Prognostic Significance of mRNA Expression RBBP8 or its Methylation in Gliomas

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Abstract

Retinoblastoma-binding protein 8 (RBBP8) affects the prognosis of patients with malignancies through various mechanisms. However, its function in gliomas is unknown. Our study explored the effects of RBBP8 on the prognosis of glioma patients, as well as its regulatory role in the glioma immune microenvironment. We used various bioinformatics methods to analyze the transcriptional profiles and methylation data of RBBP8 in gliomas from multiple databases. Our results showed that the mRNA and protein expression of RBBP8 in gliomas was higher than that in normal tissues and positively correlated with malignant clinical features such as age and WHO grade. A Kaplan–Meier analysis showed that patients with high RBBP8 expression had a poor prognosis. Cox regression demonstrated that RBBP8 was an independent risk indicator and had good diagnostic value for the poor prognosis of glioma. A meta-analysis confirmed the carcinogenic effect of RBBP8 on glioma, while the Tumor Immune Estimation Resource analysis showed that RBBP8 was positively correlated with the infiltration of six immune cell types in low-grade glioma, but only with dendritic cells in glioblastoma multiforme. Importantly, RBBP8 was positively correlated with many well-known immune checkpoints (e.g., CTLA4 and PDL-1). Finally, a gene set enrichment analysis revealed that RBBP8 can promote the activation of cancer-related pathways such as cell cycle, DNA replication and so on. In conclusion, this study is the first to elaborate on the value of RBBP8 in the pathological process of glioma for anti-tumor immunotherapy. In addition, the expression of RBBP8 and its methylation site, cg05513509, may provide potential targets for glioma therapy.

Introduction

Glioma is the most common primary intracranial tumor in adults and is characterized by high heterogeneity, recurrence rates, and mortality (Guan et al. 2018; Hu et al. 2021). Surgical resection is the main treatment for gliomas, regardless of its grade. Despite undergoing surgery, radiotherapy, and temozolomide chemotherapy, the prognosis of glioma patients remains unsatisfactory, especially in glioblastoma multiforme (GBM), which is the most common type of glioma (Reni et al. 2017). The median survival time of GBM patients is less than two years after standard treatment (Gusyatiner and Hegi 2018). Although some new treatments have been used in clinical trials, their effects are not satisfactory. Therefore, more studies are needed to determine the prognosis of glioma patients and to develop new therapeutic targets.

Immunotherapy is currently the most popular field in cancer treatment research, and is also one of the most promising treatment methods for eliminating cancers (Schumacher and Schreiber 2015; Desrichard et al. 2016; Lu and Robbins 2016; Steven et al. 2016; De Mattos-Arruda et al. 2020). Immune checkpoint inhibitors have achieved great success in the treatment of cancer and have become the main research direction in the therapy of cancer patients. However, treatment with immunosuppressive agents for immune checkpoints has shown sustained response rates in only a small percentage of patients (De Mattos-Arruda et al. 2020). Furthermore, immunotherapy is accompanied by off-target side effects and cytotoxicity. Hence, the development of new targets and a better understanding of the glioma immune

microenvironment have broad prospects and challenges for guiding individualized immunotherapy in cancer patients.

Retinoblastoma binding protein 8 (RBBP8) encodes a nucleoprotein that binds to the retinoblastoma protein. RBBP8 interacts with the MRN complex and participates in homologous recombination repair of DNA double-strand breaks (Yu and Chen 2004). Previous studies have shown that high expression of RBBP8 in plasma cell myeloma predicts poor prognosis and a high recurrence rate in these patients (Zhang et al. 2020). Liu and Lee (2006) showed that RBBP8 promotes G1/S progression by releasing the retinoblastoma protein from E2F responsive promoters. Furthermore, RBBP8 interacts with proliferating nuclear cell antigen and participates in DNA replication (Gu and Chen 2009). However, the regulatory mechanism of RBBP8 in the tumor microenvironment has not yet been confirmed. Determining whether RBBP8 will become a new immune checkpoint and play an important role in anti-tumor immunotherapy has high research value. More importantly, the relationships between RBBP8 and the clinical molecular characteristics and prognosis of glioma remain to be elucidated.

In the present study, we explored the value of RBBP8 as a potential immunotherapy target in glioma by analyzing the relationship between RBBP8 mRNA expression, DNA methylation, clinical characteristics, and prognosis in patients with glioma. A variety of bioinformatic approaches, including Kaplan-Meier, receiver operating characteristic (ROC) curve, univariate Cox, and multivariate Cox analyses, were used to identify the prognostic value of RBBP8 in gliomas through multiple datasets. Importantly, we combined gene set enrichment analysis (GSEA), co-expression analysis, and immune cell infiltration to explore the mechanism of RBBP8 on the poor prognosis of glioma. These studies help reveal the regulatory mechanism of RBBP8 in glioma and improve our understanding of its molecular mechanism.

Methods

Data sources

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) is the most reputable web tool for visualizing data from The Cancer Genome Atlas (TCGA), and helps researchers explore the expression profile of selected genes in tumors (Tang et al. 2017). Another important advantage of GEPIA is the inclusion of many matched normal tissues that aids in obtaining more accurate information on differentially expressed genes. Hence, we explored the expression status of RBBP8 in 33 common tumor types. We then compared the expression levels of RBBP8 in 163 GBM cases with 207 matched normal brain tissues, and the expression difference between 518 low-grade glioma (LGG) cases and 207 matched normal brain tissues.

TCGA (https://portal.gdc.cancer.gov/) is an online database dedicated to cancer data mining. It contains sequencing data from over 11,000 patients, 33 tumors, and their paired normal tissues (Zhang et al. 2019). TCGA contains a large amount of bioinformatics data, such as mRNA expression profiles, mutations, and methylation of relevant cancer genes. We downloaded gene expression profile data and corresponding clinical information for 652 glioma patients from the Xena portal

(https://xenabrowser.net/) to explore the expression and prognostic value of RBBP8 in gliomas. Detailed information of the included samples is provided in Supplementary Table S1.

The Chinese Glioma Genome Atlas (CGGA) dataset (http://www.cgga.org.cn/) is a specialized glioma research platform created by the world's leading neuro-oncology research institutions, and provides an important information resource platform for basic and clinical research on glioma (Zhao et al. 2021). The CGGA contains extensive histological data and complete clinical information on over 2000 cases of primary and recurrent gliomas. In this study, we obtained gene sequencing data of 748 glioma patients and microarray sequencing data of 268 glioma patients from the CCGA to validate the results of TCGA. Supplementary Table S2 lists the detailed information of the included patients from the CGGA.

We also downloaded five different datasets (GSE43378: 50 patients, GSE4412: 85 patients, GSE50025: 34 patients, GSE74187: 60 patients, and GSE83300: 50 patients) from the Gene Expression Omnibus (GEO) database, including 279 glioma samples (https://www.ncbi.nlm.nih.gov/geo/). These data were used to validate the expression of RBBP8 and perform a meta-analysis to confirm its impact on the prognosis of patients. Finally, the Human Protein Atlas database (HPA) was used to compare RBBP8 protein expression levels between normal brain tissues and gliomas (https://www.proteinatlas.org/). The database of HPA is an internationally well-known protein expression database, which can provide free authorized image acquisition of data for researcher and search this link for detailed guide information (https://www.proteinatlas.org/about/licence).

Cell culture and the reverse transcription-polymerase chain reaction (RT-PCR)

Human astrocytes and the glioma cell line, A172, were purchased from ProCell Life Science & Technology (Wuhan, China). Cells were cultured in Dulbecco's modified Eagle's medium (Procell Life Science & Technology, PM150210) containing 10% fetal bovine serum (Gibco, Gaithersburg, MD, USA, lot: 10099-141c) in an incubator containing 5% CO_2 at 37 °C. Total RNA was extracted using Total RNA Kit I (Thermo Fisher Scientific, Waltham, MA, USA) and reverse transcribed to cDNA using NovoScript cDNA Synthesis SuperMix (Hofmann-La Roche, Basel, Switzerland). RT-PCR was conducted using NovoStart SYBR qPCR SuperMix Plus (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GADPH) was used as the internal reference. The primers for RBBP8 and GAPDH were: RBBP8-forward: 5'-CGATTCCGCTACATTCCACC-3'; RBBP8-reverse: 5'-TCTTCTGCTCCTTGCCTTTT-3'; GAPDH-forward: 5'-CAAGGTCATCCATGACAACTTTG-3'; GAPDH-reverse: 5'-GTCCACCACCCTGTTGCTGTAG-3'.

GSEA analysis

GSEA is a very common and reliable calculation method that can be used to assess trends in the distribution of a predefined set of genes to determine their phenotypes (Li et al. 2019; Joly et al. 2020; Li et al. 2020). In our study, we divided glioma patients into high and low RBBP8 expression groups according to the median value, and then used GSEA 4.0jar software to identify the signaling pathways in which RBBP8 was significantly involved. The number of gene permutations was set at 1000. $P < 0.05$ and a false discovery rate (FDR) < 0.25 were applied to select the most significantly enriched pathways.

Tumor Immune Estimation Resource (TIMER) database analysis

Tumor-infiltrating immune cells are crucial for tumor treatment and prognosis. TIMER (https://cistrome.shinyapps.io/timer/) is a professional online research tool that explores the association between cancer and tumor-infiltrating immune cells (Li et al. 2017). Here, we used TIMER to analyze the relationship between RBBP8 mRNA expression levels and the tumor immune microenvironment of gliomas. First, we investigated the correlation between the expression level of RBBP8 and infiltration of six immune cells including CD4+ T cells, CD8+ T cells, B cells, macrophages, neutrophils, and dendritic cells. A Kaplan-Meier analysis was performed to explore the effect of these six immune cells on the prognosis of glioma patients. In addition, the correlation between alterations in the RBP8 somatic copy number and the abundance of immune infiltrates was explored using the SCNA module. Finally, the correlation module was used to reveal the relationship between RBBP8 and the immune checkpoints, PD-1, PD-L1, and PD-L2.

Meta-analysis

Several well-known database platforms (i.e., PubMed, Web of Science, and Embase) were used to assess the relationship between RBBP8 expression and prognosis. However, there were no relevant articles that revealed the prognostic value of RBBP8 in glioma. Therefore, a large number of samples from eight public datasets, including the CGGA mRNA microarray, CGGA RNA-seq, GSE43378, GSE4412, GSE50025, GSE74187, GSE8300, and TCGA RNA-seq, were included in our study to explore the prognostic significance of RBBP8. Q tests showed there was high heterogeneity across the eight studies. Thus, a random-effects model was applied.

Statistical analyses

R software (version 3.6.1) was used to conduct data analyses and processing. A one-way analysis of variance or t-test was used to clarify the difference in RBBP8 expression between gliomas and normal tissues. The chi-square test was used to explore the association of RBBP8 mRNA or its methylation sites with different clinical molecular characteristics. Univariate and multivariate regression analyses were performed to identify whether the RBBP8 mRNA level could be used as an independent risk factor for the prognosis of glioma. The ROC curve was plotted to assess the prognostic significance of RBBP8. The co-expression relationship between the RBBP8 mRNA level and its related methylation sites was established using the Spearman method. Finally, a Kaplan-Meier survival analysis was performed to detect the impact of RBBP8 or its methylation site on the overall survival of patients. P-values of all statistical analysis results that were less than 0.05 were considered significant.

Results

RBBP8 expression was significantly elevated in glioma

Results of the RT-PCR showed that the mRNA expression level of RBBP8 in the glioma cell line, A172, was significantly higher than that in the human astrocyte cell line (Fig. 1a). We next investigated RBBP8 expression levels in 33 common tumor types and found that aberrantly high expression of RBBP8 was common in tumors such as cervical squamous cell carcinoma, endocervical adenocarcinoma, lymphoid neoplasm diffuse large B-cell lymphoma, and lung squamous cell carcinoma (Fig. S1). The box plot shows the expression of RBBP8 in GBM and LGG (Fig. 1b). Specifically, the expression level of RBBP8 in 163 GBM and 518 LGG patients was significantly higher than the expression of RBBP8 in 207 normal brain tissues. Furthermore, the high expression of RBBP8 was confirmed in two GEO datasets (GSE35493 and GSE50161) (Fig. 1c and d). Finally, we identified a trend of increasing RBBP8 protein expression by comparing the extent of protein staining between immunohistochemical sections of normal brain tissue, LGG, and high-grade glioma (Fig. 1e-g).

Association of RBBP8 expression with clinical features in glioma

The relationship between RBBP8 expression and various clinical features was investigated based on TCGA and CGGA databases. RBBP8 expression was significantly correlated with age, and high expression of RBBP8 was more evident in older patients, which was confirmed in three datasets (TCGA RNA-seq, CGGA RNA-seq, and CGGA microarray) (Fig. 2a, d, and g). In addition, RBBP8 expression was higher in male than in female patients (Fig. 2b and 2h). Furthermore, there was a significant association between RBBP8 expression and one parameter double-hybrid molecules in the CGGA RNA-seq dataset (Fig. 2e). We also found a significant correlation between RBBP8 expression and clinical grade in all three datasets, which increased with increasing malignancy (Fig. 2c, f, and i).

Survival outcomes and diagnostic value of RBBP8 in glioma patients

We compared the difference in survival outcomes between the RBBP8 high and low expression groups to clarify the association between prognosis and RBBP8 expression. Thousands of samples were analyzed from TCGA RNA-seq, CGGA RNA-seq, and CGGA microarray datasets. Surprisingly, the results from the three datasets were consistent, indicating that patients with high expression of RBBP8 had a poor prognosis compared to those with low expression of RBBP8 (Fig. 3a-c). Moreover, we used the ROC curve to verify the prognostic value of RBBP8 in predicting survival in glioma. The area under the curve was greater than 0.7 in years 1, 3, and 5, except for the 1-year area under the curve in CGGA RNA-seq. This suggests that RBBP8 overexpression had a high predictive value for poor prognosis of glioma patients (Fig. 3d-f).

High expression of RBBP8 is a dependent risk factor for glioma patients

In TCGA RNA-seq dataset, a univariate Cox hazard analysis identified that RBBP8 ($p < 0.001$; hazard ratio (HR) = 1.047; 95% confidence interval (CI) = 1.036-1.058), and several clinical characteristics such as age ($p < 0.001$; HR = 4.701; 95% CI = 3.524-6.271), WHO grade ($p < 0.001$; HR = 4.688; 95% CI = 3.769-5.832), and cancer status ($p < 0.001$; HR = 2.034; 95% CI = 1.649-2.510), as risk factors related to survival time (Fig. 4a). However, to eliminate the deviation caused by other factors, multivariate Cox regression models

were applied to confirm that RBBP8 ($p < 0.001$; HR = 1.040; 95% CI = 1.016-1.064), age, WHO grade, and cancer status were strikingly and dependently related to the overall survival of patients (Fig. 4b). Overall, RBBP8 expression is related to the prognosis of glioma, and this independent correlation was confirmed in the CGGA RNA-seq and CGGA microarray datasets (Fig. 4c-f).

The prognostic significance of RBBP8 methylation in glioma

Methylation modifications of genes can regulate gene expression with the potential to become tumor markers. Thus, we explored the methylation of RBBP8 in gliomas. There were eight methylation sites in the RBBP8 gene (cg15770728, cg02863476, cg0818828, cg14157947, cg06299186, cg05305025, cg05513509, and cg16801093), among which cg15770728 had the highest methylation level (Fig. 5a). Furthermore, cg05513509, cg06299186, and cg15770728 had positive correlations with RBBP8; the remaining methylation sites had no relationship with the expression of RBBP8 (Fig. 5b-d). Moreover, cg05513509 affected the prognosis of LGG, patients; those with hypomethylation at the cg05513509 site had a longer survival time than those with hypermethylation at this site (Fig. 5e). Finally, the methylation level of RBBP8 was significantly correlated with age, KPS, person neoplasm cancer status, postoperative rx-tx, PRS type, and WHO grade based on TCGA RNA-seq dataset (Fig. 6a-f).

Relationships of RBBP8 with immune cells and immune checkpoints

The TIMER database was used to identify the relationship between RBBP8 expression and the six types of infiltrating immune cells in the tumor microenvironment. In GBM, there was a significant positive correlation between the expression of RBBP8 and the infiltration of dendritic cells. Notably, this positive correlation in LGG was present between all six immune cells (B cells, CD8+T cells, CD4+T cells, macrophages, neutrophils, and dendritic cells) and RBBP8 expression (Fig. 7a). Kaplan-Meier survival curves showed that patients in the RBBP8 low expression group had a significantly better prognosis than those in the RBBP8 high expression group in the setting of six immune cell infiltrates. However, in LGG, RBBP8 expression was closely associated with survival in the infiltration of dendritic cells (Fig. 7b). Finally, we explored the correlation between the mRNA expression levels of RBBP8 and genes coding immune checkpoints (i.e., PD-1 (PDCD1), PD-L1 (CD274), PD-L2 (PDCD1LG2), CTLA4, and (TIM-3) HAVCR2). The results showed that the expression level of RBBP8 was positively correlated with PD-1 in GBM (Fig.S2a-e), and RBBP8 was positively correlated with PD-1, PD-L1, PD-L2, CTLA4, and TIM-3 in LGG (Fig. S2f-j).

RBBP8-related signaling pathways in glioma

To explore the mechanism of RBBP8 in gliomas, we used GSEA to analyze two groups of samples with high and low RBBP8 expression. Analysis of TCGA data showed that the cell cycle P53 signaling pathway, homologous recombination, mismatch repair, leukocyte transendothelial migration, and antigen processing and presentation were the most significantly enriched signaling pathways (FDR < 0.25, $P < 0.05$, NES > 1.5) (Fig. 8). For a better understanding of RBBP8 functions, data from the CGGA were used to perform a GSEA analysis. The P53 signaling pathway, base excision repair, homologous

recombination, mismatch repair, protein export, and DNA replication (FDR < 0.25, P < 0.05, NES > 1.5) were the top six signaling pathways in the CGGA microarray dataset (Fig. S3). Meanwhile, cell cycle, the P53 signaling pathway, DNA replication, homologous recombination, mismatch repair, and antigen processing and presentation were the top six signaling pathways in the CGGA RNA-seq dataset (Fig. S4).

Meta-analysis of RBBP8 expression on the prognosis of glioma

Results were acquired from TCGA, CGGA, and GEO databases and used in a meta-analysis because no previous studies had revealed the relationship between high RBBP8 expression and overall survival among glioma patients. No significant heterogeneity between the two databases (I^2 = 0%, p = 0.98) was observed, and a fixed-effect model was applied. The pooled HR for the correlation between high RBBP8 expression and patient overall survival was 2.24 (95% CI: 1.37-3.65) (Fig. 9). Thus, we can conclude that RBBP8 high expression is an independent predictor of unfavorable overall survival in glioma patients.

Discussion

The high incidence rate and wide heterogeneity of glioma make it the most important disease among central nervous system tumors. Although scientists have been trying to find new treatments for gliomas, the results are still unsatisfactory. The emergence and development of sequencing technology has greatly promoted our understanding of the genetic and molecular characteristics of gliomas. The 2016 WHO classification of central nervous system tumors used histology combined with molecular parameters to define tumor entities for the first time (Louis et al. 2016). Although this classification facilitates clinical, experimental, and epidemiological research for gliomas, we still need to improve the prognosis of glioma patients.

RBBP8 plays an important role in a variety of tumors and is considered a candidate oncogene involved in cell cycle regulation (Yun and Hiom 2009; Bothmer et al. 2013; Kremer et al. 2015). However, the role of RBBP8 in gliomas has not yet been reported. In this study, we found that the mRNA and protein levels of RBBP8 in tumor samples were higher than those in normal tissues (Fig. 1). Furthermore, Kaplan-Meier analyses with data from TCGA, CGGA-seq, and CGGA-array datasets showed that glioma patients with low expression of RBBP8 had a better prognosis than those with high expression of RBBP8. Consistent with these findings, Zhang et al. (2020) showed that high expression of RBBP8 was related to poor prognosis in plasma cell myeloma. In addition, we used univariate and multivariate Cox regression analyses to identify RBBP8 as an independent prognostic indicator. Our correlation analyses showed that RBBP8 mRNA expression was related to age, sex, and IDHmutation status (Fig. 2). Interestingly, these clinical characteristics are closely related to the progression of malignant gliomas (Chen et al. 2017). Significantly, RBBP8 mRNA expression was positively correlated with WHO grade, indicating that RBBP8 might participate in glioma progression. Hence, we speculate that RBBP8 promotes the progression of malignant glioma. Moreover, a meta-analysis including eight datasets comprehensively evaluated the prognostic value of RBBP8. Collectively, these results suggest that RBBP8 can not only be used as a biomarker to predict the prognosis of glioma patients, but also is an oncogene involved in the malignant

progression of glioma. However, the role of RBBP8 in the malignant progression of tumors, and whether it can become an anti-tumor therapeutic target, remains to be explored.

Tumor immunotherapy is a new treatment that has shown good curative effects with many solid tumors (Odunsi 2017; Rodríguez-Cerdeira et al. 2017; Rolfo et al. 2017). Glioma cells are characterized by secreting inhibitory cytokines such as transforming growth factor-β and interleukin-10, and contain the cell surface inhibitor, PD-L1, as well as affecting the blood-brain barrier (Li et al. 2016; Gieryng et al. 2017). Multiple strategies to overcome immunosuppression and exploit anti-tumor immune responses have been utilized in glioma patients. In this study, we found that RBBP8 was positively correlated with infiltration of six immune cells in LGG (Fig. 7a). However, in GBM, RBBP8 was only positively associated with dendritic cell infiltration. These differing results reflect a huge variation in the immune microenvironment between LGG and GBM. The Kaplan-Meier analysis showed that patients with low infiltration levels of dendritic cells lived longer than those with high infiltration levels (Fig. 7b). In addition, RBBP8 was significantly associated with PDL2 expression, which may be used as a collaborative target.

RBBP8 is an important immune-related gene and may itself be used as a therapeutic target for glioma treatment. To further explore the function of RBBP8 in glioma, we performed a GSEA analysis (Subramanian et al. 2005). The results showed that cell cycle, P53 signaling pathway, homologous recombination, mismatch repair, leukocyte transendothelial migration, and antigen processing and presentation were the most enriched signaling pathways. The cell cycle is the most important physiological process regulating cell division and is controlled by the cell cycle checkpoint. An abnormal cell cycle often leads to cancer (Williams and Stoeber 2012). Interestingly, we found two signaling pathways related to immunity (leukocyte transendothelial migration, and antigen processing and presentation), which further indicates that RBBP8 has a strong correlation with immunity.

Although our study used extensive information from multiple databases to confirm the importance of RBBP8 in the prognosis of glioma, and its molecular function in the malignant progression of glioma, there are still some limitations to be addressed. Specifically, the number of normal samples used for control was far less than that of tumor samples, and the imbalance in sample size may have affected the results. Therefore, we examined balanced datasets of samples from two GEO databases; these results also showed that RBBP8 was highly expressed in tumor samples. The immune microenvironment of glioma is formed by complex cell-cell interactions. Our study only recognized the role of RBBP8 in glioma immunity from the perspective of immune cell infiltration and immune checkpoints, and more evidence is needed to support its role. Nevertheless, this study provides direction for the combined immunotherapy of gliomas.

Conclusion

This study is the first to partial reveal the relationship between the aberrant expression of RBBP8 or its methylation, and the clinical molecular characteristics of glioma patients. This helps explain that disorders on multiple etiologic levels of glioma are involved in its pathological progression. More

importantly, this is the first time that the increased mRNA expression of RBBP8 can promote the infiltration of six different immune cells in the glioma microenvironment especially in low-grade glioma. This can broaden our understanding of the molecular function of RBBP8 as an important oncogene. The highlight of this study is the discovery of potential clinical application value for research into, and development of, new inhibitors targeting RBBP8 for anti-tumor immunotherapy in glioma.

Declarations

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Availability of data and material Data will be made available on reasonable request.

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

Ethics approval (include appropriate approvals or waivers)

Consent to participate (include appropriate statements)

Consent for publication (include appropriate statements)

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Figures

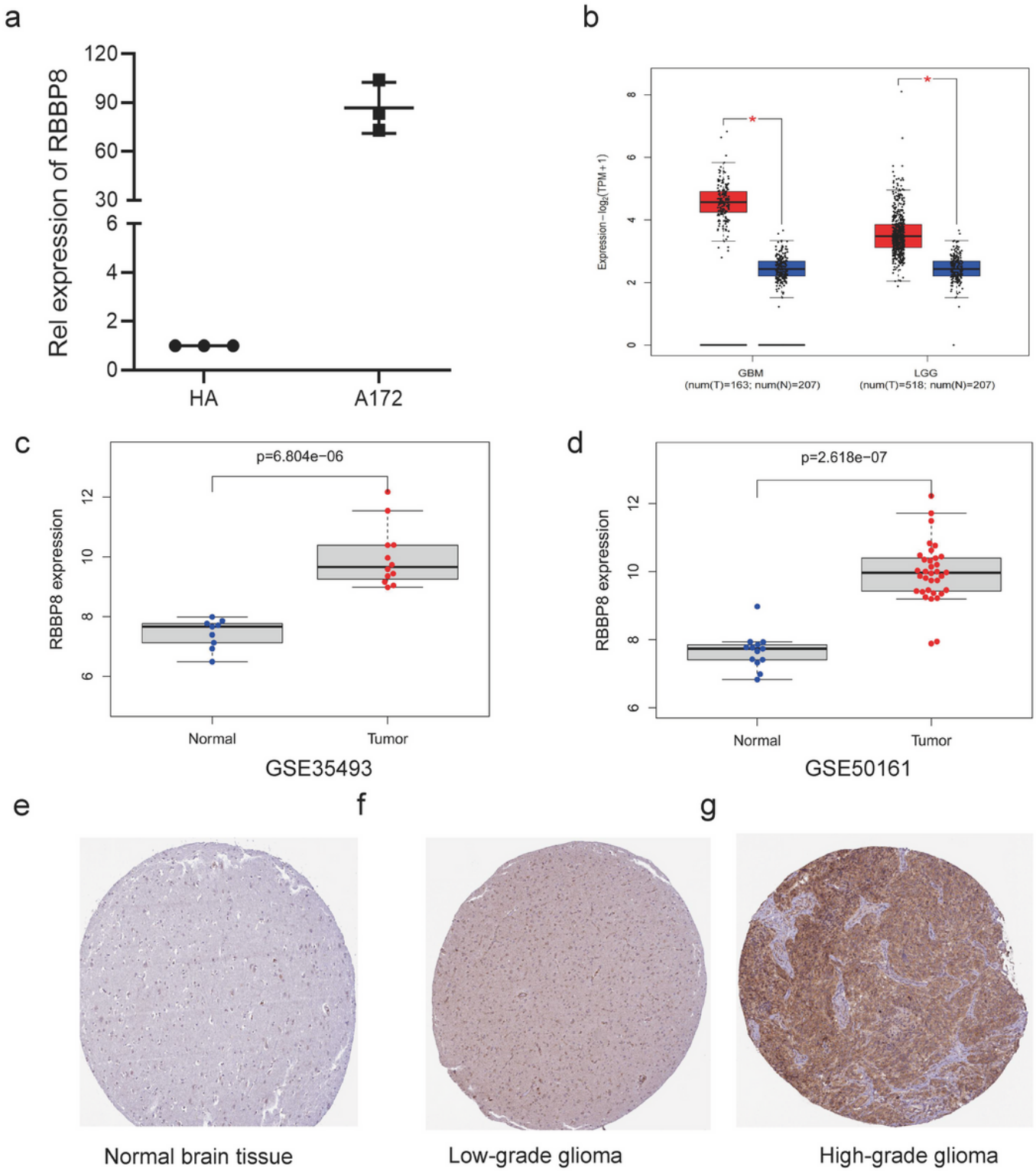

Figure 1

The expression of RBBP8 between tumor and non-tumor. (a) The expression level of RBBP8 in human glioma cells (A172) and human astrocytes (HA). (b) RBBP8 was significantly upregulated in GBM and LGG. (c) GSE35493. (d) GSE50161. (e-g) The expression of RBBP8 in protein level in normal brain tissue, low-grade glioma and high-grade glioma.

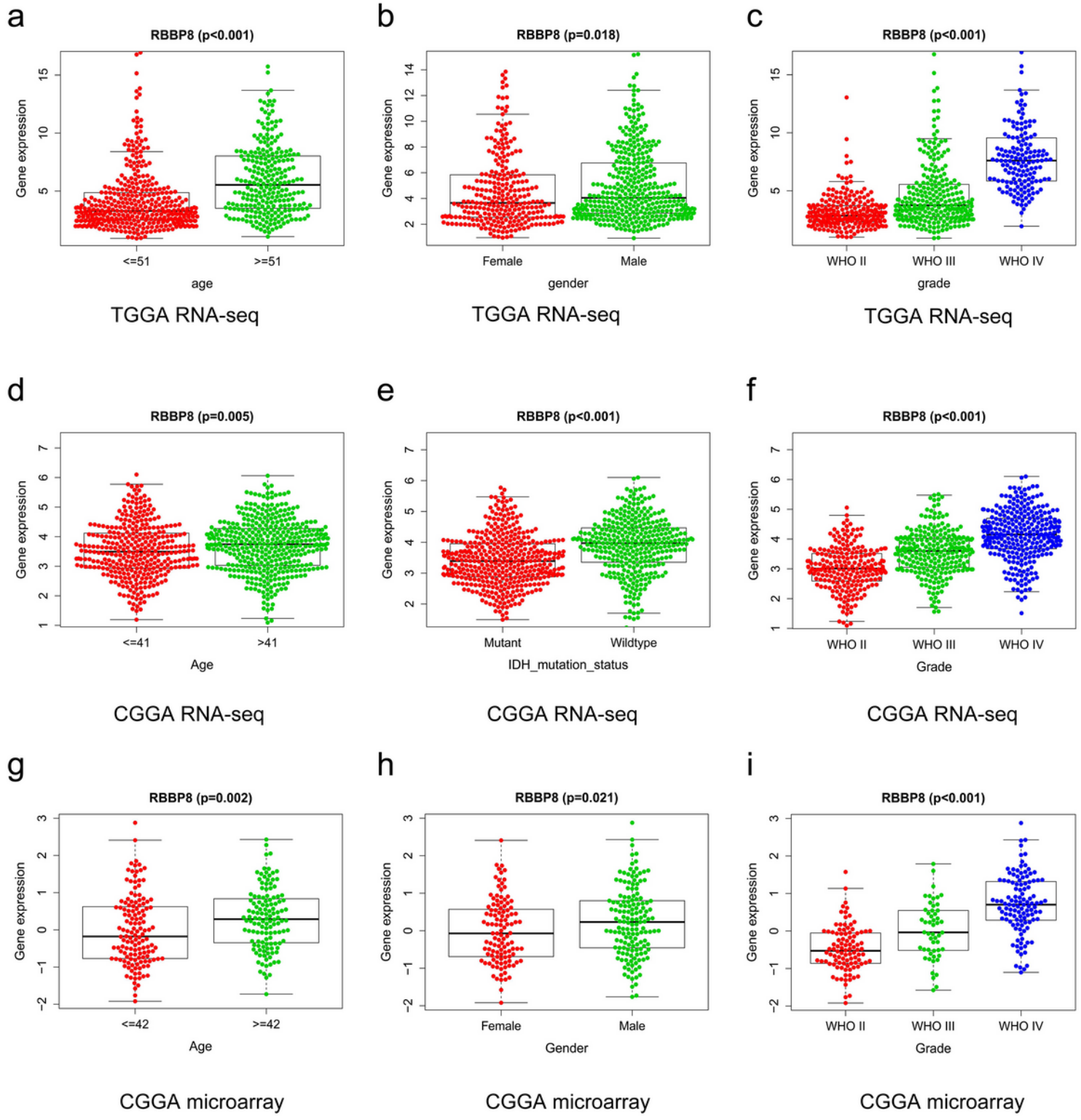

Figure 2

Correlation analysis between RBBP8 expression and clinical features of glioma patients. TCGA RNA-seq: (a) age, (b) gender, (c) WHO grade; CGGA RNA-seq: (d) age, (e) IDH mutations, (f) WHO grade; CGGA microarray: (g) age, (h) gender, (i) WHO grade.

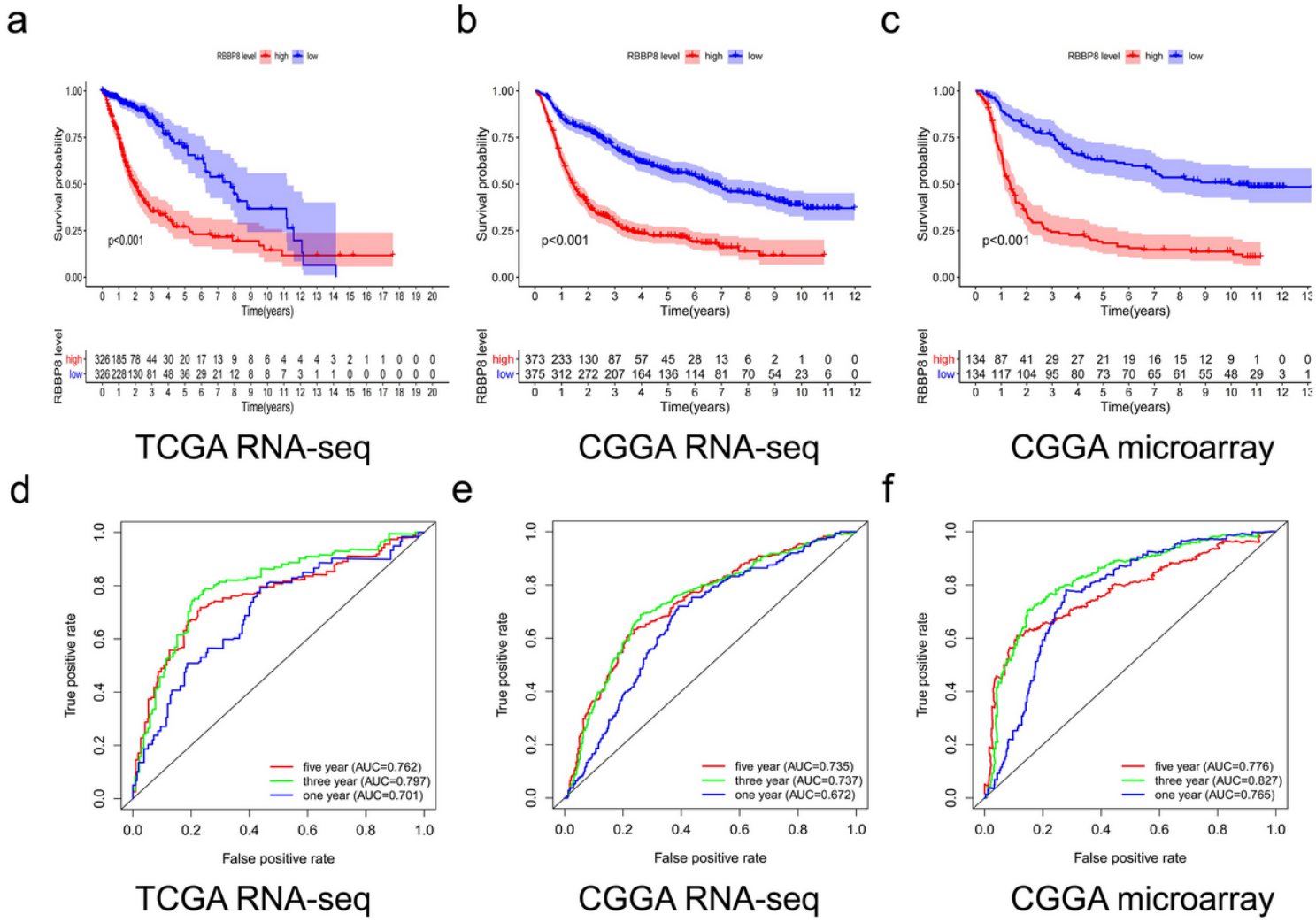

Figure 3

Survival analysis of RBBP8 using. Kaplan-Meier survival curve of RBBP8, (a) TCGA RNA-seq, (b) CGGA RNA-seq, (c) CGGA microarray; ROC curve analysis of RBBP8, (d) TCGA RNA-seq, (e) CGGA RNA-seq, (f) CGGA microarray

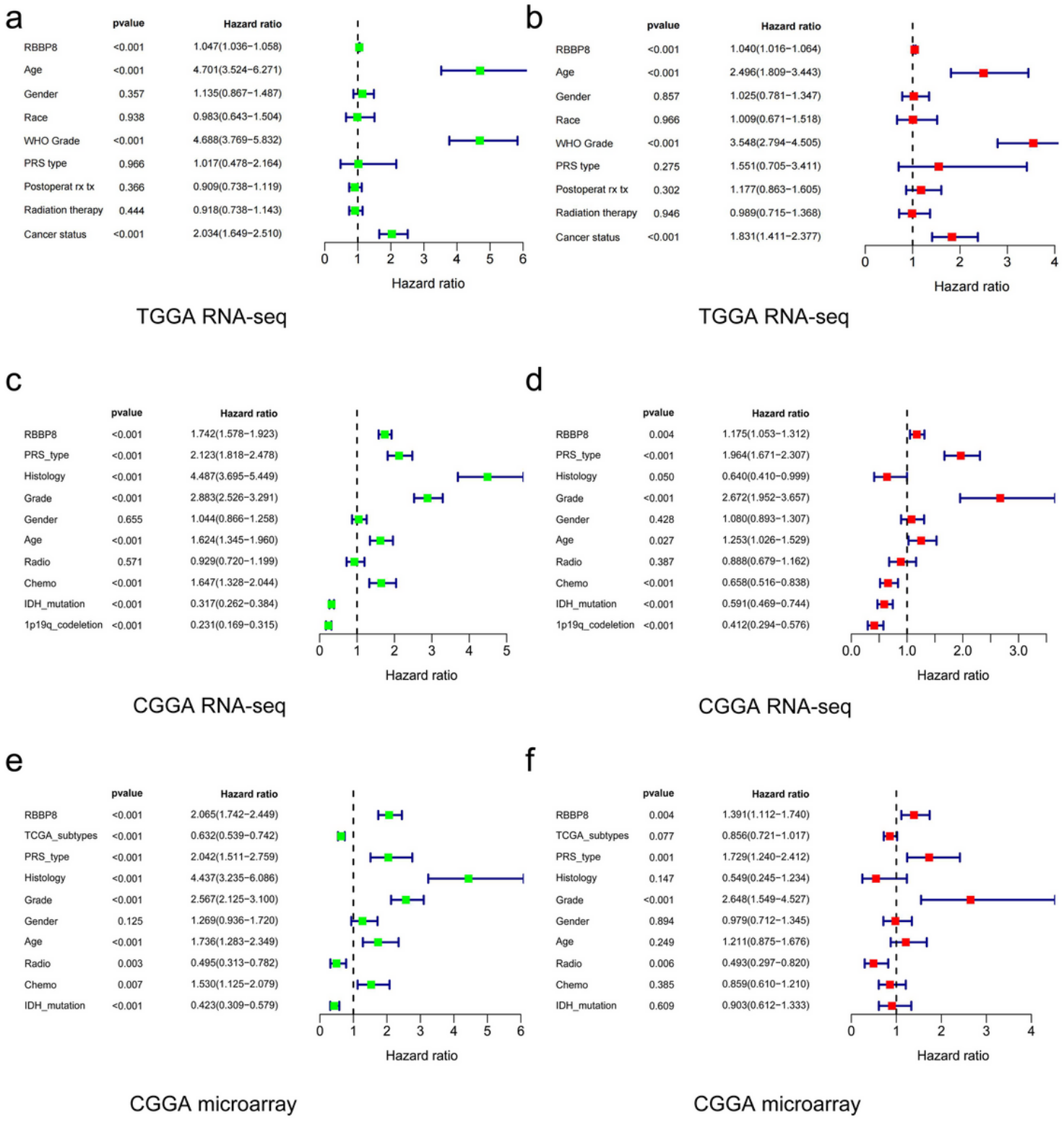

Figure 4

RBBP8 expression can be used as an independent marker of the prognosis of glioma. Univariate analysis (a) of RBBP8 and multivariate analysis (b) of RBBP8 based on TCGA RNA-seq; Univariate analysis (d) of RBBP8 and multivariate analysis (e) of RBBP8 based on CGGA RNA-seq; Univariate analysis (f) of RBBP8 and multivariate analysis (g) of RBBP8 based on TCGA microarray.

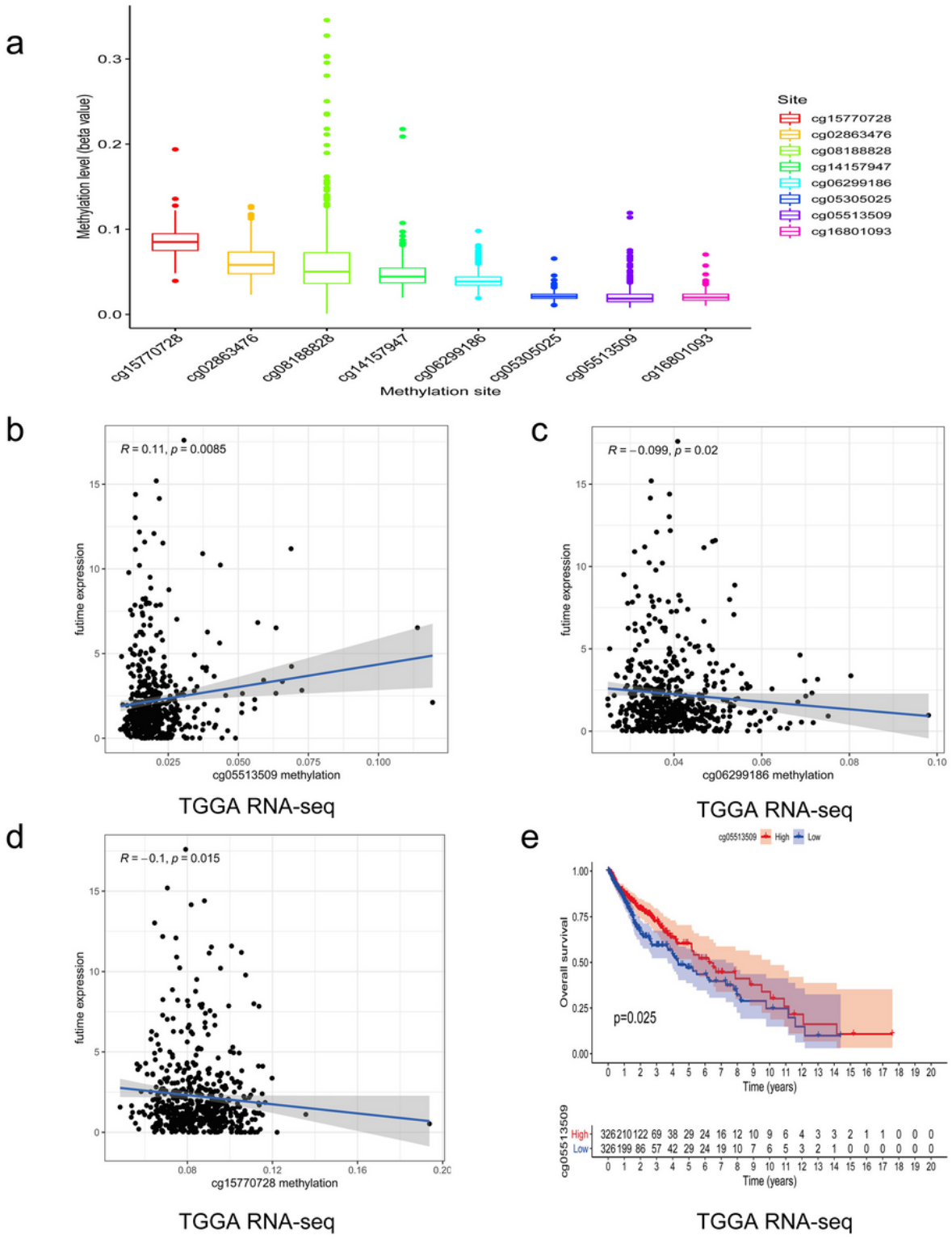

Figure 5

The expression and methylation of RBBP8 in glioma. (a) The methylation sites of RBBP8 in TCGA database; The expression of RBBP8 was positively regulated RBBP8 CpG sites methylation cg05513509 (b) and negatively regulated by RBBP8 CpG sites methylation cg06299186 (c) and cg15770728 (d); Kaplan-Meier survival curve of RBBP8 CpG sites methylation cg05513509.

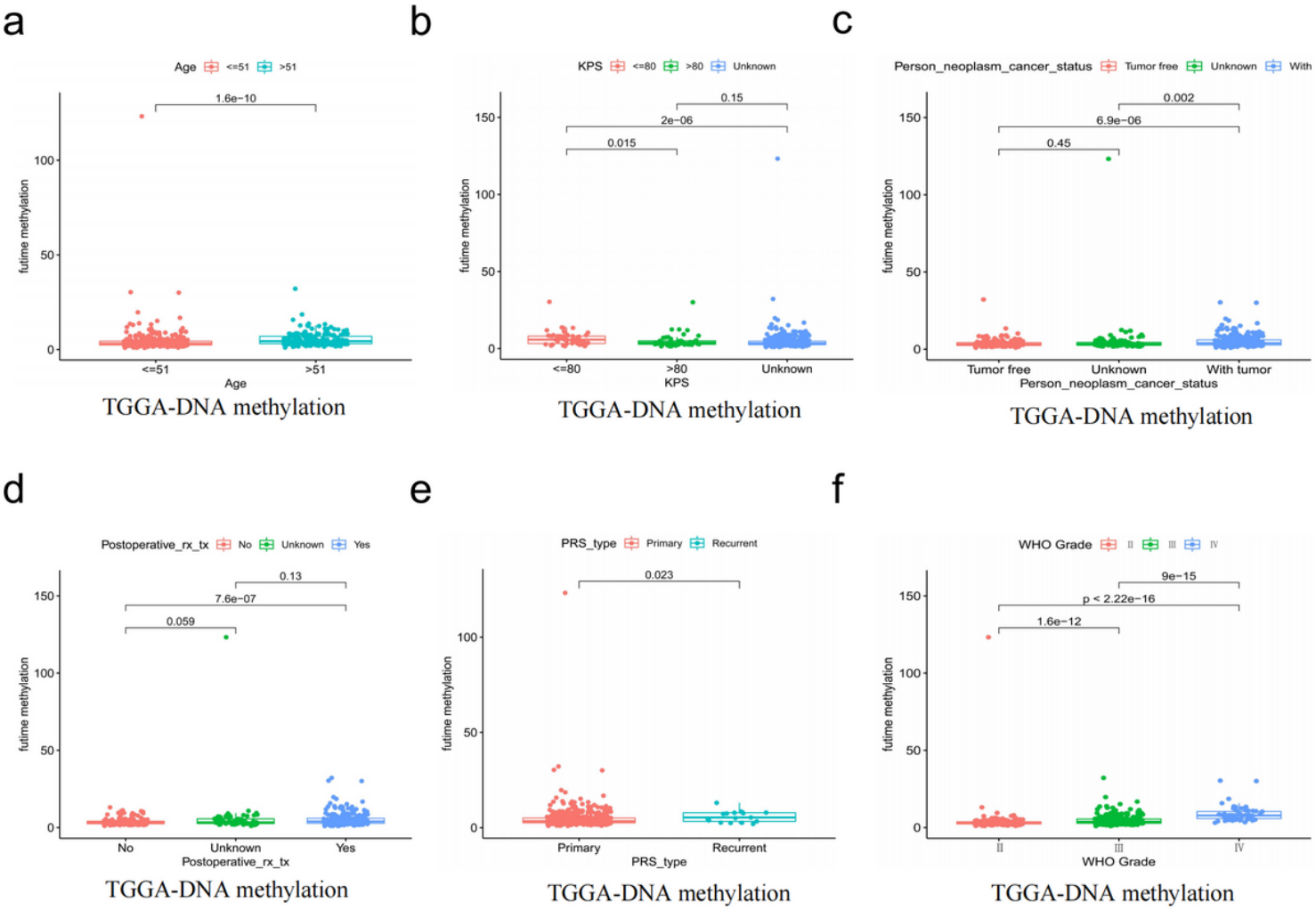

Figure 6

Correlation analysis between RBBP8 methylation level and clinical features of glioma patients, including Age, KPS, neoplasm status, postoperative_rx_tx, PRS type and WHO grade.

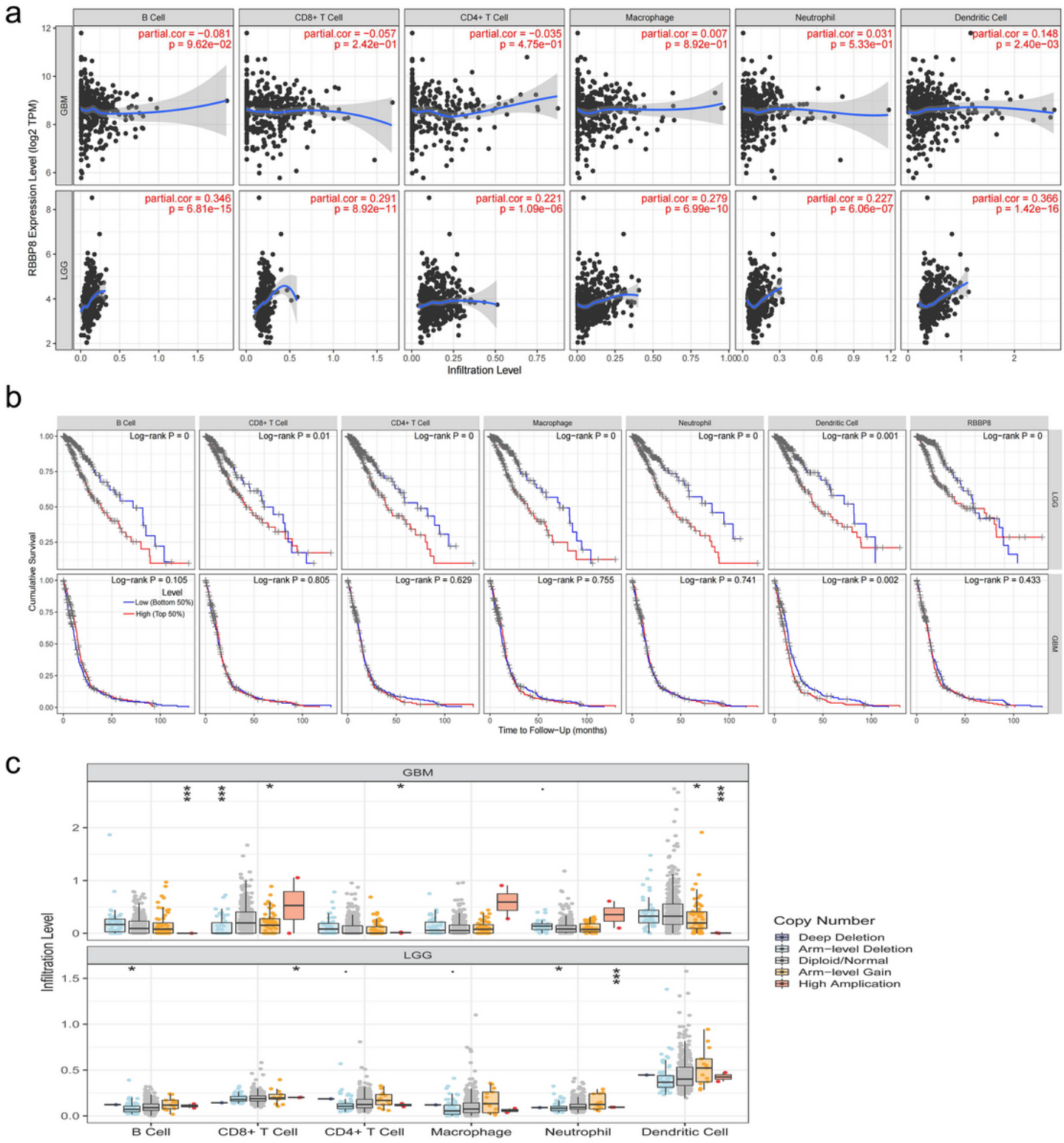

Figure 7

Correlation between RBBP8 expression and immune infiltration. (a) Relationship between RBBP8 expression and six immune cells infiltration in GBM and LGG, including B cell, CD8+ T CELL, CD4+ T cell, Macrophage, Neutrophil, and Dendritic cell; (b) Kaplan-Meier survival analysis of RBBP8 and six immune cells in GBM and LGG; (c) The relationship between somatic copy number alterations of RBBP8 and immune cell infiltration in GBM and LGG

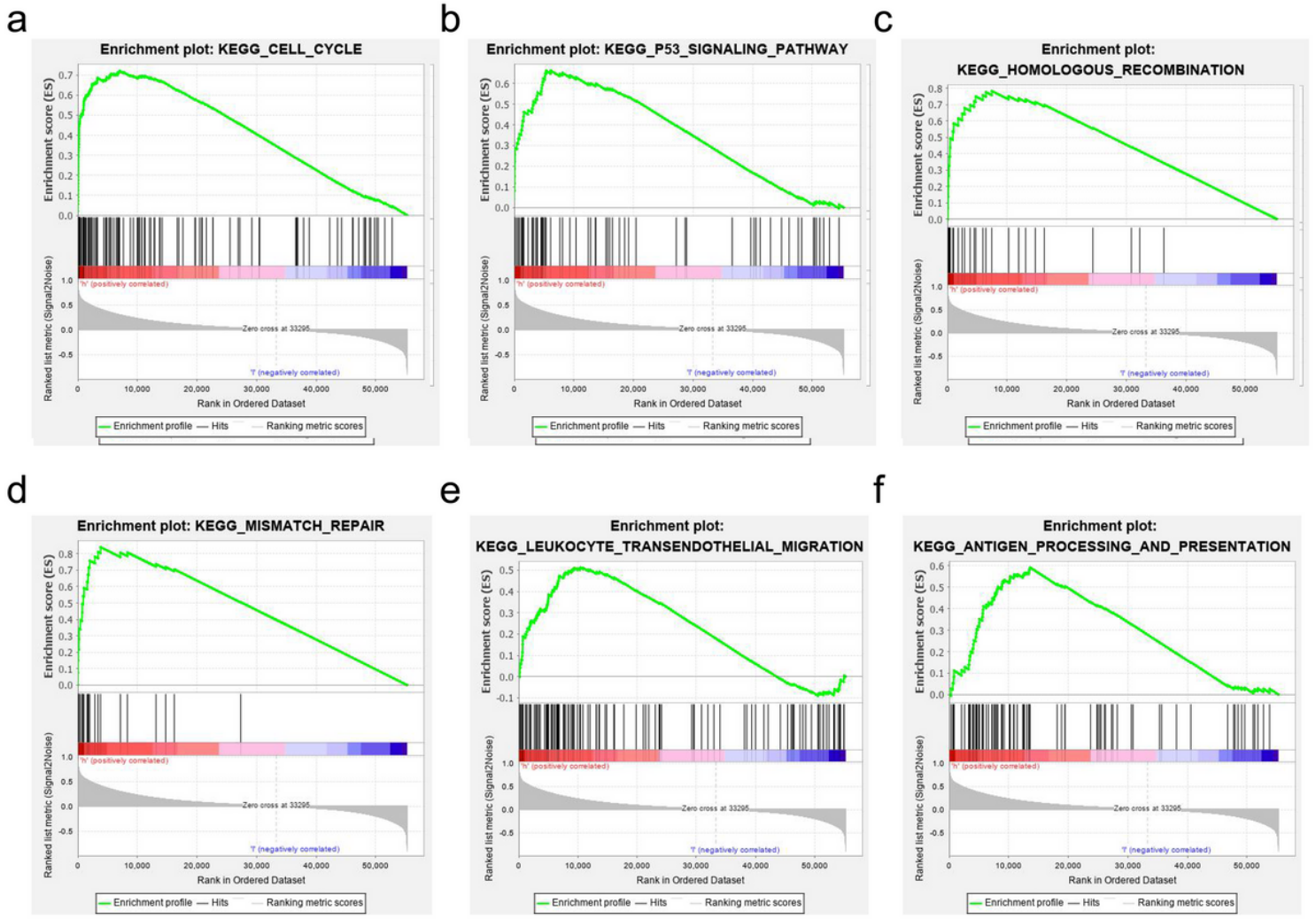

Figure 8

GSEA analysis of RBBP8 in glioma using TCGA RNA-Seq dataset. (a) cell cycle signaling pathway, (b) P53 signaling pathway, (c) homologous recombination, (d) mismatch repair, (e) leukocyte transendothelial migration, (f) antigen processing and presentation.

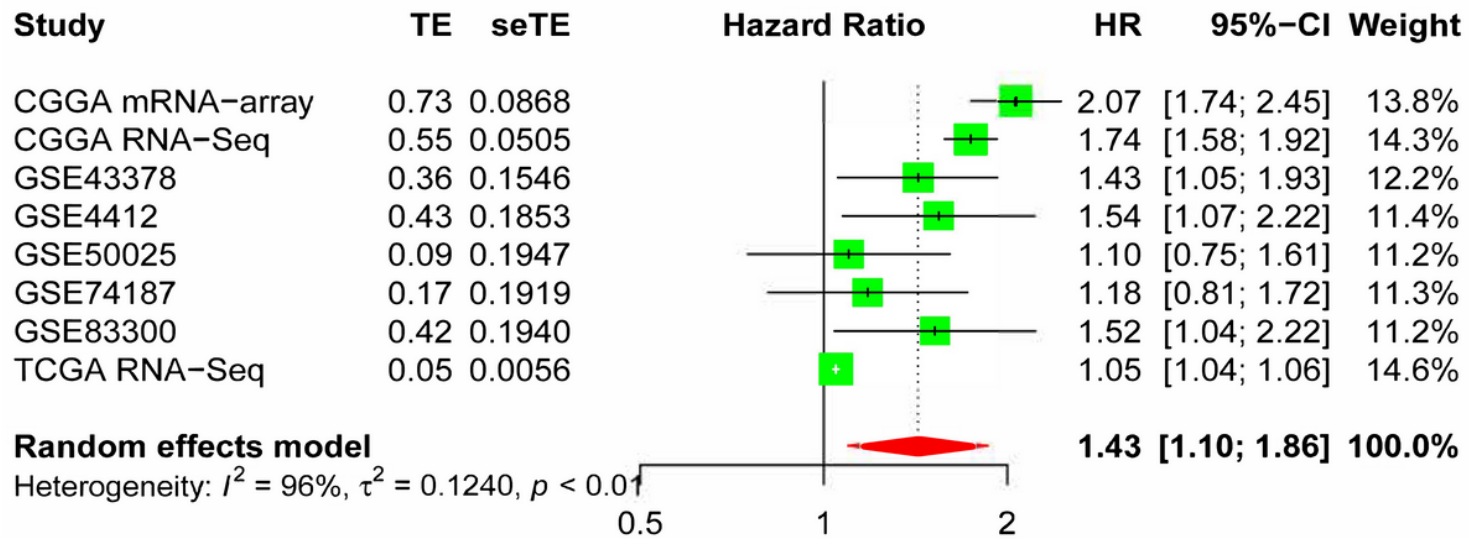

Figure 9

Forest plot of high RBBP8 expression with poor OS in glioma patients from eight datasets.

Supplementary Files

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