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ORIGINAL ARTICLE

CANCER INNOVATION

An integrated analysis of *C5AR2* related to malignant properties and immune infiltration of gliomas

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Abstract

Background: *C5AR2* is recognized as a proinflammatory molecule and activates the inflammatory response in multiple disorders. However, little has been reported on *C5AR2* in glioma. This study sought to explore its expression, biological function, and association with clinical pathological indicators, prognosis, and immune infiltration levels in glioma through glioma cohorts.

Methods: A cohort of 657 patients was screened from the Chinese Glioma Genome Atlas (CGGA). χ^2 test was performed to calculate the difference of classified variables. Cox proportional hazard regression modeling was used to identify independent prognostic indicators of glioma patients. A survival plot was generated by the Kaplan–Meier method. The immune cell infiltration score of glioma patients was calculated by TIMER algorithm.

Results: We observed that high expression of *C5AR2* was strongly associated with malignant clinical indicators in 657 patients with glioma, and patients with high *C5AR2* expression had worse prognoses. Multivariate Cox analysis showed that *C5AR2* could be a new independent prognostic indicator for glioma patients. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis revealed that *C5AR2* overexpression correlated with multiple inflammatory and immune biological processes. Additionally, high *C5AR2* expression was strongly associated with higher abundance and marker gene expression of multiple tumor immune cells in low-grade glioma. Finally, a model was constructed to improve the prognostic evaluation of glioma patients.

Conclusions: The *C5AR2* gene is highly expressed in gliomas and is significantly associated with clinical indicators of malignant progression in glioma patients. In glioma, patients with high *C5AR2* expression displayed a

Abbreviations: BP, biological process; CC, cellular component; CGGA, Chinese Glioma Genome Atlas; CNS, central nervous system; DCs, dendritic cells; GBM, glioblastoma; LGG, low grade glioma; MF, molecular function; OS, overall survival; ROC, receiver operating characteristic; SD, standard deviation.

The authors Chengying Huang, Ouwen Qiu, and Chaofu Mao are co-first authors on this work and contributed equally.

worse outcome. In glioma tissues, the expression level of *C5AR2* highly correlated with the abundance of tumor immune cell infiltration. Additionally, GO and KEGG enrichment analysis revealed that *C5AR2* expression may be involved in a variety of immune and inflammatory biological processes.

KEYWORDS
biomarker, glioma, immunotherapy, microenvironment, prognosis

1 | INTRODUCTION

Human brain gliomas are the most frequent types of central nervous system (CNS) tumors, and the most dangerous primary malignant tumors [1]. Based on the epidemiological data on brain tumors in 2019, approximately 106,207 new cases of glioma are diagnosed and there are 59,120 deaths due to glioma each year in China [2]. Although glioma treatment has improved, the overall survival (OS) of glioma patients is still not satisfactory [3, 4], and the OS of glioblastoma (GBM) patients is less than 14 months [5]. Thus, it is especially important to find new molecular targets and prognostic indicators for glioma patients to improve the outcome of glioma.

The deepening of research into the pathogenesis of glioma has revealed that the tumor immune microenvironment plays a key role in immune escape. At present, it is generally believed that CNS tumors have also been infiltrated by peripheral lymphocytes, which can have a therapeutic effect on existing CNS tumors [6]. For instance, glioma, especially glioblastoma, is thought to secrete many factors, such as TGF-β and IDO molecules, which could suppress the immune response in the tumor microenvironment and contribute to treatment failures. Meanwhile, Treg cell polarization is a key process leading to malignant progression and an unfavorable prognosis of glioma. Hence, the tumor immune microenvironment is expected to be a new potential target for solid tumors [7]. Recently, immunosuppressants have achieved remarkable success in melanoma, and clinicians have begun to test the use of immunosuppressants with other types of solid tumors [8]. Unfortunately, the response rates of glioma to immunosuppressants are not high [9]. Excessive use of immunosuppressants not only has no effect on some glioma patients, but also increases some common complications. Therefore, precise assessment of the immune status in the tumor microenvironment is particularly important for screening suitable patients for immunotherapy [10]. Although several immune-related markers have been identified for screening patients, these markers do not function well in a clinical setting [11–17]. Moreover, as opposed to other types of tumors, no reliable markers currently have been found for glioma. Thus, discovering new biomarkers for assessment and therapy is key.

Previous studies have confirmed that complement active peptide C5a is a proinflammatory-related molecule, which can interact with its corresponding receptors, such as C5AR1 and C5AR2 (GPR77 and G5L2) [18, 19]. In certain inflammatory diseases, C5a can lead to disease occurrence by increasing inflammation. C5AR2, the C5a receptor, was first reported by Ohno et al. in 2000 [20]. C5AR2 leads to NLRP3 inflammasome activation by amplifying protein kinase R expression [18]. The NLRP3 inflammasome initiates innate immunity and promotes an inflammatory response. However, the expression pattern and biological significance of *C5AR2* in glioma have not been reported.

Thus, our study aims to explore its expression, biological function, and association with clinical pathological indicators and outcome of patients. We then focus on the relationship between the *C5AR2* level and the abundance of multiple immune cells. Finally, we construct a predictive model based on vital prognostic markers in glioma. Through the above in-depth analysis, we assess the potential of *C5AR2* as a therapeutic target and prognostic biomarker, and provide a reliable basis for individualized treatment of patients.

2 | MATERIALS AND METHODS

2.1 | Glioma patient cohort

The data used in this study were obtained from the CGGA database (http://www.cgga.org.cn, Data set ID: mRNA-seq_693) on January 1, 2022. Patients without complete follow-up data were excluded. Finally, this study enrolled 657 glioma patients. The demographics of the 657 enrolled glioma patients are summarized in Table S1. In addition, the immune score of each patient was calculated by the TIMER method based on the sequencing data from each tumor. The Ethics Committee of Nanfang Hospital supplied ethical approval for this study.

2.2 | GEPIA database analysis

GEPIA (http://gepia.cancer-pku.cn/) provides online analysis of TCGA database. We analyzed the difference in *C5AR2* mRNA expression between glioma samples and normal brain tissue samples from TCGA database through the GEPIA website. Additionally, to confirm the results of the CGGA cohort, the relationship between *C5AR2* expression and OS and disease-free survival (DFS) in glioma patients was analyzed using GEPIA in 673 glioma patients.

2.3 | Tumor immune estimation resource (TIMER) analysis

TIMER (https://cistrome.shinyapps.io/timer/) is an online visual analysis tool for comprehensive analysis of tumor-infiltrating immune cells in different types of cancer using the data from TCGA database. We used this tool to assess the association between the *C5AR2* level and the infiltration abundance of immune cells including B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells (DCs) in different tumors. Moreover, the correlation coefficient between *C5AR2* expression and immune cell marker genes was obtained by the Spearman method.

2.4 | Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

First, using the co-expression score, we extracted the first 1000 genes co-expressed with *C5AR2*. GO and KEGG pathway analyses were conducted using the DAVID database (http://david.abcc.ncifcrf.gov/). The top 15 KEGG and GO terms were extracted to draw the figure. The GeneMANIA database supplies information on physical interactions, co-expression, predicted proteins, colocalization, genetic interactions, and shared protein domains. We constructed a network of 20 potential proteins interacting with *C5AR2* using the GeneMANIA database. In addition, a String network of the *C5AR2* protein was developed using the String database.

2.5 | Immunohistochemistry (IHC) and western blot analysis

IHC assays were carried out on tissue samples to detect C5AR2 expression. The western blot method was used to detect C5AR2 expression of cell lines. Western blot was performed as described [21]. The detailed steps of the IHC and western blot experiments are detailed in Supplementary Materials section. The primary antibody for the IHC assay was C5AR2 antibody (catalog no., TD2762S; dilution, 1:400). The primary antibodies for the western blot were C5AR2 antibody (catalog no., TD2762S; dilution, 1:2000) and GAPDH antibody (catalog no., 5174; dilution, 1:10,000).

2.6 | Statistical analysis

Statistical analyses were performed by SPSS (Version 23.0) and R software (Version 3.6.1). The statistical significance between diverse groups was determined using t-test. The Chi-squared test was performed to calculate the difference of classified variables. Independent prognostic factors were identified by univariate and multivariate Cox regression analyses. Survival plot was performed by the Kaplan–Meier method. A time-dependent receiver operating characteristic (ROC) curve was constructed using R software. All tests were two-sided, and p-values < 0.05 were considered to be statistically significant.

3 | RESULTS

3.1 | High expression of *C5AR2* impacts the prognosis of patients with glioma

First, the differential *C5AR2* expression between normal brain tissue and glioma was compared based on mRNA sequencing samples from TCGA. We found elevated mRNA expression levels of *C5AR2* in glioma (Figure S1). Additionally, the IHC results revealed that C5AR2 overexpression was observed in glioma compared with normal brain tissue (Figure 1a). The overexpression of C5AR2 in glioma tissues was concordant with the results in glioma cell lines (Figure 1b).

To understand the role of *C5AR2* in glioma patients, the relationship between the *C5AR2* level and clinicopathological indicators was statistically analyzed in a cohort of 657 glioma patients. Based on the median *C5AR2* expression value, all patients were classified into either the low or high *C5AR2* expression group. Chi-squared test showed that elevated *C5AR2* expression was associated with the WHO grade, histopathology, IDH, 1p/19q status, tumor recurrence, and immune score, but not with gender, radiotherapy, or chemotherapy (Table 1). The above findings indicate that *C5AR2* overexpression is a potential indicator of poor prognosis. As expected, a Kaplan–Meier curve also confirmed that high *C5AR2* expression predicted shorter OS (Figure S2A).

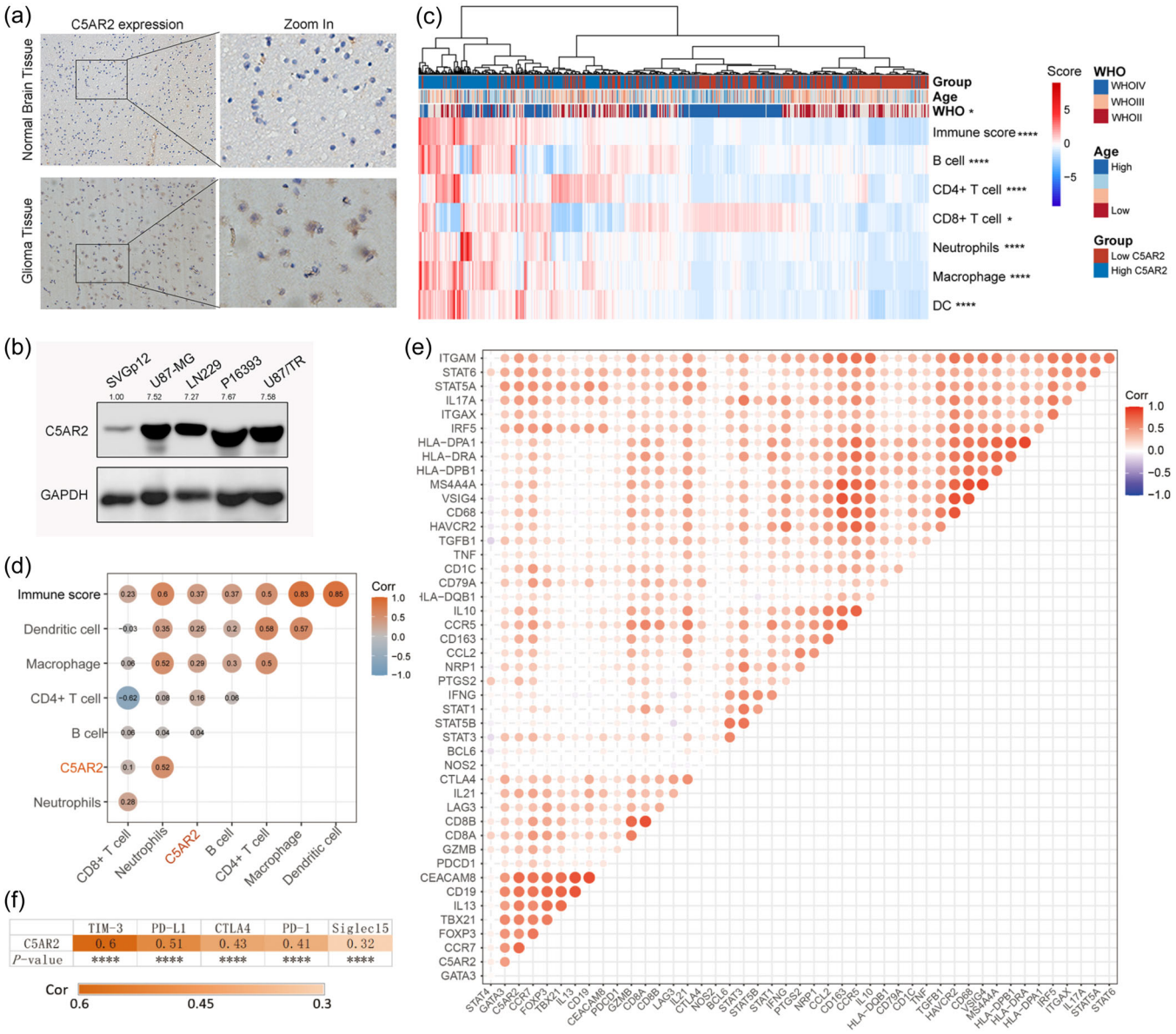

FIGURE 1 *C5AR2* expression was upregulated in glioma and was significantly associated with the infiltration levels of immune cells. (a) C5AR2 protein was enhanced in glioma compared with normal brain tissues by immunohistochemistry. (b) *C5AR2* was also upregulated in glioma cell lines (U87-MG, LN229, P16393, and U87/TR) compared with normal astroglia cells (SVGP12). (c and d) Heatmap revealing the correlation between *C5AR2* expression and infiltrating immune cells. (e) This heatmap presents the correlation between *C5AR2* expression and immune cell marker genes. (f) *C5AR2* expression is strongly associated with common immune checkpoints including TIM-3, PD-L1, CTLA-4, PD-1, and Siglec-15. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

3.2 | *C5AR2* overexpression is associated with elevated immune infiltration abundance in glioma

After exploring the characteristics of *C5AR2* in glioma, we further assessed the distribution of total immune infiltration levels of the diverse glioma patterns. As shown in Figure S2B–E, THE immune score was higher in glioma for HGG, wildtype of IDH, noncodeletion status of 1p/19q, and recurrence. The Kaplan–Meier curve also indicated that elevated immune score was related to shorter OS (Figure S2F). Previous studies have reported that *C5AR2* is a powerful proinflammatory mediator [18]. We further investigated whether *C5AR2* expression could reflect the overall immune infiltration levels. Spearman correlation analysis between *C5AR2* and immune score was performed and revealed a strong positive correlation ($r = 0.54$, $p < 0.0001$, Figure S2G). We assessed the relevance of *C5AR2* expression and immune infiltration levels in glioma through the abundance of

TABLE 1 The correlation between *C5AR2* expression level and clinicopathological features

Features	*C5AR2* expression level		χ^2	*p*-value
	Low expression	High expression		
Age				
≥60	39	41	0.015	0.903
<60	285	291		
NA	1	0		
Gender				
Male	167	177	2.012	0.132
Female	158	155		
WHO grade				
WHO II	83	89	7.819	0.020
WHO III	139	109		
WHO IV	103	134		
Histopathology				
O	39	19	43.090	<0.001
OA	4	5		
A	40	65		
AO	61	18		
AOA	12	8		
AA	66	83		
GBM	103	134		
IDH mutation				
Mutation	186	147	10.363	0.001
Wildtype	118	158		
NA	21	27		
1p/19q codeletion				
Codel	106	31	68.964	<0.001
Non-codel	168	286		
NA	51	15		
Radiotherapy				
Yes	240	261	2.069	0.150
No	72	59		
NA	13	12		
Chemotherapy				
Yes	237	243	0.020	0.887
No	76	80		
NA	12	9		
Recurrence				
Yes	191	141	4.450	0.035
No	213	112		
Immune score				
High score	90	238	127.150	<0.001
Low score	235	94		

Abbreviations: A, astrocytoma; AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; GBM, glioblastoma; NA, not appliable; O, oligodendroglioma; OA, oligoastrocytoma.

glioma-infiltrating immune cells. The results revealed that expression of *C5AR2* was positively associated with the levels of infiltrating B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and DCs (Figure 1c,d). These findings strongly show that *C5AR2* is significant in immune infiltration in glioma.

To better verify the correlation between *C5AR2* and different infiltrating immune cells, we continued to focus on *C5AR2* expression and immune gene markers of diverse immune cells. Figure 1e shows that the expression of *C5AR2* correlates with marker genes of the different immune cells in glioma, such as Treg cells, tumor-associated macrophages (TAMs), and Th2 cells. Treg cells play a significant role in glioma to produce the immunosuppressive microenvironment. These results show that *C5AR2* might influence the regulation of macrophages, TAM infiltration, and immune escape in glioma.

In addition, *C5AR2* was positively related to immune checkpoints involving molecules including TIM-3, PD-L1, CTLA-4, PD-1, and Siglec-15 in glioma (Figure 1f).

3.3 | *C5AR2* is involved in biological processes

To further validate the potential functions of *C5AR2*, we used DAVID to perform gene set enrichment analysis for the top 1000 genes co-expressed with *C5AR2*. Figure 2a,b shows the top 15 KEGG pathway enrichment and GO terms, respectively. *C5AR2* was significantly involved in activation of the cytokine–cytokine receptor interaction, NF-KB signaling pathway, TNF signaling pathway, inflammatory bowel disease, and intestinal immune network for IgA production. GO analysis indicated that *C5AR2* is involved in inflammatory and immune-related biological processes (BP), cellular components (CC), and molecular functions (MF), in particular, cytokine activity, external side of plasma membrane, inflammatory response, immune response, immune response, and T cell stimulation.

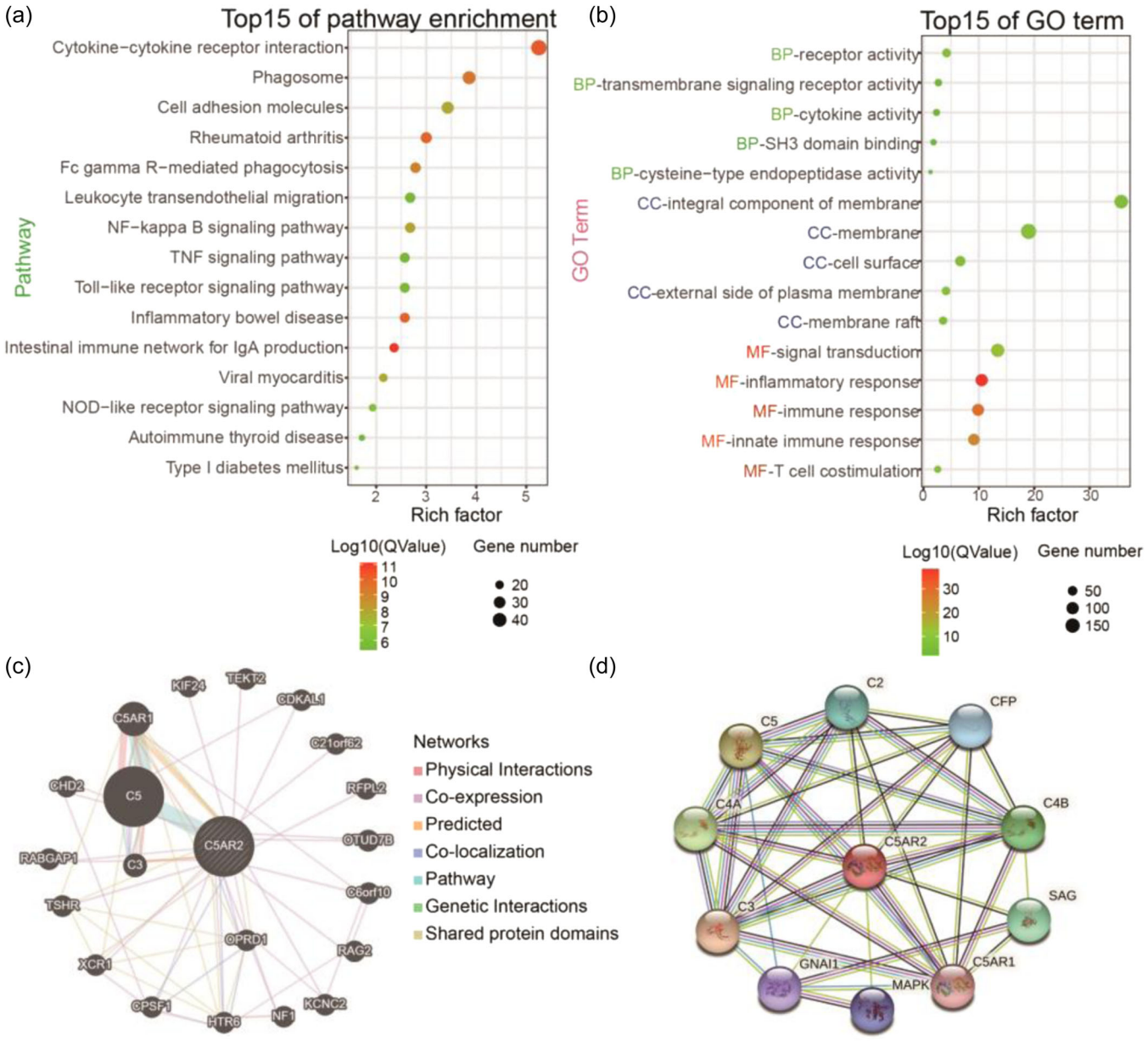

FIGURE 2 The functional annotation of the *C5AR2* gene was related to inflammation and immune biological processes. (a) The top 15 KEGG pathway enrichments. (b) The top 15 GO enrichments. (c) Gene network analysis between the *C5AR2* gene and 20 predicted associated genes using GeneMANIA. (d) The network showing the known or predicted proteins that interact with C5AR2 protein. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes

This also verifies that *C5AR2* is crucial in the regulation of the inflammatory and immune response.

To gain a comprehensive view, we performed protein–protein interaction (PPI) analysis through the GeneMANIA and String databases. Of the 20 proteins, 13.51% proteins shared similar co-expression, 6.35% were predicted, and 67.64% possessed physical interaction characteristics. Figure 2c shows the shared protein domains, colocalization, and genetic interactions. Figure 2d shows the known and predicted protein–protein interactions.

3.4 | Construction of nomogram

To construct a prognostic model predicting the outcome of patients with glioma, we used univariate and multivariate Cox regression analyses to verify the independent prognostic factors. As shown in Table 2, eight factors (age, WHO grade, histopathology, IDH, 1p/19q, recurrence, *C5AR2* expression, and immune score) were significant risk factors for survival in glioma, seven of which were further verified as independent prognostic factors through multivariate analysis.

TABLE 2 Univariate and multivariate analysis in patients with glioma

	Univariate analysis			Multivariate analysis		
Features	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
Age	1.03	1.01–1.04	<0.001	1.01	1.01–1.02	0.002
Gender	0.94	0.77–1.15	0.560			
WHO grade	2.67	2.30–3.09	<0.001	1.21	0.79–1.87	0.382
Histopathology	1.58	1.47–1.69	<0.001	1.29	1.05–1.57	0.013
IDH mutation	0.32	0.26–0.40	<0.001	0.62	0.47–0.81	<0.001
1p/19q codeletion	0.27	0.19–0.37	<0.001	0.54	0.34–0.87	0.001
Radiotherapy	1.24	0.95–1.62	0.110			
Chemotherapy	1.24	0.98–1.59	0.081			
Recurrence	2.18	1.79–2.67	<0.001	2.13	1.71–2.66	<0.001
C5AR2 level	1.07	1.03–1.12	0.003	0.67	0.53–0.85	0.001
Immune score	1.88	1.54–2.30	<0.001	1.42	1.10–1.84	0.008

Abbreviations: HR, hazard ratio; CI, confidence interval.

Next, we constructed a prognostic nomogram for predicting the patient's survival using age, histopathology, IDH, 1p/19q, recurrence, and immune score (Figure 3a). Calibration curves indicated a good match for predicting the 1-, 3-, and 5-year survival between the nomogram-predicted probability (solid line) and the ideal reference line (dashed line) (Figure 3b–d). Then we evaluated the predictive discrimination of this nomogram using the C-index, which quantifies the level of agreement between the nomogram-derived possibilities and the actual outcome. The C-index of this prognostic nomogram reached 0.785. A time-dependent ROC curve was applied to appraise the performance of this nomogram (Figure 3e). The area under the curve (AUC) of the 1-, 3-, and 5-year survival reached 0.849, 0.859, and 0.860, respectively.

3.5 | External validation of *C5AR2* in TCGA

First, to further validate the prognostic value of *C5AR2* expression, we used mRNA sequencing glioma samples from TCGA database. Kaplan–Meier curves showed comparable results, that higher *C5AR2* was associated with shorter OS and disease-free survival (Figure 4a,b).

Second, the relationship between *C5AR2* and infiltrating immune cells in different glioma subtypes was also verified. *C5AR2* was associated with B cells ($r = -0.008$, $p = 0.867$; $r = 0.504$, $p = 3.59 \times 10^{-32}$), $CD8^+$ T cells ($r = -0.038$, $p = 0.443$; $r = 0.156$, $p = 6.13 \times 10^{-4}$), $CD4^+$ T cells ($r = -0.091$, $p = 0.063$; $r = 0.542$, $p = 1.28 \times 10^{-37}$), macrophages ($r = -0.08$, $p = 0.103$; $r = 0.471$, $p = 1.85 \times 10^{-27}$), neutrophils ($r = -0.161$, $p = 9.59 \times 10^{-4}$; $r = 0.605$, $p = 8.67 \times 10^{-49}$), and dendritic cells ($r = 0.014$, $p = 0.774$; $r = 0.559$, $p = 2.0 \times 10^{-40}$) in GBM and low grade glioma (LGG), respectively (Figure 4c). Compared with GBM, the relationship between *C5AR2* and tumor immune cell infiltration was stronger in low grade gliomas. These results suggest that *C5AR2* could be an immune-related biomarker to assess the tumor infiltration levels in LGG. We also further verified the positive association between *C5AR2* expression and immune cell marker genes in LGG (Table S2). Similarly, we also confirmed that *C5AR2* was positively correlated with immune checkpoint-related molecules, including TIM-3, PD-L1, CTLA-4, PD-1, and Siglec-15 in LGG (Figure 4d).

In addition, to examine the applicability of *C5AR2* to other tumors, we investigated the correlation between *C5AR2* expression and infiltrating immune cells in kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), lung adenocarcinoma (LUAD), and uterine corpus endometrial carcinoma (UCEC). We found that *C5AR2* was not highly expressed in all tumors (Figure S3). In comparison with the normal control group, *C5AR2* expression was downregulated in LUAD and UCEC. *C5AR2* was more correlated with tumor-infiltrating immune cells in LGG and KIRC (Figure 4e).

4 | DISCUSSION

This study mainly focuses on the following two points: (1) *C5AR2* expression in glioma and its prognostic value, and (2) whether *C5AR2* could be a possible biomarker

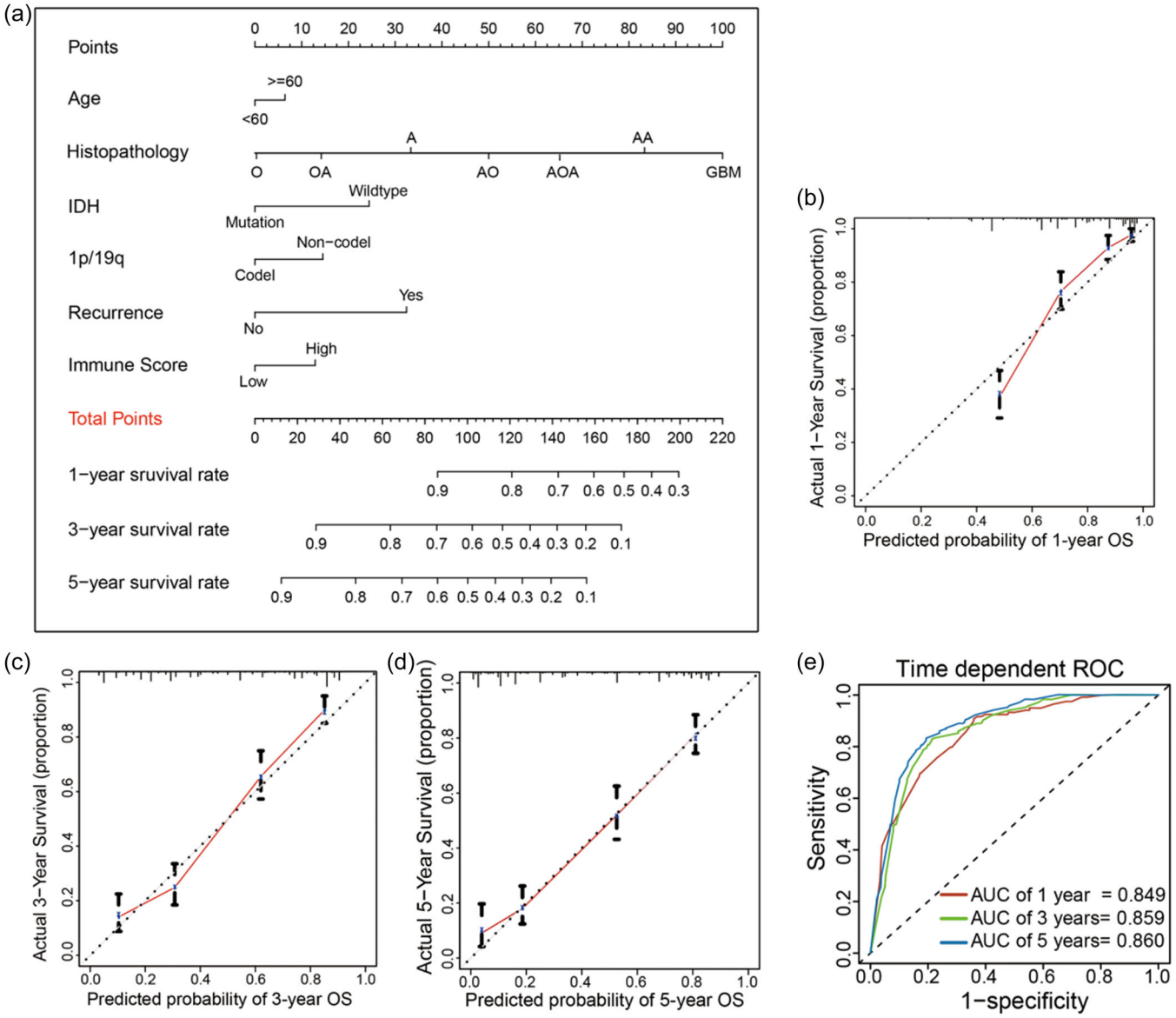

FIGURE 3 The construction and assessment of the nomogram in glioma. (a) The nomogram was constructed based on the clinical factors and immune score. (b–d) Calibration curves of the nomogram model. (e) Time-dependent ROC curves evaluating the efficiency of the nomogram for predicting 1-, 3-, and 5-year survival.

and target for glioma patients. We also developed a prognostic model which supplies a more individualized outcome prediction. *C5AR2*, a novel C5A chemokine receptor mediating the inflammatory response, was discovered by Ohno et al. for the first time in 2000 [20]. Based on the *C5AR2* mRNA level in many kinds of human cells and tissues reported in different studies, the *C5AR2* expression is very low, which is consistent with the *C5AR1* mRNA level [22]. However, *C5AR2* expression is significantly upregulated in some tissues, including bone marrow, spleen, and immune cells [23, 24]. Although it is generally accepted that *C5AR1* has broadly proinflammatory functions, the role of C5AR2 is still controversial [25]. C5AR2 can activate NLRP3 inflammatory bodies and initiate innate immunity [18, 26, 27]. However, several researchers believe that C5AR2 acts as a bait receptor for C5A, reducing the binding of C5A to C5AR1 [28]. The potential function of *C5AR2* in the occurrence and development of disease is still unclear, as well as the expression and prognostic value of *C5AR2* in glioma and its correlation with infiltrating immune cells.

Many prognostic biomarkers in glioma have been reported in the past few years [29, 30]. We first reported that the expression of *C5AR2* is upregulated in glioma compared with normal tissue, and that *C5AR2* overexpression is related to malignant behavior of glioma and poor prognosis, confirming it as a novel prognostic biomarker. Meanwhile, Zhu et al. also observed that

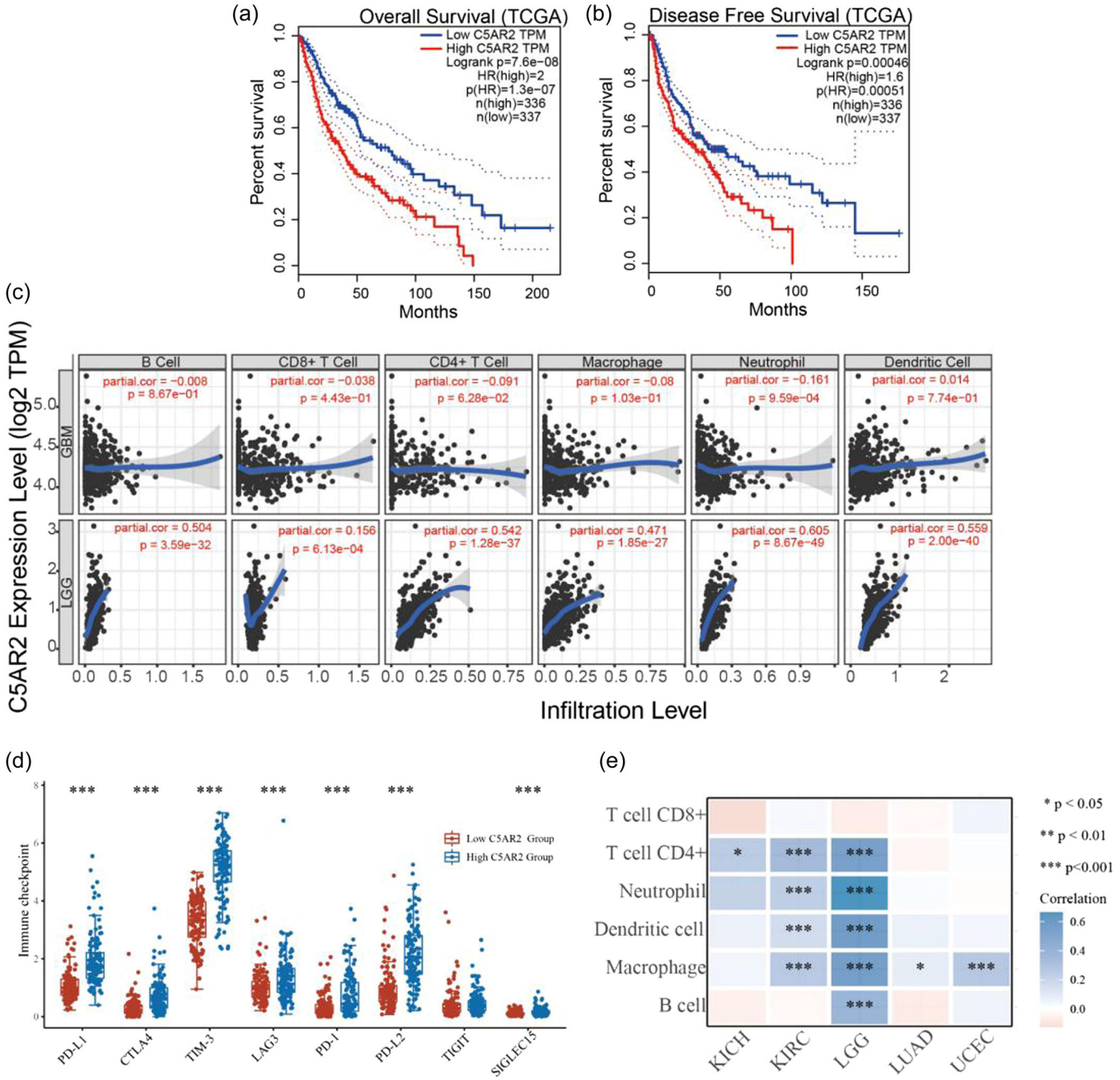

FIGURE 4 Verification of the correlation between *C5AR2* expression, prognosis, and immune infiltration levels (TCGA database). (a and b) Glioma patients with higher *C5AR2* expression had shorter OS and DFS. (c) The correlation between *C5AR2* expression and immune cell infiltration in LGG and GBM (TIMER). (d) *C5AR2* is significantly associated with common immune checkpoints including PD-L1, CTLA-4, TIM-3, LAG3, PD-1, PD-L1, PD-L2, TIGIT, and Siglec-15. (e) The correlation between *C5AR2* expression and infiltration levels of immune cells in other kinds of tumors.

upregulation of C5AR2 could enhance the proliferation and invasion of breast cancer cells, so C5AR2 is a possible prognostic biomarker for breast cancer [31]. Additionally, we also found that *C5AR2* expression is involved in activation of cytokine-cytokine receptor interaction, NF-KB signaling pathway, TNF signaling pathway, inflammatory bowel disease, and intestinal immune network for IgA production. These findings indicate that *C5AR2* might play an essential role in glioma inflammation. Inflammation is an important driving factor for tumorigenesis and development, and inflammatory cells and cytokines in tumors may be involved in tumor immunosuppression [32, 33]. *C5AR2* was initially considered an important molecule in regulation of the inflammatory response and plays a key role in a variety of inflammatory diseases [34, 35]. Yu

et al. reported that *C5AR2* activates NLRP3 inflammatory bodies and increases the HMGB1 levels of macrophages by upregulating protein kinase R [18]. However, there are very few reports on the role of *C5AR2* in tumors. Su et al. first found that $CD10^+GPR77^+$ (*C5AR2*) cancer-associated fibroblasts (CAF) can promote breast cancer formation and chemotherapy resistance, and that targeting $CD10^+GPR77^+$ CAF is an effective way to treat solid cancers [36]. These results are consistent with those we have obtained. The NF-KB signaling pathway, an inflammation-related pathway, plays a key role in many solid cancers. For instance, NF-KB, a key transcription factor, can enter the nucleus to promote the expression of many cytokines, and then can recruit the infiltration of immune cells in many cancers [37]. Increasing studies have shown that inflammatory responses and tumor immune infiltration affect each other [38, 39].

The second important finding is that there is a strong relationship between *C5AR2* expression and tumor-infiltrating immune cells in glioma, including B cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells. Furthermore, there is a significant relationship between *C5AR2* and common immune checkpoints, such as PD-L1, PD-1, TIM-3, CTLA-4, and Siglec-15. These data suggest that *C5AR2* is not only an independent prognostic factor, but also a biomarker for assessing the immune status in gliomas. Treg cells are another T cell subtype that regulate the tumor immune response [40, 41]. Verghese et al. recently discovered that *C5AR2* can enhance the formation of Treg cells, supported by the upregulation of *C5AR2* expression during Treg cell generation in a GFP knock-in *C5AR2* reporter mouse [42]. This is consistent with the significant correlation between *C5AR2* and infiltration levels and Treg cell gene markers in this study. We found that the expression of *C5AR2* was substantially related to the expression of marker genes of exhausted T cells. *C5AR2* could trigger a persistent inflammatory microenvironment in the tumor. The exhaustion of T cells into nonfunctional T cells is an important mechanism of immune escape. Exhausted T cells gradually lose effector function because of long-term exposure to a persistent inflammatory microenvironment [43]. Whether the overexpression of *C5AR2* causes T cell exhaustion still needs further experimental confirmation.

Although we discovered an immune-related biomarker and target in glioma patients, there are several limitations to this study. The analysis of gene transcription levels can reflect certain characteristics of the immune status, but not all alterations. Our finding that *C5AR2* expression is associated with the malignant progression of glioma and TME is only preliminary, and the related biological mechanism still needs to be elucidated experimentally. In addition, although this study has been verified by another independent data set, glioma patients from a single center of our hospital need to be collected for further verification.

In summary, we are the first to report that the expression of *C5AR2* is upregulated in glioma in comparison with normal tissue, and elevated *C5AR2* expression is substantially correlated with glioma malignant behavior. We verified this as a poor prognostic factor. In glioma, overexpression of *C5AR2* was confirmed to be involved in various biological processes containing the inflammatory and immune response. High *C5AR2* expression was in strong correlation with elevated levels of immune cell infiltration (including infiltration of B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and DCs) in LGG. In addition, *C5AR2* expression was significantly related to the expression of immune cell marker genes, including exhausted T cells, TAMs, and Th2 in LGG. These discoveries indicate that *C5AR2* is not only a prognostic biomarker but may also play a significant role in controlling the tumor immune microenvironment in LGG. These discoveries indicate that *C5AR2* is a possible prognostic marker and immunotherapeutic target in glioma. Furthermore, the prognostic nomogram could precisely predict the prognosis of glioma patients, which supplies a basis for individualized treatment of glioma patients.

AUTHOR CONTRIBUTIONS

Chengying Huang: Investigation (equal); methodology (equal); project administration (equal); resources (equal); validation (equal); visualization (equal); writing – original draft (lead). **Ouwen Qiu**: Supervision (equal); writing – original draft (equal); writing – review & editing (equal). **Chaofu Mao**: Data curation (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); software (equal); writing – review & editing (equal). **Zhicheng Hu**: Formal analysis (lead); software (equal); supervision (equal); validation (equal). **Shanqiang Qu**: Conceptualization (lead); data curation (lead).

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are accessible from the corresponding author upon reasonable request (qushq3@163. com).

ETHICS STATEMENT

This study was approved by an institutional review board of Nanfang hospital.

INFORMED CONSENT

The CGGA and TCGA databases are open public database, and the release of data from them do not require informed patients consent. Tissue samples were collected from patients who provided informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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