High expression of fibronectin 1 indicates poor prognosis in gastric cancer

YANG SUN[1,2], CHUNLIN ZHAO[1], YANWEI YE[1], ZHEN WANG[1], YUANHANG HE[1], YULIN LI[1] and HAOXUN MAO[1]

[1]Department of Gastrointestinal Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052; [2]Department of Breast and Thyroid Surgery, Nanyang Central Hospital, Nanyang, Henan 473000, P.R. China

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Abstract. Fibronectin 1 (*FN1*) is involved in the occurrence and development of various tumors and is upregulated in multiple cancer types. *FN1* has been demonstrated to promote cell proliferation and migration in gastric cancer cell lines. However, the relationship between the expression of *FN1* and clinicopathological factors and prognosis is not clear in gastric cancer (GC). The aim of the present study was to investigate the association between *FN1* expression and clinicopathology and prognosis of gastric cancer. In this study, 17 publicly available GC cohorts (n=2,376) with gene expression data from the Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA) and Oncomine databases were tested. In addition, *FN1* protein expression was validated by immunohistochemistry in a separate cohort (n=190). The meta-analysis results demonstrated an increase in *FN1* expression at the protein and mRNA level in GC tissues, and the *FN1* gene was highly expressed at the mRNA level in the advanced T stage (T2 + T3 + T4) group compared with that in the early T stage (T1) group. In addition, the expression of epithelial *FN1* at the protein level was positively correlated with tumor size. *FN1* expression at the protein and mRNA level was a predictor of poor prognosis following radical resection of GC. In conclusion, the expression of *FN1* in GC tissues is upregulated compared with adjacent normal tissues, and it is a potential biomarker of poor prognosis in patients with GC.

Introduction

Gastric cancer is the fifth most commonly diagnosed cancer (5.7% of total cases) and the third leading cause of cancer mortality (8.2% of total cancer mortality) worldwide (1). Gastric cancer is also the third leading cause of cancer-related mortality in China (2). Although surgery combined with radiotherapy, chemotherapy and targeted therapy prolongs survival, the 5-year overall survival rate of patients with advanced gastric cancer remains poor. The 5-year overall survival rates of patients with pathological T stage 2, 3 and 4 disease were 68.3, 33.0 and 24.0% respectively (3,4). Therefore, new biomarkers of gastric cancer to determine prognosis are necessary.

Fibronectin 1 (*FN1*) mediates the interaction between cells and the extracellular matrix and serves an important role in cell adhesion, migration, growth and differentiation (5). *FN1* is a ligand for numerous members of the integrin receptor family (6). *FN1* is involved in the occurrence and development of various tumors. *FN1* activates the PI3K/Akt pathway by binding to its integrin receptor α5β1 in breast cancer (7). In addition, *FN1* has been demonstrated to promote cell proliferation and migration in esophageal squamous cell carcinoma, oral squamous cell carcinoma (OSCC), nasopharyngeal carcinoma, colorectal, ovarian, renal and thyroid cancer (8-14). However, little is known about the expression of *FN1* in gastric cancer. *FN1* is upregulated in GC tissues compared with normal gastric tissues (15). *FN1* knockdown inhibits cell migration and invasion *in vitro*, and FOXF1 adjacent non-coding developmental regulatory RNA and microRNA-200c promote the proliferation, migration and invasion of GC cells by negatively targeting *FN1* (15-17). Overall, *FN1* is a potential biomarker candidate for GC prognosis, but the relationship between *FN1* expression and clinical factors and prognosis has not been reported, and thus it is necessary to verify and clarify the role of *FN1* in GC.

The aim of the present study was to investigate *FN1* gene expression in GC and its association with clinicopathological factors and prognosis by examining 17 publicly available GC cohorts. Furthermore, *FN1* protein expression was validated by immunohistochemistry in a separate cohort. The results demonstrated that *FN1* may serve as a new prognostic marker for GC.

Materials and methods

Data collection. Microarray data were downloaded from the following datasets in the Gene Expression Omnibus (GEO;

Correspondence to: Professor Chunlin Zhao, Department of Gastrointestinal Surgery, The First Affiliated Hospital of Zhengzhou University, 1 East Jianshe Road, Erqi, Zhengzhou, Henan 450052, P.R. China
E-mail: doctorzhaochunlin@126.com

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http://www.ncbi.nlm.nih.gov/geo/), The Cancer Genome Atlas (https://xenabrowser.net/datapages/?cohort=TCGA) and Oncomine (https://www.oncomine.org/resource/login.html): GSE13861, GSE13911, GSE14208, GSE15456, GSE15459, GSE19826, GSE26253, GSE26899, GSE26901, GSE29272, GSE34942, GSE35809, GSE54129, GSE66229, GSE79973, Chen Gastric and TCGA STAD. Several of these datasets have been previously published (18-32). The 17 datasets comprised 2,376 cancer tissues and 294 adjacent normal tissues. Datasets with no clinical data (GSE13861, GSE13911, GSE19826, GSE54129, GSE79973 and Chen Gastric), GSE29272 and TCGA STAD were used to analyze the differences between tumor and adjacent tissues. The remaining datasets were used to analyze the relationship between *FN1* expression and clinicopathological factors. Clinical information for the cohorts with respective clinical data included in this study is presented in Table I.

Validation dataset. Immunohistochemistry (IHC) was used for validation. Gastric cancer tissues and adjacent normal gastric tissues were obtained during surgery from 190 randomly selected patients between June 2011 and June 2012 at the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China). The study was approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all patients. The tissues were fixed with formalin and embedded in paraffin for subsequent experiments. All patients were followed up for ≥5 years, and 102 succumbed to any cause during the follow-up period.

IHC. Formalin-fixed paraffin-embedded tissue samples from the IHC cohort were sliced into 4-μm sections. A mouse monoclonal antibody against *FN1* (cat. no. 66042-1-Ig; ProteinTech Group, Inc.) was used at a 1:600 dilution at pH 9.0. The immunohistochemical staining of the specimens was performed as previously described (16). The results of *FN1* expression were separately scored in epithelial cancer cells and intertumoral stroma. The scoring method described by Sung *et al* (33) was used. For epithelial *FN1* (E-*FN1*) expression, staining intensity and the proportion of stained tumor cells were considered. Staining intensity was classified as follows: 1, weak; 2, moderate; and 3, strong. Positive cells were quantified as a percentage of the total number of tumor cells and assigned to one of the following categories: 0, <5%; 1, 5-24%; 2, 25-49%; 3, 50-74%; and 4, ≥75%. The percentage of positive tumor cells and staining intensity were multiplied to generate an immunoreactivity score (IS) for each case. IS values ranged from 0 to 12; IS≥3 was considered positive, whereas IS<3 was considered negative. Stromal *FN1* (S-*FN1*) expression was graded into three categories: No or weak staining, no staining or a low number of *FN1*-positive strands; moderate staining, fine *FN1*-positive strands; and strong staining, coarse *FN1*-positive strands (34).

Statistical analysis. When >1 *FN1* probe was present in a group, the probe with the highest variance was selected for statistical analysis (35). All *FN1* gene expression data normalization and probe summarization were performed by Robust Multichip Analysis and transformed by log2. SPSS 22.0 (IBM Corp.) and RevMan 5.3 (Cochrane Community) were used to perform all statistical analyses.

Independent sample t-tests were used in SPSS for continuous data analysis and Pearson's χ^2 tests were used for categorical data analysis. The gene expression value was equal to three, ≥1/3 were defined as high expression and the <1/3 as low expression. Overall survival (OS) rate was analyzed using Kaplan-Meier plots and the log-rank test or Gehan-Breslow-Wilcoxon test. When the two survival functions were parallel, the log-rank test was used, whereas the Gehan-Breslow-Wilcoxon test was used if the data crossed over. A Cox regression model was used to assess the hazard ratio (HR) and perform multivariate analysis. All tests were two-sided, and $P<0.05$ was considered to indicate a statistically significant difference.

Meta-analyses were performed using RevMan 5.3. First, the heterogeneity between the results of each study was analyzed by the χ^2 test. The threshold was set to $\alpha=0.100$, and the extent of heterogeneity was assessed by combining I^2. If $P>0.10$ and $I^2\leq50\%$, the homogeneity between the results was considered high, and the fixed effect model was used; if $P\leq0.10$ or $I^2>50\%$, the random effects model was used.

Results

Patient cohorts. Data from 17 independent GC cohorts were downloaded from the Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA) and Oncomine, including 2,670 samples, which comprised 2,376 cancer tissues and 294 adjacent normal tissues. Eight of the 17 cohorts included tumor and normal samples. The IHC cohort comprised 190 GC samples and 20 adjacent tissue samples. The clinicopathological characteristics of the patients are presented in Table I.

FN1 expression in gastric cancer. A total of eight independent cohorts that included expression data from cancer and normal samples were analyzed; the results revealed upregulated *FN1* mRNA levels in tumor tissues compared with normal tissues (Fig. 1A). Meta-analysis of all the cohorts revealed a significant combined mean difference of 1.99 ($P<0.001$; Fig. 2A). These results indicated that *FN1* expression was significantly higher in GC tissues compared with that in adjacent normal tissues.

Association between FN1 expression and clinicopathological factors. Compared with that in the early T stage (T1) group, the expression of *FN1* was significantly increased in the advanced T stage (T2+T3+T4) group ($P=0.002$; Fig. 1B) in one cohort, which was further confirmed by meta-analysis in all examined cohorts ($P<0.001$; Fig. 2B). The expression of *FN1* was not associated with differentiation in any cohort (Figs. 1C and 2C). Only two cohorts exhibited increased *FN1* expression in patients with high clinical Tumor-Node-Metastasis (TNM) stage (36) (III + IV) compared with that in patients with low clinical TNM stage (I + II) (Fig. 1D). No significant differences between patients with high and low TNM stage were observed in the meta-analysis of all cohorts (Fig. 2D).

High FN1 expression level indicates poor clinical outcomes. Kaplan-Meier survival analysis was performed using clinical data. OS analysis demonstrated that high *FN1* expression was associated with unfavorable prognosis compared with low *FN1*

Table I. Clinicopathological characteristics of patients in different datasets.

Characteristic	IHC cohort, n (%)	GSE13861, n (%)	GSE15456, n (%)	GSE15459, n (%)	GSE26253, n (%)	GSE26899, n (%)	GSE26901, n (%)	GSE29272, n (%)	GSE34942, n (%)	GSE35809, n (%)	GSE66229, n (%)	TCGA, n (%)
Sex												
Total	190 (100)	65 (100)	30 (100)	192 (100)	432 (100)	92 (100)	109 (100)	134 (100)	56 (100)	70 (100)	300(100)	415(100)
Male	144 (75.8)	46 (70.8)	17 (56.7)	125 (65.1)	280 (64.8)	73 (79.3)	69 (63.3)	103 (76.9)	36 (64.3)	48 (68.6)	199 (66.3)	268 (64.6)
Female	46 (24.2)	19 (29.2)	13 (43.3)	67 (34.9)	152 (35.2)	19 (20.7)	40 (36.7)	31 (23.1)	20 (35.7)	22 (31.4)	101 (33.7)	147 (35.4)
Median age, years (min, max)	59 (25, 85)	63 (32, 83)	73 (53, 83)	66 (23, 92)	53 (23, 74)	59 (36, 83)	58 (28, 74)	59 (23, 73)	69 (43, 84)	67 (32, 85)	64 (24, 86)	67 (30, 90)
T stage												
1	15 (7.9)	2 (3.1)	-	8 (4.2)	-	-	-	-	-	-	2 (0.7)	22 (5.3)
2	32 (16.8)	23 (35.4)	-	45 (23.4)	-	-	-	-	-	-	186 (62)	88 (21.2)
3	24 (12.6)	34 (52.3)	-	107 (55.7)	-	-	-	-	-	-	91 (30.3)	181 (43.6)
4	119 (62.6)	1 (1.5)	-	1 (0.5)	-	-	-	-	-	-	21 (7)	115 (27.7)
Unknown	0 (0)	5 (7.7)	-	31 (16.1)	-	-	-	-	-	-	0 (0)	9 (2.2)
TNM stage												
I	29 (15.3)	12 (18.5)	6 (20)	31 (16.1)	68 (15.7)	11 (12.0)	38 (29.5)	5 (3.7)	11 (19.6)	13 (18.6)	31 (10.3)	57 (13.7)
II	60 (31.6)	2 (3.1)	4 (13.3)	29 (15.1)	167 (38.7)	18 (19.6)	40 (31)	5 (3.7)	11 (19.6)	16 (22.9)	97 (32.3)	123 (29.6)
III	91 (47.9)	35 (53.8)	15 (50)	72 (37.5)	130 (30.1)	27 (29.3)	36 (27.9)	115 (85.8)	19 (33.9)	33 (47.1)	95 (31.7)	169 (40.7)
IV	10 (5.3)	16 (24.6)	5 (16.7)	60 (31.3)	67 (15.5)	36 (39.1)	15 (11.6)	9 (6.7)	13 (23.2)	7 (10)	77 (25.7)	41 (9.9)
Unknown	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3.6)	1 (1.4)	0 (0)	25 (6.0)
Tumor grade												
Low	48 (24)	20 (30.8)	2 (6.7)	6 (3.1)	-	-	-	1 (0.7)	-	2 (2.9)	-	12 (2.9)
Intermediate	66 (33)	15 (23.1)	13 (43.3)	53 (27.6)	-	-	-	48 (35.8)	-	22 (31.4)	-	148 (35.7)
High	76 (38)	6 (9.2)	15 (50)	86 (44.8)	-	-	-	85 (63.4)	-	26 (37.1)	-	246 (59.3)
Undifferentiated	10 (5)	24 (36.9)	0 (0)	47 (24.5)	-	-	-	0 (0)	-	20 (28.6)	-	9 (2.2)
Follow-up endpoint (death)												
Occurred	101 (50.5)	-	-	95 (49.5)	-	-	-	31 (23.1)	27 (48.2)	-	159 (53)	144 (34.7)
Not occurred	99 (49.5)	-	-	97 (50.5)	-	-	-	95 (70.9)	29 (51.8)	-	141 (47)	214 (51.6)
No data	0 (0)	-	-	0 (0)	-	-	-	8 (6)	0 (0)	-	0 (0)	57 (13.7)

-, data not available; TNM, Tumor-Node-Metastasis.

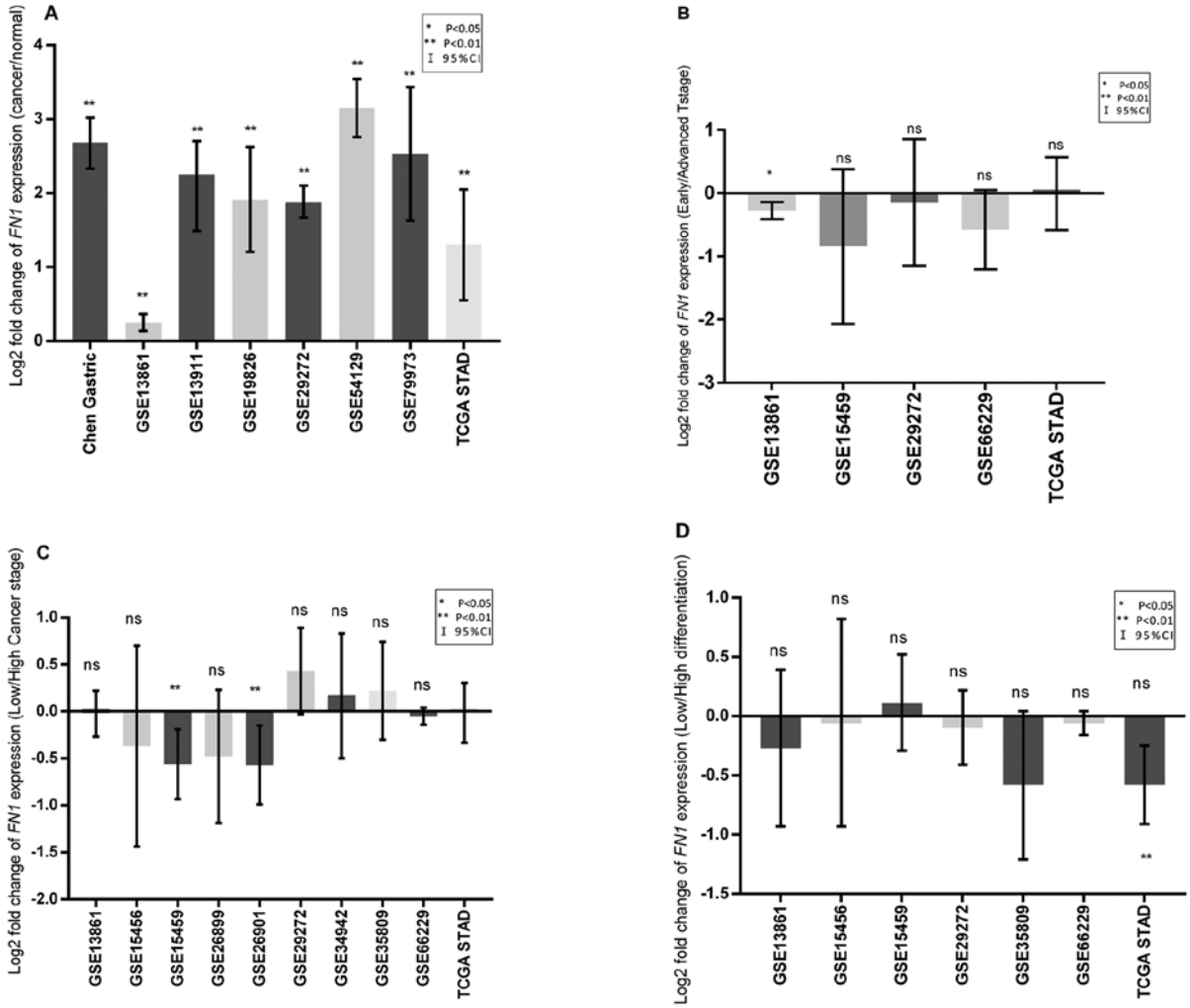

Figure 1. Log2 fold change in *FN1* gene expression in GC tissues. (A) *FN1* expression in tumor tissues compared with that in normal tissues. (B) *FN1* expression in the advanced T stage (T2 + T3 + T4) group compared with that in the early T stage (T1) group. (C) *FN1* expression in the low differentiation group (high tumor grade) compared with that in the high differentiation group (intermediate and low tumor grade). (D) *FN1* expression in the high clinical TNM stage (III+IV) group compared with that in the low clinical TNM stage (I+II) group. Error bars represent 95% confidence interval. *P<0.05 and **P<0.01 vs. normal tissue. *FN1*, fibronectin 1; TNM, Tumor-Node-Metastasis; ns, non-significant.

expression in four of the six cohorts containing prognostic information (Fig. 3). A meta-analysis of all cohorts validated this result, as it exhibited a significant combined *FN1* hazard ratio (HR) of 1.67 (P<0.001; Fig. 2E). This indicated that the expression of *FN1* is a potential indicator of clinical outcome in patients with GC.

FN1 immunohistochemistry. *FN1* is expressed in cancer cells and the intratumoral matrix in GC (Fig. 4). In the IHC cohort, normal epithelial cells exhibited no *FN1* expression. E-*FN1* expression was positive in 85 of the 190 cases (44.7%). S-FN expression was graded as no/weak in 11 (5.8%), moderate in 71 (37.4%) and strong in 108 (56.8%) cases (Table II). No association was identified between E-*FN1* and S-*FN1* expression (P=0.112; Table III). E-*FN1* expression in GC exhibited a significant association with tumor size (P=0.037), whereas S-*FN1* expression was associated with sex (P=0.027) (Table II).

E-*FN1*-positive patients with GC in the IHC cohort exhibited worse OS compared with E-*FN1*-negative patients (P=0.009; Fig. 5A). S-*FN1* expression exhibited no significant effect on OS (P=0.075, Fig. 5B). In addition, in patients with high clinical TNM stage (III + IV), E-*FN1* positivity was strongly associated with OS; however, in patients with low clinical TNM stage (I + II), no difference was observed in overall survival between patients with low and high E-*FN1* expression (Fig. 5C and D). E-*FN1* was also confirmed as an independent predictor of overall survival in GC by multivariate analysis (HR, 2.115; 95% CI, 1.343-3.333; P=0.001; Table IV).

Discussion

In this study, *FN1* gene expression was analyzed in 17 independent GC cohorts. The results demonstrated an increase in *FN1* expression in GC compared with normal tissues and a possible increase in the advanced T stage (T2+T3+T4) group compared with that in the early T stage (T1) group in one cohort; however, no association between *FN1* expression levels and differentiation or clinical TNM stage was identified. In addition, upregulation of the *FN1* gene may be a predictor of poor prognosis following radical gastrectomy for GC. In summary, the results of the present study support *FN1* as a biomarker of poor prognosis in GC.

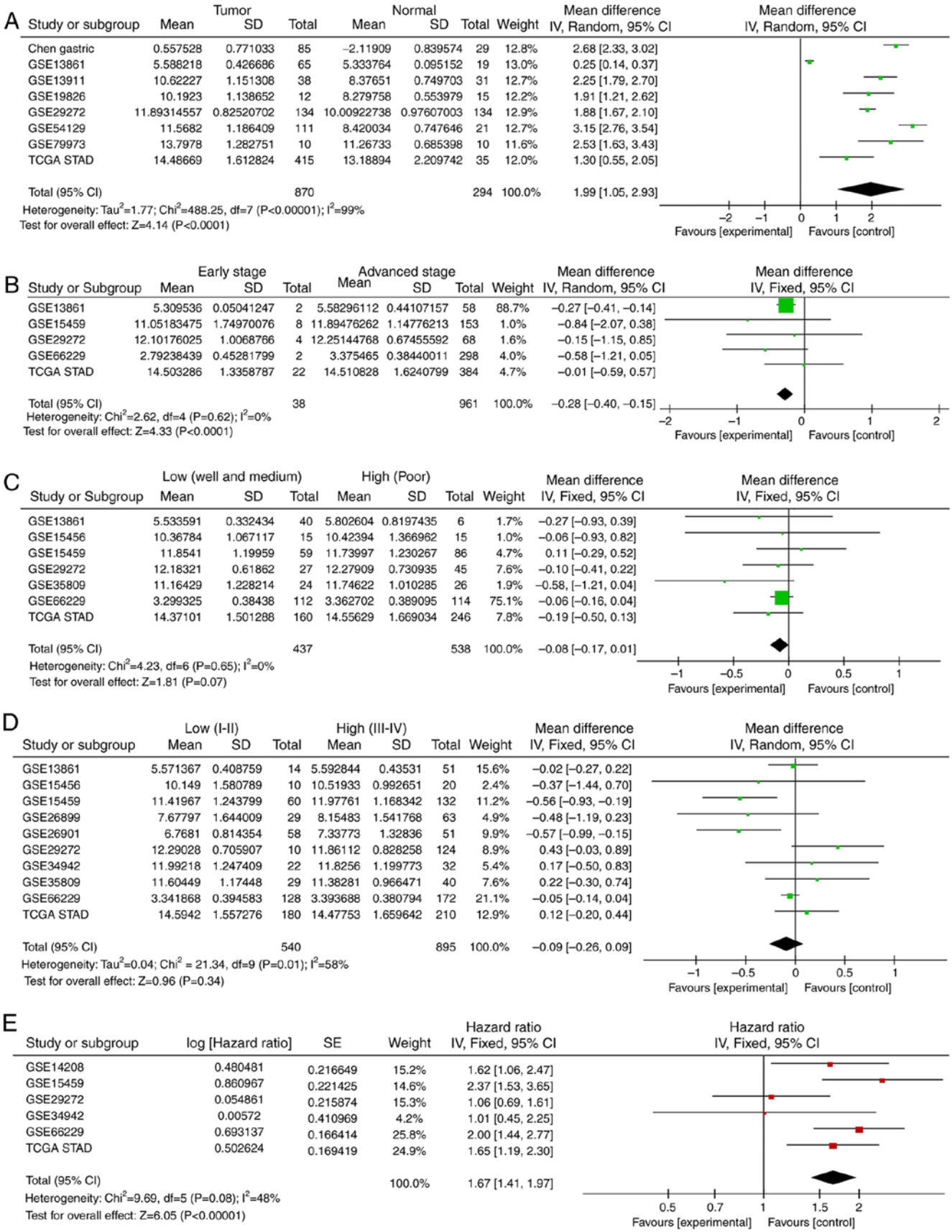

Figure 2. Forest plot and meta-analysis of *FN1* expression in gastric cancer. (A) Forest plot of the log2 fold change in *FN1* expression in tumor tissues compared with that in normal tissues. (B) Forest plot of the log2 fold change in *FN1* expression in the advanced T stage group (T2 + T3 + T4) compared with that in the early T stage group (T1). (C) Forest plot of the log2 fold change in *FN1* expression in the low differentiation group (high tumor grade) compared with that in the high differentiation group (intermediate and low tumor grade). (D) Forest plot of the log2 fold change in *FN1* expression in the high clinical TNM stage (III+IV) group compared with that in the low clinical TNM stage (I+II) group. (E) Forest plot of the comparison of overall survival in patients with gastric cancer with high and low *FN1* expression (The gene expression value was equal to three, the first two-thirds were defined as high expression and the last one-third as low expression). *FN1*, fibronectin 1; TNM, Tumor-Node-Metastasis; CI, confidence interval.

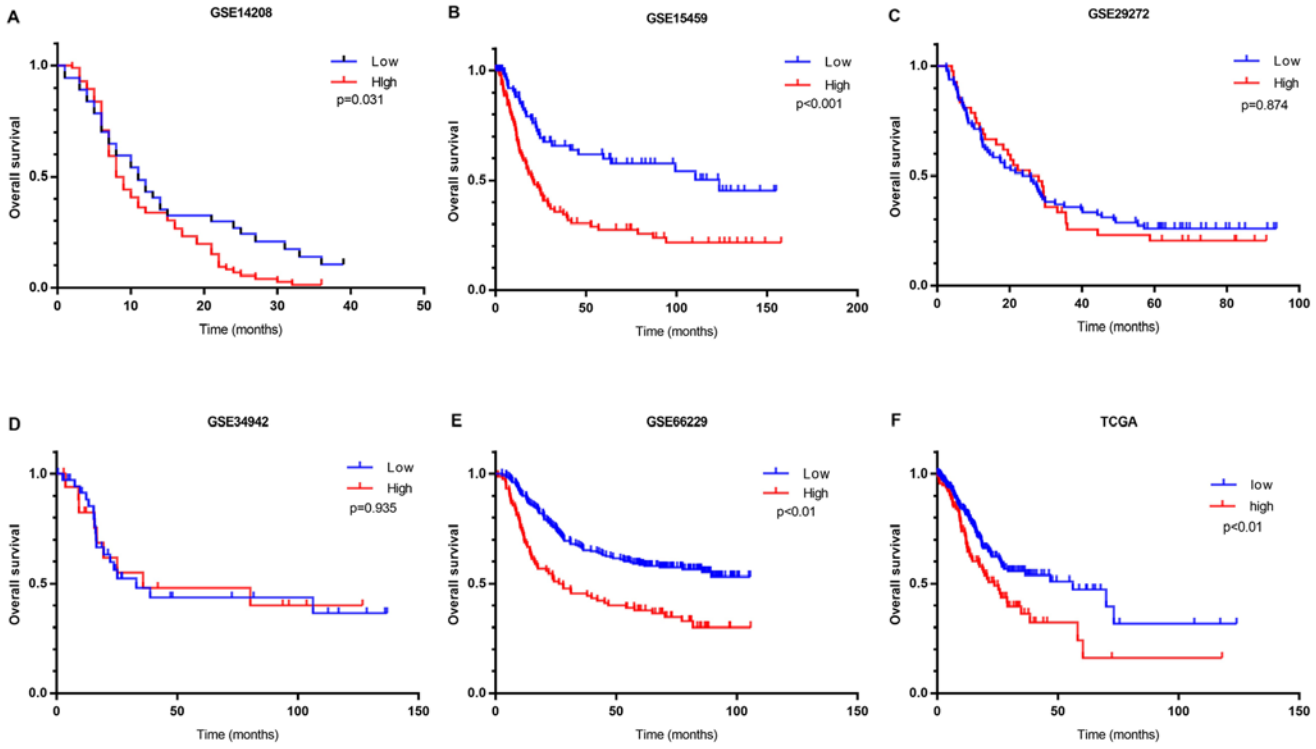

Figure 3. Kaplan-Meier survival curves of the high- and low-*FN1* expression groups in six cohorts. Survival curves for the following datasets: (A) GSE14208; (B) GSE15459; (C) GSE29272; (D) GSE34942; (E) GSE66229 and (F) TCGA. *FN1*, fibronectin 1; TCGA, The Cancer Genome Atlas.

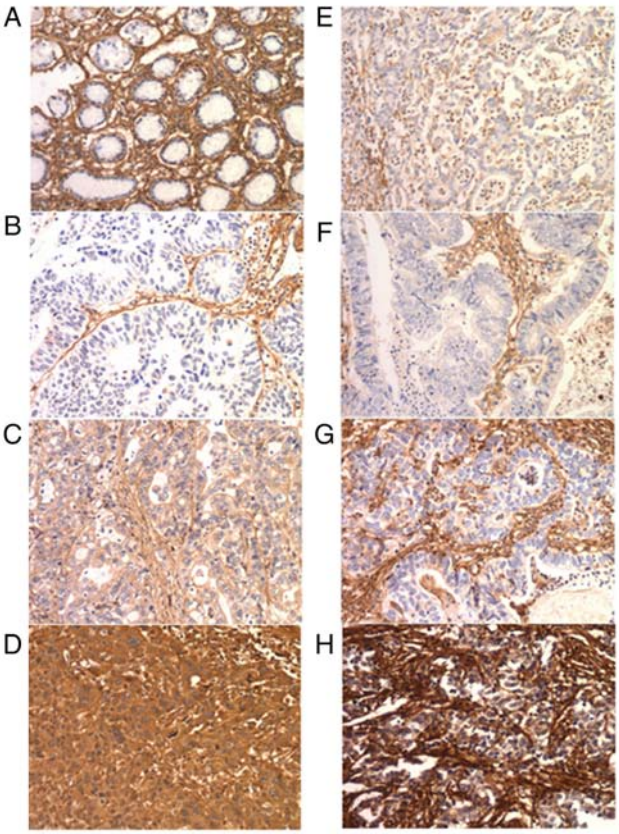

Figure 4. Immunohistochemical analysis of *FN1* in GC. (A) Normal gastric mucosa exhibited negative staining for *FN1*. (B-D) E-*FN1* expression in GS tissues. (B) Expression score, 0; (C) expression score, 6; and (D) expression score, 12. (E-H) S-*FN1* expression in in GS tissues. (E) No expression; (F) low expression; (G) medium expression; and (H) high expression. Magnification, x200. GC, gastric cancer; *FN1*, fibronectin 1; S-*FN1*, stromal *FN1*; E-*FN1*, epithelial *FN1*.

FN1, which is an extracellular matrix glycoprotein, is involved in cell proliferation, embryogenesis, wound healing, host defense, epithelial-mesenchymal transition (EMT) and metastasis, as well as oncogenic transformation (5). *FN1* is involved in the occurrence and development of various tumors and is upregulated in multiple cancer types, such as esophageal squamous cell carcinoma, colorectal cancer, OSCC, and thyroid cancer (8-10,14). For instance, *FN1* is upregulated in OSCC with lymph node metastasis (LNM); *FN1* increases the expression of vascular endothelial growth factor C, lymphangiogenesis and LNM through FAK activation and promotes EMT in SAS human OSCC cells (37). *FN1* is a key mediator of glioma progression, as its inhibition delays tumor progression and immunosuppression through a mechanism that involves the maintenance of integrin β1 FN receptors (38). In GC, *FN1* is highly expressed in tumor tissues compared with that in non-tumor tissues, and knockdown of *FN1* represses GC cell proliferation, adhesion and metastasis *in vitro* (15). The present study aimed to analyze the relationship between *FN1* expression in GC and clinicopathological factors and prognoses.

The results of the present study demonstrated that the *FN1* gene was upregulated in gastric cancer tissues compared with that in normal tissues in eight cohorts, and these data were confirmed by meta-analysis of combinations of all datasets. This result was consistent with the results of Xu *et al* (15) and Zhang *et al* (16), who used immunohistochemical methods to analyze tumor and normal tissue specimens from 40 and 52 patients with gastric cancer, respectively. In summary, previous studies have reported that the expression of the *FN1* gene was increased in GC tissues compared with that in normal gastric tissues, but the studies were all small-scale. The present

Table II. Patient characteristics based on the immunohistochemistry results of *FN1* expression in gastric cancer.

Characteristic	No. of patients (n=190)	Expression of E-*FN1* (%)		P-value	Expression of S-*FN1* (%)			P-value
		Negative (n=85)	Positive (n=105)		No/weak (n=11)	Moderate (n=71)	Strong (n=108)	
Sex				0.380				0.027[a]
Female	46	18 (39.14)	28 (60.9)		0 (0.0)	13 (28.3)	33 (71.7)	
Male	144	67 (46.5)	77 (53.5)		11 (7.6)	58 (40.3)	75 (52.1)	
Age (years)				0.508				0.361
<60	100	47 (47.0)	53 (53.0)		4 (4.0)	41 (41.0)	55 (55.0)	
≥60	90	38 (42.2)	52 (57.8)		7 (7.8)	30 (33.3)	53 (58.9)	
Tumor diameter (cm)				0.037[a]				0.639
<5	114	58 (50.9)	56 (49.1)		8 (7.0)	41 (36.0)	65 (57.0)	
≥5	76	27 (35.5)	49 (64.5)		3 (3.9)	30 (39.5)	43 (56.6)	
T stage				0.742				0.962
T1 + T2	47	22 (46.8)	25 (53.2)		3 (6.4)	18 (38.3)	26 (55.3)	
T3 + T4	143	63 (44.1)	80 (55.9)		8 (5.6)	53 (37.1)	82 (57.3)	
N stage				0.080				0.616
N0 + N1	112	56 (50.0)	56 (50.0)		8 (7.1)	42 (37.5)	62 (55.4)	
N2 + N3	78	29 (37.2)	49 (62.8)		3 (3.8)	29 (37.2)	46 (59.0)	
TNM stage				0.352				0.510
I + II	89	43 (48.3)	46 (51.7)		7 (7.9)	32 (36.0)	50 (56.2)	
III + IV	101	42 (41.6)	59 (58.4)		4 (4.0)	39 (38.6)	58 (57.4)	

[a]P<0.05, *FN1*, fibronectin 1; S-*FN1*, stromal *FN1*; E-*FN1*, epithelial *FN1*; TNM, Tumor-Node-Metastasis.

Table III. Association between epithelial and stromal expression of *FN1* in gastric cancer.

Expression of S-*FN1*	Expression of E-*FN1*		Total (%)	P-value
	Negative (%)	Positive (%)		
No/Weak	7 (63.6)	4 (36.4)	11 (5.8)	
Moderate	34 (47.9)	37 (52.1)	71 (37.4)	
Strong	44 (40.7)	64 (59.3)	108 (56.8)	
Total (%)	85 (100.0)	105 (100.0)	190 (100.0)	0.112

FN1, fibronectin 1; S-*FN1*, stromal *FN1*; E-*FN1*, epithelial *FN1*.

study used multiple cohorts to provide substantial validation of increased *FN1* expression in GC.

To the best of our knowledge, the association between *FN1* expression and clinicopathological features or patient prognosis, have not been reported previously. In the present study, compared with that in the early T stage group, the expression of *FN1* was significantly increased in the advanced T stage group, which was further confirmed by meta-analysis in all the examined groups. OS analysis revealed that high *FN1* expression was associated with unfavorable prognosis in four of the six cohorts containing prognostic information. A meta-analysis of all cohorts further validated this finding. These results indicated that the expression of *FN1* may be a potential indicator of clinical outcomes in patients with GC.

FN1 is expressed in cancer cells and the intratumoral matrix in GC. Hanamura *et al* (39) reported that the expression of S-*FN1* mRNA was positively correlated with deep invasion and LNM of colon cancer. Bae et al (34) reported that E-*FN1*-positive patients exhibited lower OS and disease-free survival compared with *FN1*-negative breast cancer patients. E-*FN1* was an independent predictor for survival in breast cancer in multivariate analysis, but the expression of S-*FN1* had no significant effect on patient survival (34). In the present study, E-*FN1*-positive patients with GC exhibited worse OS compared with E-*FN1*-negative patients, whereas S-*FN1*

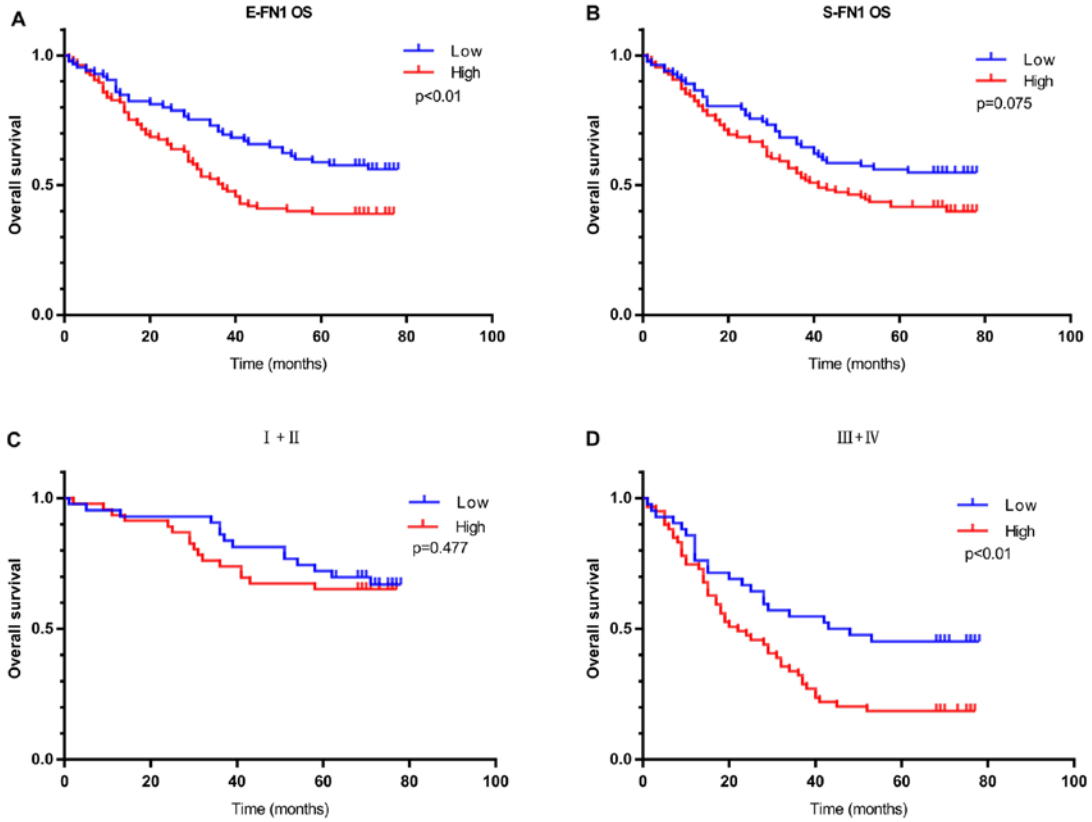

Figure 5. The prognostic significance of *FN1* in patients with gastric cancer based on immunohistochemistry. (A) Kaplan-Meier analysis of OS based on E-*FN1* expression in 190 patients. (B) Kaplan-Meier analysis of OS based on S-*FN1* expression in 190 patients. (C and D) Kaplan-Meier analysis of OS based on S-*FN1* expression in patients with (C) TNM stage I+II (D) and III+IV gastric cancer. *FN1*, fibronectin 1; S-*FN1*, stromal *FN1*; E-*FN1*, epithelial *FN1*; TNM, Tumor-Node-Metastasis; OS, overall survival.

Table IV. Multivariate analysis of overall survival in 190 patients with gastric cancer.

		95.0% CI for HR		
Characteristic	HR	Lower	Upper	P-value
E-*FN1* expression				0.001[b]
Negative vs. positive	2.115	1.343	3.333	
Tumor size				0.083
<5 cm vs. ≥5 cm	1.442	0.954	2.181	
Tumor grade				0.024[a]
Low + intermediate vs. high	1.286	1.034	1.601	
Depth of invasion				0.028[a]
T1+T2 vs. T3+T4	2.352	1.097	5.043	
TNM stage				0.005[b]
I + II vs. III + IV	2.124	1.258	3.585	

[a]P<0.05, [b]P<0.01, HR, hazard ratio; E-*FN1*, epithelial fibronectin 1; TNM, Tumor-Node-Metastasis.

expression had no significant effect on OS. In addition, in patients with high clinical TNM stage (III + IV), E-*FN1* positivity was strongly associated with OS. *FN1* was also confirmed as an independent predictor of overall survival in patients with GC by multivariate analysis.

Xu *et al* (15) and Zhang *et al* (16) demonstrated no *FN1* expression in the stroma of gastric cancer. In the IHC cohort of the present study, *FN1* was expressed in tumor cells and stromal cells, but not in normal epithelial cells. No association was observed between E-*FN1* and S-*FN1*. E-*FN1* expression in GC was significantly associated with tumor size. Soikkeli *et al* (40) reported that *FN1* is required for tumor and stromal cell growth. It may be speculated in large tumors, the central region is likely to be necrotic, and the expression of *FN1* may promote the migration of tumor cells and reduce necrosis.

In the present study, increased expression of the *FN1* gene at the protein and mRNA level in GC tissues was observed; *FN1* was highly expressed at the mRNA level in the advanced T stage group compared with that in the early T stage group, and the expression of *FN1* at the protein level was positively associated with tumor size. In addition, *FN1* expression at the protein and mRNA level was a predictor of poor prognosis following radical resection of GC. In conclusion, the expression of *FN1* in GC tissues may be upregulated, and *FN1* may be a biomarker of poor prognosis in patients with GC.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

YS wrote the manuscript and performed the majority of the experiments. CZ and YS participated in the study design, data acquisition and revision of the manuscript. YL and YH performed immunohistochemistry scoring, followed up the patients and collected clinical information. YY, HM and ZW analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all patients, and the study was approved by the Biomedical Ethics Committee of The First Affiliated Hospital of Zhengzhou University.

Patient consent for publication

Patients provided their consent for publication.

Competing interests

The authors declare that they have no competing interests.

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