CORRELATION BETWEEN EXPRESSION OF MATRIX METALLOPROTEINASE-2, MATRIX METALLOPROTEINASE-9 AND ANGIogenesis IN GASTRIC CANCER

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ABSTRACT

Objective: To investigate the relationship between matrix metalloproteinase-2 (MMP-2), MMP-9 as well as microvessel density (MVD) and their clinicopathological features in gastric cancer. Methods: The expression of MMP-2, MMP-9 and MVD were detected by immunohistochemistry SP method on 65 cases of gastric cancer tissue and 32 adjacent gastric mucosa. Results: The positive expression of MMP-2, MMP-9 and MVD in cancer group were significantly higher than those of adjacent mucosa group (P<0.01). The positive expression of MMP-2 and MMP-9 had closely correlation with clinical features of lymphatic metastasis, pathological type and stages (P<0.05), and the high level of CD 34 had correlation with metastasis and stages (P<0.01). The positive expression of MMP-2 and MMP-9 showed significant correlation with the level of MVD. Prognosis was mostly affected by lymphatic metastasis and stages of clinical features. The low cumulative survival rate was showed in the groups of positive expression of MMP-2, MMP-9 and high level of MVD. Conclusion: MMP-2, MMP-9 and MVD play an important role in metastases, invasion and prognosis of gastric cancer, which is a valuable maker to evaluate the prognosis.

Key Words: Gastric cancer; Matrix metalloproteinase; Microvessel density; Clinicopathological feature

The incidence of gastric cancer is relatively high in the carcinoma of digestive system, its malignant behavior mainly depends on the capability of invasion and metastasis of cancer cells. After the components of the extracellular matrix (ECM) degraded, tumor cells invaded the surrounding tissue and the vascular or lymphatic vessels to form metastatic colonies at distant sites. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes with the ability of degrading extracellular matrix (ECM) components as well as numerous secreted and membrane-bound cell modulators, which accelerate the invasion of carcinoma to blood and lymphatic vessels. MMPs are considered as a key in the tissue rearrangements associated with malignancy, which play important roles in the process of embryonic development, aging, angiogenesis, tumor metastasis and prognosis\(^{[11-14]}\). However, the relationship between their expression and microvessel density (MVD) as well as the clinicopathological features of gastric cancer was rarely reported.

In this study, we investigated the relationship between the expression of MMP-2, MMP-9 as well as the microvessel density (MVD) and the clinicopathological features of gastric cancer to evaluate the possible effects of these genes in the progression of gastric cancer.

MATERIALS AND METHODS
Patient Selection

Sixty-five cases of gastric carcinoma underwent surgery in the Second Affiliated Hospital of Dalian Medical University from 1990 to 1995 were selected for the study. These cases comprised 50 males and 15 females. The median age was 60.08 years with a range from 31 to 81 y. All studied patients had not been accepted for radiation therapy and chemotherapy before the operation. The histological findings, lymph node metastasis and TNM stage were evaluated based on World Health Organization Classification of Tumors. 32 cases of adjacent stomach mucous were selected as control. Of 65 cases of gastric cancer, 10 were early cancer and 55 were advanced, 38 with tumor diameter less than 5 cm, and 27 more than 5 cm. 31 with lymph node metastasis, and 24 without lymph node metastasis. 56 cases were provided with follow-up recorder and 13 cases (23.2%) with end check value in an average of 32.25 m of follow-up (from 4 to 112 m).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue specimens were stained by SP immunohistochemistry technique for MMP-2, MMP-9 and CD34 detection. Four-micron sections were dewaxed in xylene, incubated in 3% hydrogen peroxide for 5 min to eliminate intrinsic peroxidase, washed in phosphate-buffered saline (PBS) for 3 min, bathed in 0.01 mol/L sodium citrate buffer (pH 6.0) after bringing the solution to a boil in a pressure cooker, washed in PBS after refrigeration and quenched in normal goat serum for 30 min. The sections were then incubated overnight at 4°C with monoclonal antibody raised against MMP-2, MMP-9 (Santa Cruz Biotechnology Inc) and CD34 (Novocastra Laboratories Ltd.). Then second Abs and streptavidin conjugated to horseradish peroxides were incubated with sample for 15 min at 37°C, respectively. Finally, 3,3'-diaminobenzidine was used for color development, and hematoxylin was used for counter staining. Negative control sample was set up every time and reaction condition was controlled strictly.

Judge Criterion

MMP-2 and MMP-9 were mainly stained in cytoplasm and partly stained in nucleus with brown. Their immunoreactivity was evaluated semi-quantitatively according to the multiplication of the percentage of positive cells and staining intensity. Staining intensity was set as: no color as 0; light staining as 1 and heavy staining as 2 (depth of color confirm by the contrast with background). The percent of positive cells was assigned a scores as follows: 0, <5% of cell stained; 1, 5%-25; 2, 26%-50%; 3, >50%. The expression of MMP-2 and 9 was finally defined according to the score obtained from the grade of intensity multiplied by the score of cell immunoreactivity, i.e. negative (-, score 0–1), positive (+, score 2–3), and strong positive (++, score 4 or above).[5]

Microvessel density was assessed as below: Any brown endothelial or cell cluster being different from background was a blood vessel. The framework of branch was considered a vessel so far as structure was not continued. First, specimens were observed at low magnification (100X) for ensuring hotspots of blood vessel in tumor generally, and then microvessels were counted in four hot spots at magnification of 200 (0.739 mm²/view) for a mean value namely as MVD.

Statistical Analysis

All statistical analysis was performed using the SPSS 10.0 software. Spearman test, Manning-Whitney U test, student-t test, Log-Rank test, single and multivariate Cox regression were applied. A value of P<0.05 was considered to indicate significance.

RESULTS

MMP-2, MMP-9 and CD34 immunostaining were rarely expressed in adjacent mucosa tissue but obviously in gastric cancer. Positive staining for MMP-2 and MMP-9 were restricted to cytoplasm of gastric cancer at the invasive edge of the tumor, with no staining of interstitial stroma or basement membrane. There were significant difference between the gastric cancer and adjacent mucosa in positive expression of MMP-2, MMP-9 and CD34 (P<0.01) (Table 1). The positive expression of MMP-2 and MMP-9 had closely correlation with clinical features of lymphatic metastasis, pathological type and stages (P<0.05), and the positive expression of CD34 had correlation with metastasis and stages (P<0.01). The intensity and distribution of MMP-2 and MMP-9 had significant correlation with the positive expression of MVD (P<0.01) (Table 2, 3). Prognosis was mostly affected by lymphatic metastasis and stages of clinical features. Low survival rate was showed in the group with positive expression of MMP-2, MMP-9 and high level of MVD. Kaplan-Meier analysis revealed significant difference in cum survival rate between the positive expression and negative expression of MMP-2 and MMP-9 in gastric cancer (log rank test P<0.01), and difference in cum survival rate between the group with MVD more than median count and the group of MVD less than median count (log rank test P<0.01) (Figure 1—3).