

Maspin expression and its clinicopathological significance in tumorigenesis and progression of gastric cancer

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Abstract

AIM: To investigate maspin expression in tumorigenesis and progression of gastric cancer and to explore its relevant molecular mechanisms.

METHODS: Formalin-fixed and paraffin-embedded tissues from normal mucosa ($n=182$), dysplasia ($n=69$), cancer ($n=113$) of the stomach were studied for maspin expression by immunohistochemistry. Microvessel density (MVD) in gastric cancer was labeled using anti-CD34 antibody. Maspin expression was compared with clinical parameters and MVD of tumors. Caspase-3 expression was also detected in gastric carcinoma by immunohistochemistry. The relationship between Caspase-3 and maspin expression was concerned as well.

RESULTS: The positive rates of maspin expression were 79.8% (145/182), 75.4% (52/69) and 50.4% (57/113) in normal mucosa, dysplasia and cancer of the stomach, respectively. Cancer less frequently expressed maspin than normal mucosa and dysplasia ($P<0.05$). Maspin expression showed a significantly negative association with invasive depth, metastasis, Lauren's and Nakamura's classification ($P<0.05$), but not with tumor size, Borrmann's classification, growth pattern or TNM staging ($P>0.05$). The positive rate of Caspase-3 was significantly lower in gastric cancer than in normal gastric mucosa ($P<0.05$, 32.7% vs 50.4%). It was noteworthy that maspin expression was negatively correlated with MVD, but positively correlated with expression of Caspase-3 in gastric cancer ($P<0.05$).

CONCLUSION: Down-regulated maspin expression is a late molecular event in gastric carcinogenesis. Reduced expression of maspin contributes to progression of gastric cancer probably by inhibiting cell adhesion, enhancing cell mobility, decreasing cell apoptosis and facilitating angiogenesis. Additionally altered expression of maspin underlies the molecular mechanism of differentiation of gastric cancer and supports the different histogenetic pathways of intestinal and diffuse gastric cancers. Maspin expression can be considered as an effective and objective marker to reveal biological behaviors of gastric cancer.

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INTRODUCTION

Mammary serine protease inhibitor (maspin) was identified by subtractive hybridization as a candidate tumor suppressor protein in normal mammary epithelial cells^[1]. Maspin gene maps to human chromosome 18q21.3-q23, whose cDNA consists of 2 584 nucleotides encoding for a 42 ku peptide^[2]. A number of findings support its inhibitory effects on tumors. Levels of maspin expression showed an inverse correlation with progression of malignancies. Mammary carcinoma cells transfected with maspin showed a reduction of tumor growth and metastasis in nude mice, the addition of recombinant maspin decreased the migration potential of breast and prostate cancer cells across a reconstituted basement membrane. More recently, maspin has been shown to inhibit angiogenesis by blocking *in vitro* migration of vascular endothelial cells and by *in vivo* inhibition of rat cornea neovascularization^[1-8]. It has been documented that maspin transgene expression in mouse mammary gland inhibited Simian virus 40 (SV40) large T-antigen induced breast carcinogenesis and was correlated with increased apoptosis of mammary gland cells^[9].

Gastric cancer is one of the commonest malignancies in China, and even in the world. However, the molecular aspects of carcinogenesis and progression of gastric cancer remain elusive^[10-19]. The purposes of this study were to examine maspin expression in normal gastric mucosa, gastric dysplasia, gastric cancer, and to compare its expression with clinicopathological features of gastric cancer, and to analyze the correlation of maspin expression with Caspase-3 expression and MVD in gastric cancer.

MATERIALS AND METHODS

Patients and samples

Normal mucosa ($n=182$), dysplasia ($n=69$), cancer ($n=113$) of the stomach were collected from the Second Affiliated Hospital of China Medical University between Sept. 1996 and Feb. 2002. All tissues were fixed in 40 g/L formaldehyde, embedded in paraffin and incised into 4 μ m sections. These sections were stained by hematoxylin-and-eosin method to confirm their histological diagnosis and other microscopic characteristics. All patients did not undergo chemotherapy and radiotherapy before operation.

Evaluation of clinicopathological parameters of gastric cancer

Clinicopathological staging for each gastric carcinoma was evaluated according to the TNM system. Gross appearance of the tumors was described according to Borrmann's classification. Histomorphological architecture of the tumor samples was expressed according to Lauren's and Nakamura's classifications. Growth patterns of gastric cancer were classified into mass-,

nest-, or diffuse- type. Furthermore, tumor diameter, invasive depth and metastasis were determined.

Immunohistochemistry

Representative and consecutive sections were studied with streptavidin-biotin-peroxidase immunohistochemistry (SABC kit from Boster Biotech.). Anti-maspin, anti-Caspase-3 and anti-CD34 antibodies were purchased from Novocastra, Zhongshan (China) and DAKO respectively. All procedures were implemented according to the instructions of the product. For negative controls, sections were processed as above but treated with PBS (0.01 mol/L, pH7.4) instead of primary antibodies.

Evaluation of maspin and Caspase-3 immunostaining

The immunoreactivity to maspin and Caspase-3 was localized in cytoplasm. One hundred cells were selected and counted from 5 representative fields of each section by two independent observers. The positive percentage of counted cells was graded semi-quantitatively into one of the four-tier scoring system: negative(-), 5%; weakly positive (+), 5-25%; moderately positive(++), 25-50%; and strongly positive(+++), 50%.

Microvessel density counting

CD34 antibodies were distributed in cell membranes and cytoplasm of vascular endothelial cells. Modified Weidner's method was used to calculate the microvessel density, which was described as follows. Microvessels in tumor were considered as hot points in vessel counts. Any brown staining endothelial cell or endothelial cell cluster was regarded as a single, countable microvessel. Observers selected such five areas and counted individual microvessels at 400 \times magnification (*i.e.* 40 \times objective lens and 10 \times ocular lens, 0.1885 mm² per field).

Statistical analysis

Statistical evaluation was performed using *chi*-square test to differentiate the rates between different groups, using Spearman correlation test to analyze the rank data, and using one-way ANOVA to differentiate means of different groups. $P < 0.05$ was considered as statistically significant. SPSS 10.0 software was employed to analyze all data.

RESULTS

Maspin expression in normal mucosa, dysplasia, and cancer of the stomach

Figures 1A-D show the positive immunoreactivity to maspin in cytoplasm of epithelial, dysplasia and cancer cells of the stomach. As summarized in Table 1, the positive rates of maspin expression were 79.8% (145/182), 75.4% (52/69), and 50.4% (57/113) in normal mucosa, dysplasia and cancer of gastric cancer, respectively. Normal mucosa and dysplasia more frequently expressed maspin than cancer ($P < 0.05$).

Table 1 Maspin expression in normal mucosa, dysplasia, and primary cancer of the stomach

Groups	n	Maspin expression			
		-	++	+++	%
Normal mucosa	182	37	145		79.8 ^b
Dysplasia	69	17	52		75.4 ^d
Primary cancer	113	56	57		50.4

Compared with primary cancer ^b $P = 0.000$ ($\chi^2 = 27.589$, Pearson' $r = 0.306$), ^d $P = 0.000$ ($\chi^2 = 11.075$, Pearson' $r = 0.247$).

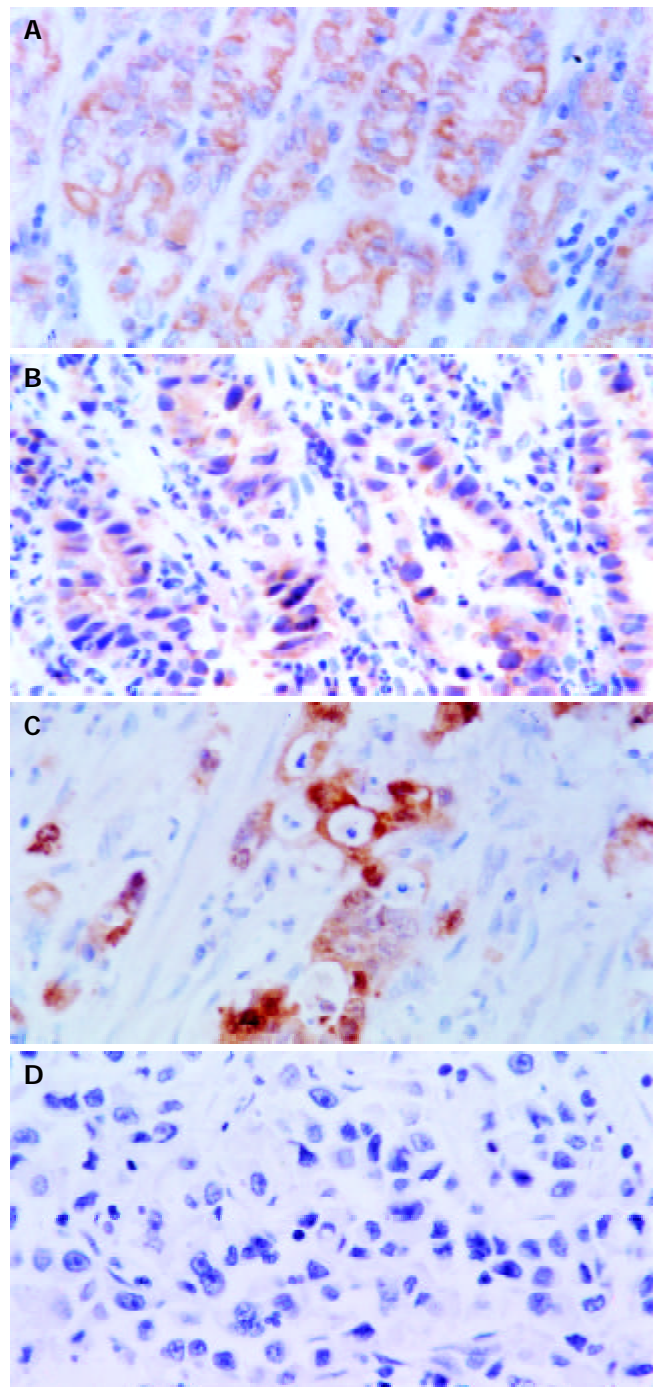


Figure 1 A: Localization of maspin in cytoplasm, and strong expression in gastric epithelial cells (SABC, $\times 400$), B: Strong immunoreactivity of gastric dysplastic cells to maspin (SABC, $\times 400$), C: Strong immunostaining of maspin in gastric papillary adenocarcinoma cells (SABC, $\times 400$), D: Negative expression of maspin in poorly-differentiated gastric adenocarcinoma cells (SABC, $\times 400$).

Relationship between maspin expression and clinicopathological features of gastric cancer

Table 2 shows that maspin expression had a significantly negative association with invasive depth, metastasis, Lauren's and histological classifications ($P < 0.05$), but not with tumor size, Borrmann's classification, growth pattern or TNM staging ($P > 0.05$).

Relationship between maspin and Caspase-3 expression, MVD in gastric cancer

The positive rate of Caspase-3 expression was lower in gastric

cancer than in normal gastric mucosa ($P < 0.05$, 32.7% vs 50.4%). It was noticeable that maspin expression was positively correlated with Caspase-3 expression, but negatively with MVD in gastric cancer ($P < 0.05$) (Tables 2-3, Figures 2,3).

Table 2 Relationship between maspin expression and clinicopathological features of gastric cancer

Clinicopathological features	n	Maspin expression					rs	P value
		-	+	++	+++	%		
Tumor size							0.000	0.999
<4 cm	47	22	13	7	5	53.2		
≥4 cm	66	34	11	15	6	48.5		
Borrmann's classification							0.014	0.896
I, II	28	12	9	5	2	57.1		
III, IV	59	30	10	13	6	49.2		
Invasive depth							0.280	0.003
Above submucosa	26	10	4	6	6	61.5		
Muscularis propria	34	12	11	7	4	64.7		
Below subserosa	53	34	9	9	1	35.8		
Metastasis							0.208	0.027
-	75	31	18	19	7	58.7		
+	38	25	6	3	4	34.2		
TNM staging							0.004	0.967
O, I	46	22	11	10	3	52.2		
II, III, IV	67	34	13	12	8	49.3		
Growth pattern							0.032	0.767
Mass	23	13	4	5	1	43.5		
Nest	30	11	6	8	5	63.3		
Diffuse	34	18	9	5	2	47.1		
Lauren's classification							0.228	0.015
Intestinal	36	12	8	11	5	66.7		
Diffuse	57	33	12	8	4	42.1		
Mixed	20	11	4	3	2	45.0		
Nakamura's classification							0.212	0.024
Differentiated	53	20	14	12	7	62.3		
Undifferentiated	60	36	10	1	4	10.0		
Caspase-3 expression							0.246	0.009
-	78	44	17	12	5	56.4		
+~+++	35	12	7	10	6	65.7		

Table 3 Relationship between maspin expression and MVD in primary gastric cancer

Maspin expression	n	MVD (mean±SD)	F value	P value
-	56	51.72±26.87	4.911	0.029
+~+++	57	41.70±20.92		
Total	113	46.67±24.47		

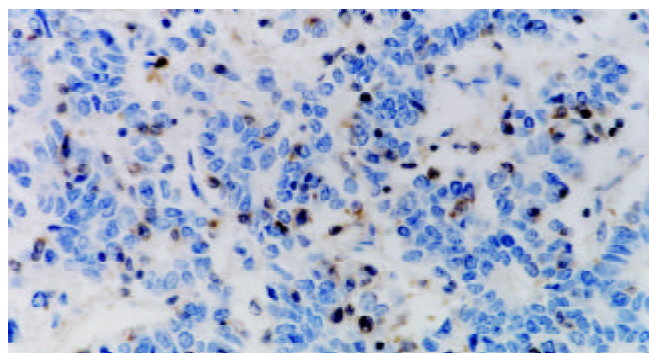


Figure 2 Negatively immunostaining of Caspase-3 in poorly-differentiated gastric adenocarcinoma cells and positive expression in infiltrating lymphocytes (SABC, ×400).

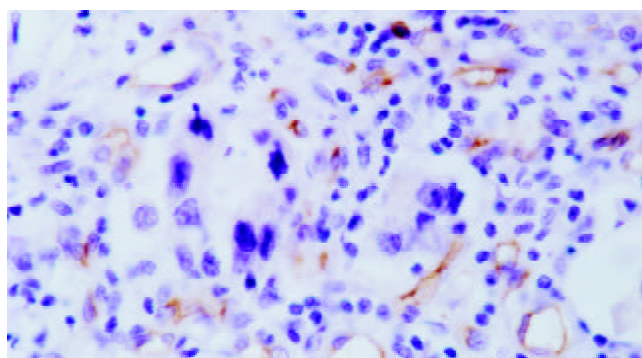


Figure 3 Localization of CD34 antigens in cell membranes and cytoplasm of vascular endothelial cells (SABC, ×400).

DISCUSSION

Carcinogenesis and progression of malignancies are a complicated multistage process that requires the coordination of multiple genes, including oncogenes and tumor suppressor genes. Genomic instability is one of the driving forces for tumor development. Among all genetic alterations, inactivation of metastasis suppressor genes has been found to be an important factor because of their contribution to malignant change of normal cells and metastasis of tumor cells^[20,21].

It is known that maspin is highly expressed in various kinds of normal cells and lowly expressed in cancer cells. Our data showed that maspin expression was gradually reduced from normal mucosa, through dysplasia to cancer of the stomach. The positive rate of maspin expression was lower in gastric cancer than in normal gastric mucosa and gastric dysplasia with no significant difference in the latter two. These results suggested that reduced maspin expression contributed to malignant change of gastric epithelial cells. The down-regulated expression of maspin could be considered as a late molecular event of gastric carcinogenesis. Previous reports indicated that DNA methylation, histone deacetylation or LOH was partially responsible for the silencing of maspin gene expression^[22-24]. Decreased expression of maspin might be attributable to these genetic alterations in gastric carcinogenesis as mentioned above.

Our study showed that maspin expression was negatively associated with invasion and metastasis of tumors, suggesting its inhibitory effects on progression of gastric cancer. Biliran *et al*^[25] found that maspin could specifically inhibit cell surface-associated urokinase-type plasminogen activator and fibrinogen-bound tissue-type plasminogen activator, which was correlated with significantly decreased cell invasion potential and motility *in vitro*. Blacque *et al*^[26] reported that interaction between recombinant maspin and some collagens might contribute to cell adhesion, cell migration and angiogenesis. Seftor *et al*^[27] demonstrated that maspin was able to regulate integrin expression, indicating that maspin could reduce the invasive phenotype of cancer cells by altering their integrin profile. These findings suggested that maspin expression could inhibit tumor progression *in vivo*, likely through a combination of increased cell adhesion, decreased angiogenesis, and inhibition of tumor cell migration.

Additionally, it was found that undifferentiated gastric carcinomas had a lower expression of maspin as compared with the differentiated ones, suggesting that down-regulated expression of maspin was closely associated with the differentiation of gastric cancer. Diffuse-type gastric cancers had less expression of maspin as compared with the intestinal-type ones. It supported that there were different tumorigenic pathways between diffuse-type and intestinal-type gastric carcinomas. Diffuse-type gastric cancer, the main part of which was undifferentiated

carcinoma, displayed a diffusely invasive growth pattern. It is possible that down-regulation of maspin expression could influence mobility and adhesion of cancer cells.

Our study also showed a negative correlation between maspin expression and MVD in gastric cancer. Song *et al*^[28] found that maspin-positive colonic adenocarcinomas showed less MVD than maspin-negative ones. Zhang *et al*^[8] reported that maspin might act directly on vascular endothelial cells to stop their migration towards basic fibroblast growth factor and vascular endothelial growth factor and to limit mitogenesis and tube formation, which could dramatically reduce tumor-associated MVD. These *in vivo* and *in vitro* data suggest that the tumor suppressor activity of maspin may depend in large part on its ability to inhibit angiogenesis and raise the possibility that maspin and similar serpins may be excellent targets for the development of drugs that modulate angiogenesis.

Furthermore, it was found that Caspase-3 expression was increased in gastric cancer with maspin positively expressed. Jiang *et al*^[29] reported that endogenous maspin expression could enhance staurosporine-induced apoptosis of carcinoma cells as judged by the increased fragmentation of DNA, increased proteolytic inactivation of poly-[ADP-ribose]-polymerase, as well as the increased activation of Caspase-8 and Caspase-3. Li *et al*^[30] showed that apoptosis induced by manganese-containing superoxide dismutase was associated with elevated maspin expression level. These results indicated that it was possible that maspin expression might inhibit progression of gastric cancer by inducing apoptosis.

In summary, down-regulated maspin expression is a late molecular event in gastric carcinogenesis. Reduced expression of maspin can contribute to progression of gastric cancer by inhibiting cell adhesion, enhancing cell mobility, decreasing cell apoptosis and facilitating angiogenesis. Additionally altered expression of maspin underlies the molecular mechanism of differentiation of gastric cancer and supports the different histogenetic pathways of intestinal and diffuse gastric cancers. Maspin expression can be considered as an effective marker to reveal biological behaviors of gastric cancer.

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