Original article

Matrix metalloproteinase 9 in mucosally invasive gastric cancer

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Abstract:

Background. Matrix metalloproteinase 9 (MMP-9) facilitates tumor invasion and metastasis via basement membrane degradation. Control of MMP-9 production by cancer-stromal cell interactions in these processes has been observed.

Methods. We measured plasma MMP-9 concentrations, using a one-step sandwich enzyme immunoassay, and also immunohistochemically localized MMP-9 in patients with small gastric cancers limited to the mucosa. The cancers were classified as intraepithelial tumor (Tis) and T1 disease according to the tumor-node-metastasis (TNM) classification of the International Union Against Cancer.

Results. Patients with T1 disease had a higher positivity rate for and mean value of MMP-9 than patients with Tis disease. In T1 tumors, MMP-9 expression determined immunohistochemically, was greater in cells in cancer stroma than in cells in noncancerous stroma, a situation found in only a few Tis tumors.

Conclusions. These results suggest that MMP-9 is related to the initial step of gastric cancer invasion.

Key words: extracellular matrix, stomach, neoplasm, metastasis, tumor-stromal cell interaction

Introduction

The major characteristic of cancer cells is their ability to invade the surrounding tissues and metastasize so as to form secondary tumors. For a tumor cell to metastasize, it must break away from its neighbors, force its way through the surrounding stroma, and penetrate extracellular matrices in order to enter the circulation. The basement membrane, one form of the extracellular matrix, is a strong barrier to the movement of tumor cells

[1–4]. Type IV collagen, the major protein of the basement membrane, provides the tight structural network that constitutes the basement membrane [5,6]. Much attention has focused on matrix metalloproteinase 9 (MMP-9, a 92-kDa gelatinase/type IV collagenase, gelatinase B) in the digestion of basement membrane type IV collagen [7–9], as an important factor in cancer invasion and metastases [10–12].

MMP-9 is secreted as the precursor form of proMMP-9, which can be activated by organomercurial compounds such as *p*-aminophenylmercuric acetate (APMA). Incubation of proMMP-9 with APMA processes it to an amino-terminal truncated intermediate form of 83-kDa and then converts it to an active 67-kDa species that lacks both the amino- and carboxy-terminal domains of the proMMP-9 molecule [13]. Although MMP-9 is expressed by a wide range of cells, including macrophages and neutrophils [14–16], it has been reported that, in human colon cancer, MMP-9 mRNA may be of macrophage origin [5], and that mRNA-expressing cells were located adjacent to invading malignant glands [17]. Recent studies have also indicated that MMP-9 production is controlled by tumor-stromal cell interaction [18–20]. We have shown previously [21] that preoperative plasma MMP-9 concentrations in gastric cancer patients correlated closely with International Union Against Cancer (UICC) tumor-node-metastasis (TNM) stage [22], and this may reflect increased MMP-9 production by stromal cells adjoining cancer cells. In the present study, we examined whether MMP-9 was related to mucosal invasion in small gastric cancers limited to the mucosa.

Patients and methods

Plasma was obtained from 40 patients with gastric mucosal cancers (less than 4 cm in diameter) who underwent gastrectomy at Aichi Cancer Center between

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August 1994 and September 1996. These 40 tumors were stratified as Tis (carcinoma in situ, intraepithelial tumor without invasion of lamina propria; $n = 14$) or T1 (tumor invading lamina propria; $n = 26$) tumors according to the UICC TNM classification [22]. Of the 28 male patients (mean age, 60.4 years; range, 26–78 years), 8 had Tis tumors; of the 12 female patients (mean age, 62.7 years; range, 39–78 years), 6 had Tis tumors. No patients had regional lymph node metastasis or distant metastasis. Twenty-five of the 40 patients had been asymptomatic and the disease was detected by screening. Written informed consent was obtained from each patient before enrollment in the study.

Plasma MMP-9 concentrations were measured using a previously reported one-step sandwich enzyme immunoassay employing monoclonal antibodies [23]. The sensitivity limit of the assay was 0.24 ng/ml (2.2 pg/assay), and a linear response was seen from 0.24 to 250 ng/ml (2.2–2280 pg/assay). The intra-assay coefficients of variation (CV) were 1.8%–3.9%, while the inter-assay CV were 1.8%–6.2%. This assay detected proMMP-9, the 83-kDa intermediate form of MMP-9, and their complex forms with tissue inhibitor of metalloprotease (TIMP)-1; the assay did not detect the 67-kDa active form. An MMP-9 concentration exceeding 62.7 ng/ml was defined as positive, as previously reported [21].

Immunohistochemical examination was performed in deparaffinized surgical specimens from all patients, by the peroxidase-labeled streptavidin-biotin technique, with a slight modification, as previously reported [24,25]. A monoclonal antibody for human MMP-9 (clone, 56-2A4; dilution, 1:100) [26]; was used which reacted with peptides in the COOH-terminal domain of 92-kDa MMP-9.

Student's *t*-test was employed for comparison of mean values, and a χ^2 test was performed for comparison of frequencies. A *P* value of <0.05 was considered significant.

Results

Plasma MMP-9 concentration

The mean plasma MMP-9 concentration in the 26 patients with T1 disease was 77.5 ± 33.8 ng/ml, significantly higher than that in the 14 patients with Tis disease (57.5 ± 19.6 ng/ml; $P = 0.049$, Fig. 1). MMP-9 was positive in 19 (73%) of the 26 patients with T1 disease, compared with only 5 (36%) of the 14 with Tis disease ($P = 0.021$).

Immunohistochemistry

Our immunohistochemical study showed greater MMP-9 expression in polymorphonuclear cells and

Fig. 1. Concentrations of plasma matrix metalloproteinase (MMP) 9 in patients with Tis and T1 (according to TNM classification [22]) gastric cancer

macrophage-like cells in cancer stroma than in noncancerous stroma in 20 (77%) of the 26 patients with T1 disease (Fig. 2a), but in only 5 (36%) of the 14 patients with Tis disease (Fig. 2b; $P < 0.05$). MMP-9 was immunostained within cancer cells in 16 (40%) of the 40 specimens. No difference in plasma MMP-9 concentration was recognized between the 16 patients with MMP-9 expression in cancer cells and the 24 without MMP-9 expression in cancer cells (64.4 ± 23.6 vs 74.6 ± 34.6 ng/ml). MMP-9 was expressed in the cancer cells, of 8 of the 14 patients with Tis disease (57%), and in 8 of the 26 with T1 disease (31%). Although MMP-9 expression was more frequent in Tis than in T1 tumors, the difference was not significant.

Discussion

Human plasma MMP-9 concentrations have been quantified [27,28] and are higher in patients with various cancers than in healthy controls [29,30]. We have previously reported that the measurement of plasma MMP-9 levels appeared useful for the detection of primary or recurrent gastric cancer, and for the assessment of tumor extent [21]. In that report, we also indicated that there were fewer MMP-9-positive stromal cells in noncancerous stroma than in the cancer stroma, and suggested that this increase in MMP-9 production in stromal cells near cancer cells may reflect elevated plasma MMP-9 concentrations in gastric cancer. Stromal cells have been shown to participate actively in human cancer invasion by the production of proteolytic enzymes such as MMP-1 and stromelysin-3 [31]. Recent studies have shown that the induction of MMP-9 expression in stromal cells may result from

Fig. 2a,b. Immunohistochemical staining with anti-MMP-9 antibody (peroxidase-labeled streptavidin-biotin technique). **a** In T1 tumors more MMP-9-positive stromal cells were observed in cancer stroma than in noncancerous stroma. **b** In Tis

tumors no difference in MMP-9 staining of stromal cells was recognized between cancer and noncancerous stroma. *Scale bar*, 0.1 mm

the release of one or several cytokines or growth factors from the invading cancer cells [17]. Several studies have also reported MMP-9 production to be controlled by tumor-stromal cell interaction [18–20].

Epithelial invasion within the lamina propria beyond the basement membrane seems to be the first step in cancer invasion, which we focused on in the present study of small mucosal cancers. Patients with mucosally invasive (T1) disease had a higher positivity rate for and plasma level of MMP-9 than patients with Tis disease. In 77% of T1 tumors, more cells stained immunohistochemically for MMP-9 in cancer stroma than in noncancerous stroma, a finding observed in only 36% of Tis tumors. In cancer cells, however, MMP-9 was observed in more patients with Tis disease than patients with of T1 disease, and plasma MMP-9 concentration was not related to immunostaining patterns. These results lead us to speculate that MMP-9 may be related to the first step of gastric cancer invasion. Although there is insufficient evidence to conclude that the elevation of plasma MMP-9 concentrations is caused by the presence of gastric cancer, our previous report showed that the concentrations were lowered after surgery [21]. Although we assume that the elevation was caused by MMP-9 release from stromal cells around tumor cells, further study is necessary to confirm the source of the elevated plasma MMP-9 concentrations in gastric cancer.

In conclusion, plasma MMP-9 concentrations and stromal cell immunohistostaining for MMP-9 were examined in small gastric mucosal cancer. MMP-9 seemed to be involved in the initial step of gastric cancer invasion.

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