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• GASTRIC CANCER •

Pathobiological significance of vascular endothelial growth factor and Maspin expressions in human gastric carcinoma

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Abstract

AIM: To investigate the correlation between expression of vascular endothelial growth factor (VEGF) and cell differentiation, invasion, metastasis and Maspin expression in gastric carcinoma.

METHODS: Formalin-fixed paraffin-embedded tissue specimens from 73 cases of gastric carcinoma were studied with SP immunohistochemistry, using anti-VEGF monoclonal antibody, and thirty-nine of them were studied using anti-Maspin monoclonal antibody. VEGF expression was compared with the clinical stage, lymph node metastasis, and Borrmann's and WHO's classification of gastric carcinoma.

RESULTS: The positive rate of VEGF expression was significantly higher in adjacent non-carcinoma epithelia (ANCE) than in non-metaplastic, non-carcinoma gastric epithelia (NMNCE), which were at least 4 cm distant from the primary tumor ($P = 0.000$, $\chi^2 = 73.03$). The positive rate of VEGF expression was significantly higher in advanced gastric carcinoma (AGC) than in early gastric carcinoma (EGC) ($P = 0.032$, $\chi^2 = 4.62$). The positive rate of VEGF expression in gastric carcinomas with lymph node metastases was significantly higher than that in those without metastasis ($P = 0.006$, $\chi^2 = 7.47$). Maspin was weakly expressed in 16 out of 39 cases of NMNCE, and the positive immunoreaction was limited to gland cells of the stomach body. There was no significant correlation between the expression of VEGF and histological or gross classifications, and correlation between the expressions of VEGF and Maspin in gastric carcinoma ($P = 0.648$, $\chi^2 = 0.21$).

CONCLUSION: Expression of VEGF is significantly correlated to the malignant biological behaviors of gastric carcinoma, but there is no significant correlation between the expression of VEGF and Maspin.

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INTRODUCTION

Tumor angiogenesis is one of the most important biological features. It has been shown that tumor angiogenesis plays an important role in its growth, invasion, metastasis and recurrence[1-3]. Among the factors contributing to angiogenesis, VEGF is recognized as one of the most important molecules in the formation of new blood vessels. There is clinical and experimental evidence that VEGF plays a role in the progression of solid tumors, and its clinical significance in solid tumors has been demonstrated both immunohistochemically and quantitatively[4]. Many studies demonstrated that over-expression of VEGF participated the growth and metastasis of malignant tumors depended on angiogenesis[2,5]. VEGF increased the incidence rate of tumor metastasis by inducing tumor angiogenesis[5]. Some studies demonstrated that the level of VEGF expression was of prognostic value in predicting metastasis of various malignant solid tumors and the level of VEGF expression correlated with tumor progression in human brain cancers and experimental tumor models[6,7]. Hence, most studies in the field have focused on the regulation and inhibition of angiogenesis. The tumor suppressor gene Maspin, a unique member of the serpin super family, could inhibit cell motility, invasion, and metastasis in some cancers[8-10]. Although at present the molecular and biological mechanisms of the function of Maspin remain unknown, there is evidence that Maspin interacts with the p53 tumor suppressor pathway and may function as an inhibitor of angiogenesis *in vitro* and *in vivo*[11]. Pemberton *et al*.[12] demonstrated the presence of Maspin in epithelia of several normal human organs (such as prostate, thymus, testis, small intestine, and colon). We are interested whether the tumor suppression function of Maspin in mammary or pancreatic carcinoma can be also detected in gastric carcinoma.

In this study, VEGF expression was immunohistochemically investigated in non-metaplastic, non-carcinoma gastric epithelia (NMNCE), which were at least 4 cm distant from the primary tumor, adjacent non-carcinoma epithelia (ANCE) and gastric carcinoma, and compared with the pathobiological behaviors of gastric carcinoma in order to clarify the clinical and pathobiological significance of the expression of VEGF. The relationship between the expressions of VEGF and Maspin was also explored.

MATERIALS AND METHODS

Tissue specimens

Seventy-three surgically removed specimens of gastric carcinoma were collected from Cancer Institute, China Medical University. The age of patients ranged from 32 to 80 years, mean age was 55.2 years; Forty-eight were males and 25 females. Carcinomas were staged according to pathological characteristics including depth of tumor invasion, tumor location, Borrmann's classification, and status of lymph node metastasis. According to clinical staging, 24 cases were in early stage (early gastric carcinoma, EGC), 49 cases in advanced stage (advanced gastric carcinoma, AGC). According to metastasis status, 40 cases had lymph node metastasis, of them 1 had ovary metastasis, 1 had liver metastasis (without lymph node metastasis), and 32 had not any metastasis. Seventy-three cases of gastric carcinoma were

studied with SP immunohistochemistry, using anti-VEGF monoclonal antibody, and thirty-nine of them were studied using anti-Maspin monoclonal antibody. Each specimen was classified according to the Borrmann's classification and WHO's histological classification criteria.

Immunohistochemistry

All specimens were fixed in 40 g/L formaldehyde solution and embedded in paraffin. Five μm Sections were cut and mounted onto glass slides. Mouse anti-human monoclonal antibody against VEGF (ready to use) was from Maixin Biotech (Fuzhou, China) and mouse anti-human monoclonal antibody against Maspin was from Novo Castro (Newcastle, England). Immunohistochemical staining was performed using SP method. For control, sections were proceeded with PBS (0.01 mol/L, pH 7.4) instead of the primary antibodies. Counterstaining was performed with haematoxylin.

Evaluation of VEGF and Maspin expression

Clearly brown staining restricted to cytoplasm was considered as positive reaction for VEGF or Maspin. Two experienced pathologists assessed the positive rate according to the percent of positive cells in counted cells from 5 randomly selected representative fields. To evaluate the expression of VEGF and Maspin, immunostaining was classified into two groups, corresponding to the percentage of immunoreactive cells. The cut-off point to distinguish negative from positive VEGF or Maspin expression was 20% of positive cells.

Statistical analysis

Statistical evaluation was performed by χ^2-test to differentiate the rates between two groups. $P<0.05$ was considered statistically significant.

RESULTS

None of NMNCE expressed VEGF. VEGF expression was significantly higher in ANCE than in NMNCE ($P = 0.000$, $\chi^2 = 73.03$) (Table 1). Immunohistochemically, VEGF expression was significantly higher in AGC than in EGC ($P = 0.032$, $\chi^2 = 4.62$). There was no correlation between expression of VEGF and histology typing or gross typing (Table 2). VEGF expression in gastric carcinoma with lymph node metastases was significantly higher than that in those without metastasis ($P = 0.006$, $\chi^2 = 7.47$) (Table 3). Sixteen (41.0%) out of thirty-nine cases of NMNCE showed a weak Maspin expression that was limited to gland cells of the stomach body, while all gastric normal epithelia with intestinal metaplasia (GNEIM) strongly expressed Maspin (14/14) (Table 4). The positive rate of Maspin was 53.6% (15/28) in specimens of positive VEGF expression, whereas the positive rate of Maspin was 45.5% (5/11) in specimens of negative VEGF expression (Table5). There was no significant correlation between the expressions of VEGF and Maspin in gastric carcinoma ($P = 0.648$, $\chi^2 = 0.21$) (Table5).

Table 1 VEGF expression in NMNCE, ANCE and gastric carcinoma ($n = 73$)

Tissue origin	*n*	VEGF expression		Positive rate (%)
		-	+	
NMNCE	73	73	0	-
ANCE	73	23	50	68.5[b]
Gastric carcinoma	73	14	59	80.8[1]

[b]$P = 0.000$ *vs* NMNCE (Yates corrected: $\chi^2 = 73.03$), [1]$P = 0.086$ *vs* ANCE (Yates corrected: $\chi^2 = 2.93$).

Table 2 Relationship between VEGF expression and gross and histological types of gastric carcinoma ($n = 73$)

Type	*n*	VEGF expression		Positive rate (%)
		-	+	
Gross types				
EGC[a]	24	8	16	66.7
I	4	0	4	100.0
II	12	6	6	50.0
III	7	2	5	71.4
SS[1]	1	0	1	100.0
AGC	49	6	43	87.8
Bor. 0	3	0	3	100.0
Bor. I	1	0	1	100.0
Bor. II	6	0	6	100.0
Bor. III	36	5	31	86.1
Bor. IV	3	1	2	66.7
Histological type				
Papillary adenocarcinoma	8	1	7	87.5
Well-differentiated adenocarcinoma	3	2	1	33.3
Moderately-differentiated adenocarcinoma	11	3	8	72.7
Poorly-differentiated adenocarcinoma	30	5	25	83.3
Undifferentiated carcinoma	3	1	2	66.7
Signet-ring cell carcinoma	10	1	9	90.0
Mucinous adenocarcinoma	7	0	7	100.0
Carcinoid	1	1	0	-

[a]$P = 0.032$ *vs* AGC ($\chi^2 = 4.62$), There was no correlation between the expression of VEGF and histology typing or gross typing ($P>0.05$).
[1]EGC SS (early gastric carcinomas of superficial spreading type).

Table 4 VEGF and Maspin expressions in NMNCE, GNEIM and gastric carcinoma

Histological type	*n*	VEGF		+ %	Maspin		+ %
		+	-		+	-	
NMNCE	39	0	39	0	16[a]	23	41.0
GNEIM	14	-	-	-	14	0	100.0
Gastric carcinoma							
Papillary adenocarcinoma	3	3	0	100.0	2	1	66.7
Well-differentiated adenocarcinoma	3	2	1	66.7	2	1	66.7
Moderately-differentiated adenocarcinoma	6	5	1	83.3	2	4	33.3
Poorly-differentiated adenocarcinoma	21	14	7	66.7	11	10	52.4
Undifferentiated adenocarcinoma	3	2	1	66.7	1	2	33.3
Signet ring-cell carcinoma	3	2	1	66.7	2	1	66.7
Total of gastric carcinoma	39	28	11	71.8	20	19	51.3

Maspin was weakly expressed in gland cells of the stomach body, while it was not expressed in superficial epithelial cells and pyloric gland of the stomach.

Table 3 Relationship between VEGF expression and metastasis of gastric carcinoma ($n = 73$)

Metastasis status	*n*	VEGF expression		Positive rate (%)
		-	+	
No metastasis	32	12	20	62.5[b]
Lymph node metastasis	39	4	35	89.7
Liver metastasis	1	1	0	0
Ovary metastasis	1	0	1	100.0

[b]$P = 0.006$ *vs* lymph node metastasis ($\chi^2 = 7.47$).

Table 5 Relationship between VEGF and Maspin expressions in gastric carcinoma

Gastric carcinoma	Maspin +	Maspin -	Total
VEGF +	15	13	28
VEGF -	5	6	11
Total	20	19	39

There was no significant correlation between the expression of VEGF and Maspin in gastric carcinoma ($P = 0.648$, $\chi^2 = 0.21$).

DISCUSSION

Ferrara[13] and his colleagues found that bovine pituitary follicular cells secreted a novel heparin-binding growth factor specific for vascular endothelial cells in 1989 and named it VEGF. VEGF is known to be a highly specific mitogen for endothelial cells which is almost specifically expressed in endothelial cells. VEGF might act as an autocrine and paracrine growth factor to induce the proliferation of tumor cells as well as tumor angiogenesis of tumor cells[14].

Tumors require blood vessels for nutrient and oxygen supply to maintain their viability. In the first stage of growth, cloning proliferative phase does not need angiogenesis. To continue tumor expansion, additional blood supply was prerequisite, which was significantly correlated to tumor invasion, metastasis and recurrence[15-18]. It has been widely accepted that tumor angiogenesis was one of the most crucial steps in tumor invasion and metastasis. There was a close relationship between VEGF expression and depth of invasion, lymph node metastasis and five-year survival rate of patients, which was an independent prognostic factor. Our study showed that there was no significant relationship between VEGF expression and histological or gross types of gastric carcinomas.

Yonemura further demonstrated the correlation between VEGF-C expression and lymphatic invasion or lymph node metastasis[19]. Tumors with high expression of VEGF-C had more remote lymph node involvement than those with low VEGF-C expression[7,19,20]. These results strongly suggested that cancer cells producing VEGF-C might induce proliferation and dilation of lymphatic vessels, resulting in the development of invasion of cancer cells into lymphatic vessels and lymph nodes. These results were consistent with recent reports that showed a positive correlation of VEGF-C levels with lymph node metastasis in gastric carcinoma.

A number of observations and animal trials have spurred extensive investigations of VEGF inhibitors as possible therapies for cancer. In tumor cell lines VEGF was an autocrine growth factor, so that inhibitors of VEGF or VEGF receptors (VEGFR) compromised the viability of tumor cells. Lastly, inhibition of VEGF or VEGFR signaling would inhibit both tumor angiogenesis and tumor cell growth and viability when there was evidence that VEGFR was expression in tumor cells[21,22].

Our study showed that VEGF was positively expressed in 76.7% of gastric carcinomas, which was significantly higher than that in NMNCE. The result indicated that VEGF was up-regulated and there might exist an autocrine mechanism of VEGF in gastric carcinoma. VEGF could promote tumor growth and metastasis by both direct and indirect pathways[23].

Maspin, a member of the serpin family of protease inhibitors, is expressed in normal human mammary and prostate epithelial cells, and down-regulated during cancer progression. Biological studies demonstrated a tumor-suppressive role of Maspin, acting at the levels of tumor invasion and metastasis[8,12]. Maass[24] did not detect Maspin expression in any of 6 gastric cancer cells. Son[25] studied Maspin expression in 30 cases of human gastric adenocarcinoma using immunohistochemistry and reverse transcripted-polymerase chain reaction. Twenty-seven cases (90%) of gastric adenocarcinoma, regardless of histological type, and all cases of GNEIM showed diffuse and strong immunoreactivity to Maspin. Eighteen of 26 cases (69.2%) of NMNCE showed weak and focal immunoreactivity. The level of Maspin expression was higher in GNEIM and lower in NMNCE than in adenocarcinoma cases. Akiyama[26] examined Maspin expression and/or allele-specific methylation status in four gastric cancer cell lines, as well as normal, metaplastic, and carcinoma epithelia obtained from 50 gastric cancer patients. Three gastric cancer cell lines exhibiting Maspin overexpression showed hypomethylation on both alleles or a haploid allele. Dense and diffuse immunoreactivity to Maspin was observed in 40 (80%) of 50 gastric carcinomas and all GNEIM, but not in GNE without IM. Maspin gene promoter region of all GNE without IM was hypermethylated on both alleles whereas those with IM frequently represented the haploid type of hypomethylation

status. Maspin mRNA was amplified from GNEIM and cancerous crypts but not from GNE without IM. These results suggested that demethylation at the Maspin gene promoter disrupted the cell-type-specific gene repression in both GNE and gastric cancer. In our study, 41.0% (16/39) of NMNCE showed a weak Maspin expression that was limited to gland cells of the stomach body, and 51.3% (20/39) of gastric carcinomas expressed Maspin. The positive rate of Maspin expression in NMNCE and in gastric carcinoma in our study was significantly lower than that in Son and Akiyama's study. We considered that the cut-off point made the different results. The reason why all GNEIM showed immunoreactivity to Maspin in all studies should been studied further. In addition, the role of Maspin gene and its encoding protein in tumorigenesis and progression of gastric cancer need to be investigated further.

In our study, Maspin and VEGF showed no correlation in gastric carcinomas. The precise roles of VEGF and Maspin in cancer tumorigenesis, invasion, and metastasis should be studied further. The relationship between expression of VEGF and Maspin in gastric cancer needs to be proved by amplifying samples.

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