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ORIGINAL ARTICLE

Correlation between the progression of cancer and the expression of matrix metalloproteinase-9 in metastatic spinal tumor

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Summary

Purpose: *To explore whether the expression levels of matrix metalloproteinase-9 (MMP-9) are related to spinal metastasis of gastric carcinoma.*

Methods: *Fifty tissue specimens were collected from gastric carcinoma with spinal metastasis and set as test group A; 30 tissue specimens of primary gastric carcinoma were collected and set as control group B; 30 healthy paracancerous gastric tissue specimens were collected and set as control group C. The expression levels of MMP-9 and vascular endothelial growth factor (VEGF) in the specimens were analyzed by immunohistochemistry.*

Results: *The positive expression rates of MMP-9 in the three groups were 82.0, 63.33 and 16.67% respectively, and the positive expression rates of VEGF were 78.00, 56.67 and 13.33%, respectively. The positive expression rates of MMP-9 and VEGF in the test group A were statistically significantly higher than those of control group B and C ($p<0.05$). These findings suggest a positive correlation between MMP-9 and VEGF expression.*

Conclusion: *Increased expression of MMP-9 is associated with the spinal metastasis of gastric carcinoma.*

Key words: *immunohistochemistry, metastatic spinal carcinoma, mmp-9, vascular endothelial growth factor*

Introduction

Tumors, whose cancer cells metastasize to the spine via blood flow and then grow into secondary sites [1] are called metastatic spinal tumors and their incidence accounts for 15% [2]. Spinal tumors require differentiation between benign and malignant, therefore histopathological and inumohistochemical examination is required [3,4] The malignant tumors that most commonly metastasize to the spinal axis are cancers of the lung, prostate, kidneys, and especially cancers of the ovaries with genetic and endocrine cause as hormonal disorders, polycystic ovaries, refractory acne [5,6]. Due to their location, metastatic spinal tumors may cause direct damages to the vertebral body and surrounding tissues, affect the nerve roots and bone marrow [7], and even result in neurological dysfunctions, psychotic disorders requiring differential diagnosis with true psychosis [8], paralysis and death.

Their treatment is based on radiotherapy, classical chemotherapy does not have the desired effect but has multiple side effects such as vitamin K coagulation disorders [9], immunosuppression favoring infections with resistant germs [10,11] or with fungi of the Fuasarium or Aspergillus genuses [12].

Alternative therapies such as plant extracts with proven cytotoxic effects or the intake of poly-

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phenols with certain anticancer protective effects have been tried [13,14].

Previous studies suggested that MMP-9 expression was correlated to cancer cell metastasis and could provide a valuable biomarker for the prevention and treatment of metastatic cancer. MMPs, which are zinc-ion-dependent endopeptidases, play a critical role in the invasion and metastasis of malignant tumors [15].

The expression of MMP-9 has been related to various tumors. In 1998, Gokaslan et al. [16] studied the expression of MMPs using immunohistochemical localization, and the content of MMPs using enzyme-linked immunosorbent assay (ELISA) and Western blotting. They suggested that MMPs may contribute to the metastasis of tumors to the spine and the overexpression of the enzymes may be correlated to the enhanced invasion of primary and metastatic spinal tumors. Choi et al. [17] reported that MMP-9 was important in the invasion and metastasis of tumor cells. They found that the development of decreased metastatic potential with the aqueous extract separated from Prunella vulgaris (PVAE) was mediated through suppression of MMP-9 expression. Gao et al. [18] found that the expression of MMP-9 was in close correlation with the invasion and metastasis of tumor through detecting the expression of MMP-9 in urinary bladder epithelial cells.

This study aimed to investigate the relationship between MMPs and tumor metastasis by detecting the expression levels of MMP-9 in tissues obtained from gastric carcinoma that were metastasized to the spine, non-metastatic gastric carcinoma and normal gastric tissue.

Methods

Experimental subjects

Fifty tissue samples, which were obtained from patients diagnosed with gastric carcinoma with spinal metastasis and resected between June 2014 and November 2016, were set as test group A; 30 tissue samples which were obtained from non-metastatic gastric carcinoma tissues were set as control group B; 30 tissue samples which were collected from normal paracancerous gastric tissues were set as control group C. Patients did not receive chemoradiotherapy before surgery.

Main reagents and equipment

The main reagents included rabbit anti-human MMP-9 monoclonal antibody (Juneng Shiji Information Technology (Suzhou Co., Ltd., China), rabbit anti-human VEGF monoclonal antibody (Thermo Fisher Scientific Co., Ltd., China), citrate buffer powder (Shanghai Yuesen Biotechnology Co., Ltd., China), DAB (3,3'-diaminobenzidine) (Shanghai Huzhen Industrial Co., Ltd., China) and neural resin (Shanghai Guyan Science and Technology Co., Ltd., China). The equipment included decoloration table (Jiangsu Tianling Equipment Co., Ltd., China), hematoxylin (Shanghai Baoman Biotechnology Co., Ltd., China) and eosin (Beijing Solarbio Co., Ltd., China).

Staining method

The paraffin sections were stained using hematoxylin-eosin. According to the conventional experimental procedures, the sections were processed using gradient alcohol dehydration, hematoxylin staining, color separation, dehydration, eosin staining, dehydration, transparency, and sealing.

The expression of MMP-9 and VEGF in gastric carcinoma and normal tissues was detected using streptavidin-peroxidase (S-P) immunohistochemical method [19]. According to the conventional experimental procedures, the paraffin sections were dewaxed using dimethylbenzene and hydrated using ethyl alcohol. After antigen was retrieved using citrate buffer, one drop of 5% sheep serum was added. Then the sections were incubated at room temperature for 25 min. Primary antibody was incubated at room temperature overnight and then rewarmed at 38 °C for 45 min. Secondary antibody was incubated at room temperature for 35 min and then processed at 38 °C for 35 min. Sections were added with SP and incubated at room temperature for 15 min and then dried using an oven at 37 °C for 35 min. Tissue staining was observed under a microscope, after one drop of DAB solution was added to each sample. Dye liquid was then removed. Color development was assessed by observation using a microscope. Sections were washed with distilled water for 4 min, redyed using hematoxylin, sealed using neutral balsam, and observed under the microscope.

Phosphate buffer saline (PBS) replaced primary antibody as the negative control, and the sections which had been known positive were taken as positive control.

Observation and determination criteria

After conventional specimen preparation, each section was observed in 5 400 x microscopic fields. Positive expression of MMP-9 and VEGF was characterized if the cytoplasm or membrane had yellow and brown-yellow particles.

Score was given according to the staining degree; colorless was scored as 0 point, weak staining was scored as 1 point, and strong staining was scored as 2 points. One-25% of staining cells was scored as 1 point, 26-50% was scored as 2 points, 51-75% was scored as 3 points, and 75-100% was scored as 4 points. The specimens were classified according to the product of the two scores; 0-2 points was determined as negative and 3-8 points was determined as positive.

Statistics

Data was processed by SPSS 22.0 software. The comparison between data was performed using Chi-square test. Spearman's correlation analysis was also carried out. $p<0.05$ indicated statistically significant difference.

Results

Correlation between MMP-9 and VEGF and clinical data

There was no remarkable difference in the expression of MMP-9 and VEGF in the tissues with different clinical characteristics, suggesting that the positive expression rate of these markers did not correlate with gender and age, as shown in Table 1.

Expression of MMP-9 and VEGF in the tissue specimens in different groups

The expression levels of MMP-9 were markedly elevated and concentrated brown-yellow particles were observed in the test group A; there was a high expression of MMP-9 and moderately concentrated light yellow particles in the control group B; there was low or even no expression of MMP-9 and sparse light yellow or non-stained particles in the control group C (Figure 1).

VEGF expression was extremely high and the particles were brown-yellow and densely distributed in the test group A. The expression levels of VEGF were high and the particles were light yellow and distributed moderately densely in the control group B. VEGF had low expression or nearly no expression and the particles were, light yellow sparsely distributed, or had no color staining in the control group C. The expression results were

Table 1. Correlation between MMP-9 and VEGF expression in gastric carcinoma with spinal metastasis and clinical data

Factor		*Gender*		*Age (years)*		*Pathological features*	
Group		Female	Male	≤ 50	>50	Adenocarcinoma	Squamous cell carcinoma
Number of cases		27	23	20	30	24	26
	+	24	18	17	25	19	22
	-	3	5	3	5	5	4
MMP-9	Positive rate (%)	88.9	78.3	85.0	83.3	79.2	84.6
	x^2	1.13		0.06		0.64	
	p	>0.05		>0.05		>0.05	
	+	21	16	15	22	20	19
	-	6	7	5	8	4	7
VEGF	Positive rate (%)	77.8	69.6	75.0	73.3	83.3	73.1
	x^2	0.86		0.21		0.57	
	p	>0.05		>0.05		>0.05	

Table 2. The expression of MMP-9 and VEGF in the three groups of tissue specimens

Group		*Test group A*	*Control group B*	*Control group C*
Number of cases		50	30	30
	+	41	19	5
	-	9	11	25
MMP-9	Positive rate (%)	82.00	63.33	16.67
	x^2		28.17	
	p		<0.05	
	Pairwise comparison of positive rate: A and B: x^2=6.38, p<0.05; A and C: x^2=28.30, p<0.05; B and C: x^2=11.60, p<0.05.			
	+	39	17	4
	-	11	13	26
VEGF	Positive rate (%)	78.00	56.67	13.33
	x^2		26.77	
	p		<0.05	
	Pairwise comparison of positive rate: A and B: x^2=3.48, p<0.05; A and C: x^2=25.30, p<0.05; B and C: x^2=12.06, p<0.05.			

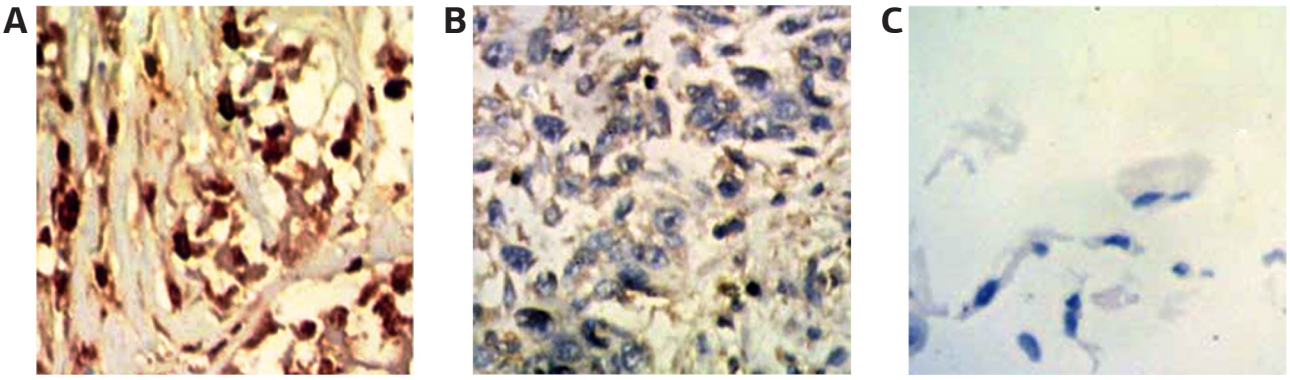

Figure 1. MMP-9 expression in different tissue specimens. **(A)** Expression of MMP-9 in the tissues from gastric carcinoma with spinal metastasis. **(B)** Expression of MMP-9 in gastric carcinoma tissues. **(C)** Expression of MMP-9 in normal gastric tissues.

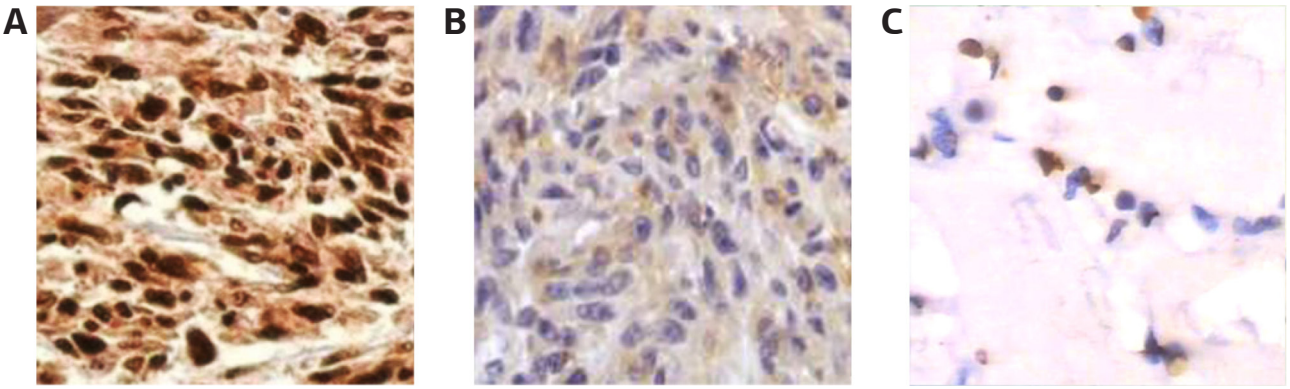

Figure 2. VEGF expression in different tissue specimens. **(A)** Expression of VEGF in tissues from gastric carcinoma with spinal metastasis. **(B)** Expression of VEGF in gastric carcinoma tissues. **(C)** Expression of VEGF in normal gastric tissues.

consistent with MMP-9. Details are shown in Figure 2.

As shown in Table 2, MMP-9 had a positive expression in 41 tissue specimens from 50 cases of gastric carcinoma with spinal metastasis (test group A) (82.0% ;41/50)); 19 out of 30 tissue specimens from cases of gastric carcinoma (control group B) had positive expression of MMP-9 (63.33% ;19/30)); 5 out of 30 normal gastric tissue specimens had positive expression of MMP-9 (16.67% ;5/30)). Differences in the expression rate between the three groups were statistically significant ($p<0.05$).

VEGF had a positive expression in 39 tissue specimens from 50 cases of gastric carcinoma with spinal metastasis (test group A) (78.0%; 39/50); 17 out of 30 tissue specimens from cases of gastric carcinoma (control group B) had positive expression of VEGF (56.67%; 17/30); 4 out of 30 normal gastric tissue specimens had positive expression of VEGF (13.33%; 4/30). Differences in the expression rate between the three groups were statistically significant ($p<0.05$).

Discussion

MMP-9, the enzyme with the largest molecular weight [20-22], is secreted from the inside of interstitial cells such as neutrophils to the outside of cells in the form of zymogen [23]. It has many active substrates [24,25] such as IV, V, VII, X and XI collagen and gelatin. Cytokines and corresponding receptors are also active substrates of MMP-9 [26]. MMP-9 expression is positively correlated with mechanisms of growth, invasion and metastasis in some cancers [27,28].

The processes of tumor growth and metastasis are highly complex. Tumor angiogenesis plays a pivotal role in growth, invasion and metastasis of the tumor. VEGF can accelerate the growth of endothelial cells and blood vessels, which provides the matrix for the development of blood vessel endothelium and tumor cells. Malignant cells can generate large amounts of VEGF to promote tumor neovascularization and formation of tumor stroma.

In this study, we showed that the positive expression rate of MMP-9 and VEGF had no correla-

tion with clinical data such as age and gender, and the differences among different tissue groups were not statistically significant ($p > 0.05$).

The positive expression of MMP-9 and VEGF in the tissues from gastric carcinoma with spinal metastasis was much higher than that in the tissues from gastric carcinoma and normal gastric tissues. Moreover, the positive expression of MMP-9 and VEGF in the gastric carcinoma tissues was higher than that in the normal gastric tissues ($p < 0.05$). These findings indicate that MMP-9 and VEGF expression is related to metastasis of gastric carcinoma, and MMP-9 expression is positively correlated with VEGF expression.

Collectively, we demonstrated the possible link between MMP-9 expression and spinal metastasis of gastric carcinoma and identified MMP-9 as a valuable marker for determining the spinal metastasis of gastric carcinoma.

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Conflict of interests

The authors declare no conflict of interests.

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