CD10-positive Stromal Cells in Gastric Carcinoma: Correlation with Invasion and Metastasis

Wen-Bin Huang, Xiao-Jun Zhou, Jie-Yu Chen, Li-Hua Zhang, Kui Meng, Heng-Hui Ma and Zhen-Feng Lu

Department of Pathology, Clinical School of Medical College of Nanjing University/Nanjing Jinling Hospital, Jiangsu Nanjing, People’s Republic of China

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Background: CD10 is a cell surface metalloproteinase expressed by a variety of normal cell types, including lymphoid precursor cells, germinal center B lymphocytes and some epithelial cells. Although accumulating data indicate that CD10 expression by stromal cells is involved in colorectal carcinogenesis and it is a novel prognostic factor in breast carcinoma, CD10-positive stromal cells and their correlation with invasion and metastasis have not been studied in gastric carcinoma. The aim of this study was to immunohistochemically investigate the rate of CD10 production in the stromal cells in our gastric carcinoma collection and clarify its correlation with invasion and metastasis.

Methods: One hundred and sixteen cases of gastric carcinoma were analyzed immunohistochemically using a monoclonal CD10 antibody (clone 56C6).

Results: The expression of CD10 by stromal cells was significantly higher in the primary gastric carcinomas than in normal and dysplasia mucosas (P = 0.014). More frequent expression of CD10 by stromal cells was detected in differentiated carcinoma than in undifferentiated carcinoma (P < 0.001). CD10 expression by the stromal cells was associated with depth of invasion and lymph node metastasis (P < 0.05). Stromal CD10 expression was lower in gastric carcinoma without vessel invasion than in those with vessel invasion (P = 0.001). However, no association was observed between stromal CD10 expression and TNM stage. In differentiated carcinoma, stromal CD10 expression was associated with the depth of invasion, lymph node metastasis, vessel invasion and TNM stage (P < 0.01).

Conclusion: These results indicate that stromal cells expressing CD10 may play an important role in gastric carcinogenesis. CD10 expression by stromal cells seems to promote invasion and metastasis of differentiated gastric carcinoma.

Key words: CD10 – gastric cancer – immunohistochemistry – invasion – metastasis – stromal cell

INTRODUCTION

Gastric cancer is the most common cancer in East Asia and South America. Although incidence rates in the West are much lower than in Asia, it still remains a significant worldwide health burden, second only to lung tumors as a leading cause of cancer death (1). Several human cancers have been shown to induce a stromal reaction or desmoplasia as a component of carcinoma progression. The reactive stroma in cancer is characterized by stromal cell phenotypic switching, extracellular matrix remodeling, increased growth factor bioavailability, elevated protease activity, increased angiogenesis and an influx of inflammatory cells (2). In cancers, reactive stroma comprises fibroblasts, myofibroblasts, endothelial cells and immune cells. Although all of these cells potentially affect tumorigenesis, myofibroblasts are of particular interest. Myofibroblasts in reactive stroma synthesize extracellular matrix (ECM) components such as collagen I, collagen III, fibronectin isoforms, tenasin and versican (3–7). In addition, myofibroblasts express proteases, including urokinase, plasminogen activator, fibroblast activation protein (FAP) and matrix metalloproteinases (MMPs) (8–10). Production of these components results in ECM remodeling, which could stimulate cancer cell growth and migration. Therefore, myofibroblasts appear to play a key role in creating the tumor-promoting reactive stroma environment.

CD10 is a 90- to 110-kDa cell surface zinc-dependent metalloprotease that has been called neutral endopeptidase (NEP) (EC 3.4.24.11), enkephalinase, neprilysin and common acute
lymphoblastic leukemia antigen (11–13). Physiologically, CD10 is widely expressed in various tissues, e.g. granulocytes, lymphoid progenitor cells, enterocytes, placental trophoblast, granular epithelium of prostate and gallbladder, myoepithelial cells, Schwann cells and renal tubules epithelium (14,15). CD10 is known to be useful for the categorization of acute leukemias and the subclassification of malignant lymphomas (16). In addition, CD10 has been demonstrated to be expressed by the stromal cells of the normal bone marrow and endometrium (17,18). Recent reports indicate that CD10-positive stromal cells belong to the myofibroblast group, are correlated with poor prognosis in breast carcinoma, and are also involved in colorectal carcinogenesis (19,20). However, association between CD10-positive stromal cells and invasion and metastasis in gastric carcinoma have not been reported.

In this study, we aimed to immunohistochemically investigate the correlation between CD10-positive stromal cells and invasion and metastasis of gastric carcinoma, especially focusing on cases with differentiated carcinoma.

SUBJECTS AND METHODS

PATIENTS

Retrospective analysis was performed on gastric carcinoma patients who had undergone gastrectomy between August 2003 and July 2004 in our department of Nanjing Jinling Hospital. The mean age of the patients was 57 years, ranging from 31 to 81 years. There were 82 males and 34 females (2.41:1). According to tumor differentiation, there were 54 (46.6%) differentiated tumors and 62 (53.4%) undifferentiated tumors. The depth of tumor invasion was classified as submucosa, muscularis propria, serosa and subserosa, and there were seven (6.0%), 46 (39.7%), 38 (32.7%) and 25 (21.6%) patients, respectively, with each type. Seventy-three (61.1%) patients were positive for lymph node metastasis. There were 24 (20.7%) patients with vessel invasion (blood vessel or lymphatic vessel or both) and 92 (79.3%) without. According to TNM stage, there were nine (7.7%) stage I, 57 (49.1%) stage II, 41 (35.3%) stage III and nine (7.7%) stage IV patients. Of 54 differentiated carcinomas, there were three (5.6%) submucosa, 27 (50.0%) muscularis propria, 15 (27.8%) serosa and nine (16.7%) subserosa invasion. Thirty-three (61.1%) patients were positive for lymph node metastasis, while 21 (38.9%) were negative. There were nine (16.7%) patients with vessel invasion and 45 (83.3%) without. According to TNM stage, there were three (5.5%) stage I, 17 (31.5%) stage II, 32 (59.3%) stage III and two (3.7%) stage IV patients. No adjuvant radiotherapy or chemotherapy was administered before surgery. Multiple sections were examined microscopically to confirm the degree of differentiation, the depth of invasion, lymph node metastasis, vessel involvement and TNM stage. One representative block was then selected for immunohistochemical study.

IMMUNOHISTOCHEMISTRY

Immunoperoxidase staining of formalin-fixed, paraffin-embedded tissue sections was performed using an ordinary biotin–streptavidin method. Briefly, the sections were deparaffinized in xylene. After hydration, the sections were heated in a pressure cooker for 5 min in 10 mM citrate buffer (pH 6.0) and washed with phosphate-buffered saline (PBS, pH 7.3). The sections were then immersed in 0.3% hydrogen peroxide (H2O2) in methanol for 20 min at room temperature to block endogenous peroxidase activity. After non-specific sites had been blocked with 10% normal calf serum in phosphate-buffered saline (PBS) for 10 min, one section was incubated with an anti-CD10 mouse monoclonal antibody (56C6, Novo Castra, Newcastle, UK) diluted to 1:40, another with anti-α-smooth muscle actin mouse monoclonal antibody (1A4; Dako, USA) diluted to 1:100 overnight at 4°C in a humid chamber. After incubation with the secondary antibody and avidin–biotin complex reagent, the color reaction was developed in 0.02% 3,3′-diaminobenzidine (DAB) hydrochloride and 0.02% H2O2 in Tris buffer pH 8.0. Hematoxylin was used for counterstaining. In the immunohistochemical staining, we performed additional staining without primary antibody in parallel to ascertain that no staining was seen.

EVALUATION OF IMMUNOSTAINING

When more than 10% of the stromal cells around the neoplastic tubules or glands were positive for CD10, the expression was judged to be positive.

STATISTICAL ANALYSIS

Correlation between CD10 expression of stromal cells and clinicopathological factors was evaluated using the chi-squared test or Fisher’s exact test. P-values <0.05 were considered to be significant.

RESULTS

IMMUNOSTAINING ANALYSIS OF CD10 EXPRESSION BY THE STROMAL CELLS IN NORMAL MUCOSA AND TUMOR

Among the 116 tumor samples, we examined 20 normal mucosa (≥10 cm distant from cancer), which showed no presence of enlarged proportion of nuclear/cytoplasm, hyperchromatism and loss of polarity, and 38 dysplastic mucosa, which showed the enlarged proportion of nuclear/cytoplasm, hyperchromatism and loss of polarity, for CD10 expression by the stromal cells, using immunostaining. Stromal CD10 expression was confined to the spindle cells around the neoplastic tubules or glands (Fig. 1A and B), especially at the invasion front. As shown in Table 1, 0% (0/20) were positive for stromal CD10 expression in normal gastric mucosa and 8% (3/38) were positive for stromal CD10 expression in dysplastic mucosa. In contrast, however, 19.0% (22/116) exhibited stromal CD10 expression in gastric carcinomas. The frequencies of CD10
expression by the stromal cells were increased markedly from normal to tumor tissue.

**Correlation Between Stromal CD10 Expression and Invasion and Metastasis**

Differentiated histology, subserosa invasion, lymph node metastasis and vessel invasion were positively correlated with stromal CD10 expression. According to TNM stage, III and IV stage gastric carcinomas seemed to display more positive stromal CD10 expression than I and II stage carcinomas, but the difference according to the stage was not statistically significant (P > 0.05) (Table 2).

**Correlation Between Stromal CD10 Expression and Invasion and Metastasis in Well-differentiated Carcinoma**

Of the 54 differentiated carcinomas, serosa and subserosa invasion, lymph node metastasis and vessel invasion were positively correlated with stromal CD10 expression. According to TNM stage, III and IV stage gastric carcinomas had more positive stromal CD10 expression than I and II stage carcinomas (Table 3).

**Distribution of the Stromal Cells with CD10 Expression and those Expressing α-smooth Muscle Actin**

To determine whether the stromal cells positive for CD10 are the same cells as those expressing α-smooth muscle actin, we performed immunohistochemistry of CD10 and α-smooth muscle actin on the serial sections of gastric carcinoma. We found that α-smooth muscle actin was expressed in the stromal cells and in the smooth muscle cells of the vessel walls, and CD10 was positive in the stromal cells and granulocytes. The distribution of CD10-positive stromal cells corresponded to that of α-smooth muscle actin-positive stromal cells (Fig. 2A and B). Fifty-nine cases of gastric carcinoma were positive for

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**Table 1.** Expression of CD10 by the stromal cells in normal and displastic gastric mucosa, and cancer

<table>
<thead>
<tr>
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<th>CD10 positive (%)</th>
<th>CD10 negative (%)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Normal mucosa</td>
<td>0 (0)</td>
<td>20 (100)</td>
<td></td>
</tr>
<tr>
<td>Dysplasia mucosa</td>
<td>3 (8)</td>
<td>35 (92)</td>
<td>0.014</td>
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<tr>
<td>Cancer</td>
<td>22 (19)</td>
<td>94 (81)</td>
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**Table 2.** Expression of CD10 by the stromal cells in relation to differentiation, depth of invasion, metastasis, vessel involvement and TNM stage in patients with gastric cancer

<table>
<thead>
<tr>
<th></th>
<th>CD10 positive (%)</th>
<th>CD10 negative (%)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Histological type</td>
<td></td>
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<tr>
<td>Differentiated</td>
<td>20 (37)</td>
<td>34 (63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>2 (3)</td>
<td>60 (97)</td>
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<td>Depth of invasion</td>
<td></td>
<td></td>
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<tr>
<td>Submucosa</td>
<td>1 (14)</td>
<td>6 (86)</td>
<td>0.003</td>
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<tr>
<td>Muscularis propria</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Serosa</td>
<td>7 (18)</td>
<td>31 (82)</td>
<td></td>
</tr>
<tr>
<td>Subserosa</td>
<td>11 (44)</td>
<td>14 (56)</td>
<td></td>
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<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19 (26)</td>
<td>54 (74)</td>
<td>0.011</td>
</tr>
<tr>
<td>Negative</td>
<td>3 (7)</td>
<td>40 (93)</td>
<td></td>
</tr>
<tr>
<td>Vessel invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13 (54)</td>
<td>11 (46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>9 (10)</td>
<td>83 (90)</td>
<td></td>
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<td>TNM stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II</td>
<td>10 (15)</td>
<td>56 (85)</td>
<td>0.229</td>
</tr>
<tr>
<td>III, IV</td>
<td>12 (24)</td>
<td>38 (76)</td>
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a-smooth muscle actin, which included 22 cases of tumors with CD10-positive expression.

**DISCUSSION**

Tumorigenesis is a multistep process accompanied by genetic alterations of precancerous cells and, simultaneously, by building up of the microenvironment that promotes transformation (21–23). Consequently, epigenetic contributions from stromal cells surrounding cancer cells play important roles in the formation of progressive neoplasm (24–26). Previous studies have indicated that this new reactive stroma environment enhances tumorigenesis by supporting cancer cell survival, proliferation and migration, and also by inducing angiogenesis. The most common marker of reactive stroma in cancer is the appearance of activated stromal cells with myofibroblastic characteristics. Many studies have shown that the invasive potentials of several types of cancer cells are regulated through tumor–stromal interactions, which involve stimulatory and inhibitory factors that regulate such functions as cellular adhesion, migration, and gene expression (27–29).

In vitro studies have demonstrated that tumor-activated myofibroblasts may prevent physical contact between cancer cells and immune cells, the essential effectors of cancer cell destruction by the host defense system (30). In colorectal cancer, myofibroblasts have been reported to proliferate at the invasive front, which can alter the adhesive and migratory properties of colon carcinoma cells (31). Our study shows that the distribution of CD10-positive cells corresponds to that of the stromal cells expressing α-smooth muscle actin. This suggests the possibility that CD10-positive stromal cells and myofibroblasts in the invasive front are the same cells, because α-smooth muscle actin has become the marker most often used to identify myofibroblasts by immunohistochemistry (32).

To date, frequent CD10 expression has been found in renal cell carcinoma, hepatocellular carcinoma, non-small cell lung cancer, pancreatic solid pseudopapillary tumor, urinary bladder and prostate carcinoma, gestational trophoblastic diseases (GTDs), breast, colon carcinoma and endometrial stromal sarcoma (14,33–35). Most studies measured epithelial CD10 expression by immunohistochemistry and cDNA array, but few studies measured stromal CD10 expression by immunohistochemistry. Iwaya et al. (18), investigating CD10 expression by the stromal cells in 123 cases of breast cancer by immunohistochemistry, showed that 18% of tumors exhibited stromal CD10 expression, which was undetectable in all non-invasive ductal carcinomas or normal breast tissue. They also showed that the frequency of positive CD10 stromal staining was positively correlated with the axillary lymph-node metastasis and prognosis. These results suggest that stromal expression of CD10 is an important novel prognostic factor in...
breast cancer. Ogawa et al. (19) also showed that the stromal expression of CD10 is an integral part of colorectal carcinogenesis. However, stromal expression of CD10 in gastric carcinoma has not been reported so far.

The present study showed that CD10 was overexpressed in patients with primary gastric cancer compared with dysplasia and normal gastric mucosa. We demonstrated a significant correlation between stromal CD10 expression and differentiation, invasion, metastasis and vessel invasion. Stromal CD10 expression, however, did not show any significant correlation with TNM stage. This suggests that CD10 expression by the stromal cells may play an important role in the pathogenesis of gastric cancer and also that the proliferation of CD10-positive stromal cells is part of the mechanism of invasive growth and metastasis in gastric cancer. In addition, we found frequent stromal expression of CD10 with differentiated cancer, which suggests that stromal cell fibroblast differentiation is necessary for the invasion and metastasis of differentiated cancer cells.

Sato et al. (35) examined CD10 expression by the epithelial cells in the malignant and adjacent non-invaded tissues of the human stomach and colon (n = 27). They showed that all of the 27 normal and 18 well- or moderately differentiated adenocarcinoma tissue specimens were positive for CD10, whereas the expression level was clearly decreased in all of the nine specimens of poorly differentiated adenocarcinoma. These findings suggest that CD10/NEP is expressed in normal epithelial cells of the human stomach and colon, whereas its expression level is decreased in the poorly differentiated adenocarcinoma. The present study has shown that CD10-positive epithelial cells were higher in differentiated adenocarcinoma than in undifferentiated adenocarcinoma (data not shown). The findings suggest that the tumor cells can degrade extracellular matrix by secreting CD10 and promote invasion and metastasis in differentiated adenocarcinoma. To further determine the role of stromal expression of CD10 in differentiated carcinoma, we also analyzed the correlation between stromal CD10 expression and invasion and metastasis. We have shown, based on our results on the relationship between CD10 expression in the differentiated carcinoma and the clinicopathological variables, that invasion, lymph node metastasis, vessel invasion and TNM stage correlate with CD10 expression. These findings strongly suggest that stromal expression of CD10 promotes invasion and metastasis in differentiated carcinoma.

Recently, Pan et al. (36) demonstrated that CD10 is capable of cleaving CPI-0004Na and related peptide prodrugs such as N-succinyl-b-alanyl-L-isoleucyl-L-alanyl-L-leucyl-Dox (sAJAL-Dox), which have an improved antitumor efficacy profile with reduced toxicity compared with Dox. Therefore, our data may be applied to a new cancer therapy that blocks the induction of CD10-positive stromal cells in gastric cancer tissue. This approach may reduce the activities of the CD10-positive stromal cells, which accelerate tumor aggressiveness. Further studies on the molecular basis of CD10 expression in stromal–cancer interaction will be required to pursue such new therapeutic strategies.

In conclusion, our data demonstrate that stromal expression of CD10 in primary gastric cancer is closely correlated with invasion and metastasis and it may play an important role in the pathogenesis of gastric cancer. Although CD10 expression appears to be an integral part of gastric cancer carcinogenesis, its precise mechanisms and significance remain undetermined. Further study in animal models will probably clarify the significance of CD10 expression in the progression of gastric cancer.

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References


