

# EFNA1 is a potential key gene that correlates with immune infiltration in low-grade glioma

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## Abstract

EFNA1 is a key gene that is associated with the pathogenesis of several human cancers. However, the prognostic role of EFNA1 in many cancers and the relationship between EFNA1 and tumor-infiltrating lymphocytes in different cancers remain unclear.

The expression levels of EFNA1 in 33 types of cancer in the TCGA (The Cancer Genome Atlas) database were collected via the UCSC Xena browser. The clinical data of LGG (low grade glioma) patients were downloaded from the TCGA database. The glioma data from the CGGA (Chinese Glioma Genome Atlas) database were also downloaded to verify the results. Kaplan–Meier and Cox regression analyses were used to investigate the prognostic value of EFNA1 in different cancers using R software. We verified the differential expression of EFNA1 in glioma and normal brain tissue via gene expression profiling interactive analysis. We evaluated the relationship between the expression level of EFNA1 and the clinicopathological features of LGG patients via the Wilcoxon signed-rank test. The immune infiltration levels were evaluated via tumor immune estimation resource (TIMER) and CIBERSORT, and the correlations between EFNA1 and immune cell levels were investigated via TIMER. Finally, we conducted gene set enrichment analysis (GSEA) to explore the potential mechanisms.

Data from the TCGA database showed that EFNA1 was differentially expressed in many kinds of cancers when compared with normal tissues. Upregulated EFNA1 expression in esophageal carcinoma (ESCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), and LGG correlated with shorter patient overall survival (OS) times. The Cox regression analysis revealed that the expression of EFNA1 was also a risk factor for the disease-specific survival (DSS) and progression-free interval (PFI) of LGG patients. The multiple Cox regression analysis revealed that EFNA1 was an independent prognostic factor for LGG patients. In addition, EFNA1 expression was increased in the WHO grade III group and the 1p19q non-codeletion group. Moreover, EFNA1 expression was positively correlated with the levels of infiltrating CD4+ T cells, myeloid dendritic cells and neutrophils in LGG. GSEA suggested that several GO and kyoto encyclopedia of genes and genomes (KEGG) items associated with nervous system function and apoptotic pathway were significantly enriched in the EFNA1-low and EFNA1-high expression phenotypes.

EFNA1 may play a pivotal role in the development of LGG and may serve as a potential marker for LGG prognosis and therapy.

**Abbreviations:** CESC = cervical squamous cell carcinoma and endocervical adenocarcinoma, CGGA = Chinese Glioma Genome Atlas, DSS = disease-specific survival, ESCA = esophageal carcinoma, GBM = glioblastoma, KEGG = kyoto encyclopedia of genes and genomes, LGG = low grade glioma, NES = normalized enrichment score, OS = overall survival, PFI = progression-free interval, PRAD = prostate adenocarcinoma, READ = rectum adenocarcinoma, TCGA = the cancer genome atlas, TIMER = tumor immune estimation resource, UCEC = uterine corpus endometrial carcinoma.

**Keywords:** EFNA1, immune infiltration, low-grade glioma, prognosis, the cancer genome atlas

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Gene transcriptome profiles of patients with LGG of the central nervous system could be downloaded in The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>) and Chinese Glioma Genome Atlas (CGGA <http://www.cgga.org.cn/>).

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The datasets generated during and/or analyzed during the current study are publicly available.

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## 1. Introduction

Cancer is a major threat to human health, and cancer treatment is a worldwide problem. In 2020, there were 1,806,590 new cases of cancer and 606,520 cancer-related deaths.<sup>[1]</sup> It is critical to investigate the molecular mechanisms of tumorigenesis and identify novel biomarkers to develop individual therapeutic strategies and new treatments to improve patient outcomes.

Gliomas account for the largest proportion of brain malignancies,<sup>[2]</sup> and the median survival rate is 4.7 to 9.8 years.<sup>[3]</sup> In the US, approximately 25,000 people are affected by gliomas each year.<sup>[4]</sup> At present, the treatment for glioma is traditional surgery, adjuvant radiotherapy and chemotherapy. However, the aggressive nature of the tumor and the growth pattern by which gliomas infiltrate into the surrounding normal brain tissue makes the complete resection of gliomas difficult, and the blood-brain barrier makes chemotherapeutic drugs less effective.<sup>[2]</sup>

In addition to traditional histological classification, molecular parameters correlated with treatment response and patient survival, including 1p/19q codeletion status and isocitrate dehydrogenase 1 mutation, are included in the 2016 WHO glioma classification guidelines. The discovery of these biomarkers enables us to understand more about the mechanism of glioma development, thus aiding the clinical diagnosis and treatment of glioma. The biomarkers suggest that the prognosis of tumors is complex and cannot be predicted accurately by a single index. The combined analysis of multiple indicators will improve the accuracy of prognosis prediction. Since the survival status of different glioma patients is also variable, individualized therapy is one of the goals of therapy. Therefore, more studies are warranted to investigate more novel biomarkers and potential molecular mechanisms of LGG (Low-Grade Glioma) to predict patient prognosis as well as their potential response to specific therapies,<sup>[2]</sup> allowing clinicians to identify the best treatment option for each patient.

EFNA1 (ephrin A1), a member of the EFNA family, was first found in 1990 as a TNF-induced protein in human umbilical vein endothelial cells.<sup>[5]</sup> The similarity between EFNA1 and other EFN members is approximately 30% to 40%.<sup>[6]</sup> It is a transmembrane protein, the extracellular receptor-binding domain of which is approximately 20 kDa, and it is anchored on the cell membrane by glycosyl phosphatidylinositol linkage.<sup>[6,7]</sup> EFNA1 binds many EphA family receptors (EphA1–5).<sup>[8–10]</sup> Previous studies revealed that the expression level of EFNA1 is upregulated in many human cancers (e.g., renal cancer,<sup>[11]</sup> gastric cancer<sup>[12]</sup> and colorectal cancer<sup>[13]</sup>) compared to the level in corresponding normal tissue and that its expression is correlated with the patient prognosis. High EFNA1 expression is a prognostic factor for gastric,<sup>[12]</sup> ovarian,<sup>[14]</sup> cervical<sup>[15]</sup> and esophageal cancer<sup>[16]</sup> and is an independent prognostic factor for hepatic carcinoma.<sup>[17]</sup> EFNA1 can be induced by hypoxia<sup>[18,19]</sup> and participates in angiogenesis,<sup>[20]</sup> tumorigenesis<sup>[21]</sup> and tumor metastasis.<sup>[22,23]</sup> However, the role of EFNA1 in many other cancers and the relationship between EFNA1 and tumor immunology are still unknown.

Bioinformatics is a flourishing study approach. Through data analysis, many potential tumor markers can be found for the study of antitumor treatments. In the present study, we investigated EFNA1 expression in 33 types of human cancers and corresponding normal tissue and determined the prognostic value of EFNA1 in low-grade glioma (LGG) based on data from the cancer genome atlas (TCGA) and Chinese Glioma Genome

Atlas (CGGA) databases. Moreover, we evaluated the correlation between EFNA1 and immune cell infiltration in the LGG microenvironment via CIBERSORT and tumor immune estimation resource (TIMER). Finally, gene set enrichment analysis was used to find gene sets enriched in the EFNA1-high and EFNA1-low expression groups. The findings of this study indicate that EFNA1 expression is a prognostic factor and associated with the clinicopathological features of LGG patients. Moreover, we identified a potential relationship between EFNA1 and immune cell infiltration in LGG and the biological processes associated with EFNA1.

## 2. Materials and methods

### 2.1. Data collection

To investigate the role of EFNA1 in different cancers and the corresponding normal tissue, we downloaded the expression data and survival data of 33 kinds of human cancers in TCGA dataset via the UCSC Xena browser (<https://xenabrowser.net>). The clinical data of LGG patients were also downloaded from TCGA for the analysis of the relationship between clinicopathological characters and EFNA1 expression level. To verify our analysis results based on data from TCGA, gene expression data and corresponding clinical data of datasets mRNAseq\_693 and mRNAseq\_325 were downloaded from Chinese Glioma Genome Atlas (CGGA <http://www.cgga.org.cn/>), including LGG and Glioblastoma (GBM) samples. The 2 sets of gene expression data from glioma samples were normalized via the “limma”<sup>[24]</sup> and “sva”<sup>[25]</sup> packages in R software (R version 3.6.2) to remove the batch effects. After excluding samples that are GBM (n=388) or recurrent LGG (n=199) or without histological type (n=5), a total of 426 samples were included in the CGGA cohort.

### 2.2. Differential expression of EFNA1 in normal brain tissue and LGG

Gene expression profiling interactive analysis (<http://gepia.cancer-pku.cn/>) is an online database including the data of TCGA and GTEx project<sup>[26]</sup> and it was used to further confirm the different expression of EFNA1 in normal brain tissue and LGG.

### 2.3. Immune cell infiltration analysis via TIMER

Tumor Immune Estimation Resource 2.0 (TIMER2.0) provides comprehensive analysis and visualization functions of tumor infiltrating immune cells based on TCGA or user-provided tumor profiles (<https://cistrome.shinyapps.io/timer/>).<sup>[27]</sup> We investigated the association between the EFNA1 expression in LGG and the abundance of immune cells infiltration, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells via TIMER2.0. The association analysis is performed using the partial Spearman’s correlation and it is considered statistically significant when *P* value <.05.

### 2.4. Identifying EFNA1-associated immune cells

CIBERSORT is a useful approach for the estimation of the abundance ratio of diverse cell types, such as tumor infiltrating leukocytes, from complex tissues requiring gene expression data. We ran CIBERSORT in R software.<sup>[28]</sup> The expression data of

529 LGG samples from TCGA was analyzed to obtain the abundance ratio matrix of 22 immune cells. Finally, 356 samples were selected with  $P < .05$  for further identification of the EFNA1-associated immune cells.

**2.5. Gene set enrichment analysis**

Gene set enrichment analysis (GSEA) is a computational method that tests whether a set of genes is differentially expressed in 2 groups.<sup>[29]</sup> To investigate the biological processes associated with EFNA1, the samples of LGG from TCGA were divided into EFNA1-high group and EFNA1-low group according to the median EFNA1 expression level and then GSEA analysis was performed using GSEA 3.0 software. In this study, the “c2.cp.kegg.v6.2.symbols.gmt,” “c5.go.bp.v7.2.symbols.gmt,” “c5.go.cc.v7.2.symbols.gmt,” “c5.go.mf.v7.2.symbols.gmt” gene sets were analyzed using GSEA 3.0 software. The number of gene set permutations was set as 1000. The analysis results were ordered by normalized enrichment score (NES), and the items with a nominal  $P$  value of  $< .05$  and a false discovery rate  $q$ -value of  $< .05$  were considered significantly enriched gene sets.

**2.6. Statistical analysis**

The Wilcoxon signed-rank test was used to compare the expression of EFNA1 in different kinds of tumors with that in normal tissues, to investigate the relationship between clinicopathological factors

and EFNA1 expression level and to explore the infiltrated immune cells associated with EFNA1 expression. The relationship between EFNA1 expression and overall survival (OS) was evaluated using Kaplan–Meier survival curves via “Survminer” and “Survival” packages in R software. Univariate Cox regression analysis and multivariate Cox regression analysis were used to evaluate the relationship between clinicopathological characteristics, EFNA1 expression level and OS rate. All statistical analyses were performed using R 3.6.2.

**2.7. Ethical statement**

The data analyzed in this paper was obtained from the public databases. So the ethical approval was not applicable.

**3. Result**

**3.1. The mRNA expression levels of EFNA1 in different types of human cancer**

To investigate the differential expression of EFNA1 in 33 types of tumor and the corresponding normal tissues, the expression data of 33 types of tumor were collected via the UCSC Xena browser (<https://xenabrowser.net>). Differential analysis of EFNA1 expression between normal and malignant tissue was carried out using R software. The results suggested that EFNA1 expression was higher in bladder urothelial carcinoma, breast invasive

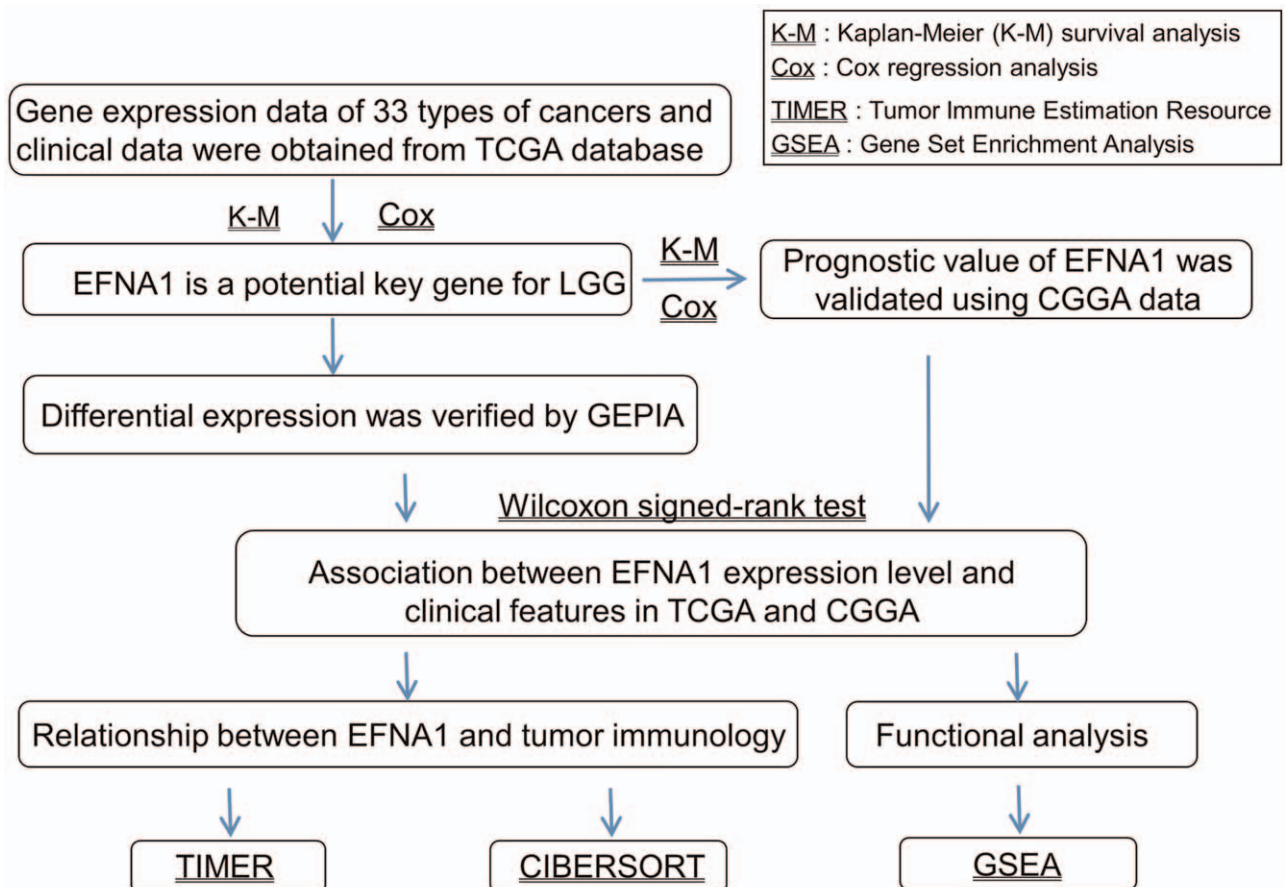
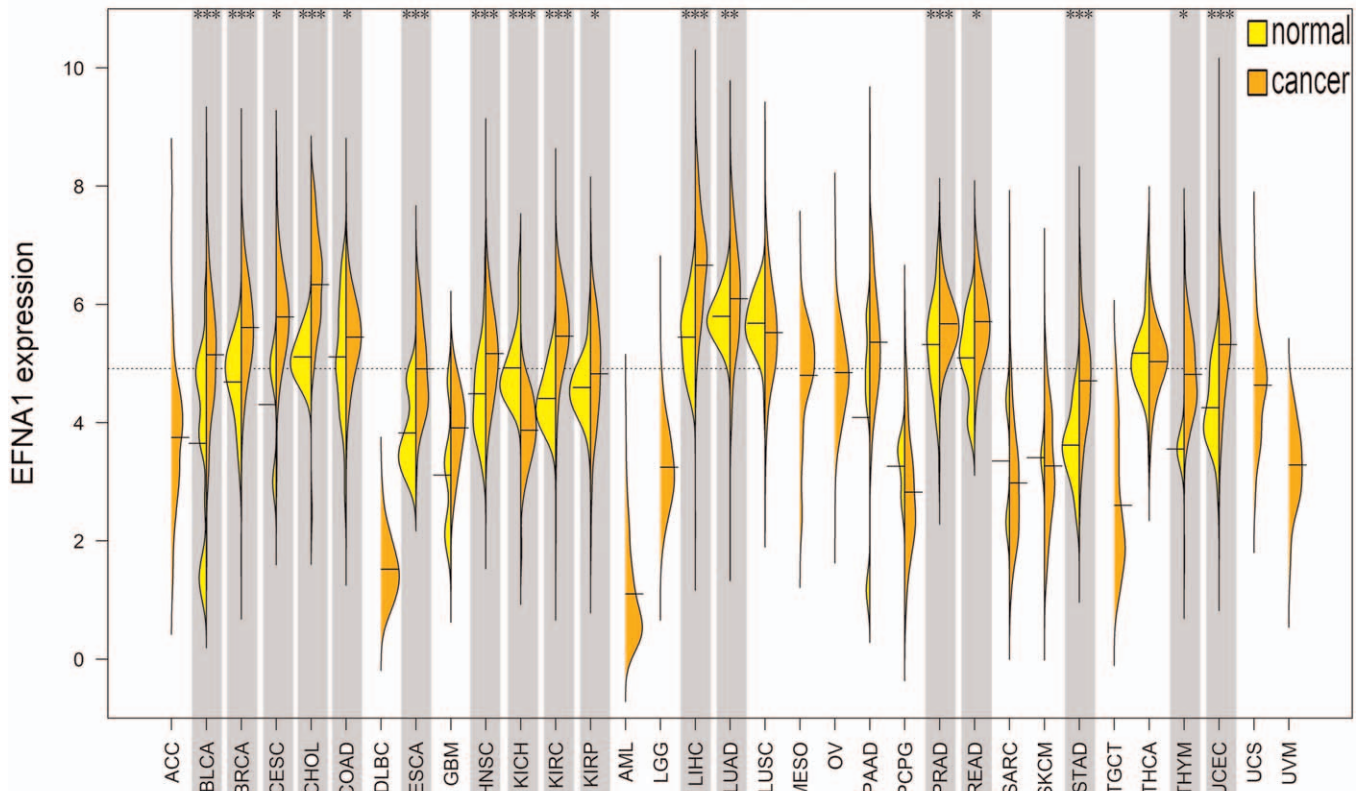


Figure 1. Flowchart, the overview of data collection and data analysis methodology in this study.

## EFNA1 expression in human cancers



**Figure 2.** The expression levels of EFNA1 in different kinds of cancers compared to normal tissue, \*  $P < .05$ , \*\*  $P < .01$ , \*\*\*  $P < .001$ .

carcinoma, CESC(cervical squamous cell carcinoma and endocervical adenocarcinoma), cholangiocarcinoma, colon adenocarcinoma, ESCA (esophageal carcinoma), head and neck squamous cell carcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma; PRAD (prostate adenocarcinoma), READ (rectum adenocarcinoma), stomach adenocarcinoma, thymoma, UCEC (uterine corpus endometrial carcinoma) than in normal tissues (Fig. 2). However, lower expression of EFNA1 was observed in kidney chromophobe. In addition, EFNA1 expression data in normal tissue corresponding to adrenocortical carcinoma and lymphoid neoplasm diffuse large B-cell lymphoma, acute myeloid leukemia, LGG (brain low grade glioma), mesothelioma, ovarian serous cystadenocarcinoma, testicular germ cell tumors, uterine carcinosarcoma, UVM (uveal melanoma) were not available.

### 3.2. Potential prognostic value of EFNA1 in cancers

We then investigated whether EFNA1 expression was correlated with the prognosis of cancer patients. Kaplan–Meier survival analysis was used to evaluate the impact of EFNA1 expression on survival rates. The detailed results of EFNA1 expression and prognosis analysis of different cancers are shown in Table 1. Using Kaplan–Meier survival analysis and univariate Cox regression analysis, we found that high EFNA1 expression was associated with worse OS rates in patients with 3 types of cancers, including ESCA (OS: KM  $P < .001$ , HR = 1.51, 95% CI = 1.08–

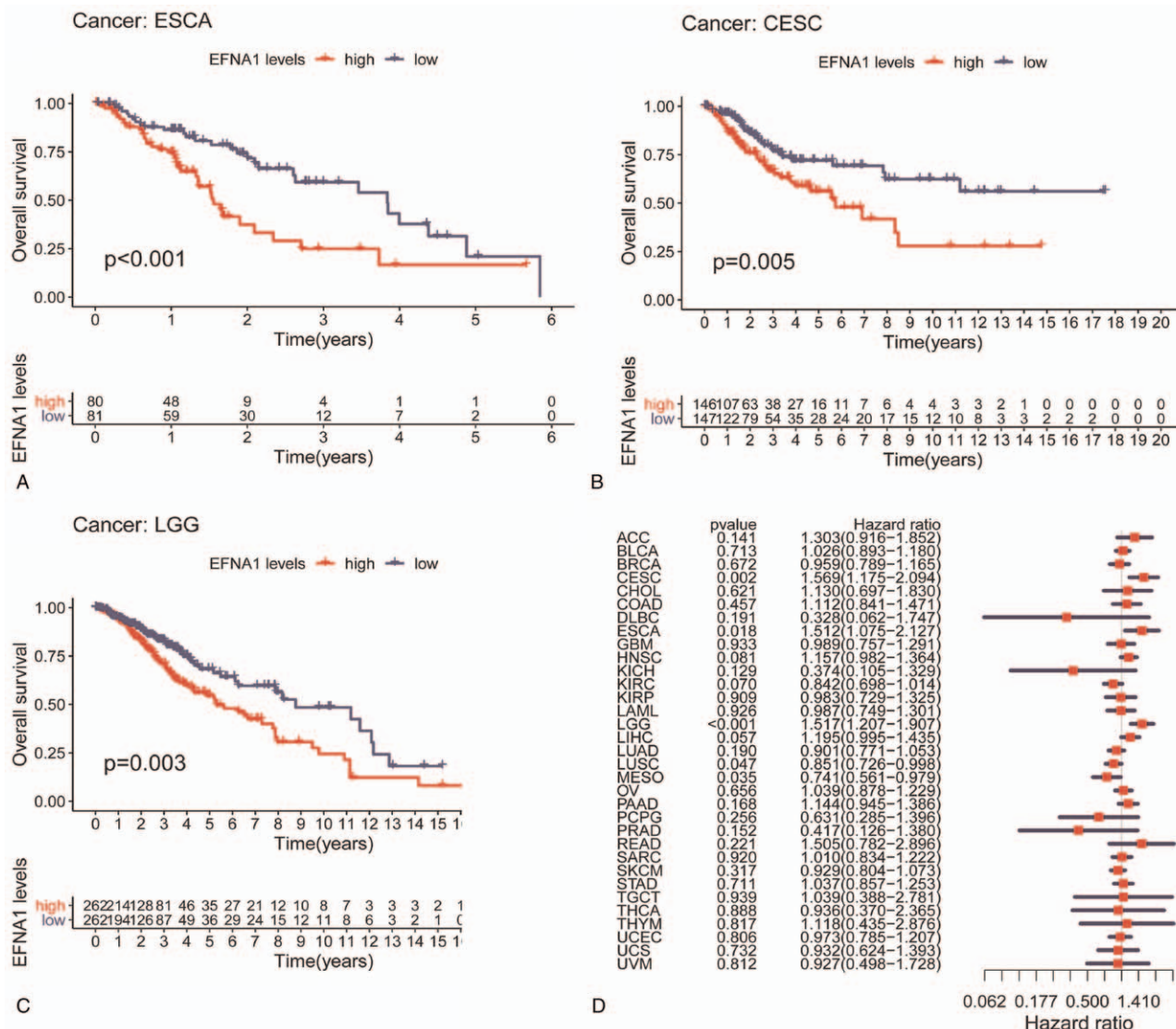
2.13, Cox  $P = .018$ , CESC (OS:KM  $P = .005$ , HR = 1.57, 95% CI = 1.17–2.09, Cox  $P = .002$ ), and LGG (OS:KM  $P = .003$ , HR = 1.52, 95% CI = 1.21–1.91, Cox  $P < .001$ ), as shown in Figure 3.

**Table 1**  
Kaplan–Meier survival analysis results.

cancer type	P value	cancer type	P value
ACC	.105324107	LUSC	.404297787
BLCA	.692865023	MESO	.950581483
BRCA	.476000399	OV	.991780791
CEC*	.004722262	PAAD	.213799865
CHOL	.495039742	PCPG	.289772773
COAD	.586989771	PRAD	.299811278
DLBC	.753590058	READ	.189866802
ESCA***	.000935056	SARC	.932581075
GBM	.993099434	SKCM	.128612495
HNSC	.124605806	STAD	.852296848
KICH	.743642217	TGCT	.327577625
KIRC	.500705724	THCA	.779487783
KIRP	.66115189	THYM	.249767102
LAML	.480451589	UCEC	.604605836
LGG**	.002941414	UCS	.50189169
LIHC	.077345941	UVM	.426249566
LUAD	.445517896		

\*  $P < .05$ .  
\*\*  $P < .01$ .  
\*\*\*  $P < .001$ .





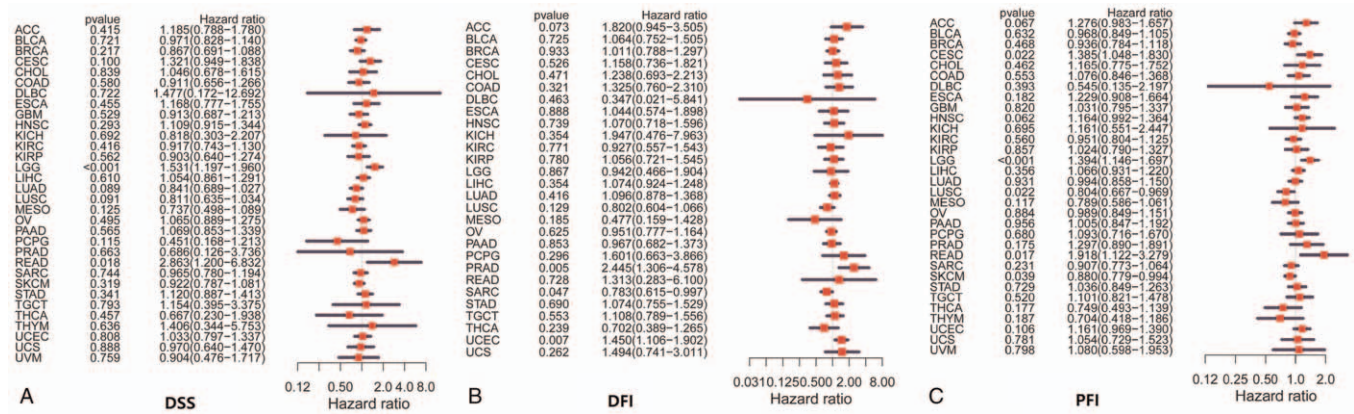
**Figure 3.** Higher EFNA1 expression level is a risk factor for ESCA, CESC, and LGG patients. (A–C) Survival curves of OS in ESCA, CESC and LGG patients. (D) Cox regression analysis revealed the relationship between EFNA1 expression level and OS of cancer patients. CESC = cervical squamous cell carcinoma and endocervical adenocarcinoma, ESCA = esophageal carcinoma.

To further examine the prognostic value of EFNA1 in different cancers, we downloaded the supplemental survival information of cancer patients from the UCSC Xena browser (<https://xenabrowser.net>), which included the disease-specific survival (DSS) rate, disease-free interval and progression-free interval (PFI) data. Higher expression of EFNA1 is associated with a poor DSS rate in patients with LGG (HR = 1.53, 95% CI = 1.20–1.96,  $P < .001$ ) and READ (HR = 2.86, 95% CI = 1.20–6.83,  $P = .018$ ), a poor disease-free interval in patients with PRAD (HR = 2.45, 95% CI = 1.31–4.58,  $P = .005$ ) and UCEC (HR = 1.45, 95% CI = 1.11–1.90,  $P = .007$ ), and a poor PFI in patients with CESC (HR = 1.39, 95% CI = 1.05–1.83,  $P = .022$ ), LGG (HR = 1.39, 95% CI = 1.15–1.70,  $P < .001$ ), and READ (HR = 1.92, 95% CI = 1.12–3.28,  $P = .017$ ). Interestingly, higher EFNA1 expression indicates a better PFI in skin cutaneous melanoma (HR = 0.88, 95% CI = 0.78–0.99,  $P = .039$ ) patients. These results confirmed the prognostic value of EFNA1 in several types of cancer, as shown in Figure 4.

Survival analysis suggested that EFNA1 expression correlated more tightly with the OS rate, DSS rate and PFI of LGG patients than of those with other cancers. We noticed that EFNA1 expression was associated with the prognosis of patients with LGG but not those with GBM. Moreover, it has been well documented that the EFNA protein family is involved in neurodevelopment.<sup>[30,31]</sup> Therefore, we investigated the potential function of EFNA1 in LGG.

### 3.3. EFNA1 expression is upregulated and is an independent prognostic factor in LGG

To assess whether EFNA1 expression was upregulated in LGG compared to normal tissue, we validated our findings using the gene expression profiling interactive analysis website, which integrated the data from the TCGA and GTEx databases. The results suggested that EFNA1 expression was significantly upregulated in LGG (Fig. 5).

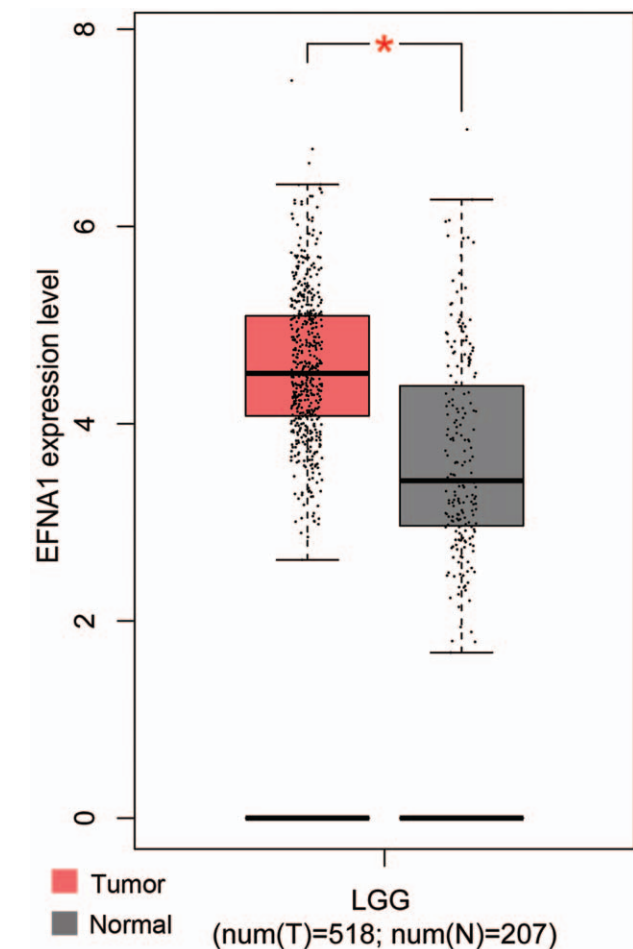


**Figure 4.** Univariate Cox regression analysis revealed the prognostic value of EFNA1 expression level in different cancers. (A)Cox result for DSS rate in different cancers.EFNA1 is a risk factor for the DSS rate of CESC, LGG and READ. (B) EFNA1 expression is a risk factor for the DFI of PRAD and UCEC patients. (C) EFNA1 expression level is a risk factor for the DSS of CESC, LGG, and READ patients, but it is associated with better PFI in SKCM. DFI = disease-free interval, DSS = disease-specific survival, SKCM = skin cutaneous melanoma.

**3.4. Prognostic value of EFNA1 in patients from the CGGA database**

Since EFNA1 expression was associated with the prognosis and clinical features of LGG patients, we then verified its role using

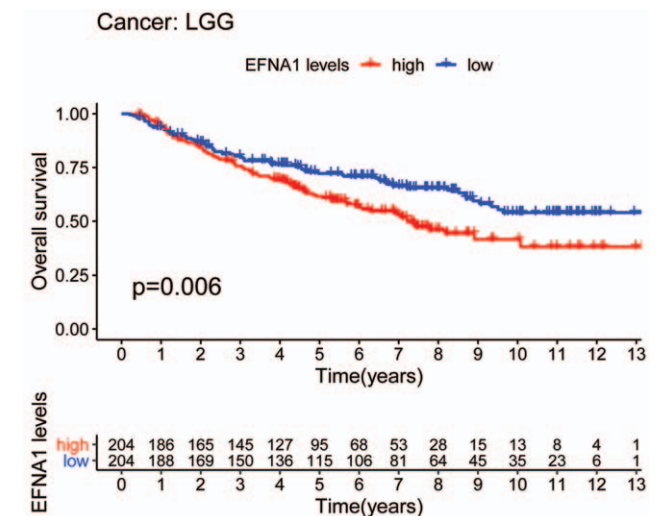
patient data from the CGGA database. Two datasets (mRNA-seq\_693 and mRNAseq\_325) were downloaded, including both LGG and GBM data. After excluding samples that were GBM or recurrent glioma or without historical type, a total of 426 primary LGG samples were included from the CGGA cohort. Survival analysis was performed using the K-M and univariate Cox methods. Higher expression of EFNA1 led to a shorter overall survival time in patients with LGG (Fig. 6). Moreover, multiple Cox regression analysis revealed that EFNA1 is an independent prognostic factor for LGG patients (Table 2).



**Figure 5.** GEPIA result showed that EFNA1 is upregulated in LGG (P < 0.05). GEPIA = gene expression profiling interactive analysis.

**3.5. Association between EFNA1 expression and clinicopathological features of patients with LGG**

We then downloaded the clinical data of LGG patients from the TCGA database (Table 3). The associations between EFNA1 expression levels and clinicopathological variables were analyzed using the Wilcoxon signed-rank test. As shown in Figure 7, higher



**Figure 6.** The median value of EFNA1 expression was considered as the cut-off value. The correlation between EFNA1 expression and overall survival (OS) was estimated using Kaplan-Meier survival curves.

**Table 2**

**Association between clinical features and overall survival of patients from CGGA database using Cox regression analysis. (A) Univariate Cox regression. (B) Multivariate Cox regression.**

clinical features	HR	HR.95L	HR.95H	P value
<b>(A)</b>				
Grade	2.68131236	1.854540253	3.876667526	1.58E-07
Gender	1.203030748	0.839344989	1.724300495	.314214836
Age	1.024087314	1.006109382	1.04238649	.008438701
IDH_mutation_status	0.312283314	0.21625824	0.450946368	5.36E-10
1p19q_codeletion_status	0.180820959	0.106493577	0.30702527	2.43E-10
MGMTp_methylation_status	0.622200152	0.436318404	0.887271831	.008779951
EFNA1	1.481117971	1.200393004	1.827493526	.000248836
<b>(B)</b>				
Grade	2.281847102	1.559981821	3.337747997	2.12E-05
Gender	1.30329856	0.902696084	1.88168218	.15746207
Age	1.023768096	1.006913691	1.040904622	.005546554
IDH_mutation_status	0.575194986	0.38245246	0.865072936	.007905957
1p19q_codeletion_status	0.229504065	0.131233473	0.4013619	2.46E-07
MGMTp_methylation_status	0.703550912	0.480010929	1.03119295	.071466565
EFNA1	1.250520522	1.012793082	1.544048439	.037697437

EFNA1 expression was associated with WHO grade III in LGG patients ( $P < .001$ ). However, no association was observed between EFNA1 expression and age ( $P = .461$ ), sex ( $P = .684$ ) or IDH mutation status ( $P = .197$ ).

We verified these results in the CGGA database with both EFNA1 expression data and clinical data (Table 4). As shown in Figure 8C and 8E, high expression of EFNA1 was significantly associated with histological grade ( $P < .001$ ) and 1p19q codeletion status ( $P < .001$ ).

### 3.6. EFNA1 expression is correlated with immune infiltration levels in low-grade glioma

It is well known that tumor-infiltrating immune cells are closely associated with the cancer development.<sup>[32]</sup> Therefore, we evaluated the correlation between EFNA1 expression and the infiltration levels of 6 types of immune cell in LGG via TIMER. The results showed that EFNA1 expression was positively correlated with the infiltration levels of CD4+ T cells, myeloid dendritic cells and neutrophils in LGG. Moreover, we investigated the prognostic value of different immune cells via TIMER.

**Table 3**

**Characteristics of LGG patients in the TCGA database.**

Clinical characteristics	number (Total N = 510)
<b>age</b>	
<=41	266
>41	244
<b>gender</b>	
male	282
female	228
<b>grade</b>	
G2	248
G3	261
unknown	1
<b>IDH mutation</b>	
YES	127
NO	292
unknown	91

Kaplan–Meier survival curves were generated with a 50% split infiltration percentage, which divided samples into high-level and low-level groups. The results showed that levels of infiltrating B cells, CD4+ T cells, myeloid dendritic cells, macrophages and neutrophils were related to the cumulative survival rate of LGG patients (Fig. 9).

### 3.7. Relationship between EFNA1 expression and tumor-infiltrating immune cells

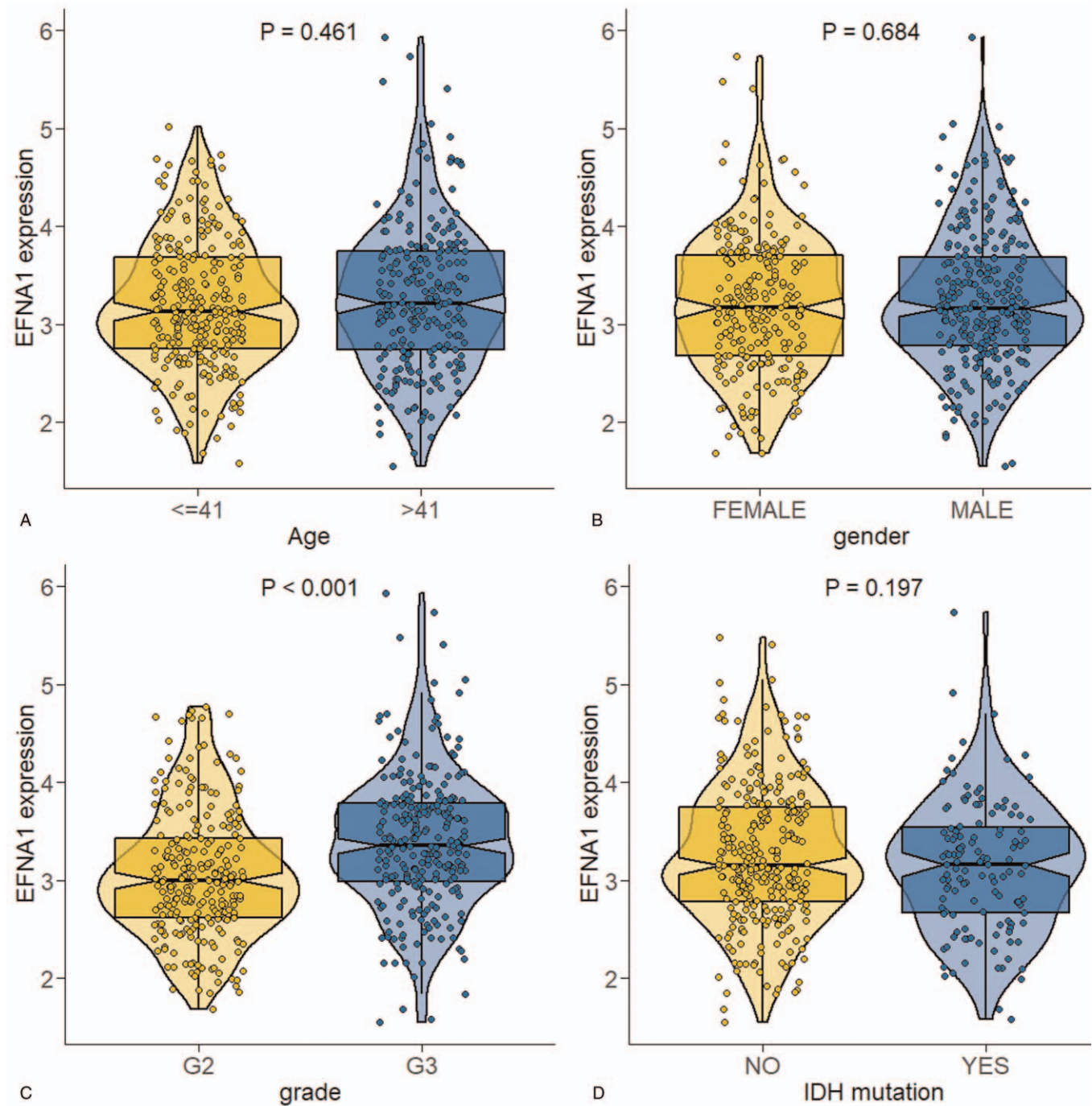
CIBERSORT is a computational method to estimate the abundance ratios of tumor infiltrating leukocytes in samples according to gene expression data. We ran CIBERSORT within R software.<sup>[28]</sup> The gene expression data of LGG samples were analyzed to determine the abundance ratios of 22 types of immune cell. Ultimately, 356 samples were selected with a  $P$  value  $< .05$  and then divided into 2 groups according to the EFNA1 expression median value. The Wilcoxon signed-rank test was then used to evaluate the different concentrations of immune cells in the EFNA1-high and EFNA1-low expression groups. As shown in Figure 10, naive B cells ( $P = .022$ ), resting memory CD4 T cells ( $P = .037$ ), activated memory CD4 T cells ( $P = .014$ ), regulatory T cells (Tregs) ( $P = .029$ ), and neutrophils ( $P = .043$ ) were the main immune cells affected by EFNA1 expression. Among them, naive B cells ( $P = .022$ ) were apparently increased, but resting memory CD4 T cells ( $p = 0.037$ ) were decreased in the EFNA1-high group compared with the EFNA1-low group.

### 3.8. Gene set enrichment analysis of EFNA1

Gene set enrichment analysis was conducted to explore the signaling pathways involved in LGG between the low and high EFNA1 expression groups. The most significantly enriched gene ontology and kyoto encyclopedia of genes and genomes (KEGG) items were selected according to NES.

Due to the limited space, several items of KEGG and GO significantly enriched in the high and low EFNA1 expression groups are shown in Figure 11. Detailed analysis results are listed in Table 5. Several biological process items associated with





**Figure 7.** The relationship between EFNA1 expression level and clinicopathological features of LGG patients. Grade III LGG has higher expression level of EFNA1, but no significant relationship was found between EFNA1 expression level and other features.

apoptotic pathway, cellular component items including rough endoplasmic reticulum and ficolin 1 rich granule and 1 molecular function item were enriched in the high EFNA1 expression group based on the NES, NOM P value, and false discovery rate value (Fig. 11). Several GO and KEGG items associated with nervous system function including dendrite morphogenesis and trans-synaptic signaling were enriched in the low EFNA1 expression group.

#### 4. Discussion

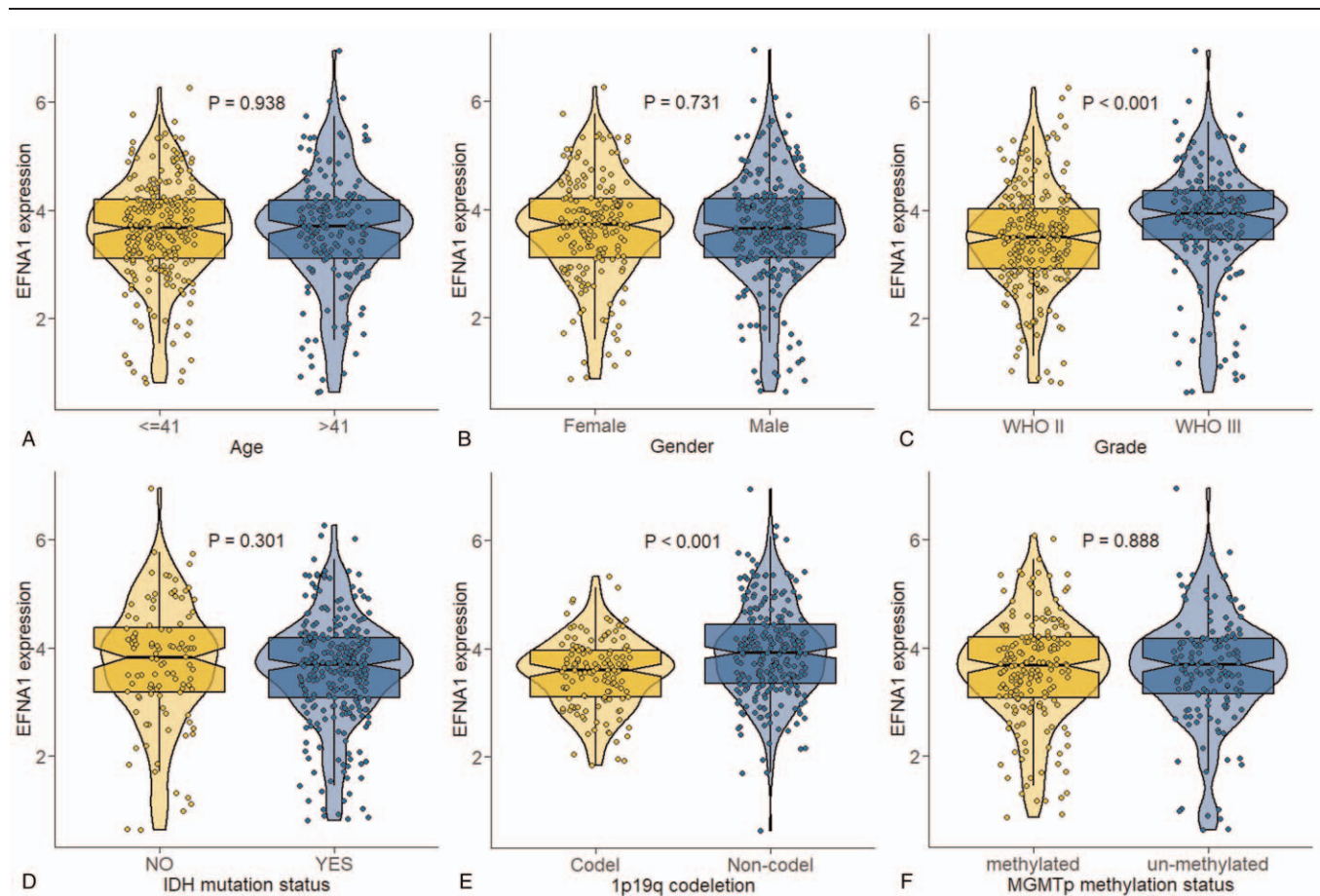
EFNA1 is a cell membrane protein and the common ligand of the EphA2 receptor.<sup>[6]</sup> EFNA1 is clearly involved in embryonic development<sup>[33]</sup> and is closely associated with the pathogenesis of many malignant tumors. The expression of EFNA1 affects tumor angiogenesis,<sup>[20]</sup> growth<sup>[34]</sup> and metastasis.<sup>[22,35]</sup> In the present study, we investigated the expression levels of EFNA1 and systematically showed its prognostic value in 33 types of cancer



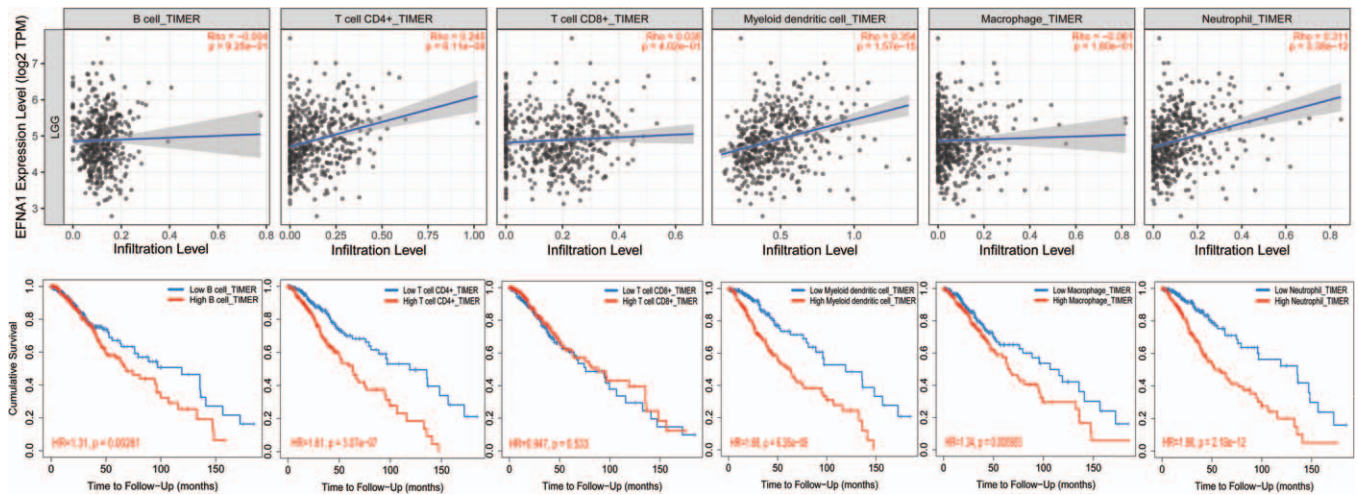
**Table 4**  
**Characteristics of primary LGG patients in the CGGA database.**

Clinical characteristics	number (Total N=426)
age	
<=41	239
>41	186
unknown	1
gender	
male	247
female	179
grade	
G2	232
G3	194
IDH mutation	
YES	289
NO	104
unknown	33
1p19q codeletion	
YES	137
NO	254
unknown	35
MGMTp_methylation_status	
methyalted	199
un-methyalted	155
unknown	72

using TCGA data via the Xena UCSC browser. We found that EFNA1 was highly expressed in bladder urothelial carcinoma, breast invasive carcinoma, CESC, cholangiocarcinoma, colon adenocarcinoma, ESCA, head and neck squamous cell carcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, PRAD, READ, stomach adenocarcinoma, thymoma, and UCEC compared to normal tissues, while EFNA1 had a lower level of expression in kidney chromophobe (Fig. 2). Our results are similar to those of previous studies.<sup>[11-13,36]</sup> We explored the prognostic value of EFNA1 for assessing the survival rate of human malignancies, and upregulated EFNA1 expression in ESCA, CESC, and LGG correlated with shorter patient OS times. These results are consistent with those of previous studies.<sup>[15,16]</sup> In addition, Cox analysis revealed that the expression of EFNA1 is a risk factor for the DSS rate and the PFI of LGG patients. The multiple Cox regression analysis revealed that EFNA1 expression is an independent prognostic factor for LGG patients. Regarding clinicopathological features, EFNA1 expression was significantly upregulated in grade III tumors compared with grade II tumors. Moreover, EFNA1 expression is upregulated in the 1p19q non-codeletion group, since EFNA1 is located on chromosome 1.<sup>[37]</sup> It is well established that tumor-infiltrating immune cells play a critical role in tumor development and control, but there is still some controversy.<sup>[32]</sup> One previous study showed that EFNA1-Fc treatment could promote CD8+ T cell recognition of EphA2+



**Figure 8.** The Wilcoxon signed-rank test was utilized to investigate the relationship between clinicopathological features and the expression of EFNA1 in LGG samples. (A) Age, (B) gender, (C) grade, (D) IDH mutation status, (E) 1p19q codeletion status, (F) MGMTp methylation status.

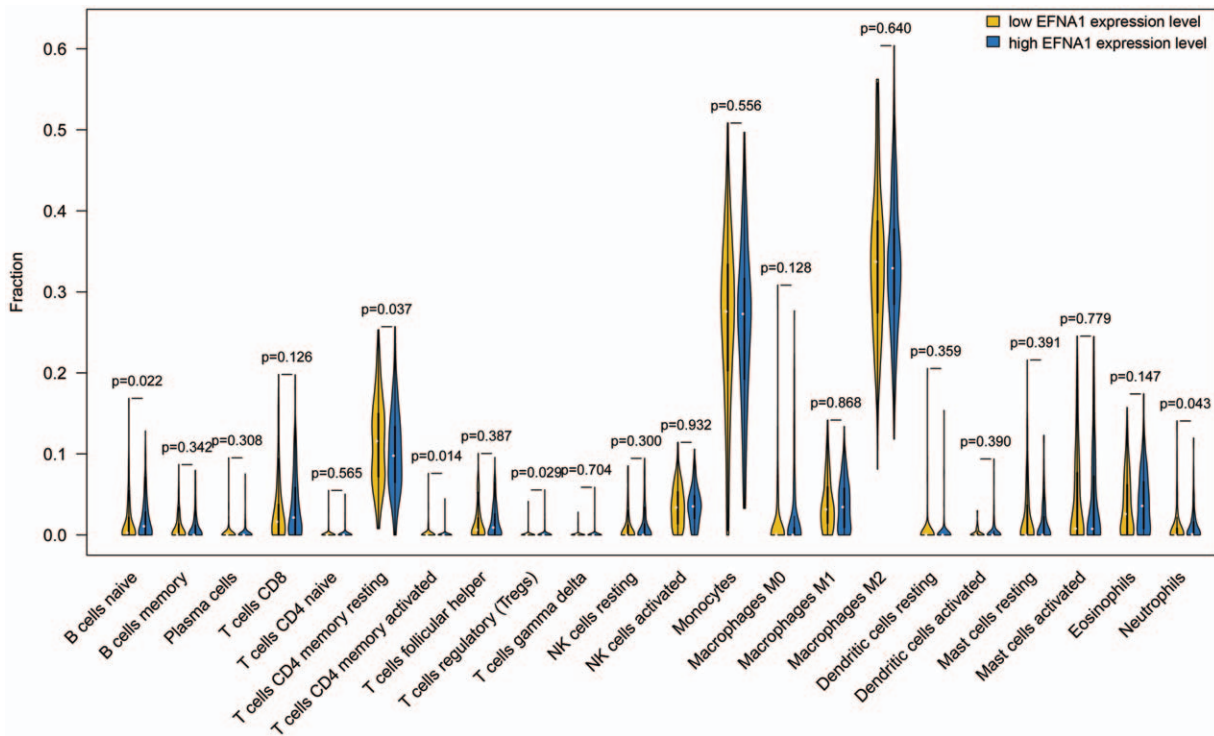


**Figure 9.** EFNA1 expression was positively correlated with the infiltration levels of CD4+ T cells, myeloid dendritic cells and neutrophils in LGG. Kaplan–Meier analysis showed that infiltrating levels of B cells, CD4+ T cells, myeloid dendritic cells, macrophages and neutrophils were related to the cumulative survival rate of LGG patients.

malignant cells both in vitro and in a HuSCID tumor model.<sup>[38]</sup> To examine the potential relationship between EFNA1 expression and immune cell infiltration in LGG, we used both TIMER and CIBERSORT. TIMER online analysis revealed that EFNA1 expression is positively correlated with the infiltration levels of CD4+ T cells and that a higher infiltration level of CD4+ T cells is correlated with the cumulative survival rate. This is similar to the

previous finding that a high level of CD4+ T cells supports tumor growth.<sup>[39]</sup>

Apoptosis is programmed cell death and plays an important role in maintaining homeostasis during biological development. Apoptosis involves 2 pathways, intrinsic and extrinsic. The intrinsic pathway is also referred to as the mitochondrial pathway since mitochondria play a key regulatory role in this process. In



**Figure 10.** The abundance ratios of 22 types of immune cells in EFNA1-high and EFNA1-low groups. B cells naive, resting memory CD4 T cells, activated memory CD4 T cells, regulatory T cells (Tregs), Neutrophils were associated with EFNA1 expression. B cells naive ( $P = .022$ ) was apparently increased but resting memory CD4 T cells ( $P = .037$ ) was decreased in EFNA1-high group compared with EFNA1-low group.

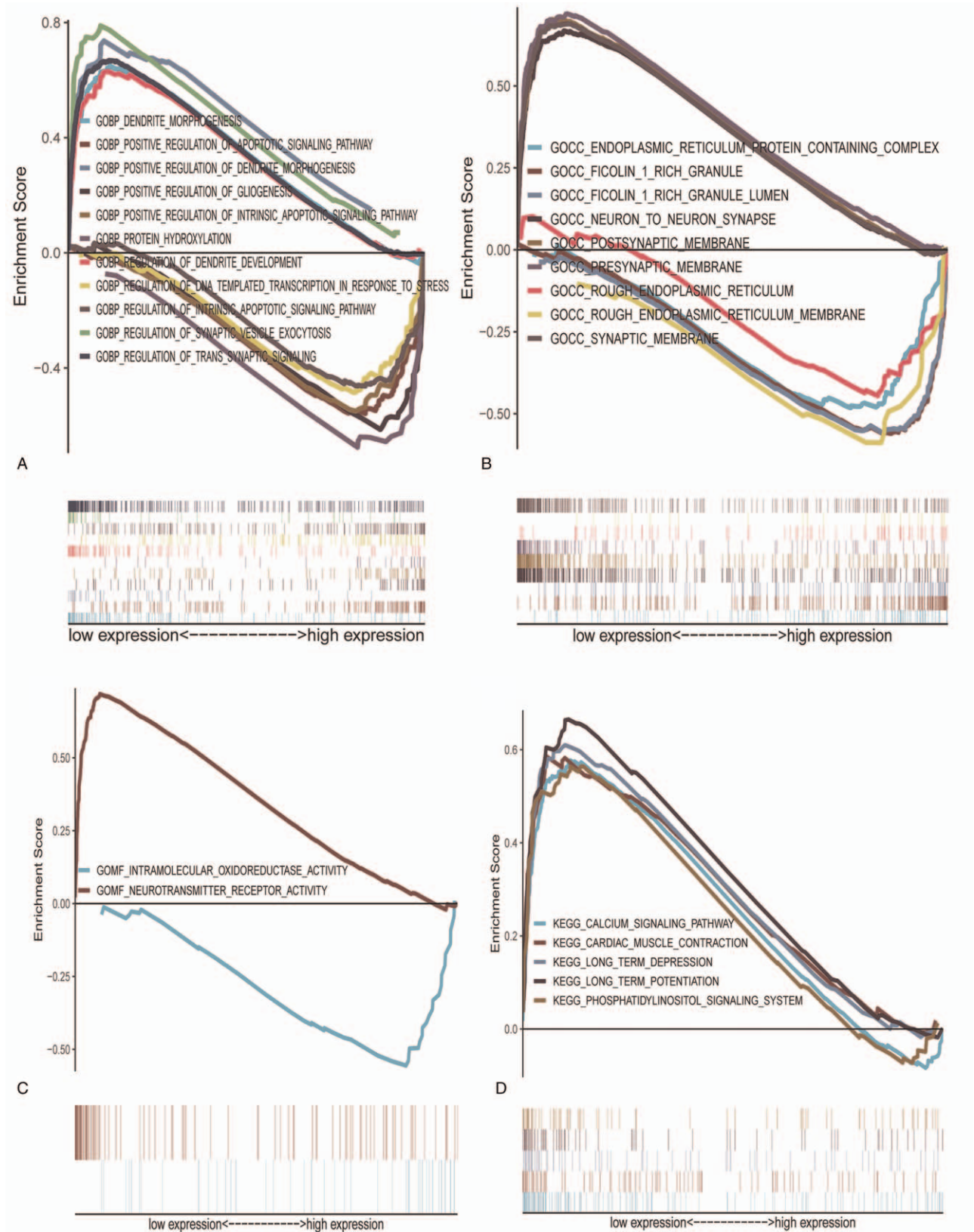


Figure 11. GSEA revealed the GO (A-C) and KEGG (D) items enriched in EFNA1-high and EFNA1-low groups.



**Table 5**  
**GO and KEGG items enriched in EFNA1-high and EFNA1-low expression group.**

NAME	NES	NOM P value	FDR q-value
EFNA1 high expression			
GOBP_POSITIVE_REGULATION_OF_INTRINSIC_APOPTOTIC_SIGNALING_PATHWAY	-2.3138468	<.001	0.044029858
GOBP_POSITIVE_REGULATION_OF_APOPTOTIC_SIGNALING_PATHWAY	-2.2724833	<.001	0.02217925
GOBP_REGULATION_OF_INTRINSIC_APOPTOTIC_SIGNALING_PATHWAY	-2.2109559	<.001	0.029042864
GOBP_REGULATION_OF_DNA_TEMPLATED_TRANSCRIPTION_IN_RESPONSE_TO_STRESS	-2.210729	<.001	0.025020886
GOBP_PROTEIN_HYDROXYLATION	-2.1933506	<.001	0.02816608
GOBP_POSITIVE_REGULATION_OF_GLIOGENESIS	-2.1829705	<.001	0.029549599
GOCC_ROUGH_ENDOPLASMIC_RETICULUM_MEMBRANE	-2.2332995	.002066116	0.030233005
GOCC_FICOLIN_1_RICH_GRANULE_LUMEN	-2.216532	.002024292	0.031349164
GOCC_ENDOPLASMIC_RETICULUM_PROTEIN_CONTAINING_COMPLEX	-2.168786	.002096436	0.031654213
GOCC_FICOLIN_1_RICH_GRANULE	-2.138414	.002004008	0.043375626
GOCC_ROUGH_ENDOPLASMIC_RETICULUM	-2.1138983	<.001	0.052571848
GOMF_INTRAMOLECULAR_OXIDOREDUCTASE_ACTIVITY	-2.308332	<.001	0.022756176
EFNA1 low expression			
GOBP_POSITIVE_REGULATION_OF_DENDRITE_MORPHOGENESIS	2.1522028	<.001	0.04428665
GOBP_REGULATION_OF_SYNAPTIC_VESICLE_EXOCYTOSIS	2.1490028	<.001	0.039780173
GOBP_DENDRITE_MORPHOGENESIS	2.1407454	<.001	0.03932195
GOBP_REGULATION_OF_DENDRITE_DEVELOPMENT	2.1399865	<.001	0.032448564
GOBP_REGULATION_OF_TRANS_SYNAPTIC_SIGNALING	2.1368196	<.001	0.03049569
GOCC_SYNAPTIC_MEMBRANE	2.1272392	<.001	0.028805934
GOCC_POSTSYNAPTIC_MEMBRANE	2.1244895	<.001	0.025740024
GOCC_PRESYNAPTIC_MEMBRANE	2.1161785	<.001	0.025564382
GOCC_NEURON_TO_NEURON_SYNAPSE	2.1123252	<.001	0.02492334
GOMF_NEUROTRANSMITTER_RECEPTOR_ACTIVITY	2.1401203	<.001	0.035520807
KEGG_LONG_TERM_DEPRESSION	2.2189817	<.001	0.009844807
KEGG_LONG_TERM_POTENTIATION	2.1982136	<.001	0.00527287
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	1.980789	<.001	0.035550725
KEGG_CARDIAC_MUSCLE_CONTRACTION	1.9510839	<.001	0.035533823
KEGG_CALCIIUM_SIGNALING_PATHWAY	1.9433945	<.001	0.030962013

FDR = false discovery rate, NES = normalized enrichment score, NOM = nominal. Gene sets with NOM *P* value <.05 and FDR q-value <.05 are considered as significant.

cancers, however, the apoptotic pathway is usually inhibited, enhancing the proliferation and invasiveness of malignant cells. Targeting proteins in the apoptotic pathway to regulate the apoptotic process of cancer cells by upregulating proapoptotic protein expression and downregulating antiapoptotic protein expression is a future direction of cancer therapy.<sup>[40]</sup> In the case of malignant glioma, apoptosis resistance is also one of the reasons for its unsatisfactory response to therapy.<sup>[41,42]</sup> Several studies have revealed the mechanisms underlying the resistance of glioma to therapy.<sup>[41]</sup> Our study showed that the intrinsic apoptotic pathway was significantly enriched in the EFNA1 high-expression phenotype. These results suggest that EFNA1 may regulate the occurrence and development of LGG by modulating the apoptotic pathways.

In conclusion, in the present study, we analyzed integrated data from the CGGA and TCGA databases to investigate the expression of EFNA1 in different cancers and its ability to predict glioma patient survival. Our results suggested an emerging role for EFNA1 in the evaluation of LGG survival rates and revealed the association between EFNA1 expression and the clinical characteristics of LGG. TIMER and CIBERSORT analyses demonstrated that EFNA1 was associated with immune cell infiltration. GSEA suggested that EFNA1 may participate in LGG regulation via apoptotic pathways. Taken together, our findings identified that EFNA1 may play a pivotal role in the development of LGG and may serve as a potential marker of LGG prognosis and therapy. However, due to technological limitations, we did not carry out further experimental verification. More *in vitro* and *in vivo* studies are

warranted to reveal the mechanisms of EFNA1's function in LGG in future studies.

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