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*Original Research*

# Identification of a novel immune-related lncRNA signature to predict prognostic outcome and therapeutic efficacy of LGG

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**Abstract**

**Background**: Recent studies have shown that the prognosis of low-grade glioma (LGG) patients is closely correlated with the immune infiltration and the expression of long-stranded non-coding RNAs (lncRNAs). It's meaningful to find the immune-related lncRNAs (irlncRNAs). **Methods**: The Cancer Genome Atlas (TCGA) data was employed in the study to identify irlncRNAs and Cox regression model was applied to construct the risk proportional model based on irlncRNAs. **Results**: In the study, we retrieved transcriptomic data of LGG from TCGA and identified 10 lncRNA signatures consisting of irlncRNAs by co-expression analysis. Then we plotted 1-year receiver operating characteristic (ROC) curves and calculated the area under the curve (AUC). LGG patients were divided into high-risk and low-risk groups according to the risk model. We found there were differences in survival prognosis, clinical characteristics, degree of immune cell infiltration, expression of immune gene checkpoint genes, and sensitivity to the commonly used chemotherapeutic agents of high-risk and low-risk groups. **Conclusions**: IrlncRNA-based risk assessment model can be used as a prognostic tool to predict the survival outcome and clinical characteristics of LGG and to guide treatment options.

**Keywords:** Low-grade gliomas; lncRNA; Immune cell infiltration; Prognosis; Chemotherapy sensitivity; Biomarker

## 1. Introduction

Low-grade gliomas (LGG) are grade I-II gliomas, mainly classified as oligodendrogliomas and astrocytomas. The prognosis of LGG is better than that of high-grade gliomas, suggesting that pathogenesis of low-grade gliomas differs from that of high-grade gliomas [1]. LGGs are inert cancers that almost always develop into high-grade aggressive tumors, such as glioblastomas, but the time course of disease-specific progression could vary widely, from as few as several months to as many as 10 years, depending on the molecular characteristics and the tumor location in the brain [2,3]. Previous research had revealed that the immune infiltrate microenvironment of LGG is closely associated with the prognosis of patients [4]. The current treatment of LGG still favors a combination of surgical-based treatments [5,6]. Although immunotherapy for gliomas has been developed for a long time, the results have not been satisfactory [7]. Therefore, it is important to study the immune molecular mechanism of LGG and discover new potential immune checkpoints to get therapeutic targets for the treatment of LGG.

Long non-coding RNA (lncRNA) is a type of non-coding RNA with more than 200 nucleotides in length, which plays important roles in epigenetic regulation, post-transcriptional regulation, alternative splicing and other gene regulations in gliomas. LncRNAs have a significant impact on tumor proliferation, migration, apoptosis, immunity, and autophagy [8]. For example, lncRNA maternally expressed gene 3 was found by Zhao *et al.* [9] to proliferation, apoptosis, and autophagy in gliomas, thus affecting the prognosis of patients. It has also been shown that the expression of lncRNA growth arrest-specific transcript 5 in LGG is associated with prognosis and its potential functions include the regulation of ribosome biogenesis and translation [10]. These studies suggest lncRNAs have a promising future as a marker of tumor prognosis in LGG.

Many studies have shown that bioinformatics-based tumors immune-related prognostic models are good predictors for tumor diagnosis, assessment, and treatment [11–13]. In our study, we integrated analysis of the lncRNA expression dataset of LGG patients in The Cancer Genome Atlas (TCGA), screened immune-related lncRNAs, constructed a clinical prognostic model of LGG, and explored the relationship between LGG immune-related irlncRNAs and indicators of immune infiltration, prognosis, and chemotherapy sensitivity.

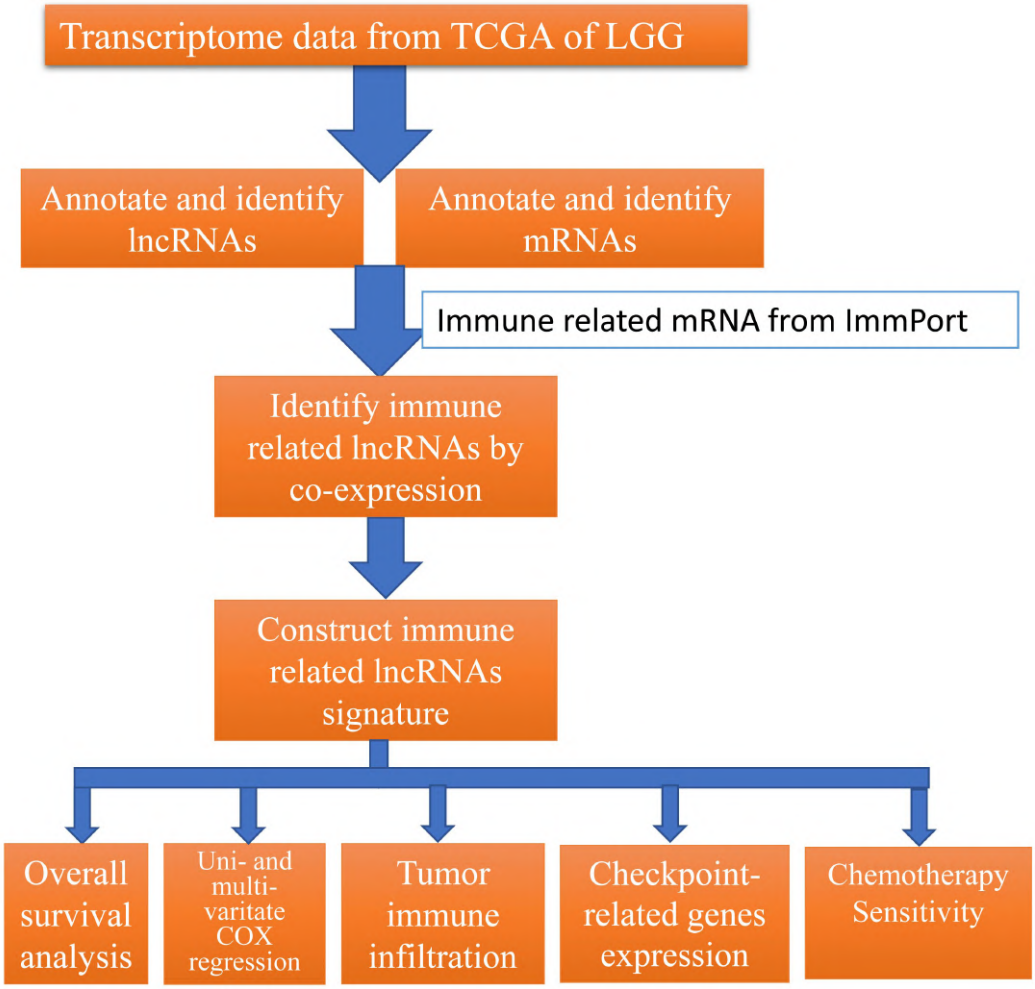


**Fig. 1. The overall workflow of the study.**

## 2. Methods

*2.1 Data, preparation, and differentially expressed analysis*

LGG transcriptome analysis (RNAseq) data was downloaded from TCGA (https://tcga-data.nci.nih.gov/tcga/) along with patient clinical data. GTF (Gene transfer format) files were downloaded from Ensembl (http://asia.ensembl.org) for annotation for mRNAs and lncRNAs. The immune-related genes list was downloaded from the ImmPort database (http://www.immport.org), and irlncRNAs were identified by co-relation analysis by using the R, with the conditions of $p \leq 0.001$ and correlation coefficient $\geq 0.4$. All the data was downloaded from public database of TCGA which was allowed to be used in other studies (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/using-tcga/citing-tcga) and is approved by the local ethics committee.

*2.2 Construction of differentially expressed irlncRNAs and establishment of a risk assessment model*

We combined the irlncRNAs with clinical survival information and performed univariate and multivariate survival analyses on the irlncRNAs by the R package "survival" ($p \leq 0.001$). Then, to verify the accuracy of the constructed model, we constructed the 1-year ROC curves of the risk model, and the area under curve (AUC) of the model were also calculated. We used its median value as a cut-off point to divide patients into a high-risk group or low-risk group based on the risk score. To increase the reliability of the data, we performed cross-validation by a method of bootstrap (n = 1000) [14].

*2.3 Association of risk model with clinical characters*

To validate the accuracy and feasibility of the risk model, Kaplan-Meier analysis was performed for patients in the LGG cohort from the TCGA database, and the different survival rates between the high-risk and low-risk groups were analyzed by "survival" package. The specific risk scores and survival status of each patient were also presented by R based on the risk model. The R packages of

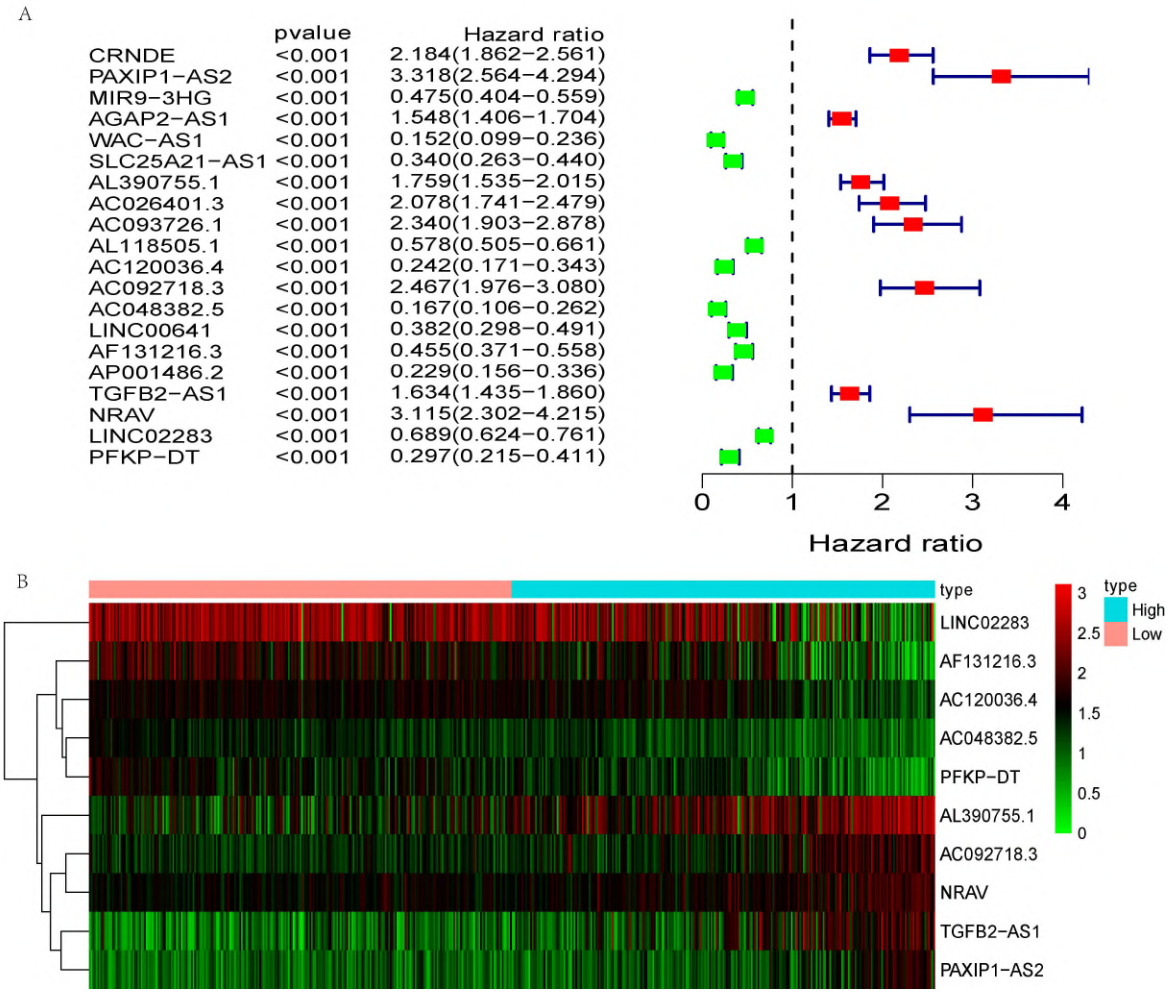


**Fig. 2. Identification of an immune related lncRNAs signature.** (A) The top 20 immune- and survival- related RNAs. (B) The expression pattern of immune related lncRNAs in risk model.

survival, survivalROC, survminer, and pHeatmap were utilized in the analysis. To confirm whether the model could be used as an independent predictor of clinical prognosis, univariate and multifactorial Cox regression analyses of risk scores and clinicopathological characteristics were performed and the results were presented by forest plots.

### *2.4 Association of the expression immune checkpoint genes and tumor-infiltrating immune cells with risk model*

The relationship between the risk model and immune microenvironment was explored. We verified the difference in immune infiltration between high-risk and low-risk groups by Wilcoxon singed-rank test and visualized with box plot. Correlation analysis demonstrated the relationship between risk score values and immune infiltration by box plots with a significance threshold set at $p < 0.05$.

### *2.5 Exploration of the significance of the model in the clinical treatment*

To determine whether the model can be used to guide clinical chemotherapy regimens, we calculated the IC50 of the commonly used antitumor drugs (Vinblastine, Cisplatin, Imatinib and Sunitinib) in clinical patients in the LGG cohort of the TCGA. Wilcoxon test was used to compare the IC50 of the different drugs to assess the difference in drug sensitivity between the high-risk and low-risk group. The algorithm was implemented by the R package pRRophetic. $p$-values $< 0.05$ were considered statistically significant.

## 3. Results

### *3.1 Identification of irlncRNAs, establishment of irlncRNAs risk assessment signature in LGG*

Our workflow is shown in Fig. 1. First, we downloaded transcriptomic data and relevant clinical data of LGG from TCGA database, including 529 tumor samples. Immune-related gene expression profiles were constructed after dividing the expression profiles into mRNA and lncRNA matrices based on gene annotation files. 1087 irlncRNAs were identified by co-correlation analysis (condition of correlation coefficient $\geq$0.4 and $p \leq 0.001$), while one-way COX regression analysis ($p \leq 0.001$) was performed on irlncRNAs and 464 irlncRNAs were identified

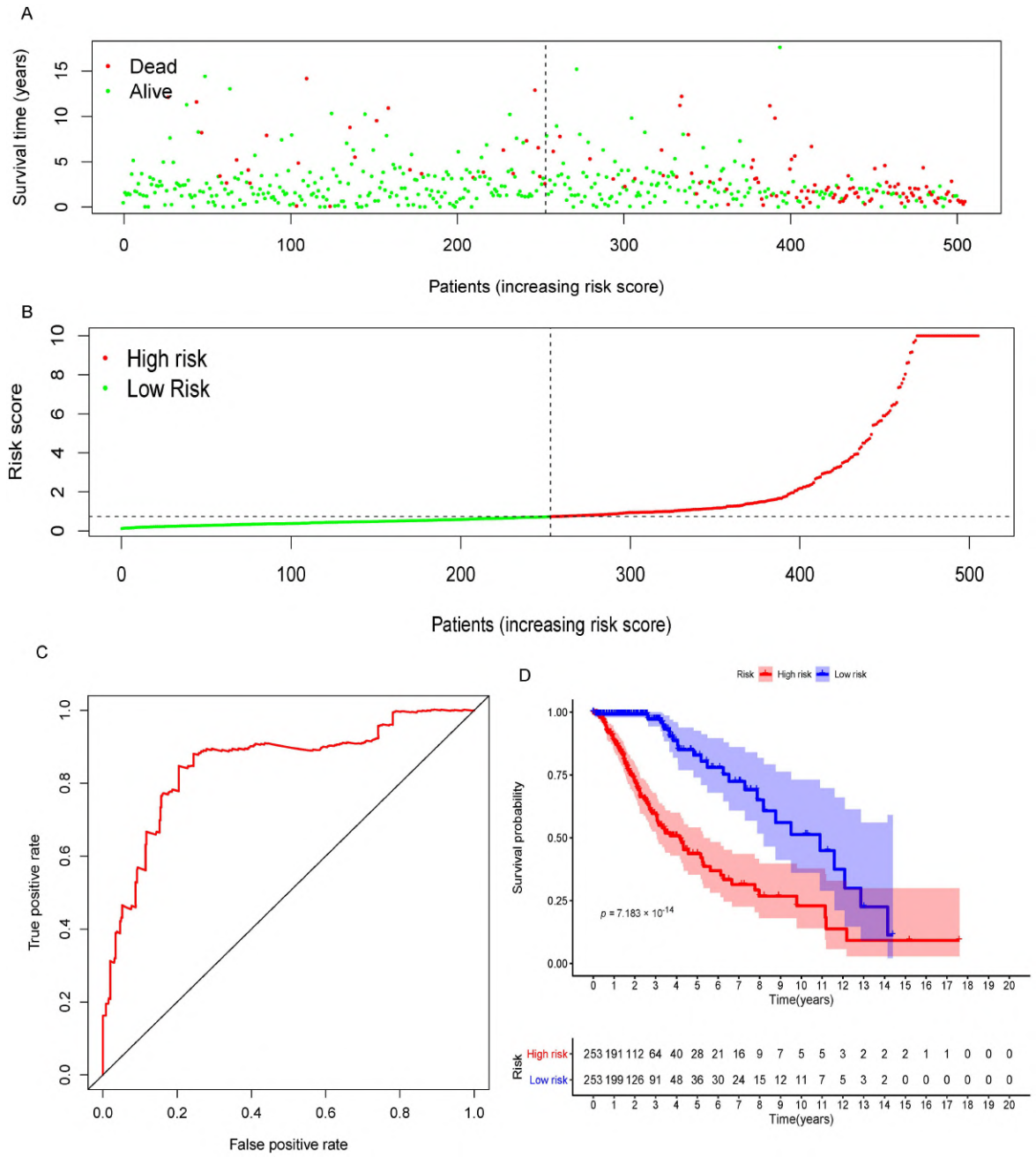


**Fig. 3. Prediction value of the risk signature for survival.** (A) Distribution of patients' survival time. (B) Risk score of patients. (C) The ROC curve of signature. (D) Survival analysis of two groups based on risk signature.

to be associated with LGG prognosis. The top 20 irlncRNAs among them were selected for inclusion in the analysis (Fig. 2A) and a multivariate COX regression analysis was conducted to identify an irlncRNA signature consisting of 10 irlncRNAs (Fig. 2B). IrlncRNA signature: 0.36 × AL390755.1 + 0.43 × AC120036.4 + 0.53 × AC092718.3 + (–0.83) × AC048382.5 + (–0.23) × AF131216.3 + 0.25 × TGFB2-AS1 + 0.50 × NRAV + 0.16 × LINC02283 + (–0.400708824748904) × PFKP-DT + 0.41 × PAXIP1-AS2.

### *3.2 Clinical prognostic assessment by risk assessment model*

We plotted the 1-year ROC curve of the model and calculated the area under the curve (AUC) as 0.849. The C-index of the model was 0.846 and the C-index by cross validation was 0.841. Thus, the model we constructed could reflect the overall survival of LGG patients with high accuracy (Fig. 3A). Based on the median value of the integrated model, we specified the cut-off value of 0.7384 and divided the LGG patients in the TCGA database into high-risk group or low-risk group. The Survival time, RiskScores

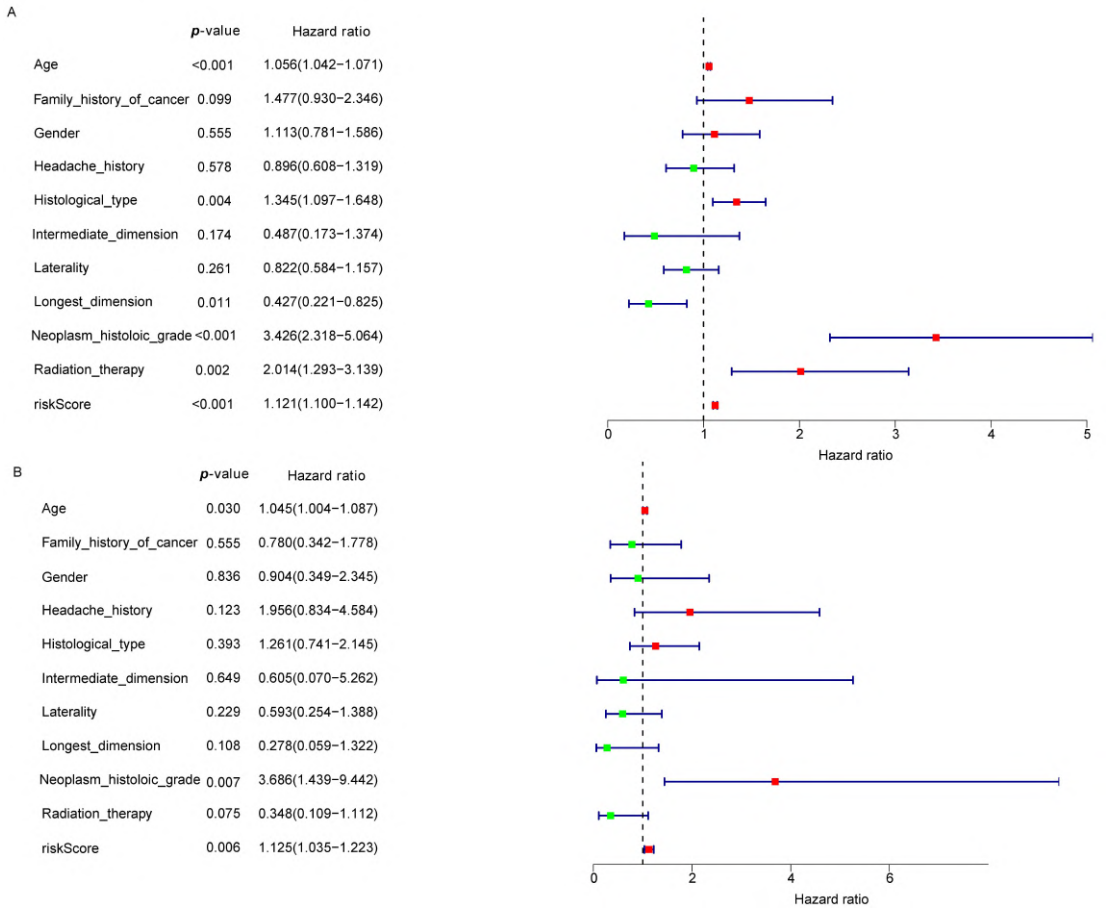


**Fig. 4. Uni- and multi- variate COX regression analysis of the model.** (A) Univariate Cox regression analysis. (B) Multivariate Cox proportional hazards model.

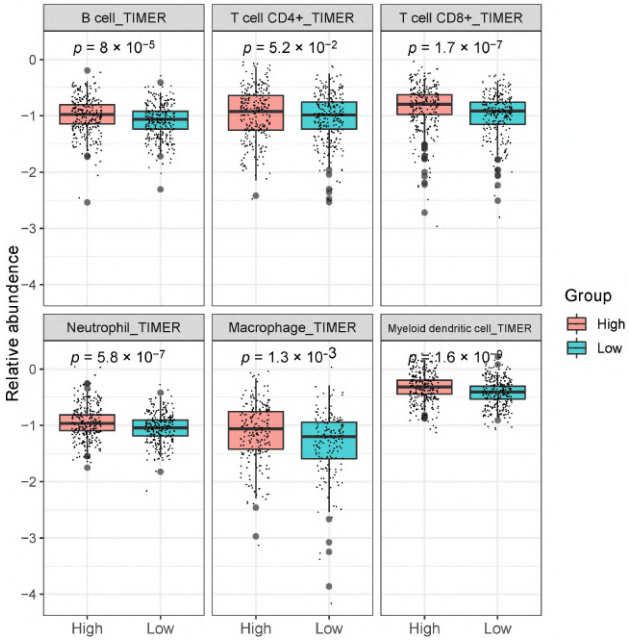


**Fig. 5. Evaluation of Tumor-Infiltrating Cells by risk signature.**

and Kaplan-Meier analysis for each case are in Fig. 3B–D, showing that the patients in the low-risk group had significantly better clinical prognostic outcomes than those in the high-risk group ($p = 7.183 \times 10^{-14}$).

Next, univariate Cox regression analysis (Fig. 4A) indicated that Age ($p < 0.001$, HR = 1.056, 95% CI [1.042–1.071]), Histological type ($p = 0.004$, HR = 1.345, 95% CI [1.097–1.648]), and Longest dimension ($p = 0.011$, HR = 0.427, 95% CI [0.221–0.825]), Neoplasm histologic grade ($p < 0.001$, HR = 3.426, 95% CI [2.318–5.064]), Radiation therapy ($p = 0.002$, HR = 2.014, 95% CI [1.293–3.139]) and riskScore ($p < 0.001$, HR = 1.121, 95% CI [1.100–1.142]) showed a difference in statistic. Finally, multivariate Cox proportional hazards model also showed that Age ($p = 0.030$, HR = 1.045, 95% CI [1.004–1.087]), Neoplasm histologic grade ($p = 0.007$, HR = 3.686, 95% CI [1.439–9.442]) and riskScore ($p < 0.006$, HR = 1.125, 95% CI [1.035–1.223]) had statistical differences, indicating that they could be used as independent prognostic predictors for LGG patients (Fig. 4B).


**Fig. 6. Assessment of expression of immune checkpoint genes by risk signature.**

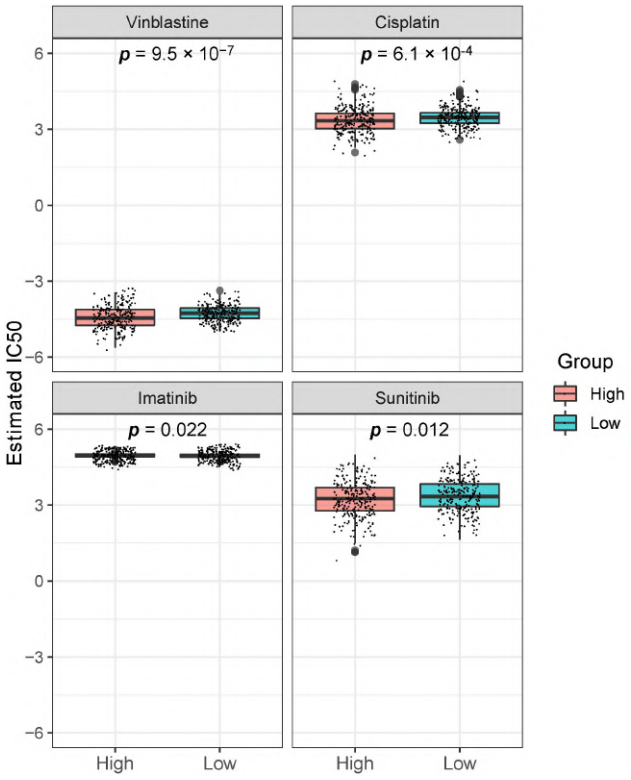


**Fig. 7. The signature served as a potential predictor for chemosensitivity.**

### *3.3 Assessment of the risk model with tumor immune microenvironment and the immune checkpoint genes*

To investigate whether the model could reflect the level of tumor immune cell infiltration in LGG, we counted six types of immune cells (B cell, T cell CD4+, T cell CD8+, Neutrophil, Macrophage, Myeloid dendritic cell) in both two risk groups of LGG patient infiltration, and the results are shown in Fig. 5. All the immune cells were significantly different in terms of the different subgroups except T cell CD4+ ($p < 0.01$).

Then we counted the distribution of differential expression characteristics of immune checkpoint genes between the two groups to further investigate whether this risk model was associated with immune checkpoint-related genes. The results showed that CD44, TNFRSF9, NRP1, CD40, CD40LG, CD276, CD86, HHLA2, CD48, CD160, TNFSF4, CD274, CD80, CD244, TNFSF9, CD70, TNFSF14, ADORA2A, IDO1 VTCN1, HAVCR2, TNFRSF14, ICOSLG, CTLA4, ICOS, CD200R1, LAIR1, CD28, TNFRSF4, TNFRSF18, PDCD1, PDCD1LG2, BTNL2 were significantly different between the two groups ($p < 0.05$) (Fig. 6).

### *3.4 Association of risk model with chemotherapeutics*

Finally, we determined the correlation between our risk model and the sensitivity of common chemotherapeutic agents by analyzing the IC50 of different chemotherapeutic agents between the high-risk and low-risk groups. We vali-

dated four agents commonly used in the clinic for LGG including Vinblastine, Cisplatin, Imatinib and Sunitinib. The results showed that higher risk scores were associated with lower IC50 ($p < 0.05$), and Vinblastine was the most significant ($p = 9.5 \times 10^{-7}$), suggesting that our risk model can be used to effectively predict chemotherapy outcomes in patients with LGG (Fig. 7).

## 4. Discussion

LGG histological typing is divided into astrocytomas and oligodendrogliomas [15], both of which are relatively insensitive to chemotherapeutic agents. Compared with oligodendrogliomas, astrocytomas show up-regulated expression of inflammation-related genes [16], and consequently the overall survival of these patients is correspondingly different. Indeed, specific inflammatory chemokines, like chemokine (C-X-C motif) ligand 12 [17], have been reported to be closely related with reduced time to tumor progression in LGG. All these related studies suggest a potential role of immune infiltration in the malignant tumor transformation of LGG. It has also been shown that multiple lncRNAs are differently expressed between astrocytomas and oligodendrogliomas, and that astrocytomas can be distinguished from oligodendrogliomas by these differentially expressed lncRNAs [18]. By analyzing the data of patients in the TCGA database, authors previously suggested that six lncRNAs were closely associated with the overall survival of patients, namely KIAA0495, GAS5, PART1, MGC21881, MIAT, and PAR5 [19]. Meanwhile, many lncRNAs actually have crucial roles on the immune microenvironment of gliomas [20]. For example, lncRNA H19 can affect the level of immune infiltration in gliomas and it affects the prognosis of patients as a result [21]. All of the above studies suggest that lncRNA has an important value in the diagnosis and prognosis prediction of gliomas. Immunotherapy of LGG has become a hot topic in recent years [22]. Therefore, it is meaningful to study the immune-related factors of LGG and construct an immune-related lncRNA clinical prognostic model to select its treatment and improve the prognosis of patients.

In our research, we developed an irlncRNA-based risk prediction model using transcriptomic data and relevant clinical data of LGG patients from the TCGA database. Firstly, we collected transcriptomic data from TCGA for LGG and identified lncRNA signatures consisting of 10 irlncRNAs by co-expression analysis, univariate and multifactorial COX regression analysis. Next, we plotted 1-year receiver operating characteristic (ROC) curves, and got the area under the curve (AUC). Based on the median values, patients with LGG were classified into high-risk or low-risk groups. Finally, we evaluated the risk assessment model by correlating the risk score with clinical characteristics including survival, pathological grade, tumor-infiltrating immune cells, and drug sensitivity.

Our results showed that patients in the high-risk group screened according to this risk model had a higher degree of immune cell infiltration, most significantly in myeloid dendritic cells ($p = 1.6 \times 10^{-9}$). Gliomas are known to have many specific barriers to antitumor immunotherapy, including the blood brain barrier (BBB) and lack of classical antigen presenting cells (APC) in the central nervous system, which limit immunotherapy in gliomas. Our model suggests that antigen-presenting cells are elevated in the high-risk group, which may expand the therapeutic ideas for the high-risk group by suggesting that the immunosuppressive microenvironment of the tumor can be reversed to generate an effective antitumor immune response, such as the use of ADV vectors expressing the Fms-like tyrosine kinase 3 (Flt3) ligand to recruit immune cells to the dendritic cell (DC) and other APCs cells (DCs) and other APCs into brain tumors. Combining this approach with methods to enhance the immunogenicity of glioma antigens is a potential strategy to generate an effective anti-tumor immune response [23,24].

In addition to chemotherapy, radiotherapy, surgery and other targeted therapies, tumor immunotherapy (also known as immuno-oncology) is now considered to be the fifth pillar of oncology treatment which is mainly due to the rapid development of immune checkpoint inhibitors [25–27]. Our results also showed a significant increase in the expression of many important immune checkpoints in the high-risk group, including familiar markers such as TNFSF14, CD274 (PD-L1), TNFRSF14, CD276 (B7-H4), CD40L ($p < 0.0001$), etc., many of which have been well studied in LGG, such as PD-L1 and the main ligand of PD-1. The immune checkpoint inhibitors targeting PD-1/PD-L1 have been put into clinical use in other cancers (e.g., melanoma and non-small cell lung cancer) and have achieved promising results, which will not be discussed here. Moreover, significant effects have also been seen in gliomas, for example, studies have shown that PD-1 inhibitors combined with radiotherapy, dendritic cell vaccine or temozolomide chemotherapy slowed tumor growth and significantly increased survival in mice [28,29]. The combination with Toll-like receptor 3 (TLR3) agonists for gliomas was reported to increase dendritic cell activation and T-cell proliferation [30]. Most of the anti-PD-1/PD-L1 drugs targeting glioma are currently in phase I or II clinical studies. Our risk model can screen the patient population with high expression of classical immune checkpoints, which provides a very meaningful basis for population selection and precision treatment of monoclonal antibodies against specific targets in the future.

The strength of our research lies in the advantages of the feasibility and generalizability of this novel model to clinical practice across patients. Age and tumor histological grade could be used as independent prognostic factors with a reduced IC50 for commonly used chemotherapeutic agents and a significantly worse overall prognosis

than in the low-risk group, which was consistent and in line with the trend of similar related previous studies, suggesting that our prediction model is highly accurate and practical. The establishment of the immune-related lncRNA prediction model may provide new ideas for the study of molecular mechanisms for the treatment of LGG.

However, there are still some limitations in this study. For example, the construction of our risk assessment model was entirely based on the original dataset of TCGA, which was not validated by basic experiments and clinical data. In addition, this risk model based on lncRNA can only be used as a biomarker to predict LGG survival outcome, tumor microenvironment status and treatment sensitivity, without determining the specific expression and biological role of individual lncRNAs involved in the model. Therefore, the risk model constructed in this study needs more samples for in-depth validation before clinical application, although it passed the cross-validation.

In summary, this study developed a validated irlncRNA-based risk assessment model for LGG. It is not only related to the survival outcome of LGG patients, but also strongly correlated with the tumor microenvironment and chemotherapy resistance. The development of this model provides a strong theoretical basis for predicting individual differences in LGG and guiding clinical treatment protocols.

## Author contributions

DW, XW and MZ conceived and designed the study; DW and XW performed the experiments; DW, YX and XW analyzed the data; DW, XW and CS wrote this paper. CS and MZ checked the proof of this paper.

## Ethics approval and consent to participate

This study did not contain any animal experiments or human tissue. All molecular and clinical data used in the study were collected from the public depository and ready for academic research use.


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## Conflict of interest

The authors declare no conflict of interest.

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