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• GASTRIC CANCER •

Expression of 1A6 gene and its correlation with intestinal gastric carcinoma

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Abstract

AIM: To investigate the expression of 1A6 gene in the lesions during the development of intestinal gastric carcinoma.

METHODS: One hundred and thirty-six cases of intestinal metaplasia (IM) from surgical resections and biopsy were classified by mucous staining. Expression of 1A6 in all cases was detected using immunohistochemical S-P method.

RESULTS: The positive rates of 1A6 in normal and superficial gastritis (SG), severe atrophic gastritis (SAG), type I, II, III IM, dysplasia (Dys) and intestinal gastric carcinoma (IGC) were 12.2 %, 16.7 %, 7.1 %, 22.6 %, 47.8 %, 46.9 % and 60.8 %, respectively. A significant difference among type III IM and SG, SAG, type I and II IM was found ($P<0.01$), the difference between type III and Dys, IGC being not significant.

CONCLUSION: As a new tumor-related gene, expression of 1A6 may be an effective parameter to predict the malignant transformation of precancerous lesion to gastric carcinoma.

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INTRODUCTION

Gastric cancer is a major health problem in the world[1-3]. Clinical statistics have shown that 50 % of gastric cancer in China are intestinal type adenocarcinoma[4]. The phenotypic events in intestinal gastric carcinoma have been well recognized as normal gastric mucosa, chronic superficial gastritis, severe atrophic gastritis, intestinal metaplasia, dysplasia, intramucosal ("early") carcinoma and invasive carcinoma[5,6]. A considerable number of oncogenes and antioncogenes involved in this process have been described, such as c-met, p53, c-erBb-2, APC, PCNA, p16, akt etc[7-12]. Unfortunately, they are not gastric carcinoma-specific. No significant progress has been made in the existing studies concerning special genotypic abnormalities in the premalignant lesions of gastric carcinoma. 1A6 is a novel tumor-related gene. In this study, immunohistochemistry was used to detect the expression of 1A6 protein in IGC, its premalignant lesions and normal counterparts.

MATERIALS AND METHODS

Patients and specimens

Paraffin-embedded archival materials from surgical resections and biopsy performed during 1998 to 2001 in our department were retrieved. There were 92 cases of gastric carcinoma (62 men and 30 women; aged 29-81 years), among them 51 cases were IGC, and 41 cases were diffuse gastric carcinoma (DGC). The criterion for classifying gastric carcinoma was derived largely from the observations of Lauren[13]. The 23 sarcoma patients consisted of 15 men and 8 women, their ages ranged from 20 to 81 years, the histopathologic types included malignant fibrohistiocytoma, leiomyosarcoma, liposarcoma, fibrosarcoma, malignant stromal tumor and malignant peripheral nerve sheath tumor. According to the updated Sydney system[14] and the Padova International Classification for Gastric Dysplasia[15], 198 cases from biopsy (109 men and 89 women; age ranged from 27-82 years) were divided into four groups : normal mucosal and chronic superficial gastritis (41 cases), severe atrophic gastritis without IM (24 cases), IM (136 cases) and dysplasia (32 cases).

Mucin histochemistry

We performed mucin histochemical studies on 136 cases of IM to classify them using HID/AB/PAS staining described previously[16-19]. The variants fell into three main groups: type I (complete type) shows features of mature small intestinal epithelia, type II (incomplete small intestinal type) had features of both gastric and small intestinal epithelia, whereas type III (incomplete colonic type) has characteristics of large intestines. Neutral mucins appeared magenta in color (PAS positive), sialomucins, blue color (AB positive) and sulphomucins brown (HID positive), respectively. The criteria for classifying IM are given in Table 1.

Table 1 Classification of IM

	PAS	AB	HID
Type I	-	+	-
Type II	+	+	-
Type III	+	+	+

Immunohistochemical staining

To investigate the expression of 1A6 protein, we adopted the standard S-P immunohistochemical method[20]. The 1A6 monoclonal antibody was provided by the Laboratary of Genetics, Beijing Institute of Cancer Research, its dilution was 1:500. The SP kit was provided by Beijing Zhongshan Biotechnology Company. All the slides were treated with PBS and were microwaved for antigen retrieval. 1A6 protein was located in nucleus. Only the samples with more than 10 % cells stained could be considered positive.

Statistical analysis

The difference of 1A6 protein expression among different lesions was analyzed by χ^2 square test. A P value of less than 0.05 was considered statistical significantly.

RESULTS

Expression of 1A6 protein in gastric carcinoma and sarcoma

The positive expression rate of 1A6 protein in all types of gastric carcinoma was 48.9 % (45/92). In contrast, 1A6 protein was negative in all cases of sarcoma (Figure 1). In comparison of IGC and DGC, there was significant difference (P<0.01). In IGC, the positive rate was 60.8 % (31/51), whereas the positive rate in DGC was 34.1 % (14/41). Most positive cells showed a nuclear and plasmatic staining (Figure 2).

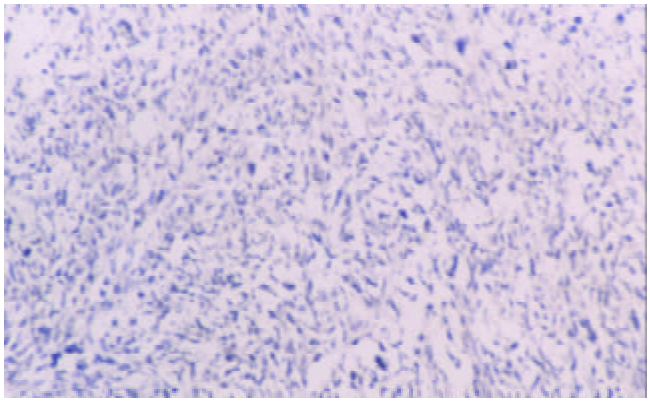

Figure 1 The expression of 1A6 protein in sarcoma. The cells were negative staining. Immunohistochemical stain, ×100.

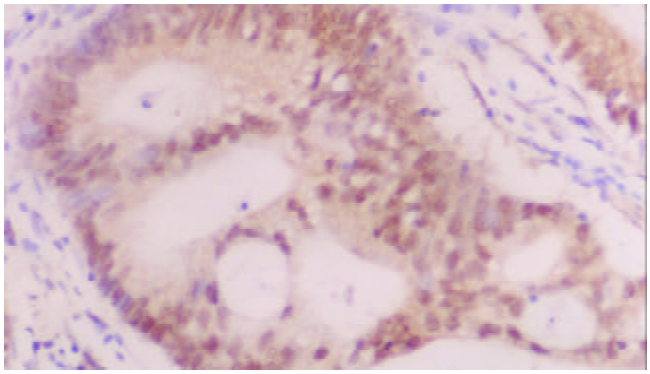

Figure 2 The expression of 1A6 protein in intestinal type gastric carcinoma. The tumor cells showed a nuclear staining. Immunohistochemical stain, ×400.

Expression of 1A6 protein in intestinal metaplasia

According to the mucin histochemistry, there were 14 cases of type I IM, 53 cases of type II IM and 69 cases of type III IM. The expression of 1A6 protein in all kinds of IM is shown in Table 2 (Figures 3,4,5). The positive rate of type III was significantly higher than that of the other two types. A nuclear and plasmatic staining was also found in most positive cases (Figure 6).

Table 2 Expression of 1A6 protein in IM

	n	1A6(+) (%)	1A6(-) (%)
Type I	14	1 (7.1)	13(92.9)
Type II	53	12 (22.6)	41(77.4)
Type III	69	33 (47.8)	39(52.2)[a]

[a]P<0.005 (χ^2=7.965, 8.167), *vs* type I IM, type II IM, respectively.

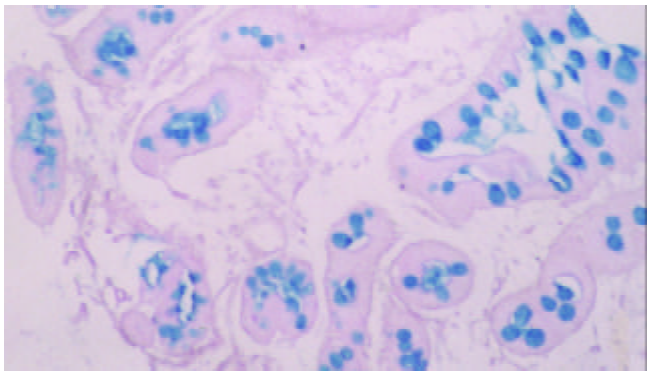

Figure 3 Type I intestinal metaplasia. The mucins in cells were stained blue. HID / AB / PAS stain, ×200.

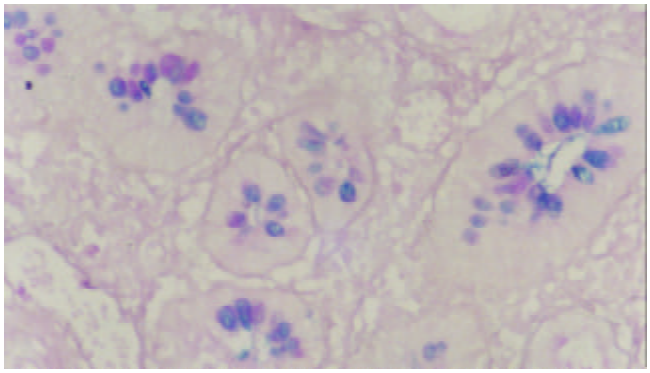

Figure 4 Type II intestinal metaplasia. The mucins in cells were stained blue and magenta. HID / AB / PAS stain, ×200.

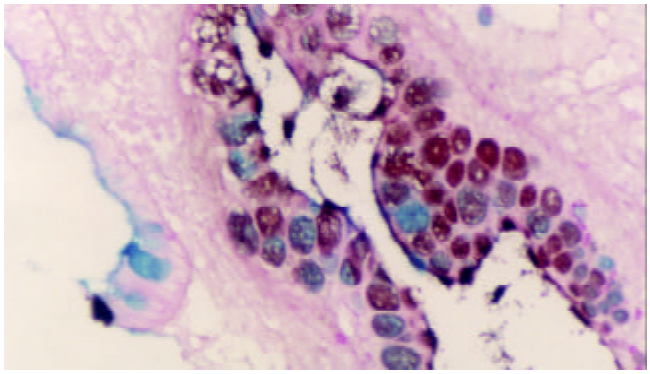

Figure 5 Type III intestinal metaplasia. The mucins in cells were stained blue and brown. HID / AB / PAS stain, ×200.

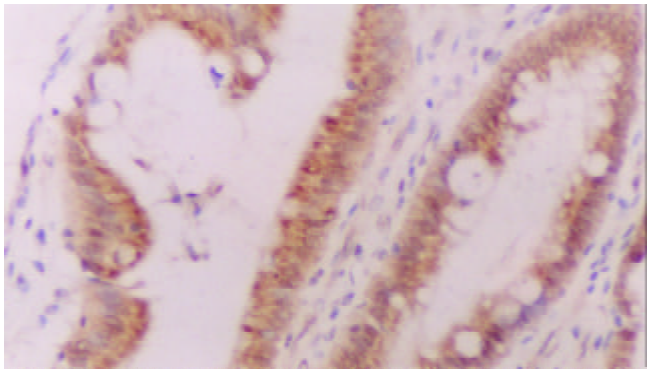

Figure 6 The expression of 1A6 protein in type III intestinal metaplasia. The metaplastic cells showed a nuclear and plasmatic staining. Immunohistochemical stain, ×200.

Expression of 1A6 protein in normal mucosa and SG, SAG, type III IM, Dys and IGC

As the stomach mucosa changed from benign lesion to malignant tumor, the positive expression of 1A6 protein became higher and higher (Table 3). The positive rate of type III, Dys and IGC were higher than SG and SAG, the difference being statistically significant ($P<0.01$). On the contrary, there were no significant differences among type III IM, Dys and IGC.

Table 3 Expression of 1A6 in different lesions of stomach

	n	1A6 + (%)
Normal mucosa and SG	41	5(12.2)
SAG	24	4(16.7)
Type III IM	69	33(47.8)[a]
Dys	32	15(46.9)[b]
IGC	51	31(60.8)[c]

[a]$P<0.01$ ($\chi^2=7.494$), *vs* normal mucosa and SG; [b]$P<0.01$ ($\chi^2=10.067$), *vs* normal mucosa and SG; [c]$P<0.01$($\chi^2=16.298$), *vs* normal mucosa and SG.

DISCUSSION

Recently, many investigations concerning intestinal metaplasia of stomach have been made in different aspects such as epidemiology, pathology and molecular biology[21-23]. Although there were controversies about the phenotypic and genotypic events of IM, it has been widely accepted that the incomplete colonic type IM plays an important role in the histogenesis of gastric carcinoma and it is considered as the premalignant lesion of intestinal gastric carcinoma[21, 24-28].

Gene 1A6 is a novel tumor-associated gene cloned from malignant cell line MC, which is an MNNG-treated human fetal gastric epithelial cell line. 1A6 is located in chromosome 12q23.2-23.3. Its cDNA is about 3kb. There are HEAT motif and nucleus-located sequence in 1A6 protein by molecular sequence analysis. 1A6 protein nucleus localization was further confirmed by immunofluorescence and immuno-electron microscopy. It was found in nucleole and heterochromatin. It suggested that 1A6 protein could interact with other proteins and may play a role as a nuclear transcription factor. Immunohistochemical studies in different tumors indicated that 1A6 was expressed not only in gastric carcinoma, but also in esophageal and breast cancers[29-31].

As mentioned above, type III IM is thought to be the premalignant lesion of gastric carcinoma[32-35]. In our study, the positive expression rate of 1A6 protein was very high in type III IM, which suggests that the overexpression of gene 1A6 maybe an early event in gastric malignant transformation. It can be an important index for predicting the carcinogenesis of stomach. How 1A6 protein functions in transformation of the intestinal metaplasia tissue into malignant tumor and the interaction between other oncogenes and antioncogenes need further investigation and the work is undergoing.

In this study, it was found that although the positive expression rate of 1A6 protein in DGC was much lower than that of IGC, one third of them were positive too. These results indicate that as gastric carcinoma, IGC and DGC may share some common characteristics, although their biological characteristics and etiological molecular mechanism are very different. In 23 cases of sarcoma, the positive rate was 0 %, it is no doubt that 1A6 protein is epithelial-tumor-specific.

In this study, many positive cells showed both nuclear and plasmatic staining. There are three possibilities: (1) The synthesis of nuclear proteins takes place in cell plasma, and then transmit into the nucleus, the plasmatic positivity might reflect the newly synthesized 1A6 protein. (2) Non-specific binding of 1A6 Ab with other plasmatic proteins. (3) Non-specific secondary Ab binding with the proteins in the plasma.

In conclusion, 1A6 is a new tumor-related gene, its expression may play an important role in the early stage of gastric carcinogenesis. The detection of 1A6 protein in the premalignant lesions of intestinal gastric carcinoma may be a very valuable index to predict the malignant transformation of these lesions.

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