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

Jian-Lin Ren, Jin-Yan Luo, Ya-Pi Lu, Lin Wang, Hua-Xiu Shi

Jian-Lin Ren, Jin-Yan Luo, Ya-Pi Lu, Lin Wang, Hua-Xiu Shi, Department of Gastroenterology, The Second Hospital, Xian Jiaotong University, Xi’an 710004, Shaanxi Province, China
Jian-Lin Ren, Ya-Pi Lu, Lin Wang, Hua-Xiu Shi, Department of Gastroenterology, Zhongshan Hospital, Xiamen University, Xiamen 361004, Fujian Province, China
Correspondence to: Dr. Jian-Lin Ren, Department of Gastroenterology, Zhongshan Hospital, Xiamen University, Xiamen 361004, Fujian Province, China. jianlinr@msn.com

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 normal gastric antrum mucosa, which showed normal gastric mucosa 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.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

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

Ren JL, Luo JY, Lu YP, Wang L, Shi HX. Relationship between trefoil factor 1 expression and gastric mucosa injuries and gastric cancer. World J Gastroenterol 2005; 11(17): 2674-2677
http://www.wjgnet.com/1007-9327/11/2674.asp

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. Eleven specimens were middle and highly differentiated adenocarcinoma, in which there was no expression of TFF1. Twenty-seven specimens were low-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).

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), spasmyloptide (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 mammary 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 &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 up of TFF1 and some protein and they played a great activity than monomer in cell migration and mucosa: monomer, dimer and TFF1 compound whose concentration of TFF1 compound was the highest while molecular weight was about 25 ku, among which 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

REFERENCES

1 Masiakowski P, Breathnach R, Bloch J, Gannon F, Kurst A, Chambon P. Cloning of cDNA sequences of hormone-regulated genes from the MCF-7 human breast cancer cell line. Nucleic Acids Res 1982; 10: 7895-7903

2 Chadwick MP, Westley BR, May FE. Homodimerization and hetero-oligomerization of the single-domain trefoil protein pN2/pS2 through cysteine 58. Biochem J 1997; 327(Pt 1): 117-123

3 Lefebvre O, Wolf C, Kedinger M, Chenard MP, Tomasetto C, Chambon P, Rio MC. The mouse one P-domain (pS2) and two P-domain (mSP) genes exhibit distinct patterns of expression. J Cell Biol 1993; 122: 191-198

4 Ribieras S, Tomasetto C, Rio MC. The pS2/TFF1 trefoil factor, from basic research to clinical applications. Biochim Biophys Acta 1998; 1378: F61-F77
Polshakov VI, Williams MA, Gargaro AR, Frenkel TA, Westley BR, Chadwick MP, May FE, Feeney J. High-resolution solution structure of human pNR-2/pS2: a single trefoil motif protein. J Mol Biol 1997; 267: 418-432

Wright NA, Poulsom R, Stamp G, Van Noorden S, Sarraf C, Elia G, Ahnen D, Jeffery R, Longcroft J, Pike C. Trefoil peptide gene expression in gastrointestinal epithelial cells in inflammatory bowel disease. Gastroenterology 1993; 104: 12-20

Newton JL, Allen A, Westley BR, May FE. The human trefoil peptide, TFF1, is present in different molecular forms that are intimately associated with mucus in normal stomach. Gut 2000; 46: 312-320

Longman RJ, Douthwaite J, Sylvester PA, Poulsom R, Casfield AP, Thomas MG, Wright NA. Coordinated localisation of mucins and trefoil peptides in the ulcer associated cell lineage and the gastrointestinal mucosa. Gut 2000; 47: 259-262

Lefebvre O, Chenard MP, Masson R, Linares J, Dierich A, LeMeur M, Wendling C, Tomasetto C, Chambon P, Rio MC. Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein. Science 1996; 274: 792-800

Calnan DP, Westley BR, May FE, Floyd DN, Marchbank T, Playford RJ. The trefoil peptide TFF1 inhibits the growth of the human gastric adenocarcinoma cell line AGS. J Pathol 1999; 188: 312-317