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The high expression of glial cell line-derived neurotrophic factor receptor alpha II (*GFRA2*) as a predictor of poor prognosis in gastric cancer patients: A survival and regression analysis approach

Shaoyu Yang [a,b,1], Juan Li [c,1], Xiaohui Cai [a,*]

[a] College of Marine Sciences, Guangxi Key Laboratory of Beibu Gulf Marine Biodiversity Conservation, Beibu Gulf University, Qinzhou 535011, China
[b] Nanchang Institute of Technology, Nanchang, 330044, China
[c] School of Basic Medicine, Guizhou University of Traditional Chinese Medicine, Guiyang, 550025, China

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ABSTRACT

Gastric cancer has high mortality rates worldwide. Therefore, there is a need to identify prognostic biomarkers. This study evaluated the association between *GFRA2* expression levels with clinicopathological features and prognosis in gastric cancer using data extracted from The Cancer Genome Atlas (TCGA) database and a series of algorithms. Survival analysis was performed using the Kaplan-Meier method. Univariate and multivariate Cox regression analyses were used to analyze the association between different clinical features and survival. Single-sample gene set enrichment analysis (GSEA) was used to examine the correlation between *GFRA2* expression and immune infiltration. The results showed that the expression of *GFRA2* in tumor samples was significantly lower than that in normal samples. High expression of *GFRA2* was significantly associated with histological type, histologic grade, and worse overall survival, disease-specific survival, and progression-free survival. The univariate Cox analysis showed that the expression of *GFRA2* was significantly correlated with T stage, N stage, M stage, and age. The multivariate analysis identified *GFRA2* expression as an independent prognostic factor for gastric cancer. GSEA showed that *GFRA2* might regulate the calcium signaling pathway, focus adhesion, olfactory conduction, the extracellular matrix glycoproteins, and response to the Leishmania parasitic infection. *GFRA2* showed a significant moderate positive correlation with the infiltration of mast cells. In summary, a high expression of *GFRA2* may contribute to poor survival in gastric cancer patients and could be used as a potential prognostic biomarker.

1. Introduction

Gastric cancer is one of the deadliest malignancies worldwide and is highly prevalent in Asia and some South American countries [1]. It has become a serious public health issue, with more than 1 million new cases diagnosed worldwide in recent years [2,3]. Even

* Corresponding author. No. 12, Binhai Avenue, Qinzhou, Guangxi, PR China,
E-mail address: caixiaohui66@163.com (X. Cai).
[1] These authors contributed equally.

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though the incidence of stomach cancer is expected to decline in most countries over the next decade [4], survival from this disease remains poor worldwide [5]. Gastric cancer is a diverse multifactorial disease. The risk factors for the development of this disease include a genetic predisposition, heavy alcohol consumption, a diet high in excessive salt and smoked food [6], and *Helicobacter pylori* [7,8] or *Epstein Barr virus* [9] infections. The World Health Organization (WHO) classifies gastric cancer into 4 main subtypes; papillary, tubular, indurated, and mucinous [10]. The TNM staging system is used to classify the extent of the disease based on the size of the tumor, lymph node involvement, and the presence of distant metastasis [11].

The early symptoms of gastric cancer include poor digestion and acid reflux. As the disease progresses, patients often experience gastrointestinal bleeding, vomiting, and other adverse gastrointestinal symptoms [12]. Most patients are diagnosed at an advanced stage, and as a result, the survival from this disease worldwide is low, with only 25% of patients with advanced gastric cancer surviving more than 5 years [6]. The treatment options for patients diagnosed with advanced disease are limited. Surgery is generally not a viable option for patients with advanced gastric cancer. Furthermore, these tumors are highly resistant to chemotherapy [13–15]. Therefore, an early diagnosis of gastric cancer is essential to improve treatment outcomes.

The current diagnostic workout for gastric cancer involves an endoscopic examination combined with computed tomography (CT), magnetic resonance imaging (MRI), and other radiographic imaging techniques [16,17]. However, the sensitivity and specificity of these imaging techniques in identifying lymph node metastases and peritoneal disease are limited, eventually leading to an approximately 20% false positive rate [18–21]. Therefore, there is an urgent need to identify biomarkers that can detect and characterize the disease at various stages.

Currently, a few biomarkers are available to diagnose and predict the prognosis of gastric cancer. Carbohydrate antigen 19–9 (*CA19-9)* and Herceptin-2 (*HER2)* are among the most commonly used biomarkers clinically [22]. However, these biomarkers have several limitations. For example, the sensitivity and specificity of *CA19-9* as a biomarker for the early recurrence of gastric cancer need to be improved [23]. Furthermore, not all patients with gastric cancer showed a *HER2*-positive phenotype [24]. Therefore, there is a need to identify new potential diagnostic markers for gastric cancer.

Glial cell line-derived neurotrophic factor receptor alpha 2 (*GFRA2*) is a receptor of the glial cell-derived neurotrophic factor family (GDNF) that has received attention for its role in the treatment of Parkinson's disease [25]. Recent studies have shown that overexpression of *GFRA2* can promote cell proliferation in neuroblastoma [26] and can also play an important role in the growth of pancreatic cancer [27]. However, the expression pattern of *GFRA2* in gastric cancers is still poorly understood. Moreover, the impact of *GFRA2* on prognosis and survival in gastric cancer is still unclear. Further studies are required to evaluate its role as a potential prognostic marker for gastric cancer. Recent advances in bioinformatics have paved the way for identifying potential prognostic markers for gastric cancer. Therefore, in this study, we used The Cancer Genome Atlas (TCGA) and extensive bioinformatics methods to identify potential prognostic biomarkers associated with specific gastric cancers that could be used to guide treatment interventions.

2. Materials and methods

2.1. Evaluation of the differential expression of GFRA2 in pan-cancer and gastric cancer

Ribose nucleic acid sequencing (RNAseq) data were downloaded in the TPM format from the USCS XENA database (https://xenabrowser.net/datapages/), log2-transformed, and then grouped for comparison for the pan-cancer analysis of *GFRA2*. A total of 32 paraneoplastic and 375 gastric tumor samples were collected. The data were first collected in the level 3 HTSeq-FPKM format, then converted into the TPM format, and log2-transformed. Subsequently, we calculated the differential expression pattern of *GFRA2* in unpaired and paired gastric cancer samples using the Wilcoxon rank sum test and paired samples *t*-test. All the data were analyzed using R software version 3.6.3 and visualized using the ggplot2 software version 3.3.3.

2.2. Correlation of GFRA2 expression with clinicopathological features

The Wilcoxon signed rank tests, Kruskal-Wallis tests, Dunn's tests, logistic regressions, Fisher's exact tests, and Chi-square tests were used to analyze the relationship between *GFRA2* expression and clinicopathological features. The TNM stage, pathological stage, sex, race, age, histological grade, and histological type were chosen as the clinicopathological features. All analyses were done using the online tool (https://www.xiantaozi.com/).

2.3. Functional enrichment analysis of GFRA2 and its related genes

Gene set enrichment analysis (GSEA) was performed to identify the genes co-expressed with *GFRA2* in gastric cancer. The GSEA was carried out using the ClusterProfiler R package 3.14.3 [28]. The reference gene was set to c2.cp.v7.2.symbols.gmt, and each gene set had between 10 and 500 genes. The number of calculations for GSEA analysis was set to 1000. A gene set was considered significantly enriched if it had a false discovery rate (FDR) below 0.25 and an adjusted p-value below 0.05. The Benjamini and Hochberg (BH) method was used to correct the *p*-value.

2.4. Kaplan-Meier and receiver operating characteristics (ROC) curve analyses

The median *GFRA2* expression value was used to categorize gastric cancer samples into high and low expression groups. The disease-specific survival (DSS), progress-free interval (PFI), and OS were evaluated using Kaplan-Meier curves. The curves were plotted

using the Survival version 3.3 and the Survminer version 0.4.9 packages available in the R software. In addition, the area under the curve (AUC) of a ROC curve was calculated to evaluate the efficacy of *GFRA2* in predicting prognosis in gastric cancer. The ROC analysis was performed using the pROC package version 1.17 available on the R software [29].

2.5. Correlation analysis of GFRA2 expression and immunological infiltration

The correlation between the *GFRA2* mRNA expression levels in gastric cancer and the relative abundance of the 24 immune cells was assessed using the single-sample GSEA algorithm and Spearman's rank correlation coefficient with the GSVA R package [30]. The 24 immune cells were derived from previously published literature [31]. The differences in immune cell infiltration between the high and low *GFRA2* expression groups were calculated.

3. Results

3.1. Differential expression of GFRA2 in pan-cancer and gastric cancer

When compared with normal tissue samples, the *GFRA2* gene expression level was significantly lower in most tumor samples and higher in nine cancer types, including diffuse Large B-cell Lymphoma (DLBC), kidney renal clear cell carcinoma (KIRC), acute myeloid leukemia (LAML), low-grade glioma (LGG), liver hepatocellular carcinoma (LIHC), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), thymoma (THYM), and uterine carcinosarcoma (UCS) ($p < 0.001$) (Fig. 1A).

The differential expression pattern of *GFRA2* in the unpaired samples revealed significantly lower *GFRA2* expression levels in the tumor samples than in normal tissue samples (Fig. 1B). Similarly, in the paired sample analysis, the expression of *GFRA2* was significantly lower in tumor samples than in normal tissue samples (Fig. 1C).

3.2. Association between GFRA2 expression and clinicopathological features

GFRA2 expression was significantly associated with the histological type ($p = 0.004$), histological grade ($p = 0.005$), and OS event ($p = 0.007$) in gastric cancer (Table 1). As shown in Fig. 2, the *GFRA2* expression levels were associated with histological subtype (diffuse type versus tubular type, $p < 0.001$; mucinous type versus tubular type, $p = 0.011$, Fig. 2A), histologic grade (G2 vs. G3, $p < 0.001$, Fig. 2B), and OS event (alive versus dead, $p < 0.01$, Fig. 2C). High and low levels of *GFRA2* expression were also correlated with the histological type ($p < 0.05$) (Table 1).

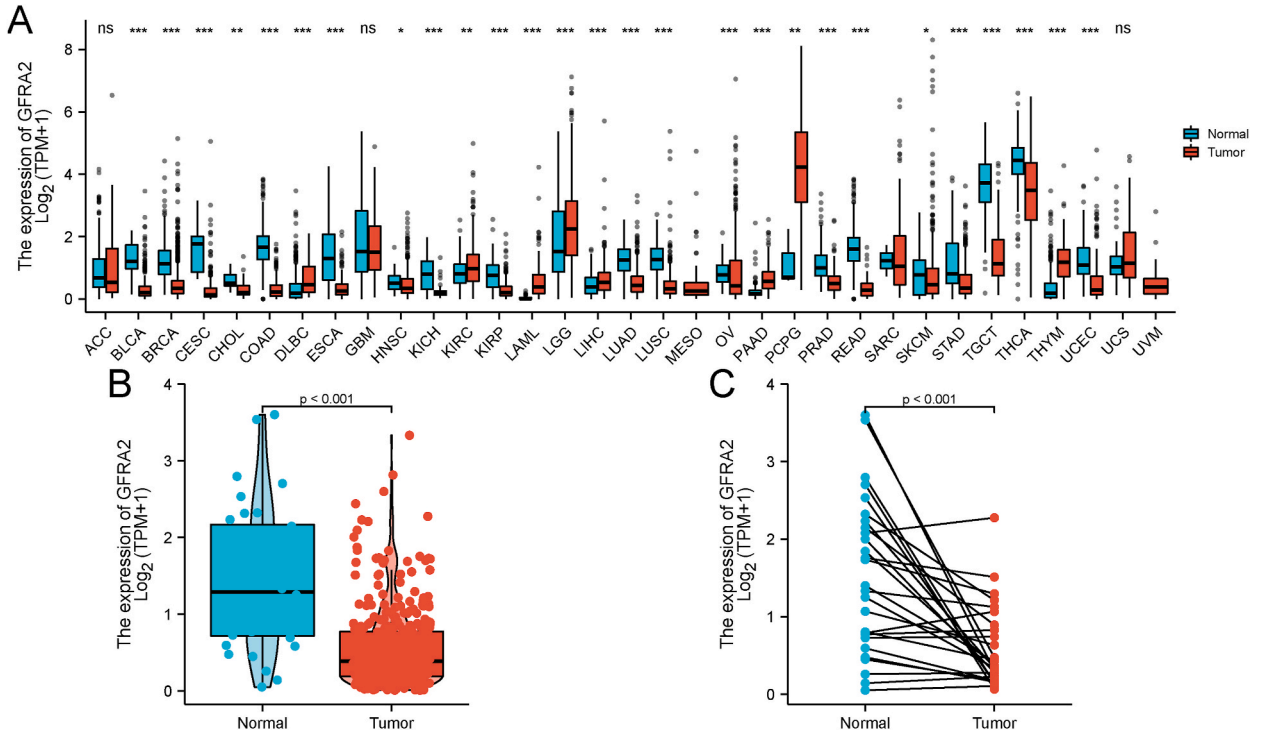

Fig. 1. Differential expression patterns of *GFRA2* in pan-cancer and gastric cancer. (A) *GFRA2* expression profiles in pan-cancer. (B) *GFRA2* expression patterns in unpaired samples of gastric cancer tissues and noncancerous tissues. (C) *GFRA2* expression patterns in paired gastric cancer and noncancerous samples. The blue color represents normal samples, and the red color represents cancer samples.

The logistic regression analysis using the high and low expression levels of *GFRA2* as dichotomous variables revealed a significant correlation between the expression of *GFRA2* and clinical characteristics, including histological type (tubular type versus diffuse type, odds ratio (OR) = 0.351, 95% confidence interval (CI): 0.170–0.708, $p < 0.01$) and histologic grade (G3 vs. G2, OR = 1.933, 95% (CI): 1.256–2.995, $p < 0.01$) (Table 2). Conversely, patients with low *GFRA2* expression were more likely to develop G3 and the diffuse histological subtype than those with high *GFRA2* expression levels.

Table 3 illustrates the results of the univariate Cox analysis. The findings of this analysis indicate that a higher T stage (HR = 1.719, 95% confidence interval (CI): 1.131–2.612, $p = 0.011$), N stage (HR = 1.925, 95% confidence interval (CI): 1.264–2.931, $p = 0.002$), M stage (HR = 2.254, 95% confidence interval (CI): 1.295–3.924, $p = 0.004$), age (HR = 1.620, 95% confidence interval (CI): 1.154–2.276, $p = 0.005$), and *GFRA2* expression levels (HR = 1.710, 95% confidence interval (CI): 1.225–2.386, $p = 0.002$) resulted in a worse prognosis. Notably, a higher T stage was linked with a worse prognosis. The *GFRA2* expression level was also identified as a significant factor influencing prognosis in the multivariate Cox analyses ($p < 0.001$). Therefore, *GFRA2* was identified as an independent prognostic factor for gastric cancer. Likewise, N stage, M stage, and patient age were also identified as independent factors affecting the prognosis of gastric cancer.

3.3. Functional enrichment analysis of GFRA2 and its related genes

As shown in Fig. 3, based on the GSEA, 5 metabolic pathways were found to be significantly enriched by *GFRA2,* including; the calcium signaling pathway (Normalized Enrichment score (NES) = 1.708, p.adj = 0.033, FDR = 0.025), focus adhesion (NES = 1.498, p.adj = 0.033, FDR = 0.025), olfactory conduction (NES = 1.498, p.adj = 0.033, FDR = 0.025), Extracellular matrix (ECM) glycoprotein (NES = 1.832, p.adj = 0.033, FDR = 0.025), and a favorable response for the Leishmania Parasitic infection (NES = 1.914, p.adj = 0.033, FDR = 0.025).

3.4. High expression of GFRA2 affects the prognosis of patients with gastric cancer

Patients in the high *GFRA2* expression subgroup had a significantly worse OS than those in the *GFRA2* low expression subgroup

Table 1
Association of *GFRA2* expression with clinicopathological features in gastric cancer.

Characteristic	Low expression of GFRA2	High expression of GFRA2	p	statistic	method
N	187	188			
T stage, n (%)			0.267	3.95	Chisq.test
T1	13 (3.5%)	6 (1.6%)			
T2	38 (10.4%)	42 (11.4%)			
T3	89 (24.3%)	79 (21.5%)			
T4	46 (12.5%)	54 (14.7%)			
N stage, n (%)			0.104	6.17	Chisq.test
N0	59 (16.5%)	52 (14.6%)			
N1	56 (15.7%)	41 (11.5%)			
N2	37 (10.4%)	38 (10.6%)			
N3	29 (8.1%)	45 (12.6%)			
M stage, n (%)			0.188	1.74	Chisq.test
M0	171 (48.2%)	159 (44.8%)			
M1	9 (2.5%)	16 (4.5%)			
Pathologic stage, n (%)			0.184	4.84	Chisq.test
Stage I	29 (8.2%)	24 (6.8%)			
Stage II	59 (16.8%)	52 (14.8%)			
Stage III	78 (22.2%)	72 (20.5%)			
Stage IV	13 (3.7%)	25 (7.1%)			
Histological type, n (%)			0.004	17.11	Chisq.test
Diffuse Type	27 (7.2%)	36 (9.6%)			
Mucinous Type	5 (1.3%)	14 (3.7%)			
Not Otherwise Specified	102 (27.3%)	105 (28.1%)			
Papillary Type	2 (0.5%)	3 (0.8%)			
Signet Ring Type	3 (0.8%)	8 (2.1%)			
Tubular Type	47 (12.6%)	22 (5.9%)			
Age, n (%)			0.280	1.17	Chisq.test
≤65	77 (20.8%)	87 (23.5%)			
>65	110 (29.6%)	97 (26.1%)			
Histologic grade, n (%)			0.005		Fisher.test
G1	3 (0.8%)	7 (1.9%)			
G2	83 (22.7%)	54 (14.8%)			
G3	97 (26.5%)	122 (33.3%)			
OS event, n (%)			0.007	7.34	Chisq.test
Alive	127 (33.9%)	101 (26.9%)			
Dead	60 (16%)	87 (23.2%)			
Age, meidan (IQR)	68 (59, 74)	66 (57, 73)	0.150	18689.5	Wilcoxon

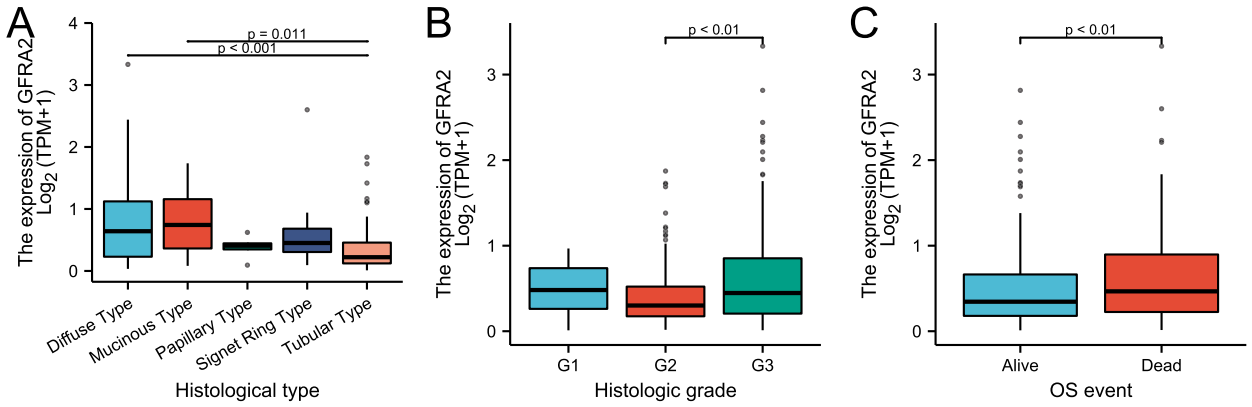

Fig. 2. Correlation between *GFRA2* expression and clinicopathological features. (A) Histological type, (B) histologic grade, (C) OS event.

Table 2
Logistic regression analysis of *GFRA2* expression and clinicopathological features.

Characteristics	Total(N)	Odds Ratio (OR)	P value
T stage (T3&T4 vs. T1&T2)	367	1.047 (0.660–1.662)	0.846
N stage (N1&N2&N3 vs. N0)	357	1.153 (0.736–1.810)	0.534
M stage (M1 vs. M0)	355	1.912 (0.837–4.633)	0.133
Gender (Male vs. Female)	375	1.333 (0.873–2.040)	0.183
Race (Black or African American vs. Asian)	85	0.520 (0.107–1.961)	0.362
Age (>65 vs.≤65)	371	0.780 (0.517–1.176)	0.237
Histological type (Tubular Type vs. Diffuse Type)	132	0.351 (0.170–0.708)	0.004
Histologic grade (G3 vs. G2)	356	1.933 (1.256–2.995)	0.003
Residual tumor (R1&R2 vs. R0)	329	1.971 (0.928–4.395)	0.084
H pylori infection (Yes vs. No)	163	0.928 (0.325–2.495)	0.883

Table 3
Univariate and multivariate Cox analysis of *GFRA2* expression.

		Univariate analysis		Multivariate analysis	
Characteristics	Total(N)	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
T stage	362				
T1&T2	96	Reference			
T3&T4	266	1.719 (1.131–2.612)	**0.011**	1.404 (0.889–2.219)	0.146
N stage	352				
N0	107	Reference			
N1&N2&N 3	245	1.925 (1.264–2.931)	**0.002**	1.708 (1.087–2.682)	**0.020**
M stage	352				
M0	327	Reference			
M1	25	2.254 (1.295–3.924)	**0.004**	2.365 (1.329–4.211)	**0.003**
Age	367				
≤65	163	Reference			
>65	204	1.620 (1.154–2.276)	**0.005**	1.935 (1.348–2.778)	**<0.001**
Histological type	132				
Diffuse Type	63	Reference			
Tubular Type	69	0.929 (0.534–1.614)	0.793		
Histologic grade	144				
G1	10	Reference			
G2	134	1.615 (0.391–6.671)	0.507		
GFRA2	370				
Low	185	Reference			
High	185	1.710 (1.225–2.386)	**0.002**	1.898 (1.340–2.688)	**<0.001**

(HR = 1.71, 95% (CI): 1.23–2.39, $p = 0.002$) (Fig. 4A). Similarly, patients in the high *GFRA2* expression subgroup had significantly worse DSS (HR = 1.75, 95% (CI): 1.15–2.68, $p = 0.009$) and PFS (HR = 1.61, 95% (CI): 1.13–2.30, $p = 0.009$) than those in the low expression group (Fig. 4B and C). The AUC of *GFRA2* in predicting OS in gastric cancer was 0.807 (95%CI 0.720–0.894), which indicates that *GFRA2* has good prediction accuracy.

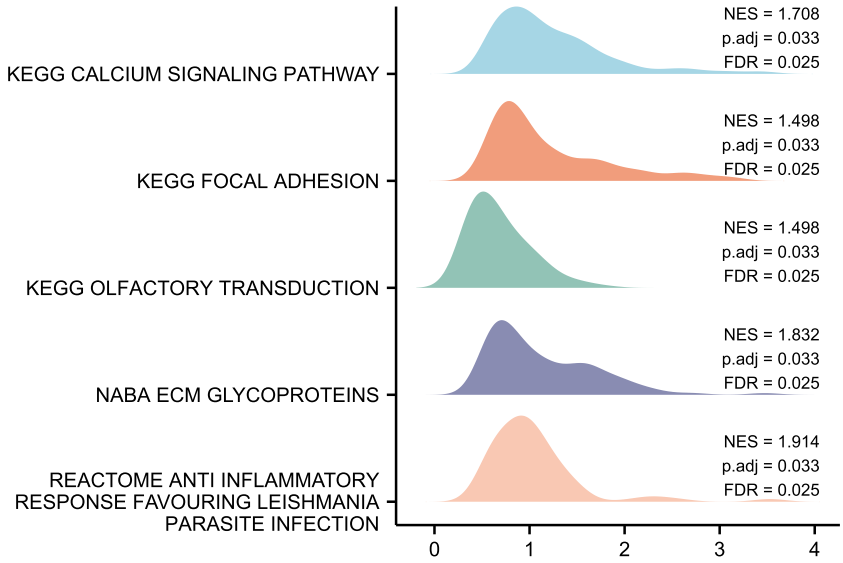

Fig. 3. Functional enrichment analysis of *GFRA2* and its related genes. The different colors represent the signaling pathways enriched with *GFRA2* and its related genes.

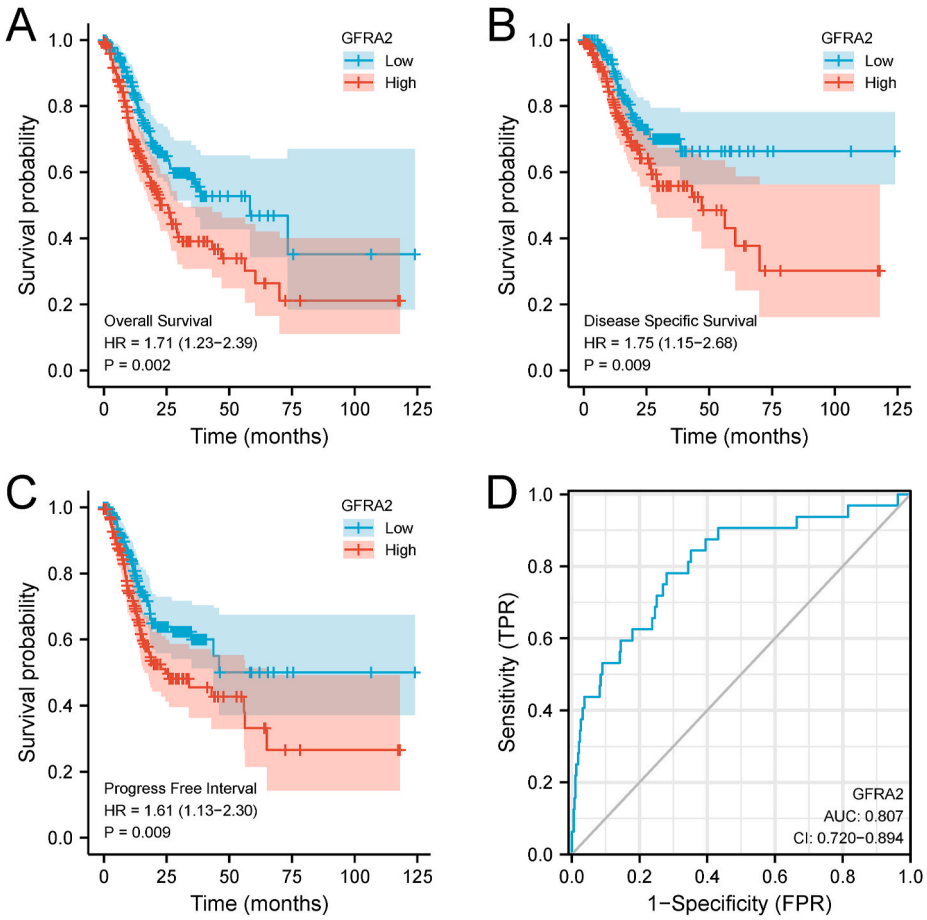

Fig. 4. Kaplan-Meier curves for high- and low-*GFRA2*-expression subgroups of gastric cancer patients and ROC curves for *GFRA2*. DSS (disease-specific survival), PFI (progression-free interval), OS (overall survival) were considered for survival analysis. (A) Overall survival, (B) disease-specific survival, (C) progression-free interval, (D) ROC curves.

3.5. Correlation analysis of GFRA2 expression and immune infiltration

The correlation between *GFRA2* expression and immune infiltration of the 24 immune cells using the ssGSEA algorithm is illustrated in Fig. 5A. The results revealed a significantly moderate correlation between *GFRA2* expression and the infiltration of mast cells ($r = 0.551$, $p < 0.001$) (Fig. 5B). *GFRA2* also showed a significant moderate positive correlation with DCs ($r = 0.528$, $p < 0.001$) (Fig. 5C), iDCs ($r = 0.517$, $p < 0.001$), NK cells ($r = 0.515$ $p < 0.001$), Tems ($r = 0.491$, $p < 0.001$), TFHs ($r = 0.488$, $p < 0.001$), B cells ($r = 0.461$, $p < 0.001$), pDCs ($r = 0.445$, $p < 0.001$) and eosinophils ($r = 0.426$, $p < 0.001$). In addition, *GFRA2* also showed a significantly weak positive correlation with macrophages ($r = 0.375$, $p < 0.001$), T cells ($r = 0.374$, $p < 0.001$), Tgds ($r = 0.367$, $p < 0.001$), CD8 T cells ($r = 0.354$, $p < 0.001$), Tcms ($r = 0.306$, $p < 0.001$), cytotoxic cells ($r = 0.299$, $p < 0.001$), Th1 cells ($r = 0.272$, $p < 0.001$), Tregs ($r = 0.204$, $p < 0.001$). Conversely, *GFRA2* showed a significantly weak negative correlation with Th2 cells ($r = -0.190$, $p < 0.001$) (Fig. 5D).

A weak correlation was noted between *GFRA2* expression levels and DCs ($r = 0.143$), Th17 cells ($r = -0.154$), neutrophils ($r = 0.140$), T helper cells ($r = 0.091$), NK CD56dim cells ($r = 0.072$), and NK CD56bright cells ($r = -0.002$) (Table S1). Moreover, the *GFRA2* was significantly more expressed in the mast cells, DCs, and Th2 cells in patients in the high *GFRA2* expression group than those in the low *GFRA2* expression group ($p < 0.001$) (Fig. 5E–G).

4. Discussion

Currently, the number of biomarkers that could be used to determine prognosis in gastric cancer is limited. Public databases could be used to identify new biomarkers for diagnosing and treating gastric cancer. In this study, we used the TCGA data to identify potential prognostic markers associated with gastric cancer. We examined the *GFRA2* differential expression in pan-cancer samples and in unpaired and paired gastric cancer samples. Furthermore, we conducted a correlation analysis of clinicopathological factors associated with *GFRA2* expression. We then evaluated the impact of the *GFRA2* expression levels on the prognosis of gastric cancer and its predictive power. Subsequently, GSEA was used to analyze the possible metabolic pathways associated with *GFRA2* and their possible biological functions in the development of gastric cancer. Finally, we examined the correlation between *GFRA2* expression and immune infiltration levels of 24 immune cells.

The study of gastrointestinal disorders increasingly focuses on neurotrophic factors because of their ability to modulate innervation, sensation, and neuroplasticity [27]. In particular, the glial cell-derived neurotrophic factor (GDNF) receptor family members are increasingly being studied due to their links with the development, survival, and maintenance of differentiation of peripheral autonomic neurons, enteric neurons, and sensory neurons. This receptor family contains four members, GDNF, neurturin (NTN), artemin, and persephin [32]. GDNF and its cognate protein NTN are both growth factors that promote the survival of motor and sensory neurons. GDNF and NTN preferentially bind to the co-receptor proteins GFRA1 and GFRA2, leading to the activation of the ret proto-oncogene (RET) tyrosine kinase receptor [33–36]. The RET tyrosine kinase receptor is activated to recruit different downstream effector molecules to perform various biological functions [37]. The absence of RET signaling has been linked with Hirschsprung disease [38], while the overstimulation of RET has been linked with cancer development [39,40]. Studies have shown that the GFRA/RET complex can activate the PI3K/AKT complex that has been linked with cancer development [41,42].

The glial cell-derived neurotrophic factor receptor GFRA2 is normally bound to NTN to form a complex involved in the survival of neurons [43]. Mutations in *GFRA2* are associated with diseases such as diabetic neuropathic pain [44], as well as defects in the innervation of gastrointestinal neurons and growth retardation [45]. In mice, GFRA2 and NTN were found to work together and promote the growth of axons in neurons. When *GFRA2* deficient neurons were stimulated by NTN alone, axon initiation did not occur [46]. *Cis*-activation of RET by GFRA2/NTN impacted projection nerve survival and central projection growth in rapidly adapting mechanoreceptors in mice [37]. This mechanoreceptor is an important neuron that mediates the discrimination of tactile sensations [47]. Similarly, studies in pancreatic cancer have shown that *GFRA2* expression was associated with tumor size, severe pain, and neuroinvasiveness. GFRA2, together with NTN, was also found to be upregulated in pancreatic cancer [48]. Conversely, our findings showed lower expression levels of *GFRA2* in gastric cancer. In addition, our survival analysis confirmed that a high expression level of *GFRA2* is predictive of poor prognosis in gastric cancer. However, in our study, it was not possible to compare the difference in *GFRA2* expression between patients with severe gastric cancer pain and those without pain. Therefore, further research is required to evaluate the impact of *GFRA2* expression and pain in gastric cancer.

This study has some limitations that have to be acknowledged. In this study, we could not precisely elucidate the pathogenic mechanisms of GFRA2 in gastric cancer. Although we identified that the activation of the PI3K/AKT oncogenic pathway, *GFRA2* overexpression, and the activation of RET might have a role in the pathogenesis of gastric cancer, other pathways may be involved. For example, *GFRA2* also plays an important role in pituitary plasticity and neuronal axon growth [49] and the maintenance of cellular ecological niche homeostasis [50]. In the pituitary gland, RET regulates the production of growth hormones. However, cancers can lead to an overexpression of *GFRA2,* which in turn causes the overactivation of RET and the abnormal secretion of hormones. Excessive hormone production can destabilize the intracellular ecological niche, eventually leading to cancer progression. Therefore, further research is recommended to evaluate the molecular pathways involved in cancer progression linked with *GFRA2* overexpression. The current study involves the identification of potential markers for gastric cancer through the analysis of large-scale sequencing data, providing the advantage of bulk marker mining at the genomic level over traditional experimental techniques. Multi-omics technologies and artificial intelligence are increasingly used to identify suitable predictive cancer biomarkers. It is believed that in the future, the integration of multi-omics data and more sophisticated algorithms will play a crucial role in identifying gastric cancer markers and the pathways involved in carcinogenesis. However, current omics studies have several limitations. First, omics studies

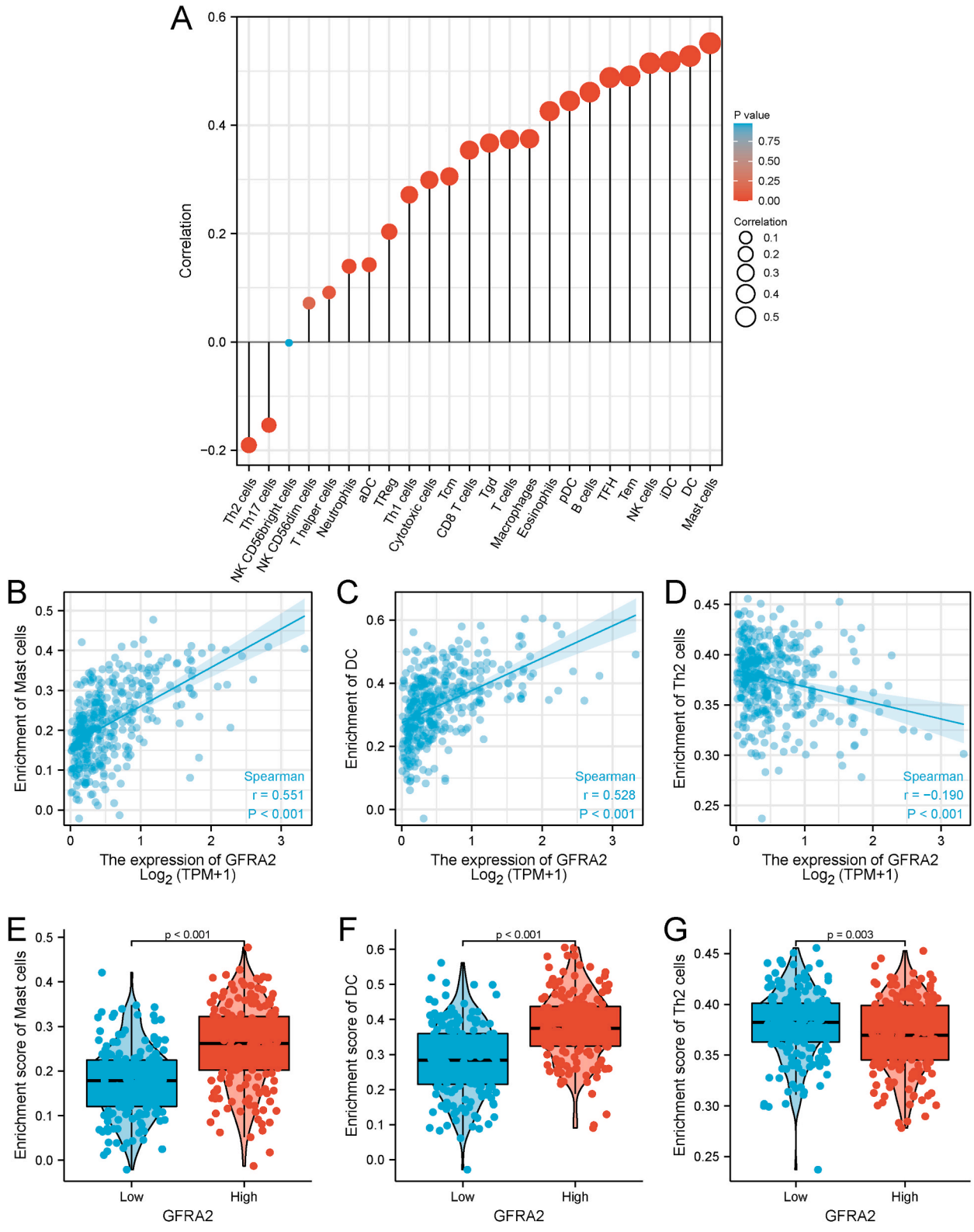

Fig. 5. The correlation between *GFRA2* expression and immune infiltration levels of 24 immune cells, and the significance of mast cells, DCs, and Th2 cells with high and low expression levels of *GFRA2*. (A) The 24 immune cells analyzed. (B–D) The correlations between mast cells, DCs, Th2 cells, with *GFRA2* expression. (E–G) The significance of mast cells, DCs, and Th2 cells in high- and low-*GFRA2*-expression subgroups of gastric cancer patients.

rely solely on computational analysis and do not involve any experimental validation of the identified potential markers. While computational methods are useful for identifying potential markers, experimental studies are necessary to confirm the biological relevance of these markers and their potential as diagnostic or prognostic tools. Additionally, the accuracy of the computational analysis is dependent on the quality and completeness of the sequencing data used, which may not always be available or accurate. Experimental studies can also provide additional information on the functional role of the identified markers, which is difficult to ascertain through computational analysis alone.

5. Conclusion

High expression of *GFRA2* was associated with poor survival in gastric cancer patients. *GFRA2* expression was significantly associated with histological type and grade. In addition, *GFRA2* expression was strongly positively correlated with the infiltration of mast cells and weakly negatively correlated with that of Th2 cells. Overall, our findings indicate that *GFRA2* could be used as a potential prognostic marker for gastric cancer. Further experimental studies are required to confirm the results of this data mining study.

Author contribution statement

Shaoyu Yang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote paper.

Juan Li: Performed the experiments; Analyzed and interpreted the data; Wrote paper.

Xiaohui Cai: Conceived and designed the experiments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18291.

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