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RAPID COMMUNICATION

Expression of trefoil factors 1 and 2 in precancerous condition and gastric cancer

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Abstract

AIM: To study the expression of trefoil factor 1 (TFF1) and TFF2 in precancerous condition and gastric cancer and to explore the relationship between TFFs and tumorigenesis, precancerous condition and gastric cancer.

METHODS: The expression of TFF1 and TFF2 was immunohistochemically analyzed in paraffin-embedded samples from 140 patients including 35 cases of chronic superficial gastritis (CSG), 35 cases of gastric ulcer (GU), 35 cases of chronic atrophic gastritis (CAG) and 35 cases of gastric cancer (GC).

RESULTS: TFF1 and TFF2 were located in cytoplasm of gastric mucous cells. In CSG, GU, CAG and GC, the level of TFF1 expression had a decreased tendency ($P < 0.05$). The expression of TFF2 was higher in GU than in CSG, but the difference was not significant. The expression of TFF2 also had a decreased tendency in GU, CAG, and GC ($P < 0.05$).

CONCLUSION: The reduced expression of TFF1 and TFF2 in precancerous conditions and gastric cancer may be associated with the proliferation and malignant transformation of gastric mucosa. More investigations are needed to explore the mechanism of TFFs and the relationship between TFFs and gastric cancer.

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Key words: Trefoil factor; Gastric cancer; Immunohistochemistry

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INTRODUCTION

Trefoil factor (TFF) is a newly discovered protective factor of gastrointestinal mucous, which has attracted more and more attention. This protein family consists of three members: TFF1 known as breast cancer-associated peptide (PS2), TFF2 as spasmolytic polypeptides (SP), and TFF3 as intestine trefoil factor (ITF). Human TFF1 and TFF2 contain only one trefoil structure domain, while human TFF3 contains two Trefoil structure domains[1]. pS2 (mpS2) gene is inactivated in the mouse, the antral and pyloric gastric mucosae of mpS2-null mice are dysfunctional and exhibit severe hyperplasia and dysplasia, while all homozygous mutant mice develop antropyloric adenoma, and 30% of them develop multifocal intraepithelial or intramucosal carcinomas[2]. Welter *et al*[3] found that TFF2 does not express in the pancreas but in pancreatic tumor of humans. Moreover, patients with pancreatic tumor expressing TFF2 have better prognosis. All these findings indicate that TFF is not only the repairing factor for inflammatory mucous and gastrointestinal ulcer, but also correlates with tumor development in digestive system. So far the functions and mechanism of TFF in gastrointestinal tract are unclear. The relationship between TFF and gastrointestinal tumors is still unclear. The aim of this study was to observe the features of the expression of TFF1 and TFF2 in four kinds of gastric mucosal lesion by immunohistochemistry and to explore the relationship between TFF1, TFF2 and tumorigenesis, progression of precancerous condition and gastric cancer.

MATERIALS AND METHODS

Materials

Thirty-five specimens of chronic superficial gastritis (CSG), chronic atrophic gastritis (CAG), gastric ulcer (GU) and gastric cancer (GC) were randomly taken from 150 patients in 1999-2000 (98 males and 42 females, the median age was 52.5 years). All the specimens were paraffin-embedded.

Immunohistochemistry staining

The streptavidin-peroxidase method was applied to immunohistochemistry. The anti-TFF1 protein antibody and the immunohistochemistry test kit (Kit-9710) were the products of Fuzhou Maixin Biotechnology Development

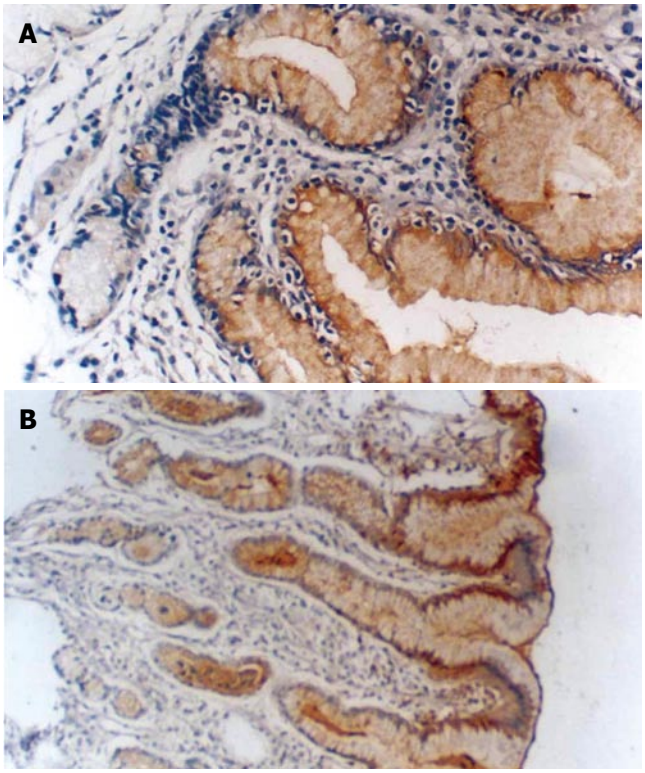

Figure 1 Strong yellow staining of TFF1 in superficial (**A**) and deeper (**B**) glands (SP100).

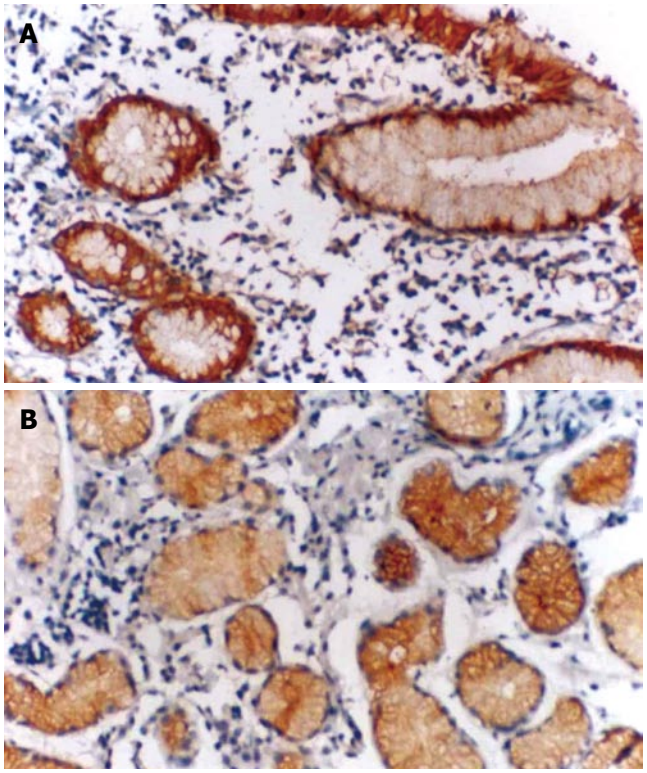

Figure 2 Strong brown-yellow staining of TFF2 in superficial (**A**) and deeper (**B**) glands (SP200).

Company. Anti-TFF2 protein antibody was provided by professor A.Giraud (Western Hospital, Australia). TBS (0.01 mol/L, pH7.4) was used as first antibody for contrast. The immunohistochemistry staining was performed according to the instructions of Fuzhou Maixin Biotechnology Development Company. DAB displayed the color. Yellow or brown-yellow pigmentation could be seen in cytoplasm and cell mucosa. According to the intensity of pigmentation and the range of positive reaction , we made the following scores: 0 = no staining or the staining intensity similar to the background; 1 = weakly positive or little deeper than the background, 2 = moderately positive or significantly deeper than the background, 3 = densely stained or deeply brown stained; According to the positive range, 0 = <10%, 1 = 10%-25%, 2 = 25%-50%, 3 = 50%-75%, 4 = >75%. Four classifications were made: 0-1 as (-), 2 as (+), 3-4 as (++) and above 5 as (+++).

Statistical analysis

All statistical analyses were carried out by SAS 8.0. Wilcox test was used according to the data. $P < 0.05$ was considered statistically significant.

RESULTS

Expression pattern of TFF1 in four kinds of gastric lesion

The expression of TFF1 could be detected in gastric mucosa. TFF1 was yellow or brown-yellow in positive regions and mainly located in cytoplasm. The expression of TFF1 could also be detected in most epithelia, glands and mucus around epithelial cell mucosa. Yellow staining in gastric foveola and superficial glands was relatively strong (Figure 1A). Yellow staining was stronger in superficial glands than in deeper ones and in basal gastric glands (Figure 1B). The level of TFF1 expression had a decreased tendency in different lesions of CSG, GU, CAG and GC ($P < 0.05$) and there was a significant difference between them ($P < 0.05$) (Table 1).

Expression pattern of TFF2 in four kinds of gastric lesion

Brown-yellow TFF2 was mainly located in cytoplasm. The expression of TFF2 could be detected in the mucus around epithelia, superficial and deep glands. However, the expression level was not significantly different between superficial and deep glands (Figure 2A). Yellow pigmentation was stronger in basal gastric glands (Figure 2B). The expression level of TFF2 in different gastric lesions was significantly different (Table 2). In different lesions, the expression level was GU > CSG > CAG > GC. There is no significant difference between CSG and GU. However, both of them were higher than CAG and GC ($P < 0.05$).

Relation between TFF1 and TFF2 expression and clinicopathological characteristics of GC

The level of TFF1 and TFF2 expression was lower in poorly-differentiated carcinoma than in moderately-and well-differentiated carcinoma ($P < 0.0001$, Table 3).

DISCUSSION

TFF is a small molecular protein with rich cysteine, and

Table 1 Expression of TFF1 protein by immunohistochmistry staining

Group	*n*	TFF1 expression				Mean score
		-	+	+ +	+ + +	
CSG	35	0	2	24	9	83.2
GU	35	1	6	20	8	73.7
CAG	35	2	3	27	3	68.1
GC	35	7	5	19	4	57

Table 2 Expression of TFF2 protein by immunohistochmistry staining

Group	*n*	TFF1 expression				Mean score
		-	+	+ +	+ + +	
CSG	35	0	0	22	13	80
GU	35	2	0	18	15	80.7
CAG	35	3	3	21	8	61.2
GC	35	2	5	21	7	60.2

Table 3 Expression of TFF1 and TFF2 in different GC

Type	*n*	TFF1 expression				Positive rate (%)	TFF2 expression				Positive rate(%)
		-	+	+ +	+ + +		-	+	+ +	+ + +	
Moderately-and well-differentiated carcinoma	21	0	2	15	4	81	0	0	14	7	100
poorly- differentiated carcinoma	14	7	3	4	0	50	2	5	7	0	85.7

is characterized by its sub-grade structures containing the trefoil like structure domain. This structure domain consists of 6 cysteine residues linked with 3 intramolecular disulfide chains. TFF is widely expressed in mucosa of endoblasts, especially in the gastrointestinal tract. In the normal gastrointestinal tract, TFF1 expresses mainly in gastric mucosa, TFF2 expresses mainly in gastric and intestinal mucosa, while TFF3 expresses mainly in intestinal mucosa. While the mucosa is acutely injured, this distinctive distribution disappears. TFF can protect against such injuries induced by stress[4], aspirin[5], indomethacin[6], frozen probe[7], *etc*. Alison *et al*[7] showed that when gastrointestinal mucosa is acutely injured, the expression of TFF increases around the injured mucosa. These findings suggest that TFF is a factor for acutely injured gastrointestinal mucosa. However, in this study, the level of TFF1 expression in chronic ulcer tissue was lower than that in chronic superficial gastritis ($P = 0.2587$), while the level of TFF2 expression in GU was slightly higher than that in CSG, but neither of which had any statistical significance, indicating that TFF can recover acute lesion of the mucosa but not chronic injury. The high expression of TFF1 and TFF2 in normal mucosa indicates that TFF1 and TFF2 are related with the physiological renewal of normal gastric mucosa cells.

TFF1 is first extracted from breast cancer cells. In breast cancer, the expression of TFF1 is estrogen-dependent. Recently, it was reported that the estrogen receptor is expressed in normal gastric membrane[8], gastric cancer[9] and pancreatic cancer[10,1]. However, Luqmani *et al*[12] found that the expression of TFF1 in normal gastric membrane and gastric cancer is non-estrogen dependent. The function of TFF1 in gastrointestinal tract and its relationship with estrogen are unclear. The decreased expression of TFF1 in gastric cancer and precancerous status indicates that TFF1 may be one of the inhibitory factors of gastric tumor and the decreased expression of TFF1 may be a sign of the deterioration of gastric membrane.

Human TFF2 contains two "trefoil" structure domains and does not express in breast cancer[13]. Pia Azarschab *et al*[14] found that aspirin (1-2mM) can significantly increase the expression of TFF2. However it is unclear whether this increasing can prevent gastric cancer. In this study, the expressive level of TFF2 in GU was higher than that in CSG, suggesting that TFF2 may play a more important role than TFF1 in the recovery of the gastric membrane. The expressive level of TFF2 in CAG and GC was lower than that in CSG ($P < 0.05$), suggesting that the expressive level of TFF2 decreases in gastric cancer and precancerous status. In this study, the common expression of TFF1 and TFF2 in mucous cells and epithelial mucous indicated that TFF interacts with mucous. TFF2 forms homodimers through cysteine in mucous gel layer[15,16], while TFF1 forms heterodimers in the same area[17,18]. Therefore, the viscosity of mucous is increased and the mucosa defensive barrier is stabilized. Because EGF, TFF1, and TFF2 are expressed in normal mucosa around the small intestinal ulcer, Wright *et al*[19] suggested that the expression of TFF1 is probably controlled by EGF. Yu *et al*[20] found that hydrotalcite increases expression of TFF2 mRNA, suggesting that hydrotalcite regulates TFF2 mRNA expression through the increase of epidermal growth factor

The expressive level of TFF1 and TFF2 was higher in gastric cancer but lower in poorly-differentiated carcinoma than in well-differentiated carcinoma, indicating that decreased expression of TFF1 and TFF2 not only has a close relationship with the occurrence of gastric cancer, but also relates to the malignancy of gastric cancer. So far the specific mechanism of these two proteins in normal gastric mucosa and in the process of malignancy is unclear. Decreased expression of TFF1 and TFF2 may be associated with the proliferation and malignant transformation of gastric mucosa. The results of this study may bring some enlightenments to the prophylaxis and treatment of gastric cancer.

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