P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wcjd@public.bta.net.cn www.wjgnet.com

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• GASTRIC CANCER •

Expression of gastric cancer-associated MG7 antigen in gastric cancer, precancerous lesions and *H. pylori*-associated gastric diseases

Dong-Li Guo, Ming Dong, Lan Wang, Li-Ping Sun, Yuan Yuan

Dong-Li Guo, Ming Dong, Lan Wang, Li-Ping Sun, Yuan Yuan, Cancer Institute, First Affiliated Hospital, China Medical University, Shenyang, 110001, Liaoning Province, China
Supported by The National Basic Research Program (973) of China, No.G1998051203
Correspondence to: Dr. Yuan Yuan, No.3 gastric cancer laboratory, Cancer Institute, First Affiliated Hospital, China Medical University, 155 Northern Nanjing Street, Heping District, Shenyang 110001, Liaoning Province, China. yyuan@mail.cmu.edu.cn
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Abstract

AIM: To investigate the relationship between the antigen MG7 antigen expression and gastric cancer as well as precancerous condition; to study the relationship between the MG7 antigen expression and *H. pylori* infection in benign gastric lesions in order to find out the effect of *H. pylori* infection on the process of gastric cancer development.

METHODS: The level of MG7 antigen expression was determined by immunohistochemical method in 383 gastric biopsied materials. The intestinal metaplasia was determined by histochemistry method. The *H. pylori* infection was determined by HE stain, PCR and ELISA in 291 specimens, among which only 34 cases of *H. pylori*-associated gastric lesions were followed up.

RESULTS: The positive rate of MG7 expression in normal gastric mucosa, intestinal metaplasia, dysplasia and gastric cancer increased gradually in ascending order ($P<0.01$). The positive rate of MG7 antigen expression in type III intestinal metaplasia of gastric mucosa was higher than that of type I and II intestinal metaplasia, being highly significant ($P<0.05$). The positive rate of MG7 antigen expression in superficial gastritis, atrophic gastritis and gastric cancer increased gradually (11.9 %, 64.8 %, 91.2 %, $P<0.01$). There was no significant difference between *H .pylori*-negative and *H. pylori*-positive intestinal metaplasia, atrophic gastritis and dysplasia of gastric epithelium in the positive rate of MG7 antigen expression. There was no expression of MG7 antigen in *H. pylori*-negative superficial gastritis. The positive rate of MG7 expression in *H. pylori*-positive superficial gastritis was 20.5 %, and the difference between them was significant ($P<0.05$). During following up, one of the three *H. pylori* negative cases turned positive again, and its MG7 antigen expression turned to be stronger correspondingly. 3 of 31 *H. pylori* positive cases were detected as early gastric cancer, among which one with "+++" MG7 antigen expression was diminished after *H. pylori* eradication.

CONCLUSION: MG7 antigen expression is highly specific in gastric cancer and can be used as a good marker for screening of gastric cancer; type III intestinal metaplasia, atrophic gastritis and dysplasia should be followed up and MG7 antigen expression has high clinical value in the dynamic follow-up study; although the positive -MG7 in positive -*H. pylori* superficial gastritis show benign morphology in features, there is still the potential risk of developing into gastric cancer, hence special attention should be paid to those showing increasing MG7 antigen expression.

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INTRODUCTION

Gastric monoclonal antibody MG7 was first gotten by immunizing the BALB/C mice directly with poor-differentiated adenocarcinoma gastric cancer cell line MKN-46-9[1]. By immunohistochemistry method the MG7 antigen is distinguished limited in the gastric cancer tissue[2-5], which is specific and serves as a marker of gastric cancer. *Helicobacter pylori (H. pylori)* infection is established as a major cause of gastritis, peptic ulcer disease and gastric cancer[6-8]. The current study is to investigate the dynamic expression of MG7 antigen in different gastric mucosa, including the normal gastric tissue, *H. pylori*-associated gastric lesions as well as other precancerous conditions and gastric cancer, also to investigate the influence of *H. pylori* on MG7 antigen expression.

MATERIALS AND METHODS

Clinical data

The gastric cancer-associated antigen MG7 expression was studied in 383 gastric mucosal biopsied materials, including 26 normal gastric mucosa, 67 superficial gastritis, 21 gastric ulcer, 71 atrophic gastritis, 82 intestinal metaplasia, 59 dysplasia, and 57 gastric cancerous tissue, among which 29 were differentiated and 28 undifferentiated.

Reagents

The MoAb MG7 was gifted by Professor Fan Daiming in No. 4 China Military Medical University; The ABC kit was the product of American Vecter Company.

HID-ABpH2.5-PAS mucin histochemistry stain

82 IM were categorized into three types according to the morphology, degree of differentiation and mucin-protein secreted. These were stained with Alican blue pH 2.5/ periodic acid Schiff (AB/PAS) to visualize neutral mucin and some acidic mucins, and with high iron diamine/Alcian blue pH 2.5 (HID/AB) to identify sulphomucin and sialomucin[9]. Type I

(complete) was characterized by mature absorptive and goblet cells, the latter secreted sialomucin. Paneth's cells were often present. Type Ⅱ (incomplete) showed few or no absorptive cells, but with 'intermediate' columnar cells in various differentiated stages, secreting neutral and sialomucins, while Paneth's cells might not be present. In type III (incomplete), cell dedifferentiation was more obvious than that in type Ⅱ, with 'intermediate' cells secreting predominantly sulphomucin and goblet cells containing sialo- and/or sulphomucin. Paneth's cells were usually absent. A variable degree of disorganized architecture was often present in type III IM.

H. pylori examination

The *H. pylori* infection was detected by HE stain, PCR and ELISA. *H. pylori* was considered positive if two of the above three methods were positive. *H. pylori* could be found in the gastric epithelium or in the mucus by histological examination. Detection of *H. pylori* with *H. pylori*-DNA-PCR method followed the protocol of kit. The band in the same position as the positive control was defined as positive. When ELISA method was performed, the sample with OD value/ OD average value of negative controls ≥2.1 was defined as positive.

Immunohistochemistry stain (ABC method) of gastric cancer-associated antigen MG7

4 μm thick sections were cut from paraffin wax blocks, mounted on acid cleaned glass slides, and heated at 55 ℃ for 60 minutes. The slides were dewaxed and dehydrated, then the endogenous peroxidase activity was inhibited by incubation with 3 % H_2O_2 (20 minutes at room temperature). To reduce the non-specific background staining, the slides were incubated with 2 % horse serum (20 minutes at room temperature), then were incubated with MG7 antibody in a moist chamber at 4 ℃ overnight. The avidin-biotin-peroxidase complex procedure was then performed as described by ABC immunohistochemistry kit. Peroxidase activity was detected with diaminobenzidine as substrate. Finally, the sections were weakly counterstained with Harris's haematoxylin. Negative controls with PBS replacing specific primary antibodies were included in each run. Positive controls were cases of undifferentiated gastric cancer with MG7 expression. The sections were considered positively stained only when unequivocal cellular membrane and cytoplasm staining for MG7 were present. Diagnosis was made by brown coloration with varied intensities and the number of cells with brown coloration[10] . score 1: light brown; score 2: brown; score 3: deep brown. score 1: stained cells <30 %; score 2: stained cells 30-70 %; score 3: stained cells > 70 %. According to the sum of the two index, as that comprehensive scores were made. Comprehensive score 0 was defined as negatively expressed, comprehensive scores 2-4 were defined positively expressed, the cases and that above 4 was defined as over-expressed.

Statistical analysis

The results were analyzed by χ^2 test.

RESULTS

The expression of gastric cancer-associated MG7 antigen in different gastric mucosal tissues

The coloration in gastric cancerous tissue was often brown or deep brown, which was mainly located in the cellular membrane, cytoplasm and the glandular lumen, but not in the nucleus. The brown coloration was usually diffusely and non-polar distributed in the cytoplasm of cancer cells and might also be present in the luminal surface of the glands, sometimes it was located in some cancer cell nests or glands. The coloration in benign gastric lesions was often light brown, which was mainly located at the apex of the cytoplasm, the luminal surface membrane, none was seen in the cell nucleus. There were less positive cells in benign gastric lesions.

There was no expression of MG7 antigen in normal gastric mucosa. The positive rate of MG7 antigen expression in gastric cancer was 91.2 %. The level of MG7 antigen expression in undifferentiated gastric cancer was higher than that of differentiated gastric cancer (89.7 %, 92.3 %, $P>0.05$). From the viewpoint of histology, the positive rates of MG7 expression in normal gastric mucosa, metaplasia/dysplasia and gastric cancer increased gradually ($P<0.01$), see Table 1. From clinical viewpoint the positive rates of MG7 expression in superficial gastritis, atrophic gastritis and gastric cancer also increased gradually ($P<0.01$), see Table 2. The expression rates of MG7 antigen in the increasing order were normal gastric mucosa, superficial gastritis, intestinal metaplasia, atrophic gastritis, dysplasia and gastric cancer, there was significant difference between gastric cancer and other benign gastric lesion groups ($P<0.05$), see Table 1 and 2.

Table 1 The expression of MG7 antigen in various gastric lessions

Gastric lessions	*n*	No. of cases with MG7expression				Positive rate(%)	Over-expression rate(%)
		–	+	++	+++		
Normal gastric mucosa	26	26	0	0	0	0.0[b]	0.0[b]
Intestinal metaplasia	82	34	43	4	1	58.5[bd]	6.1[b]
Dysplasia	59	30	23	4	2	49.2[bd]	10.2[b]
Gastric cancer	57	5	23	18	11	91.2[d]	50.9[d]

[b]$P<0.01$ *vs*: compared with gastric cancer; [d]$P<0.01$ *vs*: compared with normal gastric mucosa

Table 2 The expression of MG7 antigen in different gastric diseases

Gastric lessions	*n*	No. of cases with MG7expression				Positive rate(%)	Over-expression rate(%)
		–	+	++	+++		
Superficial gastritis	67	59	7	0	1	11.9 [b]	1.5 [b]
Atrophic gastritis	71	25	39	7	0	64.8 [bd]	9.9 [b]
Gastric cancer	57	5	23	18	11	91.2 [d]	1 50.9 [d]

[b]$P<0.01$ *vs*: compared with gastric cancer; [d]$P<0.01$ *vs*: compared with superficial gastritis

The expression of gastric cancer-associated MG7 antigen in different types of intestinal metaplasia

According to different mucin secreted, the intestinal metaplasia of gastric mucosa could be categorized into three types. The positive rate of MG7 antigen expression in type III intestinal metaplasia of gastric mucosa was significantly different as compared with type I and type Ⅱ intestinal metaplasia ($P<0.05$), but was close to gastric cancer ($P>0.05$). The over-expression rate of MG7 antigen in type I, type Ⅱ, type III intestinal metaplasia and gastric cancer increased gradually, and there was significant difference between gastric cancer group and other groups respectively ($P<0.05$), see Table 3.

Table 3 The expression of MG7 in different types of intestinal metaplasia and gastric cancer

Types of intestinal metaplasia	*n*	No. of cases with MG7expression				Positive rate(%)	Over-expression rate(%)
		–	+	++	+++		
type Ⅰ and Ⅱ intestinal metaplasia	53	28	24	1	0	47.2[bb]	1.9[b]
type Ⅲ intestinal metaplasia	29	6	19	3	1	79.3[dd]	13.8[b]
gastric cancer	57	5	23	18	11	91.2[dd]	50.9[d]

[b]$P<0.01$ *vs* compared with gastric cancer; [bb]$P<0.05$ *vs* compared with gastric cancer; [d]$P<0.01$ *vs* compared with superficial gastritis; [dd]$P<0.05$ *vs* compared with superficial gastritis

The expression of MG7 antigen in H. pylori-associated gastric diseases

On examination, *H. pylori* infection was detected in 291 specimens of different gastric mucosa tissues including 66 superficial gastritis, 20 gastric ulcer, 70 atrophic gastritis, 80 intestinal metaplasia and 55 dysplasia. The MG7 antigen expression was also examined in *H. pylori*-positive and *H. pylori*-negative groups of different gastric diseases. It was found that the positive rates of MG7 antigen expression in *H. pylori*-positive and *H. pylori*-negative cases of superficial gastritis, gastric ulcer, atrophic gastritis, intestinal metaplasia and dysplasia were 20.5 %/0, 25 %/12.5 %, 62.5 %/65.8 %, 52 %/66.7 %, 30.3 %/54.5 %, respectively. The positive rate of MG7 expression in *H. pylori*-positive superficial gastritis (20.5 %) was significantly higher than that in *H. pylori*-negative superficial gastritis ones (0), ($P<0.05$). There was no significant differences between *H. pylori*-negative and *H. pylori*-positive atrophic gastritis, intestinal metaplasia and dysplasia of gastric epithelium in positive rates of MG7 antigen expression, see table4. In the MG7 antigen expression positive ones, positive-*H. pylori* in superficial gastritis, intestinal metaplasia, atrophic gastritis and dysplasia of gastric epithelium was counted as 8/8, 26/46, 20/45, 10/22 respectively. Among the 8 superficial gastritis with MG7 expression, the rate of *H. pylori* infection was 100 %.

Table 4 The expression of MG7 antigen in *H. pylori*-associated gastric lesions

Gastric lesions	*H. pylori*-positive			*H. pylori*-negative		
	n	MG7 expression		No.of cases	MG7 expression	
		No. of cases	rate (%)		No.of cases	rate (%)
superficial gastritis	39	8	20.5	27	0	a
gastric ulcer	12	3	25	8	1	12.5
atrophic gastritis	32	20	62.5	38	25	65.8
intestinal metaplasia	50	26	52.0	30	20	66.7
dysplasia	33	10	30.3	22	12	54.5

[a]$P<0.05$ *vs* compared with *H. pylori*-positive cases of the same gastric lesion

The follow-up of cases with H. pylori-associated gastric diseases

34 cases were followed up for 2 years, among which 3 cases without *H. pylori* infection and 31 cases with *H.pylori* infection. There were 19 with negative MG7 antigen expression (-); 13 with weakly positive MG7 antigen expression (+); (++) and (+++) each. One year later, among the 3 without *H. pylori* infection, 1 case was found newly *H. pylori* infected accompanied by increased MG7 expression. Among 31 cases of *H. pylori*-positive diseases, early gastric cancer was detected in 3 with MG7 antigen expression, of which one with weakly positive MG7 antigen expression (+) was atrophic gastritis, one with MG7 antigen expression (++) was also atrophic gastritis but one case with MG7 antigen expression (+++) was superficial gastritis, see Table 5. After surgical operation and drug treatment, the reduced MG7 expression with *H. pylori* eradication was found in a case of superficial gastritis.

Table 5 The follow-up results of 34 cases with *H. pylori*-associated gastric diseases

Change of *H. pylori* status	No.of cases	MG7 antigen expression			
		mitigated	unchanged	aggravated	malignant change
H. pylori infection unchanged	19	4	13	1	1
H. pylori eradicated	14	4	7	1	2
H. pylori newly infected	1	0	0	1	0

DISCUSSION

Gastric cancer is still a major health problem and the leading cause of cancer mortality despite a worldwide decline in incidence. Early detection and early diagnosis are important in prevention and treatment of gastric cancer. The antigen recognized by gastric monoclonal antibody MG7[1] is different from other gastrointestinal tumor markers as reported[11,12]. It has been taken as a promising index in gastric cancer screening because of its specificity[1-4,13,14]. In this study, we found that the expression of MG7 antigen in different gastric tissue was different. The positive rate of MG7 antigen expression in gastric cancer is 91.2 %.The positive rate of MG7 expression in normal gastric mucosa, metaplasia/dysplasia and gastric cancer increased in ascending order ($P<0.01$). The positive rate of MG7 expression in superficial gastritis, atrophic gastritis and gastric cancer increased in succession ($P<0.01$). The expression rate of MG7 antigen in undifferentiated gastric cancer was higher than that of differentiated gastric cancer ($P>0.05$), cases with positive expression cells are more above 70 % in undifferentiated gastric cancer than that in differentiated gastric cancer ($P<0.05$), showing the presence of somewhat tendency of certain histopathologic type. The dynamic changes in expression of MG7 antigen in normal gastric mucosa, precancerous lesions and gastric cancer implicated that in the gastric precancerous conditions, MG7 antigen increased gradually with the development and progression of gastric cancer and its sensitivity was higher in gastric cancer, and could be used as a marker for screening.

This study showed that among the benign gastric lesions the positive rate of MG7 expression in atrophic gastritis and dysplasia were significantly higher than that in superficial gastritis but significantly lower than that in gastric cancer, implicating there were more gastric cancer-associated antigens in the cell membrane and cytoplasm of atrophic gastritis and dysplasia which was in access to gastric cancer. Intestinal metaplasia is taken as gastric precancerous lesion[16,17]. It was found that type Ⅲ (incomplete) intestinal metaplasia had cancerous potential, our study demonstrated the positive rate of MG7 antigen expression in type Ⅲ intestinal metaplasia of gastric mucosa was significantly different compared with type Ⅰ and type Ⅱ intestinal metaplasia ($P<0.05$), and was

analogous, even more closed to gastric cancer (P<0.05). Since the dynamic changes in of MG7 antigen expression was closely related with the development and progression of gastric cancer, cases with atrophic gastritis, dysplasia and type III intestinal metaplasia should be closely followed up for the early detection of gastric cancer.

The development of gastric cancer is a multistep process that is multifactorial. Several factors may act in stages of development of cancer[9]. Epidemiology data show the close relationship between *H. pylori* and gastric cancer[18,19]. *H. pylori* has been assigned as a class I carcinogen by WHO, and acts as the initiating agent. It virulence factors can damage gastric epithelial cells[20-22], break the balance between proliferation and apoptosis[23-33]. but it is still unclear weather *H. pylori* plays a role after development of atrophic gastritis and intestinal metaplasia[34,35].

Our study found that the positive rate of MG7 expression in *H. pylori*-positive superficial gastritis was higher than that in *H. pylori*-negative cases (P<0.05). There were 8 cases of positive *H. pylori* superficial gastritis with MG7 antigen expression, which suggested that *H. pylori* infection was directly stated to MG7 antigen expression. But there was no significant difference between *H. pylori*-negative and *H. pylori*-positive atrophic gastritis, intestinal metaplasia and dysplasia in the positive rate of MG7 antigen expression. This might be due to the change of the environment in atrophic gastritis, intestinal metaplasia and dysplasia, which was unsuitable for the growth of *H. pylori.*

By following up of the 34 cases of *H. pylori*-associated disease, we detected early gastric cancer in 3 *H. pylori*-positive cases with MG7 antigen expression, (2 cases of atrophic gastritis, one case of superficial gastritis). After surgical operation and drug treatment with *H. pylori* eradication reduced MG7 expression was found in the case of superficial gastritis. Among the 3 cases without *H. pylori* infections, 1 case had newly emerged *H. pylori* infection accompanied by increased MG7 expression. These implicated the close relationship between the *H. pylori* infection and the expression of MG7 antigen in gastric mucosa, although some *H. pylori*-positive gastric lesions with MG7 antigen expression showed benign morphology, there is still the potential risk of developing into gastric cancer, hence follow up study is essential; more attention should be paidto those with increased MG7 antigen expression.

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