Expression of Pepsinogen C in Gastric Cancer and Precancerous Diseases and its Clinical Significance

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OBJECTIVE To investigate the active expression of pepsinogen C (PGC) and its value in detection of precancerous diseases and gastric cancer.

METHODS Immunohistochemistry was used to examine the expression of pepsinogen C in 424 specimens of gastric mucosa collected by gastroscopy.

RESULTS The positive rate of PGC expression in 54 cases of normal gastric mucosa was 100 % and 2.4% in 124 cases of gastric cancer. The positive rate of PGC expression in superficial gastritis, gastric ulcer or erosion, atrophic gastritis or gastric dysplasia and gastric cancer decreased significantly in the sequence indicated ($P<0.05$).

CONCLUSION The expression of PGC is negatively correlated with the degree of malignancy of gastric mucosa and with development of gastric lesions. PGC expression has a high sensitivity and specificity for diagnosis of precancerous diseases which can lead to gastric cancer and may be a good indicator for screening and diagnosis of gastric cancer and precursors of gastric cancer.

KEYWORDS: pepsinogen C, gastric cancer, precursor of gastric cancer, diagnosis.

Valid screening for precancerous gastric diseases is essential to decrease the mortality rate of gastric cancer.[1] Atrophic gastritis and dysplasia are regarded as important gastric precancerous lesions and have a close relationship with gastric cancer. In recent years, many gastrointestinal tumor markers have been used to diagnose gastric cancer and its precancerous diseases. Pepsinogen C (PGC) also known as progastrin, is a mature marker for stomach cells.[2,3] Recent studies have shown that a change of PGC expression can reflect the degree of gastric lesions or differentiation of gastric cells.[4-7] The level of PGC and the ratio of PGA/PGC (PGA, pepsinogen A) in serum decreased in chronic atrophic gastritis and gastric cancer. It is unclear whether PGC can be a valid marker for gastric cancer and its precancerous diseases. In the present study, biopsied specimens of gastric mucosa obtained from a screening program by the Cancer Institute of China Medical University in the region of Zhuanghe, Liaoning province (a high risk area of gastric cancer), were investigated by assaying active expression of PGC antigen in gastric cancer and precancerous lesions. The data were used to evaluate the value of determination of PGC expression for

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gastric cancer diagnosis.

MATERIALS AND METHODS

Subjects

A total of 424 gastric mucosal biopsied specimens, which came from endoscopic screening from 1997 to 2002 were studied. Each of the biopsies included the gastric corpus, antrum and angulus and were diagnosed separately by 2 pathologists. The cases included 54 cases of normal gastric mucosa, 52 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. In the normal control group there were 32 males and 22 females with an average age of 51. In the diseased group there were 215 males and 155 females with an average age of 53. There were no significant differences between the normal and diseased groups in age or gender (P>0.05).

Reagents

The anti-pepsinogen C antibody was a gift from the Japanese Clinical Inspection Institute. The 2-step SP kit (Lot No: Kit-9801D2) was a product from the Maxin Company in Fujian, China.

Methods

Immunohistochemical staining of pepsinogen C

SP-two step immunostaining was performed according to the instructions in the kit. The measurement of PGC expression was conducted based on brown coloration which developed with varied intensities and on the number of cells which stained brown. [8] Intensities of staining in the cytoplasm were graded as score 1, light brown; score 2, brown; score 3, deep brown. The number of positively stained cells in the total cells were categorized as score 1, stained cells <30% ; score 2, stained cells 30-70% ; score 3, stained cells >70% . Based on the sum of the 2 indexes, comprehensive scores were developed. A comprehensive score of 0 was defined as negative expression (-), comprehensive scores 2-3 were defined as weakly positive expression (+), comprehensive score 4 was defined as moderately positive expression (++) and comprehensive scores 5-6 were defined as strongly positive expression (+++). The cases with scores above 4 were defined as over-expression.

Statistical analysis

The results were analyzed by χ^2 test. P values < 0.05 were considered to be statistically significant.

RESULTS

The active expression of pepsinogen C antigen in different gastric mucosal tissues

The antigen PGC was mainly expressed in the cytoplasm and plasma membrane of chief cells of the gastric mucosa, concentrating in the cardia, pylorius and Brunner's glands and none in the nucleus. The positive rate of PGC expression in the normal gastric mucosa and in a condition of superficial gastritis was 100% (Fig.1). The coloration in superficial gastritis and gastric ulcers or erosion tissue was often deeper and widely distributed, but often negative in dysplastic and gastric cancer tissue (Fig.2). All the patients with the atrophic gastritis mucosa were accompanied with intestinal metaplasia (IM) in which PGC was completely negative, while the positive rate of PGC expression was 14.3% in atrophic gastritis in the areas without IM. The positive rates of PGC expression decreased in a sequence of superficial gastritis , gastric ulcer or erosion, atrophic gastritis or gastric dysplasia and gastric cancer (P<0.05)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 a sequence of normal gastric mucosa, superficial gastritis, gastric ulcer or erosion, atrophic gastritis or gastric dysplasia or gastric cancer (P<0.01).

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 1. Expression of PGC antigen in different gastric mucosa

Gastric lesions	n	No. 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	52	0	12	22	18	100.0[b]	76.9[abc]
Gastric ulcer or erosion	37	4	20	13	0	89.2[bcd]	35.1[ab]
Atrophic gastritis	91	79	11	1	0	14.3[abe]	1.1[ae]
Intestinal metaplasia	91	91	0	0	0	0.0[acf]	0.0[ae]
Dysplasia	66	56	9	1	0	15.2[abe]	1.5[ae]
Gastric cancer	124	121	3	0	0	2.4[ae]	0.0[ae]

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

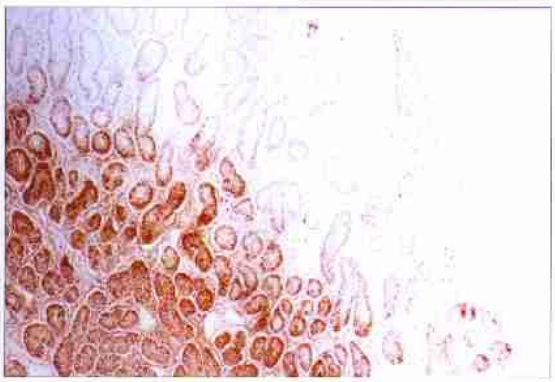

Fig.1. Positive expression of PGC in normal gastric mucosa (SP × 100).

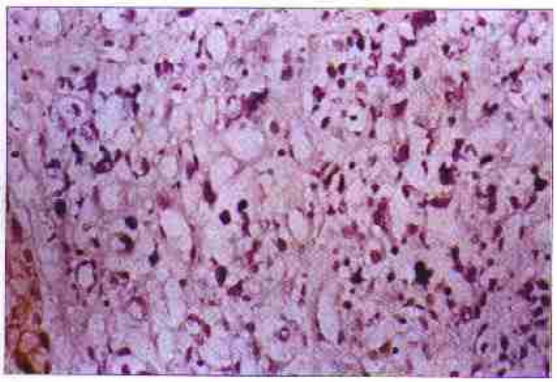

Fig.2. Negative expression of PGC in signet cell gastric cancer (SP × 400).

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

Type	n	PGC expression (cases)	Positive rate(%)
Well-differentiated	32	3	9.4
Moderately- differentiated	31	0	0.0
Poorly- differentiated	61	0	0.0*

*P<0.05 χ^2=0.015

Analysis of pepsinogen C antigen serving as a diagnostic index for gastric cancer and precancerous diseases

Based on different comprehensive scores of PGC antigen expression, we built a ROC-Curve. When a score of 2 was used as a cut-off value for gastric precancerous disease diagnosis, its sensitivity was 90.7%, specificity was 97.2%, false positive rate was 2.8%, false negative rate was 9.3%, positive predictive value was 98.5%, negative predictive value was 84.2% and accordance rate was 92.9%. If a score of 3 was used as a positive threshold, its sensitivity was 95.4%, specificity was 91.6%, false positive rate was 8.4%, false negative rate was 4.6%, positive predictive value was 95.7%, negative predictive value was 91.0% and accordance rate was 94.1% (Table 3). As a criterion of gastric cancer diagnosis, the sensitivity of PGC was 97.6%, specificity was 53.7% and accordance value was 66.5%.

Table 3. Analysis of PGC antigen serving as an diagnosic index for gastric cancer precursors (%)

Index	2 scores	3 scores	4 scores	5 scores	6 scores
Sensitivity	90.7	95.4	98.6	100.0	100.0
Specificity	97.2	91.6	74.1	39.9	9.8
Accordance rate	92.9	94.1	90.3	79.7	69.6

DISCUSSION

Pepsinogen (PG) is the precursor of pepsin or gastrin and is activated under acid conditions. Human pepsinogens can be divided into 2 groups: pepsinogen A (PGA) and pepsinogen C (PGC).[9] PGA is mainly distributed in the gastric fundus and PGC is present throughout the stomach and proximal duodenum.[10] PGC is mainly secreted by chief cells of the gastric mucosa, also by cardiac cells and pyloric cells and Brunner's glands. PGC expression is a mature marker for stomach cells,[2,3] the changes of which can reflect degrees of gastric lesions having a close relationship with gastric atrophy, intestinal metaplasia and dyspasia.[4]

In our study, the expression of the PGC antigen was varied in different gastric diseases. The positive rate of PGC expression in normal gastric mucosa was 100% and 2.4% in gastric cancer which was lower than that of any other gastric disease ($P<0.01$) with the exception of intestinal metaplasia. The coloration produced by PGC in gastric cancer cells was lighter and less than that formed in other conditions. PGC was mainly secreted by chief cells in the gastric fundus mucosa. More than 80 percent of the gastric cancers were associated with intestinal metaplasia, which can destroy many chief cells and decrease their secretion of PGC. Furthermore, due to suppressed differentiation in tumor cells, carcinogenic agents may induce mutations in the PGC gene and therapy decrease the mature expression product, i.e. the PGC antigen.[11] We also found that the positive rate of PGC antigen expression in well-differentiated gastric cancer was higher than that of moderately or poorly-differentiated gastric cancer, showing that the PGC level varied with certain histopathologic types. The presence of PGC in cancer cells indicated that they maintained some mature secretion function. The decrease of PGC expression suggests dedifferentiation or malignancy of cancer cells, and was also closely related to prognosis and metastasis.[12,13]

The positive rates of PGC expression decreased gradually in a sequence of benign lesions, precancerous lesions and gastric cancer, especially from benign lesions to precancerous lesions. We found that the PGC antigen was negative in most of the cases of dysplasia and gastric cancer mucosa while in all of intestinal metaplastic mucosa there was no production of PGC. These results are in accordance with the report of Busby-Earle et al.[14] Zhang et al.[15] found that adults with a serum PG (pepsinogen) level abnormality were accompanied with a higher risk of precancerous lesions (intestinal metaplasia and epithelia dysplasia) in the gastric mucosa than those with normal serum PG level. PGC serves as a preproteinase involved in the digestion of proteins in the stomach, its level significantly decreased in atrophic gastritis and dysplasia implicating poorly differentiated cells in these 2 precancerous diseases making them more susceptible to gastric cancer. The PGC levels of the above 2 lesions, however, were still higher than that in gastric cancer ($P<0.01$). The active expression of PGC in different gastric mucosa suggests that the PGC antigen has a close relationship with malignancy of the gastric mucosa and may well be used to recognize benign from malignant gastric lesions.

Atrophic gastritis and dysplasia are regarded as gastric precancerous changes.[14,15] It is difficult to determine whether a precancerous condition will develop into cancer or not if one only depends on pathologic or morphologic examinations. In our study, as a criterion of gastric precancerous disease and gastric cancer diagnosis, the PGC sensitivity and specificity of PGC were both high. So the PGC antigen has a significant value in detection of precancerous diseases, which can improve the early detection and prevent death from gastric cancer.

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