RNF208 is a Biomarker Associated with Immune Infiltration in Low-Grade Gliomas

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Research

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Abstract

Background: Gliomas that contain common tumors originating in the central nervous system include low-grade gliomas (LGGs) and high-grade gliomas (HGGs). RNF208 is a gene that has not been researched in LGGs.

Methods: Our study appraised the function of RNF208 in LGGs using data from The Cancer Genome Atlas (TCGA) database. The RNF208 expression level was analyzed via the Oncomine and TCGA database. The association between RNF208 expression levels and the clinical survival outcomes was evaluated by using COX regression and Kaplan-Meier plotting analysis. CIBERSORT was applied to investigate the correlation between RNF208 expression levels and cancer immune infiltrating cells. To explore relevant biological processes, we carried out Gene Set Enrichment Analysis. A protein network interacting with RNF208 was also established using the STRING tool.

Results: A group of data of LGGs patients based on the TCGA database revealed that high RNF208 expression level was relevant to a favorable prognosis. Besides, RNF208 expression that served as an independent factor was significantly correlated with WHO grade groups in univariate analysis. Furthermore, RNF208 expression was negatively correlated with the immune infiltration level of 22 species of immune infiltrating cells and immune checkpoints including PD1, PDL2, TIM3, and CTLA4. GSEA showed that 20 biological pathways were discriminatively enriched in the RNF208 low expression level.

Conclusions: Our findings revealed that RNF208 would be regarded as a promising prognostic biomarker in LGGs.

1 Introduction

Glioma is a type of tumor of the central neural system that affects the brain and spinal cord(1). It accounts for 81% of craniocerebral malignancies and is the greatest frequent primary tumor of the central nervous system(2). It causes a high incidence and mortality rate, with an annual incidence of five point two six of one hundred thousand, which are usually associated with poor prognosis and low quality of life(3). 2016 WHO of the central nervous system tumors classified gliomas into low-grade gliomas (LGGs) and high-grade gliomas based on whether the tissue is isocitrate dehydrogenase 1 mutant or wild-type(4–6). Although LGGs patients have a better survival rate than those with high-grade glioma, all LGGs will eventually develop high-grade glioma. Survival of LGGs patients hasn't been improved in the last few decades. Although age was considered as a well prognostic factor in LGGs, there are few other prognostic elements related to LGGs(7).

More and more studies have shown that many biological processes involved Ring finger protein 2 (RNF2), such as tissue development, tumorigenesis, cell proliferation, and apoptosis. Current studies have shown that RNF2, the component of polycomb repressor complexes (PRC1) has E3 ligase activity(8). It is involved in catalyzing single ubiquitin of histone H2A 119 lysine residue (H2AK119Ub1), protein degradation, promoting the group by different molecular signaling pathways(9). It also affects cell proliferation, cell apoptosis, and tumor migration, and has a significant effect on the process of tumor progression(10). Researches have shown that overexpression of RNF208 has an effect on the inhibition of tumor formation and metastasis, and is considered as a prognostic biomarker(11). Whereas, the role of RNF208 in LGGs has been rarely reported, which still needs to be further explored and clarified.

Presently, we explored the relevance of RNF208 expression with the prognosis of LGGs via COX regression. Moreover, we evaluated the relation between RNF208 expression and immune infiltrating levels of tumor cells using CIBERSORT. Ultimately, GSEA showed that there are some signaling pathways relevant to RNF208 low expression phenotype in LGGs. We also constructed a protein network interacting with RNF208.

2 Results

2.1 The expression of RNF208

We used the Oncomine database to investigate the mRNA expression levels of RNF208 in different tumors and the corresponding normal tissues to determine the differential expression between the tumor and normal tissues. RNF208 expression was higher in the breast, colorectal, gastric, and prostate cancers and lower in brain and CNS, esophageal, head and neck, kidney, pancreatic cancers, leukemia, and sarcoma (Fig. 1A). To further verify the expression of RNF208, we analyzed the expression of RNF208 in common tumors to determine the differential expression among the different cancers. The expression of RNF208 in normal tissues of LGGs and glioblastoma multiforme (GBM) was prominently higher than in tumor tissues (Fig. 1D). Simultaneously, RNF208 expression in Grade II was higher than Grade III (Fig. 1B). The HPA database also confirmed that the expression of RNF208 in brain tissue was higher than that in LGGs (Fig. 1C). According to the WHO grading system, gliomas were divided into four categories depending on how malignant they are, i.e. Grade I, Grade II, Grade III, Grade IV (12). We assessed the expression of RNF208 in WHO-grade groups in LGGs by using the data that was downloaded in the TCGA database. Cox regression was performed to display that high expression of RNF208 was significantly related to tumor grade (which divided into Grade II and Grade III, $p = 0.001$) and gender (**Table 1**, $p = 0.015$). To confirm this conclusion, we additionally conducted cox regression using data downloaded from the CGGA database. Similarly, high RNF208 expression was notably correlated with tumor grade (**Table 1**, $p < 0.001$).

2.2 Survival outcomes

As our above results indicated that RNF208 expression was significantly correlated with grade groups, we accessed the prognostic effect of RNF208 in LGGs using data downloaded from the TCGA database. High expression of RNF208 had a better prognosis than low RNF208 expression in GBM and LGGs (Fig. 2A, $P < 0.001$). Whereafter, we performed the survival curve of RNF208 in LGGs in GEPIA. Similarly, low expression of RNF208 had a poor prognosis (Fig. 2B, $P = 0.029$). As exhibited in **Table 2**, we executed univariate survival analysis using COX regression. Some elements, involving WHO grade system (HR = 3.085, P-value < 0.001) which was divided into Grade II and Grade III, age (HR = 5.548, P-value < 0.001) that was divided into groups of 60 or older and 60 or younger and RNF208 expression (HR = 0.454, P-value = 0.004), are markedly related to cumulative survival (Fig. 2D). In multivariate analysis, grade group is an independent prognostic element of a positive prognosis. Receiver operating characteristic (ROC) curves were used to evaluate the accuracy of survival (Fig. 2C). The area under the curve (AUC) for population survival was 0.747. These results demonstrate the accuracy of RNF208 in predicting survival in LGGs. RNF208 is a biomarker for good prognosis in LGGs.

2.3 Immune-related analysis

Studies showed that tumor immune microenvironment was critical to gliomas(13). Subsequently, we explored the immune cell infiltration levels in LGGs in TIMER database to research the relation between RNF208 expression level and immune infiltrating cells. B cell, CD8 + T cell, CD4 + T cell, macrophage, neutrophil, and dendritic cell showed significantly negative correlations with RNF208 (Fig. 3A). Moreover, the infiltration of B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells and the expression of RNF208 were significantly correlated with the prognosis of LGGs (Fig. 3B). This suggests that RNF208 plays an important role in the regulation of immune cell infiltration in LGGs. To understand the relationship between RNF208 and various immune cells in more detail, we evaluated the proportion of 22 immune cells in LGGs with high or low expression of RNF208. T cells CD4 memory activated, NK cells activated, Macrophages M1 and Neutrophils were significantly different. (Fig. 3C). Correlation heat map of the proportions of 22 immune cells also showed varying degrees of correlations (Fig. 3E). TIMER database was applied to assess the correlation between RNF208 expression and marker levels in specific cell subpopulations including $CD8^+$T cells, monocytes, M1 and M2 macrophages, total T cells, NK cells, B cells, TAMs, Tfh cells, neutrophils, DCs, Th17 cells, Th1 cells, Th2 cells, Tregs and exhausted T cells. We adjusted for tumor purity and found that RNF208 expression was associated with all of these markers (**Table 3**). Subsequently, we evaluated the correlation between RNF208 and immune checkpoints including PD1(PDCD1), PD-L1(CD274), PD-L2(PDCD1LG2), TIM-3(HAVCR2), and CTLA4 in GEPIA. We found that RNF208 had a negative correlation with all above, especially TIM-3 and PD-L2(Fig. 3D). Furthermore, the relation between RNF208 and checkpoints was evaluated in CGGA. In general, RNF028 was negatively associated with these immune checkpoints, especially PD-L2 (**Supplementary Fig. 1**).

2.4 RNF208-related genes

For further researching on the molecular mechanism of RNF208 in the occurrence of LGGs, we attempted to screen out the targeted RNF208 binding protein and RNF208 expression-related genes. A total of 52 RNF208 binding proteins supported by experimental evidence are obtained using the STRING database (Fig. 4A). We then used the UALCAN database to obtain all genes related to RNF208 expression in LGGs. The top 10 RNF208-related genes were compared in different clinical phenotypes (Fig. 4B). RNF208 expression is positively related to HSD11B1L, FBXL15, PIN1, AGAP3, and PPFIA3 (Fig. 4C). The heat map indicated that these five genes were positively related to RNF208 in most detailed cancer types (Fig. 4D). The cross-analysis of the above two groups shows that there are four common members, namely, H3F3C,HFE□LITAF□NANOS3(Fig. 4E).

2.5 Downstream signaling pathways in GSEA

To investigated related signaling pathways of different expression levels of RNF208 in LGGs, we performed GSEA analysis, which determine whether the gene sets show statistically significant (Fig. 5A). We partitioned the data sets into two sets-high expression and low expression with the median expression of RNF208 as a limit to analyze the differences among the groups. The most important pathways for down-regulated gene sets in the significance requirement (NOM p-value < 0.05, FDR q-value < 0.05) are listed in our study (**Table 4)**. Twenty signaling pathways have discrepantly enriched in the RNF208 low expression phenotypic traits including JAK-STAT signaling pathway, apoptosis, Toll-like signaling pathway, B cell receptor signaling pathway, TGF-β signaling pathway,cell adhesion molecules CAMs, and so on (Fig. 5B).

3 Discussion

Recently, RNF208 has been reported to play a major role in inhibiting the metastasis of triple-negative breast cancer(11). However, there have been few studies on gliomas. In this study, we found that changes in the expression of RNF208 may influence the prognosis of LGGs. Upregulated expression of RNF208 was an individual prognostic element with a fine prognosis. Simultaneously, the increased expression of RNF208 was memorably correlated with WHO grade groups. Also, considering that there are few studies on RNF208, we adopted functional enrichment analysis. The analysis results showed that there are many related signaling pathways. The study also manifested that some immune checkpoints and disparate levels of cellular immunity infiltration were related to RNF208 expression in LGGs.

In this study, we examined the expression of RNF208 in diverse carcinomas and normal structures in Oncomine and TCGA database. We found that RNF208 expression level was elevated in breast, colorectal, gastric, and prostate cancers while had a low expression level in brain and CNS, esophageal, head and neck, kidney, pancreatic cancers, leukemia, and sarcoma. Based on the TCGA dataset, RNF208 expression was higher in normal tissues in LGGs. Subsequently, we analyzed RNF208 expression levels in WHO grade groups based on the TCGA and CGGA database, which showed that RNF208 was highly significant in WHO grade groups. Besides, we found the correlation of RNF208 expression with the prognosis of LGGs patients by using Kaplan-Meier survival analysis. It also could be found in the UALCAN dataset and SPSS20.0. RNF208 upregulated expression was associated with a favorable prognosis. Based on the TCGA data set, we further investigated the expression mechanism of RNF208 in LGGs cells and its relationship. Statistical analysis by SPSS20.0 revealed that RNF208 expression was related to WHO grade groups. In multivariate analysis, RNF208 expression was an independent prognostic element in the prognosis of LGGs. Another momentous thing of this study relates to the relationship between RNF208 expression and the level of LGGs immune infiltration which manifests an apparent relationship of RNF208 expression with Neutrophil, B cell, $CD8^+$cell, Macrophage, $CD4^+$cell, and Dendritic cell especially macrophage. Most grade I and II astrocytoma contain microglia and macrophages(14). Tumor-associated macrophages are recruited in the immune microenvironment of glioma and have immune function. It interacts with certain cytokines released by cancer cells to promote tumor proliferation and migration(15). Likewise, we also found that there were some connections between RNF208 expression and immune checkpoints including PD1, PD-L1, PD-L2, TIM-3, and CTLA4. RNF208 was negatively correlated with all these immune checkpoints, suggesting that RNF208 is a potential prognostic biomarker. Meanwhile, RNF208 has been found to have a crucial effect on the inhibition of tumor metastasis in several studies, but few studies have been performed on LGGs(11). Hence, we performed GSEA analysis which showed that many signaling pathways were distinguishingly enriched in RNF208 high and low expression phenotype, including toll-like reporters (TLRs) signaling pathways. TLR may play a role in tumor immune surveillance by binding to some endogenous molecules produced by the body itself. Since some endogenous ligands that can be recognized by TLR can be produced in the process of tumor development, TLR may play a role in tumor immune surveillance(16). Studies clarified that TLRs play a critical role in the activation of microglia cells, the congenital immune cells of the central nervous system(17). Microglia are another feature of the central nervous system. They cluster in macrophages and have the role of immune monitoring and debris removal(18, 19). But it has not been researched in LGGs which needs us to go further study. Another remarkable signaling pathway is apoptosis, a kind of programmed cell death, may improve the effectiveness of cancer

treatment(20). The occurrence of the tumor is due to the disorder of the balance between cell proliferation and apoptosis, and apoptosis plays a negative supervisory role in the process of tumor growth(21). Therefore, it is a new idea for tumor therapy to study the proliferation of various tumor cells and induce cell apoptosis with little effect on normal cells. TGF-β belongs to the TGF-β superfamily that regulates cell growth and differentiation(22). TGF-β mRNA can be detected in almost all tumor cells. Glioma can secrete higher levels of TGF-β in the body(23). Low expression of RNF208 inhibits TGF-β signaling pathways, which insinuates RNF208 is a prognostic biomarker of low-grade gliomas.

Although we preliminarily explored the biological process of RNF208 in LGG by enrichment analysis, further biomedical experiments are needed to investigate the specific mechanism of RNF208 expression, immune microenvironment of RNF208 in LGG and LGG progression. Overall, however, it is noteworthy that RNF208 is a promising prognostic biomarker in LGG.

4 Conclusions

We investigated the relationship between RNF208 and the pathological features, prognosis, and immune infiltration of LGGs. RNF208 may be a promising prognostic biomarker for LGG.

5 Methods

5.1 Data acquisition

TCGA (The Cancer Genome Atlas)(https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga), a public database that researchers around the world can access the data on the molecular characteristics of various cancers they need, was full exploited to download clinical information and gene expression profiles of 668 cases including over survival time, survival status, age, gender and WHO grade classification(12). CGGA (Chinese Glioma Genome Atlas), data memory and analysis to survey brain carcinomas datasets more than 2,000 samples among Chinese cohorts, was similarly used to analyze the above.

5.2 Expression and survival analysis

Oncomine(https://www.oncomine.org/resource/login.html), a cancer microarray database and integrated data-mining platform, was applied to analyze the expression of RNF208 in different cancer tissues(13). The threshold was set to the following values: P-value = 0.05, fold change = 2, and gene ranking of all. We also investigated RNF 208 expression in different human tissues and a variety of cancers at RNA and protein levels using TCGA database and STRING tool, a protein interaction tool that can query genome-related genes and form visualized results(14). The prognostic value of RNF208 was verified by Human Protein Atlas (HPA) using clinical tissue samples.

5.3 Immune-related analysis

TIMER (Tumor Immune Estimation Resource) (https://cistrome.shinyapps.io/timer/) was used for accessing the relationship between RNF208 and immune cells. The Kaplan-Meier survival curve was also included. CIBERSORT was utilized to examine the components of 22 immune cells in LGGs. We also accessed the

association of RNF208 with several immune checkpoints such as PD1(PDCD1), PD-L1(CD274), PD-L2(PDCD1LG2), TIM-3(HAVCR2), and CTLA4.

5.4 Gene set enrichment analysis and related genes

GSEA (Gene Set Enrichment Analysis), a knowledge-based approach for interpreting genome-wide expression profiles, which including three pivotal elements: calculation of enrichment score (ES), estimation of the significance level of ES, and adjustment for multiple hypothesis testing, was used to explore the potential molecular mechanisms of different RNF208 expression levels in LGGs(15). We investigated the differences in biological functions and pathways in LGGs between groups with low and high expression in a median value of RNF208. STRING tool and UALCAN database were applied to search RNF208-related genes.

5.5 Statistical analysis

The SPSS 20.0 statistical software was utilized to perform all statistical analyses. The COX regression and Pearson's chi-square test were used to determine the parameters significantly correlated with overall survival in LGGs. R 4.0.2 software was used to analyze the expression of RNF 208 in WHO grade groups.

Declarations

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8. Availability of data and materials

All data generated or analyzed during this study are included in this article. All data have been deposited in The Cancer Genome Atlas database(https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) and Chinese Glioma Genome Atlas(http://www.cgga.org.cn/).

9. Author Contributions

"Tong Chen and MYX contributed to conception and design of the study. STJ organized the database. QQW performed the statistical analysis. TC wrote the first draft of the manuscript. TC, BWW, MYX, QQW, STJ, and XJZ wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version".

10. Ethics approval and consent to participate

Not applicable.

11. Consent for publication

Not applicable.

12. Declarations of interest: none

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Tables

Table I. Relationship between the expression of RNF208 and clinicopathological

Characteristic	TCGA				CGGA			
	n	Low expression (%)	High expression (%)	P	n	Low expression (%)	High expression (%)	P
Total	508	380(74.80)	128(25.20)		174	74(42.53)	100(57.47)	
Gender				0.015*				0.525
Male	281	222(79.00)	59(21.00)		100	44(60.27)	56(55.45)	
Female	227	158(69.60)	69(30.40)		73	29(39.73)	45(44.55)	
Age				0.667				0.464
≤ 60	447	333(74.50)	114(25.50)		161	46(41.82)	64(58.18)	
>60	61	47(77.05)	14(22.95)		13	28(43.75)	36(56.25)	
Grade				0.001*				
II	247	168(68.02)	79(31.98)			65(55.56)	52(44.44)	
III	261	212(81.23)	49(18.33)			9(15.79)	48(84.21)	<0.001*
*P<0.05								

Table II. Univariate and multivariate analysis of prognostic factors in Low-grade gliomas

	Univariate analysis				Multivariate analysis			
	HR	P	95%CI		HR	P	95%CI	
RNF208 expression	0,454	0.004*	0.266	0.776	0.511	0.016*	0.296	0.881
Age	5,548	< 0.001*	3.517	8.751	4.116	< 0.001*	2.592	6.536
(≤ 60>60)								
Gender	1.058	0.769	0.725	1.545				
Male vs Female								
Grade	3.085	< 0.001*	2.038	4.670	2.562	< 0.001*	1.664	3.943
II vs III								
*P < 0.05								

Table III. Correlation analysis between RNF208 expression and gene markers in TIMER

Description	Gene markers	LGG			
		None		Purity	
		Cor	P	Cor	P
CD8 + T cell	CD8A	-0.334	***	-0.019	***
	CD8B	-0.219	***	-0.243	***
T cell (general)	CD3D	-0.258	***	-0.252	***
	CD3E	-0.297	***	-0.292	***
	CD2	-0.334	***	-0.326	***
B cell	CD19	-0.146	***	-0.143	***
	CD79A	-0.217	***	-0.177	***
Monocyte	CD86	-0.413	***	-0.413	***
	CD115 (CSF1R)	-0.303	***	-0.3	***
TAM	CCL2	-0.319	***	-0.305	***
	CD68	-0.391	***	-0.371	***
	IL10	-0.369	***	-0.35	***
M1 Macrophage	INOS (NOS2)	0.14	***	0.136	***
	IRF5	-0.284	***	-0.282	***
	COX2(PTGS2)	-0.056	***	-0.06	***
M2 Macrophage	CD163	-0.345	***	-0.321	***
	VSIG4	-0.368	***	-0.356	***
	MS4A4A	-0.383	***	-0.407	***
Neutrophils	CD66b (CEACAM8)	0.003	***	0.025	***
	CD11b (ITGAM)	-0.327	***	-0.329	***
	CCR7	-0.232	***	-0.227	***
Natural killer cell	KIR2DL1	-0.054	***	-0.081	***
	KIR2DL3	-0.138	***	-0.149	***
	KIR2DL4	-0.234	***	-0.232	***
	KIR3DL1	-0.077	***	-0.084	***
	KIR3DL2	-0.087	***	-0.098	***
	KIR3DL3	-0.015	***	-0.029	***

Description	Gene markers	LGG			
	KIR2DS4	-0.089	***	-0.106	***
Dendritic cell	HLA-DPB1	-0.339	***	-0.32	***
	HLA-DQB1	-0.254	***	-0.236	***
	HLA-DRA	-0.413	***	-0.397	***
	HLA-DPA1	-0.396	***	-0.378	***
	BDCA-1(CD1C)	-0.252	***	-0.238	***
	BDCA-4(NRP1)	-0.316	***	-0.301	***
	CD11c (ITGAX)	-0.223	***	-0.203	***
Th1	T -bet (TBX21)	-0.119	***	-0.099	***
	STAT4	0.194	***	0.181	***
	STAT1	-0.381	***	-0.366	***
	IFN-γ (IFNG)	-0.184	***	-0.182	***
	TNF-α (TNF)	-0.133	***	-0.117	***
Th2	GATA3	-0.268	***	-0.258	***
	STAT6	-0.148	***	-0.176	***
	STAT5A	-0.348	***	-0.34	***
	IL13	0.207	***	0.21	***
Tfh	BCL6	-0.146	***	-0.126	***
	IL21	-0.094	***	-0.074	***
Th17	STAT3	-0.547	***	-0.521	***
	IL17A	-0.056	***	-0.046	***
Treg	FOXP3	0.072	***	0.053	***
	CCR8	-0.154	***	-0.162	***
	STAT5B	-0.21	***	-0.205	***
	TGFβ (TGFB1)	-0.22	***	-0.205	***
T cell exhaustion	PD-1 (PDCD1)	-0.234	***	-0.197	***
	CTLA4	-0.161	***	-0.146	***
	LAG3	0.027	***	0.039	***
	TIM-3 (HAVCR2)	-0.384	***	-0.379	***

Description	Gene markers	LGG			
	GZMB	-0.108	***	-0.112	***
***P < 0.05					

Table IV. Gene sets enriched in the low RNF208 expression phenotype

Gene set name	NES	NOM p-value	FDR q-value
KEGG_SMALL_CELL_LUNG_CANCER	-2.115	0	0.004
KEGG_JAK_STAT_SIGNALING_PATHWAY	-2.076	0	0.004
KEGG_ADHERENS_JUNCTION	-1.987	0	0.019
KEGG_PANCREATIC_CANCER	-1.962	0.002	0.020
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	-1.957	0.002	0.017
KEGG_PATHWAYS_IN_CANCER	-1.939	0	0.020
KEGG_FOCAL_ADHESION	-1.912	0	0.026
KEGG_APOPTOSIS	-1.897	0.004	0.027
KEGG_CELL_ADHESION_MOLECULES_CAMS	-1.890	0.006	0.027
KEGG_PROSTATE_CANCER	-1.889	0.004	0.024
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	-1.883	0.002	0.024
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	-1.849	0.012	0.033
KEGG_VIRAL_MYOCARDITIS	-1.832	0.012	0.039
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	-1.831	0.012	0.036
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	-1.812	0.010	0.039
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	-1.815	0.016	0.038
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	-1.815	0.006	0.036
KEGG_TGF_BETA_SIGNALING_PATHWAY	-1.811	0.002	0.035
KEGG_AUTOIMMUNE_THYROID_DISEASE	-1.810	0.018	0.033
KEGG_NICOTINATE_AND_NICOTINAMIDE_METABOLISM	-1.787	0.008	0.039

Figures

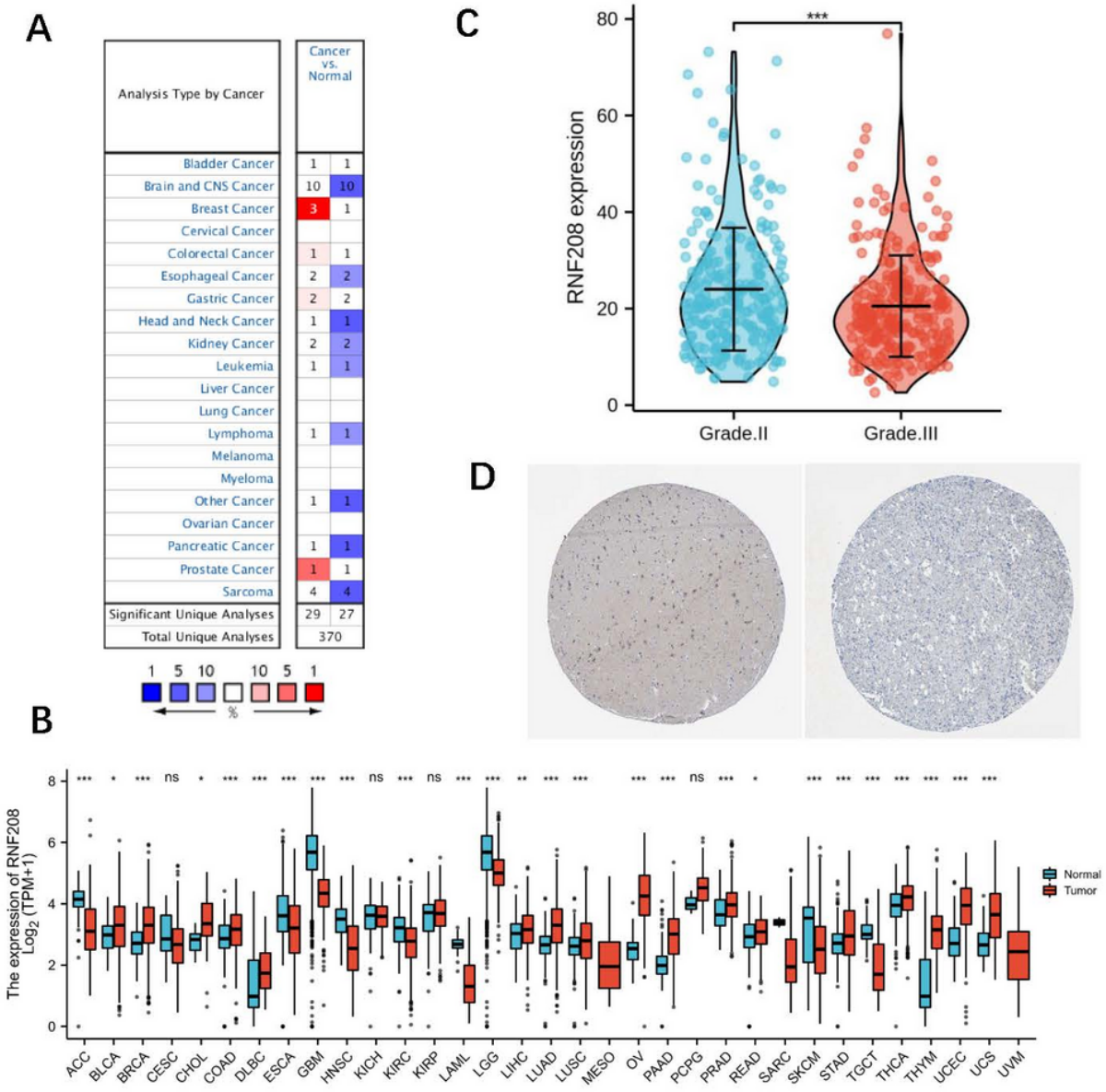

Figure 1

RNF208 expression in different cancers and gliomas. (A)RNF208 expression in different cancers and normal tissues. (B)RNF208 expression in Grade II and Grade III. (C)The level of RNF208 protein in LGGs tissue was lower than that in cerebral cortex in the Human Protein Atlas (Antibody HPA021256, 10X). (D)RNF208 expression in common cancers.

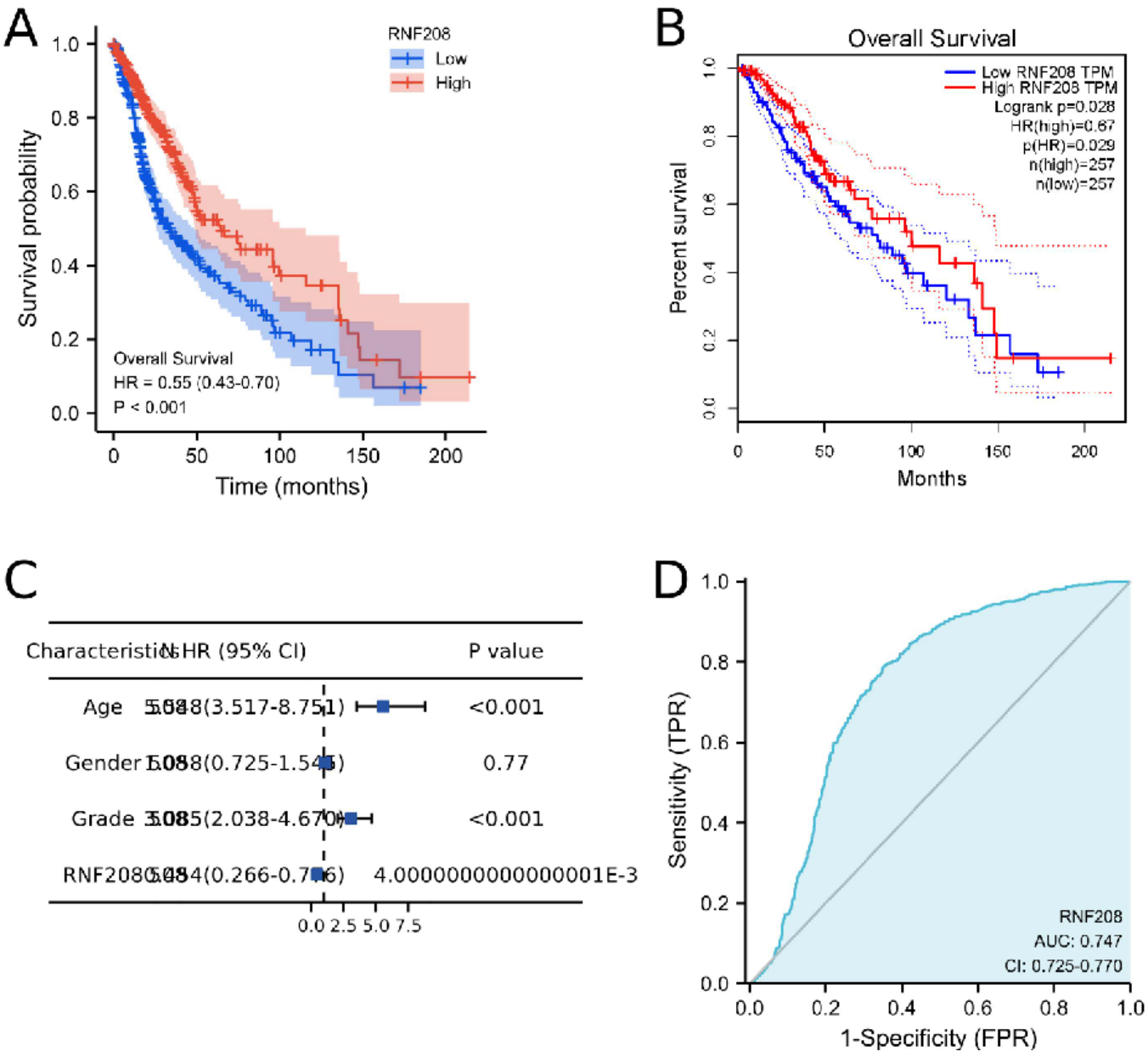

Figure 2

Survival outcomes comparing the high and low expression of RNF208 in LGGs. (A)Survival curves of high expression and low expression of RNF208 in LGGs in TCGA database. (B)Survival outcome and expression difference analyzed by GEPIA. (C)ROC curve was used to evaluate the accuracy of survival. (D)Forest map of the prognostic role of RNF208.

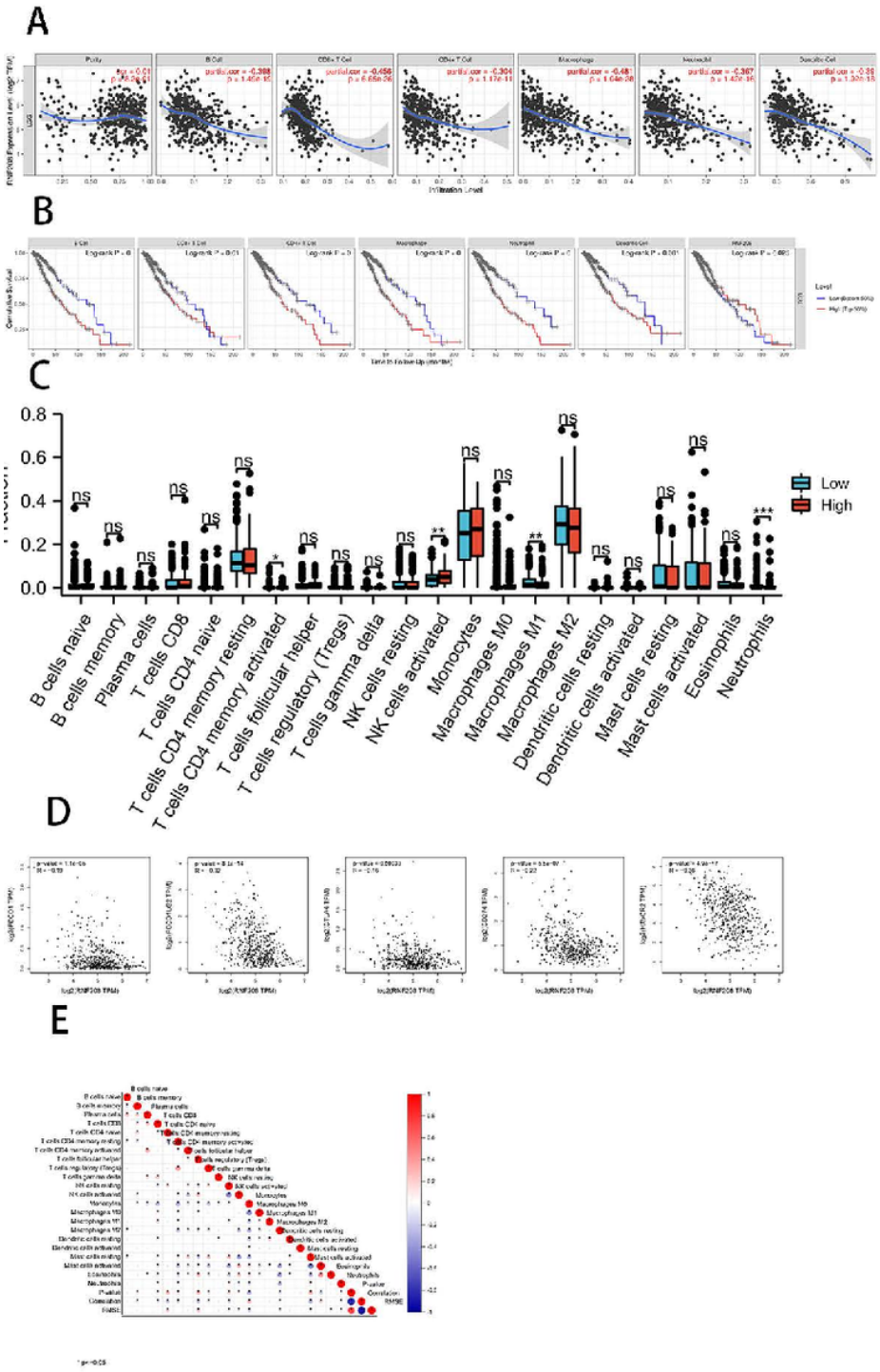

Figure 3

Correlation of RNF208 expression with immune infiltration level in LGGs. (A)The expression of RNF208 in LGGs was correlated with the level of immune infiltration. (B)Kaplan-Meier curves of immune infiltration and RNF208 expression levels in LGGs. (C)Immune infiltration of 22 kinds of immune cells in LGGs. (D)The relationship between RNF and immune checkpoints in the CGGA database. (E)Correlation heat map of 22 immune cells in LGGs. (F)RNF208 is negatively correlated with immune checkpoints, including PD1, PDL1, PDL2, TIM3, and CTLA4 in GEPIA.

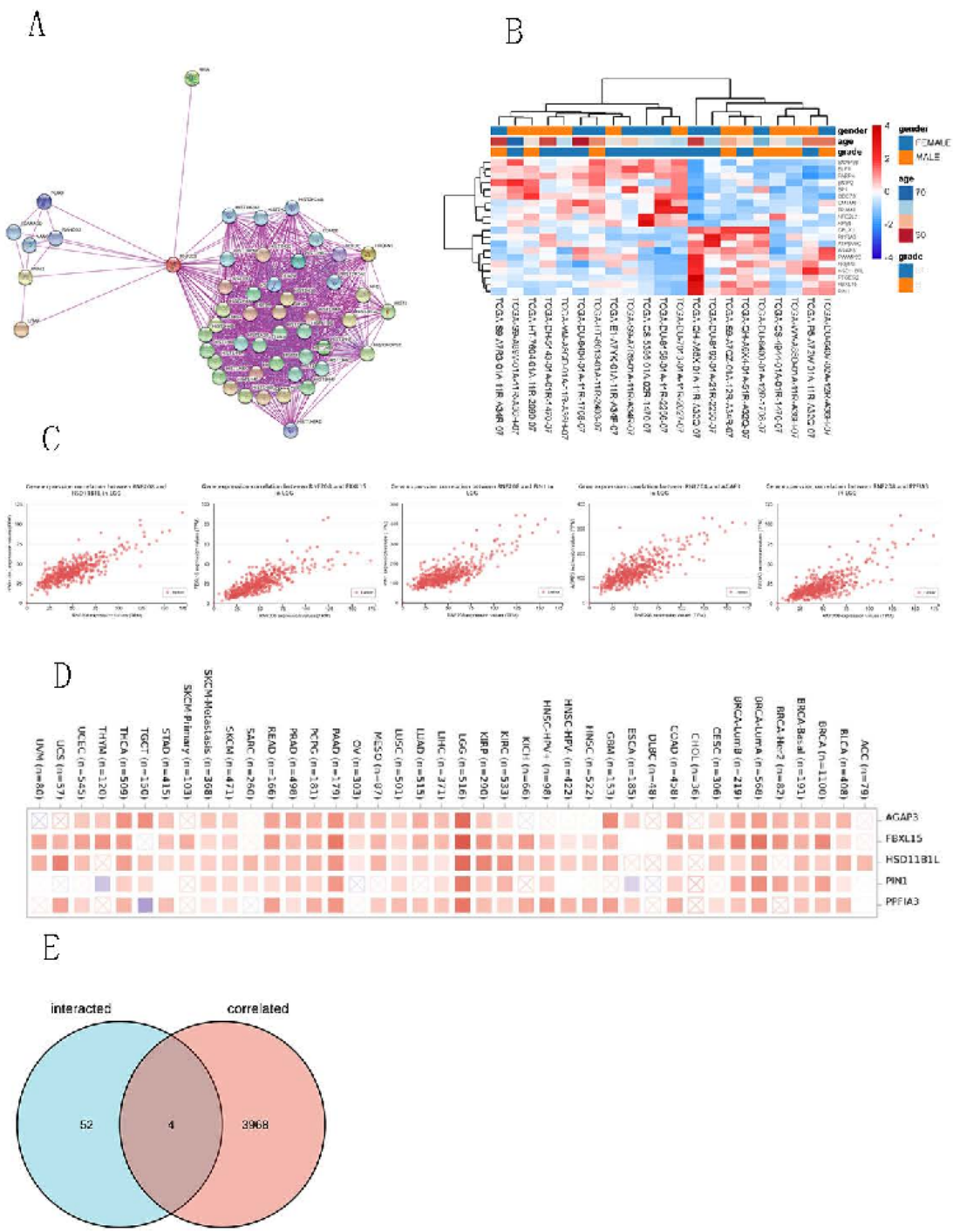

Figure 4

RNF208-interaction proteins and related genes in LGGs tissue. (A)The available experimentally determined RNF208 binding protein was obtained using the STRING tool. (B)The top five target genes associated with RNF208 in the UALCAN database. (C)An intersection analysis of the RNF208-binding and correlated genes was conducted. (D)Heat map data for five genes in detailed cancer types. (E) Venn diagram showing common genes associated with RNF208.

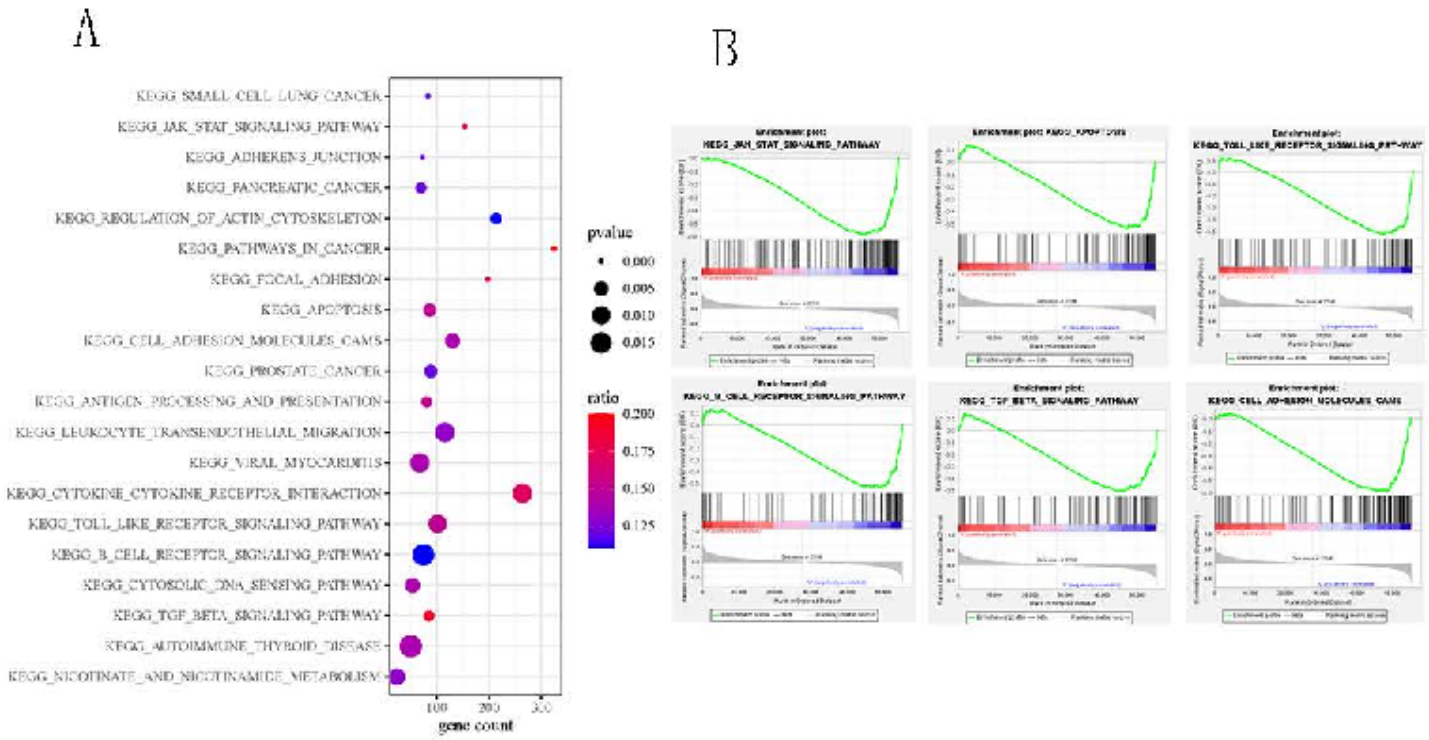

Figure 5

Enrichment plots from gene set enrichment analysis. (A)Bubble chart of RNF208-related biological processes in LGGs. (B)GSEA analysis including JAK-STAT signaling pathway, apoptosis, Toll-like receptor signaling pathway, B cell receptor signaling pathway, TGF beta signaling pathway, Cell adhesion molecules CAMs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.pdf