

Original Article

Effects of fascin1 on epithelial-mesenchymal transition and metastasis of human gastric cancer cells

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Abstract: Objective: The goal of this study was to investigate the role of fascin1 on epithelial-mesenchymal transition (EMT) and metastasis of gastric cancer cells. Methods: Expression of fascin1, E-cadherin, and vimentin in gastric cancer and adjacent tissues was assessed by immunohistochemistry and their correlation with clinicopathological parameters was analyzed. The relationship between their expression levels was also assessed. Regulatory effects of fascin1 on E-cadherin and vimentin and the relationship between fascin1 and invasion and migration of gastric cancer cells were further examined using the gastric cancer cell lines SGC-7901 and MGC-803. Results: Fascin1 and vimentin expression were significantly higher in the gastric cancer tissues than in adjacent tissues, whereas E-cadherin expression was significantly lower. Expression of fascin1, E-cadherin, and vimentin was significantly correlated with TNM stage, lymph node metastasis, and depth of invasion. Fascin1 knockdown in the gastric cancer cell lines led to a significant increase in the expression of E-cadherin, and a significant decrease in vimentin expression. Invasion and migration ability of gastric cancer cells decreased significantly upon fascin1 knockdown. Conclusion: Fascin1 demonstrates a negative regulatory effect on E-cadherin and a positive regulatory effect on vimentin. Fascin1 may participate in EMT of gastric cancer cells and regulate metastasis of gastric cancer.

Keywords: Fascin1, E-cadherin, vimentin, EMT, metastasis, gastric cancer

Introduction

Gastric cancer is currently one of the most common malignant tumors worldwide, causing approximately 841,000 deaths in 2013 [1]. In China, the incidence and mortality rates of gastric cancer are second only to lung cancer [2]. An important cause of the high mortality is that gastric cancer is prone to metastasis. Recent studies have shown that fascin1—a cytoskeletal protein—is expressed in many human tumors, but not in most normal epitheliums [3]. Fascin1 promotes cell invasion and metastasis by upregulating cellular processes and the formation of filamentous pseudopodia, which has been reported to occur in gastric cancer [4-6]. Metastasis of gastric cancer is related to factors such as EMT, tumor stem cells, and vascular endothelial growth factor [7-9].

EMT is an early process in tumor invasion and metastasis, and refers to the process of the transformation of epithelial cells into mesen-

chymal cells under the stimulation of several factors. Epithelial cells exhibit characteristics of interstitial cells after EMT. Through the EMT process, cell adhesion ability is reduced, and the ability of tumor cells to migrate, invade, resist apoptosis, and degrade extracellular matrix is improved [10, 11].

Presently, the role of fascin1 in the EMT process of gastric cancer cells is unclear. E-cadherin and vimentin are markers of EMT. Activation of EMT is accompanied by expression changes of specific molecules, including decrease or loss of E-cadherin and the upregulation of vimentin [12]. Therefore, expression of fascin1, E-cadherin, and vimentin was studied in gastric cancer tissues and adjacent tissues. The regulatory relationship between fascin1 and E-cadherin or vimentin in gastric cancer cells was assessed, revealing the role of fascin1 in gastric cancer cells EMT and metastasis.

Fascin1 regulates EMT to affect metastasis of gastric cancer

Materials and methods

Antibodies and reagents

Human tissue microarrays of gastric cancer were obtained from Xian Alina. Human gastric cancer cell lines, SGC-7901 and MGC-803, were purchased from Shanghai Cell Bank of Chinese Academy of Sciences. Fascin1 antibody was obtained from R&D Systems. E-cadherin antibody, vimentin antibody, and beta-actin antibody were from Cell Signaling Technology. Fascin1-siRNA and control-siRNAs were purchased from Santa Cruz Biotechnology. Lipo6000™ transfection reagent was from Beyotime Biotechnology. SABC immunohistochemical kit was from BOSTER Biological Technology.

Immunohistochemistry and determination of results

This study was approved by the Institutional Ethics Committee of Taian Central Hospital. Gastric cancer tissue microarrays contain both gastric cancers tissues and their corresponding adjacent mucosal tissues were used, representing 90 patients including 67 males and 23 females, with an average age of 50.02 ± 10.35 years. None of the patients had undergone chemotherapy or radiotherapy before surgery. Expression of fascin1, E-cadherin, and vimentin was detected using an SABC kit according to the manufacturer's instructions. Positive expression was determined by the combined scoring of the proportion of stained cells and staining intensity. Positive cell scoring: < 5%, 0; 5-25%, 1; 26-75%, 2; and > 75%, 3. Scoring of dye intensity: 0, colorless; 1, light yellow; 2, yellow; and 3, brown. The points were added and < 6 was considered negative, whereas ≥ 6 was positive.

Cell culture and siRNA transfection

SGC-7901 and MGC-803 cells were cultured in a constant temperature incubator. The cell culture medium consisted of DMEM-F12, 10% fetal bovine serum, and 1% penicillin streptomycin. Cell transfections were carried out in a 6-well plate using Lipo6000™ transfection reagent, according to the manufacturer's instructions. Upon reaching a cell density of 30%, fascin1-siRNA and control-siRNA were transfected.

Western blotting

Total cell protein was extracted 72 hours post transfection. Protein concentrations were determined using the BCA method. Lysates (20 μg) were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes. Membranes were blocked for 1.5 hour at room temperature with 5% skim milk powder. The corresponding primary antibodies were incubated for 12 hours at 4°C. Secondary antibodies were incubated for 1 hour at room temperature. Protein bands were analyzed using the ECL method on a Bio-Rad imaging analysis system.

Real-time PCR

Total RNA was extracted using Trizol 48 hours post transfection, and RNA concentration was determined. The complementary DNA (cDNA) was reverse transcribed and real-time PCR was performed to determine relative mRNA expression levels. Primer sequences: Fascin1 forward 5'-GCCAGGGTATGGACCTGTCTG-3' and reverse 5'-CACGCCACTCGATGTCAAAGTA-3'; E-cadherin forward 5'-TTAAACTCCTGGCCTCAAGCAATC-3' and reverse 5'-TCCTATCTTGGGCAAAGCAACTG-3'; Vimentin forward 5'-AAATGGCTCGTCACCTTC-3' and reverse 5'-ACCTGAGGCTTTGGATTC-3'; β -actin forward 5'-AGCGAGCATCCCCCAAGTT-3' and reverse 5'-GGGCACGAAGGCTCATCATT-3'.

Transwell migration and invasion assays

Migration assay: Cells (200 μL at $0.5 \times 10^6/\text{mL}$) were diluted in serum-free medium and added to the upper layer of the chamber, and 600- μL serum-containing medium was added to the lower chamber. Invasion assay: Performed similarly to the migration assay, except that Matrigel glue was used to coat the chamber. Cells were cultured for 24 hours. The chambers were removed and fixed with 4% paraformaldehyde. After crystal violet staining, ten microscope fields (400 \times) were randomly selected and the cells that passed through the membrane were counted.

Statistical analysis

All data was analyzed using SPSS 17.0. Pairwise comparisons were performed using the *t*-test, and one-way ANOVA was used to compare the

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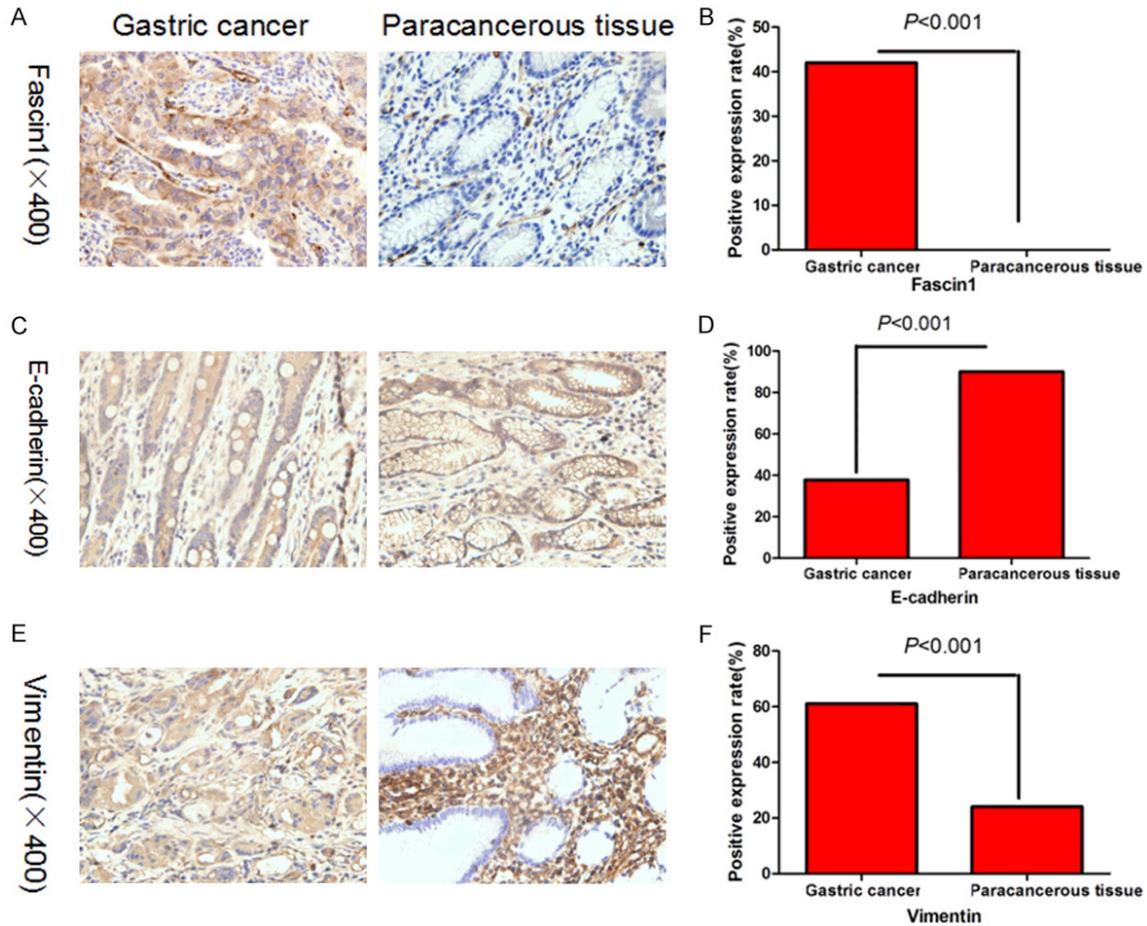


Figure 1. Immunohistochemistry was done to analysis the expression of fascin1, E-cadherin, and vimentin in gastric cancer and adjacent tissues ($\times 400$). (A and B, E and F) Fascin1 and vimentin expression levels were significantly higher in the gastric cancer tissues than in adjacent tissues ($P < 0.001$). (C and D) Expression of E-cadherin in gastric cancer tissues was significantly lower than that in adjacent tissues ($P < 0.001$).

three groups. The counting data was analyzed by Chi-square test. Correlation between expression of two proteins was analyzed by Spearman's rank correlation coefficient, and $P < 0.05$ was considered statistically significant. All experiments were repeated three times.

Results

Expression of fascin1, E-cadherin, and vimentin protein in gastric cancer and adjacent tissues

Fascin1 was mainly expressed in the cytoplasm and its positive expression rate was 42.2% (38/90) in gastric cancer tissues; however, fascin1 expression was not observed in paracancerous tissues (**Figure 1A, 1B**). E-cadherin was mainly expressed in the cell membrane and cytoplasm, with a positive expression rate of

37.8% (34/90) in gastric cancer tissues and 90% (81/90) in adjacent tissues (**Figure 1C, 1D**). Vimentin was mainly expressed in the cytoplasm, and the positive expression rates in the gastric cancer and adjacent tissues were 61.1% (55/90) and 24.4% (22/90), respectively (**Figure 1E, 1F**). All differences were statistically significant ($P < 0.05$).

Expression of fascin1, E-cadherin, and vimentin in gastric carcinoma and their relationship with clinicopathological parameters

Fascin1, vimentin, and E-cadherin expression in gastric cancer tissues showed no correlation with the patients' sex or age. Expression of fascin1 and vimentin positively correlated with TNM stage, lymph node metastasis, and depth of invasion, whereas E-cadherin exhibited a

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Table 1. Expression of fascin1 and E-cadherin and vimentin in gastric carcinoma and their relationship with clinicopathological parameters

Parameters	n	Fascin1		P	E-cadherin		P	Vimentin		P	
		+	-		+	-		+	-		
Gender	Male	67	28	39	0.888	25	42	0.877	41	26	0.978
	Female	23	10	13		9	14		14	9	
Age (years)	> 60	40	17	23	0.962	18	22	0.206	26	14	0.498
	≤ 60	50	21	29		16	34		29	21	
TNM stage	I+II	72	26	46	0.019	32	40	0.019	38	34	0.003
	III+IV	18	12	6		2	16		17	1	
Lymph node metastasis	Yes	20	13	7	0.019	3	17	0.034	17	3	0.026
	No	70	25	45		31	39		38	32	
Infiltration depth	T1+T2	25	6	19	0.030	16	9	0.001	11	14	0.039
	T3+T4	65	32	33		18	47		44	21	

negative correlation with these parameters (Table 1).

Correlation analysis of expression of fascin1, E-cadherin, and vimentin in gastric cancer

Fascin1 and E-cadherin were co-expressed in 7 patients with gastric cancer; 31 patients were fascin1-positive and E-cadherin-negative, and 27 patients were fascin1-negative and E-cadherin-positive. Fascin1 and E-cadherin expression was not observed in 25 patients. Overall, a significant negative correlation was observed between the expression of fascin1 and E-cadherin in gastric cancer tissues ($r=-0.341$, $P=0.001$).

Fascin1 and vimentin were co-expressed in 30 patients. Eight patients were fascin1-positive and vimentin-negative, and 25 patients were fascin1-negative and vimentin-positive. Fascin1 and vimentin expression was not observed in 27 patients. Overall, a positive correlation was observed between the expression of fascin1 and vimentin in gastric cancer tissues ($r=0.313$, $P=0.003$).

Fascin1 regulates E-cadherin and vimentin expression in gastric cancer cells

To investigate whether fascin1 regulates E-cadherin and vimentin, fascin1-siRNA and control-siRNA were transfected into gastric cancer cell lines, SGC-7901 and MGC-803. The mRNA and protein levels of fascin1, E-cadherin, and vimentin were determined by real-time PCR and Western blotting, respectively. Upon siRNA knockdown of fascin1, the mRNA and protein

expression of E-cadherin was significantly increased in both cell lines. Conversely, the mRNA and protein expression of vimentin was significantly decreased (Figure 2). These results indicate that fascin1 could negatively regulate E-cadherin expression and positively regulate vimentin expression.

Effects of fascin1 on migration and invasion of gastric cancer cells

SGC-7901 and MGC-803 cells were both transfected with fascin1-siRNA and control-siRNA. Compared with the normal and control-siRNA group, the migration and invasion ability of both cell lines after fascin1-siRNA treatment was significantly decreased. There were no significant differences between the normal and control-siRNA treated cells (Figure 3). Thus, fascin1 knockdown significantly reduced the migration and invasion capability of gastric cancer cells.

Discussion

Gastric cancer is the fourth most common cancer in the world, accounting for 1×10^6 diagnoses and 7×10^5 deaths annually, mostly related to metastases [13, 14]. Therefore, understanding the escape of gastric cancer cells from the primary tumor, invasion of surrounding tissues, lymphatic dissemination, and distant metastasis has become the focus of gastric cancer research. In order to infiltrate and metastasize locally, tumor cells need high mobility and invasiveness. Tumor cells possess obvious morphological characteristics (such as elongation, polarization, the emergence of cell processes, etc.) and disordered cell-cell contacts [15]. All

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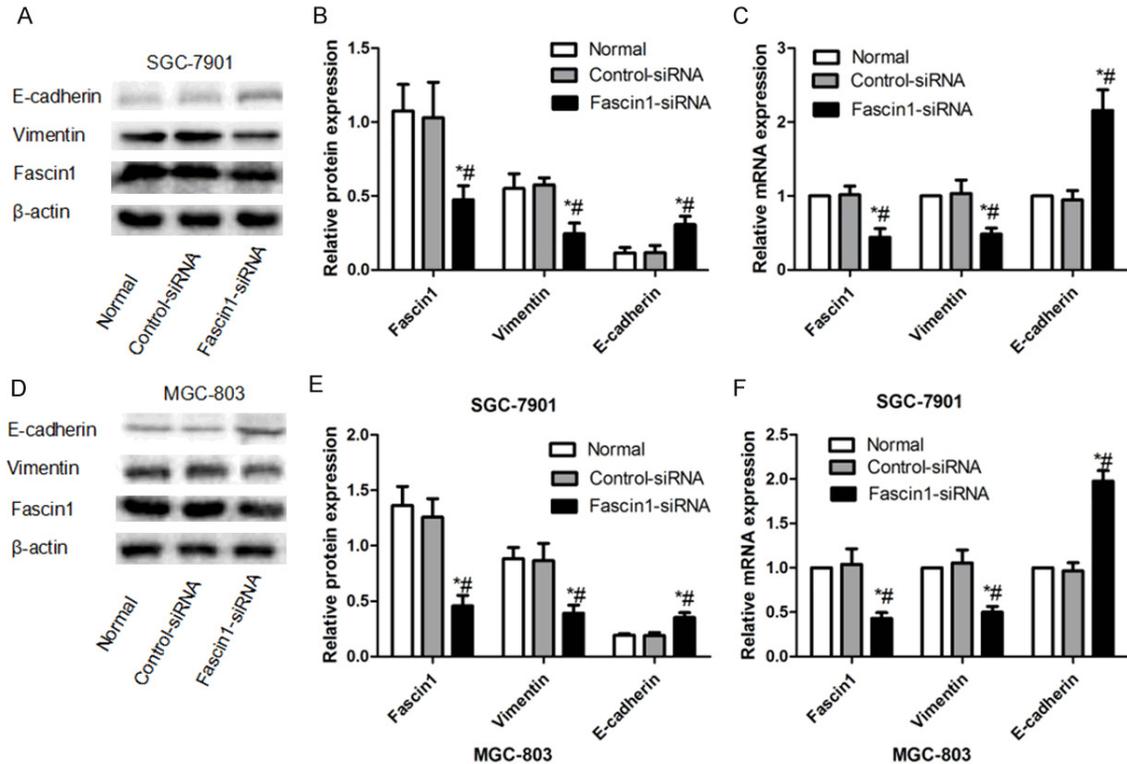


Figure 2. Fascin1 regulates E-cadherin and vimentin expression in gastric cancer cells. (A) SGC-7901 cells were transfected with fascin1-siRNA and control-siRNA. (B and C) Fascin1, E-cadherin and vimentin protein and mRNA levels were detected by real-time PCR and Western blot analysis. (D) MGC-803 cells were transfected with fascin1-siRNA and Control-siRNA. (E and F) Fascin1, E-cadherin and vimentin protein and mRNA levels were detected by real-time PCR and Western blot analysis. β -actin was used as loading control. *: Compared with normal group, $P < 0.05$; #: Compared with control-siRNA group, $P < 0.05$.

of these changes are related to alterations and rearrangement of the cytoskeleton (especially actin binding protein) [16]. As a filamentous actin binding protein, fascin1 is associated with several cellular biological behaviors, such as filamentous pseudopodia formation, cell adhesion, and motion and migration, and these affect the invasion and metastasis of tumor cells [17].

Fascin1 is an actin cluster protein encoded by *FSCN1*, whose expression is positively correlated with the proliferation, migration, and metastasis of malignant tumors. Fascin1 expression in gastric cancer is significantly higher than that in normal mucosa [18]. In this study, fascin1 was highly expressed in gastric cancer, and was associated with invasion and metastasis. This was confirmed by knocking down the expression of fascin1 in SGC-7901 and MGC-803 cells, which decreased their invasion and migration capability. At present, the specific

molecular mechanism of fascin1 in gastric cancer metastasis is not clear.

EMT is a process in which epithelial cells adopt the characteristics of mesenchymal cells [19]. During EMT, the polarity of epithelial cells is lost and the reduction in the number of cell contacts results in remodeling of the cytoskeleton. Therefore, the expression of E-cadherin is downregulated and vimentin expression is upregulated [20]. EMT is related to tumor metastasis and invasion in gastric cancer [21, 22]. Therefore, it is important to study the molecular mechanism of EMT in gastric cancer and understand its regulation in order to deter metastasis and improve treatment. During EMT, synthesis of the epithelial marker E-cadherin is decreased, and cytokeratin filaments with an epithelial phenotype are replaced by vimentin, which changes the cell morphology into a spindle shape [23]. Upregulation of vimentin, a marker of stromal cells, increases

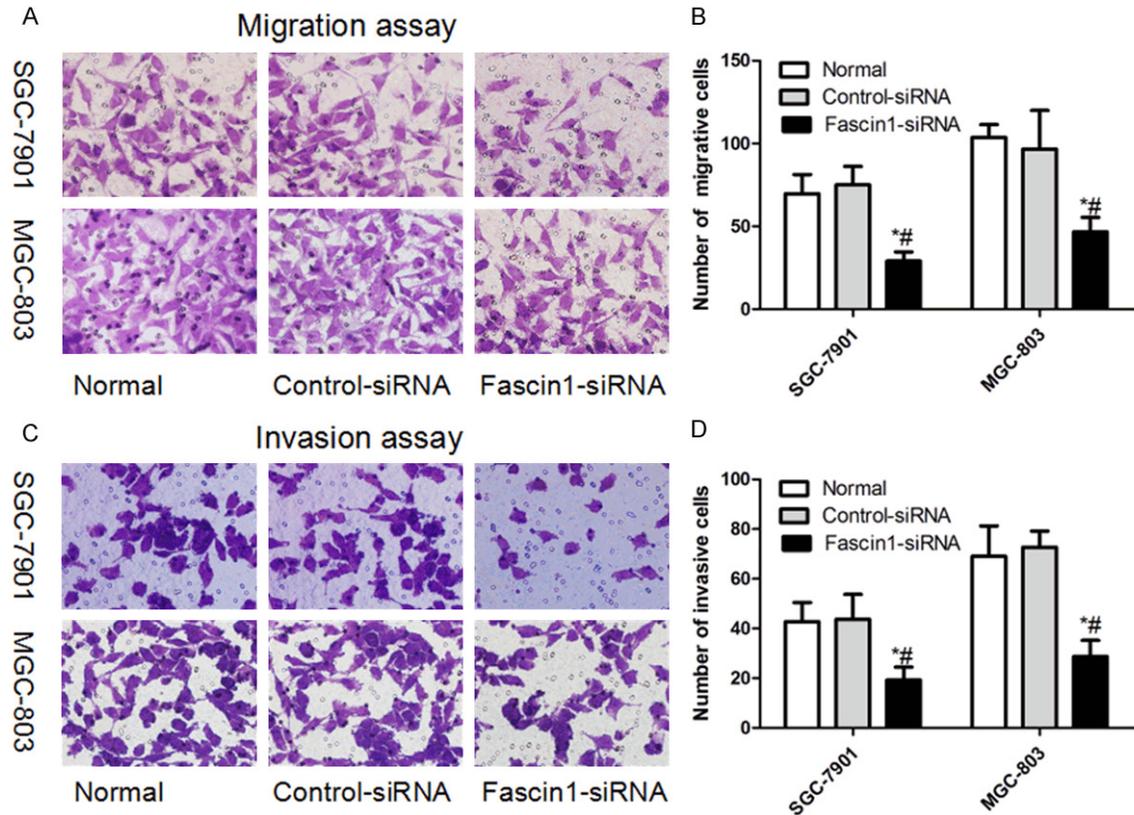


Figure 3. Knockdown of fascin1 inhibits migration and invasion of gastric cancer cells. (A and B) knockdown of fascin1 inhibited migration of SGC-7901 and MGC-803 cells. (C and D) knockdown of fascin1 inhibited invasion of SGC-7901 and MGC-803 cells. *: Compared with normal group, $P < 0.05$; #: Compared with control-siRNA group, $P < 0.05$.

the migration and invasion of cancer cells [24]. In this study, E-cadherin expression was significantly lower in gastric cancer than in adjacent tissues, and its expression was negatively correlated with TNM stage, lymph node metastasis, and depth of invasion. Conversely, vimentin was highly expressed in gastric cancer when compared with the adjacent tissues, and its expression was positively correlated with TNM stage, lymph node metastasis, and depth of invasion.

These results suggest that fascin1 may be related to EMT in tumor cells, but whether it participates in EMT and its molecular mechanism are still unclear. Therefore, expression levels of fascin1, E-cadherin, and vimentin were studied in gastric cancer and adjacent tissues and found to be significantly correlated with the TNM stage, lymph node metastasis, and depth of invasion. Expression of fascin1 correlated negatively with that of E-cadherin and positively with that of vimentin. Furthermore, knock down of fascin1 in SGC-7901 and MGC-803 cells

showed that E-cadherin expression was significantly increased, while vimentin expression was significantly decreased. This further confirmed that fascin1 could regulate E-cadherin and vimentin expression, and cell experiments showed that fascin1 knockdown decreased invasion and migration ability of gastric cancer cells. Therefore, fascin1 may promote the invasion and metastasis of gastric cancer cells by regulating EMT.

In conclusion, fascin1 is closely related to gastric cancer metastasis. Downregulation of fascin1 can reduce the motility of gastric cancer cells and inhibit EMT. Fascin1 is thus a potential therapeutic target for gastric cancer.

Disclosure of conflict of interest

None.

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