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

Guo-Yong Hu, Bao-Ping Yu, Wei-Guo Dong, Mu-Qi Li, Jie-Ping Yu, He-Sheng Luo, Zong-Xue Rang

Guo-Yong Hu, Bao-Ping Yu, Wei-Guo Dong, Mu-Qi Li, Jie-Ping Yu, He-Sheng Luo, Zong-Xue Rang, Gastroenterology Department, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China. yubaoping62@yahoo.com.cn

INTRODUCTION

The effect of Helicobacter pylori infection was detected by Warthin-Starry staining. Decrease of TFF2 expression in CAG was significantly lower than that without H. 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 H. 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).

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.

H. pylori infection diagnosis and with 3, 3’-diaminobenzidine (DAB) as the chromogen (Ultrasureactive SP Kit, DAB, FuZhou Maixin Biotechnology Co). Sections was incubated for one hour at 37 °C 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 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).

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

| Classification | Cases | -  | +  | ++ | +++ | 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[11], immunohistochemical expression of TFF2 was not observed at the surface epithelium, although expression of the corresponding mRNA was previously detected by in situ hybridization[12]. We observed, as did others, the expression of TFF2[12] in the mucus 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 mucus 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, Schenidt et al[11] found the SPEG (SP-expressing metaplasia) lineage was detected in 91 % of gastric carcinoma, typically located in mucosa adjacent to the carcinoma or areas of dysplasia. And others studies[12] also discovered that SPEG 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 SPEG 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[21-23], more and more studies suggested that Helicobacter pylori infection was significantly associated with gastric carcinoma and was a risk factor for gastric carcinoma[24-32]. It has been reported that an increase in the number of mucus neck cells expressing TFF2 has been observed in both Helicobacter infected human patients[22] and Helicobacter-infected mice with both preneoplastic[33] and neoplastic[34] 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 with 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.

REFERENCES

1. Tomasetto C, Rio MC, Gautier C, Wolf C, Hareuveni M, Chambon P, Lathe R. HSP, the domain-duplicated homolog of pS2 protein, is co-expressed with pS2 in stomach but not in breast carcinoma. EMBO J 1990; 9: 407–414
2. Hanby AM, Poulsom R, Singh S, Elia G, Jeffery RE, Wright NA. Spasmolytic-polypeptide is a major antral peptide: distribution of the trefoil peptides human spasmolytic polypeptide and pS2 in the stomach. Gut 1993; 105: 1110–1116
3. McKenzie C, Thin L, Parsons ME. Topical and intravenous administration of the trefoil peptides protect the gastric mucosa from ethanol-induced injury in the rat. Aminonut Pharmocol Ther 2000; 14: 1033–1040
4. Tran CP, Cook GA, Yeomans ND, Thin L, Giraud AS. TFF2 peptide TFF2 (spasmolytic polypeptide) potently accelerates healing and reduces inflammation in a rat model of colitis. Gut 1999; 44: 636–642
5. Cook GA, Familiar M, Thin L, Giraud AS. The trefoil peptides TFF2 and TFF3 are expressed in rat lymphoid tissues and participate in the immune response. FEBS Lett 1999; 456: 155–159
6. Playford RJ, Marchbank T, Chinery R, Evison R, Pignatelli M, Boulton RA, Thin L, Hanby AM. Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. Gastroenterology 1995; 108: 108–116
7. Dignass A, Lynch-Davey K, Kindon H, Thin L, Podolsky DK. Trefoil peptides promote epithelial migration through a trans-
forming growth factor beta-independent pathway. J Clin Invest 1994; 94: 376-383

8 Tanaka S, Podolsky DK, Engel E, Guth PH, Kaunitz JD. Human spasmolytic polypeptide decreases proton permeation through gastric mucus in vivo and in vitro. Am J Physiol 1997; 272(6 Pt 1): G1473-1480

9 Poulsen SS, Thulesen J, Christiansen L, Nexø E, Thilm L. Metabolism of oral trefoil factor 2 (TFF2) and the effect of oral and parenteral TFF2 on gastric and duodenal ulcer healing in rats. Gut 1999; 45: 516-522

10 Machado JC, Nogueira AM, Carneiro F, Reis CA, Sobrinho-Simoes M. Gastric carcinoma exhibits distinct types of cell differentiation: an immunohistochemical study of trefoil peptides (TFF1 and TFF2) and mucins (MUC1, MUC2, MUC5AC, and MUC6). J Pathol 2000; 190: 437-443

11 Schmidt PH, Lee JR, Joshi V, Playford RJ, Poulsom R, Wright NA, Goldwring JR. Identification of a metastatic cell lineagie associated with human gastric adenocarcinoma. Lab Invest 1999; 79: 639-646

12 Yamaguchi H, Goldenring JR, Kannimuthi M, Lee JR. Identification of spasmolytic polypeptide expressing metastasis (SPEM) in remnant gastric cancer and surveillance postgastrectomy biopsies. Dig Dis Sci 2002; 47: 573-578

13 Terris B, Blavet E, CmoporacJuricic T, Jones M, Missaglia E, Ruzsniewski P, Sauvant A, Lemoine NR. Characterization of gene expression profiles in intraductal papillary-mucinous tu-

14 azars P, Al-Azeh E, Kornberger W, Gott P. Aspinin pro-

15 Ohshio G, Suwa H, Kawaquchi Y, Imamura M, Yamaoka Y, Yamaeh H, Matsumoto M, Yoshikawa H, Hashimoto Y, Takeda H. Differential expression of human spasmolytic polypeptide (trefoil factor family-2) in pancreatic carcinomas, ampullary carcinomas, and mucin-producing tumors of the pancreas. Dig Dis Sci 2000; 452: 659-664

16 Nogueira AM, Machado JC, Carneiro F, Reis CA, Gott P, Sobrinho-Simoes M. Patterns of expression of trefoil peptides and mucins in gastric cancer with and without malignant transformation. J Pathol 1999; 187: 541-548

17 Labouvie C, Machado JC, Carneiro F, Sarbha M, Vieth M, Porschen R, Setz G, Bln N. Differential expression of mucins and trefoil peptides in native epithelium, Barrett’s metaplasia and squamous cell carcinoma of the oesophagus. J Cancer Res Clin Oncol 1999; 125: 71-76

18 Efstathiou JA, Liu D, Wheeler JM, Kim HC, Beck NE, Ilyas M, Karayanakiss A, Mertosten M, Kmriot W, Playford RJ, Pignatelli M, Bodmer WF. Mutated epithelial cadherin is associated with increased tumorigenicity and loss of adhesion and of responsiv-

19 dos Santos Silva E, Kayademir T, Regakari F, Machado JC, Savas S, Dolast S, Bln N, Gott P. Variable distribution of TFF2 (Spasmolytic polypeptide) in European does not indicate predomin-

20 Farrell JI, Taupin D, Koh TJ, Chen D, Zhao CM, Podolsky DK, Wang TC. TFF2/ SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibil-

21 Cai L, Yu SZ, Zhan ZF. Cytochrome P450 2E1 genetic polymor-

22 Xiao HB, Cao WX, Yin HR, Lin YZ, Ye SH. Influence of L-meth-

23 He XS, Su Q, Chen ZC, He XT, Long ZF, Ling H, Zhang LR. Expression, deletion [was deleton] and mutation of p16 gene in human gastric cancer. World J Gastroenterol 2001; 17: 709-715

24 Cai L, Yu SZ, Zhan ZF, Glutathione S-transferases M1, T1 genotypes and the risk of gastric cancer: a case-control study. World J Gastroenterol 2001; 7: 506-509

25 Liu DH, Zhang XY, Fan DM, Huang XQ, Zhang JS, Huang WQ, Zhang YQ, Huang QS, Ma WY, Chai YB, Jin M. Expression of vascular endothelial growth factor and its role in oncogenesis of human gastric carcinoma. World J Gastroenterol 2001; 7: 500-505

26 Niwu XQ, Qin XY, Liu H, Wang CP. Clinicopathological analysis of patients with gastric cancer in 1200 cases. World J Gastroenterol 2001; 7: 281-284

27 Xin Y, Li XL, Wang YP, Zhang SM, Zheng HC, Wu DY, Zhang YC. Relationship between phenotypes of cell-function differen-

28 Guo CQ, Wang YP, Li W, Du QX, Liu WW. A study on relationship between infection of Helicobacter pylori and inactivating of antioncogenes in cancer and pre-cancerous lesion. Shijie Huan Xiaohua Zazhi 2000; 8: 386-388

29 Wang DX, Fang DC, Li W, Du QX, Liu WW. A study on relationship between infection of Helicobacter pylori and inactivating of antioncogenes in cancer and pre-cancerous lesion. Shijie Huan Xiaohua Zazhi 2000; 8: 505-508

30 Lu W, Chen LY, Gong HS, PCNA and c-erbB-2 expression in gastric mucosal intestinal metaplasia with Helicobacter pylori infection. Shijie Huan Xiaohua Zazhi 1999; 7: 111-113

31 Liu WW, Fang DC, Li W, Du QX, Liu WW. A study on relationship between infection of Helicobacter pylori and inactivating of antioncogenes in cancer and pre-cancerous lesion. Shijie Huan Xiaohua Zazhi 2000; 8: 500-504

32 Lu SU, Pan XD, Peng XW, Shi ZL, Lin L, Chen MH. Effect of Hp infection on gastric epithelial cell kinetics in stomach diseases. Shijie Huan Xiaohua Zazhi 1999; 7: 313-315

33 He XX, Wang JML, Wu JL, Yuan SY, Ai L. Telomerase expression, Hp infection and gastric mucosal carcinogenesis. Shijie Huan Xiaohua Zazhi 2000; 8: 505-508

34 Liang HJ, Gao JH, Liu WW, Feng DC, Men RR. Long-term effects of concentrated Helicobacter pylori culture supernatant on gastric mucosa of rats. Shijie Huan Xiaohua Zazhi 1999; 7: 881-883

35 Gao JH, Liang HJ, Liu WW, Feng DC, Wang ZH. Expression of C-myc gene protein and epidermal growth factor receptor in gastric mucosa pre and post Helicobacter pylori clearance. Shijie Huan Xiaohua Zazhi 1999; 7: 1018-1019

36 Peng ZS, Liang ZC, Liu MC, Ouyang NT. Studies on gastric epithelial cell proliferation and apoptosis in Hp associated gastric ulcer. Shijie Huan Xiaohua Zazhi 1999; 7: 218-219

37 Liu HF, Liu WW, Feng DC, Gao JH, Wang ZH. Apoptosis and proliferation induced by Helicobacter pylori and its association with p53 protein expression in gastric epithelial cells. Shijie Huan Xiaohua Zazhi 2001; 9: 1265-1268

38 Mauda S, Yoshida H, Sato M, Ito Y, Harata Y, Yamai Y, Akamura M, Shiratori Y. Omatama N. H. pylori activates NF-kappB through a signaling pathway involving IkappB kinase, NF-

39 Ekstrom AM, Heid M, Hansson LE, Engstrand L, Nyren O. Helicobacter pylori in gastric carcinomas established by CagA immuno blot as a marker of past infection. Gastroenterology 2003;
Xue FB, Xu YY, Wan Y, Pan BR, Ren J, Fan DM. Association of H. pylori infection with gastric carcinoma: a Meta analysis. World J Gastroenterol 2001; 7: 801-804

Zhang Z, Yuan Y, Gao H, Dong M, Wang L, Gong YH. Apoptosis, proliferation and p53 gene expression of H. pylori associated gastric epithelial lesions. World J Gastroenterol 2001; 7: 779-782

Yao YL, Xu B, Song YG, Zhang WD. Overexpression of cyclin E in Mongolian gerbil with Helicobacter pylori-induced gastric precancerosis. World J Gastroenterol 2002; 8: 60-63

Morgner A, Miehlke S, Stolte M, Neubauer A, Alpen B, Thiede C, Klann H, Hierlmeier FX, Ell C, Ehninger G, Bayerdorffer E. Development of early gastric cancer 4 and 5 years after complete remission of Helicobacter pylori associated gastric low grade marginal zone B cell lymphoma of MALT type. World J Gastroenterol 2001; 7: 248-253

Miehlke S, Kirsch C, Dragosics B, Gschwantler M, Oberhuber G, Antos D, Dite P, Lauter J, Labenz J, Leodolter A, Mafertheiner P, Neubauer A, Ehninger G, Stolte M, Bayerdorffer E. Helicobacter pylori and gastric cancer: current status of the Austrian Czech German gastric cancer prevention trial (PRISMA Study). World J Gastroenterol 2001; 7: 243-247

Cai L, Yu SZ, Zhang ZF. Helicobacter pylori infection and risk of gastric cancer in Changle County, Fujian Province, China. World J Gastroenterol 2000; 6: 374-376

Wang TC, Goldenring JR, Dangler C, Ito S, Mueller A, Jeon WK, Koh TJ, Fox JG. Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with Helicobacter felis infection. Gastroenterology 1998; 114: 675-689

Wang TC, Dangler CA, Chen D, Goldenring JR, Koh T, Raychowdhury R, Coffey RJ, Ito S, Varro A, Dockray GJ, Fox JG. Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer. Gastroenterology 2000; 118: 36-47