

Research Paper

Overexpression of FNDC1 in Gastric Cancer and its Prognostic Significance

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Received: 2018.06.05; Accepted: 2018.09.10; Published: 2018.11.13

Abstract

Objectives: The aims of this study were to compare the expression of fibronectin type III domain containing 1 (FNDC1) in gastric cancer (GC) and normal gastric tissue, to explore the prognostic significance of FNDC1 expression in patients with gastric adenocarcinoma, and to analyze FNDC1-related signaling pathways.

Methods: The expression level of FNDC1 was initially predicted using the Oncomine and Cancer Genome Atlas databases. A Kaplan–Meier plotter database was mined to examine the clinical prognostic significance of FNDC1 mRNA in patients with GC. Subsequently, immunohistochemistry was used to measure FNDC1 protein expression levels in tissue from 90 cases of GC and paired adjacent normal tissue. Kaplan–Meier univariate and Cox multivariate survival analyses were used to determine the prognostic role of FNDC1 expression.

Results: Bioinformatic data indicated that FNDC1 mRNA expression levels were significantly highly expressed in GC compared with normal gastric tissue (all $P < 0.05$), and patients with GC with high FNDC1 mRNA expression levels had remarkably lower overall survival (all $P < 0.01$). Immunohistochemical results revealed that expression levels of FNDC1 protein were significantly increased in GC compared with normal gastric tissue ($P < 0.001$). Additionally, Kaplan–Meier univariate and Cox multivariate survival analyses indicated that increased expression of FNDC1 was an independent predictor of poor prognosis in patients with GC (all $P < 0.05$).

Conclusions: FNDC1 was highly expressed in GC, and high expression of FNDC1 was an independent predictor of poor prognosis in patients with GC. FNDC1 co-expressed genes were largely enriched in extracellular matrix–receptor interactions, which are closely related to tumor metastasis.

Key words: stomach neoplasms, prognosis, bioinformatics, FNDC1

Introduction

Gastric cancer (GC) is the fifth most common malignant tumor of the digestive tract and the third most common cause of cancer-related death worldwide[1, 2]. Most patients with GC are diagnosed at advanced stages of the disease, and thus have lost the best opportunities for treatment[3]. Despite gradual declines in the incidence of and mortality from GC in recent years, the overall prognosis of GC has not improved significantly[4]. Thus, exploring

highly specific and sensitive biomarkers and identifying new molecular targets may not only help clinicians predict prognosis, but also help clarify the mechanism underlying gastric tumorigenesis, which has profound significance for the development of new drug targets.

Fibronectin type III domain containing 1 (FNDC1) is a protein-coding and disease-related gene, also known as AGS8, FNDC2, or MEL4B, which

contains the conserved fibronectin type III domain of fibronectin (FN)[5-7]. FN is an important type of extracellular matrix (ECM) protein and a well-known regulator of tumorigenesis that is involved in cell proliferation, migration, and invasion in several human tumors[8-10]. Liu et al.[11] reported that FN may play a critical role in the modulation of cell proliferation and invasion via mTOR signaling activation in gallbladder cancer progression. Moreover, Vizoso and colleagues[12] found that breast cancer cells with distant metastases frequently expressed intracellular FN, and that FN expression by tumor cells was associated strongly with matrix metalloprotease expression by stromal mononuclear inflammatory cells, which may represent crosstalk that is of prognostic relevance in breast cancer. However, a limited number of studies have examined the function of FNDC1. Although reports have described the potential role of FNDC1 in inflammation and hypoxia-induced apoptosis of cardiomyocytes[13, 14], the expression levels of FNDC1 in malignant tumors, especially in GC, have not been well defined.

Therefore, in the present study, the Cancer Genome Atlas (TCGA) and OncoPrint databases were used to compare the expression levels of FNDC1 mRNA in GC and normal gastric tissue. In addition, the Kaplan-Meier plotter database was used to evaluate the prognostic significance of FNDC1 expression in patients with GC. Moreover, a tissue microarray (TMA) was used to examine the expression level of FNDC1 protein in 90 GC cases, and its prognostic value was further investigated using Kaplan-Meier and Cox survival analyses.

Materials and Methods

Bioinformatic mining methods

The cancer-related public databases OncoPrint and TCGA were used to predict the expression levels of FNDC1 in GC and normal gastric tissue. In the OncoPrint database, we entered the gene name "FNDC1" and chose the differential gene analysis module (GC versus normal) to retrieve the results. Expression of FNDC1 between primary GC and normal gastric tissue was also reviewed using the GEPIA browser (<http://gepia.cancer-pku.cn/>), which is an online tool for the analysis of data provided by TCGA and the GTEx projects[15]. The association between FNDC1 expression and overall survival (OS) was analyzed by data mining in the Kaplan-Meier plotter database (<http://kmplot.com>), which is an online database that enables validation of survival-associated GC biomarkers using transcriptomic data from 1065 patients[16]. The median FNDC1

expression was used as the cutoff. Hazard ratios with 95% confidence intervals and log-rank *P* values were calculated.

KEGG pathway analysis using the cBioPortal database

The genes co-expressed with FNDC1 in GC (absolute Pearson's *r* \geq 0.5) were identified using the cBioPortal database (<http://www.cbioportal.org/>). Then, the co-expressed genes were loaded into ClueGo in Cytoscape for analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways[17]. Only pathways with *P* values \leq 0.05 were included.

Table 1. Relationship between FNDC1 levels and clinicopathological parameters of GC patients.

Clinicopathological parameters	Cases(N)	FNDC1 expression level		χ^2	<i>P</i> value
		Low	High		
Age at surgery (years)					
<60	27	9	18		
\geq 60	63	16	47	0.593	0.441
Gender					
Female	22	10	12		
Male	68	15	53	4.535	0.033
Tumor location					
Antrum	38	11	27		
Other sites	52	14	38	0.045	0.832
Tumor size (cm)					
<5	36	13	23		
\geq 5	53	12	41	1.926	0.165
Histological differentiation					
Well	29	8	21		
Moderate/poor	61	17	44	0.001	0.978
Neural/vascular invasion					
No	72	19	53		
Yes	18	6	12	0.346	0.556
Lymph node metastasis (n)					
<3	38	14	24		
\geq 3	52	11	41	2.694	0.101
TNM stage					
I-II	36	16	20		
III-IV	54	9	45	8.308	0.004

GC tissue and patient clinical information

A human TMA (cat no. HStmA180Su09) containing paired tumor and normal tissue samples from 90 cases was purchased from Shanghai Outdo Biotech Co., Ltd. (Shanghai, China). Detailed clinical characteristics, including patient age and sex, tumor size, histologic differentiation, and TNM stage, are shown in Table 1. All patients with GC received radical surgery between December 2009 and June 2010, with ultimate follow-up through June 2016. Patients who underwent chemotherapy or radiotherapy before the surgery were excluded from the study.

Immunohistochemistry and interpretation of the results

Immunohistochemical staining was performed according to the manufacturer's protocol to examine the expression levels of FNDC1 in GC and matched normal tissue. An FNDC1 rabbit polyclonal antibody was purchased from Absin Corporation (abs127634a; Absin, China) and used at a dilution of 1:500. Two pathologists independently evaluated immunostaining of each tissue section in a double-blind manner. The immunoreactive score (IRS) for each slice was calculated by multiplying the staining intensity in four gradations (0, negative; 1, weak; 2, moderate; 3, strong) with the percentage of positive cells in five gradations (0, negative; 1, < 10%; 2, 10%-50%; 3, 51%-80%; 4, > 80%), each specimen was measured in three different magnification fields[18]. IRS ranged from 0 to 12, with IRS > 4 indicating high FNDC1 expression and IRS ≤ 4 indicating low FNDC1 expression.

Statistical analysis

The SPSS 24.0 software (SPSS, Inc., Chicago, IL, USA) was used to analyze the experimental data. Quantitative data were presented as means ± standard deviations. The differential expression levels of FNDC1 mRNA between the GC group and the

normal tissue group from TCGA and Oncomine databases were analyzed using the independent-samples *t* test. The chi-squared test was performed to analyze the relationship between FNDC1 expression level and clinicopathological parameters. IRS scores of FNDC1 staining in tumor and adjacent non-tumor gastric tissues were compared by Wilcoxon rank-sum test. Kaplan-Meier survival analyses were performed using the log-rank test, and a Cox regression model was established for a multivariate survival analysis to determine prognostic factors using variables that were significant in the univariate analysis. *P* values < 0.05 were considered to indicate statistical significance.

Results

Overexpression of FNDC1 mRNA and protein levels in GC

TCGA and Oncomine database data were used to examine the differential expression levels of FNDC1 mRNA between GC and normal gastric tissue. The FNDC1 mRNA expression level was dramatically higher in GC tissue than in normal gastric tissue (all *P* < 0.05, Figure 1A-H). To confirm the predictive results, immunohistochemical assays were performed to examine FNDC1 protein levels in 90 cases of GC with paired adjacent tissue (Figure 2A,

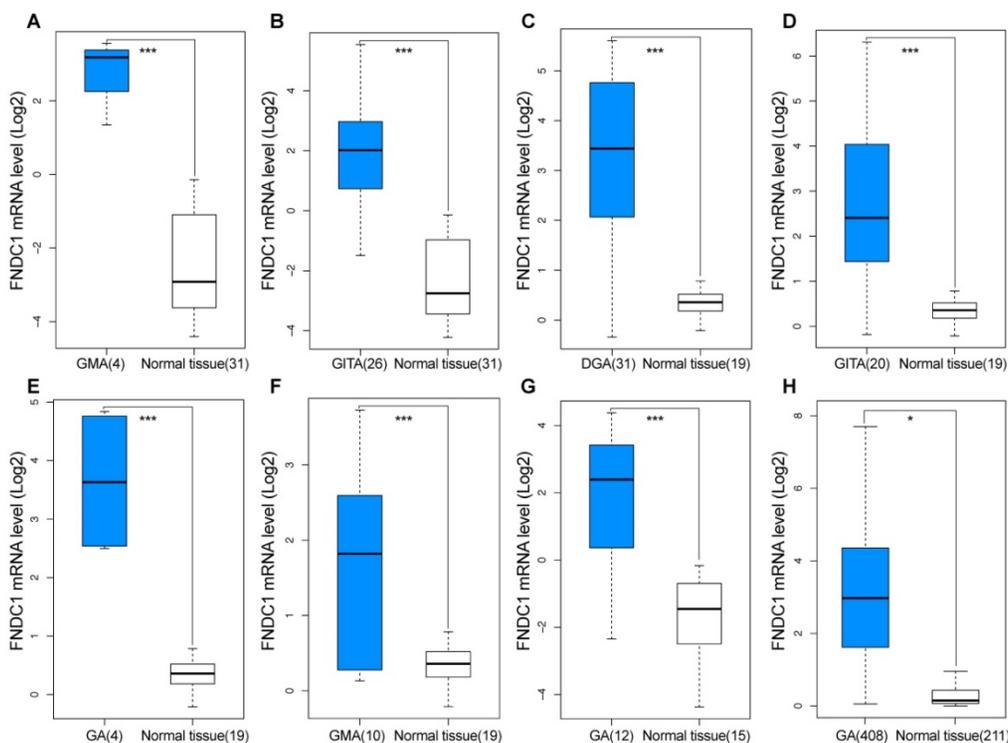


Figure 1. Overexpression of FNDC1 in gastric cancer predicted using GEO and TCGA data. FNDC1 mRNA levels of GC versus normal gastric tissue in (A, B) DErrico Gastric (GEO: GSE13911), (C-F) Cho Gastric (GEO: GSE13861), (G) Wang Gastric (GEO: GSE19826), and (H) TCGA and GTEx databases. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. DGA, diffuse gastric adenocarcinoma; GMA, gastric mixed adenocarcinoma; GITA, gastric intestinal type adenocarcinoma; GA, gastric adenocarcinoma.

B). The mean \pm standard deviation IRS scores of FNDC1 in cancerous tissue and adjacent normal tissue were 5.822 ± 2.368 and 4.526 ± 2.219 , respectively. The IRS scores of FNDC1 in tumors were much higher than those in adjacent non-tumor gastric tissues ($P < 0.001$; Figure 2C and Supplementary Table S1-S2), indicating that the expression level of FNDC1 protein was significantly higher in GC tissues than in paired normal tissues.

Relationships between FNDC1 and clinicopathological parameters of patients with GC

Relationships between FNDC1 protein expression and clinicopathological parameters of patients with GC (age, sex, tumor site, tumor size, histological grade, neural/vascular invasion, lymph node metastasis, and TNM stage) were investigated. The differential expression level of FNDC1 protein (low versus high) was associated significantly with sex and TNM stage in patients with GC (all $P < 0.05$; Table 1).

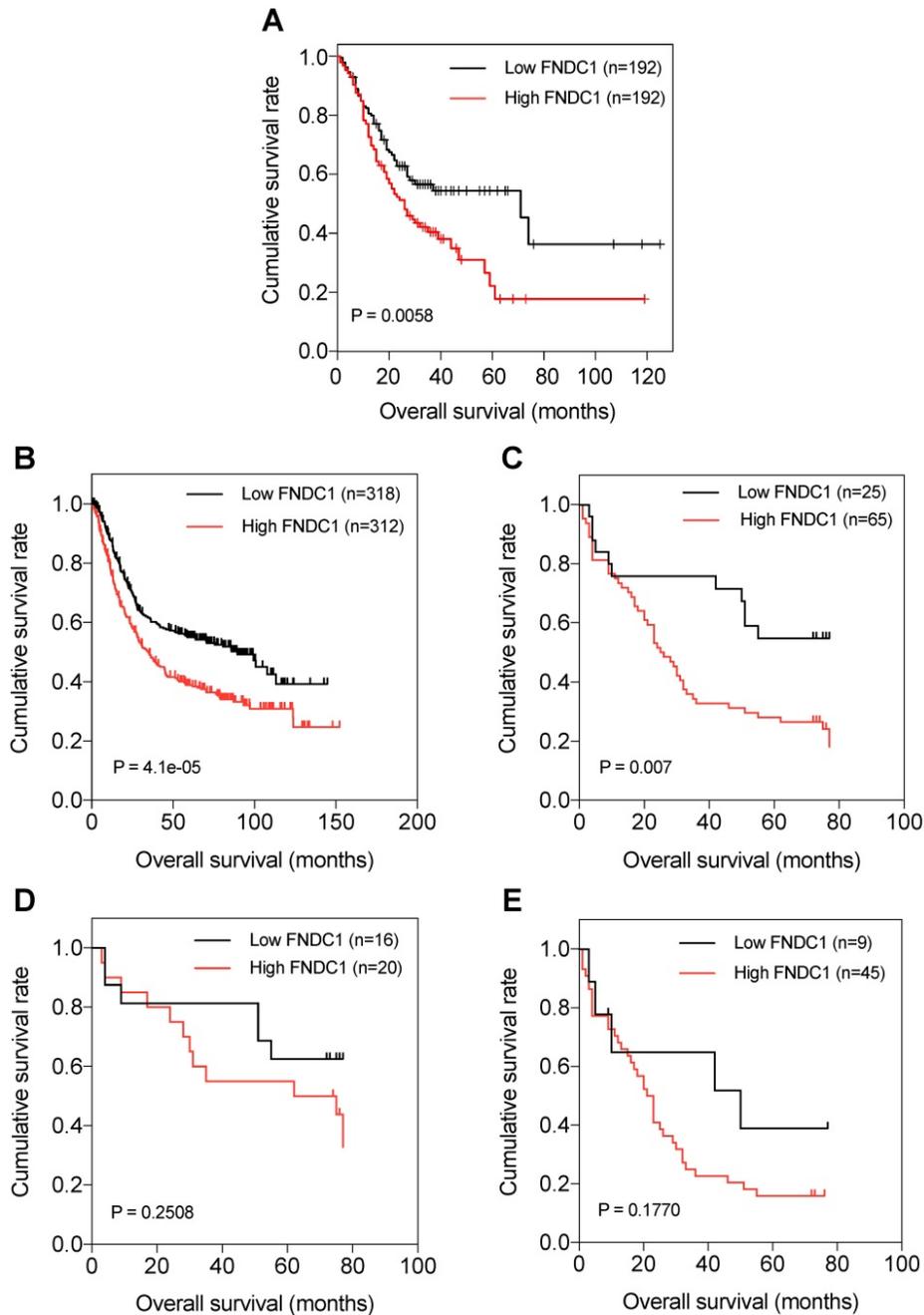


Figure 2. FNDC1 immunoreactivity was higher in gastric cancer tissues than in adjacent normal tissues. (A) Representative high expression of FNDC1 in GC tissue (HE, 200 \times); (B) Representative low expression of FNDC1 in matched adjacent normal tissue (HE, 200 \times). (C) The IRS in tumor tissues were statistically higher than that in adjacent normal tissues. *** $P < 0.001$. IRS, immunoreactive score; GC, gastric cancer; NT, normal tissue; HE, hematoxylin and eosin.

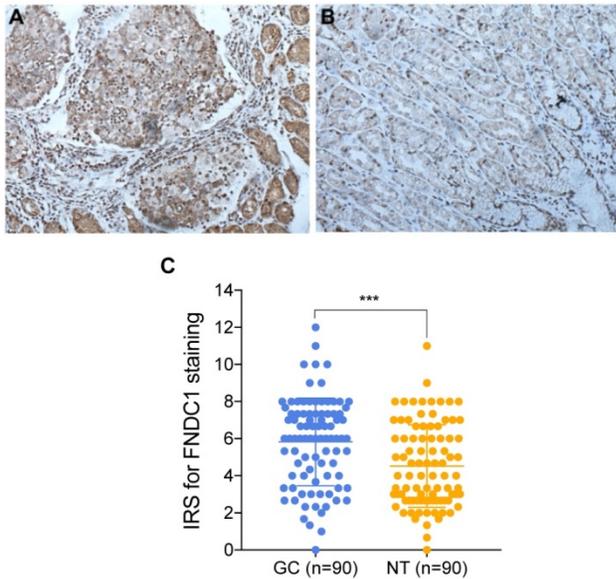


Figure 3. Kaplan–Meier curves of OS of patients with gastric adenocarcinoma based on FNDC1 mRNA and protein expression (low versus high). (A) Relationship between FNDC1 mRNA expression level and OS of patients with GC based on TCGA data. (B) Relationship between FNDC1 mRNA expression level and OS of patients with GC based on GEO data (GSE22377, GSE14210, GSE51105). (C) Relationship between FNDC1 protein expression level and OS of patients with GC based on immunohistochemical staining. (D) Relationship between FNDC1 protein expression level and OS of GC stage I-II. (E) Relationship between FNDC1 protein expression level and OS of GC stage III-IV. OS, overall survival; GC, gastric cancer.

High FNDC1 expression is associated with poor OS of patients with GC

The prognostic significance of FNDC1 expression levels in patients with GC was investigated. Through data mining in TCGA database and the Kaplan–Meier plotter, we found that OS was significantly lower among patients with GC with high expression of FNDC1 mRNA than among those with low expression, using the median as the cutoff value (all $P < 0.01$; Figure 3A, B). As FNDC1 is a protein-coding gene, to confirm the predictive results, we also analyzed the immunohistochemical staining results and found that OS was significantly lower among patients with GC with high expression of FNDC1 protein than among those with low expression ($P = 0.007$; Figure 3C). As shown in Table 1, the differential expression level of FNDC1 protein (low versus high) was significantly associated with TNM stage, so we further investigated the relationship between FNDC1 protein expression level and OS of GC patients stratified by clinical stage, but found no statistical significance ($P = 0.2508$ for stage I-II, Figure 3D; $P = 0.1770$ for stage III-IV, Figure 3E). In addition, a Cox univariate survival analysis revealed that FNDC1 expression, tumor location, tumor size, lymph node metastasis, and TNM stage were significant parameters affecting the survival

time of patients with GC (Table 2). These five significant factors were subsequently substituted into the multivariate Cox survival analysis, which indicated that high FNDC1 expression level and large tumor size were independent predictors of unfavorable prognosis in patients with GC (all $P < 0.05$; Table 3).

Table 2. Cox univariate survival analysis of FNDC1 and other clinicopathological parameters in GC patients.

Clinicopathological parameters	Mean survival time (months)	95% CI	P value
FNDC1 expression			
Low	54.053	42.469-65.636	0.006
High	34.522	27.582-41.461	
Age (years)			
<60	49.119	37.251-60.986	0.081
≥60	35.719	28.607-42.831	
Gender			
Female	42.182	28.761-55.603	0.521
Male	38.984	31.985-45.983	
Tumor location			
Antrum	46.184	35.984-56.384	0.043
Other sites	34.978	27.396-42.561	
Tumor size (cm)			
<5	54.435	44.577-64.294	0.000
≥5	28.918	22.085-35.751	
Lymph node metastasis (n)			
<3	51.588	41.985-61.190	0.002
≥3	30.963	23.591-38.335	
Neural/vascular invasion			
No	41.345	34.489-48.202	0.517
Yes	33.374	18.867-47.881	
Histological differentiation			
Well	48.069	37.481-58.657	0.133
Moderate/poor	35.780	28.274-43.286	
TNM stage			
I-II	54.287	44.729-63.845	0.000
III-IV	29.908	22.781-37.036	

Table 3. Cox multivariate analysis of FNDC1 and other clinicopathological parameters in GC patients.

Covariates	HR	95% CI for HR	P value
FNDC1 expression level (low vs. high)	2.143	1.069-4.297	0.032
Tumor location (antrum vs. other sites)	1.452	0.841-2.508	0.181
Tumor size (<5 vs. ≥5 cm)	2.259	1.129-4.522	0.021
Lymph node metastasis (<3 vs. ≥3)	1.212	0.515-2.853	0.660
TNM stage (I-II vs. III-IV)	1.232	0.492-3.086	0.656

KEGG pathway analysis of FNDC1 and its co-expressed genes in TCGA-STAD

Data mining using cBioPortal for TCGA was used to identify 1212 genes significantly co-expressed with FNDC1. A total of 265 genes were selected by setting absolute Pearson’s $r \geq 0.5$ as a threshold. Then, the genes were subjected to enrichment analysis with the KEGG pathways; we found that the genes were enriched in the following: steroid hormone biosynthesis, malaria, proteoglycans in cancer, Wnt signaling pathway, protein digestion and absorption, hedgehog

Table 4. KEGG pathway analysis of FNDC1 and its co-expressed genes in TCGA-STAD.

GOID	GO Term	Term PValue	Group PValue	% Associated Genes	Nr. Genes	Associated Genes Found
00140	Steroid hormone biosynthesis	4.62E-02	4.62E-02	5.17	3.00	[CYP11B1, CYP7B1, HSD11B1]
05144	Malaria	5.46E-04	5.46E-04	10.20	5.00	[LRP1, TGFB3, THBS1, THBS2, THBS3]
05205	Proteoglycans in cancer	1.94E-03	1.94E-03	4.43	9.00	[DCN, FN1, FZD1, LUM, MMP2, THBS1, TIMP3, TWIST2, WNT2]
04310	Wnt signaling pathway	3.60E-03	3.60E-03	4.90	7.00	[FZD1, PRICKLE1, SERPINF1, SFRP2, SFRP4, SOX17, WNT2]
04974	Protein digestion and absorption	1.47E-11	1.47E-11	15.56	14.00	[COL10A1, COL12A1, COL15A1, COL18A1, COL1A1, COL1A2, COL3A1, COL4A2, COL5A1, COL5A2, COL6A1, COL6A2, COL6A3, ELN]
04340	Hedgehog signaling pathway	4.49E-04	9.75E-05	10.64	5.00	[EVC, GAS1, GLI1, GLI2, GLI3]
04390	Hippo signaling pathway	5.40E-03	9.75E-05	4.55	7.00	[CTGF, DLG4, FZD1, GLI2, LATS2, TGFB3, WNT2]
05217	Basal cell carcinoma	1.73E-03	9.75E-05	7.94	5.00	[FZD1, GLI1, GLI2, GLI3, WNT2]
04151	PI3K-Akt signaling pathway	1.21E-06	4.05E-05	5.13	18.00	[COL1A1, COL1A2, COL4A2, COL6A1, COL6A2, COL6A3, FGF7, FN1, ITGA11, LAMA2, LAMA4, LAMB1, PDGFRA, PDGFRB, THBS1, THBS2, THBS3, VEGFC]
04510	Focal adhesion	1.42E-09	4.05E-05	8.54	17.00	[COL1A1, COL1A2, COL4A2, COL6A1, COL6A2, COL6A3, FN1, ITGA11, LAMA2, LAMA4, LAMB1, PDGFRA, PDGFRB, THBS1, THBS2, THBS3, VEGFC]
04512	ECM-receptor interaction	3.93E-12	4.05E-05	17.07	14.00	[COL1A1, COL1A2, COL4A2, COL6A1, COL6A2, COL6A3, FN1, ITGA11, LAMA2, LAMA4, LAMB1, THBS1, THBS2, THBS3]
04926	Relaxin signaling pathway	9.17E-03	4.05E-05	4.62	6.00	[COL1A1, COL1A2, COL3A1, COL4A2, MMP2, VEGFC]
04933	AGE-RAGE signaling pathway in diabetic complications	8.17E-06	4.05E-05	9.09	9.00	[COL1A1, COL1A2, COL3A1, COL4A2, FN1, MMP2, NOX4, TGFB3, VEGFC]
05146	Amoebiasis	7.05E-07	4.05E-05	10.42	10.00	[COL1A1, COL1A2, COL3A1, COL4A2, FN1, IL1R1, LAMA2, LAMA4, LAMB1, TGFB3]
05165	Human papillomavirus infection	1.47E-06	4.05E-05	5.33	17.00	[COL1A1, COL1A2, COL4A2, COL6A1, COL6A2, COL6A3, FN1, FZD1, ITGA11, LAMA2, LAMA4, LAMB1, PDGFRB, THBS1, THBS2, THBS3, WNT2]
05222	Small cell lung cancer	9.22E-03	4.05E-05	5.38	5.00	[COL4A2, FN1, LAMA2, LAMA4, LAMB1]

Discussion

The FNDC1 gene is located in the 6q25.3 human chromosomal region, and was first identified as a differentially expressed mRNA from human dermal fibroblasts. The biological function of FNDC1 has yet to be well defined, and the majority of studies concerning FNDC1 have shown it may play an important role in inflammation. The minor (T) allele of SNP rs2392989 was reported to confer a higher risk of acute otitis media and to be correlated with higher expression of FNDC1[6]. In addition, FNDC1 expression in the mouse middle ear was upregulated upon lipopolysaccharide treatment, which is known to be a potent inducer of inflammation through stimulation of transforming growth factor- β , tumor necrosis factor- α , and interleukin-1 signaling[19]. To date, the only functional study of FNDC1 in human cancer was reported by Das and colleagues[5]. They discovered that miR-1207-3p was significantly under-expressed in prostate cancer (PCa) cell lines in comparison with normal prostate epithelial cells, and that increased expression of microRNA-1207-3p significantly inhibited proliferation and migration, and induced apoptosis of PCa cells via direct targeting of FNDC1. They also revealed significant overexpression of FNDC1, fibronectin (FN1), and the androgen receptor in human PCa cell lines and tissue,

which was correlated positively with aggressive PCa. However, the expression level of FNDC1 and its potential clinical significance in GC have not been well defined.

In the current study, we first used bioinformatics to predict the high expression of FNDC1 in gastric adenocarcinoma tissue compared with normal gastric tissue using the Oncomine and TCGA public databases. Then, immunohistochemistry was used to verify that FNDC1 protein expression levels were significantly higher in the GC tissue group compared with the normal tissue group. These results were consistent with those of bioinformatic predictions and suggested that FNDC1 acts as an oncogene by promoting the development of gastric adenocarcinoma. Subsequently, the clinical prognostic significance of FNDC1 gene expression levels in patients with GC was examined. First, differential expression levels of FNDC1 protein were associated significantly with sex and TNM stage in patients with GC. Moreover, a Kaplan–Meier univariate survival analysis showed that, compared with those with low FNDC1 mRNA and protein expression levels, patients with GC with high FNDC1 expression levels had remarkably shorter OS. A multivariate Cox analysis showed that high expression of FNDC1 and advanced TNM stage were independent predictors of unfavorable prognosis in patients with GC. Our results also

showed that the genes co-expressed with FNDC1 in GC were most enriched in ECM-receptor interactions. It is well known that the complex interactions between tumor cells and the ECM may play important roles in tumor metastasis, including cell differentiation, migration, cytoskeletal reorganization, and morphological transition[20, 21]. According to previous reports[22, 23], FN is considered a key element in ECM components and plays an important role in cancer cell proliferation, differentiation, and migration. As FNDC1 contains the conserved fibronectin type III domain of FN, we inferred that FNDC1 may play an important role in human cancer tumorigenesis. All of these findings indicate that FNDC1 was over-expressed in GC tissue and that high levels of FNDC1 expression could indicate poor prognosis for patients with GC. Thus, this gene may be a key target involved in the growth and metastasis of GC.

However, this study is not without limitations. First, the cases used were collected non-sequentially, which may have resulted in selection bias and influenced the final statistical results. Next, tissue from only 90 cases was used in this study, which was a small sample. In addition, some clinicopathological data associated with GC, such as disease-free survival, were not included. Lastly, although KEGG pathway analysis indicated that the genes co-expressed with FNDC1 were enriched in ECM-receptor interactions, the detailed underlying molecular mechanisms have not been determined. The above limitations should be addressed in future studies.

Conclusions

In conclusion, our study revealed that FNDC1 was highly expressed in GC, and overexpression of FNDC1 was an independent predictor of reduced prognosis in patients with GC. FNDC1 co-expressed genes are largely enriched in ECM-receptor interactions, which are related to tumor metastasis. FNDC1 may be a potential prognostic biomarker and therapeutic target for GC in the future.

Supplementary Material

Supplementary tables.

<http://www.jcancer.org/v09p4586s1.pdf>

Acknowledgements

We greatly appreciate the technological help from the department of pathology (Shanghai Fifth People's Hospital, Fudan University, Shanghai, China) for the IHC staining and data analysis. We also sincerely thank Dr. Jun Hou (Department of Pathology, Zhongshan Hospital, Fudan University, Shanghai, China) for his kind help in pathology.

Funding Statement

This work was supported by Shanghai Fifth People's Hospital (Institutional Grant for Newly Imported Talents 2016WYRC01); Shanghai Minhang District Health and Family Planning Commission (Research Fund 2016MW03); Shanghai Minhang District Science and Technology Commission (Natural Science Fund 2017MHZ02); Key Medical Specialty funded by The Fifth People's Hospital of Shanghai, Fudan University (NO. 2017WYZDZK01); and Great Discipline Construction Project from the Medical System of Shanghai Minhang District. (NO. 2017MW DXK01).

Competing Interests

The authors have declared that no competing interest exists.

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