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ORIGINAL RESEARCH

A 1p/19q Codeletion-Associated Immune Signature for Predicting Lower Grade Glioma Prognosis

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Abstract

Lower grade gliomas (LGGs) with codeletion of chromosomal arms 1p and 19q (1p/19 codeletion) have a favorable outcome. However, its overall survival (OS) varies. Here, we established an immune signature associated with 1p/19q codeletion for accurate prediction of prognosis of LGGs. The Chinese Glioma Genome Atlas (CGGA) and The Cancer Genome Atlas (TCGA) databases with RNA sequencing and corresponding clinical data were dichotomized into training group and testing group. The immune-related differentially expressed genes (DEGs) associated with 1p/19q codeletion were screened using Cox proportional hazards regression analyses. A prognostic signature was established using dataset from CGGA and tested in TCGA database. Subsequently, we explored the correlation between the prognostic signature and immune response. Thirteen immune genes associated with 1p/19q codeletion were used to construct a prognostic signature. The 1-, 3-, 5-year survival rates of the low-risk group were approximately 97%, 89%, and 79%, while those of the high-risk group were 81%, 50% and 34%, respectively, in the training group. The nomogram which comprised age, WHO grade, primary or recurrent types, 1p/19q codeletion status and risk score provided accurate prediction for the survival rate of glioma. DEGs that were highly expressed in the high-risk group clustered with many immune-related pathways. Immune checkpoints including TIM3, PD1, PDL1, CTLA4, TIGIT, MIR155HG, and CD48 were correlated with the risk score. VAV3 and TNFRFSF11B were found to be candidate immune checkpoints associated with prognosis. The 1p/19q codeletion-associated immune signature provides accurate prediction of OS. VAV3 and TNFRFSF11B are novel immune checkpoints.

Keywords Lower grade gliomas · The cancer genome atlas · Chinese glioma genome atlas · Immune · Prognosis

Introduction

Glioma, which derives from glial cells, is the commonest primary intracranial malignancy and is associated with poor outcomes. Gliomas are classified into grade I, II, III, or IV (Louis et al. 2007). Those in histological grade IV, such as glioblastoma (GBM), are considered high grade gliomas, while those in grade II and III are regarded as lower grade gliomas (LGG) (Kiran et al. 2019). The median GBM survival is 1 to 2 years after diagnosis while the overall survival (OS) for LGG patients ranges between 5 and 10 years. Despite advances in cancer screening, diagnosis and treatment, LGGs often progresses into high grade glioma within years (Huang et al. 2017; Kiran et al. 2019; Stupp et al. 2005). While LGG patients experience a longer survival times and a better quality of life, progression into GBM, is associated with poor therapeutic options and significantly lower prognosis. Therefore, effective LGGs treatments are of utmost importance for improved glioma outcomes.

Conventionally, brain tumors are classified through histogenesis, by observing microscopic tumor features. However, over time, it became clear that more efficient techniques were needed, leading to the development of molecular classification features techniques (Louis 2012). Currently, the WHO recommends that molecular parameters, such as the codeletion of chromosomal arms 1p and 19q (1p/19 codeletion), and isocitrate dehydrogenase (IDH) mutation status be

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included in the histopathologic classification of brain cancers (Louis et al. 2016). Numerous studies have associated 1p/19 codeletions and IDH mutations with better glioma outcomes (Leeper et al. 2015; Park et al. 2018). However, prognoses of glioma patients harboring 1p/19 codeletion vary widely (Hu et al. 2017). Little is known about how 1p/19 codeletion affects LGG prognosis.

In glioma treatment, surgery, followed by chemotherapy and radiotherapy are associated with some improvement in therapeutic benefits relative to surgery alone. Immunotherapy is expected to improve treatment outcomes against glioma. While 1p/19 codeletion is being used in LGGs classification, little is known about the correlation between 1p/19 codeletion, the immune system and OS (Ceccarelli et al. 2016). Here, we uncovered a prognostic immune signature that correlates with 1p/19 codeletion. We hypothesize that gene expression reprogramming that follows the 1p/19 codeletion might modulate the immune system.

Materials and Methods

Identification of Immune-Related Genes Correlated with 1p19q Codeletion

Glioma RNA-seq datasets and corresponding clinical information were downloaded CGGA (https://www.cgga.org.cn/) and TCGA (https://portal.gdc.cancer.gov/). Kaplan–Meier (KM) analysis was then used to evaluate survival. Log-rank tests were used to assess the correlation between 1p19q codeletion status and OS in various WHO grade phenotypes. The 1p19q codeletion status assessed using gene expression analysis as done previously Hu et al. (2017). Next, univariate and multivariate Cox regression analyses were used to evaluate the value 1p19q codeletion as an independent prognostic factor. Differentially expressed genes (DEGs) in 1p19q codeletion vs non-deleted samples were identified using the "limma" package in R software (version 3.6.1), by imposing the following criteria: |log2 fold change, log2FC|> 1 and an adjusted $p = < 0.05$. This analysis involved data from 192 1p19q codeletion LGG samples and 394 non-deletion LGG samples. 1p19q codeletion-associated immune-related DEGs were identified from the DEGs based on immune-related gene annotation on the IMMPORT website (https://www.immport.org/) (Zhang et al. 2019a). Only genes shared by CGGA and TCGA were included in downstream analyses. Only samples for which an OS time of > 90 days were retained for downstream analyses.

Elucidation of the Prognostic Signature

Next, univariate Cox proportional hazards regression and LASSO (least absolute shrinkage and selection operator) Cox regression analyses were done on the 1p19q codeletion-associated immune-related DEGs to prognosis-associated genes. The LASSO regression algorithm is used to reduce overfitting high-dimensional prognostic genes (Castro et al. 2019; Goeman 2010). Multivariate Cox proportional hazards regression analysis was then used to establish a prognostic signature with a coefficient (β) based on all the genes included in the signature (Deng et al. 2019). The risk score was a sum value calculated in accordance with the formulate: risk score = (expression of gene A1*β1) + (expression of gene A2*β2) + (expression of gene A3*β3) + … (expression of gene An*βn) (Qian et al. 2018). All CGGA dataset samples were identified and classified as either low-risk or high-risk based on the median risk score (Liu et al. 2019). KM survival plots and log-rank tests were used to evaluate the correlation between risk scores and OS.

Validation of the Prognostic Signature

Next, internal and external validation analyses were done to verify the prognostic signature's predictive power, which was evaluated using survival plots, 1-, 3-, and 5-year time-dependent receiver operating characteristic (ROC) curves, and survival status plots were (Yang et al. 2020). Heatmaps and violin plots were used to visualize the expression profiles of the prognostic signature genes in the low and high-risk groups.

Evaluation of the Independent Value of the Prognostic Signature

Correlation between risk score and clinical information including age, sex, radiotherapy status, chemotherapy status, tumor grade, primary or recurrent tumor and IDH mutation status were analyzed. Univariate and multivariate Cox regression coupled with available clinical information were used to evaluate the independent prognostic capacity of the risk score.

Prognostic Nomogram Analysis and Validation

Independent prognostic factors emerging from the CGGA dataset were subjected to nomogram analysis to predict the 1-, 3- and 5-year survival (Long et al. 2019). 5 independent prognostic factors, age, WHO grade, primary or recurrent glioma types, 1p19q codeletion status, and risk score, were used to develop the nomogram. We then validated the nomogram's prognosis accuracy using concordance index (C-index) combined with a calibration curve plot. This analysis was done in 1000 reiterations (Duan et al. 2018; Kiran et al. 2019).

Gene Ontology Analysis

Next, we executed a GO term analysis of DEGs between the high and low-risk groups. DEGs were identified by setting the following thresholds: log2FC > 1.2 and *p-value* < 0.05. Significantly enriched GO terms were indicated by *p-value* < 0.01, *q value* < 0.01, and gene counts > 10. Results from this analysis were visualized on circle plots.

Analysis of Correlation Between Risk Score and Expression of Immune Checkpoints

Differential expression of 7 established immune checkpoint genes in the low and high-risk groups was analyzed. These are, T cell immunoglobulin domain and mucin domain 3 (TIM3), programmed cell death 1 (PD1), PD1 interacts with programmed death ligand 1 (PDL1), cytotoxic T lymphocyte antigen 4 (CTLA4), T cell immunoreceptor with Ig and ITIM domains (TIGIT), long non-coding RNA MIR 155 host gene (MIR155HG), and CD48 (Filippova et al. 2018; Hung et al. 2018; Liu et al. 2018, 2020; Peng et al. 2019). This analysis evaluated the correlation between risk score and expression of the checkpoint genes. *p*-value = < 0.05 was considered statistically significant.

Evaluation of the Candidate Immune Checkpoints

The following criteria used to elucidate underlying immune checkpoints: (1) DEGs in high-risk and low-risk groups were common in the CGGA and TCGA datasets, (2) genes correlated with OS, (3) the genes were independent of clinical prognosis parameters including age, sex, WHO grade, and IDH1 mutation status, (4) genes had an AUC > 0.7, (5) there was correlation between gene expression and risk score, (6) candidate immune checkpoints genes had a correlation value > 0.6 both in CGGA and TCGA datasets, (7) candidate immune checkpoints show correlation with familiar immune checkpoints, (8) select candidates with a correlation score > 0.4 as immune checkpoints.

Results

1p/19q Codeletion and Differentially Expressed Immune Genes

Analysis of survival in the CGGA datasets, revealed significantly lower OS in cases with 1p/19q codeletion relative

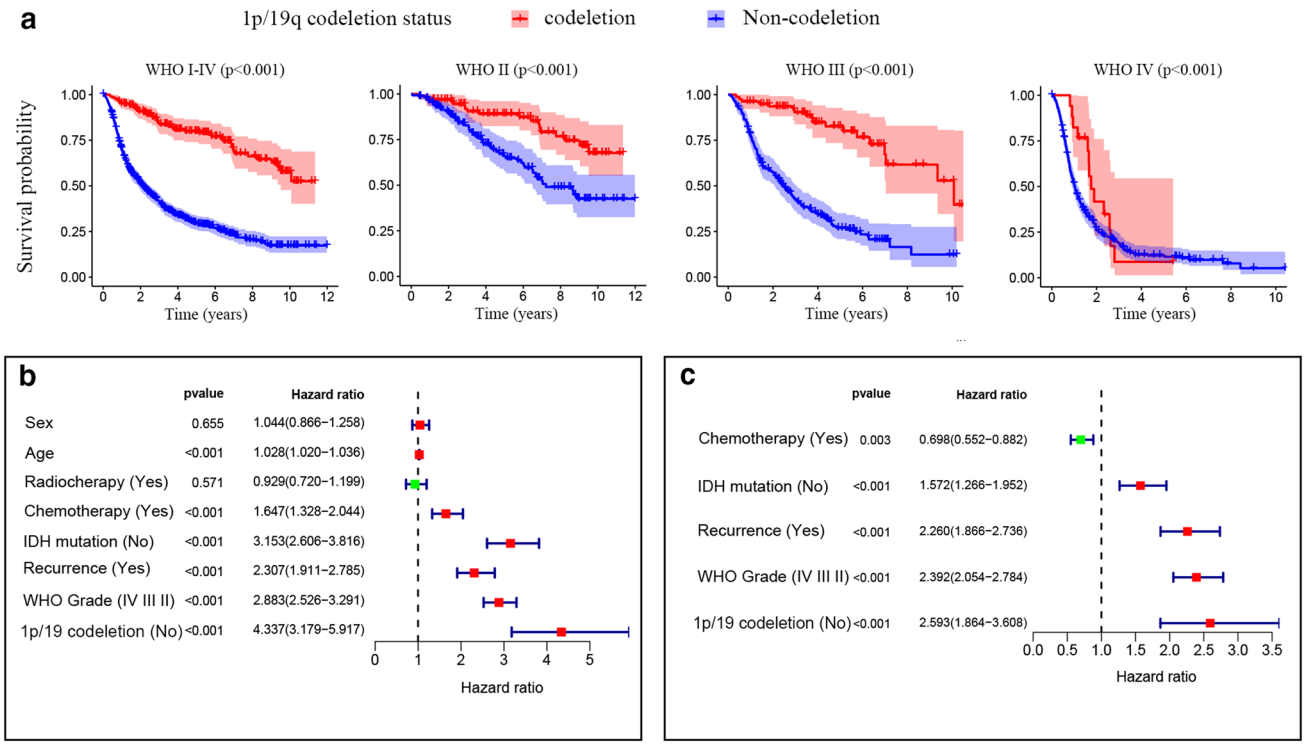

Fig. 1 Kaplan–Meier (KM) survival curves illustrate that 1p/19q codeletion predicts favorable outcomes in gliomas (**a**). 1p/19q codeletion is an independent factor for predicting OS in univariate (**b**) and multivariate (**c**) Cox analyses

to those lacking the codeletion. Interestingly, outcomes were markedly in grade II and III tumors with 1p/19q codeletion relative to grade IV tumors (Fig. 1a). Univariate and multivariate Cox analyses revealed 1p/19q codeletion as an independent prognostic factor for LGGs (Fig. 1b-c). Differential gene expression analysis revealed that 551 DEGs between 1p/19q codeletion samples ($n = 191$) and non-codeletion samples ($n = 393$). Of the 551, 56 are immune-related genes. No statistically significant differences were noted in codeletion vs non-codeletion samples with regards to sex, age, primary or recurrent type, chemotherapy or radiotherapy status (Table 1).

Table 1 The characteristics of samples in CGGA

Variables	1p19q codeletion		Value	*P* value
	Yes (191)	No (393)		
Sex			1.816	0.178
Female	88	158		
Male	103	235		
Age (mean ± SD, years)	40.7 ± 8.6	39.6 ± 10.8	− 1.034	0.181
OS (mean, years)[a]	9.1 (174)	5.6 (363)	66.680	< 0.001
PR type			2.926	0.087
Primary	137	254		
Recurrent	54	139		
Histology type			66.833	< 0.001
Astrocytoma	7	89		
Oligodendroglioma	95	81		
Mixed glioma	89	223		
WHO grade			− 20.558	< 0.001
WHO II	102	170		
WHO III	89	223		
Radio status			0.412	0.814
Yes	146	291		
No	31	69		
Na	14	33		
Chemo status			1.961	0.375
Yes	97	223		
No	69	128		
Na	25	42		
IHD mutation status			57.670	< 0.001
Yes	166	246		
No	7	124		
Na	18	23		

PR primary or recurrent, *OS* overall survival, radio radiotherapy, chemo chemotherapy, *Na* not available

[a]174 codeletion and 363 no codeletion samples were included using log-rank test according to the missing data

Elucidation and Internal Validation of the Immune-Related Prognostic Signature

Univariate Cox proportional hazards regression and LASSO regression analyses of candidate genes revealed 23 genes that were screened in multivariate Cox proportional hazards regression. Thirteen immune-related genes associated with coefficients were included in the prognostic signature (Table 2). KM analysis of the low and high-risk LGG samples using log-rank test revealed that 1-, 3- and 5-year survival rates in the low-risk group were 97%, 89%, and 79%, respectively, while in the high risk groups survival rates were 81%, 50% and 34%, respectively (Fig. 2a). The prognosis was significantly better in cases with lower risk scores, indicating that the risk score negatively correlated with OS. AUC values for the signature's prediction of 1-, 3-, and 5-year survival were 0.818, 0.793, and 0.750, respectively (Fig. 2b). Indicating high risk score correlated with decreased survival (Fig. 2c). The heatmap depicted the visual difference trends of transcript expression of genes incorporated in the signature between the high- and low-risk categories (Fig. 2d). The violin plot presented a statistically differential expression between the two categories (Fig. 2e).

External Validation of the Prognostic Signature

The 1-, 3- and 5-year survival in the TCGA database were 99%, 89%, and 76%, respectively, while in the testing cohort, the corresponding survival rates were only 84%, 51%, and 36%, respectively (Fig. 3a). The capacity of the testing cohort to predict survival was very similar to that of the training cohort. ROC curve analysis was used to validate prediction accuracy. The AUC values for 1-, 3- and

Table 2 Thirteen genes and coefficients in the prognostic signature

Gene	Coef	Gene	Coef
S100A3	0.010009781	F2RL1	0.061144932
FAM19A3	0.048040197	VAV3	0.053997988
ADM2	0.002940087	BMP8B	0.14087201
CLCF1	0.021229638	NTS	0.021282164
TNFRSF11B	0.013799876	AR	0.033614978
GLP1R	− 0.000625507	PRLHR	− 0.084033137
TRDC	0.039746687		

ADM2 adrenomedullin 2, *F2RL1* coagulation factor II (thrombin) receptor-like 1, *NTS* neurotensin, *VAV3* vav 3 guanine nucleotide exchange factor, *CLCF1* cardiotrophin-like cytokine factor 1, *S100A3* S100 calcium binding protein A3, *BMP8B* bone morphogenetic protein 8b, *FAM19A3* family with sequence similarity 19 (chemokine (C–C motif)-like), member A3, *TRDC* T cell receptor delta constant, *PRLHR* prolactin releasing hormone receptor, *AR* androgen receptor, *TNFRSF11B* tumor necrosis factor receptor superfamily, member 11b, *GLP1R* glucagon-like peptide 1 receptor, *coef* coefficient

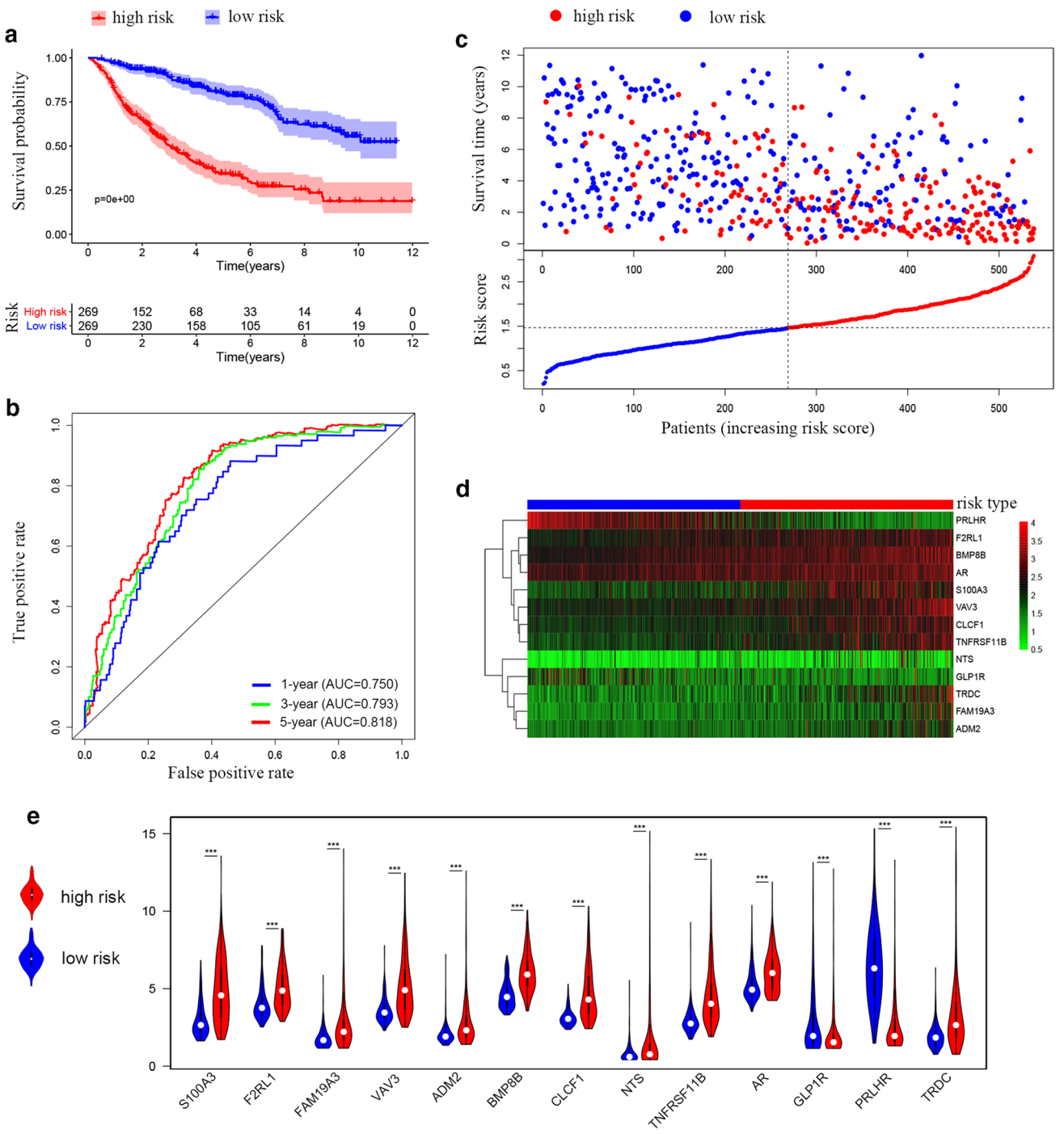

Fig. 2 The prognostic signature validated in the training cohort. The KM survival curve of OS for LGGs (**a**); The time-dependent receiver operating characteristic (ROC) curves for the 1-, 3- and 5-year survival rate (**b**); The survival status of each samples and the distribution of risk scores (**c**); The heatmap (**d**) and violin plot (**e**) of 13 genes between the low- and high-risk groups included the signature

5-year survival were 0.896, 0.785 and 0.708, respectively (Fig. 3b). A similar trend was observed in the testing cohort (Fig. 3c–e).

Evaluation of the Independent Prognostic Value

Correlation between risk score and clinical parameters, including age, sex, radiotherapy and chemotherapy status, tumor grade, primary or recurrent types and IDH mutation status, was assessed. The value of risk score was lower in

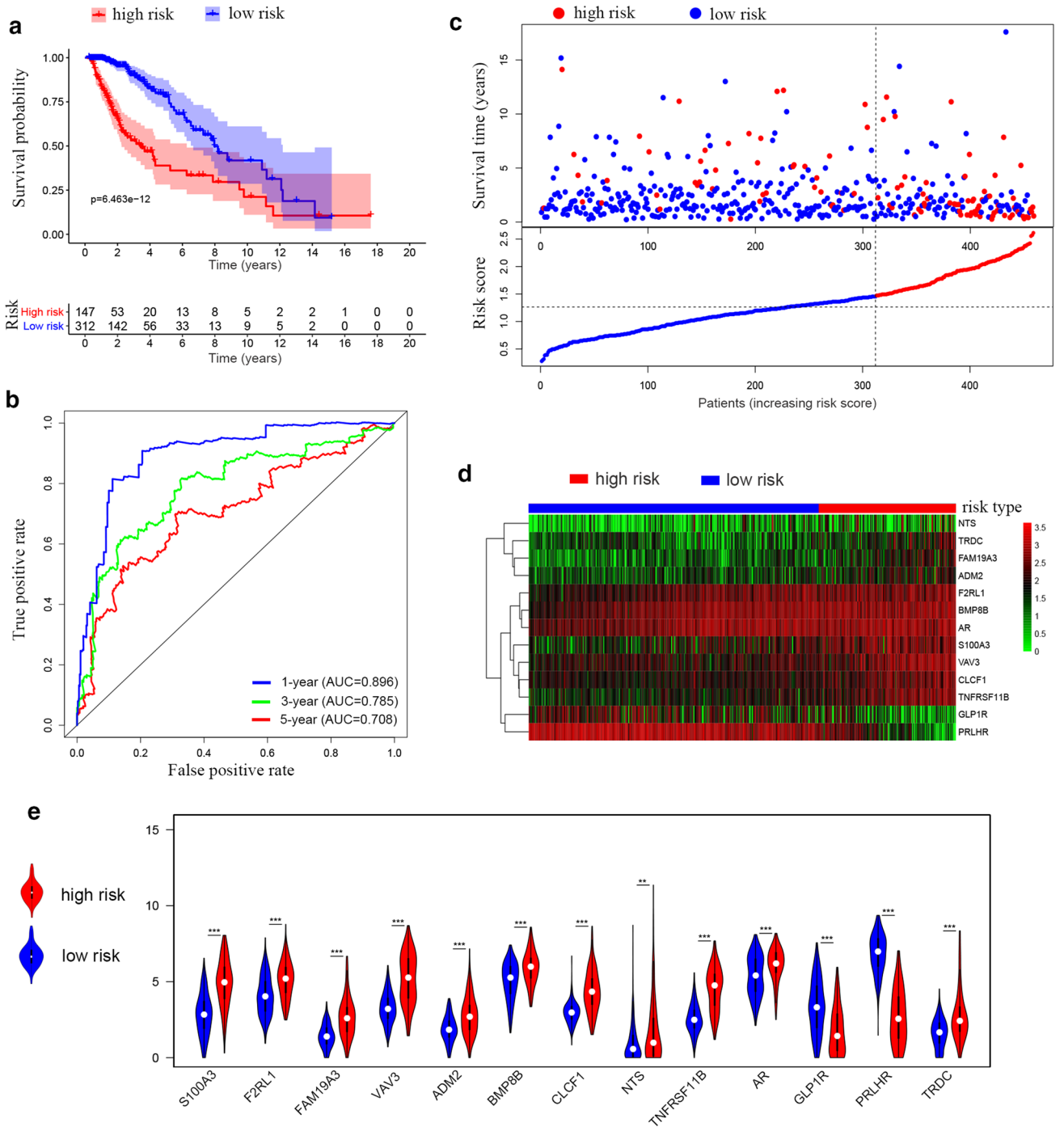

Fig. 3 The prognostic signature validated in the testing cohort. The KM survival curve of OS for LGGs (**a**); The time-dependent ROC curves for 1-, 3- and 5-year survival rate (**b**); The survival status of each samples and the distribution of risk scores (**c**); The heatmap (**d**) and violin plot (**e**) of 13 genes between the low- and high-risk groups included the signature

chemotherapy, 1p/19 codeletion, tumor grade II, primary and IDH mutation categories ($p < 0.05$, Fig. 4a). Univariate and multivariate Cox proportional hazards regression indicated that the risk score phenotype had independent prognostic value in the training testing cohort (Fig. 4b–e).

Nomogram Analysis

Nomogram analysis, using 5 prognostic markers (age, tumor grade, primary or recurrent type, risk score, and 1p/19 codeletion status), was used to predict survival in

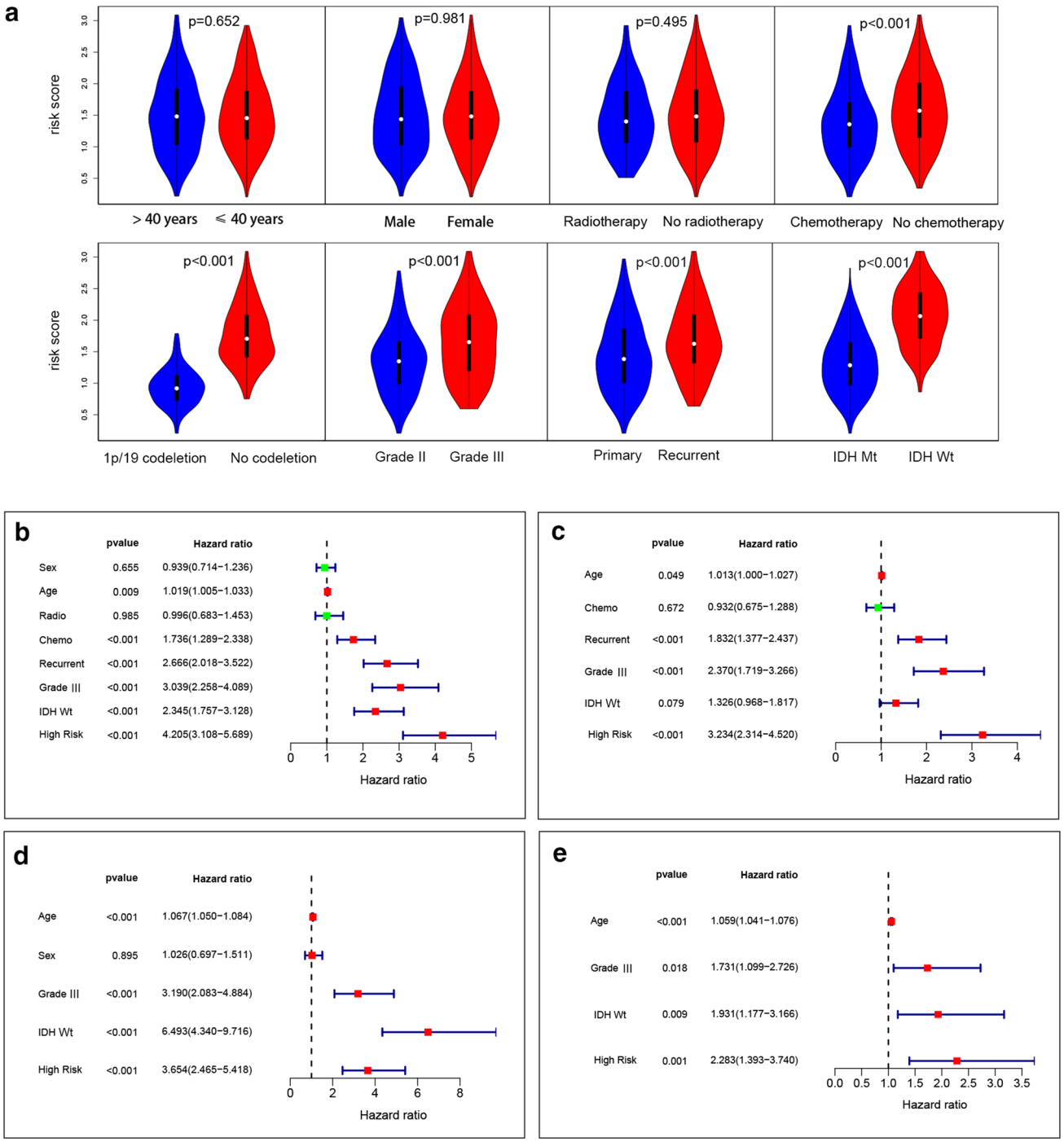

Fig. 4 Lower risk scores in patients receiving chemotherapy, with 1p/19q codeletion, WHO grade II, primary tumor, or IDH mutation. However, risk score in age > 40 years, male, or radiotherapy groups, has no statistical significance (**a**). Univariate (**b**) and multivariate (**c**) Cox proportional hazards regression analyses showing the risk score is an independent predictor of the OS

the training set. Among these prognostic factors, risk score ranked a vital proportion in the total points (Fig. 5a). To validate the accuracy of the individual assessment, concordance index (C-index) and calibration curve of the nomogram were evaluated for internal validation. The C-index of the nomogram was 0.794. The visualized calibration curve for probabilities for 1-, 3- and 5-year OS revealed good agreement between the predicted nomogram and actual survival (Fig. 5b–d). Additionally, internal validation was done by randomly sampling 50% of the CGGA samples. The C-index was 0.797, and the calibration curves had goodness-off-fit (Fig. 5e–g).

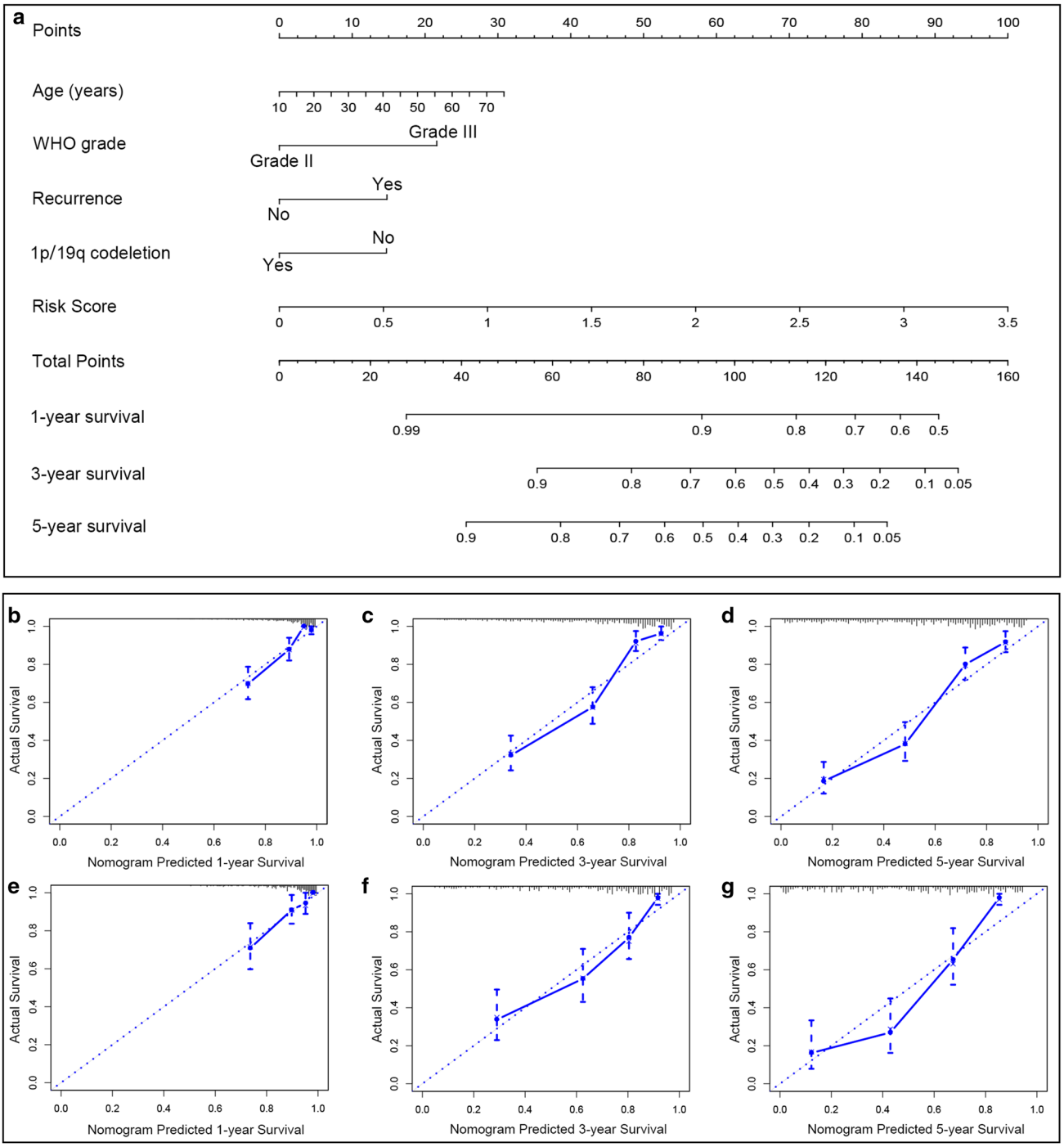

Fig. 5 Nomogram for predicting the 1-, 3-, 5-year OS for LGG according to age, WHO grade, recurrent status, 1p/19q codeletion status and risk score (**a**). Calibration plot for 1-, 3-, 5-year OS predicted by the nomogram in CGGA database (**b**–**d**) and TCGA database (**e**–**g**)

GO Term Analysis

503 genes were differentially expressed between low and high-risk groups. Of these, 255 had a log2FC > 1.2, and 166 of them were included in the term GO analysis. The GO term analysis produced 33 terms that had gene counts > 10, 12 of which (including 37 DEGs) were associated with immune-related terms, including B cell receptor signaling pathway, lymphocyte-mediated immunity and humoral immune response (Fig. 6).

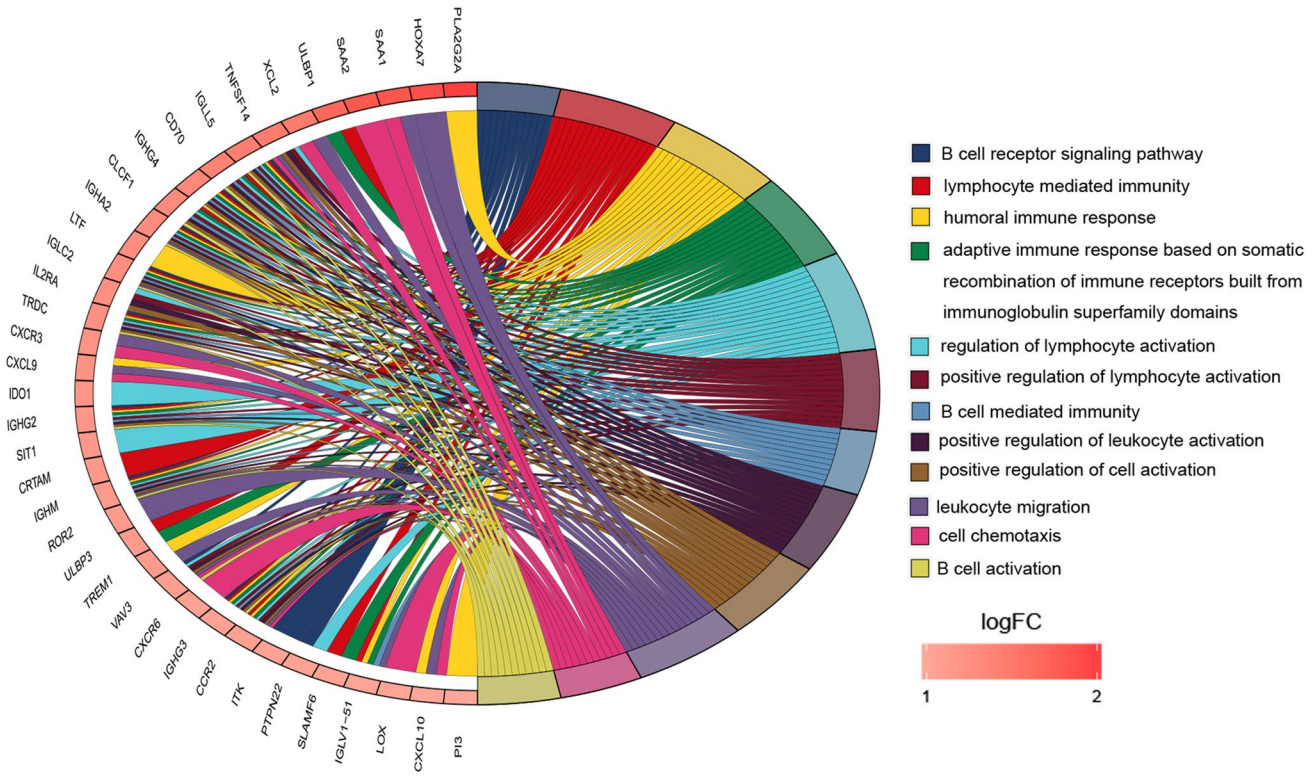

Fig. 6 Chord plot showing 37 genes included in the 12 immune-related pathways

Elucidation of Immune Checkpoints and Risk Score

Next, the relationship between 7 established immune checkpoints and risk score was evaluated (Fig. 7). Expression of immune checkpoint genes, (except TIGIT), in the low-risk vs high-risk samples was statistically significant in the training and testing sets (Fig. 7a, b). TIM3, MIR155, and CD48 exhibited the highest correlation (> 0.4) in the training set (Fig. 7c–i). In the testing set, TIM3, MIR155, PD1, and PDL1 expression positively correlated with risk score (Fig. 7g–p). Analysis of the correlation between DEGs (in low vs high-risk samples) and survival indicated that 279 and 199 genes in the CGGA and TCGA dataset, respectively, are significantly associated with OS. Further analysis revealed 42 and 73 genes in the CGGA and TCGA datasets (AUC > 0.7), respectively, that were independent of age, gender, tumor grade and IDH mutation status. Of these, 20 were common between the 2 datasets. Analysis of correlation between expression of the 20 genes and the risk score revealed 6 (colorectal neoplasia differentially expressed (CRNDE), transmembrane protein 71 (TMEM71), growth arrest specific 2 like 3 (GAS2L3), insulin like growth factor 2 mRNA binding protein 3 (IGF2BP3), vav guanine nucleotide exchange factor 3 (VAV3), TNF receptor superfamily member 11b (TNFRSF11B)) with a correlation coefficient > 0.6 in the training and he validation groups (Fig. 8a–d). VAV3 and TNFRSF11B are immune-related genes. Sankey diagram analysis revealed co-expression between the 7 established immune checkpoint genes and the 6 immune checkpoint genes we identified. CD48, MIR155HG, PDL1 showed a strong relationship (Cor > 0.4, $p < 0.05$) with other immune checkpoints in the training and testing set (Fig. 8e, f). VAV3 exhibited a close relationship with MIR155HG, while TNFRSF11B correlated with MIR155HG and PD1 in the training and testing sets.

Discussion

1p/19q codeletion is a well-established biomarker (Durand et al. 2010), currently recommended by the WHO for tumor grade classification (Ceccarelli et al. 2016). Here, we find that the 1p/19 codeletion related immune genes have prognostic potential in LGG. To design an unbiased prognostic system, we uncovered a prognostic signature by analyzing LGGs RNA-seq and clinical data from CGGA and TCGA. This prognostic signature validated the hypothesis that improved outcomes upon 1p/19q codeletion are associated with altered immunoregulation. In addition to the well-established immune checkpoints, including PD1 and TIM3, uncovered 6 novel immune checkpoint candidate.

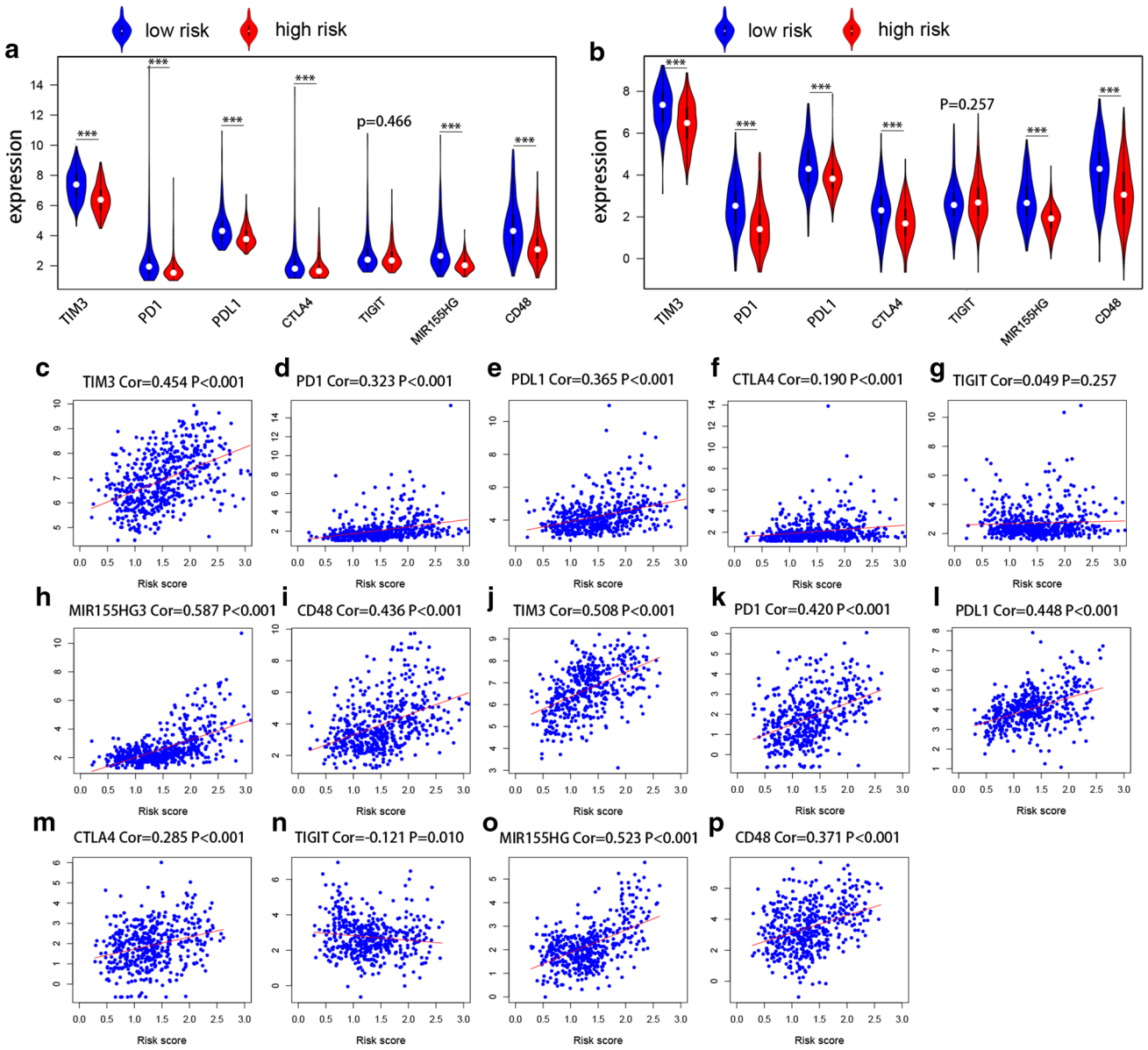

Fig. 7 The expression of seven immune checkpoints (TIM3, PD1, PDL1, CTLA4, TIGIT, MIR155HG, CD48) in low- and high-risk groups in CGGA database (**a**) and TCGA database (**b**). The correlations between the immune checkpoints and risk score in CGGA database (**c**–**i**) and TCGA (**j**–**p**) database

Numerous studies have previously described prognostic signatures for glioma (Jang and Kim 2018; Zhang et al. 2019b; Zhou et al. 2018). Additionally, there has been a growing interest in glioma immunotherapy (Chheda et al. 2018; Deumelandt et al. 2018; Kohanbash et al. 2017; Weller et al. 2017). There is evidence that 1p/19q codeletion correlates with significantly improved glioma prognosis. However, it remains unclear whether the codeletion's impact on outcomes are mediated via immune regulation. Here, we find that an immune-related prognostic signature associated with 1p/19q codeletion might influence glioma prognosis. Unlike a previous study (Zhang et al. 2019c), our prognostic signature, based on a phenotype, decreased heterogeneity and increased prediction accuracy.

Immunotherapy has generated a lot of interest as a treatment for gliomas (Srinivasan et al. 2017). Deng et al. (2019) reported an IDH1 mutation prognostic signature and its association immune-related GO terms. Here, we find that highly expressed genes in high-risk group correlated with various immune-related pathways, including, B cell receptor signaling, lymphocyte-mediated immunity, and humoral immune response. Analysis of the microenvironment has shown that immune-related pathways influence behavior of glioma cells (He et al. 2014). To evaluate the relationship between our

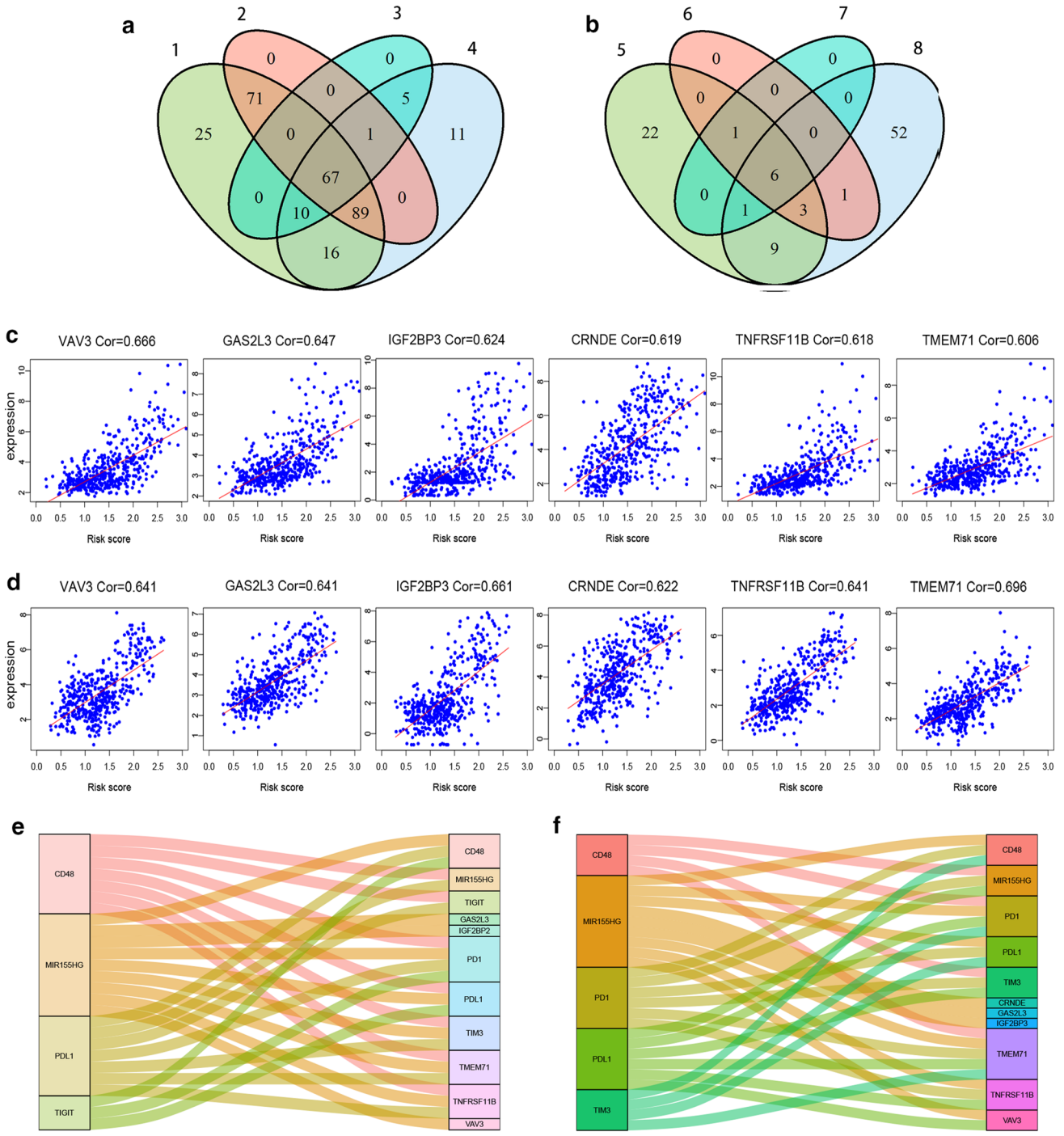

Fig. 8 Venn diagrams for identification of candidate immune checkpoints. The genes are significantly associated with the OS in CGGA dataset (2) and TCGA dataset (3). The genes are independent predictors of OS in CGGA dataset (1) and TCGA dataset (4). Genes have an AUC of > 0.7 (6, 7) and a correlation value of > 0.6 (5, 8) in CGGA dataset and TCGA dataset, respectively. The correlations between six candidate immune checkpoints and the risk score in CGGA dataset (**c**) and TCGA dataset (**d**). Sankey diagrams showing the internal and external correlations between avowed immune checkpoints and candidate immune checkpoints in CGGA dataset (**e**) and TCGA dataset (**f**)

prognostic signature and immunobiology, we evaluate its correlation with well-established immune checkpoint genes, and found that TIM3, PD1, PDL1, CTLA4, MIR155HG, and CD48, but not TIGIT (Hung et al. 2018; Liu et al. 2018; Peng et al. 2019), correlates with risk score. These findings are to some extent consistent with those by Deng et al.

(2019). Recent studies have highlighted the potential of immune checkpoints therapeutic targets. Here, we identified 6 novel immune checkpoint genes (VAV3, GAS2L3, IGF2BP3, CRNDE, TNFRSF11B, and TMEM71) that correlate with prognosis. VAV3 and TNFRSF11B had already been annotated as immune-related genes on IMMPORT (https://www.immport.org/). The expression of these genes also highly correlates with risk score and expression of 7 well-established immune checkpoints. Most of the candidate immune checkpoint genes have been previously associated with glioma (Kryvdiuk et al. 2015; Pop et al. 2018; Salhia et al. 2008). Kiang et al. (2017) reported that CRNDE is elevated in glioma and might be modulated by EGFR signaling to promote gliomagenesis. However, there is little knowledge of the role of our candidate immune checkpoints in immune regulation of glioma. Further studies are needed to experimentally validate their involvement in glioma.

Although the phenotypes of glioma were classified according to molecular biomarkers (Reifenberger et al. 2017), the OS of LGGs with 1p/19q codeletion varies widely. It is clear that the 1p/19q codeletion-associated immune prognostic signature reduces variability, making prognosis more accurate. The immune response pathways associated with high-risk group raised several important questions, including, which immune checkpoint genes might regulate these immune response pathways. Glioma prognosis remains extremely poor, suggesting that the single immune therapy in use has failed to significantly improve OS. However, it should be noted that a diversified immune therapeutic strategy may be more effective. Thus, additional immune checkpoints for LGG treatment should be made first-line treatments along with surgical resection, radiotherapy or chemotherapy. The novel candidate immune checkpoint genes identified here, especially VAV3 and TNFRSF11B, are likely to become established immune checkpoint genes.

The purpose of this study was to establish a prognostic signature for LGGs with 1p/19q codeletion that can be used in clinical settings. However, LGGs including astrocytomas and oligodendrogliomas, have great tissue heterogeneity. The utility of this prognostic signature will likely be limited by such heterogeneity. The candidate immune checkpoint genes, are in fact, prognostic related genes and had a close correlation with the risk score and well-established immune checkpoints. The immune-related function and mechanisms of candidate immune checkpoints in LGGs in our study were hypothesized and their experimental validation is necessary. We contend that immunotherapy based on multiple immune checkpoints simultaneously may provide improved outcomes in glioma.

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Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by JX, FL and LS; The first draft of the manuscript was written by JX and FL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The RNA-seq data and corresponding clinical information were observed from the TCGA (https://portal.gdc.cancer.gov/) and CGGA (https://www.cgga.org.cn). The immune-related gene list was got from the IMMPORT website (https://www.immport.org/).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants performed by any of the authors.

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