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• *BRIEF REPORTS* •

Relationship between trefoil factor 1 expression and gastric mucosa injuries and gastric cancer

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Abstract

AIM: To determine whether trefoil factor 1 (TFF1) is associated with mucosa healing and carcinoma suppression, we assess the expression of trefoil factor 1 in normal and pathologic gastric mucosa.

METHODS: TFF1 in normal and pathologic gastric mucosa was assessed by immunohistochemical method, and the average positive *A* was estimated by Motic Images Advanced 3.0 software.

RESULTS: Increased TFF1 was detected in gastritis, gastric ulcer and duodenal ulcer compared with normal mucosa. The same result could be seen in multiple and compound ulcer compared with simple ulcer. There was no significant difference between gastric ulcer and duodenal ulcer, gastritis and simple ulcer respectively. Increased TFF1 was detected in the peripheral mucosa of the gastric adenocarcinoma compared with normal mucosa. The expression of TFF1 in gastric adenocarcinoma was related to the differentiation of adenocarcinoma. The lower the differentiation of adenocarcinoma, the weaker the expression of TFF1. There was no TFF1 expressed in low-differentiated adenocarcinoma. The expression of TFF1 in middle and highly differentiated adenocarcinoma was a little lower than that in normal mucosa. But there was no significant difference. No TFF1 was assessed in esophageal squamous carcinoma and peripheral tissue. There was no significant difference between male and female.

CONCLUSION: The expression of TFF1 was higher in gastritis and peptic ulcer than that in normal mucosa, and was also higher in multiple and compound ulcer than in simple ulcer. It seems that TFF1 plays a role in gastric mucosa protection and epithelial restitution. Increased expression of TFF1 in peripheral tissue suggests that TFF1 is associated with mechanism of carcinoma suppression and differentiation. Decreased expression of TFF1 in carcinoma and its relativity to the differentiation suggests that TFF1 is related to gland and cell destruction of carcinoma.

Key words: Trefoil factor; Gastric mucosa protection; Epithelial restitution; Carcinoma suppression

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INTRODUCTION

TFF1 is one of the trefoil factor family (TFF). TFF is a group of small molecule polypeptide mainly secreted by gastrointestinal mucous cell. In normal tissue, TFF1 is expressed mainly in mucosal epithelial cell of body and antrum of stomach. If the gastrointestinal mucosa is injured, the specificity of expression disappears. TFF1 can express in the whole injured mucosal position and is much higher than normal. There is great clinical importance to explore the effect of TFF1 in gastric mucosa injury repair and carcinoma suppression. We use immunohistochemical method to assess TFF1 in physiologic and pathologic gastric mucosa to explore the effect of TFF1 in maintenance of integrity and continuity of gastric mucosa and carcinoma suppression.

MATERIALS AND METHODS

Materials

Two hundred and two specimens of paraffin obtained by gastroscopic biopsy and radical gastric carcinomectomy during 2002.1-2002.12 in the First Clinical Hospital of Medical College of Xiamen University were studied. The age of the cases range 40-70 years (50.3±7.8 years). Thirty-five specimens were normal gastric antrum mucosa, which showed normal gastric mucosa in gastroscopy and chronic and light superficial gastritis in pathologic examination. Eighteen specimens were gastric antrum mucosa obtained from patients with gastritis, which showed congestion and erosion of gastric mucosa in gastroscopy and chronic and middle-severe superficial gastritis in pathologic examination. Thirty-five specimens were peripheral mucosa obtained from patients with gastric ulcer. Thirty-seven specimens were

gastric antrum mucosa obtained from patients with duodenal ulcer. Nineteen specimens were peripheral mucosa obtained from patients with multiple and compound ulcer. Thirty-eight specimens were carcinoma and peripheral tissue obtained from patients with gastric adenocarcinoma. Twenty specimens were carcinoma and peripheral tissue obtained from patients with esophageal squamous carcinoma.

Reagents

Mice anti-human TFF1 monoclonal antibody, S-P super sensitive kit and DAB display kit were all purchased from Fuzhou Mai Xin Biotechnology Development Company.

Methods

All the specimens were fixed in 40 g/L formaldehyde, routinely dehydrated, cleaned, infiltrated with wax, embedded and made into serial sections whose thickness was 4 μm. Then the sections were dewaxed, dyed by SP method, displayed by DAB, dyed again with hematoxylin and blown dry at last. All the operations were carried out as on the direction of S-P Test Kit. PBS was negative contrast instead of the first antibody.

Evaluation of the result

Every section was photographed in 100× high-power microscope. Cytoplasm of positive cell was buffy. Motic Imaged Advanced 3.0 software was used to estimate the average positive *A* of 20 glands, which were selected randomly to reflect the intensity of expression of TFF1. The higher the *A* was, the stronger was the expression of TFF1. The results were analyzed with SPSS10.0. All data were expressed as mean±SD and statistical analysis was performed using the Student's *t* test.

RESULTS

TFF1 expressed mainly in gastric mucosal gland cytoplasm, especially around the nucleus. Positive cytoplasm was buffy and the dyeing was deeper, close to the side of the lumen than apart from it. There was no significant difference of intensity of dyeing in different position of peripheral ulcer mucosa of the same stomach in patient with multiple ulcer, which illustrated that the expression of TFF1 in gastric mucosal gland did not alter among different positions in the same stomach. The average positive *A* of TFF1 expression in normal gastric antrum mucosa was 0.44±0.06, while the average positive *A* in gastric antrum mucosa of gastritis was 0.51±0.05 and was much higher than the former. The difference was significant ($P<0.001$). The average positive *A* in gastric antrum mucosa of duodenal ulcer was much higher than that in normal gastric antrum mucosa. There was significant difference (0.50±0.06 *vs* 0.44±0.06, $P<0.001$). The average positive *A* in peripheral mucosa of multiple and compound ulcer was higher than that of single gastric ulcer or duodenal ulcer. The difference was significant (0.54±0.05 *vs* 0.50±0.06, $P<0.05$). There was no significant difference between the expression of TFF1 in peripheral mucosa of gastric ulcer and in gastric antrum mucosa of duodenal ulcer, patients with gastritis and patients with single peptic ulcer respectively ($P>0.05$). The average positive *A* in peripheral mucosa of gastric adenocarcinoma was much higher than that in normal mucosa. The difference was significant (0.51±0.07 *vs* 0.44±0.06, $P<0.001$). But the expression of TFF1 in gastric carcinoma decreased or was negative, the intensity of which was related to the differentiation of adenocarcinoma. The lower the differentiation of adenocarcinoma, the weaker the expression of TFF1. Twenty-seven specimens were low-differentiated adenocarcinoma, in which there was no expression of TFF1. Eleven specimens were middle and highly differentiated adenocarcinoma, in which the average positive *A* was a little lower than that in normal mucosa. But there was no significant difference (0.41±0.07 *vs* 0.44±0.06, $P>0.05$). There was no significant difference between the expression of TFF1 in peripheral mucosa of gastric carcinoma and single peptic ulcer, multiple and compound ulcer and gastritis respectively ($P>0.05$). There was no expression of TFF1 in 20 specimens (including peripheral tissue) obtained from patients with esophageal squamous carcinoma. There was no significant difference between male and female in all groups (Table 1).

Table 1 Expression of TFF1 in physiologic and pathologic gastric mucosa

Classification	*n*	Average positive *A*
Normal	35	0.44±0.06
Gastritis	18	0.51±0.05
Gastric ulcer	35	0.51±0.06
Duodenal ulcer	37	0.50±0.06
Single peptic ulcer	72	0.50±0.06
Multiple and compound ulcer	19	0.54±0.05
Low-differentiated carcinoma	27	None
Middle and highly differentiated carcinoma	11	0.41±0.07
Peripheral tissue of the gastric carcinoma	38	0.51±0.07
Esophageal squamous carcinoma	20	None
Peripheral tissue of the esophageal carcinoma	20	None

DISCUSSION

TFF is a group of small molecule polypeptide and mainly secreted by gastrointestinal mucous cell. At present there are three kinds of trefoil peptide found in mammal, which are breast cancer-associated peptide (pS2 or TFF1), spasmolytic polypeptide (SP or TFF2) and intestinal trefoil factor (ITF or TFF3). The common characteristic of them is that all have a special structure-P structure domain. This structure is composed of 38-39 amino acid sequence by six highly conservative cysteine residues by way of link of three intramolecular disulfide bonds (Cys1-Cys5, Cys2-Cys4, Cys3-Cys6), which makes the whole peptide chain twisted and folded. Thus, the trefoil structure takes shape and the name is formed. The stability of this trefoil structure makes the TFF be capable of resisting hydrolysis of protease and digestion of acid and have a characteristic of heat proof. So TFF could keep its biologic activity in complicated environment of digestive tract. In mammal, trefoil peptide has the function of mucosa protection, epithelial restitution, carcinoma suppression, signal conduction and apoptosis

adjustment, *etc*. TFF1 was obtained from cell line MCF-7 of human mammary carcinoma induced by estrogen by Masiakowski[1]. Every TFF1 molecule is composed of 60 amino acids and its molecular weight is 6 674 ku. Every TFF1 molecule includes seven cysteine residues, six of which take part in the constitution of P structure domain and the seventh lies in the third base to the end of carboxyl, i.e., Cys58. Mark *et al*[2], replaced Cys58 of recombinant TFF1 protein with Ser58 and analyzed TFF1 and TFF1 analog including Ser58. They found that homologous dimer could come into being in TFF1 but could not in TFF1 analog, which suggested that Cys58 was the very one which formed intermolecular disulfide bond by which dimer came into being. It has been reported that the biologic activity of TFF1 is relevant to the formation of homologous dimer or the formation of oligomer by binding with other proteins. The expression of TFF1 in mammal begins from embryonic period. Several studies revealed that the expression of TFF1 could be seen in stomach in mice embryo from 8 d after copulation to being prenatal[3]. In normal tissue, TFF1 expresses mainly in mucosal epithelial cell of body and antrum of stomach. But in pathologic tissue, the specificity of expression disappears[4].

Many internal and overseas studies have proved that trefoil peptide plays a great role in gastric mucosa protection. There are two hypotheses about its mechanism: (1) trefoil peptide could bind with mucous glycoprotein to form stable gel compound. This compound could reinforce the mucous gel layer and decrease injury of harmful substance in gastric surface and mechanical stress to mucosa. Polshakov *et al*, found that there was a fissure of 6&A ring between the second and third link of trefoil peptide. Amino acids around the fissure were highly conservative and formed a hydrophobic region, which was likely to offer a binding site for oligosaccharide chain in mucous glycoprotein or hydrophobic side chain of some proteins to accomplish its biologic function[5]. Otherwise the common secretion of TFF1 and mucin also proved this viewpoint[6]. (2) Trefoil peptide is likely to accomplish its biologic function by binding with its recipient or transport protein. Newton *et al*, found that there were three patterns of TFF1 in normal gastric mucosa: monomer, dimer and TFF1 compound whose molecular weight was about 25 ku, among which the concentration of TFF1 compound was the highest while the presence of dimer was little. Many experiments have proved that TFF1 dimer has more significant biologic activity than monomer in cell migration and mucosa protection. They also found that the amount of dimer was meager, so they assumed that TFF1 compound was made up of TFF1 and some protein and they played a great biologic role. This protein may be was TFF1 recipient or transport protein[7]. It has been illustrated by a series of studies that trefoil peptide also takes part in epithelial restitution of injured tissue. Trefoil peptide could reinforce the migration of peripheral intact epithelial cells to the surface of injured mucosa and cover it, which promotes the repair of injured mucosa. Many studies revealed that there was an ulcer-associated cell lineage around the chronic ulcer in gastrointestinal tract (for example, Crohn's disease, ulcerative colitis, peptic ulcer, *etc*.). The expression of TFF1 in this UACL obviously increased and was related to expression of mucin[8]. In our experiment, obvious increase of TFF1 expression in gastritis and peptic ulcer also validated this viewpoint. The expression of TFF1 in multiple and compound ulcer was higher than that in single peptic ulcer, which suggested that the more seriously gastric mucosa injured, the higher the expression of TFF1. Increased expression of trefoil peptide in many malignant tumors could be seen. Thus, it once was a hot spot to research whether neoplasia led to excessive expression of trefoil peptide or excessive expression of trefoil peptide led to neoplasia. In mice model from which TFF1 gene was removed, all the gastric epithelial cells showed severe hyperplasia, high dysplasia and adenoma formation in gastric antrum. Some could develop into gastric infiltrative adenocarcinoma[9]. Other researches revealed that TFF1 could restrain proliferation of gastric adenocarcinoma cell line AGS and its inhibition was dose-dependent. TFF1 dimer had more significant biologic activity than monomer[10], which suggested that TFF1 was a kind of tumor suppression factor. In our experiment, the expression of TFF1 in peripheral tissue of gastric adenocarcinoma was much higher than normal, which illustrated that TFF1 was relevant to tumor suppression. Neoplasia could promote secretion of TFF1 to restrain growth of tumor, while the expression of TFF1 in carcinoma decreased or was negative. The lower the differentiation of carcinoma, the weaker the expression of TFF1. The probable reasons are as follows: (1) some reason causes decreased expression of TFF1 and leads to formation of carcinoma; (2) necrosis of glands and cells of carcinoma leads to decreased secretion of TFF1. The lower the differentiation of carcinoma, more obvious was the destruction of glands and cells; the less the secretion of TFF1, the weaker the expression of TFF1. The expression of TFF1 in middle and highly differentiated adenocarcinoma was a little lower than that in normal mucosa, but there was no significant difference, which may be due to less sample size. Our experiment also assayed the expression of TFF1 in 20 esophageal squamous carcinoma and peripheral tissue. The results were negative. That is, there was no trefoil factor expressed in esophageal mucosa. The episode and suppression mechanism of esophageal squamous carcinoma was irrelevant to trefoil factor. The relation between trefoil peptide and tumor and its mechanism remain unclear at present. Further research is necessary to make it clear.

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