



IL-32 as a prognostic biomarker and therapeutic target in glioma: implications for tumor progression and immune modulation

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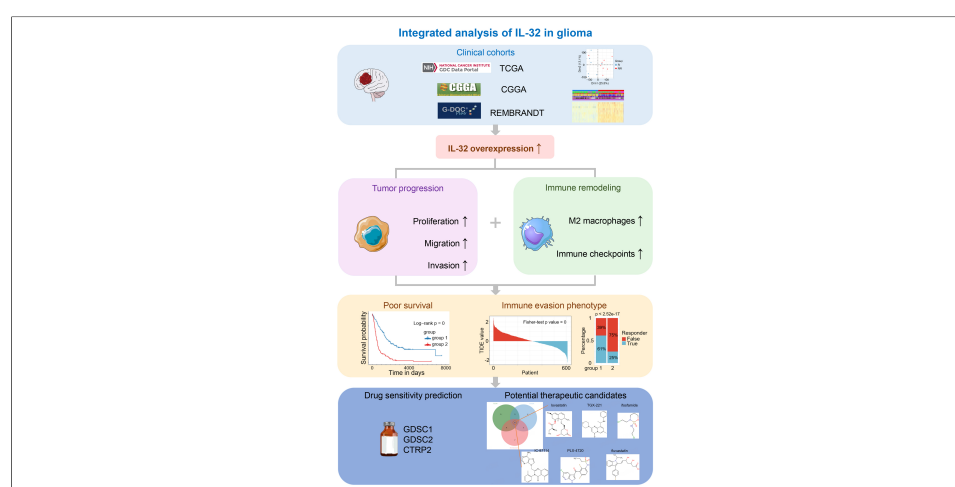
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Abstract

Aim: Interleukin-32 (IL-32), a pro-inflammatory cytokine, has been implicated in tumor progression and immune modulation. However, its function in glioma is still unclear. This study analyzed the expression of IL-32 in glioma, its prognostic significance, and its potential as a therapeutic target.

Methods: We analyzed transcriptional and clinical data from glioma patients across multiple datasets, including The Cancer Genome Atlas (TCGA), Chinese Glioma Genome Atlas (CGGA), and REMBRANDT, to explore IL-32 expression patterns, correlations with survival, immune infiltration, and drug sensitivity. A risk model was constructed and validated, and immune escape mechanisms and immunotherapy responses were

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evaluated. Experimental validation was performed using glioblastoma cell lines, employing transfection, cell proliferation assays, and Transwell assays.

Results: Elevated IL-32 expression was linked to poor overall survival and enhanced immune infiltration, particularly M2-type macrophages. Consensus clustering identified two molecular subtypes with distinct prognostic outcomes. A 10-gene IL-32-related signature (*IL13RA2*, *FCER1G*, *S100A8*, *GPNMB*, *LUM*, *ANXA1*, *EMP3*, *CHI3L1*, *PTX3*, and *IGFBP2*) demonstrated robust prognostic performance across independent cohorts and remained an independent predictor of overall survival. High-risk patients exhibited greater immune evasion and reduced predicted responsiveness to immune checkpoint inhibitors. Drug sensitivity analysis further identified six candidate compounds (lovastatin, fluvastatin, TGX-221, ifosfamide, IC-87114, and PLX-4720) that may warrant further investigation for high-risk glioma. Functional experiments further demonstrated that IL-32 promoted glioblastoma cell proliferation, migration, and invasion.

Conclusion: IL-32 serves as a prognostic biomarker in glioma, while the IL-32-related 10-gene signature enables risk stratification, predicts potential immunotherapy responsiveness, and prioritizes candidate therapeutic agents for future validation.

INTRODUCTION

As the most prevalent central nervous system tumor, glioma continues to pose a major clinical challenge due to its highly malignant nature. Despite substantial advancements in surgical resection, radiotherapy, and chemotherapy, the prognosis for glioma patients, particularly those with glioblastoma (GBM), remains exceedingly poor, with a median survival of approximately 15 months^[1]. A defining feature of glioma is its pronounced molecular and cellular heterogeneity, which underlies therapeutic resistance and tumor recurrence^[2]. Therefore, the discovery of reliable biomarkers and therapeutic candidates is crucial for improving patient stratification and enabling the development of more effective treatment strategies.

Interleukin-32 (IL-32), originally characterized as a mediator of immune and inflammatory responses, has been implicated in the pathogenesis of a broad spectrum of diseases, including autoimmune and infectious disorders^[3]. Accumulating evidence suggests that IL-32 plays a critical role in multiple cancer types, such as breast, colorectal, and lung cancer, where its expression is related to tumor progression, metastatic dissemination, and immune evasion^[4,5]. Mechanistically, IL-32 promotes tumor cell proliferation, facilitates angiogenesis, and reshapes the tumor microenvironment (TME) through interactions with immune cells^[6]. However, despite emerging evidence suggesting a potential involvement of IL-32 in glioma biology, its precise biological functions, prognostic significance, and contribution to tumor immune regulation remain incompletely characterized. Therefore, comprehensive studies integrating large-scale clinical datasets and experimental validation are needed to further clarify the role of IL-32 in glioma progression and evaluate its potential as a prognostic biomarker or therapeutic candidate.

In this study, we investigated the expression landscape, prognostic significance, and functional implications of IL-32 in glioma using comprehensive bioinformatic analyses. Using large-scale public datasets, we evaluated the relationship of IL-32 expression with survival outcomes of patients. We further characterized the immune milieu associated with IL-32 expression, with particular emphasis on macrophage infiltration and immune checkpoint (IC) regulation. Based on IL-32-associated gene signatures, a prognostic risk model incorporating IL-32-related genes was constructed to enable patient stratification and to predict potential responsiveness to immunotherapy. Furthermore, drug sensitivity analyses were performed to identify candidate therapeutic compounds for high-risk (HR) glioma patients. Collectively, our findings demonstrated IL-32's function in glioma progression and immune modulation, offering a framework for the development of personalized treatment strategies in glioma.

METHODS

Data acquisition

Transcriptional profiles and corresponding clinical data for human gliomas were retrieved from three publicly available databases: The Cancer Genome Atlas (TCGA, <https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>), the Chinese Glioma Genome Atlas (CGGA, <http://www.cgga.org.cn/>), as well as the REMBRANDT (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE108474>) dataset. The final analytical dataset comprised 702 glioma samples from TCGA, 693 from CGGA, and 475 from REMBRANDT. Additionally, RNA sequencing data from 15 patients with recurrent glioblastoma (rGBM) who received neoadjuvant anti-PD-1 immunotherapy (GSE121810, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121810>) were retrieved from the Gene Expression Omnibus (GEO) database for external evaluation of the immunotherapy response prediction model. Immunohistochemical staining data for normal brain tissue and glioma specimens were acquired from the Human Protein Atlas (<https://www.proteinatlas.org/>). Glioma subtypes were defined according to the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (4th edition).

Survival analysis

The Kaplan-Meier approach was applied to generate survival estimates, while the log-rank test served to evaluate prognostic disparities among the subgroups.

Mutation analysis

Somatic mutation data for gliomas were retrieved from TCGA and analyzed using the R package maftools (version 2.20)^[7]. The mutational landscape of the 20 most frequently altered genes, together with IL-32, was profiled and compared between gliomas stratified by IL-32 expression levels.

Immune infiltration analysis and immune checkpoints expression

Stromal and immune scores were computed for glioma samples utilizing the R package ESTIMATE (version 1.0.13) and correlated with IL-32 expression to characterize the TME. Immune cell composition was inferred from TCGA, CGGA, and REMBRANDT datasets using CIBERSORTx^[8], which deconvolutes bulk transcriptomic data based on a reference of 22 human immune cell types. This approach enabled quantitative assessment of immune cell proportions in individual gliomas, helping to dissect the link between IL-32 expression and immune infiltration patterns.

Differentially expressed genes analysis

Glioma samples were stratified into two groups according to IL-32 expression: high and low. Differentially expressed genes (DEGs) between the two groups were identified utilizing the R package limma (version 3.60.6), with thresholds of $|\log_2 \text{fold change}| > 1$ and adjusted P -value < 0.05 . A total of 51 DEGs were consistently detected across TCGA, CGGA, and REMBRANDT cohorts, which were then analyzed for their biological significance through functional enrichment.

Functional enrichment analysis

Functional enrichment of the identified DEGs was performed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses via the R package clusterProfiler (version 4.12.6). Significant enrichment in GO and KEGG analyses was determined by a corrected P -value < 0.05 .

Consensus clustering

R package ConsensusClusterPlus (version 1.68.0) was applied to the 51-DEG expression matrix, assigning each glioma specimen to one of two newly defined molecular subgroups. Cluster stability and the optimal number of clusters (K) were determined via the cumulative distribution function (CDF) plot. Principal

component analysis (PCA) was subsequently performed using the R package FactoMineR (version 2.11) to visualize variance captured by the principal components and validate subtype separation. Differential expression patterns across subtypes were illustrated with a heatmap of the 51 DEGs, confirming their biological distinctiveness. Overall survival (OS) analyses were then conducted to determine whether these subtypes hold distinct clinical significance.

Risk model development and evaluation

We developed a prognostic risk signature based on the Least Absolute Shrinkage and Selection Operator (LASSO) Cox regression algorithm via the R package glmnet (version 4.1.8). LASSO identified ten genes with non-zero coefficients from the 51 DEGs, which were used to compute a risk score for each sample as follows: Risk score = $\sum_1^n \text{Coef } i \times \text{Expression } i$. Using the median risk score as the cutoff, the TCGA glioma cohort was split into HR and low-risk (LR) groups. The independent prognostic value of the risk model was further evaluated using multivariate Cox proportional hazards regression incorporating established clinical and molecular prognostic factors, including age, WHO grade, isocitrate dehydrogenase (IDH) mutation status, 1p/19q co-deletion status, and O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status when available. The incremental predictive performance of the risk score was assessed by comparing time-dependent receiver operating characteristic (ROC) curves between the risk model and conventional clinical variables. Furthermore, decision curve analysis (DCA) was performed using the R package rmda (version 1.6) to evaluate the net clinical benefit of applying the risk model compared with traditional prognostic factors across a range of threshold probabilities. For external validation, the model was applied to the CGGA and REMBRANDT cohorts, demonstrating its robustness and clinical applicability.

Tumor immune dysfunction and exclusion analysis and immunotherapy response evaluation

Immune escape potential in glioma was evaluated using the tumor immune dysfunction and exclusion (TIDE) algorithm^[9], which predicts tumor immune evasion based on gene expression profiles. Glioma transcriptomic data were uploaded to the TIDE web portal (<http://tide.dfci.harvard.edu/>) to calculate immune-related parameters (microsatellite instability scores, PD-L1 expression, as well as myeloid-derived suppressor cell levels), enabling the estimation of immune checkpoint inhibitor (ICI) responsiveness. In addition, TIDE scores were compared between HR and LR groups using unpaired *t*-tests.

Next, we examined the risk model's relationship to immunotherapy benefit by profiling RNA-seq count data from 15 recurrent GBM patients who received neoadjuvant anti-PD-1 therapy^[10]. Patients were classified as responders [overall survival (OS) > 200 days] or non-responders (OS < 200 days), and risk scores were compared between subgroups utilizing unpaired *t*-tests ($P < 0.05$).

Drug sensitivity prediction

Drug sensitivity for individual glioma patients was predicted using the calcPhenotype function from the R package oncoPredict (version 1.2)^[11], which implements a ridge regression model trained on pharmacogenomic datasets from the Genomics of Drug Sensitivity in Cancer (GDSC1, GDSC2) and the Cancer Therapeutics Response Portal (CTRP v2). The Pearson correlation between each drug's half-maximal inhibitory concentration (IC₅₀) and the calculated risk score was computed, with $|r| > 0.50$ considered indicative of a strong association. To ensure robustness, drugs demonstrating significant correlations across the TCGA, CGGA, and REMBRANDT glioma cohorts were retained as a consensus set of candidate therapeutics.

Cell culture and transfection

The U87 and LN229 GBM cell lines, sourced from Procell (Wuhan, China), were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, ScienCell,

USA) at 37 °C under humidified conditions with 5% CO₂. U87 and LN229 cells were maintained at passages 5-10, respectively. Both cell lines were authenticated by short tandem repeat (STR) profiling (Procell, Wuhan, China) and tested negative for mycoplasma using Mycoplasma Detection Kit (Beyotime, China).

Transfections were performed using Lipofectamine 3000 (Invitrogen, USA) following the manufacturer's protocols. The reagents used in this study include siRNA (s17656, s17657, AM4611, Thermo Fisher Scientific, USA). The compounds used for IL-32 overexpression were purchased from OriGene (OR, USA). The lyophilized protein was reconstituted in sterile phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) at a concentration of 100 µg/mL as per the manufacturer's instructions, and aliquots were stored at -80 °C until use.

Cell proliferation assay

The BeyoClick EdU kit was applied to assess cellular proliferation (Cat. C0075L, Beyotime, Shanghai, China) following the manufacturer's protocol. A 20 mM working solution of Alexa Fluor 555-EdU was prepared, and cells were incubated with EdU for 1 h. Cells were then fixed with 4% paraformaldehyde for 20 min and washed three times with PBS containing 3% BSA. Permeabilization was performed with 0.1% Triton X-100 (Cat. P0096-100mL, Beyotime, Shanghai, China) for 20 min, followed by incubation with the Click-iT EdU reaction mixture for 30 min. Nuclei were counterstained with Hoechst 33342 for 5 min in the dark. Finally, images were captured using a fluorescence microscope (Olympus, Japan). The proliferation index was calculated as (EdU⁺ nuclei/total nuclei) × 100%. Three independent experiments were performed on different days with different cell passages (passages 5-10).

Transwell assay

Transwell migration assays were performed by placing 3×10^4 cells (in serum-free DMEM) into the upper insert, using 600 µL of complete medium in the lower well as the migratory stimulus. After 48 h of incubation, non-migratory cells were removed, and the inserts were washed, fixed with methanol for 20 min, and stained with 1% crystal violet for 30 min. For invasion assays, the upper chambers were pre-coated with Matrigel (Cat. 356255, BD, USA) to mimic the extracellular matrix. Migrated or invaded cells were visualized under a light microscope (Olympus, Japan), and three random fields per insert were counted using ImageJ software.

Statistical analysis

All data analyses were carried out utilizing R (version 4.4.1) and appropriate R packages as described above. Statistical significance was defined as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. All analyses adhered to established guidelines to ensure rigor, reproducibility, and reliability.

Ethics statement

This study was based on publicly available and de-identified datasets from the TCGA, CGGA, REMBRANDT, and GEO databases, including GSE121810. No new human participants were recruited and no additional human specimens were collected for this study. The original studies from which these datasets were derived obtained relevant ethical approvals and informed consent. Therefore, no additional ethical approval or informed consent was required for the present secondary analysis.

RESULTS

Evaluation of IL-32 expression and prognostic significance in glioma

To explore IL-32's function in glioma, we initially compared its mRNA levels between tumor and normal brain tissues. Relative to normal brain, IL-32 was significantly upregulated in both low-grade gliomas and GBM [Figure 1A]. Besides, its expression progressively increased with glioma WHO grade across the TCGA,

CGGA, and REMBRANDT cohorts [Figure 1B]. At the protein level, immunohistochemistry confirmed the highest IL-32 expression in GBM and minimal expression in normal cerebral cortex [Figure 1C], suggesting a correlation between IL-32 overexpression and tumor malignancy. Survival analyses demonstrated that elevated IL-32 expression was consistently linked to a worse prognosis across the three glioma cohorts [Figure 1D], supporting its potential as a prognostic biomarker. Mutation profiling of the 20 most frequently altered genes and IL-32 revealed no detectable somatic mutations in IL-32 across the TCGA glioma cohort [Figure 1E]. This finding suggests that the clinical and biological significance of IL-32 in glioma is more likely driven by transcriptional dysregulation than by recurrent genomic alterations. Together with the consistent overexpression of IL-32 across glioma grades and its association with poor prognosis, these results indicate that aberrant IL-32 expression, rather than mutation, may underlie its contribution to glioma progression. The mutation profiles are summarized in Supplementary Figure 1.

Biological function of IL-32 in glioma

To explore the biological role of IL-32 in glioma, we examined its expression across GBM subtypes in three independent cohorts. The mesenchymal subtype exhibited the highest IL-32 expression [Figure 2A], a subtype characterized by elevated tumor-associated macrophage (TAM) infiltration and an immunosuppressive TME^[12]. Consistently, higher IL-32 expression correlated positively with increased stromal and immune scores across all cohorts [Figure 2B], indicating a strong association with the immune modulation within TME. Using CIBERSORTx to deconvolute 22 immune cell types, IL-32 expression showed the strongest positive correlation with M2-type macrophages [Figure 2C], which are implicated in tumor progression and immune evasion. Gene expression profiling revealed that positively regulated DEGs in IL-32-high tumors were enriched in immune-related pathways [Figure 3A-C and Supplementary Table 1]. Based on the above results, it could be inferred that IL-32 is critical for shaping the glioma immune microenvironment, particularly through its association with M2-type TAMs and immune-related pathways, potentially contributing to immune evasion and tumor progression.

Correlation between IL-32 expression and immune checkpoints in glioma

To investigate the immunological role of IL-32 in glioma, we examined its correlation with IC genes. IL-32 was strongly and positively associated with major immune checkpoints (ICs): CD274 (PD-L1), PDCD1 (PD-1), as well as PDCD1LG2 (PD-L2) ($P < 0.05$; Figure 4). These results indicate that IL-32 plays a role in the immune modulation of the glioma TME through interactions with checkpoint-associated pathways, highlighting its potential as a regulator of immune evasion and a candidate therapeutic target.

Assessment of survival significance of intersected DEGs and glioma subtype identification

To establish a survival risk model, we first assessed the prognostic significance of the 51 DEGs consistently identified across TCGA, CGGA, and REMBRANDT cohorts [Supplementary Figure 2A]. Consensus clustering based on these DEGs divided glioma patients into two distinct molecular subtypes ($K = 2$) in all three datasets [Figure 5A], with cluster stability confirmed by CDF analysis [Supplementary Figure 2B]. PCA further validated the clustering, with the first principal component (PC1) effectively separating subtype 1 from subtype 2 [Figure 5B]. A heatmap of clinical characteristics revealed that subtype 2 exhibited higher expression of the 51 DEGs [Figure 5C] and was associated with significantly shorter OS compared with subtype 1 [Figure 5D]. These results were consistent across all cohorts, demonstrating the strong prognostic value of the 51 DEGs and establishing a robust basis for constructing a survival risk model to inform personalized therapeutic strategies in glioma.

Assessment of IL-32 in glioma cell proliferation, migration, and invasion

To evaluate the biological effects of IL-32 over a concentration-dependent range, U87 and LN229 cells were treated with recombinant human IL-32 at 0, 20, 40, and 60 ng/mL. The selected concentrations were based on previously published studies investigating IL-32-mediated regulation of macrophage function, in which

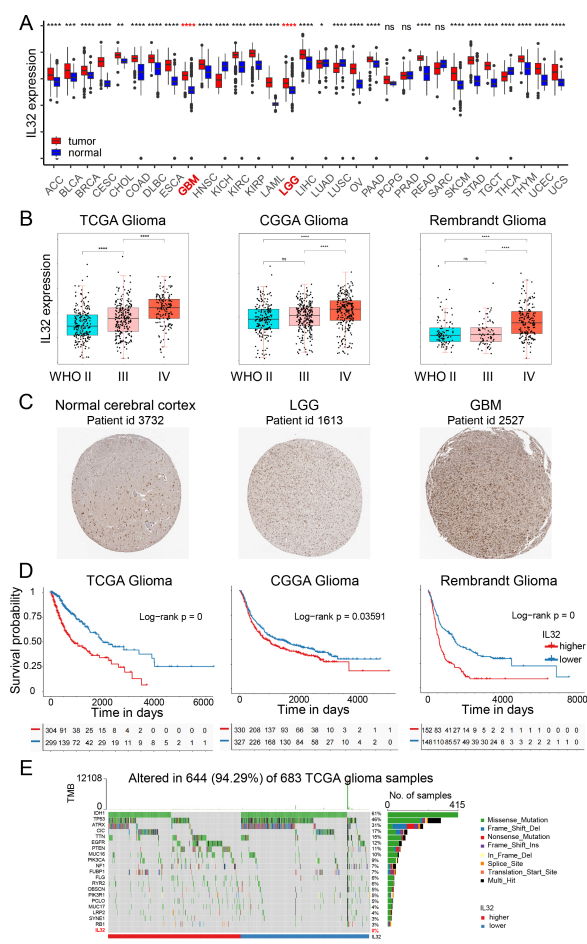


Figure 1. IL-32 expression and prognostic significance in glioma. (A) Box plots show IL-32 expression differences across various cancers and matched normal tissues. IL-32 expression is significantly higher in low-grade glioma and GBM than in normal brain tissue; (B) IL-32 mRNA levels progressively increased with WHO glioma grade across three independent datasets (TCGA, CGGA, and REMBRANDT); (C) Immunohistochemical staining confirms the highest IL-32 protein expression in GBM, with the lowest expression in normal cerebral cortex. Reproduced from the Human Protein Atlas under the CC BY 4.0 International License (Source: <https://www.proteinatlas.org/ENS00000008517-IL32/tissue/cerebral+cortex#img>, <https://www.proteinatlas.org/ENSG00000008517-IL32/cancer/glioma#img>); (D) Kaplan-Meier survival curves for the TCGA, CGGA, and REMBRANDT cohorts demonstrated significantly worse overall survival in glioma patients with high IL-32 expression; (E) Waterfall plots depict the mutational landscape of the top 20 most frequently mutated genes associated with IL-32 in glioma. Notably, no mutations in IL-32 were detected in glioma samples. ns: not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. IL-32: Interleukin-32; GBM: glioblastoma; WHO: World Health Organization; ACC: Adrenocortical carcinoma; BLCA: bladder urothelial carcinoma; BRCA: breast invasive carcinoma; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: cholangiocarcinoma; COAD: colon adenocarcinoma; DLBC: lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: esophageal carcinoma; HNSC: head and neck squamous cell carcinoma; KICH: kidney chromophobe; KIRP: kidney renal papillary cell carcinoma; LAML: acute myeloid leukemia; LGG: brain lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; OV: ovarian serous cystadenocarcinoma; PAAD: pancreatic adenocarcinoma; PCPG: pheochromocytoma and paraganglioma; PRAD: prostate adenocarcinoma; READ: rectum adenocarcinoma; SARC: sarcoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; TGCT: testicular germ cell tumors; THCA: thyroid carcinoma; THYM: thymoma; UCEC: uterine corpus endometrial carcinoma; UCS: uterine carcinosarcoma; TCGA: the cancer genome atlas; CGGA: Chinese glioma genome atlas; KIRC: kidney renal clear cell carcinoma.

recombinant IL-32 was commonly used at concentrations up to 40 ng/mL^[13,14]. An additional 60 ng/mL group was included to determine whether the biological effects of IL-32 on glioma cells remained concentration-dependent over a broader range. EdU assays demonstrated that IL-32 promoted cell proliferation in a dose-dependent manner [Figure 6A-D]. Transwell migration and invasion assays further showed that exogenous IL-32 markedly enhanced glioma cell motility and invasiveness [Figure 6E-H]. To validate these effects, siRNA-mediated knockdown of IL-32 significantly reduced proliferation, migration, and invasion compared with controls, whereas IL-32 overexpression produced the opposite effects [Figure 7

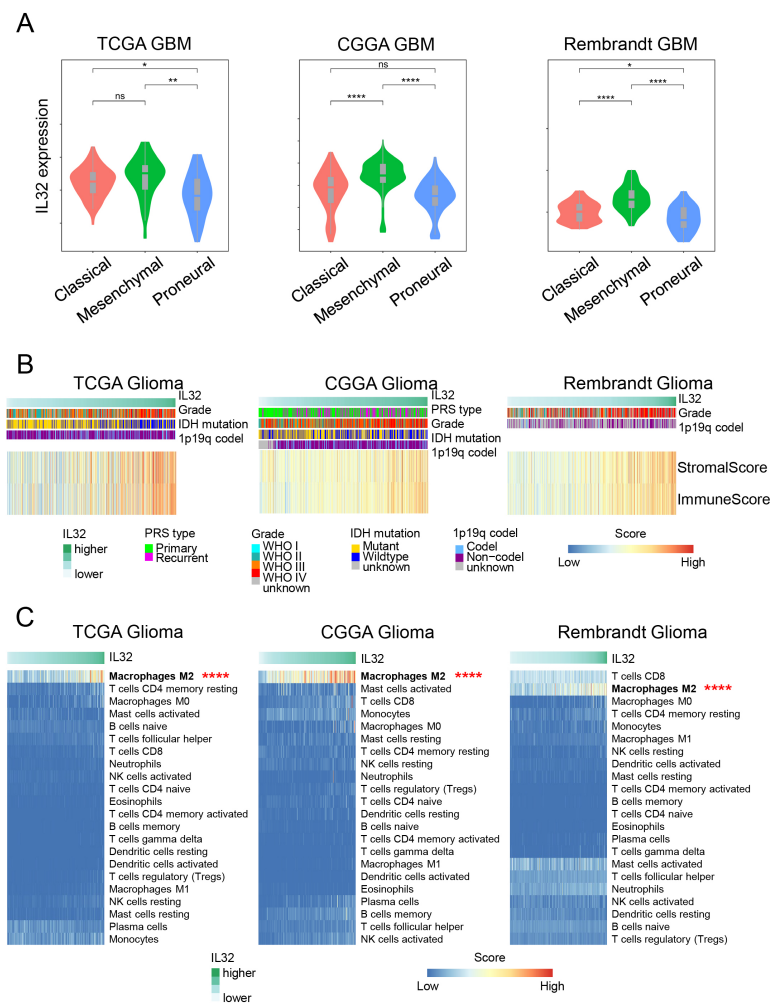


Figure 2. Functional characterization of IL-32 in glioma. (A) The mesenchymal GBM subtype exhibited markedly higher IL-32 levels compared to other molecular classifications; (B) IL-32 levels showed a positive association with the stromal score and immune score, suggesting its potential role in modulating the glioma immune microenvironment; (C) Using the CIBERSORTx pipeline, we assessed the correlation between IL-32 expression and the infiltration levels of 22 immune cell types in the glioma microenvironment. Notably, IL-32 expression showed the strongest positive correlation with M2 macrophages, indicating a potential role in promoting an immunosuppressive tumor microenvironment. ns: not significant; *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$. IL-32: Interleukin-32; GBM: glioblastoma; WHO: World Health Organization; TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas; PRS: primary-recurrent-secondary; IDH: isocitrate dehydrogenase.

]. These results collectively identify IL-32 as a pivotal regulator of glioma progression and support its potential as a therapeutic target.

Construction and validation of a 10-gene prognostic risk model for glioma

To develop a precise prognostic tool for glioma, LASSO-penalized Cox regression was applied to the core set of DEGs [Figure 8A and B]. Ten genes, namely *IL13RA2*, *FCER1G*, *S100A8*, *GPNMB*, *LUM*, *ANXA1*, *EMP3*, *CHI3L1*, *PTX3* and *IGFBP2*, were selected to construct the risk model. Each patient's risk scores were computed by multiplying LASSO-derived coefficients of each gene by its corresponding mRNA expression level, then summing the product: Risk score = $0.0240873736114369 * IL13RA2 + 0.0639986787396445 * FCER1G + 0.012863666565142 * S100A8 + 0.114384024092816 * GPNMB - 0.064292476977219 * LUM + 0.0877923634931701 * ANXA1 + 0.000136148895132336 * EMP3 + 0.0220760633277022 * CHI3L1 + 0.0696953333682331 * PTX3 + 0.297974768973838 * IGFBP2$.

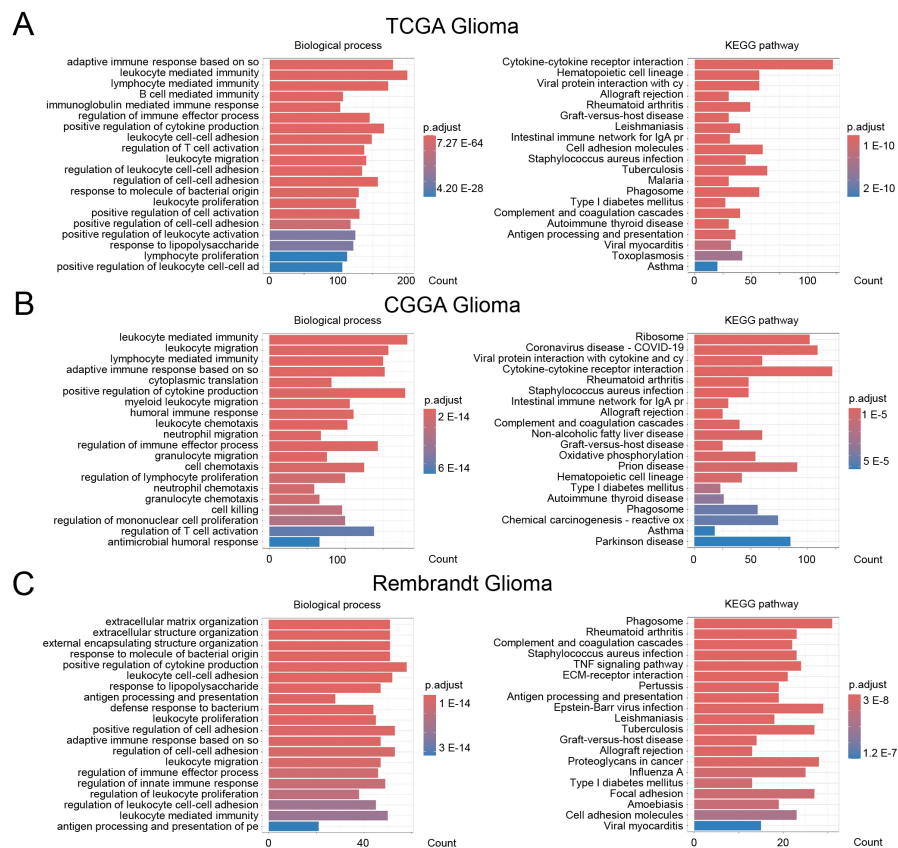


Figure 3. Functional enrichment analysis of IL-32 in glioma. We performed differential expression analysis by comparing high and low IL-32 expression glioma samples. Across three independent glioma cohorts, genes significantly elevated in the high IL-32 expression group were subjected to GO and KEGG pathway enrichment analyses: (A) TCGA, (B) CGGA, and (C) REMBRANDT. IL-32: Interleukin-32; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas; TNF: tumor necrosis factor; ECM: extracellular matrix.

The TCGA cohort served as the training set, with CGGA and REMBRANDT cohorts used for validation. Heatmaps of clinical characteristics revealed that the 10 genes were upregulated in the HR group [Figure 8C]. Patients were stratified into HR and LR groups based on median risk scores (TCGA: 5.232; CGGA: 2.898; REMBRANDT: 5.863) [Figure 8D]. HR patients exhibited a greater likelihood of mortality [Figure 8D], and OS was significantly shorter compared with LR patients [Figure 8E]. Univariate and multivariate Cox regression models were applied to determine whether the IL-32-associated risk score serves as an independent prognostic factor [Supplementary Figure 3]. In the multivariate Cox models, established prognostic factors, including age, WHO grade, IDH mutation status, 1p/19q co-deletion status, and MGMT promoter methylation status when available, were incorporated as covariates. The risk score remained independently associated with overall survival across the TCGA, CGGA, and REMBRANDT cohorts, with hazard ratios of 2.26 (95%CI: 1.67-3.00, $P < 0.001$), 1.40 (95%CI: 1.22-1.60, $P < 0.001$), and 1.50 (95%CI: 1.06-2.00, $P < 0.05$), respectively [Figure 8F], indicating that the model provided prognostic information beyond established clinicopathological and molecular features. Time-dependent ROC analysis further demonstrated that the 10-gene risk model achieved improved predictive discrimination compared with conventional prognostic variables, including WHO grade, age, IDH mutation status, 1p/19q co-deletion status, and MGMT promoter methylation [Supplementary Figure 3]. Moreover, DCA showed that the risk model provided greater net clinical benefit than individual traditional prognostic factors across a range of clinically relevant threshold probabilities [Supplementary Figure 4]. Together, these findings support the robustness of the IL-32-related risk model and suggest its potential value for individualized prognostic stratification in glioma patients.

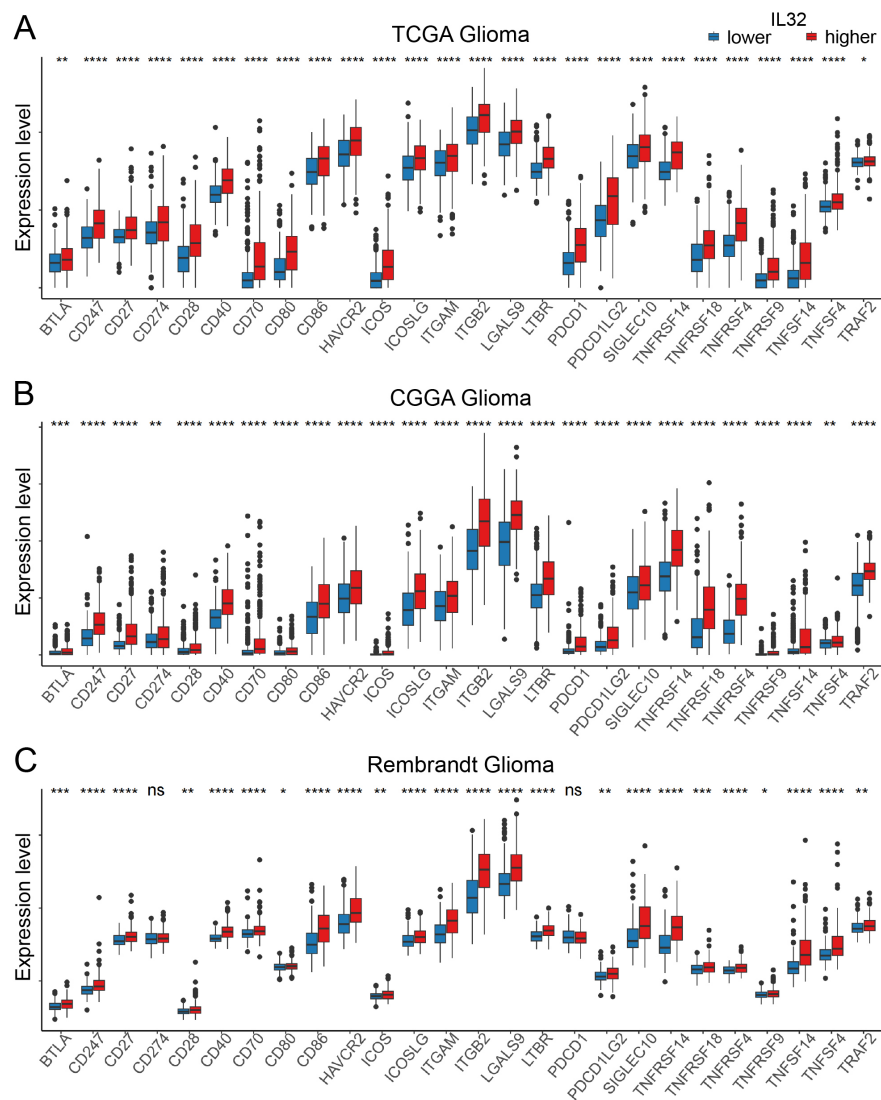


Figure 4. Association of IL-32 with immune checkpoints in glioma. Our analysis identified significant positive correlations between IL-32 expression and multiple key immune checkpoint molecules in glioma, suggesting a potential role for IL-32 in shaping the tumor immune microenvironment. These findings were consistently observed across three independent glioma cohorts: (A) TCGA, (B) CGGA, and (C) REMBRANDT. ns: not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. IL-32: Interleukin-32; TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas.

Predictive value of the 10-gene risk model for immunotherapy response in glioma

To evaluate the model's predictive power for immunotherapy, the TIDE algorithm was applied to transcriptomic data from glioma cohorts. In TCGA, LR patients had a higher proportion of predicted responders (61%) compared with HR patients (25%) (Fisher's exact test, $P < 0.0001$; [Figure 9A](#)). Similar trends were observed in CGGA and REMBRANDT cohorts [[Figure 9B](#) and [C](#)], confirming the model's robustness across independent datasets. Further validation using the independent GSE121810 cohort, which included 15 recurrent GBM patients treated with neoadjuvant anti-PD-1 therapy, showed that responders and non-responders formed distinct clusters by PCA [[Figure 9D](#)]. In contrast with responders, non-responders showed higher risk scores [[Figure 9E](#)]. These results indicate that the 10-gene risk model can stratify glioma patients by prognosis and predict their likelihood of responding to immunotherapy.

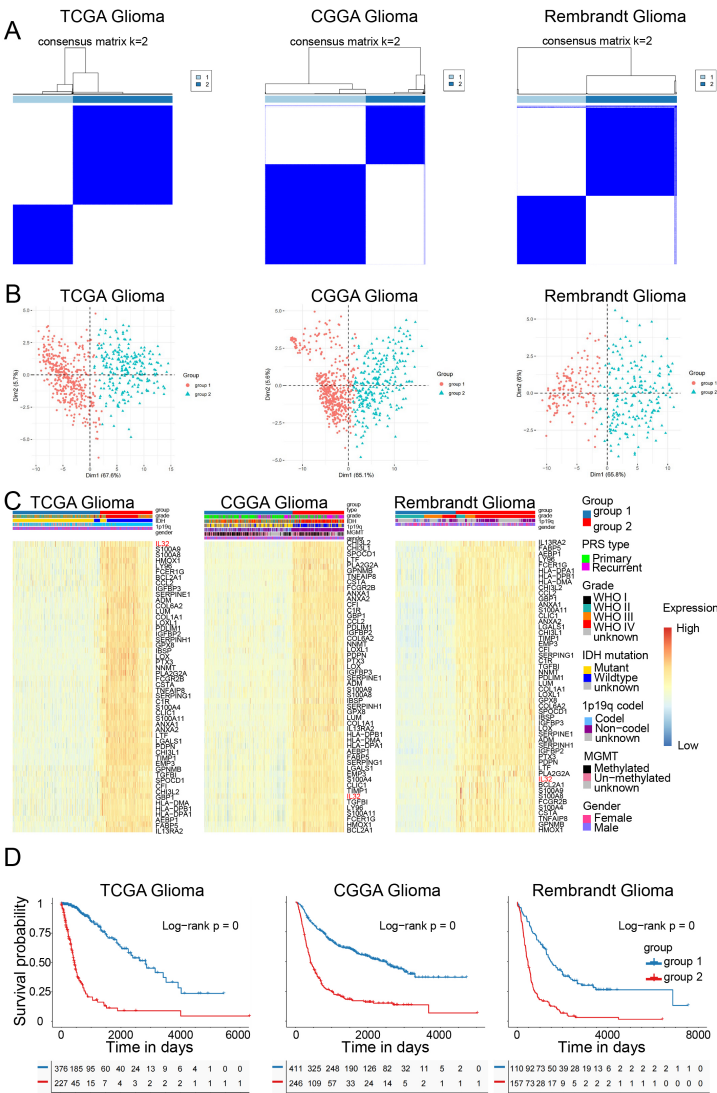


Figure 5. Survival analysis and subtype identification based on intersected DEGs in glioma. (A) Consensus clustering analysis was performed to determine the optimal number of clusters (k) for glioma patients across the TCGA, CGGA, and REMBRANDT datasets. The results identified two distinct molecular subtypes (k = 2), suggesting a robust classification scheme; (B) Principal component analysis further validated the clustering results. The first principal component effectively separated subtype 1 and subtype 2, demonstrating the distinct transcriptional landscapes of these glioma subtypes; (C) A heatmap visualization illustrated the clinical characteristics and expression profiles of 51 DEGs across the two subtypes. Notably, subtype 2 exhibited significantly higher expression of these DEGs, suggesting a more aggressive molecular phenotype; (D) Kaplan-Meier survival analysis revealed a significant reduction in OS for patients in subtype 2 compared to subtype 1, indicating the potential prognostic relevance of this classification. DEGs: Differentially expressed genes; OS: overall survival; WHO: World Health Organization; TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas.

Prediction of drug sensitivity for high-risk glioma patients

To investigate possible therapeutic strategies for HR glioma patients, drug sensitivity was estimated utilizing the oncoPredict R package^[11], with IC50 values representing sensitivity (higher IC50 indicates greater resistance). Pearson correlations between risk scores and IC50 values were calculated, focusing on drugs with $r < -0.50$ [Figure 10A]. Six compounds, namely lovastatin, TGX-221, ifosfamide, IC-87114, PLX-4720, and fluvastatin, showed consistent sensitivity across TCGA, CGGA, and REMBRANDT cohorts [Figure 10B]. These results highlight potential therapeutic options for personalized treatment in HR glioma patients.

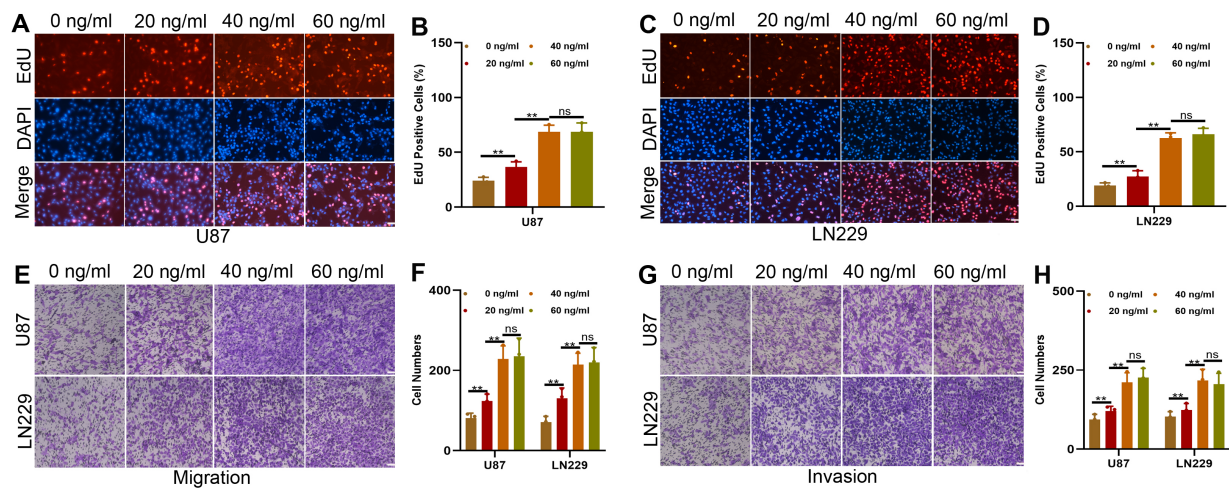


Figure 6. Exogenous IL-32 promoted glioma cell proliferation, migration, and invasion. (A-D) Glioma cells were treated with increasing concentrations of IL-32 (0, 20, 40, 60 ng/mL). Higher IL-32 levels progressively enhanced cell proliferation compared to the control group (0 ng/mL); (E and F) IL-32 stimulation at increasing doses (0, 20, 40, 60 ng/mL) significantly promoted glioma cell migration, as observed in quantitative assays; (G and H) Similarly, glioma cells exposed to IL-32 exhibited a dose-dependent increase in invasive capacity, suggesting its role in tumor aggressiveness. ns: not significant; **, $P < 0.01$. IL-32: Interleukin-32; DAPI: 4',6-diamidino-2-phenylindole. Scale bar, 100 μ m.

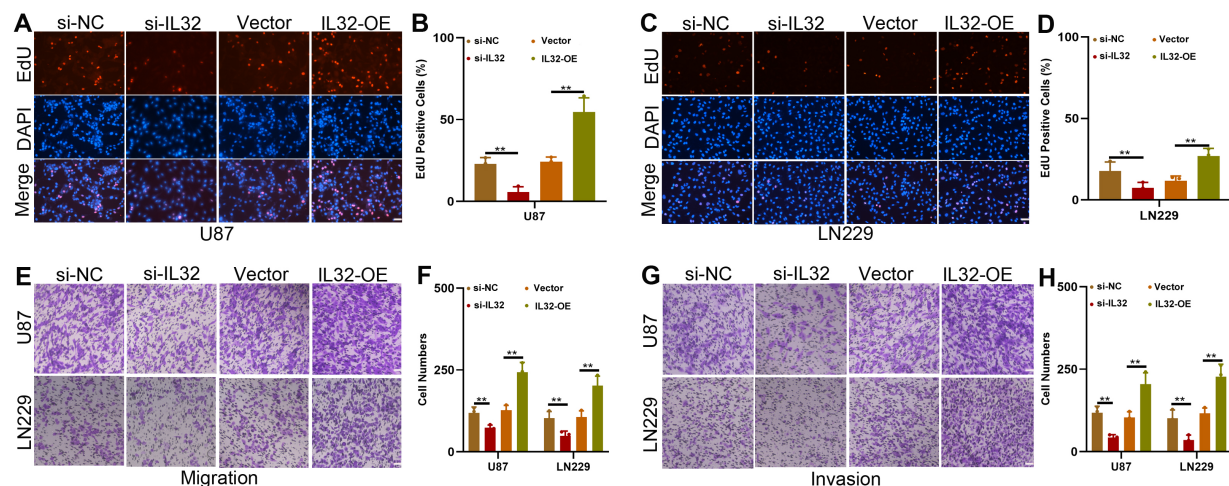


Figure 7. Endogenous IL-32 promoted glioma cell proliferation, migration, and invasion. (A-D) IL-32 knockdown led to a marked reduction in glioma cell proliferation, while its overexpression significantly enhanced proliferation, confirming its pro-tumorigenic role; (E and F) IL-32 knockdown significantly impaired glioma cell migration, whereas overexpression of IL-32 greatly enhanced migratory ability, as demonstrated in representative images; (G and H) IL-32 knockdown markedly reduced the invasive potential of glioma cells, whereas overexpression promoted invasion, further supporting its role in glioma progression. Representative images illustrate these findings. **, $P < 0.01$. IL-32: Interleukin-32. Scale bar, 100 μ m. DAPI: 4',6-diamidino-2-phenylindole.

DISCUSSION

In this study, we conducted a comprehensive bioinformatics analysis to investigate the role of IL-32 in glioma. We observed markedly elevated IL-32 levels in glioma specimens relative to normal brain tissue, and this upregulation was linked to increased tumor malignancy. Interestingly, although IL-32 was consistently overexpressed in glioma and strongly associated with patient outcome, no recurrent somatic mutations were detected. This observation suggests that IL-32 contributes to glioma biology primarily through altered transcriptional regulation rather than genomic mutation, which is consistent with its established role as an inflammation-associated cytokine. These results align with previous studies linking IL-32 to tumor

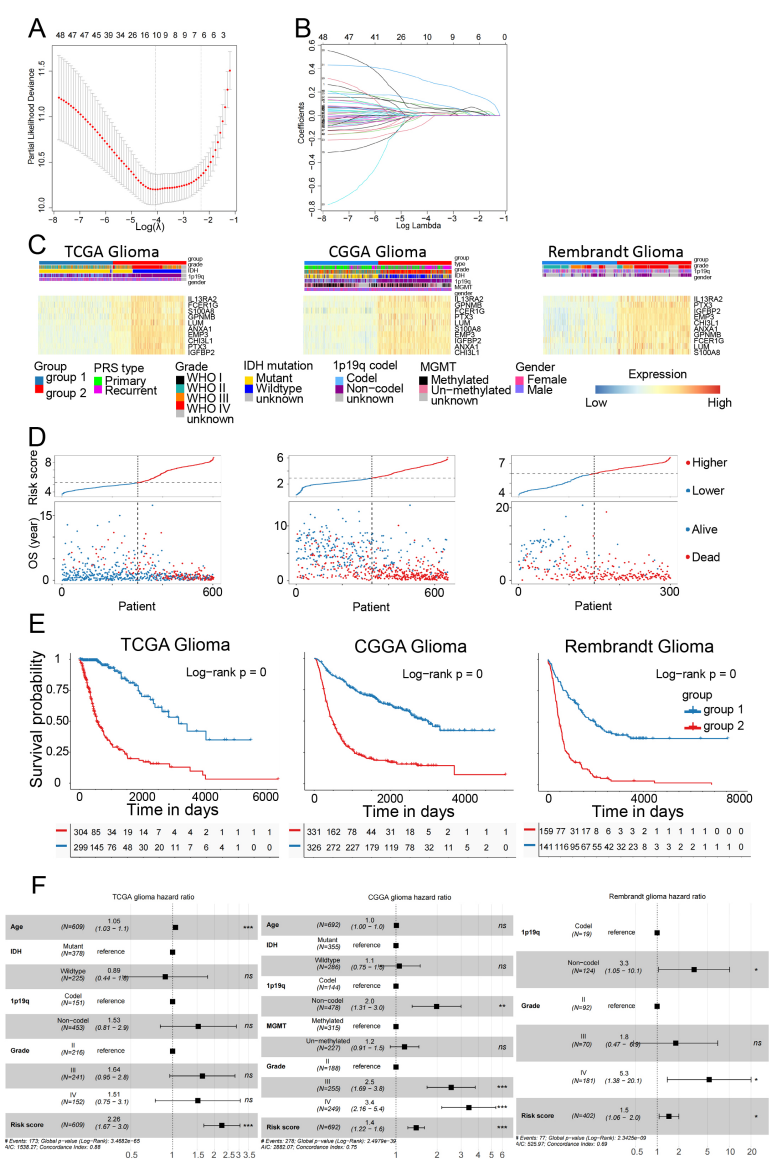


Figure 8. Development and validation of a prognostic risk model for glioma. (A) LASSO regression analysis identified ten key genes as active covariates for constructing the glioma risk prediction model; (B) LASSO regression analysis was applied to calculate the coefficients of the 10 chosen genes; (C) A heatmap visualization illustrated the clinical characteristics and expression profiles of the ten core DEGs across high-risk and low-risk glioma patient groups; (D) Risk score distribution and survival status plots revealed that high-risk patients exhibited an increased likelihood of mortality; (E) Survival analysis indicated a significant association between high-risk status and shortened overall survival in glioma patients; (F) Multivariable Cox regression validated the risk score as an independent prognostic factor for glioma, adjusting for clinical variables. DEGs: Differentially expressed genes; LASSO: least absolute shrinkage and selection operator; TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas.

progression in various cancers^[15-19], underscoring its pivotal contribution to gliomagenesis. A risk model was successfully established, which stratified glioma patients into HR and LR groups. This model showed strong predictive ability for survival outcomes, offering a potential tool for patient risk stratification. Additionally, our research highlighted the association between IL-32 and ICs, as well as its influence on the TME, suggesting that IL-32 might be a therapeutic candidate in glioma. Through drug sensitivity analysis, we identified several promising therapeutic options for HR glioma patients, providing new opportunities for personalized treatment strategies.

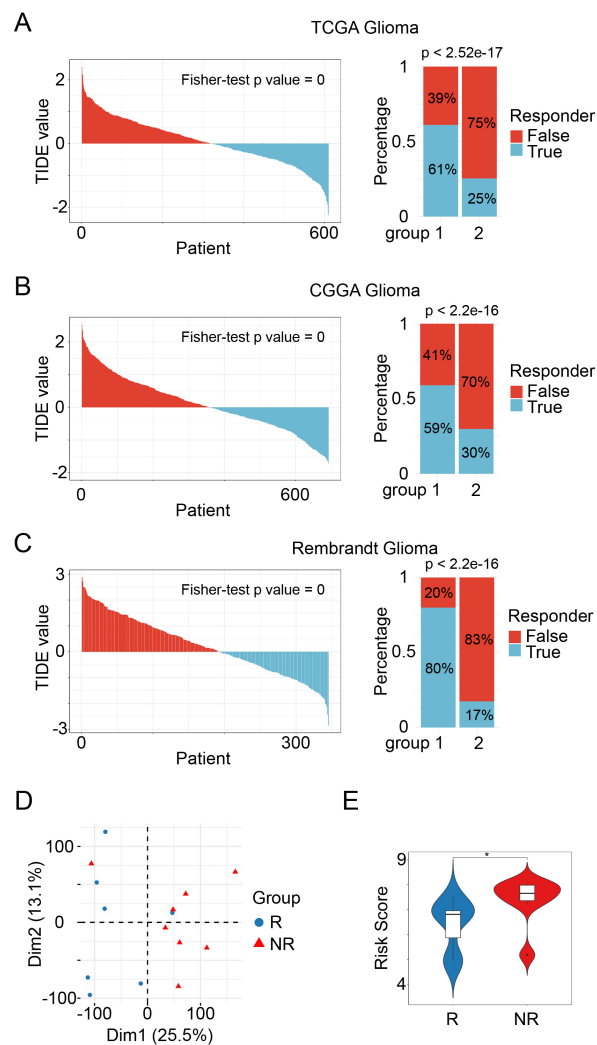


Figure 9. Predictive value of the risk model for immunotherapy response in glioma. The left column presents bar plots illustrating the distribution of TIDE scores among glioma patients. The right column depicts the proportion of potential immunotherapy responders, demonstrating a significantly higher response rate among low-risk glioma patients compared to high-risk patients across three independent glioma cohorts: (A) TCGA, (B) CGGA, (C) REMBRANDT; (D) PCA revealed distinct clustering between GBM responders and non-responders to neoadjuvant anti-PD-1 immunotherapy, emphasizing the differences in gene expression patterns associated with treatment response; (E) The risk model effectively distinguished non-responders from responders, with the former exhibiting significantly elevated risk scores, thereby underscoring its predictive power for immunotherapeutic response. *, $P < 0.05$. TIDE: Tumor immune dysfunction and exclusion; PCA: principal component analysis; GBM: glioblastoma; TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas.

Although our study identified a significant association between IL-32 expression and M2 macrophage enrichment, the underlying molecular mechanisms remain to be fully elucidated in glioma. Current evidence from other tumor models suggests that IL-32 may influence macrophage behavior through inflammatory signaling networks rather than acting as a classical macrophage-polarizing cytokine^[13]. IL-32 has been reported to activate nuclear factor kappa-B (NF- κ B) and signal transducer and activator of transcription 3 (STAT3) signaling pathways, which play critical roles in regulating cytokine production, immune cell recruitment, and macrophage functional states^[20,21]. Moreover, IL-32-mediated induction of inflammatory mediators, including IL-6 and tumor necrosis factor- α (TNF- α), may contribute to the formation of a cytokine environment that favors the accumulation and maintenance of immunosuppressive macrophage phenotypes^[20]. Therefore, IL-32 produced by glioma cells or stromal components may indirectly promote M2 macrophage enrichment by remodeling the local inflammatory landscape^[22]. In the present study, the

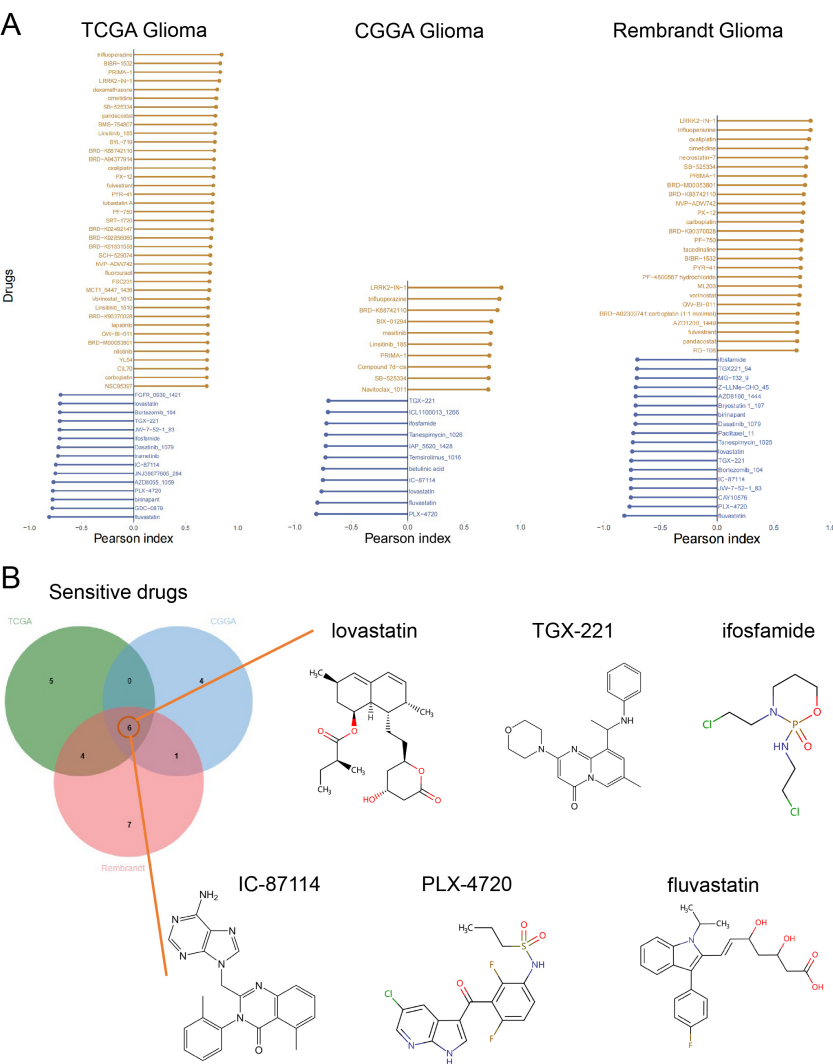


Figure 10. Identification of potentially effective drugs for high-risk glioma patients. (A) Lollipop plots illustrate the Pearson correlation coefficients between the risk score and IC50 values of various drugs across three independent glioma cohorts; (B) A Venn diagram identifies six drugs that are more likely to be effective in high-risk glioma patients, as they consistently demonstrated significant correlations with the risk score across all three cohorts. IC50: Half-Maximal Inhibitory Concentration; TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas.

relationship between IL-32 and M2 macrophages was primarily established through transcriptomic immune deconvolution analyses, and direct experimental evidence demonstrating IL-32-driven macrophage recruitment or polarization in glioma is currently lacking. However, multiple independent observations support a potential functional role of IL-32 beyond a prognostic biomarker. Across several glioma cohorts, elevated IL-32 expression was consistently associated with mesenchymal GBM characteristics, increased immune and stromal activity, enhanced M2 macrophage infiltration, and higher expression of immune checkpoint molecules. In addition, our *in vitro* experiments demonstrated that IL-32 modulation directly influenced glioma cell proliferation, migration, and invasion, suggesting that IL-32 may participate in both tumor-intrinsic malignant progression and tumor microenvironment remodeling. Nevertheless, these findings should be interpreted as evidence supporting a potential regulatory role rather than definitive proof of a causal relationship between IL-32 and immune suppression. Future investigations integrating glioma-macrophage co-culture systems, single-cell transcriptomic approaches, patient-derived models, and immune-competent animal studies will be essential to determine whether IL-32 directly regulates macrophage recruitment, polarization, functional activation, and immune escape within the glioma

microenvironment.

In addition, IL-32 was significantly correlated with key ICs, including PD-L1, PD-1, and PD-L2, suggesting its potential involvement in immune escape mechanisms. Given the critical role of ICs in glioma immunotherapy^[23], targeting IL-32 in combination with immune checkpoint immunotherapies (ICIs) may offer a novel therapeutic strategy to enhance immunotherapy responses in glioma patients.

The prognostic risk model we developed using IL-32-related genes demonstrated robust predictive power across multiple glioma cohorts. The HR group exhibited elevated expression of immune and stromal components, which is consistent with a more aggressive tumor phenotype. Importantly, our model outperformed traditional clinicopathological factors in predicting OS, highlighting its potential for improving clinical decision-making [Supplementary Figure 3]. Importantly, the prognostic value of the IL-32-related risk model remained significant after adjustment for established molecular and clinical factors, suggesting that it may capture biological features not fully represented by conventional classification systems. Nevertheless, prospective validation in larger clinical cohorts is required before routine clinical implementation.

From a clinical perspective, the IL-32-related risk model may serve as a complementary tool for individualized risk assessment rather than a replacement for established molecular classification. In a potential clinical workflow, tumor tissue obtained during surgical resection or biopsy could undergo routine transcriptomic profiling, from which the expression levels of the ten model genes would be quantified and integrated into the risk score using the predefined formula. The calculated score could then be interpreted together with established prognostic factors, including WHO grade, IDH mutation status, MGMT promoter methylation status, and 1p/19q co-deletion status, to provide a more comprehensive evaluation of patient prognosis. Patients classified as high risk may benefit from closer radiographic surveillance, enrollment in clinical trials, or consideration of individualized therapeutic strategies, whereas low-risk patients may be expected to have more favorable outcomes under current standard-of-care management. In addition, because the risk model was associated with predicted immunotherapy responsiveness and differential drug sensitivity, it may help prioritize patients for future biomarker-guided therapeutic studies. Nevertheless, prospective multicenter validation and standardized transcriptomic testing will be required before routine clinical implementation can be recommended.

The TIDE algorithm was used to test our model's capacity to predict immunotherapy response. LR glioma patients were more likely to respond to ICIs compared to HR patients. Furthermore, responders to anti-PD-1 treatment exhibited significantly lower risk scores, underscoring the relevance of our model in guiding immunotherapy strategies [Figure 9]. This finding supports the growing trend in personalized medicine, where biomarker-based models are increasingly used to guide treatment decisions^[24].

Finally, our drug sensitivity analysis identified several potential therapeutic options for HR glioma patients, including lovastatin, TGX-221, ifosfamide, IC-87114, PLX-4720, and fluvastatin [Figure 10]. These drugs demonstrated notable sensitivity across three independent glioma cohorts, suggesting their potential as targeted therapies for patients with HR profiles. The drug identification provides new avenues for personalized treatment plans and could help improve clinical outcomes for glioma patients. It should be noted that the six candidate compounds identified in this study were not selected because they directly target IL-32 itself. Rather, they were prioritized based on their predicted sensitivity in tumors characterized by the IL-32-related transcriptional signature. At present, direct evidence linking these agents to IL-32 signaling is limited. Nevertheless, several compounds have biological activities that may intersect with pathways associated with IL-32-mediated tumor progression. For example, statins have been reported to suppress NF-

κB activation and inflammatory cytokine production, processes that are closely associated with IL-32 signaling^[22,25]. Likewise, PI3K inhibitors (TGX-221 and IC-87114) and the BRAF inhibitor PLX-4720 can modulate intracellular signaling networks involved in tumor proliferation, immune regulation, and cytokine responses^[26]. Therefore, although these agents cannot currently be regarded as direct IL-32-targeted therapies, they may influence downstream biological processes associated with IL-32-high gliomas. Future mechanistic studies will be required to determine whether these compounds directly regulate IL-32 expression or interfere with IL-32-dependent signaling pathways.

However, an important consideration for translating these candidate compounds into glioma therapy is their ability to reach therapeutic concentrations within the central nervous system. Among the six compounds identified, lovastatin has relatively favorable blood-brain barrier (BBB) permeability because of its lipophilic properties, whereas fluvastatin has been reported to exhibit more limited, but detectable, central nervous system (CNS) penetration^[27]. Ifosfamide has demonstrated measurable penetration into the cerebrospinal fluid and has been incorporated into selected treatment regimens for primary brain tumors, although CNS exposure remains lower than systemic concentrations^[28]. For PLX-4720, preclinical studies have shown partial brain distribution in experimental models; however, its intracranial pharmacokinetic profile has not been fully characterized^[29]. In contrast, published evidence regarding BBB penetration of TGX-221 and IC-87114 is currently limited^[30]. Therefore, these compounds should be regarded as hypothesis-generating candidates identified through transcriptome-based drug sensitivity prediction rather than immediately translatable therapeutic options. Future studies integrating pharmacokinetic evaluation, BBB permeability assessment, and orthotopic glioma models will be necessary to determine whether these agents can achieve therapeutically relevant concentrations within the brain^[31].

Although our findings suggest that IL-32 may represent a potential therapeutic target in glioma, several important challenges need to be addressed before clinical translation. First, our current evidence supporting IL-32 targeting is mainly derived from bioinformatic analyses and *in vitro* functional experiments. Although modulation of IL-32 expression altered glioma cell proliferation, migration, and invasion, whether IL-32 inhibition can effectively suppress tumor growth and improve survival *in vivo* remains unknown. Future studies using orthotopic glioma models and genetically engineered mouse models will be essential to evaluate the therapeutic efficacy of IL-32 blockade within an intact tumor microenvironment. Second, IL-32 is a multifunctional cytokine involved in both tumor-associated inflammation and physiological immune regulation^[22]. Therefore, systemic inhibition of IL-32 may potentially affect normal immune homeostasis, and strategies to achieve tumor-specific targeting will be required. Third, the molecular diversity of IL-32 isoforms and their context-dependent functions represent additional challenges. Different IL-32 isoforms may exert distinct or even opposing biological effects, which should be carefully considered when designing therapeutic approaches^[4]. Moreover, effective delivery across the BBB remains a major obstacle for cytokine-targeted therapies in glioma. Approaches such as antibody-based therapies, nanoparticle delivery systems, or locally administered agents may provide potential solutions, but require extensive preclinical evaluation^[32]. Therefore, while IL-32 represents a promising candidate for therapeutic exploration, substantial mechanistic, pharmacological, and clinical validation is still necessary before it can be considered a feasible treatment target for glioma patients.

This study has several limitations that should be acknowledged. First, although the analyses were performed using three independent public glioma cohorts and externally validated across multiple datasets, the retrospective nature of these databases inevitably introduces potential selection bias and heterogeneity arising from differences in patient populations, sequencing platforms, clinical management, and data preprocessing procedures. Such variability may influence the generalizability of our findings. Second, while our analyses consistently demonstrated significant associations between IL-32 expression, immune

infiltration, patient prognosis, and predicted therapeutic response, these observations are primarily correlative and cannot establish causality. Additional mechanistic studies are required to determine how IL-32 regulates the glioma immune microenvironment and whether it directly contributes to immune escape. Third, although we performed in vitro functional experiments demonstrating that IL-32 promotes glioblastoma cell proliferation, migration, and invasion, these experiments were limited to established cell lines. Validation in orthotopic animal models and patient-derived glioma models will be necessary to better recapitulate the complex interactions between tumor cells and the immune microenvironment. Furthermore, the predictive performance of the IL-32-related risk model for immunotherapy response was evaluated using a single external cohort of rGBM patients treated with neoadjuvant anti-PD-1 therapy (GSE121810, $n = 15$). Although the observed association between lower risk scores and favorable treatment response was encouraging, the limited sample size substantially restricts the statistical power and generalizability of these findings. Therefore, the immunotherapy prediction results should be considered preliminary and interpreted with appropriate caution until they are validated in larger, independent clinical cohorts. Similarly, the candidate therapeutic agents identified by oncoPredict were derived from computational prediction and require further pharmacological and clinical validation before clinical application. Finally, because glioma exhibits substantial molecular heterogeneity, future studies integrating single-cell transcriptomics, spatial transcriptomics, and multi-omics data may provide a more comprehensive understanding of the cellular sources, regulatory mechanisms, and context-dependent functions of IL-32 within the glioma microenvironment. Addressing these limitations will further strengthen the clinical utility of IL-32 as a prognostic biomarker and potential therapeutic target.

Our study demonstrated IL-32 to be a significant regulator of glioma progression and immune modulation within the TME, positioning it as a robust prognostic biomarker. The IL-32-related risk model stratified glioma patients into HR and LR groups, predicted responses to immunotherapy, and identified promising drug candidates for therapeutic targeting. Findings from this study underscore the potential of IL-32 as both a biomarker and a therapeutic candidate in glioma management. However, further clinical validation and prospective trials are essential to establish IL-32-targeted therapies as a viable option for glioma treatment.

DECLARATIONS

Authors' contributions

Conceptualized and coordinated the work: Xiao Y

Prepared the acquisition, analysis, and interpretation of data: Zhang P, Lu F, Zhang Z

Conducted the experimental procedures: Wu Y, Liu L

Prepared the figures and tables: Wang R, Wang D, Xiang C, Xu C

Wrote the original draft: Qian C, Liu Y, Geng L, Chen C

Reviewed and edited the manuscript: Xiao Y, Zou Y, Liu H

All authors have read and approved the final version of the manuscript and agreed to its publication.

Availability of data and materials

Publicly available datasets were analyzed in this study. These data can be found here: TCGA database, the CGGA database, the REMBRANDT database, and the GEO database with accession numbers GSE108474 and GSE121810.

AI and AI-assisted tools statement

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was based on publicly available and de-identified datasets from the TCGA, CGGA, REMBRANDT, and GEO databases, including GSE121810. No new human participants were recruited and no additional human specimens were collected for this study. The original studies from which these datasets were derived obtained relevant ethical approvals and informed consent. Therefore, no additional ethical approval or informed consent was required for the present secondary analysis.

Consent for publication

Not applicable.

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Supplementary Materials

[Supplementary Materials](#)

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