Changes of histology and expression of MMP-2 and nm23-H1 in primary and metastatic gastric cancer

Lin-Bo Wang, Zhi-Nong Jiang, Miao-Ying Fan, Chao-Yang Xu, Wen-Jun Chen, Jian-Guo Shen

AIM: To investigate the changes of histology and expression of MMP-2 and nm23-H1 in primary and metastatic gastric cancer.

METHODS: One hundred and seventy-seven gastric cancer patients with lymph node and/or distal metastasis between 1997 and 2001 were reviewed. Differences in histology of the primary and metastatic gastric cancer were assessed. MMP-2 and nm23-H1 immunoreactivity was compared in 44 patients with tumor infiltration to the serosa layer.

RESULTS: Poorly and moderately differentiated metastatic gastric cancer was found in 88.7% (157/177) and primary gastric cancer in 75.7% (134/177) of the patients. The histological type of metastatic gastric cancer that was not completely in accordance with the preponderant histology of primary gastric cancer was observed in 25 patients (14.1%). MMP-2 immunoreactivity in metastatic gastric cancer was significantly stronger than that in primary gastric cancer, while nm23-H1 immunoreactivity showed no difference in primary and metastatic gastric cancer.

CONCLUSION: Metastatic gastric cancer presents more aggressive histological morphology and higher MMP-2 immunoreactivity than primary gastric cancer. This heterogeneity may elicit a possible mechanism of gastric cancer metastasis.

Key words: Heterogeneity; Gastric cancer; Nm23-H1; MMP-2; Histological change

INTRODUCTION

Gastric cancer, one of the most common malignant diseases in the world, has been shown to frequently metastasize. Certain studies have reported the possible mechanisms underlying its metastasis[1-3]. However, whether there is a histological difference between primary and metastatic gastric cancer is unclear and has been rarely reported.

Matrix metalloproteinases (MMPs) are defined as a family of enzymes which degrade extracellular membrane proteins, thus playing a significant role in tumor invasion and metastasis[4]. MMP-2 is one of the most extensively studied MMPs in the process of cancer. It was reported that elevated MMP-2 level is related to increased tumor metastasis and stage in the lung, breast, stomach and colon[5-8]. However, differences in MMP-2 expression between primary and metastatic gastric cancer have been rarely assessed.

The nm23 gene is a putative metastasis suppressor gene originally identified in metastatic murine melanoma cells[9]. Reduction in nm23 expression is related with a high incidence of lymph node metastasis or poor prognosis of gastric cancer[10,11]. However, there is no inverse relationship between nm23 expression and metastatic potential of gastric cancer[12]. Their relationship in gastric cancer remains controversial.

Our study was to investigate the differences in histology and expression of MMP-2 and nm23-H1 between primary and metastatic gastric cancer, and to elucidate the possible mechanism of tumor heterogeneity underlying gastric cancer metastasis.

MATERIALS AND METHODS

Patients and tissue specimens

Complete data and tissue specimens were obtained from
230 gastric cancer patients, who underwent resection of gastric cancer at Sir Run Run Shaw Hospital, Zhejiang University College of Medicine between June 1997 and January 2001. Among them, 177 patients including 124 males and 53 females, ranging in age from 20 years to 79 years with a mean age of 55.5 years, had pathologically confirmed lymph node and/or distal metastasis and were enrolled to assess the differences in histology between primary and metastatic gastric cancer. Their clinicopathological features are shown in Table 1. Disease stage was classified based on the 5th edition of the International Union against Cancer and the American Joint Committee for Cancer Staging.

Forty-four patients, who had pathologically confirmed tumor infiltration to the serosal layer (24 pts, T3; 11 pts, T4; 17 pts, T1), were recruited to evaluate the difference in MMP-2 and nm23-H1 immunoreactivity between primary and metastatic gastric cancer by immunohistochemistry.

Slides of tissue from primary and metastatic gastric cancer were observed by two pathologists. Following the criteria of World Health Organization (WHO), papillary adenocarcinoma was classified as well differentiated type, signet cell carcinoma and mucous adenocarcinoma as poorly differentiated type, tubular adenocarcinoma as well or moderately or poorly differentiated type.

We set the largest proportion of histological type of primary gastric cancer as the preponderant histological type. Thereby, percentage of the preponderant histological type of metastatic gastric cancer was as follows: - lower than 5%; +: 5%-25%; ++: 25%-50%; +++: 50%-75%; ++++: higher than 75%. Comparison of changes in histology between primary and metastatic gastric cancer was made based on the percentages of their preponderant histological type.

Immunohistochemistry

Immunohistochemical study was performed using the following antibodies: anti-nm23-H1 protein (GE-213, monoclonal, 1:100; Manxin, Fuzhou, China) and anti-MMP-2 (CA-4001, monoclonal, 1:50; Manxin, Fuzhou, China). Four-µm thick sections of 10% formalin-fixed, paraffin-embedded gastric cancer tissue were cut, mounted on glass slides coated with 3-aminopropyltrihoxysilane, and air-dried overnight at 60°C. The sections were deparaffinized in xylene and rehydrated in ethanol. Endogenous peroxidase was blocked with methanol containing 3% hydrogen peroxidase for 25 min. For staining with anti-MMP-2, sections were pretreated with citrate buffer (0.01 mol/L, pH 6.0) and heated at 100°C in a microwave oven for 20 min. For staining with anti-nm23-H1, sections were pretreated with trypsin (0.5%, pH 7.4) for 20 min at room temperature. The sections were incubated with primary antibodies at 4°C overnight, stained with a streptavidin-biotin-peroxidase kit (Manxin, Fuzhou, China), and reacted in a solution containing 3, 3′-diaminobenzidine and peroxycrilocidate substrate, and counterstained with hematoxylin. The provided sections known to react positively with nm23-H1 or MMP-2 (Manxin, Fuzhou, China) were used as a positive control. As a negative control, the primary antibody was deleted.

Evaluation

The immunoreactivity of each antibody was evaluated. MMP-2 and nm23-H1 immunoreactivity was graded as - without or with immunoreactivity in less than 5% tumor cells; +: immunoreactivity in 5%-25% tumor cells; ++: immunoreactivity in 25%-50% tumor cells; +++: immunoreactivity in 50%-75% tumor cells; ++++: immunoreactivity in over 75% of tumor cells.

Statistical analysis

All statistical analyses were conducted using the statistical program SPSS 10.0 for windows (SPSS, Chicago, IL, USA). Differences in histological morphology and expression of MMP-2 and nm23-H1 between each group were analyzed by chi-square test or by Fisher’s exact test. P < 0.05 was considered statistically significant.

RESULTS

Histological changes

We observed different histological changes in primary and metastatic gastric cancer patients (Figure 1). Poorly and moderately differentiated metastatic gastric cancer was found in 88.7% (157/177) of the patients while primary gastric cancer in 75.7% (134/177) of the patients. The preponderant histological types of primary gastric cancer, graded as +++ and ++++, were more than those of metastatic lymph nodes (170 vs 138, P < 0.01). Moreover, the preponderant histological type of the metastatic lymph nodes in 14.1% patients (25/177) was not completely in accordance with that of primary gastric cancer (Table 2).

MMP-2 immunoreactivity

MMP-2 immunoreactivity was significantly stronger in metastatic gastric cancer than in primary gastric cancer. For the 20 patients with distal metastasis, a different MMP-2 immunoreactivity was observed in primary and metastatic gastric cancer (Table 3). MMP-2 immunoreactivity was stronger in metastatic gastric cancer than in primary gastric

| Table 1  Clinicopathologic data obtained from 177 gastric cancer patients |
|---------------------------------|-----|----------|
| **Tumor size (cm) (mean ± SD)** | 6.0 ± 2.9 |
| **Location** | n | % |
| Upper or whole body | 40 | 22.6 |
| Lower or middle body | 137 | 77.4 |
| **Gross type** | n | % |
| Localized | 39 | 22.0 |
| Infiltrative | 138 | 78.0 |
| **Depth of invasion** | n | % |
| T1 | 25 | 14.1 |
| T2 | 86 | 48.6 |
| T3 | 44 | 24.9 |
| T4 | 22 | 12.4 |
| **Retrieved lymph nodes (mean ± SD)** | 22.4 ± 3.5 |
| **Stage** | n | % |
| I | 6 | 3.4 |
| II | 29 | 16.4 |
| III | 88 | 49.7 |
| IV | 54 | 30.5 |
cancer. However, the immunoreactivity was similar in metastatic lymph nodes and distal metastasis (Figure 2).

**nm23-H1 immunoreactivity**

There was no significant difference in nm23-H1 immunoreactivity between primary and metastatic gastric cancer. The immunoreactivity was quite similar in primary and metastatic lymph nodes and distal metastasis (Table 4).

**DISCUSSION**

Studies on intratumoral and intertumoral heterogeneity have provided valuable insights into the pathogenesis and progression of different tumors. Although the concept of intratumoral heterogeneity of tumors has been generally accepted, studies on it in gastric cancer are scant. Previous reports focused mainly on comparison of molecular genetic alterations in each individual. However, this study investigated the tumor heterogeneity including histological morphology changes in primary and metastatic gastric cancer.

We observed different histological changes in primary and metastatic gastric cancer. Metastatic gastric cancer showed poorer differentiation. Meanwhile, the preponderant histological type of primary and metastatic gastric cancer was not completely identical. In the present study, the preponderant histological type of primary and metastatic gastric cancer site was different in 14.1% patients. These findings may imply that not all the histological types of primary gastric cancer have potential to metastasize, poorly differentiated cancer cells may play a significant role in lymph node metastasis, which may possibly explain why a small proportion of poorly differentiated cancer cells in primary gastric cancer may be preponderant in metastatic gastric cancer.

It is widely accepted that tumor may synchronously contain multiple histological types, reflecting different tumor differentiation and biological behavior. Most tumors may contain multiple cell clones with a diverse metastatic potential. Cell clones with a high metastatic potential are apt to metastasize to lymph nodes or distal organs. This dynamic heterogeneity may give a possible explanation for the different changes in histology between primary and metastatic gastric cancer. Although the preponderant histological change in primary gastric cancer is generally considered a prognostic predictor, the other histological changes in primary gastric cancer, especially in poorly differentiated subclones, may be as important as the preponderant histological change for the prognosis of primary gastric cancer. However, few studies have been addressed this issue, further investigations are warranted.

Because of its ability to degrade the basement membrane, MMP-2 has been postulated as a potential marker of tumor progression and prognosis in different malignancies such as ovarian cancer, gastric cancer and lung carcinoma. Schwartz et al. reported that MMP-2 is expressed in SK-GT1, SK-GT5 and SK-GT6 but not in SK-GT3.

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**Table 2** Preponderant histological type of primary and metastatic lymph nodes in 177 gastric cancer patients

| Tumor type               | Grade | P value |
|--------------------------|-------|---------|
| Primary lymph nodes      | -     |         |
| Metastatic lymph nodes   | -     |         |

**Table 3** MMP-2 immunoreactivity in primary and metastatic T3 gastric cancer

| Patients (n) | Tumor type | Grade | P value |
|--------------|------------|-------|---------|
| 44 T:NxMx    | Primary lesion | 12    | 0.004   |
| Patients     | Metastatic lymph node | 7     |         |
| 20 T:NxM1    | Primary lesion | 6     | 0.019   |
| Patients     | Metastatic lymph node | 4     |         |
|              | Distal metastatic site | 3     |         |

**Table 4** nm23-H1 immunoreactivity in primary and metastatic T3 gastric cancer

| Patients (n) | Tumor type | Grade | P value |
|--------------|------------|-------|---------|
| 44 T:NxMx    | Primary lesion | 12    | 0.138   |
| Metastatic lymph node | 22    |         |
| 20 T:NxM1    | Primary lesion | 8     | 0.497   |
| Metastatic lymph node | 9     |         |
|              | Distal metastatic site | 10    |         |

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SK-GT2 and SK-GT4 gastric cancer cell lines. Ji et al\[23\] reported that MMP-2 expression is significantly higher in advanced than in early gastric cancer patients\[23\]. These findings indicate that gastric cancer cells with a greater malignant and metastatic potential may secrete much more MMP-2 protein. Moreover, it has been shown that downregulation of MMP or MMP-2 may inhibit tumor growth and metastasis, indicating that MMP-2 is correlated with gastric cancer invasion and metastasis\[21,22\]. In this study, MMP-2 immunoreactivity was significantly higher in metastatic than in primary gastric cancer. This heterogeneity may elicit one of the possible mechanisms underlying gastric cancer metastasis.

In conclusion, metastatic gastric cancer is more aggressive and has a higher expression in tumor genes than primary gastric cancer. This heterogeneity may elicit one of the possible mechanisms underlying gastric cancer metastasis.

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**COMMENTS**

**Background**

Gastric cancer metastasis occurs frequently and its possible mechanism has not been well addressed. Intratumoral and intertumoral heterogeneity plays a significant role in tumor progression. However, studies on gastric cancer are rarely reported.

**Research frontiers**

The aim of this study was to evaluate the morphology and tumor metastatic gene heterogeneity in gastric cancer.

**Innovations and breakthroughs**

This study showed the changes in morphology and expression of MMP-2 of primary and metastatic gastric cancer.

**Applications**

The heterogeneity in gastric cancer may provide a clue to the possible mechanism of cancer invasion and metastasis.

**Peer review**

This is a nice study comparing primary and metastatic gastric cancer. The expression of MMP2, one of the known metalloproteinases relating to tumor invasion and metastases, was stronger in metastatic than in primary gastric cancer. However, NM23-H1 expression did not change in primary and metastatic gastric cancer. Primary and metastatic gastric cancer were found to have different histological types.
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