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• GASTRIC CANCER •

Expression of TFF2 and *Helicobacter pylori* infection in carcinogenesis of gastric mucosa

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Abstract

AIM: To investigate the expression of TFF2 and *Helicobacter pylori* infection in carcinogenesis of gastric mucosa.

METHODS: The expression of TFF2 was immunohistochemically analyzed in paraffin-embedded samples from 119 patients with endoscopic biopsy and subtotal gastrectomy specimens of gastric mucosal lesions, including 16 cases of chronic superficial gastritis (CSG), 20 chronic atrophic gastritis (CAG), 35 intestinal metaplasia (IM), 23 gastric epithelial dysplasia (GED) and 25 gastric carcinoma (CA), and *Helicobacter pylori* infection was detected by Warthin-Starry staining.

RESULTS: 1: TFF2 was located in the cytoplasm of gastric mucous neck cell. The expression of TFF2 was 100 %, 100 %, 0, 56.5 % and 0 in CSGs, CAGs, IMs, GEDs and CAs, respectively. 2: The value of TFF2 positive cell density in CSG with *Helicobacter pylori* infection was higher than that without *Helicobacter pylori* infection. (52.89±7.27*vs* 46.49±13.04, $P>0.05$); But the value of TFF2 positive cell density in CAG and GED with *Helicobacter pylori* infection was significantly lower than that without *Helicobacter pylori* infection (18.17±4.09*vs* 37.93±13.80, $P<0.01$ and 14.44±9.32*vs* 24.84±10.22, $P<0.05$).

CONCLUSION: Increase of TFF2 expression in CSG is perhaps associated with the protective mechanism after gastric mucosal injury. Decrease of TFF2 expression in CAG possibly attributes to the decrease in the number of gastric gland cell expressing TFF2. Re-expression of TFF2 in gastric epithelial dysplasia implies that TFF2 possibly contributes to the initiation of gastric carcinoma. The effect of *Helicobacter pylori* on the expression of TFF2 depends on the status of gastric mucosa.

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INTRODUCTION

Trefoil factor family 2 (TFF2), also known as spasmolytic polypeptide (SP), is one of three known mammalian trefoil peptides. Trefoil peptides are small (7-12 kDa) protease-resistant proteins secreted by the gastrointestinal mucosa in a lineage-specific manner. While expressed and secreted preferentially by gastric mucous neck cell[1,2], TFF2 is upregulated in diverse pathological conditions of gastrointestinal tract, which involves regeneration and restitution of epithelial lineage during the epithelial cell injury, mucosal protection and healing of ulcer[3-5]. However, the relationship between TFF2 expression and gastric carcinoma is still not fully elucidated. In the present study, we evaluated the expression of TFF2 and *Helicobacter pylori* infection in a series of gastric mucosal lesions. The aim of this study was in two aspect: to characterize the expression pattern of TFF2 on carcinogenesis of gastric mucosa; and to study the relationship between the expression of TFF2 and *Helicobacter pylori* infection.

MATERIALS AND METHODS

Tissue material

The gastric mucosal lesions of all 119 patients who had undergone surgical resection or endoscopic biopsy in the Renmin Hospital of Wuhan University from March 2001 to March 2002 were studied. There were 16 cases of chronic superficial gastritis (CSG), 20 chronic atrophic gastritis (CAG), 35 intestinal metaplasia (IM), 23 gastric epithelial dysplasia (GED) and 25 gastric carcinoma (CA). Tissue fragments were fixed in 10 % formaldehyde and embedded in paraffin. Serial sections of 4 μm were stained with haematoxylin and eosin (HE) , Warthin-Starry and by immunohistochemistry.

Immunohistochemistry of TFF2 protein

A modification of the streptavidin-peroxidase method was applied to immunohistochemistry and with 3, 3'-diaminobenzidine (DAB) as the chromogen (Ultrasensitive SP Kit, DAB, FuZhou Maixin Biotechnology Co). Sections was incubated for one hour at 37 ℃ with monoclonal antibody against human TFF2 (hSP, diluted 1:35, Novocastra Laboratories Ltd). All batches of staining included positive control. Negative control was performed by replacing the primary mAbs with Tris buffer solution (TBS). The value of positive cell density was determined by image analysis system of HPIAS2000.

Warthin-Starry staining

Histological examination with Warthin-Starry staining technique was used for *Helicobacter pylori* infection diagnosis in all cases, and was identified as positive brown-black or black staining of *Helicobacter pylori*.

Scoring of immunoreactivity

Immunoreactivity were scored according to the presence of immunoreactive cells: - , none or rate positive cells (<5 %); +, 5-25 % ; ++, 25-75 %; +++, >75 %.

Statistical analysis

All results are expressed as means plus or minus SD unless otherwise stated, and analyzed by software of SPSS 10.00.

The statistical significance of difference was evaluated with the Student' s *t* test. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Expression pattern of TFF2 protein in carcinogenesis of gastric mucosa

TFF2 protein was located in the cytoplasm of gastric epithelial cells and gastric gland mucous neck cells by immunohistochemistry, and positive cells were stained brown-yellow. TFF2 protein was expressed in all the chronic superficial gastritis and chronic atrophic gastritis (Figure 1-2), but the scoring of TFF2 staining was higher in chronic superficial gastritis than in that chronic atrophic gastritis. The expression of TFF2 was partly detected (56.5 %) in the gastric epithelial dysplasia (Figure 3). There was no expression of TFF2 in the intestinal metaplasia and gastric carcinoma. (Figure 4-5) (Table 1).

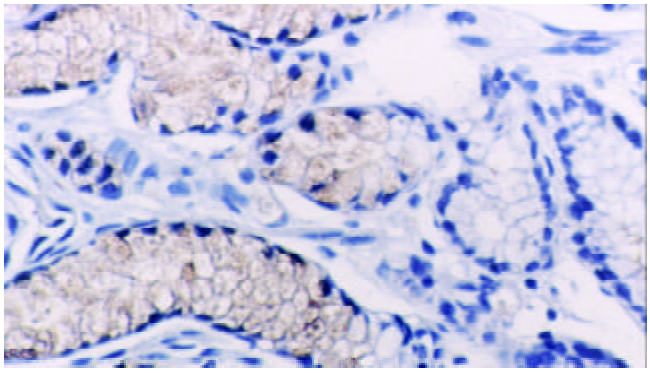

Figure 1 The expression of TFF2 protein in chronic superficial gastritis by immunohistochemistry (SP×200).

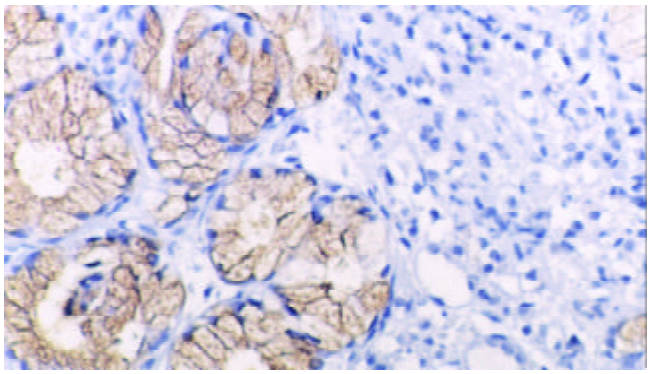

Figure 2 The expression of TFF2 protein in chronic atrophic gastritis by immunohistochemistry (SP×200).

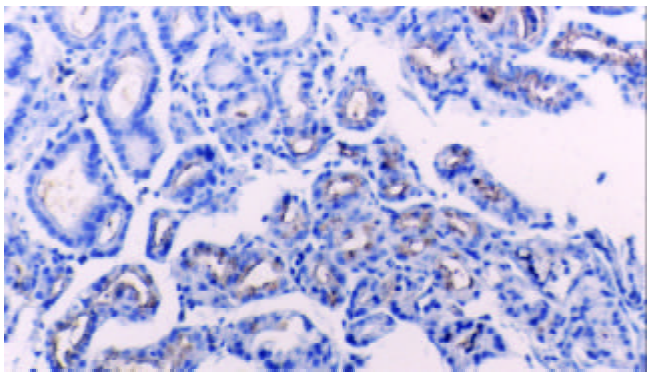

Figure 3 The expression of TFF2 protein in gastric epithelial dysplasia by immunohistochemistry (SP×100).

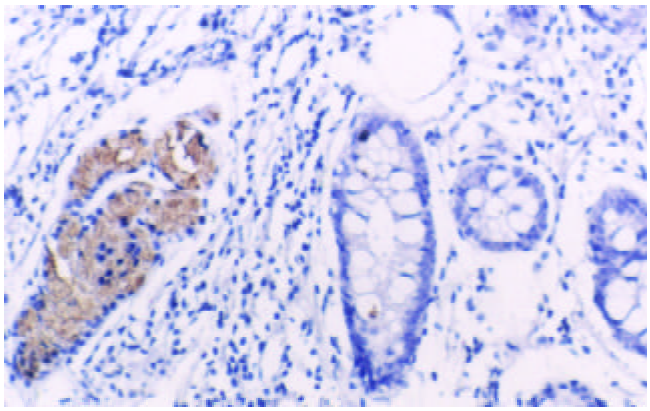

Figure 4 No expression of TFF2 protein in intestinal metaplasia, but the expression observed in its surrounding tissues (SP×100).

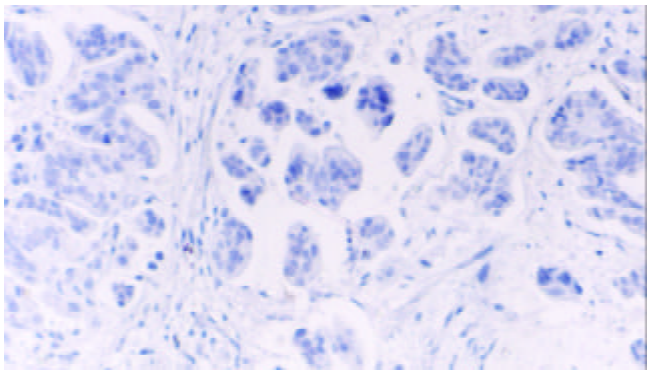

Figure 5 No expression of TFF2 protein in gastric carcinoma (SP×100).

Table 1 The expression pattern of TFF2 protein in carcinogenesis of gastric mucosa

Classification	Cases	TFF2				Rates of positivity
		-	+	++	+++	
CSG	16	0	1	5	10	100 %
CAG	20	0	5	11	4	100 %
IM	35	35	0	0	0	0
GED	23	10	9	2	2	56.5 %
CA	25	25	0	0	0	0

Table 2 The relationship between the expression of TFF2 and *H.pylori* infection

Classification	Cases	TFF2	*t* test
CSG			
H.pylori (+)	8	52.89±7.27	*P*>0.05
H.pylori (-)	8	46.49±13.04	
CAG			
H.pylori (+)	12	18.17±4.09	*P*<0.01
H.pylori (-)	8	37.93±13.80	
IM			
H.pylori (+)	16	0	
H.pylori (-)	19		
GED			
H.pylori (+)	12	14.44±9.32	*P*<0.05
H.pylori (-)	11	24.84±10.22	
CA			
H.pylori (+)	15	0	
H.pylori (-)	9		

The relationship between the expression of TFF2 and Helicobacter pylori infection

The positive rate of *Helicobacter pylori* was 53.8 % (64/119) in our cases. The value of TFF2 positive cell density in chronic superficial gastritis with *Helicobacter pylori* infection was higher than that without *Helicobacter pylori* infection (52.89±7.27 *vs* 46.49±13.04, $P>0.05$), but the difference was not statistically significant; While the value of that in chronic atrophic gastritis and dysplasia with *Helicobacter pylori* infection was significantly lower than that without *Helicobacter pylori* infection (18.17±4.09 *vs* 37.93±13.80, $P<0.01$ and 14.44±9.32 *vs* 24.84±10.22, $P<0.05$), and the difference was statistically significant (Table 2).

DISCUSSION

We undertook the present study in order to characterize the pattern of TFF2 protein expression in carcinogenesis of gastric mucosa. In conformity with data in the literature[2], immunohistochemical expression of TFF2 was not observed at the surface epithelium, although expression of the corresponding mRNA was previously detected by *in situ hybridization*[2]. We observed, as did others, the expression of TFF2[2,6] in the mucous cells of the antrum, the chief cells of the body and neck zone cells.

In this study, we have clarified the expression pattern of TFF2 protein in carcinogenesis of gastric mucosa. In chronic superficial gastritis and atrophic gastritis, the high expression of TFF2 was observed in all cases. As a cytoprotective factor, TFF2 protein was induced as a result of gastric mucosal injury. Our results confirmed an important cytoprotective and restitutive role for TFF2. The mechanism of protective and healing effect of TFF2 on the gastric mucosa is still not fully elucidated. *In vitro*,TFF2 stimulated cell migration[7]. It has recently been shown that hTFF decreased proton permeation through interacting with mucus in vivo and in vitro[8], and oral TFF2 binds to the mucus layer of the stomach[9], which accelerates the gastric ulcer healing in rat. Further, we also discovered that the scores of TFF2 staining were higher in chronic superficial gastritis than that in chronic atrophic gastritis, which might attribute to the decrease in the number of mucous neck cell expressing TFF2 in chronic atrophic gastritis. There was no expression of TFF2 protein in intestinal metaplasia and gastric carcinoma, but TFF2 protein was observed in surrounding tissues of intestinal metaplasia and gastric carcinoma, which suggested the expression of TFF2 could be associated with the phenotype of gastric epithelial cell differentiation. Although it had been reported by Machado *et al*[10], the expression of TFF2 was observed in 10 (10.4 %) of 96 cases of gastric carcinomas.

Furthermore, we also found that there were 13 out of 20 cases with re-expression of TFF2 in gastric epithelial dysplasia. It was well known that dysplasia is a precancerous lesion of stomach, and TFF2 could contribute to the initiation of gastric carcinoma. In 1999, Schemidt *et al*[11] found the SPEM (SP-expressing metaplasia) lineage was detected in 91 % of gastric carcinoma, typically located in mucosa adjacent to the carcinoma or areas of dysphasia. And others studies[12] also discovered that SPEM was identified in 88 % of the surrounding mucosa in the remnant cancers, as well as 61 % of the follow-up biopsies. In the malignant resections, 67 % of the surface dysplasia displayed SP positive cells. All findings implicated a strong association of the SPEM lineage with gastric carcinoma. At present, no confirmed relationship could be found between TFF2 and carcinoma[11-19]. However, Farrell *et al*[20] generated a model of TFF2-deficient mice, and suggested a physiologic role of TFF2 in promoting mucosal healing through the stimulation of proliferation and downregulation of gastric acid secretion. In other words, increased TFF2 expression and secretion could contribute directly to gastric cancer risk not only through stimulation of proliferation, but also through inhibition of acid secretion. Meanwhile, our results and others [11] showed there was loss of TFF2 in all gastric carcinoma cases. Therefore, it is impossible that TFF2 expression plays an important role in the progression of gastric carcinoma. Whether TFF2 can directly or indirectly contribute to carcinogenesis of gastric mucosa will be required for further studies.

Gastric carcinoma is the most common tumor in gastrointestinal tract[21-32], more and more studies suggested that *Helicobacter pylori* infection was significantly associated with gastric carcinoma and was a high risk factor for gastric carcinoma[33-52]. It has been reported that an increase in the number of mucous neck cells expressing TFF2 has been observed in both Helicobacter infected human patients[12] and Helicobacter-infected mice with both preneoplastic[53] and neoplastic[54] changes of the gastric mucosa. In all of these findings, it was likely that *Helicobacter pylori* infection would contribute to the expression of TFF2 by promoting the proliferation of gastric mucosal cell, which would be a mechanism of *Helicobacter pylori* contributing to carcinogenesis of gastric mucosa. Then, we examined retrospectively the *Helicobacter pylori* infection of all cases by Warthin-Starry staining, and found that the value of TFF2 positive cell density was higher in chronic superficial gastritis with *Helicobacter pylori* infection than that without, which suggested that TFF2 was induced in the early stage of *Helicobacter pylori* infection. Nevertheless, its value was significantly lower in chronic atrophic gastritis and dysplasia with *Helicobacter pylori* infection than that without. We inferred that low expression of TFF2 was associated with a decrease in the number of gastric mucosal cell as result of *Helicobacter pylori* infection. As a result, the effect of *Helicobacter pylori* on the expression of TFF2 could depend on the status of gastric mucosa.

In conclusion, early-stage or short-term upregulation of TFF2 appears to be helpful in healing of gastric mucosa, but prolonged upregulation may in fact contribute to carcinogenesis, Further studies will be needed to define the role of TFF2 in *Helicobacter pylori*-associated chronic gastritis and gastric carcinoma.

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