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• *GASTRIC CANCER* •

Dynamic expression of pepsinogen C in gastric cancer, precancerous lesions and *Helicobacter pylori* associated gastric diseases

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Abstract

AIM: To investigate the relationship between the expression of pepsinogen C (PGC) and gastric cancer, precancerous diseases, and *Helicobacter pylori* (*H pylori*) infection.

METHODS: The expression of PGC was determined by immunohistochemistry method in 430 cases of gastric mucosa. *H pylori* infection was determined by HE staining, PCR and ELISA in 318 specimens.

RESULTS: The positive rate of PGC expression in 54 cases of normal gastric mucosa was 100%. The positive rates of PGC expression in superficial gastritis or gastric ulcer or erosion, atrophic gastritis or gastric dysplasia and gastric cancer decreased significantly in sequence ($P<0.05$; 100%/89.2% *vs* 14.3%/15.2% *vs* 2.4%). The over-expression rate of PGC in group of superficial gastritis with *H pylori* infection was higher than that in group without *H pylori* infection ($P<0.05$; $\chi^2 = 0.032$ 28/33 *vs* 15/25). The positive rate of PGC expression in group of atrophic gastritis with *H pylori* infection was lower than that in group without *H pylori* infection ($P<0.01$; $\chi^2 = 0.003$ 4/61 *vs* 9/30), and in dysplasia and gastric cancer.

CONCLUSION: The level of PGC expression has a close relationship with the degree of malignancy of gastric mucosa and development of gastric lesions. There is a relationship between *H pylori* infection and expression of antigen PGC in gastric mucosa, the positive rate of PGC expression increases in early stage of gastric lesions with *H pylori* infection such as gastric inflammation and decreases during the late stage such as precancerous diseases and gastric cancer. PGC-negative cases with *H pylori*-positive gastric lesions should be given special attention.

Key words: *H pylori*; Pepsinogen C; Gastric cancer

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INTRODUCTION

Pepsinogen (PG) is the precursor of pepsin or gastricsin and activated in acid condition. Pepsin of humans can be divided into two groups: pepsinogen A (PGA) and pepsinogen C (PGC)[1]. PGA is mainly distributed in gastric fundus and PGC throughout the stomach and proximal duodenum. PGC is mainly secreted by chief cells of gastric gland, also by cardiac gland and pyloric gland and Brunner gland. The change of serum PGC can reflect the degree of gastric lesions or differentiation of gastric cells[2,3]. Recently studies showed that the change of PGC could reflect the degree of gastric disease and differentiation[4-7]. The level of PGC and the ratio of PGA/PGC in serum decreased in chronic atrophic gastritis and gastric cancer. There is now evidence from epidemiological studies that *Helicobacter pylori* (*H pylori*) carriers have a significantly greater risk of developing gastric cancer[8-10]. *H pylori* has been classified as a group I carcinogen by IARC, but the exact role *H pylori* plays is unclear. In the present study, we investigate the dynamic expression of PGC antigen in gastric cancer and precancerous lesions and *H pylori*-associated gastric lesions and evaluate the application value of PGC in gastric cancer diagnosis, and also the influence of *H pylori* on PGC antigen expression.

MATERIALS AND METHODS

Samples

A total of 430 gastric mucosal biopsied specimens were involved in this study which came from the endoscopic screening of subjects in the region of Zhuanghe, Liaoning Province, a high risk area of gastric cancer from 1997 to 2002. Each of the biopsies contained gastric corpus, antrum and angulus and was diagnosed by two pathologists separately, including 54 cases of normal gastric mucosa, 58 cases of superficial gastritis, 37 cases of gastric ulcer or erosion, 91 cases of atrophic gastritis (all with intestinal metaplasia), 66 cases of dysplasia, and 124 cases of gastric cancer. There was no significant difference between normal and disease groups in age and sex ($P>0.05$).

Reagents

The anti-PGC antibody was a gift from Japanese Clinical

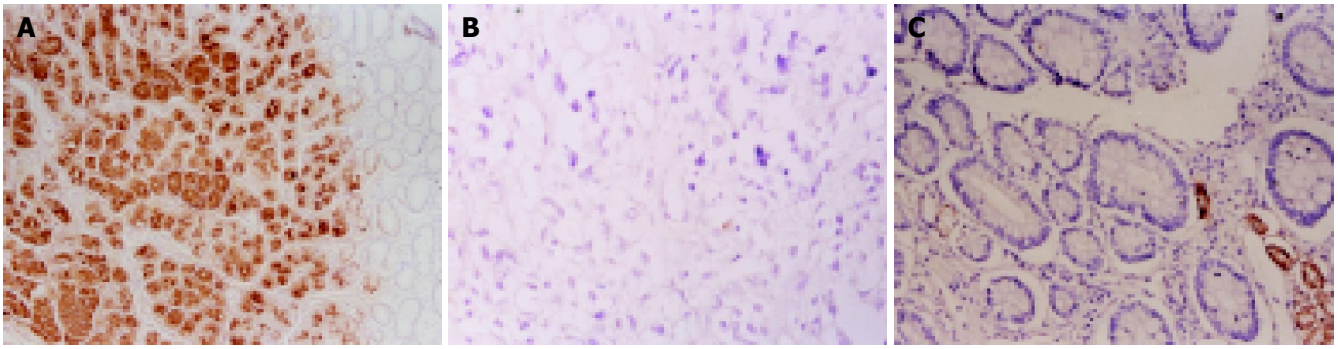

Figure 1 Expression of PGC in different gastric tissues. **A**: Positive expression of PGC in normal gastric mucosa (SP×100); **B**: Negative expression of PGC in gastric cancer (SP×400); **C**: Negative expression of PGC in atrophic gastritis with *H pylori* infection (SP×200).

Inspection Institute. The two-step SP kit (Lot No: Kit-9801D2) was a product of Maxin Company in Fujian, China; ELISA kit was from Huamei Company; and *H pylori*-DNA-PCR kit was from Fuhua Company in Shanghai, China.

H pylori examination

H pylori infection was detected by HE staining, PCR and ELISA. *H pylori* was considered positive if two of the three methods were positive. *H pylori* could be found in the gastric pit and mucus by histological examination. Detection of *H pylori* with *H pylori*-DNA PCR method followed the protocol of the kit. The bands in the same position as positive controls were defined as positive. When ELISA was performed, the samples with A value/A average value of negative controls $\geqslant$2.1 were defined as positive.

Immunohistochemistry staining of pepsinogen C

SP-two step immunostaining was performed according to the instructions of the kit. Diagnosis was made based on brown coloration with varied intensities and the number of cells stained brown[11]. Intensities of staining in cytoplasm were graded as score 1: light brown; score 2: brown; score 3: deep brown. The number of positively stained cells in total cells was categorized as score 1: stained cells<30%; score 2: stained cells 30-70%; score 3: stained cells >70%. According to the sum of the two indexes, the comprehensive scores were made. Comprehensive score 0 was defined as negative expression (-), comprehensive scores 2-3 as weakly positive expression (+), comprehensive score 4 as moderately positive expression (++), comprehensive scores 5-6 as strongly positive expression (+++). The cases with scores greater than 4 were defined as overexpression.

Statistical analysis

The data were analyzed by χ^2 test. P values less than 0.05 were considered statistically significant.

RESULTS

Dynamic expression of pepsinogen C antigen in different gastric mucosal tissues

The PGC antigen was mainly expressed in plasma and nuclei. The positive rate of PGC expression in normal gastric mucosa and superficial gastritis was 100% (Figure 1A). All the atrophic gastritis mucosae were accompanied with intestinal metaplasia (IM) in which PGC was all negative, while the positive rate of PGC expression was 14.3% in atrophic gastritis in the area of non IM. The positive rates of PGC expression decreased in sequence of superficial gastritis, gastric ulcer or erosion, atrophic gastritis or gastric dysplasia and gastric cancer (P<0.05) (Figure 1B) and decreased significantly from superficial gastritis or gastric ulcer to atrophic gastritis or gastric dysplasia (P<0.01) (Table 1). The over-expression rates decreased significantly in sequence of normal gastric mucosa, superficial gastritis, gastric ulcer or erosion, atrophic gastritis or gastric dysplasia or gastric cancer (P<0.05).

Table 1 Expression of PGC antigen in various gastric lesions

Gastric lesions	*n*	Number of cases with PGC expression				Positive rate (%)	Over-expression rate (%)
		-	+	++	+++		
Normal gastric mucosa	54	0	1	14	39	100.0[b]	98.2[b]
Superficial gastritis	58	0	15	26	17	100.0[b]	74.1[b,d,f]
Gastric ulcer or erosion	37	4	20	13	0	89.2[a,b,c]	35.1[b,c,d]
Atrophic gastritis	91	79	11	1	0	14.3[b,d,f]	1.1[d,f]
Intestinal metaplasia	91	91	0	0	0	0.0[d,f,h]	0.0[a,f]
Dysplasia	66	56	9	1	0	15.2[b,d,f]	1.5[d,f]
Gastric cancer	124	121	3	0	0	2.4[d,f]	0.0[d,f]

[b]P<0.01 *vs* gastric cancer; [d]P<0.01, [a]P<0.05 *vs* normal gastric mucosa; [c]P<0.05 *vs* superficial gastritis; [f]P<0.01 *vs* gastric ulcer or erosion; [h]P<0.01 *vs* dysplasia.

The positive rate of PGC expression in well-differentiated gastric cancer was 9.4% and 0% in moderately or poorly-differentiated gastric cancer ($P<0.05$) (Table 2).

Table 2 Expression of PGC antigen in different histopathologic types of gastric cancer

Histopathologic type of gastric cancer	*n*	No. of cases with PGC expression	Positive rate (%)
Well-differentiated	32	3	9.4
Moderately-differentiated	31	0	0.0
Poorly-differentiated	61	0	0.0[a]

[a]$P<0.05$ $\chi^2 = 0.015$ 0/61 *vs* 3/32.

Expression of PGC antigen in H pylori-associated gastric diseases

On examination, *H pylori* infection was detected in 318 cases of different gastric mucosa tissues in which 192 cases were *H pylori*-positive and 126 cases *H pylori*-negative. The positive rate of PGC expression was 100% in superficial gastritis but the over-expression rate was higher in *H pylori*-positive group than in *H pylori*-negative group ($P<0.05$). The PGC positive rate was higher in *H pylori*-positive group than in *H pylori*-negative group in the area of non IM in atrophic gastritis ($P<0.01$) (Figure 1C) and lower in groups of dysplasia and gastric cancer ($P>0.05$) (Table 3).

DISCUSSION

PGC is known as progastricsin and a mature marker of stomach cells, the change of which could reflect the degree of gastric lesions[6,7]. In our study, the expression of PGC antigen was various in different gastric diseases. The positive rate of PGC expression was 100% in normal gastric mucosa and 2.4% in gastric cancer. The positive rates of PGC expression decreased gradually in sequence of benign lesions, precancerous lesions and gastric cancer, especially from benign lesions to precancerous lesions. We found that PGC antigen was negative in most of dysplasia and gastric cancer mucosae while in all of intestinal metaplasia mucosa there was no production of PGC. Zhang *et al*[12], found the adult residents with serum PG level abnormality were accompanied with a higher risk of precancerous lesions (intestinal metaplasia and epithelia dysplasia) in gastric mucosa than those with normal serum PG level during following-up. PGC served as a proteinase involved in the digestion of proteins in the stomach, its levels significantly decreased in atrophic gastritis and dysplasia implicating poorly differentiated cells in these two precancerous diseases and were more susceptible to gastric cancer, but the PGC levels of the above two lesions were still higher than those in gastric cancer ($P<0.01$). The dynamic expression of PGC in different gastric mucosa implicated that PGC antigen had a close relationship with malignancy of gastric mucosa and could well recognize benign or malignant gastric lesions. We also found the positive rate of PGC antigen in well-differentiated gastric cancer was higher than that of moderately or poorly-differentiated gastric cancer, showing PGC expression had some tendency toward a certain histopathologic type. The presence of PGC in cancer cells implicated some mature secreting function in them. The decrease of PGC expression indicated dedifferentiation or malignancy of cancer cells, and was also closely related with prognosis and metastasis[13-15].

The cause of gastric cancer is still unclear but it is generally considered as a multifactor process. *H pylori* infection is an important factor in the pathogenesis of gastric cancer. Most atrophic gastritis are related with *H pylori* infection. Epidemiological data showed *H pylori* carriers had a 2.8 to 6.0 fold increased risk of developing gastric cancer when compared with their *H pylori*-negative counterparts[16,17]. *H pylori* has been known as a group I carcinogen[7] and acts as the initiating agent[18,19]. It is still unclear whether *H pylori* plays a role in the development of atrophic gastritis and intestinal metaplasia.

In our study, the overexpression rate of PGC was higher in *H pylori*-positive superficial gastritis than in *H pylori*-negative cases. This is consistent with serological researches[5,20]. The level of PGC was proportional to the planting density of *H pylori* and decreased after *H pylori* eradication[21,22]. Possible explanations were[23-26]: *H pylori* infection could induce the expression of PG gene, the cytokines produced by *H pylori*-associated gastritis such as TNFα and lipopolysaccharide could stimulate chief cells to secrete PGC, *H pylori* infection could increase gastric acid and gastrin while decreasing somatostatin, all of which could increase PG level. The PG released into gastric lumen was activated to pepsin, which can damage gastric mucosa, aggravate gastritis and gastric ulcer. We also found the positive rate of PGC expression in *H pylori*-positive atrophic gastritis was lower than that in *H pylori*-negative cases ($P<0.01$) and the same

Table 3 Expression of PGC in *H pylori*-associated gastric lesions

Gastric lesions	*n*	*H pylori* positive				*n*	*H pylori* negative			
		PGC positive expression		PGC over-expression			PGC positive expression		PGC over-expression	
		n	Rate (%)	*n*	Rate (%)		*n*	Rate (%)	*n*	Rate (%)
Superficial gastritis	33	33	100.0	28	84.8	25	25	100.0	15	60.0[a]
Atrophic gastritis	61	4	6.6	0	0.0	30	9	30.0[b]	1	3.3
Intestinal metaplasia	61	0	0.0	0	0.0	30	0	0.0	0	0.0
Dysplasia	40	4	10.0	0	2.5	19	6	31.6	0	0.0
Gastric cancer	58	0	0.0	0	0.0	52	3	5.8	0	0.0

[a]$P<0.05$, $\chi^2 = 0.032$; 28/33 *vs* 15/25; [b]$P<0.01$, $\chi^2 = 0.003$; 4/61 *vs* 9/30.

trend presented in dysplasia and gastric cancer. One possible explanation was that the cytotoxin and immunoinflammation response induced by *H pylori* stimulated carcinogens such as oxygen-free radical and superoxide, which can accelerate mutations of PG gene and indirectly reduce the expression of PGC antigen, affecting the balance between cell proliferation, differentiation and apoptosis, and increasing the risk of developing gastric cancer[27-29].

In conclusion, the positive rate of PGC antigen increases in *H pylori*-related gastritis and decreases in *H pylori*-related atrophic gastritis, dysplasia and gastric cancer, the latter should be followed up closely to improve the early detection, evaluation of recurrence and prognosis of gastric cancer.

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