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Original Article

FOXM1 overexpression and decreased E-cadherin expression are correlated with poor prognosis advanced gastric cancer

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Abstract: Objective: The goal of this study was to investigate expression of forkhead box M1 (FOXM1) and E-cadherin in tissues of gastric cancer (GC) to reveal a potential correlation between FOXM1, E-cadherin, and clinicopathological parameters and survival in GC patients following radical resection. Methods: Expression of FOXM1 and E-cadherin in gastric cancer and adjacent normal tissue on tissue microarray was detected using immunohistochemistry (IHC). The association between FOXM1 and E-cadherin expression and clinicopathological features was determined by Chi-square test and the association with prognosis of GC patients was determined by the Kaplan-Meier method with the log-rank test. Results: Increased expression of FOXM1 and decreased expression of E-cadherin were evident in gastric cancer and was associated with TNM stage ($P < 0.05$), lymph node metastasis ($P < 0.05$), ages ($P < 0.05$), larger tumor size ($P < 0.05$), differentiation ($P < 0.05$), vascular invasion ($P < 0.05$), and shorter overall survival (OS) after radical resection. Furthermore, the correlation between FOXM1 and E-cadherin expression in gastric cancer tissue was negative. Conclusion: Increased FOXM1 expression and decreased E-cadherin expression are correlated with advanced gastric cancer and poor prognosis.

Keywords: Gastric carcinoma, FOXM1, E-cadherin, invasion, metastasis, prognostic

Introduction

Gastric cancer (GC) remains one of the most common diseases [1, 2]. The survival rate of patients with gastric cancer has improved over the past three years due to improvements in early detection and treatment [3]. However, gastric cancer continues to be the second leading cause of cancer death in China [4], which is mainly attributed to the unsatisfied early detection rate, early tumor recurrence and high chemotherapy resistance, etc.. Therefore, it is essential to find more specific biomarkers for early detection and develop more effective treatment. FOXM1 is also identical to MPP-2 or FKHL-16, and its gene is composed of 10 exons. The helical DNA binding domain of the protein is located in the third exon, containing 110 amino acids [5]. Previous studies have shown that FOXM1 is associated with angiogenesis, invasion, and metastasis. Furthermore, it is strongly expressed in a variety of tumor cells, promoting the progression of pancreatic cancer, breast cancer and colorectal cancer [6, 7]. In an estab-

lished mouse gastric cancer model, up-regulated expression of FOXM1 contributed to the enhanced tumorigenic ability and metastasis of gastric cancer cells, while inhibition of FOXM1 expression showed the opposite effects, suggesting the importance of FOXM1 in the development of gastric cancer [8, 9]. It has been reported [8] that FOXM1 could regulate the expression of vascular endothelial growth factor (VEGF) at the transcriptional level. Another study showed that down-regulation of FOXM1 expression could inhibit proliferation and invasion of nasopharyngeal carcinoma cells via regulating epithelial-to-mesenchymal transition (EMT) [10]. In contrast, increased expression of FOXM1 improved invasion of HCC cells by suppressing expression of epithelial markers and acquisition of the EMT phenotype [11]. As an EMT-related protein, E-cadherin is an anti-metastasis gene by enhancing the adhesion between cells, and is down-regulated or deleted in a variety of malignant tumors [12, 13]. The purpose of this study was to investigate the expression of FOXM1 and E-cadherin in normal gastric tissues and gastric cancer tissues, and the association of the expression of these two proteins with clinicopathological parameters and prognosis, and importantly, to explore the association between FOXM1 and E-cadherin and their roles in gastric cancer progression.

Materials and methods

Patients and samples

Between January 2005 and December 2008, 70 cases of patients with primary gastric cancer and no evidence of malignancy in other organs were analyzed. The detailed clinic and survival data of all the cancer patients was well preserved until 2012. The 70 paraffin specimens were derived from each gastric cancer patients, and these paraffin specimens were collected and conserved by Suzhou Municipal Hospital. The ages of these gastric cancer patients were 40-83, with an average of 62. The TNM staging was according to the Seventh Edition of AJCC (2010). All patients were unreceived radiotherapy and chemotherapy before operation, and the paraffin specimens were confirmed diagnosis by two pathologists. All patients care and experimental procedures were approved by the Committee on Patients Care and the Committee on the Ethic of Suzhou Municipal Hospital. Additionally, all methods were performed in accordance with the relevant guidelines and regulations. All patients had signed the Informed Consent Form.

The preparation of tissue array

The 70 cases of gastric carcinoma tissues were fixed by 10% neutral formalin and the pathological tissues were paraffin-embedded. After stained by H&E, the tumor tissues were labeling localized within the paraffin blocks via the microscopy detection. The 3 paraffin chips containing 8 × 12 tissue arrays (Each sample contains two pieces of cancer tissue and a negative surgical margin, with a total diameter of 1.6 mm). There were 69 effective cases of gastric carcinoma tissues in this study.

Reagents, sample treatment and methods

E-cadherin was purchased from MXB Biotechnologies Company (Fuzhou, China) as the working fluid. FOXM1 was a rabbit anti-human polyclonal antibody, which was purchased from Abcam, then the antibody was diluted to 400 times by the antibody dilution buffer. The conventional paraffin-embedding were sliced into serial sections up to 4 μm thick. Detection was done by immunohistochemical methods, with PBS instead of the primary antibody as the negative control. The figure of positive control provided by Abcam was taken for the positive control.

Evaluation of immune staining

Immunohistochemical sections of FOXM1 and E-cadherin were detected by a pathologist blinded to the clinicopathological characteristics of patients. Positive expression of FOXM1 and E-cadherin was sub-located in the cytoplasm (partly in the nucleus) and the cytomembrane, respectively. Whole field inspection of the section was scored and intensities of staining were grouped as follows: 0 = no staining, 1 = weak staining (positive), 2 = strong staining (positive), staining results in **Figures 1** and **2** showed the represented non staining and positive staining pictures.

Statistical analysis

Correlation analysis between expression of FOXM1 and E-cadherin and the clinicopathological parameters (CPP) was performed using Chi-square test. The relationship analysis be-

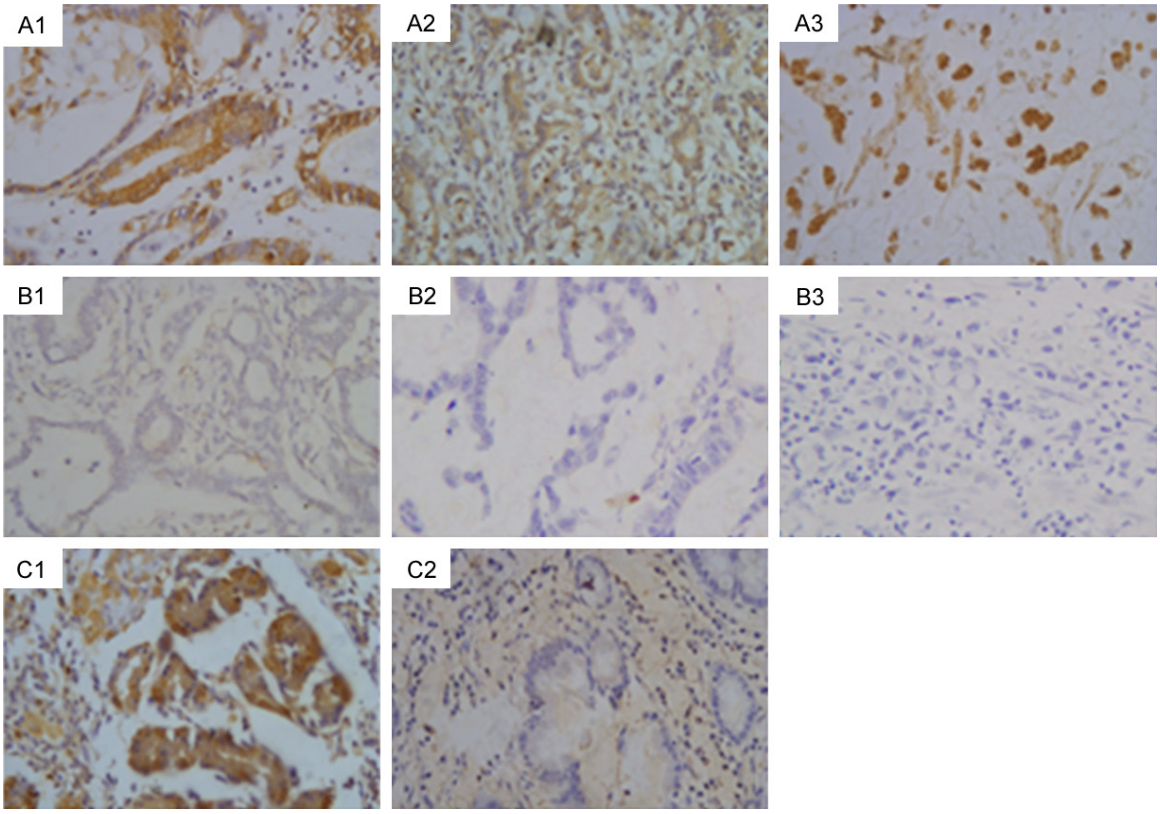

Figure 1. FOXM1 highly positive expression in the gastric cancer tissue (Sub-located in the cytoplasm, partly expressed in the nucleus, magnification ×400 for all images). A1/B1: High differentiated adenocarcinoma. A2/B2: Moderately differentiated adenocarcinoma. A3/B3: Low differentiated adenocarcinoma. C1/C2: Normal gastric tissue. A1/A2/A3/C1: FOXM1 positive expression; B1/B2/B3/C2: FOXM1 negative expression.

tween expression of FOXM1 and E-cadherin and the patients' survival period was performed using log-rank test via Kaplan-Meier method. Multivariate Cox regression was conducted to identify the independent contribution of FOXM1 and E-cadherin on overall survival (OS). The criteria for statistical significance used were *$P \leq 0.05$.

Results

Increased FOXM1 expression and decreased E-cadherin expression were correlated with advanced gastric cancer

There were 22 positive expression cases of FOXM1 in all 69 cases of gastric carcinoma tissues (31.9%, **Figure 1**), which were irrelevant to the gender of patients ($P > 0.05$) and were closely related to the tumor differentiation, lymphatic metastasis, vessel invasion, TNM staging, age and the greatest tumor diameter (GTD) ($P < 0.05$, **Table 1**). In only 2 cases, expression of FOXM1 was found partly expressed in the normal gastric mucosa, which suggested that the FOXM1 may be highly expressed in gastric cancer tissues. A total of 25 positive expression cases of E-cadherin were found in the 69 cases of gastric carcinoma tissues (36.2%, **Figure 2**), which were closely related to the lymphatic metastasis, tumor differentiation, TNM staging and GTD ($P < 0.05$, **Table 1**). Only 2 cases of E-cadherin expression were negative in the normal gastric mucosa.

Expression of FOXM1 was not inversely correlated with the expression of E-cadherin in the gastric cancer tissues

The result of Spearman correlation test showed that there were 7 positive expression cases of E-cadherin within the 22 positive expression cases of FOXM1, a cross-correlation analysis revealed that the expression of FOXM1 was not inversely correlated ($r = -0.063$, $P = 0.789$) with the expression of E-cadherin in the gastric cancer tissues (**Table 2**).

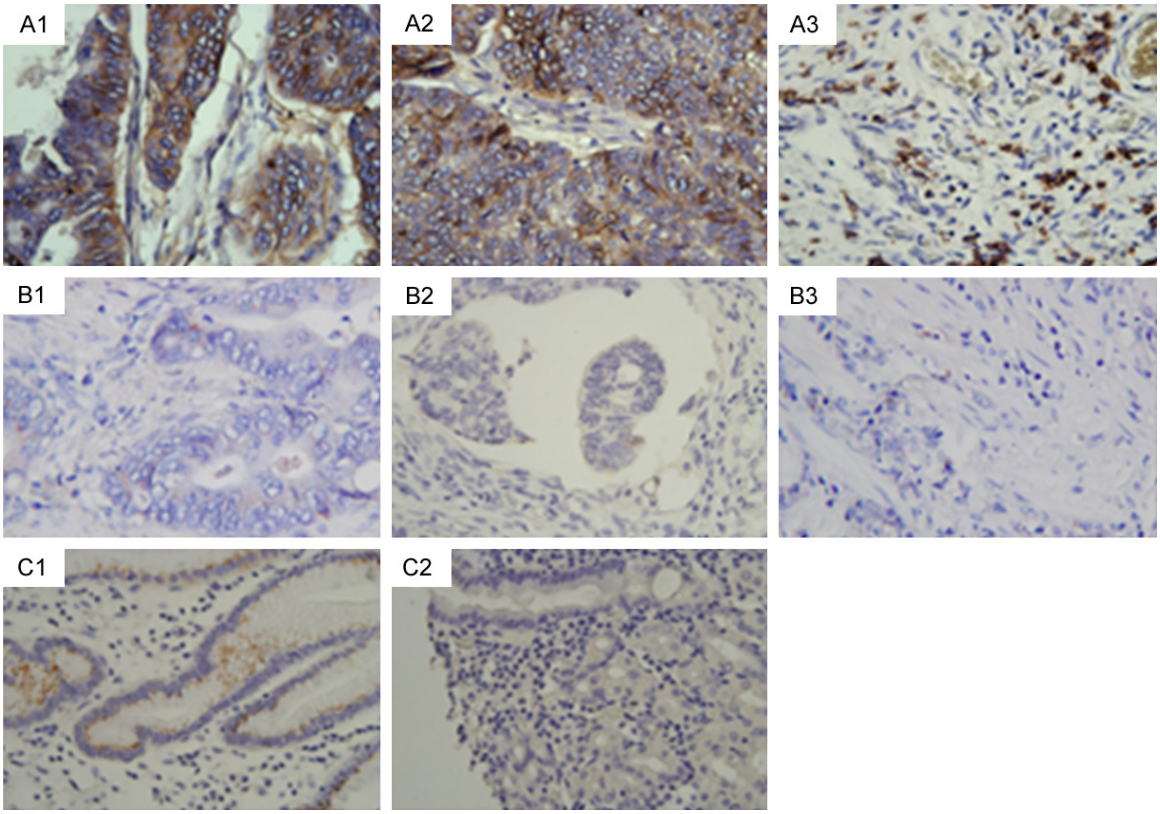

Figure 2. E-cadherin highly positive expression in the gastric cancer tissue (Sub-located in the cytoplasm, partly expressed in the nucleus, magnification ×400 for all images). A1/B1: High differentiated adenocarcinoma. A2/B2: Moderately differentiated adenocarcinoma. A3/B3: Low differentiated adenocarcinoma. C1/C2: Normal gastric tissue. A1/A2/A3/C1: E-cadherin positive expression; B1/B2/B3/C2: E-cadherin negative expression.

FOXM1 overexpression and decreased E-cadherin expression were correlated with poor prognosis

Relationship analysis between expression of FOXM1 and the patients' survival period were performed using Kaplan-Meier test ($P = 0.015$), the 5-year survival rate of patients with positive expression of FOXM1 was lower than patients with negative expression of FOXM1. According to the result of the relationship analysis based on Kaplan-Meier test, there was no obvious ($P = 0.755 > 0.05$) correlation between expression of E-cadherin and the patients' survival period (**Figure 3**).

Discussion

FOXM1 not only plays a central role in expression of genes in G1-S and G2-M phases, but is also essential for mitosis and chromosome stabilization. Bella L et al. [14] documented in their study that FOXM1 was involved in the complex process of gene expression during embryonic development and tissue homeostasis. In addition, FOXM1 is also involved in various aspects of cancer development, such as cell proliferation, tumor angiogenesis, migration, invasion, EMT, and metastasis [15]. For example, there is growing evidence that FOXM1 has a significant effect on migration, invasion, and metastasis in different types of cancers, including pancreatic cancer [16], lung adenocarcinoma [17] and prostate cancer [18], and EMT. Huang C et al. [16] reported that FOXM1 was highly expressed in pancreatic cancer cell lines with obvious metastasis. Furthermore, Kong FF et al. [19] showed that reduced expression of FoxM1 could reverse epithelial phenotype of stromal cells in non-small cell lung cancer (NSCLC). Overall, these reports further enhanced the hypothesis that FOXM1 might serve as a tumor promoter in gastric cancer development. Our experiments also confirmed that FOXM1 protein was highly expressed in gastric cancer tis-

Table 1. Expression of FOXM1 and E-cadherin and its association with the clinical pathologic characteristics of gastric carcinoma

CPP	n	FOXM1				E-cadherin			
		Positive (%)	Negative (%)	x^2	p	Positive (%)	Negative (%)	x^2	p
Age (Year)				8.026	0.005			0.230	0.632
< 65	36	6 (16.7)	30 (83.3)			14 (38.9)	22 (61.1)		
≥ 65	33	16 (48.5)	17 (51.5)			11 (33.3)	22 (66.7)		
Gender				1.622	0.203			0.026	0.873
Male	45	12 (26.7)	33 (73.3)			16 (35.6)	29 (64.4)		
Female	24	10 (41.7)	14 (58.3)			9 (37.5)	15 (62.5)		
Differentiated degree				0.432	0.511			8.435	0.004
High, medium	31	9 (42.9)	22 (57.1)			17 (54.8)	14 (45.2)		
Low	38	13 (34.2)	25 (65.8)			8 (21.1)	30 (78.9)		
Lymphatic metastasis				0.425	0.514			1.600	0.206
Negative	29	8 (27.6)	21 (72.4)			13 (44.8)	16 (55.2)		
Positive	40	14 (35)	26 (65)			12 (30)	28 (70)		
Vascular invasion				10.287	0.003			1.200	0.273
Negative	56	13 (23.2)	43 (76.8)			22 (39.3)	34 (60.7)		
Positive	13	9 (69.2)	4 (30.8)			3 (23.1)	10 (76.9)		
Lauren's pattern TNM grade				0.073	0.787			4.110	0.043
I+II grade	33	10 (30.3)	23 (69.7)			16 (48.5)	17 (51.5)		
III+IV grade	36	12 (33.3)	24 (66.7)			9 (25)	27 (75)		
Greatest tumor diameter				0.473	0.492			3.423	0.064
< 4 cm	26	7 (26.9)	19 (73.1)			13 (50)	13 (50)		
≥ 4 cm	43	15 (34.9)	28 (65.1)			12 (27.9)	31 (72.1)		

Table 2. Spearman correlation test of expression of FOXM1 and E-cadherin

E-cadherin	FOXM1		
	Positive	Negative	Total
Positive	7	18	25
Negative	15	29	44
Total	22	47	69

sues, suggesting that the increase of FOXM1 protein might be an early event of gastric cancer. Yang et al. showed that high FOXM1 expression was strongly correlated with lymph node metastasis and TNM staging [7]. Moreover, the survival period of patients with FOXM1 positive expression was significantly lower than that of negative expression group. Similarly, in our experiment, expression of FOXM1 protein was closely associated with clinical tumor staging, lymph node metastasis, age, tumor size, differentiation, and vascular invasion. Importantly, the survival rate of patients with high expression of FOXM1 was reduced compared to that of patients with low expression.

E-cadherin, a known tumor suppressor gene, is mainly expressed by epithelial cells. Down regulation of E-cadherin promotes EMT and tumor metastasis [20]. The inhibitory effect of E-cadherin on tumor cells metastasis has been verified in hepatocellular carcinoma [21], breast cancer [22] and colon cancer [23]. Zhai X et al. [24] discovered that decreased expression of E-cadherin was associated with the clinicopathological features of hepatocellular carcinoma, including the late stage of tumor, poor tumor differentiation, and lymph node metastasis, etc. In this study, expression of E-cadherin protein was also reduced in gastric cancer tissues, and indicated intimate correlation with lymph node metastasis, TNM staging, tumor differentiation and maximum diameter. These results suggest that detection of E-cadherin protein may be an important marker of prognosis in gastric cancer. Furthermore, Wierstra [25] demonstrated that transcription factor FOXM1 could bind directly to the promoter of E-cadherin gene in mice and humans, and induced EMT, thereby promoting the invasion and metastasis of cancer. In conclusion, high expres-

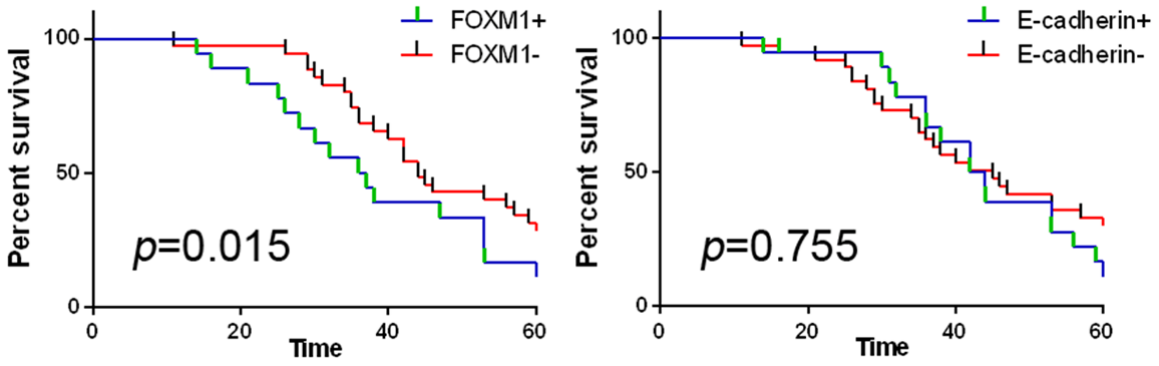

Figure 3. Relationship between expression of FOXM1, E-cadherin, and the patients' survival period (5-year survival).

sion of FOXM1 and low expression of E-cadherin may play a pivotal role in invasion, metastasis, and survival of gastric cancer, and FOXM1 may be a promising molecular target for the treatment of gastric cancer in the future.

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Disclosure of conflict of interest

None.

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