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ORIGINAL ARTICLE

Roles of Fibroblast Activation Protein and Hepatocyte Growth Factor Expressions in Angiogenesis and Metastasis of Gastric Cancer

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Abstract This study aims to explore the roles of fibroblast activation protein (FAP) and hepatocyte growth factor (HGF) expressions in the angiogenesis and metastasis of gastric cancer (GC). From May 2012 to December 2015, 110 GC patients who received surgical treatment in the First Hospital of Qinhuangdao were selected. The HGF and FAP expressions in 110 cases of GC, 130 cases of normal gastric mucosa and 115 cases of gastric ulcer were detected by streptavidin-perosidase (SP) method. Venous blood HGF level of GC patients was tested by enzyme-linked immunosorbent assay (ELISA). The micro-vessel number of the patients in the three groups were calculated and analyzed. In GC group, positive expression rates of FAP and HGF protein were 61.8% and 67.3% respectively, which were both higher than those in normal gastric mucosa and gastric ulcer groups. The micro-vessel numbers in patients of the normal gastric mucosa and gastric ulcer groups are far less than that in GC group. FAP, HGF and micro-vessel density (MVD) were significantly correlated with infiltration depth, tumor-node-metastasis (TNM) staging, lymph node metastasis (LNM) and distant metastasis. The results of ELISA showed that serum HGF level was related to tumor size, infiltration degree, TNM staging, LNM and distant metastasis. FAP and HGF expressions in GC were positively correlated with MVD, and the expressions of FAP and HGF in GC were in positive correlation. Our study provided evidence that high FAP and HGF expressions may be positively correlated with the angiogenesis and metastasis of GC.

Keywords Gastric cancer · Fibroblast activation protein · Hepatocyte growth factor · Angiogenesis · Metastasis · Microvessel density

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Introduction

Gastric cancer (GC) is a common malignant tumor in the world whose incidence rate ranked fourth among all malignant tumors; and the mortality rate of GC is second only to lung cancer, with an average annual death of 650,000 [1]. Moreover, GC is a multi-factorial disease, and many genetic and environmental factors played their parts in the development of GC, including the genetic background of the host, infection and dietary habits [2]. It has been revealed that the diagnostic rate and recovery rate of early GC patients could be remarkably increased through endoscopic examination, and, D2 radical surgery is regarded as a standard treatment for GC patients [3, 4]. Previous studies have confirmed that although the incidence and mortality of GC patients after D2 radical surgery are decreased, the reoccurrence rate remains high and there are still more than 25% of the postoperative patients develop lymph node metastasis (LNM) [4, 5]. The main treatment strategies of GC include surgery, chemotherapy, radiation therapy, gastric stent, and palliative care, among which surgery is the most commonly-used, however, the optimal strategy for treating GC patients remained to be explored [3, 6]. In recent years, fibroblast activation protein (FAP) is found to be highly-expressed in the invasion and metastasis of GC, suggesting that FAP expression may be an effective therapeutic target for GC treatment [7]. Also it had been proved

that Hepatocyte growth factor (HGF) played a key role in the occurrence and development of many tumors including GC through regulating the process of proliferation, invasion and angiogenesis [8, 9].

FAP is a type II membrane-bound glycoprotein, which belongs to the family of serine protease and is expressed by carcinoma associated fibroblasts (CAFs) [10]. Moreover, it has been identified as reaction tumor matter into fibroblasts, because it is reported to be selectively expressed in peritumoral stromal fibroblasts of multiple epithelial cancers such as basal cell carcinoma [11]. As a matrix cell marker, FAP proteolytic activity could enhance the invasion of tumor cells to the extracellular matrix, which is also a therapeutic target in tumor microenvironment [12, 13]. HGF and hepatocyte growth factor receptor (C-MET) genes was found associated in a previous study [14]. HGF is a growth and different motogenic cell type, including vascular endothelial cells and smooth muscle cells, which could promote angiogenesis effect [15]. Previous study shows that HGF is beneficial for the treatment of neovascular through direct gene transfer, so HGF could be the dominant angiogenic and protective factor secreted by ASCs [16]. HGF signal transduction pathways has been shown to trigger a variety of cellular responses and plays an important role in human normal cells and malignant cell transformation, invasion and metastasis processes [17]. There were few studies that focused on the relationship between FAP and HGF protein expression and GC angiogenesis and metastasis. Therefore, our study aims to investigate the roles of FAP and HGF expressions in GC angiogenesis and metastasis.

Materials and Methods

Ethics Statement

The study is in conformity with Chinese laws, and informed consent has been obtained from all participants. The study was approved by the Committees for the First Hospital of Qinhuangdao.

Study Subjects

A total of 110 GC patients treated with surgical therapy in the First Hospital of Qinhuangdao City from May, 2012 to October, 2015 were selected. Among them, there were 76 males and 34 females with the average age of 57.3 ± 8.6 years. Inclusion criteria: patients who were never treated with chemotherapy or radiotherapy before or during the surgery; patients who received subtotal or total gastrectomy; patients who had not take corticosteroid drugs and non-steroidal anti-inflammatory drugs for a long term; patients with detailed clinic pathological data and surgery records. Exclusion criteria: patients diagnosed with hypertension, coronary heart disease, diabetes, nephrosis or other diseases; those who were treated with drug therapy, chemotherapy, radiotherapy and immunotherapy; individuals without complete pathological data. According to the tumor-node-metastasis (TNM) staging proposed by International Union Against Cancer (UICC) [18], 110 patients with GC were divided into: 48 in stage I and II and 62 in stage III and IV; according to histological grade standard, 71 were well or moderately differentiated and 39 were poorly differentiated; in terms of infiltration degree, 78 were found serosal infiltration and 32 were not; 69 were found LNM and 41 were not; in terms of tumor diameter, 63 had ≤5 cm tumor and 47 had >5 cm; 35 were found with distance metastasis and 75 without. Besides, 130 people with normal gastric mucosa tissues and 115 people with gastric ulcer tissues were selected to compose the control group. After being detected by endoscope and mucosal biopsy in Department of Gastroenterology of the hospital, normal gastric mucosa was confirmed to be more than 5 cm apart from tumor without cancer involved; and through detection of gastric ulcer tissue by X-ray barium meal examination and endoscope, it was found that ulceration invaded muscularis mucosa with regular border and lesions such as inflammatory edema, inflammatory cell infiltrate and fibroplastic proliferation. The bottom of gastric ulcer tissues were clean and was covered by gray fibrinous exudates, no carcinogenesis detected. There was no significant difference in age and gender between patients in the control group and GC patients, which resulted in comparability between them.

Sample Collection

After subtotal or total gastrectomy, patients' tumor border was taken as the sample of GC; the far-end normal mucosa of incised diseased stomach sample was taken as the sample of normal gastric mucosa; and gastric ulcer tissue was taken in deviation of gastric antrum at the junction of the gastric body and gastric antrum. The average volume of all collected samples was $1 \times 1 \times 0.4$ cm^3. The samples were then numbered, fixed with 4% methanol, embedded with paraffin, and serially sectioned into 20 μm thick for later experiments.

Streptavidin-Perosidase (SP) Method

Streptavidin-perosidase (SP) method was adapted to detect the FAP and HGF protein expression in each group. Phosphate-buffered saline (PBS) buffer was used to replace the first antibody as blank control, and the positive control picture attached to the reagent was used as positive contract (the SP reagent kit was purchased from Beijing Zhongshan Biotechnology Co., Ltd., Beijing, China). The sections were sealed by silicone in 2% 3-triethoxysilylpropylamine (APES), pulled in 500 mL pure acetone and heated at 37 °C for 48 ~ 72 h. Afterwards, the sections were successively

deparaffinized, processed with 3% hydrogen peroxide, fully rinsed with distilled water for 5 min and with PBS buffer for 15 min, and processed with antigen retrieval citrate buffer. When the PBS buffer on the sections dried, 50 μL non-immune goat serums were added in drops, after which the sections were incubated at room temperature for 15 min. In addition, sections stained with CD34 (the mouse anti-human CD34 monoclonal antibody was obtained from Beijing ZSGB-Bio, Co., Ltd., Beijing, China) were added with normal goat serum and incubated at room temperature for 10 min. The section were treated with first antibody and incubated at 4 °C overnight and then with biotinylated secondary antibody and incubated at 37 °C for 30 min, followed by being rinsed with PBS buffer. SP complex solution was dropped onto the sections, and streptavidin solution labeled with horseradish peroxidase was added onto CD34 stained sections, which was followed by incubation at room temperature for 10 min, and FAP and HGF protein expressions were then detected. According to the specification of SP reagent kits, sections were stained with FAP and HGF, counter stained with hematoxylin, sealed with neutral balsam and colored with diaminobenzidine (DAB) (DAB coloration kit was purchased from Beijing Zhongshan Biotechnology Co., Ltd., Beijing, China). The remaining steps were also taken strictly in accordance with the specification of the reagent kit.

Brownish cells in cytomembrane and cytoplasm were determined as FAP and HGF-positive, and further analysis was done according to morphology and position of the cells. Scores were given on the basis of staining degree and percentage of positive cells: 10 exemplary fields of view were selected and 50 cells in each field were counted randomly, thus a total of 500 cells were observed. The percentage of positive cells was presented by scores: no FAP and HGF protein expression: 0 point; < 10%, 1 ~ 2 points; 10% ~ 50%, 2 ~ 3 points; > 50%, > 3 points; substantially colorless, 0 point; light color, 1 point; dark color, 2 points. The total score of the field was worked out by multiplying the two scores above, and the average of total score served as the final staining results. In terms of the final scores, 0 ~ 1 point stood for negative (−), 2 ~ 4 points, weak positive (+); 5 ~ 7 points, positive (++); 8 ~ 9 points, strong positive (+++).

Microvessel Count

The influences from factors not qualified to be involved in micro-vessel count were ruled out. Any well-stained endothelial cell or cell cluster in fields of view was considered a countable micro-vessel. Besides, the cells to be counted should be apart from micro-vessel of adjacent tumor cells and other connective tissues; when the endothelial cells in the same micro-vessel were, however, well stained and apart from each other, they could still serve as a single countable micro-vessel. Micro-vessel count was performed as follows: Firstly, the overall sections were observed at low magnification, and then 3 fields with dense micro-vessel were selected in each section to be observed at high magnification with the field area being 0.443 mm^2. Finally the average count of the 3 fields was drawn to be the micro-vessel density (MVD) count, which was presented by mean ± standard deviation (SD).

Enzyme-Linked Immunosorbent Assay (ELISA)

Blood sampling was performed on all GC patients within 24 h after admission to obtain 3 mL peripheral venous blood from each one. The serum samples were centrifuged at 3000 rpm for 15 min to separate serum, and the separated serum was preserved at −70 °C. The expression of HGF protein in peripheral venous blood was detected by ELISA (human anti-mouse antibody ELISA reagent kit was purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). The preserved serum was diluted at the ratio of 1: 2, and then placed into microplate covered by rabbit-anti human HGF polyclonal antibody. After 2 h incubation, the microplate was rinsed. Horseradish peroxidase-conjugated mouse anti-human HGF monoclonal antibody (from Beijing Zhongshan Biotechnology Co., Ltd., Beijing, China) was then added. Afterwards, the serum was incubated at 37 °C for 2 h and added with hydrogen peroxide and tetramethylbenzidine. The 450 nm absorbance (A) was measured by enzyme-linked immune detector to figure out the concentration of FAP and HGF protein.

Statistical Analysis

SPSS 21.0 software was employed for statistical analysis, in which enumeration data was shown in the form of case number and percentage; the value of FAP and HGF protein staining among the 3 groups were compared by rank-sum test; positive rates and clinic pathological features among the groups were compared by χ^2 test; measurement data was shown by $\overline{x} \pm s$, and the total average scores among the groups were compared by *t* test; the correlation between FAP, HGF and MVD was analyzed by Spearman's rank correlation method. $P < 0.05$ meant that the differences were statistically significant.

Results

Comparison of FAP and HGF Expressions and MVD in Gastric Cancer Tissues, Normal Gastric Tissues and the Gastric Ulcer Tissues

The positive expressions of FAP and HGF protein in GC cells presented the color of pale brown to deep brown (Fig. 1). The

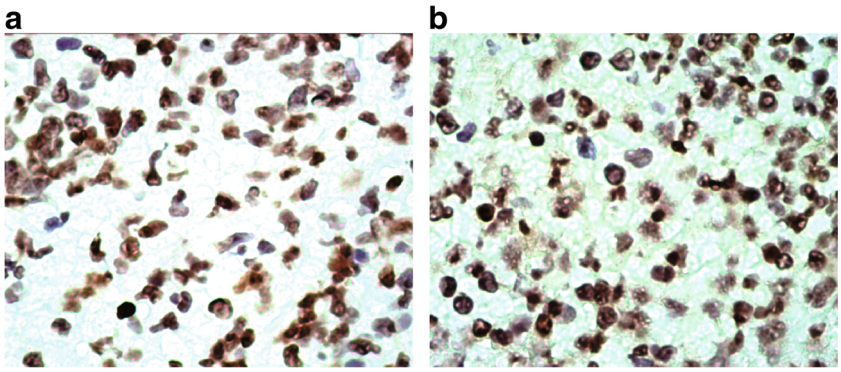

Fig. 1 Positive expression of FAP and HGF proteins in gastric cancer tissue. Notes: **a**, positive expression of FAP in gastric cancer tissue; **b**, positive expression of HGF in gastric cancer tissue; FAP, fibroblast activation protein; HGF, hepatocyte growth factor

expressions of FAP and HGF were detected in the normal gastric mucosa group and the gastric ulcer group; however, the expressions were relatively low. Specifically, the positive expressions of HGF were respectively 43.1% and 44.3%, and those of FAP were respectively 33.1% and 33.9% in the normal gastric mucosa group and the gastric ulcer group (both $P > 0.05$). In the 110 samples from GC patients, the positive expressions of FAP and HGF were 61.8% and 67.3% respectively, which were significantly higher than those in the normal gastric mucosa group and the gastric ulcer group (all $P < 0.05$). Besides, no obvious difference in FAP and HGF was observed between the normal gastric mucosa group and the gastric ulcer group ($P > 0.05$). Still, micro-vessel was seen in the control group. The MVDs of the normal gastric mucosa group, the gastric ulcer group and the GC group were 8.36 ± 2.65, 8.29 ± 2.63 and 25.14 ± 14.39, respectively, and in GC group, obvious microvascular proliferation was observed. The MVD of GC group was significantly higher than that of the normal gastric mucosa group and the gastric ulcer group (both $P < 0.05$), while there was no statistical difference between the normal gastric mucosa group and the gastric ulcer group ($P > 0.05$) (Table 1).

Correlation Between FAP and HGF Expressions and Clinicopathological Characteristics of GC Patients

Expressions of FAP and HGF were not correlated with patients' gender, age, tumor size and differentiation degree (all $P > 0.05$), but they were closely correlated with depth of tumor invasion, TNM staging, LNM and distant metastasis (all $P < 0.05$). For GC patients with depth of tumor invasion of TI/T2, the positive expression rate was 43.8% and 31.3% respectively, while for those with depth of tumor invasion of T3/T4, the positive expression rate was 69.2% and 75.6%, respectively. In terms of TNM staging, among 110 GC patients, for those of stage I and II, the positive expression rates of FAP and HGF protein were 37.5% and 50.0% respectively, while for those of stage III and IV, the positive expression rates were both more than 80%. Compared with stage I and II, the positive expressions of FAP and HGF were significantly higher in stage III and IV (all $P < 0.05$). In addition, patients with LNM and distant metastasis both had over 78% of positive FAP and HGF expression rates, while these two rates were both less than 55% for those without metastasis (all $P < 0.05$) (Table 2).

Correlation Between Serum HGF Levels and Clinicopathological Characteristics of GC Patients

The results of serum HGF level detected by ELISA showed: patients with >5 cm tumor had higher serum HGF levels than those with ≤5 cm tumor; as for TNM staging, patients of stage I and II had significantly lower serum HGF level than those of stage III and IV; and as for depth of tumor invasion, patients of T3 and T4 had higher serum HGF levels than those of T1 and T2, from which the conclusion could be drawn that serum HGF level was statistically significant in tumor size, depth of tumor invasion and TNM staging (all $P < 0.05$); there

Table 1 Comparison of FAP and HGF expressions and MVD in gastric cancer tissues, normal gastric tissues and the gastric ulcer tissues

Tissue type	N	FAP		HGF		MVD
		Positive (%)	Negative (%)	Positive (%)	Negative (%)	
Gastric cancer	110	68 (61.8)*	42 (38.2)	74 (67.3)*	36 (32.7)	25.14 ± 14.39*
Normal gastric mucosa	130	43 (33.1)	87 (66.9)	56 (43.1)	74 (56.9)	8.36 ± 2.65
Gastric ulcer	115	39 (33.9)	76 (66.1)	51 (44.3)	64 (55.7)	8.29 ± 2.63

*, compared with normal gastric mucosa and gastric ulcer, $P < 0.05$. FAP, fibroblast activation protein; HGF, hepatocyte growth factor; MVD, microvessel density

Table 2 Correlations between FAP and HGF expressions and clinicopathological characteristics of patients with gastric cancer

Characteristic	N	FAP		HGF	
		Positive (%)	*P*	Positive (%)	*P*
Age (years)			0.936		0.561
≥ 60	44	27 (61.4)		31 (70.5)	
< 60	66	41 (62.1)		43 (65.2)	
Gender			0.400		0.620
Male	76	45 (59.2)		50 (65.8)	
Female	34	23 (67.6)		24 (70.6)	
Tumor size			0.226		0.282
≤ 5 cm	63	42 (66.7)		45 (71.4)	
> 5 cm	47	26 (55.3)		29 (61.7)	
Differentiation degree			0.649		0.92
Well or moderately differentiated	71	45 (63.4)		48 (67.6)	
Poorly differentiated	39	23 (59.0)		26 (66.7)	
Depth of tumor invasion			0.013		0.004
T1/T2	32	14 (43.8)		15 (31.3)	
T3/T4	78	54 (69.2)		59 (75.6)	
TNM staging			< 0.001		0.001
I + II	48	18 (37.5)		24 (50.0)	
III + IV	62	50 (80.6)		50 (80.6)	
Lymph node metastasis			< 0.001		0.001
Yes	69	56 (81.2)		54 (78.3)	
No	41	12 (29.3)		20 (48.8)	
Distant metastasis			0.001		< 0.001
Yes	35	30 (85.7)		34 (97.1)	
No	75	38 (50.7)		40 (53.3)	

FAP, fibroblast activation protein; HGF, hepatocyte growth factor; TNM, tumor-node-metastasis

was a much higher serum HGF level in patients with LNM and distant metastasis than in those without metastasis (both $P < 0.05$), which suggested that serum HGF level was correlated with metastasis of GC (Table 3).

Correlation Between MVD and Clinicopathological Characteristics of GC Patients

MVD was not correlated with patients' gender, age, tumor size and differentiation degree (all $P > 0.05$). Of depth of tumor invasion, MVD was 21.81 ± 0.66 in patients of T1/T2, lower than those of T3/T4 (28.89 ± 0.78) ($P < 0.05$); of TNM staging, MVD was 20.30 ± 0.60 in patients of stage I and II, significantly lower than those of stage III and IV (28.89 ± 0.78) ($P < 0.05$); MVD was 28.24 ± 0.76 in patients found LNM, higher than those who were not found it (19.91 ± 0.62) ($P < 0.05$); and MVD was 31.37 ± 0.82 in patients with distant metastasis, statistically higher than those without it (22.23 ± 0.65) ($P < 0.05$) (Table 4).

Correlation Between FAP and HGF Expressions and MVD in Gastric Cancer Tissues

The MVDs in FAP and HGF-positive patients were 29.84 ± 0.81 and 29.10 ± 0.79, respectively, while those in FAP and HGF-negative patients were 17.53 ± 0.52 and 16.98 ± 0.51, respectively. The MVDs in FAP and HGF-negative patients were much lower than those in FAP and HGF-positive patients (all $P < 0.05$). Spearman rank correlation analysis showed that expressions of FAP and HGF in GC were positively related with MVD ($r = 0.693$, $P = 0.000$; $r = 0.664$, $P = 0.000$), which suggested that FAP and HGF in GC tissues could promote tumor angiogenesis. Among 110 GC patients, 65 were both FAP and HGF-positive and 33 were both FAP and HGF-negative with concordance rate of the two protein expressions being 89.1% (98/110). And correlation analysis showed that expressions of FAP and HGF in GC were positively correlated ($r = 0.768$, $P < 0.05$) with a high concordance rate. It suggested the coordinative contribution of FAP and HGF in tumor angiogenesis of GC (Tables 5 and 6).

Table 3 Correlation between serum HGF levels and clinicopathological characteristics of patients with gastric cancer

Characteristic	N	HGF	
		Serum level (μg/L)	*P*
Age (years)			0.615
≥ 60	44	4.41 ± 0.76	
< 60	66	4.34 ± 0.68	
Gender			0.785
Male	76	4.38 ± 0.71	
Female	34	4.34 ± 0.71	
Tumor size			0.025
≤ 5 cm	63	4.21 ± 0.67	
> 5 cm	47	4.52 ± 0.76	
Differentiation degree			0.726
Well or moderately differentiated	71	4.39 ± 0.73	
Poorly differentiated	39	4.34 ± 0.68	
Depth of tumor invasion			0.003
T1/T2	32	4.05 ± 0.66	
T3/T4	78	4.50 ± 0.73	
TNM staging			< 0.001
I + II	48	4.00 ± 0.62	
III + IV	62	4.66 ± 0.78	
Lymph node metastasis			< 0.001
Yes	69	4.61 ± 0.76	
No	41	3.97 ± 0.62	
Distant metastasis			< 0.001
Yes	35	4.76 ± 0.82	
No	75	4.19 ± 0.65	

HGF, hepatocyte growth factor; TNM, tumor-node-metastasis

Table 4 Correlation between MVD and clinicopathological characteristics of patients with gastric cancer

Characteristic	Case number	MVD	*P*
Age (years)			0.074
≥ 60	44	25.29 ± 0.76	
< 60	66	25.04 ± 0.68	
Gender			0.496
Male	76	25.17 ± 0.71	
Female	34	25.07 ± 0.71	
Tumor size			0.096
≤ 5 cm	63	25.04 ± 0.67	
> 5 cm	47	25.27 ± 0.76	
Differentiation degree			0.208
Well or moderately differentiated	71	25.20 ± 0.73	
Poorly differentiated	39	25.02 ± 0.68	
Depth of tumor invasion			< 0.001
T1/T2	32	21.81 ± 0.66	
T3/T4	78	26.50 ± 0.73	
TNM staging			< 0.001
I + II	48	20.30 ± 0.60	
III + IV	62	28.89 ± 0.78	
Lymph node metastasis			< 0.001
Yes	69	28.24 ± 0.76	
No	41	19.91 ± 0.62	
Distant metastasis			< 0.001
Yes	35	31.37 ± 0.82	
No	75	22.23 ± 0.65	

MVD, microvessel density; TNM, tumor-node-metastasis

Discussion

Almost 1 million cases of GC are diagnosed and over 650,000 lives are claimed by the cancer each year, establishing it as the fourth most common cancer worldwide as well as the second leading cause of cancer-related deaths [1]. Worse still, its diagnosis is often delayed due to a lack of early specific symptoms and thus most patients are not diagnosed until cancer has invaded the muscularis propria, which may account for why its 5-year survival rate is lower than 15% [19]. Therefore, it is of great urgency to find its symbolic marker in order to have a better understanding of its mechanism and development stages, which is likely to further the finding of effective therapeutic targets and prognostic indicators.

Initially, the study revealed that expressions of FAP protein and HGF protein as well as MVD are significantly increased in GC tissues. Currently, FAP has been found in a large variety of carcinomas, making it an important biomarker of malignant tumors [11]. FAP, a cell surface glycoprotein, is expressed in over 90% of human epithelial cancers such as breast, ovarian and lung cancers but not expressed in epithelial cancer cells, normal fibroblasts and other normal tissues, making it much higher in GC tissues than in its adjacent tissues and normal tissues [20]. Activation of proto-oncogene c-Met (MET)-mediated signaling pathways which were induced by HGF, a ligand of MET, was found to play an important role in the

Table 5 Correlation between FAP and HGF expressions and MVD in gastric cancer tissues

	N	MVD	r	*P*
FAP expression			0.693	0.000
Positive	68	29.84 ± 0.81		
Negative	42	17.53 ± 0.52		
HGF expression			0.664	0.000
Positive	74	29.10 ± 0.79		
Negative	36	16.98 ± 0.51		

FAP, fibroblast activation protein; HGF, hepatocyte growth factor; MVD, microvessel density

Table 6 Correlation between FAP and HGF expressions in gastric cancer tissues

FAP	HGF			r	*P*	Concordance rate
	Positive	Negative	N			
Positive	65	3	68			
Negative	9	33	42	0.768	0	89.10%
N	74	36	110			

FAP, fibroblast activation protein; HGF, hepatocyte growth factor

pathogenesis of peritoneal carcinomatosis in scirrhous GC [17]. The fact that genomic amplification of *MET* leads to the aberrant activation of GC tumors prompts us to associate MET protein over-expression or MET gene amplification with tumor progression and survival of patients with GC [21]. Consequently, its ligand of HGF is reported to be over-expressed in a large proportion of GC [17]. In addition, the growth, invasion and metastasis of malignant tumors including gastric tumors entails the formation of neovascularization, of which MVD serves as an index showing an increase in malignant tumors [22]. Du *et al.* found in their study that MVD is significantly elevated in esophageal and GC tissues than that in normal tissues, which is in accordance with our study [23].

Furthermore, it was also found in the study that FAP and HGF in GC tissues are likely to play a collaborative role in promoting the angiogenesis of GC. It has been proved that FAP, which is an important marker for CAFs, plays a predominant role in the progression of many tumor types, and FAP expression level is likely to present an important prognosis for tumors' clinical behavior [24] One study investigating the relationship between FAP expression in stroma of GC and MVD reaches the outcome that MVD positive expression rate is raised with the increase in FAP expression level, which demonstrates a consistent conclusion with ours that FAP stimulates tumor progression by enhancing tumor angiogenesis and inducing reactive stroma in carcinomas [25]. On top of that, another study on the molecular mechanism of angiogenesis and metastasis of GC reveals that angiogenesis is subjected to a wide range of factors and inhibitors, and HGF is one of them [26].When HGF is combined with c-Met, which is a transmembrane protein containing a tyrosine kinase domain, HGF/c-Met signaling pathway will be activated and further promotes tumor angiogenesis and results in increased cell growth, migration and invasion of GC cells [27]. Therefore, it is safe to speculate that MVD positive expression rate in GC tissues is positively correlated with FAP and HGF protein expressions and that FAP combined with HGF plays an important role in regulating the angiogenesis of GC.

In conclusion, our study found that FAP and HGF protein expressions may be positively correlated with the angiogenesis and metastasis of GC, thus FAP and HGF expressions may lay a theoretical foundation for GC diagnosis and treatment and help to find effective biological targets for GC treatment. However, as the mechanism of FAP and HGF in the development and prognosis of GC remains unclear, more clinical cases are still required to be collected to substantiate the conclusion.

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Compliance with ethical standards

Conflict of interest None.

References

1. Shirahata A, Sakata M, Kitamura Y, Sakuraba K, Yokomizo K, Goto T, Mizukami H, Saito M, Ishibashi K, Kigawa G, Nemoto H, Hibi K (2010) MACC 1 as a marker for peritoneal-disseminated gastric carcinoma. Anticancer Res 30:3441–3444
2. Jang BG, Kim WH (2011) Molecular pathology of gastric carcinoma. Pathobiology 78:302–310
3. Izuishi K, Mori H (2016) Recent strategies for treating stage iv gastric cancer: roles of palliative gastrectomy, chemotherapy, and radiotherapy. J Gastrointestin Liver Dis 25:87–94
4. Songun I, Putter H, Kranenbarg EM, Sasako M, van de Velde CJ (2010) Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial. Lancet Oncol 11:439–449
5. Marrelli D, De Stefano A, de Manzoni G, Morgagni P, Di Leo A, Roviello F (2005) Prediction of recurrence after radical surgery for gastric cancer: a scoring system obtained from a prospective multicenter study. Ann Surg 241:247–255
6. Marrelli D, Pedrazzani C, Neri A, Corso G, DeStefano A, Pinto E, Roviello F (2007) Complications after extended (D2) and superextended (D3) lymphadenectomy for gastric cancer: analysis of potential risk factors. Ann Surg Oncol 14:25–33
7. Wang RF, Zhang LH, Shan LH, Sun WG, Chai CC, Wu HM, Ibla JC, Wang LF, Liu JR (2013) Effects of the fibroblast activation protein on the invasion and migration of gastric cancer. Exp Mol Pathol 95:350–356
8. Lordick F (2014) Targeting the HGF/MET pathway in gastric cancer. Lancet Oncol 15:914–916
9. Graziano F, Galluccio N, Lorenzini P, Ruzzo A, Canestrari E, D'Emidio S, Catalano V, Sisti V, Ligorio C, Andreoni F, Rulli E, Di Oto E, Fiorentini G, Zingaretti C, De Nictolis M, Cappuzzo F, Magnani M (2011) Genetic activation of the MET pathway and prognosis of patients with high-risk, radically resected gastric cancer. J Clin Oncol 29:4789–4795
10. Abbas O, Richards JE, Mahalingam M (2010) Fibroblast-activation protein: a single marker that confidently differentiates morpheaform/infiltrative basal cell carcinoma from desmoplastic trichoepithelioma. Mod Pathol 23:1535–1543
11. El Khoury J, Kurban M, Kibbi AG, Abbas O (2014) Fibroblast-activation protein: valuable marker of cutaneous epithelial malignancy. Arch Dermatol Res 306:359–365
12. Tchou J, Zhang PJ, Bi Y, Satija C, Marjumdar R, Stephen TL, Lo A, Chen H, Mies C, June CH, Conejo-Garcia J, Pure E (2013) Fibroblast activation protein expression by stromal cells and tumor-associated macrophages in human breast cancer. Hum Pathol 44:2549–2557

13. Wen Y, Wang CT, Ma TT, Li ZY, Zhou LN, Mu B, Leng F, Shi HS, Li YO, Wei YQ (2010) Immunotherapy targeting fibroblast activation protein inhibits tumor growth and increases survival in a murine colon cancer model. Cancer Sci 101:2325–2332
14. Yanovitch T, Li YJ, Metlapally R, Abbott D, Viet KN, Young TL (2009) Hepatocyte growth factor and myopia: genetic association analyses in a Caucasian population. Mol Vis 15:1028–1035
15. Cai L, Johnstone BH, Cook TG, Liang Z, Traktuev D, Cornetta K, Ingram DA, Rosen ED, March KL (2007) Suppression of hepatocyte growth factor production impairs the ability of adipose-derived stem cells to promote ischemic tissue revascularization. Stem Cells 25:3234–3243
16. Nakagami H, Maeda K, Morishita R, Iguchi S, Nishikawa T, Takami Y, Kikuchi Y, Saito Y, Tamai K, Ogihara T, Kaneda Y (2005) Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. Arterioscler Thromb Vasc Biol 25:2542–2547
17. Zhao L, Yasumoto K, Kawashima A, Nakagawa T, Takeuchi S, Yamada T, Matsumoto K, Yonekura K, Yoshie O, Yano S (2013) Paracrine activation of MET promotes peritoneal carcinomatosis in scirrhous gastric cancer. Cancer Sci 104:1640–1646
18. Wang W, Xu DZ, Li YF, Guan YX, Sun XW, Chen YB, Kesari R, Huang CY, Li W, Zhan YQ, Zhou ZW (2011) Tumor-ratio-metastasis staging system as an alternative to the 7th edition UICC TNM system in gastric cancer after D2 resection–results of a single-institution study of 1343 Chinese patients. Ann Oncol 22: 2049–2056
19. Wroblewski LE, Peek RM Jr, Wilson KT (2010) Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev 23:713–739
20. Lo PC, Chen J, Stefflova K, Warren MS, Navab R, Bandarchi B, Mullins S, Tsao M, Cheng JD, Zheng G (2009) Photodynamic molecular beacon triggered by fibroblast activation protein on cancer-associated fibroblasts for diagnosis and treatment of epithelial cancers. J Med Chem 52:358–368
21. Inokuchi M, Otsuki S, Fujimori Y, Sato Y, Nakagawa M, Kojima K (2015) Clinical significance of MET in gastric cancer. World J Gastrointest Oncol 7:317–327
22. Erbersdobler A, Isbarn H, Dix K, Steiner I, Schlomm T, Mirlacher M, Sauter G, Haese A (2010) Prognostic value of microvessel density in prostate cancer: a tissue microarray study. World J Urol 28: 687–692
23. Du JR, Jiang Y, Zhang YM, Fu H (2003) Vascular endothelial growth factor and microvascular density in esophageal and gastric carcinomas. World J Gastroenterol 9:1604–1606
24. Liu F, Qi L, Liu B, Liu J, Zhang H, Che D, Cao J, Shen J, Geng J, Bi Y, Ye L, Pan B, Yu Y (2015) Fibroblast activation protein overexpression and clinical implications in solid tumors: a meta-analysis. PLoS One 10:e0116683
25. Cai F, Li Z, Wang C, Xian S, Xu G, Peng F, Wei Y, Lu Y (2013) Short hairpin RNA targeting of fibroblast activation protein inhibits tumor growth and improves the tumor microenvironment in a mouse model. BMB Rep 46:252–257
26. Guo T, Yang J, Yao J, Zhang Y, Da M, Duan Y (2013) Expression of MACC1 and c-Met in human gastric cancer and its clinical significance. Cancer Cell Int 13:121
27. Noguchi E, Saito N, Kobayashi M, Kameoka S (2015) Clinical significance of hepatocyte growth factor/c-Met expression in the assessment of gastric cancer progression. Mol Med Rep 11:3423–3431