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Original Article

Increased expression of FAM83B predicts poor prognosis in patients with gastric cancer

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Abstract: FAM83B (family with sequence similarity 83, member B) has been recently reported to be an oncogene, which is associated with the prognosis of various human cancers. However, the role of expression of FAM83B in gastric cancer (GC) is unknown. In this study, we examined the expression of FAM83B in human GC by using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), Western blotting and immunohistochemistry. The correlation between FAM83B expression and clinicopathological parameters of GC was assessed with the chi-square test. Survival analysis according to FAM83B expression was performed by Kaplan-Meier method. Univariate and multivariate Cox regression analyses were applied to reveal the prognostic value of FAM83B expression. Our results showed that FAM83B mRNA expression level was significantly higher in GC tissues than in adjacent noncancerous tissues (P<0.001). This trend in expression levels was validated by Western blotting and immunohistochemistry. Also, we found that high expression of FAM83B was significantly correlated with tumor size (P=0.026), clinical stage (P=0.018), depth of invasion (P=0.019), histological grade (P=0.004), perineural invasion (P=0.001) and vascular invasion (P=0.04). Kaplan-Meier survival curves showed that patients with higher FAM83B expression levels had poorer recurrence free survival (RFS; P<0.001) and overall survival (OS; P<0.001). Furthermore, multivariate Cox analysis indicated that an elevated FAM83B expression level was an independent poor prognosis factor for RFS and OS in GC patients. In conclusions, the expression of FAM83B in GC tends to be up regulated, and may serve an important role in assessing GC patient prognosis.

Keywords: FAM83B, gastric cancer, prognosis, biomarker

Introduction

Despite the universal use of gastroscopy and improved therapy, gastric cancer (GC) still ranked third for both cancer incidence and cancer death in developing countries [1]. Most patients with GC tend to present at an advanced stage upon diagnosis. The prognosis for advanced GC patients is very poor, and mechanisms of tumorigenesis and progression are still unclear.

FAM83B (family with sequence similarity 83, member B) is an 110 kDa member of the FAM83B protein family, which all have a common domain of unknown function (DUF1669) [2]. The DUF1669 of FAM83B is necessary for CRAF binding and oncogenic transformation of human mammary epithelial cells (HMECs). Binding of FAM83B with CRAF increased CRAF membrane localization, resulting in elevated mitogen-activated protein kinase (MAPK) signalling in FAM83B-expressing HMEC [3].

In addition, FAM83B could bind directly to the p85α and p110α subunits of phosphatidylinositol-3-kinase (PI3K), as well as AKT, and increased p110α and AKT membrane localization, consistent with elevated PI3K/AKT signalling in FAM83B-expressing cells [4]. Furthermore, elevated FAM83B expression increased phospholipase D (PLD) activity by binding and hyperactivating epidermal growth factor receptor (EGFR) [5], a bona fide oncogene. The product of activated PLD, phosphatidic acid (PA), was reported to be able to activate MAPK signalling [6], as well as mammalian Target of Rapamycin (mTOR) signalling [7]. Signalling by both PI3K/AKT/mTOR and MAPK pathways can play an important role in tumor formation, proliferation and angiogenesis. All the evidence suggests that FAM83B is a potential oncogene.

Table 1. Association between FAM83B expression and clinicopathological parameters in GC patients

Parameters	Total	FAM83B expression, n		χ^2	*p* value
		Low	High		
Gender					
Male	74	32	42	1.171	0.279
Female	33	18	15		
Age (years)					
<60	50	26	24	1.048	0.306
≥60	57	24	33		
Location of tumor					
The proximal 1/2	30	12	18	0.758	0.384
The distal 1/2	77	38	39		
Preoperative CEA (ng/ml)					
<5	84	40	44	0.124	0.724
≥5	23	10	13		
Tumor size (cm)					
<5	65	36	29	4.984	**0.026**
≥5	42	14	28		
Clinical stage					
I-II	29	19	10	5.641	**0.018**
III-IV	78	31	47		
Depth of invasion					
T1-T2	31	20	11	5.547	**0.019**
T3-T4	76	30	46		
Lymph node status					
N0-N1	32	19	13	2.933	0.087
N2-N3	75	31	44		
Distant metastasis					
No	97	47	50	1.240	0.265
Yes	10	3	7		
Histological grade					
Well	3	2	1	11.072	**0.004**
Moderately	37	25	12		
Poor	67	23	44		
Perineural invasion					
No	53	33	20	10.182	**0.001**
Yes	54	17	37		
Vascular invasion					
No	55	31	24	4.220	**0.040**
Yes	52	19	33		

The bold number indicates a significant association among the variables.

FAM83B has been demonstrated to be significantly elevated in lung, breast, ovarian, cervical, testicular, thyroid, bladder and lymphoid cancers [3]. In a variety of tumor types, elevated FAM83B expression was associated with specific cancer subtypes, increased tumor grade, and decreased overall survival [3]. Furthermore, clinical analysis of FAM83B expression in lung squamous cell carcinoma has shown it may serve as a novel prognostic and diagnostic biomarker [8]. However, no information is available regarding the role of FAM83B in tumorigenesis, progression, or metastasis of GC.

In this study, we evaluated FAM83B expression levels in GC tissue using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), Western blotting and immunohistochemistry (IHC). We then analysed the relationship between FAM83B expression and clinicopathological parameters, and examined the prognostic value of FAM83B in GC.

Materials and methods

Patients and samples

A total of 24 fresh-frozen GC tissues and their paired adjacent noncancerous gastric tissues (excised >5 cm away from the edge of the GC) were collected for the use of Western blotting and qRT-PCR analysis. The fresh tissues were preserved in liquid nitrogen within 30 min after resection. Another 107 samples of paired carcinomatous and adjacent noncancerous gastric tissues were subjected to immunohistochemical analysis. All formalin fixed paraffin-embedded tissue samples were collected from GC patients who had surgery performed at the First Affiliated Hospital of Nanchang University between 2007 and 2009. None of the patients were treated with radiotherapy, chemotherapy, or targeted agents prior to surgery, and all cases were gastric adenocarcinoma. Histopathological type classification of the excised GC

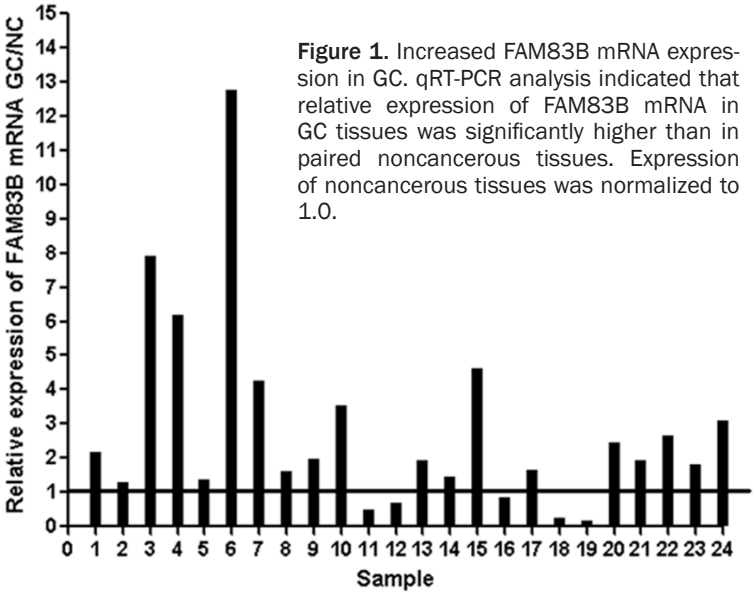

Figure 1. Increased FAM83B mRNA expression in GC. qRT-PCR analysis indicated that relative expression of FAM83B mRNA in GC tissues was significantly higher than in paired noncancerous tissues. Expression of noncancerous tissues was normalized to 1.0.

samples followed the World Health Organization system, and clinical staging was based on guidelines from the American Joint Committee on Cancer (AJCC). Informed consent was obtained from all individual participants included in the study. Clinicopathological data was collected from all patients (**Table 1**).

Quantitative real-time reverse transcription polymerase chain reaction analysis

Total RNA was extracted from 24 pairs of fresh GC tissues and paired noncancerous tissues using TRIzol reagent according to the manufacturer's instruction. The qRT-PCR was carried out to detect the expression of FAM83B mRNA by using SYBR Green qPCR SuperMix-UDG with ROX (Invitrogen). β-actin was applied as the control. The primers were as follows: FAM83B forward, 5'-CCAACGTCCAGTGAGCTTCT-3'; FAM83B reverse, 5'-GCATGTTGCTTGTTGGCTGA-3'; β-actin forward, 5'-TCACCCACACTGTGCCCATCATCGA-3'; and β-actin reverse, 5'-CAGCGGAACCGCTCATTGCCAATGG-3'. Relative expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method.

Western blotting analysis

Protein extraction from both tumor and paired noncancerous tissue used RIPA lysis buffer, followed by lysate harvested with centrifugation (13,000 rpm) at 4°C for 15 min. After quantification, 20 ug of protein was separated using 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were blocked for 1 h at room temperature with 5% non-fat dry milk, and then incubated with the rabbit polyclonal anti-FAM83B antibody (1:250; Abcam, United Kingdom) at 4°C overnight. Mouse anti-human β-actin antibody (1:1500; Abcam, United Kingdom) was applied as an internal control. After three washes with TBST (Tris-buffered saline with Tween-20) for 15 min, membranes were incubated with secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin; 1:1000; Maxin Biotechnology, Fuzhou, China) at room temperature for 1 h. Finally, secondary antibodies were detected using a chemiluminescence system.

Immunohistochemical staining

All tissue blocks were cut into 4-μm sections, which were then dewaxed with dimethylbenzene and rehydrated with a descending ethanol gradient (100%, 95%, 90%, 85%, 80%, 70% ethanol). Antigen retrieval was performed under high temperature and high pressure with citrate buffer (pH 6.0). Endogenous peroxidase activity was inactivated with 3% hydrogen peroxide and 10% normal goat serum was used to reduce nonspecific staining. Samples were then incubated with rabbit polyclonal anti-FAM83B antibody (Abcam ab122175, 1:100 dilution) at 4°C overnight. After washing three times with phosphate buffered saline (PBS), the sections were incubated with horseradish peroxidase conjugated secondary goat anti-rabbit antibody (Maxin Biotechnology, Fuzhou, China) for 30 min at room temperature. Antibody detection was performed using DAB solution (Maxin Biotechnology, Fuzhou, China). Finally, sections were counterstained with hematoxylin. Negative controls were performed by replacing the primary antibody with PBS.

Evaluation of FAM83B expression in tissue

Tissues samples were scored separately by two pathologists blinded to the patients' clinico-

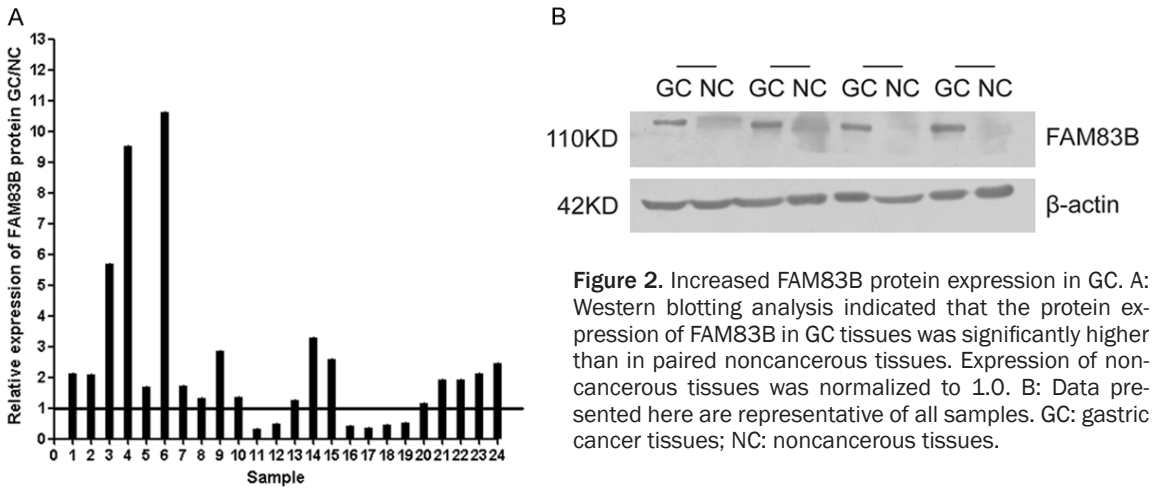

Figure 2. Increased FAM83B protein expression in GC. A: Western blotting analysis indicated that the protein expression of FAM83B in GC tissues was significantly higher than in paired noncancerous tissues. Expression of noncancerous tissues was normalized to 1.0. B: Data presented here are representative of all samples. GC: gastric cancer tissues; NC: noncancerous tissues.

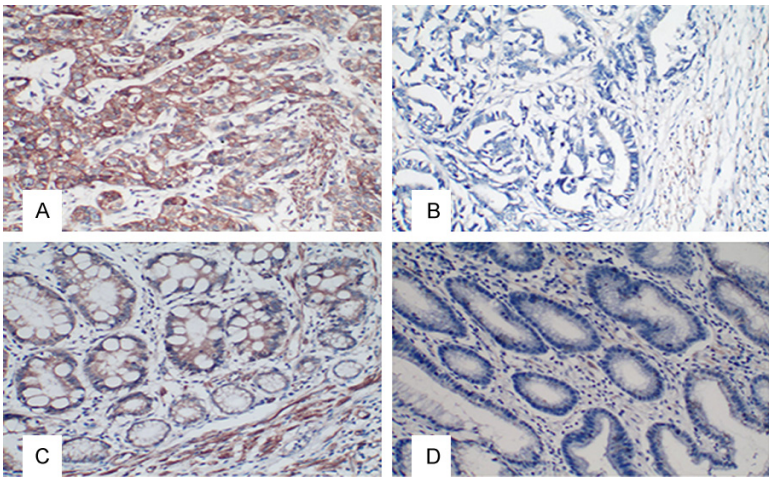

Figure 3. Immunochemistry analysis of FAM83B in GC tissues (A, B) and adjacent noncancerous tissues (C, D). (A) High expression of FAM83B in GC. (B) Low expression of FAM83B in GC. (C) High expression of FAM83B in adjacent noncancerous tissues. (D) Low expression of FAM83B in adjacent noncancerous tissues (magnification 200×).

pathological parameters. Any disagreements were arbitrated by a third pathologist. Expression of FAM83B was scored based on staining intensity and staining area as previously described [9-11]. Staining intensity was scored as "0" (no staining), "1" (weak staining), "2" (moderate staining), or "3" (strong staining). Staining area was scored as "0" (<5%), "1" (5-25%), "2" (26-50%), "3" (51-75%), or "4" (>75%), on the basis of the percentage of positively stained cells. A final score for each sample was calculated by multiplying the staining intensity by the score for the percentage of positive cells. The overall FAM83B expression level was defined as low expression with a score of 0-4 or high expression with a score of 5-12.

Statistical analysis

Data was analysed using SPSS 17.0 software. The χ^2 test was used to evaluate the association between FAM83B expression and clinicopathological parameters. Survival curves were calculated using the Kaplan-Meier method, and the log-rank test was applied to assess differences between groups. Duration of recurrence free survival (RFS) was calculated from the date of operation to any instance of neoplasm recurrence or distant metastasis. Similarly, overall survival (OS) was calculated from the date of operation to the date of patients' death or of last follow-up. Univariate and multivariate analysis was performed using a Cox regression model. Differences were deemed statistically significant with a $P<0.05$.

Results

The FAM83B mRNA and protein expression levels in GC and adjacent noncancerous tissues

We performed qRT-PCR to analyze the expression of FAM83B mRNA in 24 pairs of fresh-

frozen samples including GC tissues and adjacent noncancerous tissues. Compared with paired noncancerous tissues, 79.2% (19/24) of the GC tissues exhibited high expression of FAM83B (P<0.001, **Figure 1**).

As shown in **Figure 2A** and **2B**, the Western blotting analysis also indicated that the expression levels of FAM83B protein were significantly up-regulated in 75% (18/24) GC tissues compared with paired noncancerous tissues.

Immunochemistry staining results indicate FAM83B is localized primarily at the membrane and occasionally in the cytoplasm (**Figure 3A**, **3C**). In GC samples 53.3% (57) had a high expression level of FAM83B (**Figure 3A**), while 46.7% (50) had a low expression level (**Figure 3B**). In the adjacent noncancerous tissues, 25.2% (27) samples had a high expression level of FAM83B (**Figure 3C**), while 74.8% (80) had a low expression level (**Figure 3D**). The percentage of samples with a high expression rate of FAM83B in GC tissues was significantly greater than in the adjacent noncancerous tissues (53.3% vs. 25.2%; P<0.001).

Association between FAM83B expression and clinicopathological parameters

Based on the results from the immunochemical staining, we explored the relationship between FAM83B expression and clinicopathological parameters by using the χ^2 test, with results shown in **Table 1**. Results suggested that FAM83B expression was closely related to tumor size (P=0.026), clinical stage (P=0.018), depth of invasion (P=0.019), histological grade (P=0.004), perineural invasion (P=0.001), and vascular invasion (P=0.04).

Relationship between FAM83B expression and patients' survival

The Kaplan-Meier method and log-rank test were applied to evaluate the potential prognostic effect of FAM83B. Up to the date of the last follow-up, 77 out of 107 patients had died, with a median RFS of 37.1 months and a median OS of 52.1 months. The median RFS was shorter in patients with high expression of FAM83B compared to patients with low expression (21.2 m vs. 53.5 m; P<0.001; **Figure 4A**). In addition, median OS was shorter in patients with high expression of FAM83B compared to patients with low expression (34.9 m vs. 66.4 m; P<0.001; **Figure 4B**).

Univariate and multivariate analyses

As shown in **Tables 2** and **3**, we used Cox proportional hazards regression models to perform univariate and multivariate analyses. Results of univariate analysis indicated clinical stage, depth of invasion, lymph node metastasis, histological grade, perineural invasion, vascular invasion, and FAM83B expression were all prognostic factors for unfavourable RFS in GC patients (**Table 2**). The prognostic factors for unfavourable OS included the factors above as well as distant metastasis.

Results of multivariate analysis (**Table 3**) revealed clinical stage, depth of invasion, lymph node status. histological grade, perineural invasion, vascular invasion, and FAM83B expression were independent poor prognostic factors for RFS in GC patients. Moreover, clinical stage, depth of invasion, lymph node status, histological grade, distant metastases, perineural invasion, vascular invasion, and FAM83B expression were all found to be independent poor prognostic factors for OS.

Discussion

The tumorigenesis, progression and metastasis of gastric cancer are an unclear, complicated, multifactorial process that is regulated by intracellular and extracellular signals involving genetic and epigenetic alterations. The prognosis of patients who present with similar clinical and pathological features can be different [12, 13]. Despite advances in radiotherapy, chemotherapy and targeted therapy, the prognosis of patients with advanced stage tumors remains dismal. To improve patient outcomes, it is necessary to explore the molecular mechanisms driving tumor progression in gastric cancer, and to investigate novel biomarkers, which can play important roles in predicting prognosis and provide insights for clinical management.

In the current study, we investigated FAM83B mRNA expression in 24 pairs of fresh GC tissues and adjacent noncancerous tissues, and found that FAM83B mRNA expression levels in GC tissues were significantly higher compared with the adjacent noncancerous tissues. Moreover, this finding was validated by Western blot

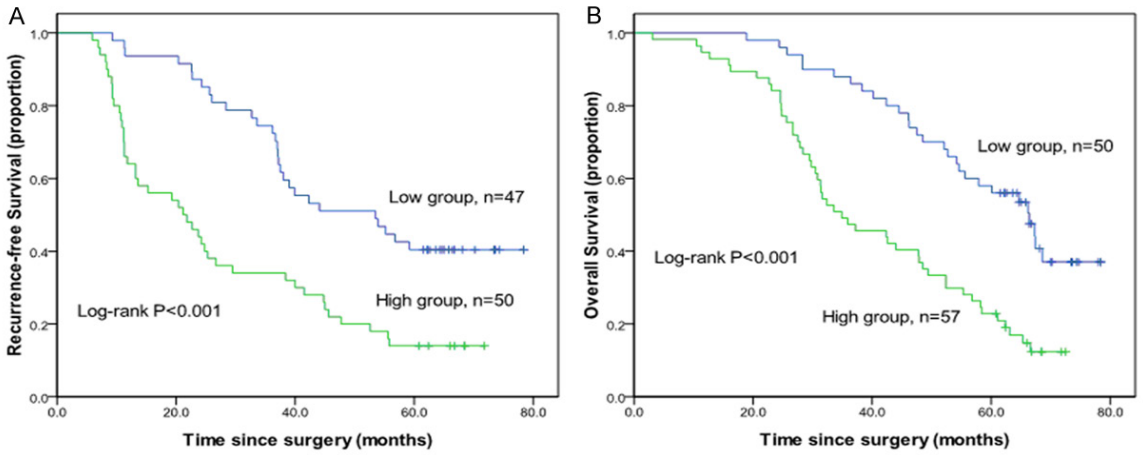

Figure 4. Kaplan-Meier curves for GC patients grouped according to FAM83B expression. A: Shorter RFS in GC patients with high FAM83B expression levels compared with patients with low FAM83B expression levels. B: Shorter OS in GC patients with high FAM83B expression levels compared with patients who had low FAM83B expression levels.

and IHC analysis. It has been reported that FAM83B overexpression could be found in several malignant tumor types, including breast, lung, ovarian, cervical, testicular, thyroid, bladder, and lymphoid cancers, and was also associated with specific cancer subtypes and increased tumor grade [3]. In our study, we also found FAM83B expression was correlated with tumor size, clinical stage, depth of invasion, histological grade, perineural invasion, and vascular invasion. All the evidence supported the conclusion that FAM83B was associated with GC tumorigenesis and progression, but our data did not indicate that FAM83B was involved in GC metastasis.

Cipriano et al. demonstrated that increased FAM83B expression in tumors was associated with decreased overall survival [3]. To investigate the utility of using FAM83B to predict the prognosis of GC patients, we performed Kaplan-Meier and Cox proportion hazard model analysis. The results showed that the median RFS and median OS in patients with high FAM83B expression were significantly reduced compared with patients who had low expression, and FAM83B was an independent poor prognostic factor for RFS and OS in GC patients. These results were similar to those from a study by Okabe et al. [8] who found that elevated FAM83B expression in lung squamous cell carcinoma was associated with shorter disease free survival (DFS). However, their results failed to confirm that FAM83B was an independent poor prognostic factor for DFS in lung squamous cell carcinoma.

To date, several targeted agents have been approved for the treatment of GC patients [14-17]. And additional signalling pathways remain as potential therapeutic targets in GC. The AKT/mTOR and MAPK signalling pathways have been shown to be involved in the proliferation, growth, invasion and migration of gastric cancer cells [18, 19]. However, there are many points of crosstalk between these two signalling pathways [20], inhibition of mTOR could activate MAPK pathway through a feedback loop [21, 22]. Therefore, inhibitors targeting single pathway tend to have limited efficacy and result in drug resistance [23]. FAM83B protein can directly activate both PI3K/AKT/mTOR and MAPK signalling pathways by binding to PI3K or CRAF [3, 4], or indirectly activate them by binding and hyperactivating EGFR, an upstream regulator of the two signalling pathways [5]. All the evidences suggest FAM83B may be a new and effective therapeutic target. Moreover, because FAM83B protein can directly activate PI3K/AKT/mTOR and MAPK signalling pathways by bypassing the block to EGF survival signalling induced by EGFR-TKIs [24], the expression level of FAM83B may predict which patients can benefit from EGFR-TKIs, and inhibition of FAM83B may reverse EGFR-TKI resistance.

In summary, our study for the first time demonstrates that the expression of FAM83B is sig-

Table 2. Univariate analyses of RFS and OS

Parameters	RFS			OS		
	HR	95% CI	p	HR	95% CI	p
Gender						
Male	0.782	0.465-1.315	0.354	0.729	0.445-1.193	0.208
Female						
Age (years)						
<60	1.006	0.627-1.614	0.979	0.829	0.534-1.286	0.402
≥60						
Location of tumor						
The proximal 1/2	0.801	0.486-1.321	0.384	0.849	0.529-1.363	0.497
The distal 1/2						
Preoperative CEA (ng/ml)						
<5	1.348	0.777-2.340	0.288	1.392	0.844-2.296	0.195
≥5						
Tumor size (cm)						
<5	1.497	0.934-2.398	0.093	1.401	0.899-2.186	0.137
≥5						
Clinical stage						
I-II	14.811	6.249-35.103	**<0.001**	14.205	6.017-33.532	**<0.001**
III-IV						
Depth of invasion						
T1-T2	3.791	2.096-6.858	**<0.001**	4.165	2.323-7.470	**<0.001**
T3-T4						
Lymph node status						
N0-N1	11.883	5.232-26.991	**<0.001**	8.554	4.181-17.499	**<0.001**
N2-N3						
Distant metastasis						
No				22.722	9.477-54.479	**<0.001**
Yes						
Histological grade						
Well + Moderately	3.280	1.917-5.610	**<0.001**	3.393	2.027-5.680	**<0.001**
Poor						
Perineural invasion						
No	3.316	2.052-5.358	**<0.001**	3.855	2.424-6.133	**<0.001**
Yes						
Vascular invasion						
No	6.517	3.872-10.968	**<0.001**	4.838	2.993-7.819	**<0.001**
Yes						
FAM83B						
Low	2.780	1.705-4.534	**<0.001**	2.930	1.836-4.677	**<0.001**
High						

HR hazard ratio, CI confidence interval. Bold number means statistically significance, P<0.05.

nificantly increased in GC tissues, and is associated with tumor size, clinical stage, depth of invasion, histological grade, perineural invasion, and vascular invasion. In addition, multivariate analysis showed that overexpression of FAM83B was an independent poor prognostic factor for RFS and OS in GC patients. FAM83B can play an important role in predicting the prognosis of GC patients, and has the potential to be an effective therapeutic target.

Table 3. Multivariate analyses of RFS and OS

Prognostic factors	RFS			OS		
	HR	95% CI	*p*	HR	95% CI	*p*
Clinical stage						
I-II	5.987	1.518-23.617	**0.011**	3.822	1.099-13.293	**0.035**
III-IV						
Depth of invasion						
T1-T2	2.693	1.308-5.547	**0.007**	2.583	1.268-5.262	**0.009**
T3-T4						
Lymph node status						
N0-N1	3.869	1.299-11.523	**0.015**	3.442	1.404-8.439	**0.007**
N2-N3						
Distant metastasis						
No				14.958	5.657-39.552	**<0.001**
Yes						
Histological grade						
Well + Moderately	3.094	1.632-5.867	**0.001**	2.379	1.306-4.337	**0.005**
Poor						
Perineural invasion						
No	6.446	2.971-13.984	**<0.001**	3.372	1.721-6.608	**<0.001**
Yes						
Vascular invasion						
No	7.185	3.277-15.750	**<0.001**	3.043	1.540-6.013	**0.001**
Yes						
FAM83B						
Low	1.777	1.055-2.993	**0.031**	1.717	1.033-2.854	**0.037**
High						

HR hazard ratio, CI confidence interval. Bold number means statistically significance, P<0.05.

Acknowledgements

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Disclosure of conflict of interest

None.

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