Research Article

Immune-Related lncRNA Correlated with Transcription Factors Provide Strong Prognostic Prediction in Gliomas

Yixin Tian [ID],[1] **Yi-Quan Ke**,[2] **and Yanxia Ma** [ID][1]

[1]*Department of Neurosurgery, Zhujiang Hospital, Southern Medical University, Guangzhou, China*
[2]*The National Key Clinical Specialty, The Engineering Technology Research Center of Education Ministry of China,
Guangdong Provincial Key Laboratory on Brain Function Repair and Regeneration, Guangzhou, China*

Correspondence should be addressed to Yanxia Ma; laoma888xin@sina.com

Glioma is the most common and deadly tumor in central nervous system. According to previous studies, long noncoding RNAs (lncRNA) and transcription factors were significant factors of gliomas progression by regulating gliomas immune microenvironment. In our study, we built two independent cohorts from CGGA and TCGA. And we extracted 253 immune-related lncRNA correlated with prognosis. After LASSO analysis and multivariate Cox regression analysis, 8 immune-related lncRNA were used to construct classifier. The effectiveness of classifier was confirmed in both CGGA (AUC = 0.869) and TCGA (AUC = 0.902) cohorts. The correlation between transcription factors and immune-related lncRNA was calculated by WCGNA. Eventually, we built a network between 8 lncRNA and transcription factors. The function of core immune-related lncRNA in gliomas immune microenvironment was also investigated by CIBERTSORT. Our research provided a strong classifier of immune-related lncRNA to predict gliomas patient outcome. We also found the correlation between core immune-related lncRNA and transcription factors. These results may stimulate new strategy of immunotherapy in gliomas patients.

1. Introduction

Glioma is the most aggressive tumor in central nervous system and patients with gliomas have poor prognosis. As the 2016 WHO classification of central nervous system tumors, immunoreactivity and molecular alterations were considered as classifiers. For example, Glioblastomas have been classified by IDH immunoreactivity as IDH-mutant, IDH-wildtype, and IDH-NOS. Oligodendroglioma diagnosis now requires an identified mutation of IDH and a codeletion of 1p19q. Thus, discovery of effective prognostic biomarkers was significant towards gliomas patients. Tumor microenvironment (TME) was illuminated as a significant player of gliomas progression, metastasis, and recurrence [1]. Recent studies have demonstrated the function of different types of immune cells in the glioma microenvironment [2, 3]. For example, M2 macrophages promoted gliomas progression while M1 macrophages played the antitumor role [4]. In addition, the discovery of molecular function of immune cells may stimulate new strategies of immunotherapy in gliomas patients [5].

Long noncoding RNAs (lncRNA) played significant role in plenty of biological processes such as regulation of transcription, translation, and protein modification [6]. And previous research studies revealed that lncRNA presented strong power of prognosis prediction in many cancer types [7, 8]. For example, GAS5 was an independent molecular cancer biomarker in bladder cancer patients [9], and a cell cycle-associated lncRNA, HOXA11-AS, was a biomarker of progression in glioma [10]. Several researches revealed the pro/antitumor function of lncRNA in different types of cancers by regulated immune responses. Neftel et al. found that lncRNA Sros1 promoted IFN-γ-mediated activation of innate immune responses by stabilizing Stat1 mRNA [11], and Zhao et al. revealed that LncRNA SNHG14/miR-5590-3p/ZEB1 positive feedback loop promoted diffuse large B cell lymphoma progression through regulating PD-1/PD-L1 checkpoint [12]. Therefore, the identification of immune-related lncRNA

provided possible mechanism in gliomas microenvironment. In addition, transcription factors affect different immune cell types that are important in the tumor progression. Atsaves V et al. found the roles of AP-1 in the regulation of antitumor immune responses and provided a new sight of immune checkpoint blockade therapy [13]. However, the relation between immune-related lncRNA and transcription factors in gliomas microenvironment was unknown.

In this study, we built two independent cohorts from CGGA and TCGA. We also extracted immune-related lncRNA correlated with prognosis. After LASSO analysis and multivariate Cox regression analysis, 8 immune-related lncRNA were used to construct classifier. The effectiveness of classifier was confirmed in both CGGA and TCGA cohorts. Eventually, we built a network between 8 lncRNA and transcription factors. The function of core immune-related lncRNA in gliomas immune microenvironment was also investigated by CIBERTSORT. Our research provided a strong classifier of immune-related lncRNA to predict gliomas patient outcome. We also found the correlation between core immune-related lncRNA and transcription factors. These results may stimulate new strategy of immunotherapy in gliomas patients.

2. Methods

2.1. Collection of RNA-Seq and Matched Clinical Data from CGGA and TCGA.
We built two independent cohorts for training set and testing set from CGGA and TCGA. The gene expression profiles were downloaded from the Chinese Gliomas Genome Atlas (CGGA http://www.cgga.org.cn/). 695 RNA-seq data of Chinese gliomas patients were obtained from CGGA database. Moreover, a series of measures were taken: (1) Genes with a variance of 0 were filtered out. (2) Complete follow-up information and samples with a follow-up time >30 days were served. Finally, 464 samples meeting the inclusion criteria were included. Each patient matched specific clinical data, which includes histology, WHO grades, age, gender, chemotherapy, radiotherapy, survival status, and survival duration in days. To build the testing cohort, the RNA-seq FPKM of gliomas including corresponding outcome data were downloaded from the Cancer Genome Atlas (TGCA). The following measures were taken: (1) the IDs were annotated on the basis of hg38 reference genome. (2) Genes with a variance of 0 were filtered out. (3) For the same gene corresponding to multiple IDs or a patient with multiple tumor samples, the average was taken. (4) Samples with a follow-up time >30 days were kept. Eventually, a total of 629 TCGA samples fulfilled our criteria.

2.2. Identification of Immune-Related lncRNA.
For extracting immune-related lncRNA, we downloaded immune-related gene set which includes immune response and immune system process from the MSigDB. The correlation analysis between lncRNA and immune-related genes was performed by *limma* package. Correlation coefficient >0.4 and P value <0.001 were included. Finally, we generated 543 immune-related lncRNA for further analysis.

2.3. Generation and Assessment of Prognostic Classifier by Multiple Assays.
To select prognostic immune-related lncRNA, univariate Cox regression analysis of continuous variables was performed by survival package. 253 prognostic immune-related lncRNA ($P < 0.01$) were generated for next analysis. Then, the Least Absolute Shrinkage and Selection Operator (LASSO) regression was performed to identify prognostic biomarkers and we set the random seed to 5 before the LASSO analysis. To generate and optimize the prognostic classifier, the multivariate Cox regression analysis was performed. The receiver operator characteristics (ROC) curves were drawn by *survivalROC* package. The area under the curve (AUC) of the ROC curve was calculated and compared to examine the performance of the classifier in both training and testing cohorts. The median risk score (RS) was determined to separate the genes into high-risk and low-risk groups. KM curves and independent test were performed by *survival* package to assess the effectiveness of classifier in gliomas patients.

2.4. Functional Enrichment Analysis of High-/Low-Risk Score Groups.
To assess the different functions between high- and low-risk groups, GO categories include biological processes (BP), molecular functions (MF), or cellular components (CC), and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways were analyzed by GSEA (Gene Set Enrichment Analysis). FDR <0.1 and $P < 0.01$ were considered significant.

2.5. Generation of Immune-Related lncRNA and Transcription Factors Network.
The transcription factors were identified by Cistrome Cancer database, and the expression data of 312 transcription factors were extracted for weighted gene coexpression network analysis (WGCNA). The risk score, clinical information, and expression modules were calculated in WGCNA. A soft-thresholding parameter of 5 was chosen to guarantee a scale-free network. Module eigengenes (MEs) were considered as the most principal component of each gene module and adopted as the representative of all genes in each module. The interesting module was identified by calculating the relevance between MEs and risk score. Gene significance (GS) represented the degree of linear correlation between gene expression of module and risk score. Besides, the average GS for all genes in the module was defined as the module significance (MS). The module with the highest MS score was chosen as the one related to risk score. Eventually, the network between prognostic immune-related lncRNA and chosen transcription factors was built by Cytoscape. In addition, the functional enrichment analysis was performed by *clusterProfiler* package.

2.6. Comparison of Gliomas-Infiltrating Immune Cells between High and Low Expressions of Hub lncRNA.
We assessed the proportions of 22 immune cell subtypes based on mRNA genes by CIBERSORT package. The perm equal to 1000 and P value <0.01 in CIBERSORT analysis result

were contained for further analysis. *Pheatmap* package, *corrplot* package, and *vioplot* package were used for plotting.

2.7. Statistical Analysis.

All analyses were carried out by *R* version 3.6.1 and corresponding packages.

3. Result

3.1. Construction of Prognostic Immune-Related lncRNA Classifier by Multiple Assays.

After screening data, a total of 464 samples from CGGA fulfilled our criteria. We extracted expression data of lncRNA and performed correlation analysis between lncRNA and immune-related genes. As a result, 543 immune-related lncRNA were generated for further analysis (correlation coefficient >0.4 and P value <0.001 were included). Next, 543 immune-related lncRNA were filtrated by univariate Cox regression analysis, and 253 prognostic immune-related lncRNA ($P < 0.01$) were generated. To construct classifier, these prognostic immune-related lncRNA were selected for LASSO analysis and optimized by multivariate Cox regression analysis (Figures 1(a) and 1(b)). Eventually, 8 immune-related lncRNA were selected to construct classifier which includes AC002454.1, AC062021.1, CRNDE, FAM181A-AS1, H19, HOXA-AS2, LINC00671, and SNAI3-AS1 (Figures 1(c)–1(f)). We also calculated the risk score (RS) of each sample based on the relative expression level and the corresponding LASSO coefficients in CGGA cohort.

3.2. Assessment of the Classifier Efficiency and Verification of the Classifier in TCGA Cohort.

We generated univariate and multivariate analysis which enrolled clinical features and risk score in the overall set. The result showed that the classifier was an independent factor for gliomas patients (Figures 2(a) and 2(b)). KM curves showed that the patients with high-risk score had lower survival rate than patients with low-risk score ($P < 0.001$) (Figure 2(c)). To assess the efficiency of the classifier, time-dependent and multiple ROC curves were performed in CGGA cohort. As a result, the AUC was 0.796 in 1 year, 0.869 in 3 years, and 0.866 in 5 years (Figure 2(e)). In addition, the classifier had better predictive power than other clinical features which includes WHO grades, histology, IDH mutation, and 1p19q codeletion (Figure 2(d)). To verify the results, time-dependent ROC curves were performed in TCGA cohort. The AUC was 0.868 in 1 year, 0.902 in 3 years, and 0.853 in 5 years. The results showed the classifier also had favorable accuracy in the validation set (Figure 2(f)).

3.3. Clinical Correlation and Functional Enrichment Analysis of High-Risk Score Group.

Clinical correlation analysis showed that each of the 8 lncRNA was correlated with WHO grades in gliomas ($P < 0.001$) (Figure 3(a)). Then, we confirmed that high-risk score group was correlated with immune-related gene sets by GSEA analysis (Figures 3(b) and 3(c)). To explore the potential mechanism in high-risk group, functional enrichment analysis was performed by GSEA (Gene Set Enrichment Analysis) (Figures 3(e)–3(g)).

3.4. Construction of Network between 8 Immune-Related lncRNA and Transcription Factors.

Based on Cistrome Cancer database, we extracted expression data of 312 transcription factors in CGGA cohort for WGCNA. The soft-thresholding power in the WGCNA was determined based on scale-free R2 ($R2 = 0.95$). Five modules were identified by the average linkage hierarchical clustering based on the soft-thresholding power. The blue module had the highest correlation with the risk score and contained 40 transcription factors (Figure 4). Then, the correlation analysis between 8 immune-related lncRNA and 40 transcription factors was performed. Then, we constructed the network by Cytoscape. The result showed that 3 high-risk immune-related lncRNA (CRNDE, HOXA-AS2, and AC002454.1) and 3 low-risk immune-related lncRNA (SNAI3-AS1, AC062021.1, and LINC00671) were correlated with transcription factors expression. We also found that CRNDE, HOXA-AS2, and SNAI3-AS1 played core roles in the network (Figure 5(a)). The functional enrichment analysis was performed to explore the potential mechanism (Figures 5(b) and 5(c)).

3.5. The Function of Core Immune-Related lncRNA in Gliomas Microenvironment.

Based on CIBERSORT algorithm, we performed the landscape of tumor infiltrating immune cell subtypes in CGGA cohort (Figures 6(a)–6(c)). Besides, the difference of immune cell subtypes between high-risk and low-risk groups was analyzed. To investigate the function of 3 core immune-related lncRNA in immune system, the median expression was determined to separate the samples into the high-expression and low-expression groups. The results indicated that multiple immune cells showed different infiltration between high-risk and low-risk groups. Plasma cells, CD8 T cells, T cells follicular helper, Tregs, T cells gamma delta, macrophages M0, and neutrophils were significantly increased in high-risk groups, while T cells CD4 naïve, T cells CD4 memory resting, monocytes, and activated mast cells were significantly decreased. Furthermore, the different glioma-infiltrating immune cells between high and low expression of core lncRNA were also calculated (Figures 6(d)–6(g)).

4. Discussion

Because of the limitations of the immune response in the central nervous system, there is no successful immunotherapy for gliomas patients currently [14, 15]. Although there have been few failures of immune therapy clinical trials in gliomas, a plenty of different immunotherapies studies are currently being performed in gliomas patients which include immune-checkpoint blockade, CAR T cell therapy, oncolytic viral therapy, and vaccination therapy [16, 17]. Thus, the new strategies of immunotherapy needed to be found in gliomas. Previous studies suggested that lncRNA play a significant role in gliomas progression by regulating gliomas immune
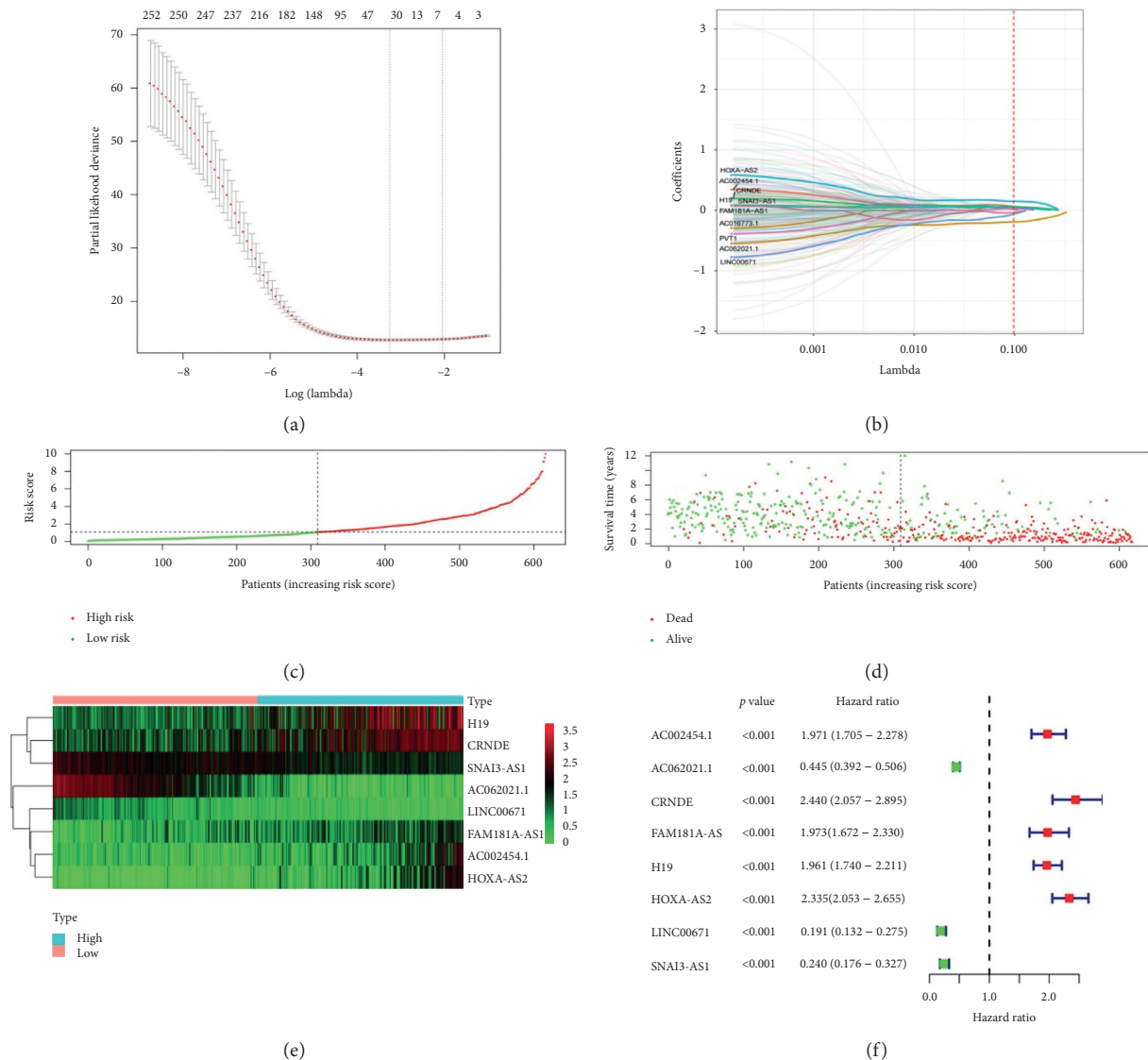
(a)

(b)

(c)

(d)

(e)

(f)

Figure 1: Construction of prognostic immune-related lncRNA classifier. (a, b) The selection of prognostic immune-related lncRNA by LASSO analysis. (c) The distribution of risk score. (d) The survival time and status of patients. (e) The heatmap of selected 8 immune-related lncRNA of the classifier. (f) The univariate Cox regression analysis of 8 immune-related lncRNA.

microenvironment. For example, Li et al. found that modulating lncRNA SNHG15/CDK6/miR-627 circuit reduces M2-polarization in glioblastoma [18]. Due to the wide expression in multiple tissues and cell lines, lncRNA was also considered as a favorable prognostic biomarker [19]. Besides, increasing evidence suggested that the transcription of lncRNA genes is regulated by multiple core transcription factors applied to protein-coding genes [20, 21]. Therefore, immune-related lncRNA regulated by transcription factors may become novel prognostic biomarkers in gliomas.

In our study, we generated two independent gliomas cohorts in CGGA and TCGA, and we extracted 253 immune-related lncRNA correlated with prognosis. After LASSO analysis and multivariate Cox regression analysis, 8 immune-related lncRNA were used to construct classifier. The classifier showed strong predictive ability in both CGGA

and TCGA cohorts. In addition, the classifier had better predictive power than other clinical features which include WHO grades, histology, IDH mutation, and 1p19q codeletion. According to previous studies, lncRNA H19 promoted gliomas progression and angiogenesis by regulating several microRNAs [22–24]. LncRNA HOXA-AS2 regulated malignant glioma behaviors via the RND3 expression and miR-373/EGFR axis [25, 26]. CRNDE promoted malignant progression of glioma by STAT3 and EGFR [27, 28]. In addition, other 5 lncRNA have not been researched in gliomas. Eventually, we built a network between 8 lncRNA and transcription factors. The result shows that CRNDE, HOXA-AS2, and SNAI3-AS1 played core roles in the network. 3 core transcription factors (EZH2, BRCA1, and E2F7) were positively correlated with CRNDE and HOXA-AS2 but negatively correlated with SNAI3-AS1. 6
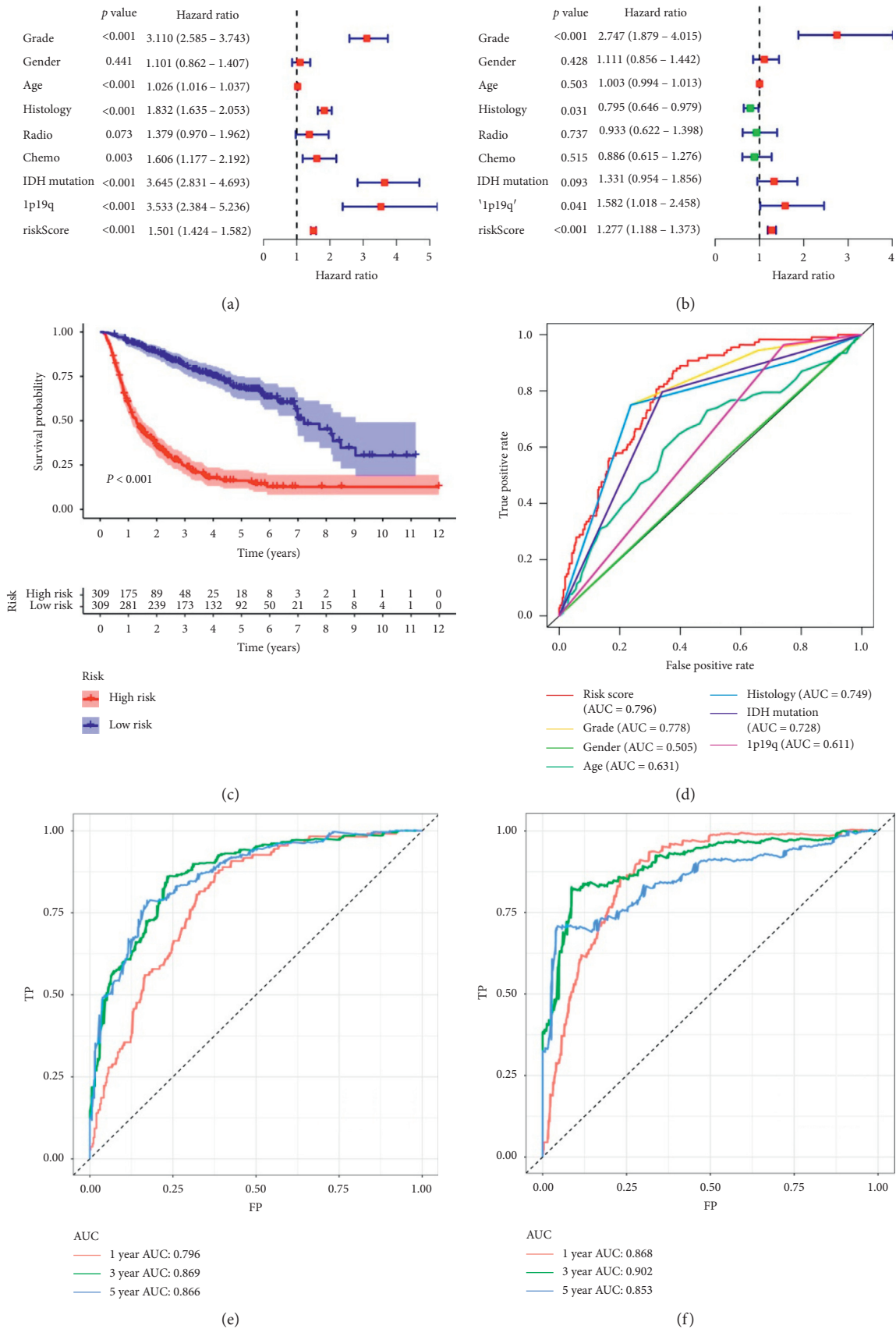
(a)

(b)

(c)

(d)

(e)

(f)

FIGURE 2: Assessment of the classifier efficiency and verification of the classifier in TCGA cohort. (a) The univariate Cox regression analysis of risk score and several clinical features in gliomas. (b) The multivariate Cox regression analysis of risk score and clinical features. (c) K–M curves of high- and low-risk score groups in CGGA cohort. (d) Multiple ROC curves of risk score and clinical features in CGGA cohort. (e) Time-dependent ROC curves of 1, 3, and 5 years in CGGA cohort. (f) Time-dependent ROC curves of 1, 3, and 5 years in TCGA cohort.
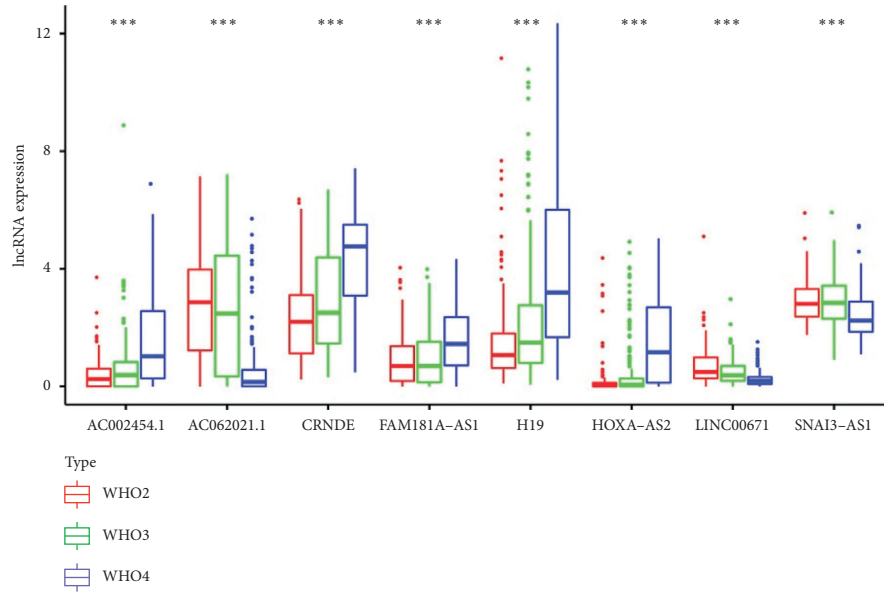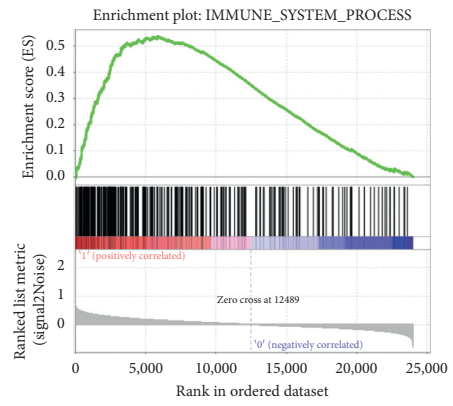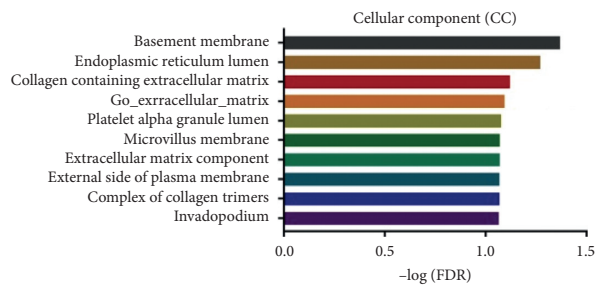
(a)
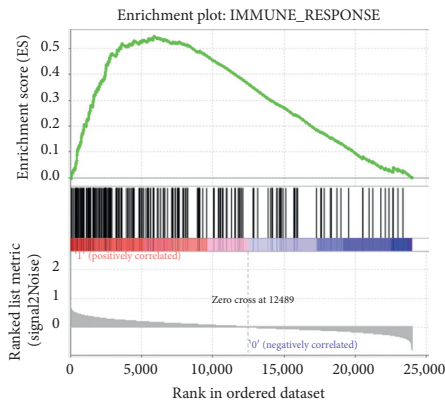
(b)

(c)
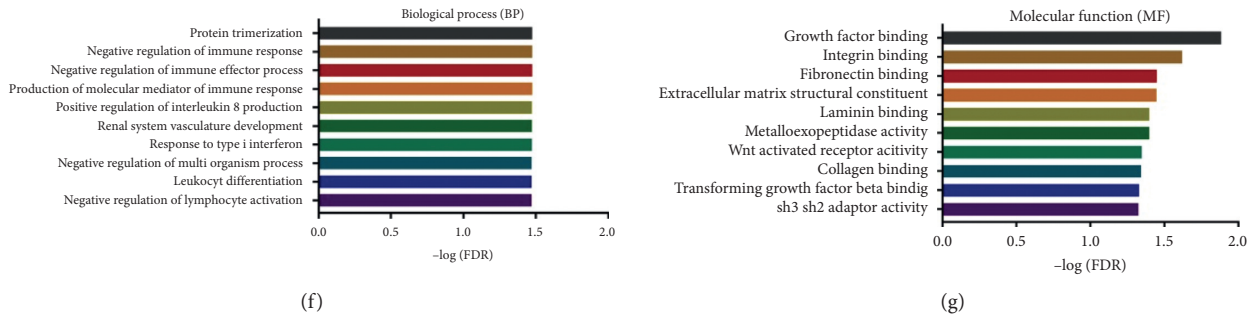

(d)
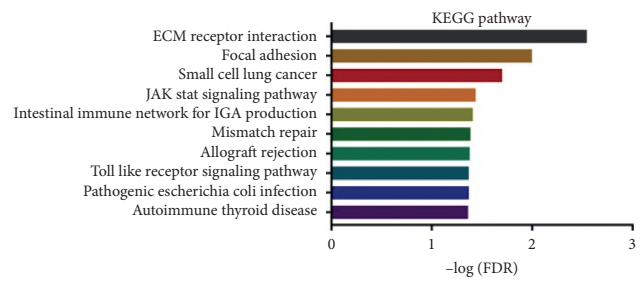
(e)

FIGURE 3: Continued.

(f)

(g)

FIGURE 3: Clinical correlation and functional enrichment analysis of high-risk score group. (a) The correlation between 8 immune-related lncRNA and WHO grades. (b, c) High-risk score group was correlated with immune response and immune system process gene sets by GSEA analysis. (f, d, g, e) Functional enrichment of high-risk score groups in biological process, cellular components, molecular function, and KEGG pathway.

(a)

(b)

(c)

(d)

FIGURE 4: Continued.

(e)

(f)

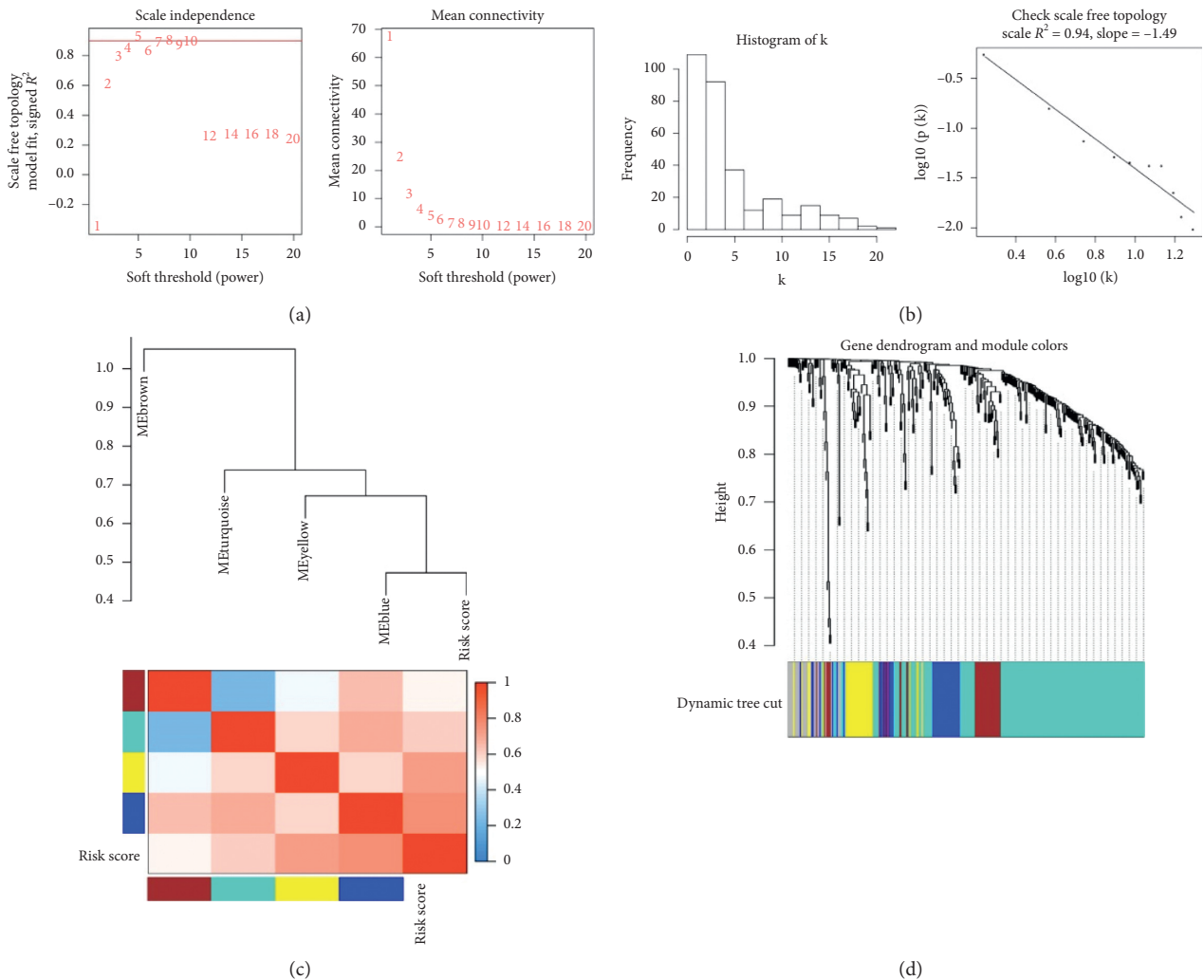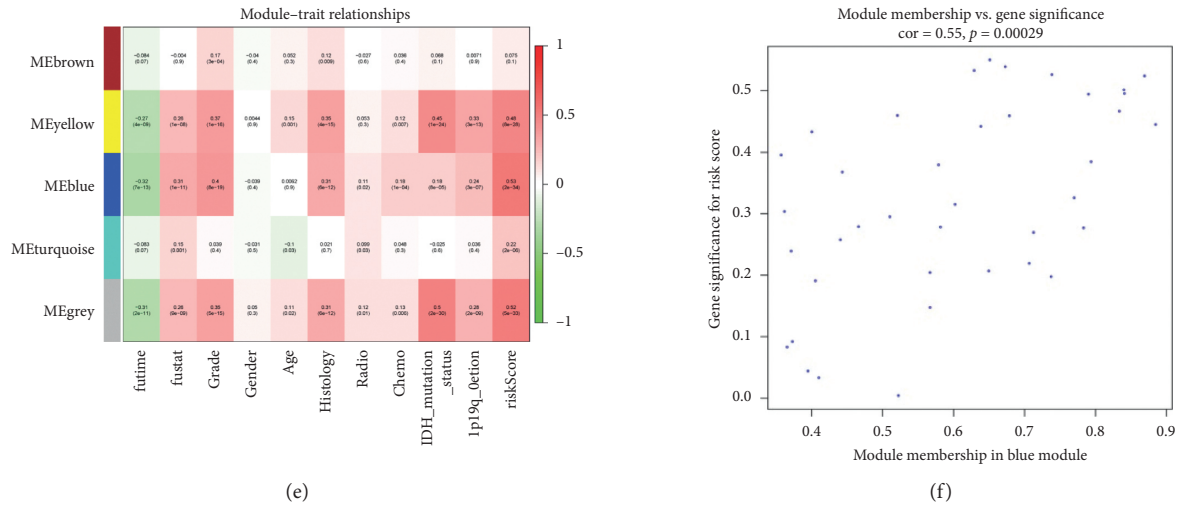Figure 4: WGCNA of 312 transcription factors in CGGA cohort. (a, b) The scale-free fit index for soft-thresholding powers. (c, d) Dendrogram of all differentially expressed transcription factors clustered based on different metrics. (e) Heatmap of the correlation between module eigengenes and clinical traits. (f) The correlation between gene expression and risk score in blue module.

(a)

Figure 5: Continued.

(b)

(c)

Figure 5: (a) The network between 8 immune-related lncRNA and transcription factors. Circles represent lncRNA, triangles represent transcription factors, red lines mean positive correlations, and green lines mean negative correlations. (b, c) Functional enrichment of selected lncRNA and transcription factors in GO and KEGG.

(a)

(b)

(c)

Figure 6: Continued.

(d)

(e)

(f)

(g)
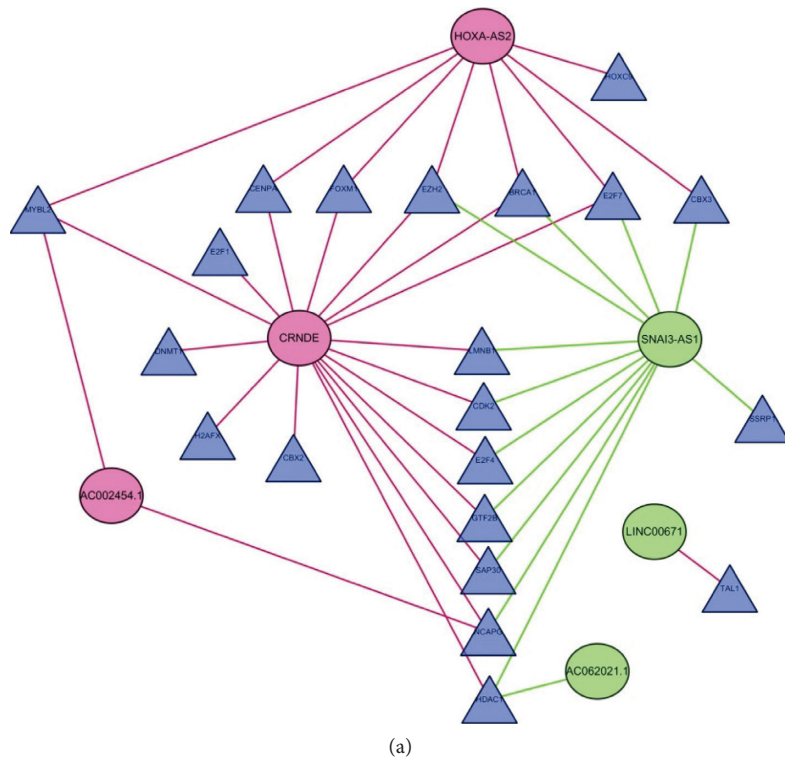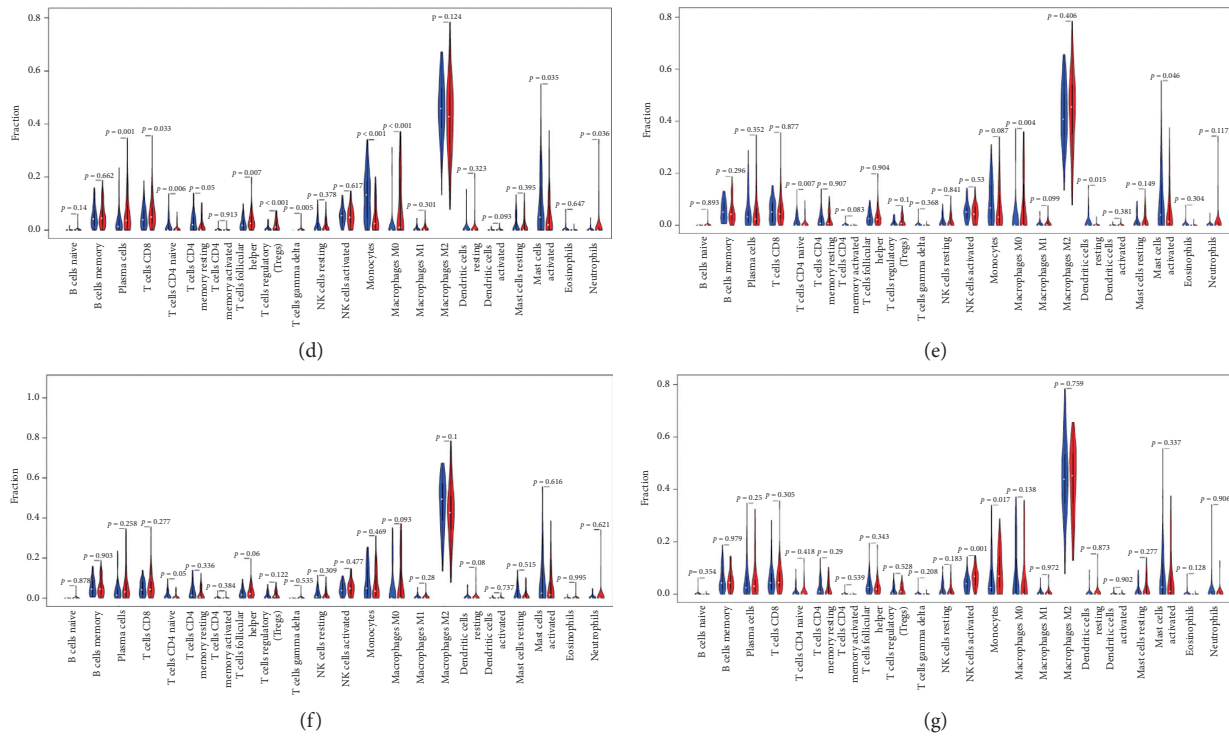
Figure 6: (a) 22 subtypes of immune cells in CGGA cohort. (b, c) Correlation analysis between 22 subtypes of immune cells. (d) The different infiltrating immune cells in high/low-risk score group. (e–g) The different infiltrating immune cells in high/low expression of 3 core lncRNA.

transcription factors were consistent with CRNDE and HOXA-AS2. 7 transcription factors were positively correlated with CRNDE but negatively with SNAI3-AS1. Besides, 4 transcription factors were positively correlated with HOXA-AS2 but negatively with SNAI3-AS1. In addition, CRNDE and HOXA-AS2 were significantly increased in high grade gliomas while SNAI3-AS1 was deceased.

To investigate the role of classifier and 3 core lncRNA in glioma immune microenvironment, we calculated 22 subtypes of glioma-infiltrating immune cells based on CIBERTSORT [29]. Tregs are correlated with unfavorable prognosis in several kinds of tumor microenvironment [30–32] (e.g., ovarian cancer, breast cancer, kidney cancer, and pancreatic cancer). Our results showed that Tregs were significantly increased in high-risk score group, which demonstrated that Tregs may promote the development of gliomas. Mast cells play an important role in the growth of tumor [33]. However, the contribution of mast cells to the microenvironment of solid malignancies remains controversial. Previous studies have illustrated that gliomas are associated with a profound accumulation of mast cells. Our results showed that activated mast cells were significantly decreased in high-risk group. Consistent with previous study, gliomas may block resting mast cell activation. We found that macrophages M0 were significantly increased in high-risk group while monocytes were decreased. In addition, high expression of CRNDE and HOXA-AS2 showed the same trend while SNAI3-AS1 had opposite trend. These results indicate that the high-risk immune lncRNA (CRNDE and HOXA-AS2) may promote gliomas progression by

transforming monocytes into macrophages while the low-risk immune lncRNA conversely inhibit macrophages infiltration. Natural killer (NK) cells are cellular components of the immune system that are more difficult to deceive by tumor cells and have greater cytotoxic activity [34]. Previous studies have found that lncRNA plays an important role in NK cell development and function [35]. Although gliomas are frequently infiltrated by natural killer (NK) cells, these are actively suppressed by the gliomas microenvironment [36, 37]. According to our results, activated NK cells showed no difference or slightly decreased in high expression of CRNDE and HOXA-AS2, but activated NK cells were significantly increased in high expression of SNAI3-AS1. This result suggested that high expression of SNAI3-AS1 may suppress gliomas progression by activating NK cells and be regulated by multiple transcription factors.

However, there were several limitations in our research. First, the calculation results from public database may show bias. Although we have verified the results in two independent cohorts, we should carry out deeper research. Second, the correlation between 3 core lncRNA and transcription factors in gliomas needs further confirmation *in vitro* and *in vivo*.

5. Conclusion

We successfully constructed a prognostic classifier based on immune-related lncRNA in gliomas. 8 selected immune-correlated lncRNA were independent prognostic factors and were significantly correlated with WHO

grades. We also built a correlation network between immune-related lncRNA and transcription factors. The landscape of gliomas immune microenvironment and the function of core immune-related lncRNA were investigated by CIBERSORT. Our research provided a strong classifier to predict gliomas patient outcome, and these results may stimulate new strategy of immunotherapy in gliomas patients.

Data Availability

The datasets including the clinical information and the gene expression data of glioma patients analyzed during the current study are available in the TCGA database (https://cancergenome.nih.gov/) and CGGA database (http://www.cgga.org.cn/).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] Q. Hu, T. Kang, J. Feng et al., "Tumor microenvironment and angiogenic blood vessels dual-targeting for enhanced anti-glioma therapy," *ACS Applied Materials & Interfaces*, vol. 8, no. 36, pp. 23568–23579, 2016.

[2] T. Xie, B. Liu, C.-G. Dai et al., "Glioma stem cells reconstruct similar immunoinflammatory microenvironment in different transplant sites and induce malignant transformation of tumor microenvironment cells," *Journal of Cancer Research and Clinical Oncology*, vol. 145, no. 2, pp. 321–328, 2019.

[3] X. Meng, C. Duan, H. Pang et al., "DNA damage repair alterations modulate M2 polarization of microglia to remodel the tumor microenvironment via the p53-mediated MDK expression in glioma," *EBioMedicine*, vol. 41, pp. 185–199, 2019.

[4] Y. Tian, Y. Ke, and Y. Ma, "High expression of stromal signatures correlated with macrophage infiltration, angiogenesis and poor prognosis in glioma microenvironment," *PeerJ*, vol. 8, Article ID e9038, 2020.

[5] M. K. Boisen, C. B. Holst, N. Consalvo, O. L. Chinot, and J. S. Johansen, "Plasma YKL-40 as a biomarker for bevacizumab efficacy in patients with newly diagnosed glioblastoma in the phase 3 randomized AVAglio trial," *Oncotarget*, vol. 9, no. 6, pp. 6752–6762, 2018.

[6] L. Petrova, P. Korfiatis, O. Petr et al., "Cerebral blood volume and apparent diffusion coefficient - valuable predictors of non-response to bevacizumab treatment in patients with recurrent glioblastoma," *Journal of the Neurological Sciences*, vol. 405, Article ID 116433, 2019.

[7] X. Qian, J. Zhao, P. Y. Yeung, Q. C. Zhang, and C. K. Kwok, "Revealing lncRNA structures and interactions by sequencing-based approaches," *Trends in Biochemical Sciences*, vol. 44, no. 1, pp. 33–52, 2019.

[8] W. X. Peng, P. Koirala, and Y.-Y. Mo, "LncRNA-mediated regulation of cell signaling in cancer," *Oncogene*, vol. 36, no. 41, pp. 5661–5667, 2017.

[9] M. Avgeris, A. Tsilimantou, P. K. Levis et al., "Loss of GAS5 tumour suppressor lncRNA: an independent molecular cancer biomarker for short-term relapse and progression in bladder cancer patients," *British Journal of Cancer*, vol. 119, no. 12, pp. 1477–1486, 2018.

[10] Q. Wang, J. Zhang, Y. Liu et al., "A novel cell cycle-associated lncRNA, HOXA11-AS, is transcribed from the 5-prime end of the HOXA transcript and is a biomarker of progression in glioma," *Cancer Letters*, vol. 373, no. 2, pp. 251–259, 2016.

[11] C. Neftel, J Laffy, M. G Filbin et al., "An integrative model of cellular states, plasticity, and genetics for glioblastoma," *Cell*, vol. 178, no. 4, pp. 835–e21, 2019.

[12] L. Zhao, Y. Liu, J. Zhang, Y. Liu, and Q. Qi, "LncRNA SNHG14/miR-5590-3p/ZEB1 positive feedback loop promoted diffuse large B cell lymphoma progression and immune evasion through regulating PD-1/PD-L1 checkpoint," *Cell Death & Disease*, vol. 10, no. 10, p. 731, 2019.

[13] D. T. Graves and T. N. Milovanova, "Mucosal immunity and the FOXO1 transcription factors," *Frontiers in Immunology*, vol. 10, p. 2530, 2019.

[14] C. M. Jackson, J. Choi, and M. Lim, "Mechanisms of immunotherapy resistance: lessons from glioblastoma," *Nature Immunology*, vol. 20, no. 9, pp. 1100–1109, 2019.

[15] M. Lim, Y. Xia, C. Bettegowda, and M. Weller, "Current state of immunotherapy for glioblastoma," *Nature Reviews. Clinical Oncology*, vol. 15, no. 7, pp. 422–442, 2018.

[16] C. T. Petersen and G. Krenciute, "Next generation CAR T cells for the immunotherapy of high-grade glioma," *Frontiers in Oncology*, vol. 9, p. 69, 2019.

[17] T. McGranahan, K. E. Therkelsen, S. Ahmad, and S. Nagpal, "Current state of immunotherapy for treatment of glioblastoma," *Current Treatment Options in Oncology*, vol. 20, no. 3, p. 24, 2019.

[18] Y. Tan, W. Mu, X.-C. Wang, G.-Q. Yang, R. J. Gillies, and H. Zhang, "Improving survival prediction of high-grade glioma via machine learning techniques based on MRI radiomic, genetic and clinical risk factors," *European Journal of Radiology*, vol. 120, Article ID 108609, 2019.

[19] Y. J. Zheng, T.-S. Liang, J. Wang et al., "Silencing lncRNA LOC101928963 inhibits Proliferation and promotes Apoptosis in spinal cord glioma Cells by Binding to PMAIP1. Molecular therapy," *Nucleic Acids*, vol. 18, pp. 485–495, 2019.

[20] N. GuX. Wang et al., "Silencing lncRNA FOXD2-AS1 inhibits proliferation, migration, invasion and drug resistance of drug-resistant glioma cells and promotes their apoptosis via microRNA-98-5p/CPEB4 axis," *Aging*, vol. 11, no. 22, pp. 10266–10283, 2019.

[21] Q. Li, C. Dong, J. Cui, Y. Wang, and X. Hong, "Over-expressed lncRNA HOTAIRM1 promotes tumor growth and invasion through up-regulating HOXA1 and sequestering G9a/EZH2/Dnmts away from the HOXA1 gene in glioblastoma multiforme," *Journal of Experimental & Clinical Cancer Research: CR*, vol. 37, no. 1, p. 265, 2018.

[22] K. M. Y. Kiang, X. Q. Zhang, and G. K.-K. Leung's, "Long non-coding RNAs: the key players in glioma pathogenesis," *Cancers*, vol. 7, no. 3, pp. 1406–1424, 2015.

[23] P. Jia, H. Cai, X. Liu et al., "Long non-coding RNA H19 regulates glioma angiogenesis and the biological behavior of glioma-associated endothelial cells by inhibiting microRNA-29a," *Cancer Letters*, vol. 381, no. 2, pp. 359–369, 2016.

[24] Q. Hu, A. Zeng, X. Jin et al., "H19 functions as a competing endogenous RNA to regulate EMT by sponging miR-130a-3p in glioma," *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*, vol. 50, no. 1, pp. 233–245, 2018.

[25] L. Bao and X. Li, "MicroRNA-32 targeting PTEN enhances M2 macrophage polarization in the glioma microenvironment and further promotes the progression of glioma,"

Molecular and Cellular Biochemistry, vol. 460, pp. 67–79, 2019.

[26] Y. Gao, H. Yu, Y. Liu et al., "Long non-coding RNA HOXA-AS2 regulates malignant glioma behaviors and vasculogenic mimicry formation via the MiR-373/EGFR axis," *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*, vol. 45, no. 1, pp. 131–147, 2018.

[27] J. Zheng, X. Liu, P. Wang et al., "CRNDE promotes malignant progression of glioma by attenuating miR-384/PIWIL4/STAT3 axis," *Molecular Therapy: The Journal of the American Society of Gene Therapy*, vol. 24, no. 7, pp. 1199–1215, 2016.

[28] K. M. Kiang, X.-Q. Zhang, G. P. Zhang et al., "CRNDE expression positively correlates with EGFR activation and modulates glioma cell growth," *Targeted Oncology*, vol. 12, no. 3, pp. 353–363, 2017.

[29] B. Chen, M. S. Khodadoust, C. L. Liu, A. M. Newman, and A. A. Alizadeh, "Profiling tumor infiltrating immune cells with CIBERSORT," *Methods in Molecular Biology (Clifton, N.J.)*, vol. 1711, pp. 243–259, 2018.

[30] K. I. Woroniecka, K. E. Rhodin, P. Chongsathidkiet, K. A. Keith, and P. E. Fecci, "T-cell dysfunction in glioblastoma: applying a new framework," *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, vol. 24, no. 16, pp. 3792–3802, 2018.

[31] L. Vandenberk and S. W. Van Gool, "Treg infiltration in glioma: a hurdle for antiglioma immunotherapy," *Immunotherapy*, vol. 4, no. 7, pp. 675–678, 2012.

[32] R. Mirzaei, S. Sarkar, and V. W. Yong, "T cell exhaustion in glioblastoma: intricacies of immune checkpoints," *Trends in Immunology*, vol. 38, no. 2, pp. 104–115, 2017.

[33] J. Põlajeva, A. M. Sjösten, N. Lager et al., "Mast cell accumulation in glioblastoma with a potential role for stem cell factor and chemokine CXCL12," *PLoS One*, vol. 6, no. 9, Article ID e25222, 2011.

[34] I. Golán, L. Rodríguez de la Fuente, and J. A. Costoya, "NK cell-based glioblastoma immunotherapy," *Cancers*, vol. 10, no. 12, 2018.

[35] C. Yang, C. Shen, T. Feng, and H. Li, "Noncoding RNA in NK cells," *Journal of Leukocyte Biology*, vol. 105, no. 1, pp. 63–71, 2019.

[36] L. Zhu, J. M. Oh, P. Gangadaran et al., "Targeting and therapy of glioblastoma in a mouse model using exosomes derived from natural killer cells," *Frontiers in Immunology*, vol. 9, p. 824, 2018.

[37] M. C. Burger, C. Zhang, P. N. Harter et al., "CAR-engineered NK cells for the treatment of glioblastoma: turning innate effectors into precision tools for cancer immunotherapy," *Frontiers in Immunology*, vol. 10, p. 2683, 2019.