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FCGR2B as a prognostic and immune microenvironmental marker for gliomas based on transcriptomic analysis

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Abstract

To explore the expression and prognosis of Fc fragment of IgG low affinity IIb receptor (FCGR2B) in glioma and its relationship with immune microenvironment, so as to provide potential molecular targets for the treatment of glioma. We analyzed the gene expression of FCGR2B using the Cancer Genome Atlas database, Chinese Glioma Genome Atlas, Gene Expression Omnibus database and other glioma related databases. Moreover, we generated survival receiver operating characteristic curve, carried out univariate and multivariate Cox analysis and nomograph construction, and analyzed the relationship between FCGR2B and prognosis. According to the median of FCGR2B gene expression value, the differential expression analysis was carried out by high and low grouping method, and the gene ontology, Kyoto encyclopedia of genes and genomes, and gene set enrichment analysis enrichment analysis were carried out to explore the possible mechanism. Then, the correlation between immune score of glioma and prognosis, World Health Organization grade and FCGR2B expression was analyzed. Finally, the correlation between FCGR2B expression and the proportion of tumor infiltrating immune cells, immune checkpoints, tumor mutation load and immune function was analyzed. The expression of FCGR2B in gliomas was higher than that in normal tissues and was associated with poor prognosis. Independent prognostic analysis showed that FCGR2B was an independent prognostic factor for glioma. The analysis of gene ontology and gene set enrichment analysis showed that FCGR2B was closely related to immune-related functions. The analysis of immune scores and prognosis, World Health Organization grade and FCGR2B expression in gliomas indicated that patients with high immune scores had significantly poorer overall survival and higher tumor pathological grade. In addition, immune scores were significantly positively correlated with the expression of FCGR2B. The analysis of tumor infiltrating immune cells suggested that the expression level of FCGR2B affected the immune activity of TME. In addition, the expression of FCGR2B was positively correlated with almost all immune checkpoint molecules including CD28, CD44, TNFSF14, PDCD1LG2, LAIR1, and CD48 and was significantly positively correlated with tumor mutation load. All immunobiological functions of the high expression group of FCGR2B were significantly inhibited. FCGR2B may play an important role in the occurrence, development and invasion of tumor by influencing the tumor microenvironment of immunosuppression. FCGR2B may be an important target for the treatment of glioma.

Abbreviations: AUC = area under curve, CGGA = Chinese Glioma Genome Atlas, CI = confidence interval, FCGR2B = Fc fragment of IgG low affinity IIb receptor, GEO = Gene Expression Omnibus, GO = gene ontology, GSEA = gene set enrichment analysis, HR = hazard ratio, KEGG = Kyoto encyclopedia of genes and genomes, ROC = receiver operating characteristic, TCGA = the Cancer Genome Atlas database, TICs = tumor infiltrating immune cells, TMB = tumor mutation load, TME = tumor microenvironment, WHO = World Health Organization.

Keywords: FCGR2B, glioma, prognosis, tumor microenvironmental

1. Introduction

Glioma is the most common malignant primary intracranial tumor, originating from glial cells, accounting for about 80% of intracranial malignant tumors.[1,2] The total incidence rate of glioma varies from 4.67 to 5.73 per 100,000 people. Glioblastoma is the most common and fatal subtype of glioma in adults, with a incidence rate of 0.59 to 3.69 per 100,000

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people.[3,4] Great progress has been made in surgical methods and other therapies for glioma, but the prognosis of patients with glioma, especially glioblastoma, is still poor.[3–7] At present, the median survival period of GBM patients is only 12 to 15 months after treatment, even though they have received comprehensive treatment including surgery, radiotherapy and chemotherapy and even electric field therapy.[8–10] Therefore, searching for new gene targets that play a role in the pathogenesis of glioma may find more effective treatment strategies for this disease.[11,12]

Fc fragment of IgG low affinity IIb receptor (FCGR2B) is immunoglobulin γ Low-affinity receptor in Fc region of complex and is a member of fragment crystallizable receptor (FcR) family. FCR is an immune cell expressed protein that binds to the Fc region of immunoglobulin (IgG) and plays a crucial role in regulating the crosstalk between innate and acquired immune responses.[13] FCGR2B is involved in a variety of effects and regulatory functions, such as the phagocytosis of immune complexes and the regulation of antibody production by B cells.[14,15] The binding with this receptor leads to the down-regulation of the activation state of cells previously triggered by the antigen receptor on B cells (BCR), T cells (TCR) or another Fc receptor.[16,17] Previous studies have shown that FCGR2B is closely related to the occurrence and development of tumors, so regulating the expression of FCGR2B may become a new way to treat tumors.[18] It has been confirmed that FCGR2 can be divided into 3 subtypes: FCGR2A, FCGR2B, and FCGR2C. The main difference between these subtypes lies in the different structure of the intracellular region.[19] Among them, FCGR2A is an activated receptor, and the intracellular region contains tyrosine receptor activation motif (ITAM), which leads to tyrosine phosphorylation and activation of cells after binding with immune complex. FCGR2B is an inhibitory receptor, and its intracellular region contains tyrosine receptor inhibitory motif (ITIM), which, when combined with immune complex, triggers a cascade reaction based on ITIM signal, inhibits cell activation, and even induces cell apoptosis. The two can coexist on the same cell surface, maintain normal physiological balance, and are related to the occurrence, development and treatment of tumors and autoimmune diseases.[20] Recent studies have found that white variety laboratory mice (BALB/c mice) lacking FCGR2B can inhibit the growth of tumor. This indicates that FCGR2B plays an important role in the occurrence and development of tumor.[21] FCGR2B is related to the mechanism of human mouse chimeric anti-CD20 monoclonal antibody treating CD20 positive lymphoma.[22] However, the role and mechanism of FCGR2B in glioma are still unknown.

In this study, we collected glioma samples from multiple public databases, including the expression sequence and clinical data of 1018 samples from the Chinese Glioma Genome Atlas (CGGA) databases, the expression data of 693 samples and the clinical data of 1114 samples from the Cancer Genome Atlas (TCGA), the 23 normal samples and 157 tumor samples from the GSE4290 datasets, the 5 normal samples and 30 glioma samples from GSE15824 datasets and the 12 glioma samples from GSE8692 datasets to explore the expression, prognosis, biological function and related mechanism of FCGR2B in glioma samples. This research aims to provide new markers and potential therapeutic targets for glioma patients.

2. Materials and Methods

2.1. Data collection and download

The expression data of GSE4290, GSE8692, and GSE15824 were obtained from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database for gene expression analysis. GSE4290 dataset included 23 normal samples from epilepsy patients and 157 tumor samples, including 26 astrocytomas, 50 oligodendrogliomas, and 81 glioblastomas.[23] The GSE8692 dataset included 2 low grade anaplastic mixed gliomas, 1 low grade oligodendroglioma, 6 high grade glioblastoma, 2 high grade gliosarcoma, and 1 high grade glioblastoma/gliosarcoma.[24] GSE15824 dataset included 5 normal samples and 30 glioma samples, including 8 astrocytomas, 7 oligodendrogliomas, and 15 glioblastomas.[25] In addition, the expression sequences and clinical data of 1018 samples were downloaded from the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn/) databases,[26] the expression data of 693 samples and clinical data of 1114 samples were downloaded from the TCGA (https://portal.gdc.ancer.gov/) databases.[27] Before further analysis, we performed log2 conversion on RNA sequencing data. All samples from databases were filtered to delete the samples with missing clinical information. Our research is based on open-source data and therefore does not require ethics committee approval for the study.

2.2. Expression analysis of the FCGR2B gene

First of all, the Interactive Bodymap of FCGR2B was obtained through the Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/). Then, The expression data of FCGR2B in 33 cancers from the TCGA databases were analyzed through the UCSCXenaShiny module of HiPlot open source network platform (https://hiplot.com.cn/advance/ucscxena) and grouped according to normal or tumor tissues. The GSE4290, GSE8692, and GSE15824 datasets were downloaded from the GEO database. The expression value of FCGR2B in glioma and normal brain tissue was imported into GraphPad Prism 8 software for analysis, and then verified by the Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) database. Finally, the protein level expression of FCGR2B was analyzed by the Human Protein Atlas (HPA, https://www.proteinatlas.org/) online analysis.

2.3. Clinical prognosis analysis of FCGR2B

The clinical prognosis of FCGR2B was analyzed according to the transcriptome data and clinical data of patients in CGGA data set and TCGA data set. The expression level of FCGR2B was divided into high and low groups according to the median. We used the "Survival" and "Survivminer" software packages in R version 4.0.5 to draw the survival curves of different expression levels of FCGR2B. The "timeROC" software package in R version 4.0.5 was used to calculate the subject characteristic (ROC) curve of FCGR2B in 1, 3, and 5 years, and the area under the curve (AUC) was calculated. The prognostic value of FCGR2B was further evaluated by univariate and multivariate COX regression analysis. Finally, based on the CGGA data, we used the "survival," "regplot," and "rms" software packages in R version 4.0.5 to construct the 1-year, 2-year, and 3-year survival nomograms using the clinicopathological features of FCGR2B expression. Then, a calibration curve was drawn to evaluate the accuracy of the match between predicted survival and actual survival.

2.4. Correlation analysis between the expression of FCGR2B and clinicopathological characteristics

According to the transcriptome data and clinical data of patients in the CGGA dataset, the "ComplexHeatmap" package in R version 2.6.2 and the "lima" and "ggpubr" package in R version 4.0.5 were used to analyze the correlation between the expression of FCGR2B and the clinicopathological characteristics and draw the graph. Subsequently, the accuracy of the analysis was verified with transcriptome data and clinical data from TCGA dataset.

2.5. Analysis of differentially expressed genes and signal transduction mechanism in high and low expression groups of FCGR2B

Through co-expression analysis, the co-expression genes with FCGR2B were found in CGGA and TCGA databases, and the representative genes significantly related to FCGR2B were mapped by R software. At the same time, according to the expression of FCGR2B gene, the CGGA samples were divided into high and low groups, and the differential genes between the high and low groups were screened with "lima" and "pheatmap" packages, and the heat map was drawn. In order to explain the potential mechanism of FCGR2B function, in the CGGA and TCGA databases, R software was used for GO and KEGG analysis of differential genes. In addition, c5.go.v7.4 symbols and c2.cp.kegg.v7.4 symbols were used for Gene Set Enrichment Analysis (GSEA) of FCGR2B. When NES > 1, $P < .05$, and FDR < 0.05, the geneset was considered as an enrichment group.

2.6. Correlation analysis between immune score of glioma and prognosis, WHO grade and FCGR2B gene expression

In order to evaluate the proportion of immune and interstitial components in tumor microenvironment (TME), the "Limma" and "Estimate" packages in R software were used to calculate the immune and interstitial fractions of each glioma sample in CGGA and TCGA databases. According to the median score obtained, the samples were divided into high score and low score groups in immune score and Stromal Score respectively. In order to verify the difference of survival rate between high and low groups, "survival" and "surviver" packages were used for survival analysis. The survival curve was drawn by Kaplan–Meier method. The correlation between immune score and tumor grade was analyzed with the "limma" and "ggpubr" packages in R software. According to the expression of FCGR2B, the 2 groups were divided into high and low groups, and the difference analysis of immune score was compared. The correlation between FCGR2B expression and immune score was analyzed by using the "limma," "ggExtra," and "ggpubr" packages in R software.

2.7. Correlation analysis between the expression of FCGR2B and tumor infiltrating immune cells (TICs)

Based on the deconvolution algorithm, we downloaded the gene annotation matrix of 22 immune cell subtypes provided by CiberSort network platform (http://cibersort.stanford.edu/) and calculated the *P* value of each sample in the CGGA and TCGA datasets,[28,29] and calculated the relative content of immune cells in each sample. Then, according to the median of FCGR2B expression, the samples were divided into high expression group and low expression group, and the differences of various immune cells between the 2 groups were compared with the "limma" package in R version 4.0, and the block diagram was drawn. In addition, the correlation between FCGR2B expression and various immune cells was analyzed using the "ggpubr" package in R version 4.0.5, and the lollipop map was drawn.

2.8. Correlation analysis of FCGR2B expression with immune checkpoints (ICs) and tumor mutation load (TMB)

The correlation analysis between FCGR2B expression and immune checkpoint was performed using the "limma," "reshape2," and "ggpubr" packages in R version 4.0.5, and the correlation graph was drawn. TMB refers to the number of genes with mutations per 1 million bases. We downloaded and sorted the glioma mutation data from TCGA database, calculated and compared the differences of TMB between the 2 groups in TCGA glioma dataset by R software, and analyzed the correlation between FCGR2B expression and TMB.

2.9. Correlation analysis between expression of FCGR2B and immune function

Using the "GSVA" and "GSEABase" packages in R version 4.0, the various immune function scores of each sample in the CGGA and TCGA data sets were calculated, and 29 immunobiological function maps were constructed. According to the median of FCGR2B expression, the samples were divided into high and low expression group, and the differences of various immune functions between the 2 groups were compared with the "lima," "reshape2," and "ggpubr" packages in R version 4.0.5, and a boxplot was drawn. At the same time, the correlation between immune functions in each sample was intuitively constructed by the "corrplot" package.

2.10. Statistical analysis

R (version 4.0.3) and GraphPad Prism 8.0 software were used for statistical analysis and chart drawing. The comparison between the 2 groups was carried out by *t* test, and the data between multiple groups were compared by analysis of variance. *P* value < .05 was statistically significant.

3. Results

3.1. Overexpression of FCGR2B in Glioma

We first analyzed the expression of FCGR2B in 31 solid tumors and adjacent tissues (or GTEx). As shown in Figure 1A and B, the expression of FCGR2B was higher in BRCA, GBM, KIRC, KIRP, LGG, LUAD, OV, PAAD, SKCM, STAD, TGCT, UCEC, and UCS ($P < .05$) than normal tissues, while the expression of FCGR2B was lower in tumor of ACC, BLCA, CHOL, COAD, ESCA, KICH, LAML, LIHC, PCPG, READ, and THYM ($P < .05$). Based on the expression data of gliomas and normal tissues in GSE4290 and GSE15824 datasets, FCGR2B gene is significantly overexpressed in gliomas (Fig. 1C and E), and the expression of FCGR2B in GBM samples is higher than that of LGG in GSE8692 datasets (Fig. 1D). In addition, these results were verified by the GEPIA and the Human Protein Atlas online analysis (Fig. 1F and G). These results showed that FCGR2B was overexpressed in gliomas, and the expression in GBM was higher than that in LGG, which supported that FCGR2B was associated with higher grade gliomas.

3.2. High expression of FCGR2B has poor prognosis

Using the sample information from the CGGA database, the Kaplan–Meier survival method was used to analyze the survival prognosis, which showed that the high expression of FCGR2B had a worse prognosis ($P < .001$, Fig. 2A). These results were verified by the analysis of the TCGA database ($P < .001$, Fig. 2B). According to CGGA and TCGA databases, ROC curves are generated. In the CGGA database, the AUC values of the ROC curves of FCGR2B are 0.697, 0.715, and 0.712 respectively for the results of 1, 3, and 5 years (Fig. 2C). The ROC curve of the FCGR2B gene was verified in the TCGA database, and the AUC values of the 1-year, 3-year, and 5-year results were 0.810, 0.811, and 0.736, respectively (Fig. 2D). Univariate Cox regression analysis showed that the expression of FCGR2B [HR = 1.275, 95% CI (1.211–1.342), $P < .001$], age, IDH mutation, 1p19q expression status and MGMT methylation level were significantly correlated with

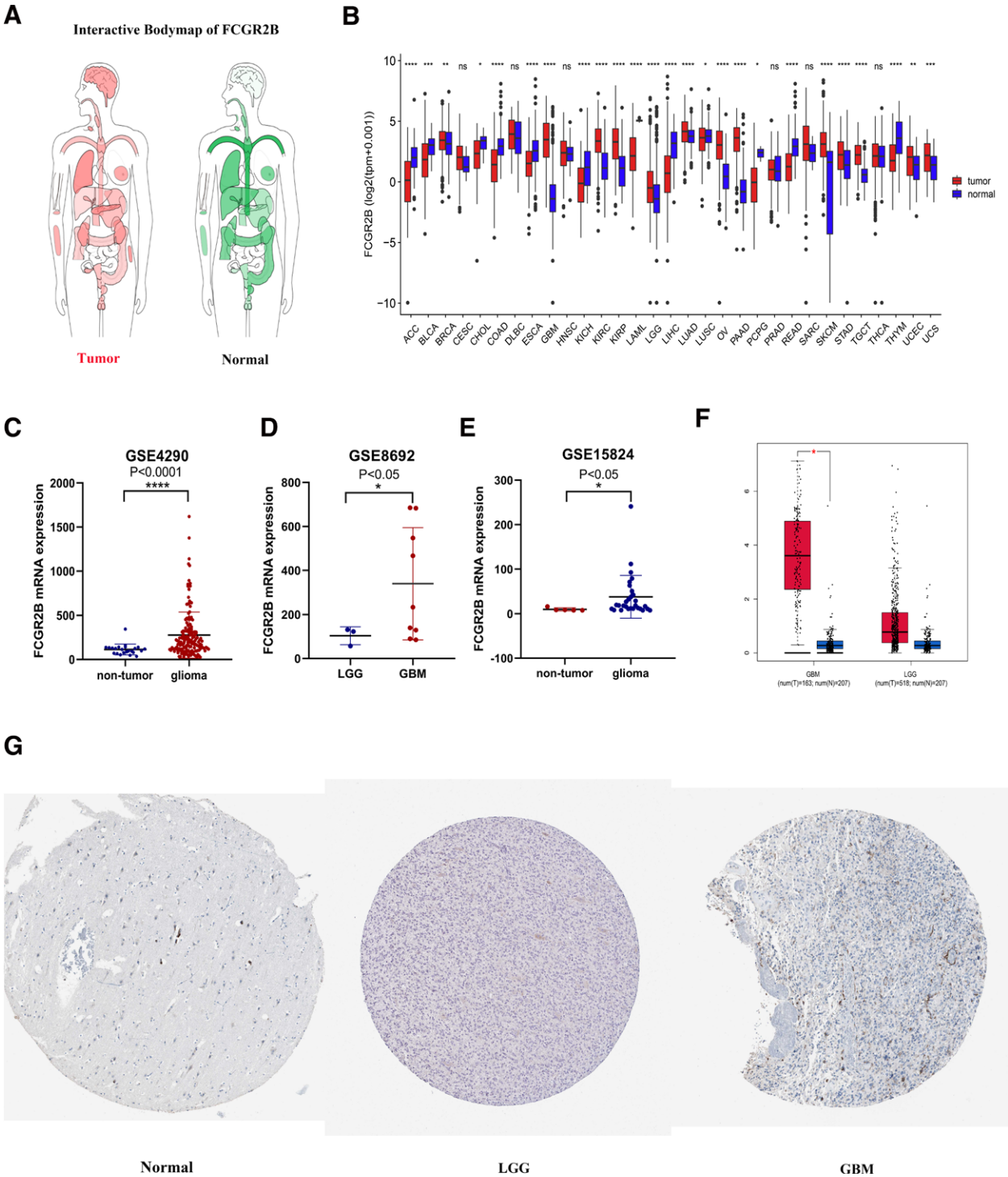

Figure 1. Expression level of the FCGR2B gene in glioma and normal tissues. A. The body map of FCGR2B mRNA in both nontumor tissues (green) and cancer tissues (red). B. The expression of FCGR2B mRNA in 31 cancers and their corresponding nontumor tissues. C. GSE4290 dataset. D. GSE8692 dataset. E. GSE15824 dataset. F. Based on the GEPIA online website, the expression level of FCGR2B in glioma and normal samples. G. Based on The Human Protein Atlas online website, the expression level of FCGR2B in the brain tissues of glioma and normal controls. FCGR2B = Fc fragment of IgG low affinity IIb receptor.

survival and prognosis (Fig. 2E). In addition, multivariate Cox regression analysis showed that the expression of FCGR2B [HR = 1.085, 95% CI (1.020–1.154), $P < .01$], age, IDH mutation and 1p19q expression status were independent factors of survival and prognosis (Fig. 2G). Univariate and multivariate regression analysis were verified in TCGA databases (Fig. 2F and H). Therefore, these findings suggest that FCGR2B is an independent prognostic indicator of glioma. Next, based on the clinical characteristics of CGGA dataset, including age, sex, chemotherapy, radiotherapy, IDH mutation, MGMT methylation level, 1p19q expression and FCGR2B expression level, we constructed a nomograph for quantitative prediction (Fig. 2I). The nomograph model constructed with FCGR2B expression has good prediction effect on the 1-year, 3-year, and 5-year survival rate of glioma (Fig. 2K). Similarly, the nomograph model constructed by combining clinical features and FCGR2B expression was verified in TCGA database (Fig. 2J and L).

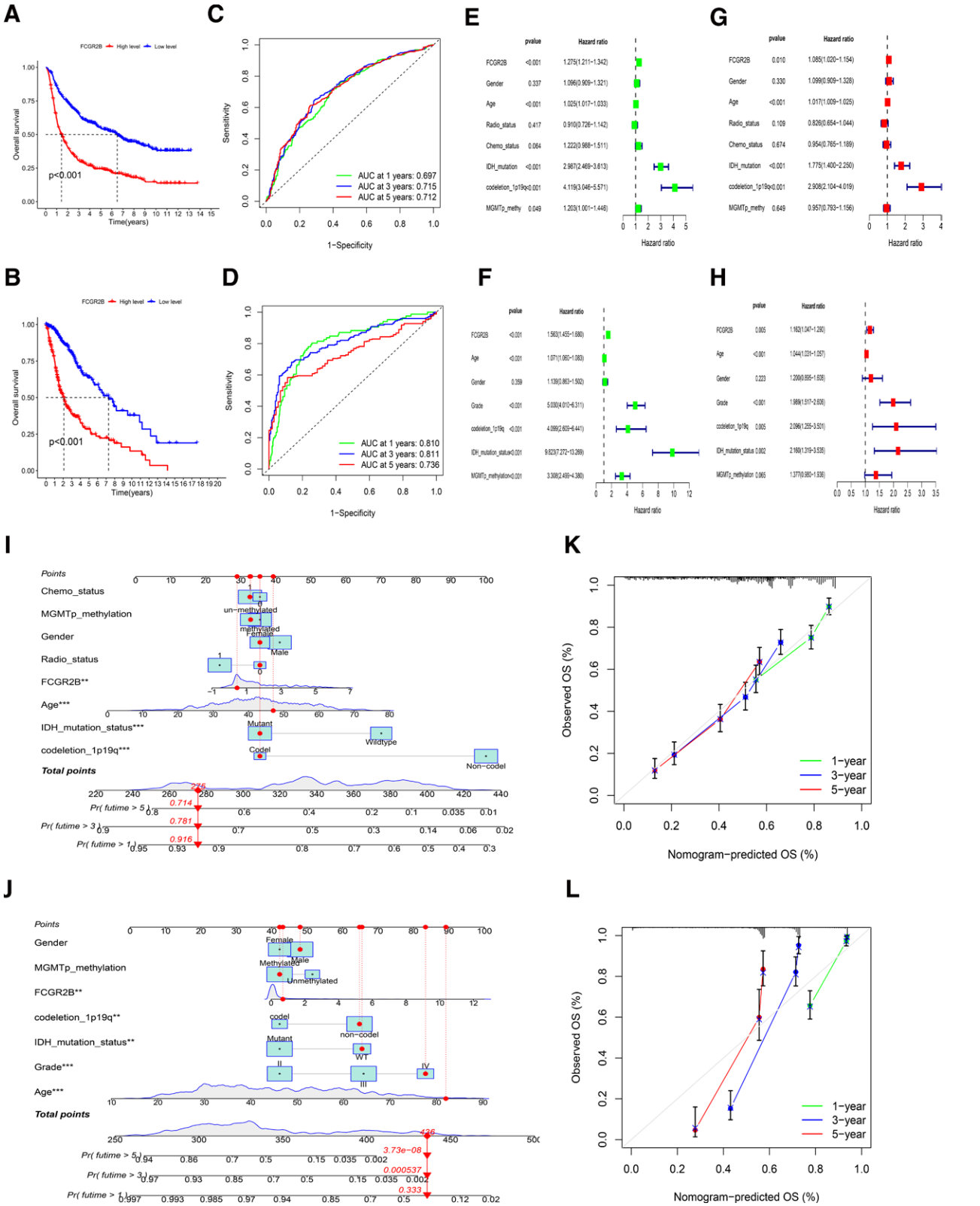

Figure 2. Survival analysis and independent prognostic analysis of the FCGR2B gene in glioma. A. Survival analysis of FCGR2B (CGGA). B. Survival analysis of FCGR2B (TCGA). C. Survival ROC curve of FCGR2B at 1, 3, and 5 years (CGGA). D. Survival ROC curve of FCGR2B at 1, 3, and 5 years (TCGA). E. Univariate analysis of FCGR2B (CGGA). F. Univariate analysis of FCGR2B (TCGA). G. Multivariate analysis of FCGR2B (CGGA). H. Multivariate analysis of FCGR2B (TCGA). I. Based on the clinical information of the CGGA database, a prognostic nomogram model was constructed. J. Based on the clinical information of the TCGA database, a prognostic nomogram model was constructed. K. Calibration curve was constructed according to the CGGA database information. L. Calibration curve was constructed according to the CGGA database information. CGGA = Chinese Glioma Genome Atlas, FCGR2B = Fc fragment of IgG low affinity IIb receptor, ROC = receiver operating characteristic, TCGA = the Cancer Genome Atlas database.

3.3. The correlation between FCGR2B gene expression and clinicopathological characteristics

The clinicopathological characteristics of the high expression group and the low expression group of FCGR2B were compared. In CGGA data, the FCGR2B high expression group was significantly associated with tumor recurrence, high pathological grade, age greater than 65 years, IDH wild type, 1p19q non-coding and MGMTp un-methylated status (Fig. 3A). In TCGA data, the FCGR2B overexpression group was significantly associated with high pathological grade, age greater than 65 years, IDH wild-type, 1p19q co-deletion and MGMTp un-methylated status (Fig. 3B). Next, further correlation analysis between FCGR2B expression and clinicopathological features showed that (Fig. 3C), based on CGGA dataset, FCGR2B was over-expressed in patients over 65 years old ($P < .0001$). In addition, the expression of FCGR2B also increased with the increase of tumor grade ($P < .0001$), and the expression of FCGR2B was lower in patients with IDH mutation, 1p19q coding and MGMTp methylated ($P < .0001$). These findings were completely consistent with the analysis results of TCGA dataset (Fig. 3D).

3.4. Co-expression, differential expression and enrichment analysis of FCGR2B gene

The circle chart showed the first 11 genes in the CGGA database that have co-expression relationship with FCGR2B. FCGR2B was positively correlated with FCGR2A, PLBD1, CD163, CSTA, FCGR2C, and HSPA7, and negatively correlated with ELFN2, MMD2, KCNIP3, JPH3, and TNR (Fig. 4A). The circle chart showed the first 11 genes in the TCGA database that have co-expression relationship with FCGR2B. FCGR2B is positively correlated with PLBD1, PLAUR, CASP4, TREM1, S100A4, and CSTA, and negatively correlated with MAPT, ZDHHC22, SOX8, TNRC6C, and MTSS1L (Fig. 4A). Respectively, the heat map showed the differentially expressed genes between high and low FCGR2B expression groups in CGGA and TCGA datasets (Fig. 4B). GO analysis showed that FCGR2B was closely related to immune-related functions, such as T cell activation and lymphocyte mediated immunity (Fig. 4C). KEGG analysis showed that signal pathways such as Cytokine-cytokine receptor interaction and Hematopoietic cell lineage may be the mechanism of FCGR2B's involvement in tumor genesis and development (Fig. 4D). GSEA analysis showed that in the high expression group of FCGR2B, the biological functions related to cell adhesion molecules (CAMS) and Hematopoietic cell lineage or the immune related biological functions mediated by them and JAK-STAT signal pathway were activated (Fig. 4E).

3.5. Correlation analysis between immune score of glioma and prognosis, WHO grade and FCGR2B gene expression

We evaluated the correlation between immune or interstitial components and FCGR2B gene expression and clinical parameters. First of all, Kaplan–Meier analysis results showed that compared with patients with low ESTIMATEScore, ImmuneScore and StromalScore, patients with high ESTIMATEScore, ImmuneSore, and StromalScore had significantly poorer overall survival in CGGA and TCGA datasets. However, tumor purity was positively correlated with overall survival (Fig. 5A and E). At the same time, with the increase of tumor pathology, the patient's ImmuneScore, StromalScore, and ESTIMATEScore increased, but the tumor purity decreased (Fig. 5B and F). As shown in Figure 5C and G, the ESTIMATEScore, ImmuneScore, and StromalScore of patients with high expression of FCGR2B were higher than those of patients with low expression of FCGR2B. In addition, ImmuneScore, StromalScore, and ESTIMATEScore were significantly positively correlated with the expression of FCGR2B mRNA. There was a significant negative correlation between tumor purity and FCGR2B gene expression (Fig. 5D and H). The above results showed that the non-tumor components (immune components and interstitial components) were closely related to the prognosis and WHO grade of glioma. FCGR2B may play a role by interacting with non-tumor components in TME.

3.6. The relationship between the expression of FCGR2B gene and the proportion of TICs

Subsequently, we further revealed the correlation between the expression of FCGR2B mRNA and the proportion of TICs. First, the results of differential analysis showed that compared with the low expression group of FCGR2B mRNA, the proportion of M0 or M1 macrophages in the high expression group of FCGR2B mRNA was significantly increased, and the proportion of T cells CD8, T cells CD4 memory activated and Neutrophils was increased. However, the proportion of NK cells activated, Monocytes and Mast cells activated in the low expression group of FCGR2B mRNA was significantly higher than that in the high expression group of FCGR2B mRNA (Fig. 6A and B). Then, correlation analysis showed that the expression of FCGR2B was significantly negatively correlated with immune cells such as NK cells activated, T cells CD4 naive, Monocytes, Mast cells activated and T cells CD4 memory resting. The expression of FCGR2B was significantly positively correlated with immune cells such as Dendritic cells resting, Neutrophils, T cells gamma delta, T cells CD4 memory activated, Macrophages M1, T cells CD8 and Macrophages M0 (Fig. 6C and D). These results showed that the expression level of FCGR2B affected the immune activity of TME. In addition, in glioma with high expression of FCGR2B, the activity of M0 and M1 tumor-associated macrophages (TAMs) in TME was significantly increased.

3.7. Correlation of FCGR2B expression with immune checkpoints (ICs) and tumor mutation load (TMB)

The growth and development of glioma are closely related to the microenvironment of immunosuppression. Tumor cells have the ability of immunosuppression by stimulating the immune checkpoint pathway.[30,31] We further confirmed that in the CGGA and TCGA datasets, the expression of FCGR2B was negatively correlated with CD200 and VTCN1, and positively correlated with almost all other immune checkpoint molecules, including CD28, CD44, TNFSF14, PDCD1LG2, LAIR1, and CD48 (Fig. 7A–D). In addition, in the TCGA glioma database, the TMB in the high expression group of FCGR2B was significantly higher than that in the low expression group, and there was a significant positive correlation with TMB (Fig. 7E and F).

3.8. Correlation between expression of FCGR2B and immune function

In order to further reveal the difference between the immunobiological functions of glioma with low and high expression of FCGR2B, 29 kinds of immunobiological function maps were constructed (Fig. 8A and B). The result of difference analysis showed that compared with the low expression group of FCGR2B, all immunobiological functions of the high expression group of FCGR2B were significantly inhibited (Fig. 8C and D). Interestingly, in the glioma samples of CGGA and TCGA, there was a positive correlation between almost all immunobiological functions (Fig. 8E and F). The above results may explain the poor prognosis of gliomas in the high expression group of FCGR2B from a certain angle.

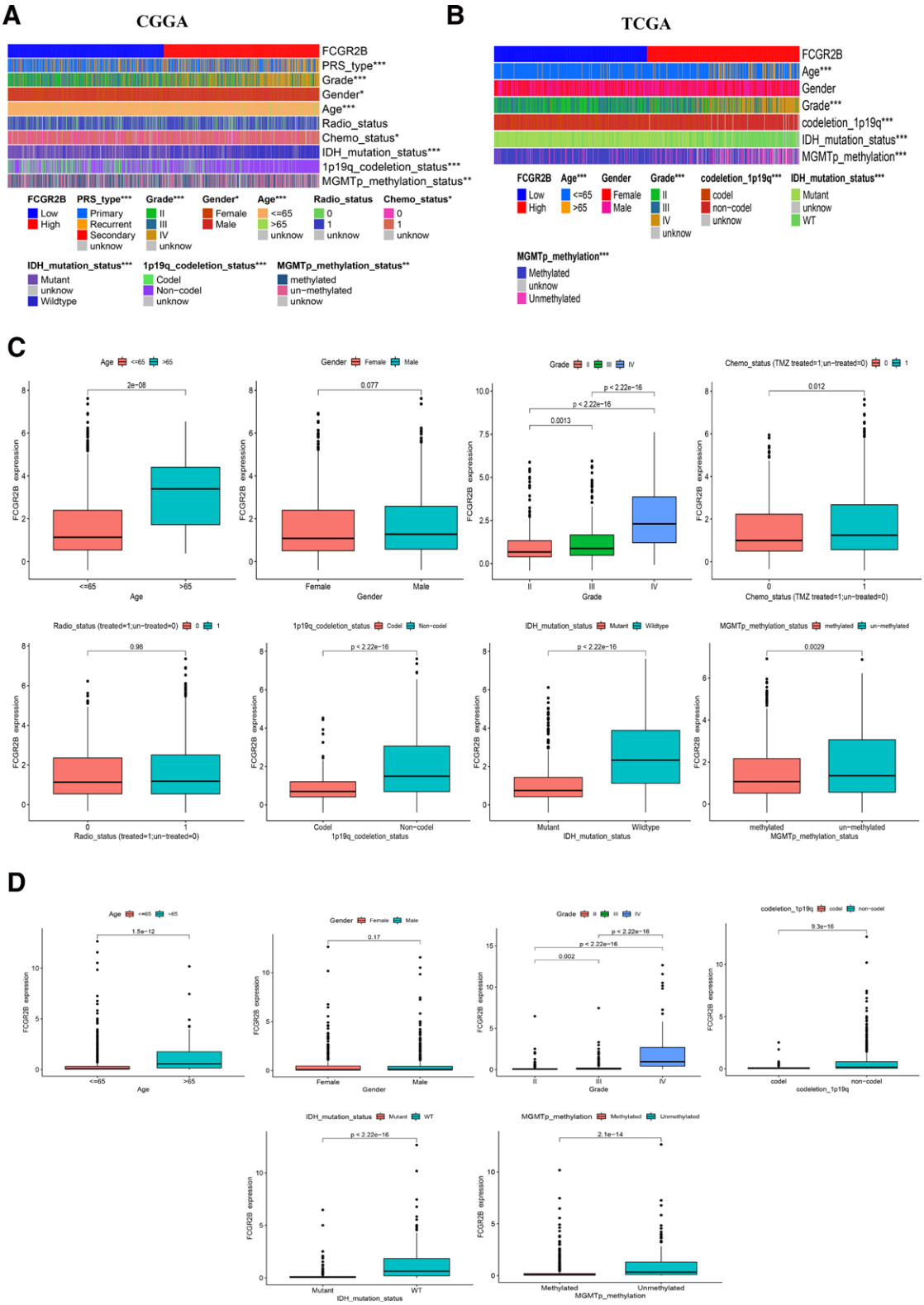

Figure 3. Correlation analysis of the FCGR2B gene and clinicopathological features. A and B. Heatmap of the correlation between FCGR2B expression and clinical features. C. Expression of FCGR2B in different age, genders, WHO grades, chemo status, radio status, 1p19q codeletion, IDH status and MGMTp methylation of glioma (CGGA). D. Expression of FCGR2B in different age, genders, WHO grades, 1p19q codeletion, IDH status and MGMTp methylation of glioma (TCGA). CGGA = Chinese Glioma Genome Atlas, FCGR2B = Fc fragment of IgG low affinity IIb receptor, TCGA = the Cancer Genome Atlas database, WHO = World Health Organization.

4. Discussion

Glioma is an aggressive growth of central nervous system malignant tumor with high recurrence rate and low survival rate, which seriously threatens human health.[8,9,32,33] In recent years, targeted therapy and immunotherapy have improved the prognosis of other common tumors. Although some glioma patients

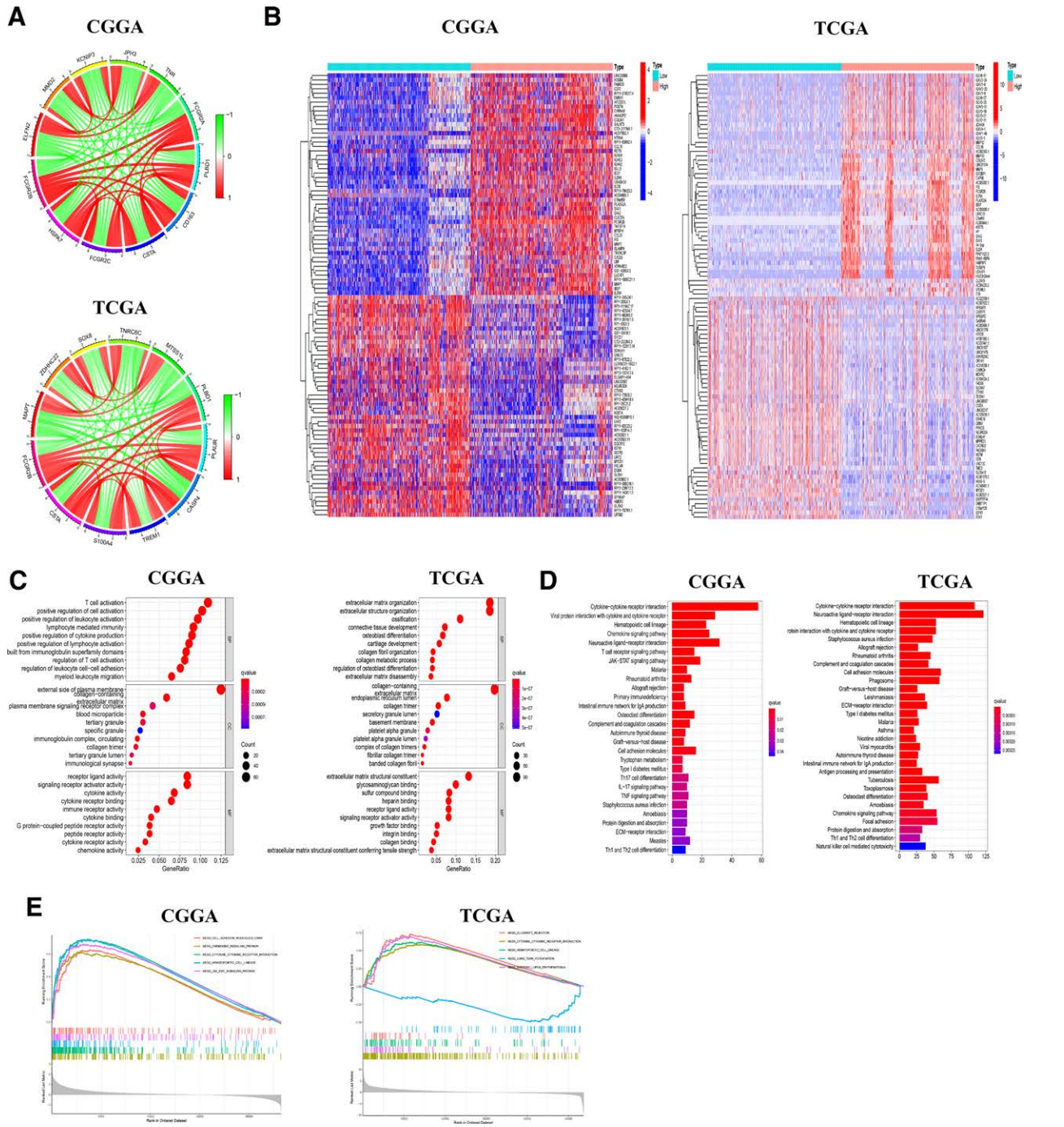

Figure 4. DEGs analysis and FCGR2B gene mechanism analysis. A. The gene circle map FCGR2B of co-expression analysis. B. Heat map of DEGs was drawn, showing DEGs that were significantly upregulated and significantly downregulated. C. The GO analysis of DEGs. D. The KEGG analysis of DEGs. E. The GSEA enrichment analysis of FCGR2B high and low expression groups. DEGs = differentially expressed genes, FCGR2B = Fc fragment of IgG low affinity IIb receptor, GO = gene ontology, GSEA = gene set enrichment analysis, KEGG = Kyoto encyclopedia of genes and genomes.

have responded to targeted therapy or immunotherapy, most glioma patients still lack obvious effects.[34,35] Therefore, it is necessary to further explore and research new predictive targets and potential therapeutic targets for glioma.

In our study, we analyzed the expression of FCRR2B in gliomas through the GEO database. The results showed that FCGR2B was highly expressed in gliomas, and FCGR2B was associated with higher grade gliomas. The Gene Expression Profiling Interactive Analysis (GEPIA) and Human Protein Atlas (HPA) online analysis confirmed these results. The survival prognosis, ROC curve, univariate and multivariate Cox regression analysis using CGGA and TCGA databases further showed that FCGR2B played an important role in the occurrence and development of glioma and supported FCGR2B as an independent prognostic indicator of glioma. Finally, based on the clinical characteristics of CGGA and TCGA databases and the expression level of FCGR2B, the nomograph and nomograph models were constructed, which had a good prediction effect on the 1-year, 3-year, and 5-year survival rates of gliomas. These findings suggest that FCGR2B is worthy of further study in glioma. Next, according to the clinical characteristics of glioma, the expression of FCGR2B was studied, and the clinical

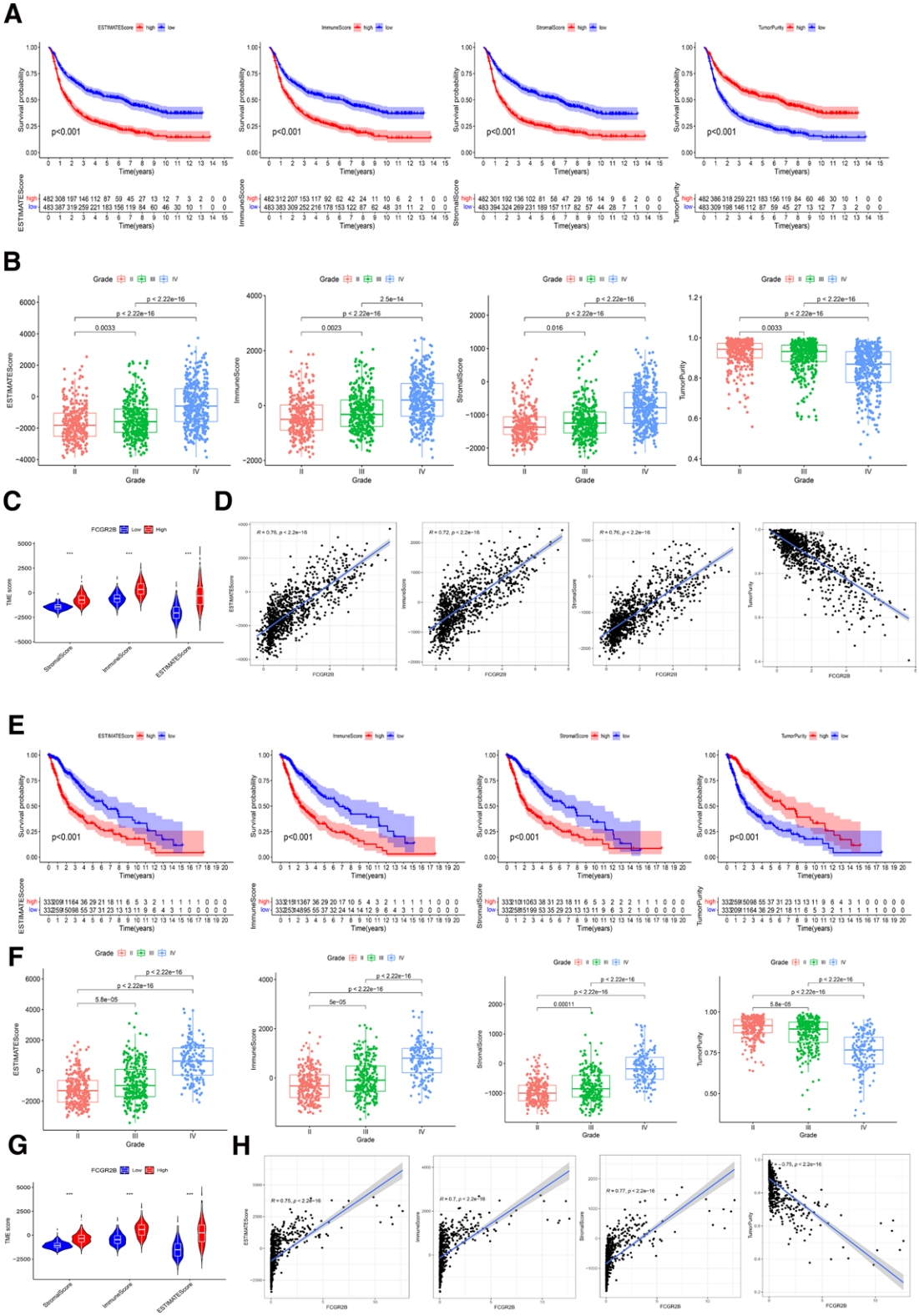

Figure 5. Scores were correlated with the prognosis, WHO grade, and FCGR2B mRNA expression in gliomas. A, E. Kaplan–Meier survival analysis for gliomas grouped into high or low score in ImmuneScore, StromalScore, ESTIMATEScore, and TumorPurity determined by the comparison with the median (A in CGGA, E in TCGA). B, F. Distribution of ImmuneScore, StromalScore, and ESTIMATEScore in different WHO grade (B in CGGA, F in TCGA). C, G. Distribution of the TME scores in high and low FCGR2B expression groups (C in CGGA, G in TCGA). D, H. Correlation of BACH1 mRNA expression with the ImmuneScore, StromalScore, ESTIMATEScore, and TumorPurity by Pearson correlation analysis (D in CGGA, H in TCGA). CGGA = Chinese Glioma Genome Atlas, FCGR2B = Fc fragment of IgG low affinity IIb receptor, TCGA = the Cancer Genome Atlas database, TME = tumor microenvironment, WHO = World Health Organization.

correlation map of FCGR2B was constructed using TCGA and CGGA databases. The results showed that the high expression group of FCGR2B was significantly associated with high pathological grade, age greater than 65 years, IDH wild type, 1p19q non-coding and MGMTp un-methylated status. This further indicates that FCGR2B is an important predictor of glioma.

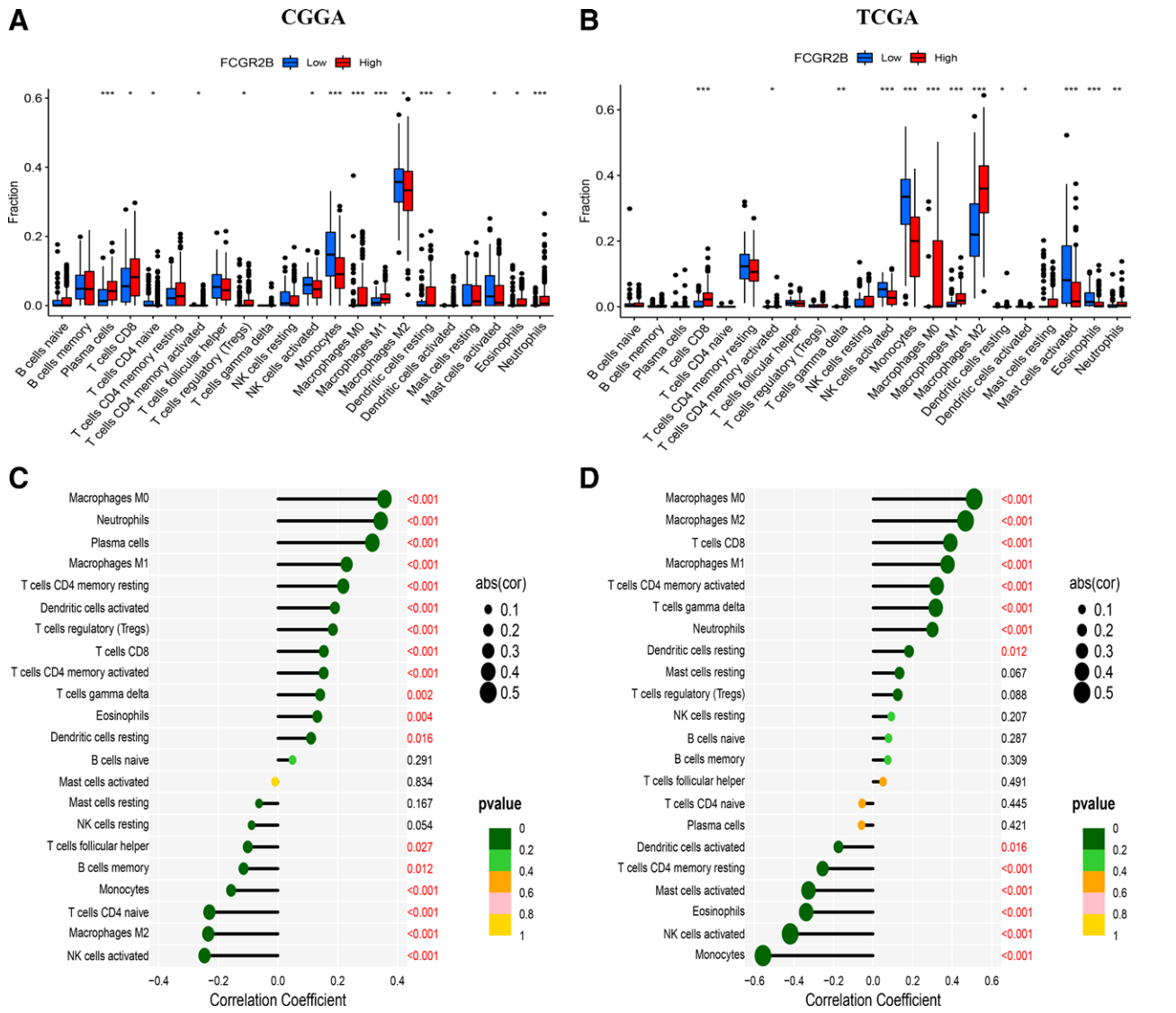

Figure 6. Correlation of FCGR2B mRNA expression with the proportion of TICs. A and B. boxplot showed the ratio differentiation of 22 TICs between glioma with low and high FCGR2B expression groups. C and D. The correlation between immune infiltrating cells and FCGR2B expression. The ordinate represents the name of the immune cell and the abscissa represents the correlation coefficient. FCGR2B = Fc fragment of IgG low affinity IIb receptor, TICs = tumor infiltrating immune cells.

Previous studies have shown that FCGR2B is closely related to the occurrence and development of tumors. BALB/c mice lacking FCGR2B can inhibit the growth of tumors. This indicates that FCGR2B plays an important role in the occurrence and development of tumor.[21] FCGR2B is related to the mechanism of human mouse chimeric anti-CD20 monoclonal antibody treating CD20 positive lymphoma.[22] FCGR2B plays an important role in regulating tumor infiltrating CD8+ T cells in experimental melanoma model.[36] The low level of FCGR2B mRNA expression in prostate tumor tissue is associated with better survival. FCGR2B is involved in various immune pathways that promote tumor.[37] Our research found that FCGR2B is closely related to immune-related functions, such as T cell activation and lymphocyte mediated immunity. GSEA analysis showed that in the high expression group of FCGR2B, the biological functions related to cell adhesion molecules (CAMS) and Hematopoietic cell lineage or immune related biological functions mediated by them and JAK-STAT signal pathway were activated. This suggests that FCGR2B can be used as a prognostic indicator and immunotherapy marker for glioma microenvironment.

Many studies have shown that TME plays an important role in the growth and invasion of glioma.[38–43] The components of tumor microenvironment include not only tumor cells, but also peripheral blood vessels, extracellular matrix and some molecular signal factors.[44] There is a two-way driving role between tumor microenvironment and the progress, invasion and metastasis of tumor cells,[45,46] and its molecular mechanism is complex. Tumor cells up-regulate the expression level of immunosuppressive factors (including PD-L1 and IDO), and limit the self-representation of antigen by down-regulating the expression of MHC.[47] Microglia secrete transforming growth factor β and IL-10 down-regulate local myeloid and lymphoid immune cells, and ultimately promote systemic immunosuppression. TAMs also play an immunosuppressive role by regulating the expression of various intracellular and extracellular mediators.[48] Tregs can mediate the immunosuppressive effect by up-regulating a variety of soluble factors, immune checkpoint molecules and metabolic pathways.[49,50] Previous studies have shown that the treatment of tumor microenvironment has achieved good results, such as anti-programmed death ligand 1 treatment[51,52] and T-cell immunotherapy.[53,54] In addition,

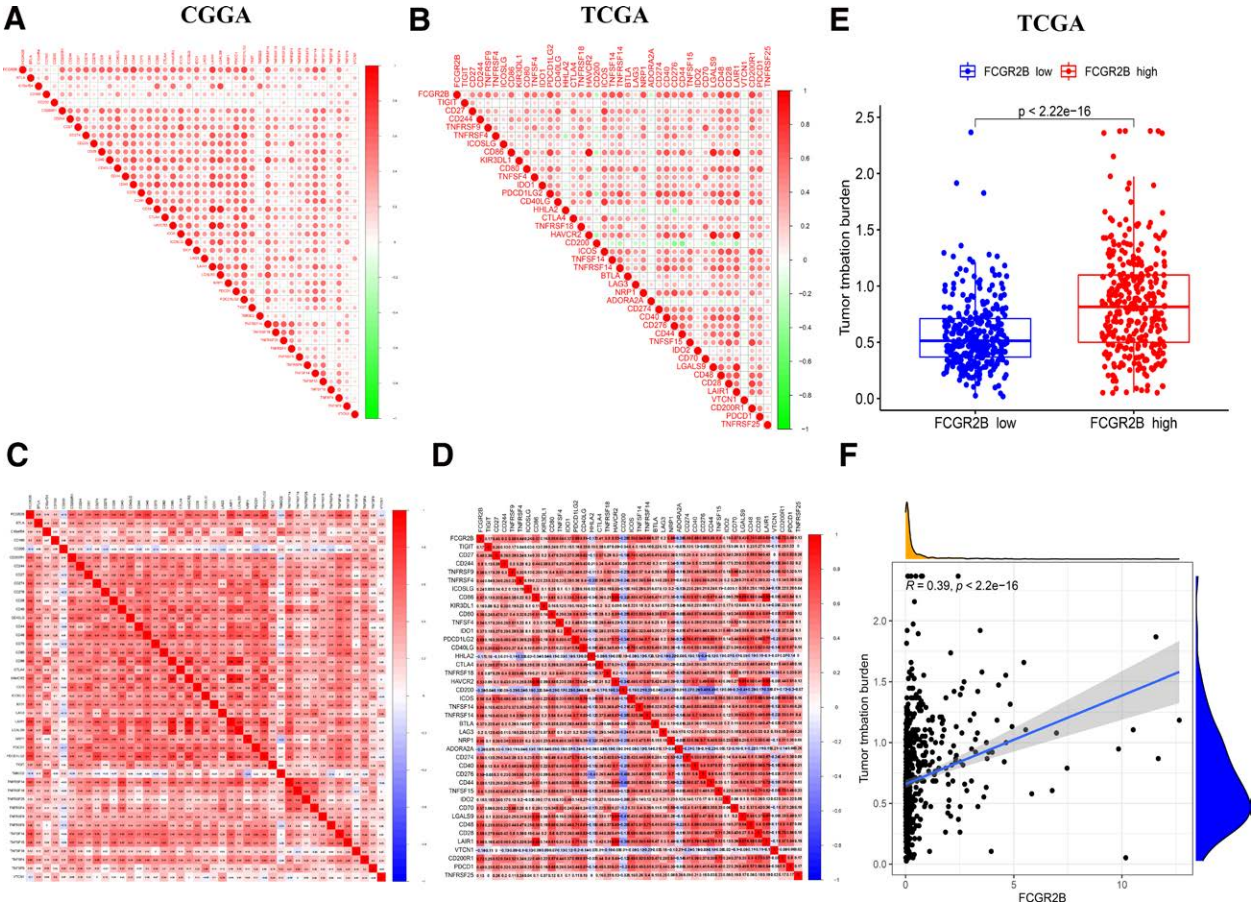

Figure 7. Correlation of FCGR2B expression with ICs and TMB. A–D. The Correlation between FCGR2B expression and ICs was presented. The value represents the correlation value, red represents the positive correlation while blue or green represents negative correlation. E. The TMB between high and low FCGR2B expression groups. F. The correlation between FCGR2B expression and TMB. FCGR2B = Fc fragment of IgG low affinity IIb receptor, ICs = immune checkpoints, TMB = tumor mutation load.

immunotherapy has been proved to be effective for lung cancer and melanoma.[55] Therefore, we further analyzed the relationship between FCGR2B expression and TME. We found that the non-tumor components (immune components and interstitial components) are closely related to the prognosis and WHO grade of glioma. In the CGGA and TCGA data sets, the overall survival rate of patients with high ESTIMATEScore, ImmuneSore, and StromalScore was significantly poor, and the tumor pathology was high. In addition, the immune score, StroMalScore and ESTIMATEScore were significantly positively correlated with the expression of FCGR2B, which also highlighted the importance of exploring the interaction between tumor cells and immune cells and researching new therapeutic targets to develop more effective therapeutic methods. FCGR2B may play a role by interacting with non-tumor components in TME.

CiberSort's method of calculating the proportion of TIC showed that the expression level of FCGR2B affected the immune activity of TME. In addition, in glioma with high expression of FCGR2B, the activity of M0 and M1 TAMs in TME was significantly increased. It suggests that FCGR2B may participate in the maintenance of TME cellular immunity. Previous studies have shown that TAMs are one of the most important immunosuppressive cells in TME and mediate tumor progression by regulating TME.[56,57] TAMs plays an important role in promoting tumor progression, including promoting tumor occurrence and development, forming immunosuppressive microenvironment to promote metastasis, establishing pre-cancer metastasis microenvironment and promoting tumor angiogenesis.[58–61]

The growth and development of glioma are closely related to the microenvironment of immunosuppression. Tumor cells have the ability of immunosuppression by stimulating the immune checkpoint pathway.[30,31] Tumor cells with high tumor mutation load (TMB) may have more new antigens, resulting in a corresponding increase in tumor microenvironment and peripheral anti-tumor T cells. Therefore, patients with high TMB are more likely to react to tumor immunotherapy.[62] We further explored the potential mechanism of FCGR2B in TME. The expression of FCGR2B was positively correlated with almost all immune checkpoint molecules including CD28, CD44, TNFSF14, PDCD1LG2, LAIR1, and CD48. In addition, in the TCGA glioma database, the expression of FCGR2B was significantly positively correlated with TMB. Furthermore, all the immunobiological functions of the high expression group of FCGR2B were significantly inhibited. This suggests that FCGR2B may affect tumor microenvironment by regulating these tumor immune genes. In general, these findings prove that the key gene FCGR2B in TME can become an effective target for the treatment of glioma.

However, this study has certain limitations. Firstly, this study is based on glioma tissue samples of online database and lacks sufficient clinical experimental data to validate the role of FCGR2B in glioma. Secondly, the mechanism by which FCGR2B affects the prognosis and immune microenvironment of glioma patients is not yet thoroughly studied. Therefore, more clinical experimental studies are needed to further explore the role and mechanism of FCGR2B in gliomas.

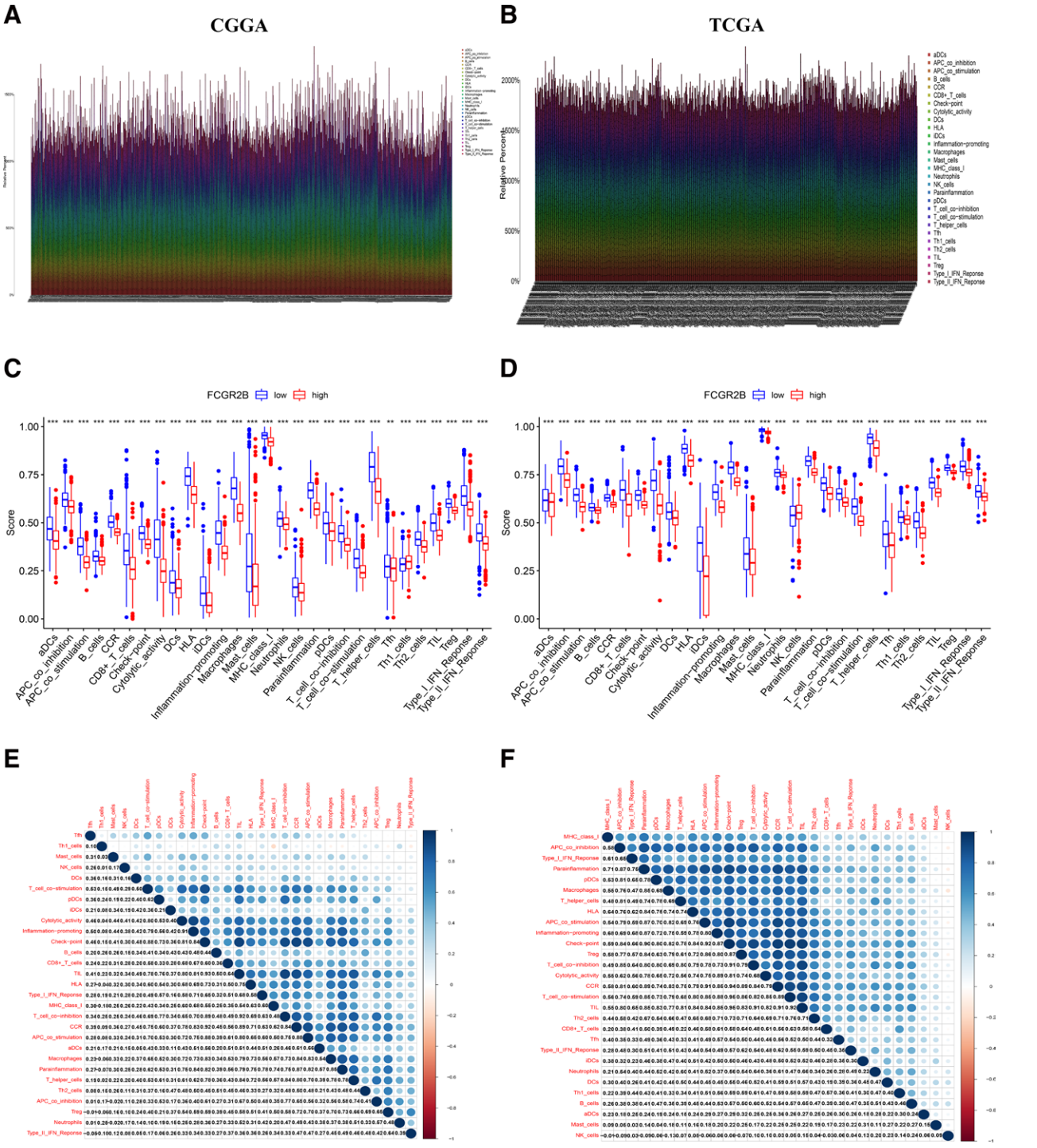

Figure 8. Correlation between expression of FCGR2B and immunobiological function. A and B. Barplot showed the proportion of 29 immunobiological in glioma. C and D. boxplot displayed the ratio differentiation of 29 immunobiological function between glioma with low and high FCGR2B expression groups. E and F. Correlation between each immunobiological function in glioma samples, blue represents the positive correlation while red represents negative correlation. FCGR2B = Fc fragment of IgG low affinity IIb receptor.

5. Conclusion

FCGR2B is overexpressed in glioma. In addition, the prognosis of glioma patients with high expression of FCGR2B is poor, and FCGR2B can be used as an independent prognostic indicator of glioma. The results of this study also show that FCGR2B can be used as a prognostic indicator and immunotherapy marker for glioma microenvironment TME. FCGR2B may play an important role in the occurrence, development and invasion of tumor by influencing the tumor microenvironment of immunosuppression. Therefore, this study suggests that FCGR2B may be an important molecular target for glioma. Blocking the overexpression of FCGR2B gene may improve the prognosis of glioma patients.

Author contributions

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Writing – original draft: Zhimin Sun, Xiaoli Sun, Yaqin Yuan.

References

[1] Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: a clinical review. JAMA. 2013;310:1842–50.
[2] Ostrom QT, Patil N, Cioffi G, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2013-2017. Neuro Oncol. 2020;22:iv1–iv96.
[3] Esquenazi Y, Friedman E, Liu Z, et al. The survival advantage of "Supratotal" resection of glioblastoma using selective cortical mapping and the subpial technique. Neurosurgery. 2017;81:275–88.
[4] Stupp R, Taillibert S, Kanner A, et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. JAMA. 2017;318:2306–16.
[5] Gu J, Wang J, You A, et al. MiR-137 inhibits the proliferation, invasion and migration of glioma via targeting to regulate EZH2. Genes Genomics. 2021;43:1157–65.
[6] Gusyatiner O, Hegi ME. Glioma epigenetics: from subclassification to novel treatment options. Semin Cancer Biol. 2018;51:50–8.
[7] Wang H, Xu T, Huang Q, et al. Immunotherapy for malignant glioma: current status and future directions. Trends Pharmacol Sci. 2020;41:123–38.
[8] Lapointe S, Perry A, Butowski NA. Primary brain tumours in adults. Lancet. 2018;392:432–46.
[9] Stupp R, Mason WP, Bent M.J. van den, et al.; R. European Organisation for, T. Treatment of Cancer Brain, G. Radiotherapy, and G. National Cancer Institute of Canada Clinical Trials. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352:987–96.
[10] Wu H, Yang L, Liu H, et al. Exploring the efficacy of tumor electric field therapy against glioblastoma: an in vivo and in vitro study. CNS Neurosci Ther. 2021;27:1587–604.
[11] Zachariah MA, Oliveira-Costa JP, Carter BS, et al. Blood-based biomarkers for the diagnosis and monitoring of gliomas. Neuro Oncol. 2018;20:1155–61.
[12] Chen TC, da Fonseca CO, Schonthal AH. Intranasal perillyl alcohol for glioma therapy: molecular mechanisms and clinical development. Int J Mol Sci. 2018;3905:19.
[13] Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat Rev Immunol. 2008;8:34–47.
[14] Nimmerjahn F, Ravetch JV. FcgammaRs in health and disease. Curr Top Microbiol Immunol. 2011;350:105–25.
[15] Anthony RM, Wermeling F, Ravetch JV. Novel roles for the IgG Fc glycan. Ann N Y Acad Sci. 2012;1253:170–80.
[16] Suzuki T, Coustan-Smith E, Mihara K, et al. Signals mediated by FcgammaRIIA suppress the growth of B-lineage acute lymphoblastic leukemia cells. Leukemia. 2002;16:1276–84.
[17] Takai T, Ono M, Hikida M, et al. Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. Nature. 1996;379:346–9.
[18] Kim HA, Choi B, Suh CH, et al. Highly expression of CD11b and CD32 on peripheral blood mononuclear cells from patients with adult-onset still's disease. Int J Mol Sci. 2017;202:18.
[19] Li F, Ravetch JV. Apoptotic and antitumor activity of death receptor antibodies require inhibitory Fcgamma receptor engagement. Proc Natl Acad Sci USA. 2012;109:10966–71.
[20] Van den Herik-Oudijk IE, Capel PJ, Bruggen T. van der, et al. Identification of signaling motifs within human Fc gamma RIIa and Fc gamma RIIb isoforms. Blood. 1995;85:2202–11.
[21] Lowe DB, Shearer MH, Jumper CA, et al. Fc gamma receptors play a dominant role in protective tumor immunity against a virus-encoded tumor-specific antigen in a murine model of experimental pulmonary metastases. J Virol. 2007;81:1313–8.
[22] Flieger D, Renoth S, Beier I, et al. Mechanism of cytotoxicity induced by chimeric mouse human monoclonal antibody IDEC-C2B8 in CD20-expressing lymphoma cell lines. Cell Immunol. 2000;204:55–63.
[23] Sun L, Hui AM, Su Q, et al. Neuronal and glioma-derived stem cell factor induces angiogenesis within the brain. Cancer Cell. 2006;9:287–300.
[24] Liu T, Papagiannakopoulos T, Puskar K, et al. Detection of a microRNA signal in an in vivo expression set of mRNAs. PLoS One. 2007;2:e804.
[25] Grzmil M, Morin PJr., Lino MM, et al. MAP kinase-interacting kinase 1 regulates SMAD2-dependent TGF-beta signaling pathway in human glioblastoma. Cancer Res. 2011;71:2392–402.
[26] Zhao Z, Zhang KN, Wang Q, et al. Chinese Glioma Genome Atlas (CGGA): a comprehensive resource with functional genomic data from Chinese Glioma Patients. Gen Proteom Bioinf. 2021;19:1–12.
[27] Blum A, Wang P, Zenklusen JC. SnapShot: TCGA-analyzed tumors. Cell. 2018;173:530.
[28] Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods. 2015;12:453–7.
[29] Bense RD, Sotiriou C, Piccart-Gebhart MJ. Relevance of tumor-infiltrating immune cell composition and functionality for disease outcome in breast cancer. J Natl Cancer Inst. 2017;djw192:109.
[30] Schalper KA, Rodriguez-Ruiz ME, Diez-Valle R, et al. Neoadjuvant nivolumab modifies the tumor immune microenvironment in resectable glioblastoma. Nat Med. 2019;25:470–6.
[31] Lu Y, Ng AHC, Chow FE, et al. Resolution of tissue signatures of therapy response in patients with recurrent GBM treated with neoadjuvant anti-PD1. Nat Commun. 2021;12:4031.
[32] Lee CH, Jung KW, Yoo H, et al. Epidemiology of primary brain and central nervous system tumors in Korea. J Korean Neurosurg Soc. 2010;48:145–52.
[33] Dobes M, Khurana VG, Shadbolt B, et al. Increasing incidence of glioblastoma multiforme and meningioma, and decreasing incidence of Schwannoma (2000-2008): findings of a multicenter Australian study. Surg Neurol Int. 2011;2:176.
[34] Chen R, Smith-Cohn M, Cohen AL, et al. Glioma subclassifications and their clinical significance. Neurotherapeutics. 2017;14:284–97.
[35] Xu S, Tang L, Li X, et al. Immunotherapy for glioma: current management and future application. Cancer Lett. 2020;476:1–12.
[36] Farley CR, Morris AB, Tariq M, et al. FcgammaRIIB is a T cell checkpoint in antitumor immunity. JCI Insight. 2021;e135623:6.
[37] Fortis SP, Goulielmaki M, Aubert N, et al. Radiotherapy-related gene signature in prostate cancer. Cancers (Basel). 2022;5032:14.
[38] Chang AL, Miska J, Wainwright DA, et al. CCL2 produced by the glioma microenvironment is essential for the recruitment of regulatory T cells and myeloid-derived suppressor cells. Cancer Res. 2016;76:5671–82.
[39] Qian J, Wang C, Wang B, et al. The IFN-gamma/PD-L1 axis between T cells and tumor microenvironment: hints for glioma anti-PD-1/PD-L1 therapy. J Neuroinflammation. 2018;15:290.
[40] Meng X, Duan C, Pang H, 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. 2019;41:185–99.
[41] Caponnetto F, Dalla E, Mangoni D, et al. The miRNA content of exosomes released from the glioma microenvironment can affect malignant progression. Biomedicines. 2020;8:564.
[42] Jung Y, Cackowski FC, Yumoto K, et al. Abscisic acid regulates dormancy of prostate cancer disseminated tumor cells in the bone marrow. Neoplasia. 2021;23:102–11.
[43] Yi K, Zhan Q, Wang Q, et al. PTRF/cavin-1 remodels phospholipid metabolism to promote tumor proliferation and suppress immune responses in glioblastoma by stabilizing cPLA2. Neuro Oncol. 2021;23:387–99.
[44] Hui L, Chen Y. Tumor microenvironment: sanctuary of the devil. Cancer Lett. 2015;368:7–13.
[45] Meurette O, Mehlen P. Notch signaling in the tumor microenvironment. Cancer Cell. 2018;34:536–48.
[46] Parker TM, Henriques V, Beltran A, et al. Cell competition and tumor heterogeneity. Semin Cancer Biol. 2020;63:1–10.
[47] Darvin P, Toor SM, Sasidharan Nair V, et al. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med. 2018;50:1–11.
[48] Roesch S, Rapp C, Dettling S, et al. When immune cells turn bad-tumor-associated microglia/macrophages in glioma. Int J Mol Sci. 2018;19:436.
[49] Woroniecka KI, Rhodin KE, Chongsathidkiet P, et al. T-cell dysfunction in glioblastoma: applying a new framework. Clin Cancer Res. 2018;24:3792–802.
[50] Miska J, Lee-Chang C, Rashidi A, et al. HIF-1alpha is a metabolic switch between glycolytic-driven migration and oxidative phosphorylation-driven immunosuppression of tregs in glioblastoma. Cell Rep. 2019;27:226–237.e4.
[51] Ruan S, Xie R, Qin L, et al. Aggregable nanoparticles-enabled chemotherapy and autophagy inhibition combined with anti-PD-L1 antibody for improved glioma treatment. Nano Lett. 2019;19:8318–32.
[52] Ene CI, Kreuser SA, Jung M, et al. Anti-PD-L1 antibody direct activation of macrophages contributes to a radiation-induced abscopal response in glioblastoma. Neuro Oncol. 2020;22:639–51.
[53] Brown CE, Alizadeh D, Starr R, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. N Engl J Med. 2016;375:2561–9.
[54] Choi BD, Maus MV, June CH, et al. Immunotherapy for glioblastoma: adoptive T-cell strategies. Clin Cancer Res. 2019;25:2042–8.

[55] Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–54.
[56] Chen Y, Zhang S, Wang Q, et al. Tumor-recruited M2 macrophages promote gastric and breast cancer metastasis via M2 macrophage-secreted CHI3L1 protein. J Hematol Oncol. 2017;10:36.
[57] Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application. J Hematol Oncol. 2017;10:58.
[58] Li L, Yang L, Wang L, et al. Impaired T cell function in malignant pleural effusion is caused by TGF-beta derived predominantly from macrophages. Int J Cancer. 2016;139:2261–9.
[59] Najafi M, Hashemi Goradel N, Farhood B, et al. Macrophage polarity in cancer: a review. J Cell Biochem. 2019;120:2756–65.
[60] Wang D, Yang L, Yue D, et al. Macrophage-derived CCL22 promotes an immunosuppressive tumor microenvironment via IL-8 in malignant pleural effusion. Cancer Lett. 2019;452:244–53.
[61] Yang L, Dong Y, Li Y, et al. IL-10 derived from M2 macrophage promotes cancer stemness via JAK1/STAT1/NF-kappaB/Notch1 pathway in non-small cell lung cancer. Int J Cancer. 2019;145:1099–110.
[62] Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348:69–74.