene signature for predicting survival and immunotherapy efficacy in hepatocellular carcinoma. Cancer Immunol Immunotherapy: CII. 2021;70(4):967–79.
13. Lyu F, Li Y, Yan Z, He Q, Cheng L, Zhang P, et al. Identification of ISG15 and ZFP36 as novel hypoxia- and immune-related gene signatures contributing to a new perspective for the treatment of prostate cancer by bioinformatics and experimental verification. J Translational Med. 2022;20(1):202.
14. Fernandez-Martinez A, Pascual T, Singh B, Nuciforo P, Rashid NU, Ballman KV, et al. Prognostic and predictive value of Immune-related gene expression signatures vs Tumor-infiltrating lymphocytes in early-stage ERBB2/HER2-Positive breast Cancer: a correlative analysis of the CALGB 40601 and PAMELA Trials. JAMA Oncol. 2023;9(4):490–9.
15. Mao X, Xu J, Wang W, Liang C, Hua J, Liu J, et al. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. Mol Cancer. 2021;20(1):131.
16. Marozzi M, Parnigoni A, Negri A, Viola M, Vigetti D, Passi A et al. Inflammation, Extracellular Matrix Remodeling, and Proteostasis in Tumor Microenvironment. Int J Mol Sci. 2021;22(15).
17. Schönherr E, Hausser HJ. Extracellular matrix and cytokines: a functional unit. Dev Immunol. 2000;7(2–4):89–101.
18. da Silva PHR, Borges BC, Uehara IA, Soldi LR, de Araújo RA, Silva MJB. Chemokines and extracellular matrix: set of targets for tumor development and treatment. Cytokine. 2021;144:155548.
19. Gong Z, Li Q, Shi J, Wei J, Li P, Chang CH, et al. Lung fibroblasts facilitate pre-metastatic niche formation by remodeling the local immune microenvironment. Immunity. 2022;55(8):1483–e5009.
20. Desbois M, Wang Y. Cancer-associated fibroblasts: Key players in shaping the tumor immune microenvironment. Immunol Rev. 2021;302(1):241–58.
21. Martinez M, Moon EK. CAR T cells in solid tumors: new strategies for finding, infiltrating, and surviving in the Tumor Microenvironment. Front Immunol. 2019;10:128.
22. Tao W, Chu C, Zhou W, Huang Z, Zhai K, Fang X, et al. Dual role of WISP1 in maintaining glioma stem cells and tumor-supportive macrophages in glioblastoma. Nat Commun. 2020;11(1):3015.
23. Elia I, Rowe JH, Johnson S, Joshi S, Notarangelo G, Kurmi K, et al. Tumor cells dictate anti-tumor immune responses by altering pyruvate utilization and succinate signaling in CD8(+) T cells. Cell Metab. 2022;34(8):1137–e506.
24. Lei X, Lei Y, Li JK, Du WX, Li RG, Yang J, et al. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. Cancer Lett. 2020;470:126–33.
25. Viallard C, Larrivée B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis. 2017;20(4):409–26.
26. Lamplugh Z, Fan Y. Vascular microenvironment, Tumor Immunity and Immunotherapy. Front Immunol. 2021;12:811485.
27. Protopsaltis NJ, Liang W, Nudleman E, Ferrara N. Interleukin-22 promotes tumor angiogenesis. Angiogenesis. 2019;22(2):311–23.
28. Paweletz N, Knierim M. Tumor-related angiogenesis. Crit Rev Oncol/Hematol. 1989;9(3):197–242.
29. Liu Y, Dai S, Wen L, Zhu Y, Tan Y, Qiu G, et al. Enhancing drug delivery for overcoming angiogenesis and improving the Phototherapy Efficacy of Glioblastoma by ICG-Loaded Glycolipid-Like Micelles. Int J Nanomed. 2020;15:2717–32.
30. Pansy K, Uhl B, Krstic J, Szmyra M, Fechter K, Santiso A et al. Immune Regulatory processes of the Tumor Microenvironment under Malignant conditions. Int J Mol Sci. 2021;22(24).
31. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013;14(10):1014–22.
32. Zheng Z, Zhang J, Jiang J, He Y, Zhang W, Mo X et al. Remodeling tumor immune microenvironment (TIME) for glioma therapy using multitargeting liposomal codelivery. J Immunother Cancer. 2020;8(2).
33. 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.
34. Kong Y, Feng ZC, Zhang YL, Liu XF, Ma Y, Zhao ZM, et al. Identification of Immune-related genes contributing to the development of Glioblastoma Using Weighted Gene Co-expression Network Analysis. Front Immunol. 2020;11:1281.
35. Wang N, Zhu L, Wang L, Shen Z, Huang X. Identification of SHCBP1 as a potential biomarker involving diagnosis, prognosis, and tumor immune microenvironment across multiple cancers. Comput Struct Biotechnol J. 2022;20:3106–19.
36. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res. 2020;48(W1):W509–14.
37. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, et al. Inferred tumour purity and stromal and immune cell admixture from expression data. Nat Commun. 2013;4:2612.
38. Huang X, Tang T, Zhang G, Liang T. Identification of tumor antigens and immune subtypes of cholangiocarcinoma for mRNA vaccine development. Mol Cancer. 2021;20(1):50.
39. Langfelder P, Horvath S. WGCNA: R package for weighted correlation network analysis. BMC Bioinf. 2008;9:559.
40. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma power differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
41. Yu G, Wang LG, Han Y. Clusterprofiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16(5):284–7.
42. Tibshirani R. The lasso method for variable selection in the Cox model. Stat Med. 1997;16(4):385–95.
43. Engebretsen S, Bohlin J. Statistical predictions with glmnet. Clin Epigenetics. 2019;11(1):123.
44. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med. 2018;24(10):1550–8.
45. Lapointe S, Perry A, Butowski NA. Primary brain tumours in adults. Lancet (London England). 2018;392(10145):432–46.
46. Naulaerts S, Datsi A, Borras DM, Antoranz Martinez A, Messiaen J, Vanmeerbeek I, et al. Multiomics and spatial mapping characterizing human CD8(+) T cell states in cancer. Sci Transl Med. 2023;15(691):eadd1016.
47. Sheng J, He X, Yu W, Chen Y, Long Y, Wang K, et al. p53-targeted lncRNA ST7-AS1 acts as a tumour suppressor by interacting with PTBP1 to suppress the Wnt/β-catenin signalling pathway in glioma. Cancer Lett. 2021;503:54–68.
48. Hu S, Cai J, Fang H, Chen Z, Zhang J, Cai R. RPS14 promotes the development and progression of glioma via the p53 signaling pathway. Exp Cell Res. 2023;423(1):113451.
49. Ge X, Xu M, Cheng T, Hu N, Sun P, Lu B, et al. TP53I13 promotes metastasis in glioma via macrophages, neutrophils, and fibroblasts and is a potential prognostic biomarker. Front Immunol. 2022;13:974346.
50. Liu B, Zhang G, Cui S, Du G. Upregulation of KIF11 in TP53 mutant glioma promotes Tumor Stemness and Drug Resistance. Cell Mol Neurobiol. 2022;42(5):1477–85.
51. Sun H, Yang B, Zhang H, Song J, Zhang Y, Xing J, et al. RRM2 is a potential prognostic biomarker with functional significance in glioma. Int J Biol Sci. 2019;15(3):533–43.
52. Saito K, Ohta S, Kawakami Y, Yoshida K, Toda M. Functional analysis of KIF20A, a potential immunotherapeutic target for glioma. J Neurooncol. 2017;132(1):63–74.
53. Wang J, Zhou F, Li Y, Li Q, Wu Z, Yu L, et al. Cdc20 overexpression is involved in temozolomide-resistant glioma cells to epithelial-mesenchymal transition. Cell Cycle (Georgetown Tex). 2017;16(24):2355–65.
54. Joel M, Mughal AA, Grieg Z, Murrell W, Palmero S, Mikkelsen B, et al. Targeting PBK/TOPK decreases growth and survival of glioma initiating cells in vitro and attenuates tumor growth in vivo. Mol Cancer. 2015;14:121.
55. Zhang H, Meng S, Chu K, Chu S, Fan YC, Bai J, et al. KIF4A drives glioma growth by transcriptional repression of Rac1/Cdc42 to induce cytoskeletal remodeling in glioma cells. J Cancer. 2022;13(15):3640–51.

56. Zhao WJ, Ou GY, Lin WW. Integrative Analysis of Neuregulin Family members-related Tumor Microenvironment for Predicting the Prognosis in Gliomas. Front Immunol. 2021;12:682415.
57. Lin WW, Ou GY, Zhao WJ. Mutational profiling of low-grade gliomas identifies prognosis and immunotherapy-related biomarkers and tumour immune microenvironment characteristics. J Cell Mol Med. 2021;25(21):10111–25.
58. Zhang H, Huang Y, Yang E, Gao X, Zou P, Sun J et al. Identification of a fibroblast-related Prognostic Model in Glioma based on Bioinformatics methods. Biomolecules. 2022;12(11).
59. Zi H, Tuo Z, He Q, Meng J, Hu Y, Li Y, et al. Compr Bioinf Anal Gasdermin Family Glioma Comput Intell Neurosci. 2022;2022:9046507.

Publisher's note