Osteonectin-expressing cells in human stomach cancer and their possible clinical significance

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Abstract

The clinical significance of osteonectin in human stomach cancer was examined immunohistochemically and molecular biologically in 31 differentiated and eight undifferentiated stomach adenocarcinomas and 19 non-cancer stomach tissues. Osteonectin-mAb-stained cells were observed in stroma of 90% differentiated and 63% undifferentiated adenocarcinomas, and of 26% non-cancer stomach tissues. Competitive reverse transcriptase polymerase chain reaction results generally coincided with immunohistochemical data. The present results suggest that osteonectin is highly expressed in reactive stroma associated with invasive differentiated adenocarcinomas and that it may serve as a useful clinical diagnostic marker for stomach cancer. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Stomach cancer is still a leading cause of cancer mortality in the world including countries such as Japan and Korea [1]. Several factors are suspected to play a role in stomach carcinogenesis including diet, exogenous chemicals, intragastric synthesis of carcinogens, infectious agents, pathological conditions in the stomach and genetic factors [2]. However, the molecular mechanisms involved in the development of stomach cancer remain unelucidated. Therefore, molecular characterization of stomach cancer tissues is useful for investigation of these mechanisms, and may lead to clues for cancer prevention, diagnosis and therapies.

We have previously studied changes in the initiation stage of experimental rat stomach carcinogenesis [3,4]. Recently, we found that osteonectin-expressing fibroblastic cells appeared in the stomach pyloric mucosa in the initiation stage of experimental rat stomach carcinogenesis and in stomach tumors.
induced with N-methyl-N\textsuperscript{0}-nitro-N-nitrosoguanidine but not in normal tissue (unpublished). The results suggest that osteonectin plays a role in stomach tumor development. Osteonectin is a non-structural component of extracellular matrix-associated matriclellular glycoprotein. It is overexpressed and down-regulated in several human cancers [5–8], however, little is known about its dynamics in stomach cancer [9].

In the present study, which aimed to investigate the clinical significance of osteonectin in human stomach cancer, osteonectin expression in a number of human stomach cancer tissues and non-cancer stomach tissues was examined immunohistochemically and molecular biologically. A high level of osteonectin was detected in reactive stroma associated with invasive differentiated adenocarcinoma, suggesting that osteonectin can be used as a prognostic marker for stomach adenocarcinoma.

### 2. Material and methods

#### 2.1. Tissue specimens

A total of 58 specimens from 39 stomach cancer patients were used in this study. All samples were collected at the Chungnam National University Hospital, Taejon, South Korea. Nineteen non-cancer stomach tissues were obtained together with the stomach adenocarcinoma specimens from the same 19 patients; the remaining 20 patients contributed only stomach adenocarcinoma specimens. The clinical stages of the malignancies were classified according to WHO classification [10].

#### 2.2. Immunohistochemistry

Tissues were frozen in OCT compound (Miles Inc., Elkhart, IN, USA) and 10 \( \mu \)m sections were prepared on a cryostat. Immunohistochemical staining of osteonectin was performed using human osteonectin mAb (Biodesign Co., Kennebunk, ME, USA). The statistical significance of differences between values in Table 1 was analyzed by \( \chi^2 \) test.

#### 2.3. RNA isolation and competitive RT-PCR

Total RNA was extracted from 27 stomach adenocarcinomas and nine non-cancer stomach tissues using TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD, USA). Competitive reverse transcriptase polymerase chain reaction (RT-PCR) with the specific primers and the first-strand cDNA was performed at high stringency. The amplified cDNAs were examined on 1% agarose gel and stained with ethidium bromide. The target osteonectin band was estimated by the ratio to the competitor. The specific oligonucleotide primers for osteonectin were as follows: 5\textsuperscript{'}-CCTGATGAGACAGAGGTGGTGG-3\textsuperscript{'} (5\textsuperscript{'} primer) and 5\textsuperscript{'}-GCTCAGAGTCCAGGCAAGG-3\textsuperscript{'}.
GG-3' (3' primer) (406 bp). The competitor for osteonectin was prepared by RT-PCR with 5' primer for osteonectin and with 43 mer-3' primer 5'-GCTCAGTCCAGGCAAGGGCCCTCCAGGTGCAC-TTTGTGGC-3' (351 bp).

3. Results

3.1. Osteonectin-mAb-stained cells in the stroma

Table 1 shows the summarized results of osteonectin-mAb-staining in 31 differentiated and eight undifferentiated stomach adenocarcinomas and 19 non-cancer stomach tissues. Dense immunohistochemical staining was observed in 85% (26 out of 31) of the differentiated adenocarcinomas, while it was observed in only 25% (two out of eight) of the undifferentiated adenocarcinomas and in 5% (one out of 19) of the non-cancer stomach tissues from stomach cancer patients. Positive staining, which included dense and sparse staining, was observed in 90% (28 out of 31) of the differentiated adenocarcinomas, in 63% (five out of 8) of the undifferentiated adenocarcinomas and in 26% (five out of 19) of the non-cancer stomach tissues. Therefore, it appears that osteonectin expression is related to the degree of differentiation of stomach cancer.

Osteonectin protein was not detected in malignant cells in any specimen. Instead, elevated levels of staining were detected in the stromal cells of differentiated adenocarcinoma specimens (Fig. 1A), particularly at the stromal-malignant cell interface. This was less often detected in undifferentiated adenocarcinomas (Fig. 1B) and much less often detected in non-cancer specimens (Fig. 1C). Immunoreactivity was also evident throughout the stromal matrix, which is consistent with the staining pattern expected for a secreted glycoprotein (Fig. 1A).

3.2. Osteonectin gene expression

Fig. 2 shows results of competitive RT-PCR of osteonectin in 22 differentiated and five undifferentiated adenocarcinomas and nine non-cancer stomach tissues. Increased gene expression was observed in 91% (20 out of 22) of the differentiated adenocarcinomas and in 60% (three out of five) of the undifferentiated adenocarcinomas and in 33% (three out of five).
nine) of the non-cancer tissues. Competitive RT-PCR results generally concurred with the immunohistochemical results.

4. Discussion

In this study we examined osteonectin expression in a considerable number of human stomach cancer tissues and non-cancer stomach tissues. Osteonectin-expressing cells were usually observed in differentiated adenocarcinomas (90%) and further up-regulated in the stroma associated with malignant cells. On the other hand osteonectin-expressing cells were less often observed in undifferentiated adenocarcinomas (63%) and much less often observed in non-cancer stomach tissues from stomach cancer patients (26%). These data suggest that appearance of osteonectin-mAb-stained cells in the stroma may parallel the development of differentiated human stomach cancer. Based on the statistical analysis (Mantel–Haenszel test, \( P > 0.05 \)), the expression of osteonectin in gastric carcinoma was not correlated to clinical stage and lymph node metastasis. Changes in cells in the stroma are suggested to be important in the development of stomach cancer. Specimens of human gastric carcinoma tissue and adjacent morphologically normal tissue were collected at the Chungnam National University Hospital. Adjacent morphologically normal tissues were collected at 10–15 cm far from carcinoma tissue and did not manifest any histological characteristics of transitional mucosa. No clear differences were observed between osteonectin-positive case and osteonectin-negative case in non-neoplastic gastric mucosa. Although examination of normal stomach mucosa from persons without stomach cancer is desirable for research, such specimens were unavailable for the present study. As we observed that osteonectin expression was low or non-discriminable in normal rat stomach pyloric mucosa (unpublished data) and was low in the present non-cancer tissue of human stomach cancer patients, it is probable that osteonectin expression in normal human stomach pyloric mucosa is at least as lower as that in the non-cancer stomach tissues reported here.

In recent studies, we found that osteonectin-expressing fibroblastic cells appeared in the stroma of stomach pyloric mucosa in the initiation stage of experimental rat stomach carcinogenesis and of stomach tumors induced with \( N \)-methyl-\( N' \)-nitro-\( N \)-nitrosoguanidine (unpublished data). Osteonectin expression was elevated in all 12 examined differentiated adenocarcinomas and adenomas. The present results are consistent with these rodent data. The present human results support our previous suggestion that osteonectin plays a role in stomach tumor
development. In the present human study, cell type of osteonectin expressing cells could not be defined.

Wewer et al. [9] reported osteonectin expression in only six human stomach adenocarcinomas. They noted that osteonectin was localized in the tumor basement membrane in well-differentiated stomach adenocarcinomas and that the cytoplasmic area was stained in undifferentiated stomach adenocarcinomas. They also noted osteonectin-Ab staining of both tumoral and stromal cells. However, we did not observe any osteonectin-mAb-stained tumor basement membrane or tumoral cells. We observed stained stromal cells in both differentiated and undifferentiated stomach carcinomas, with a lower frequency in undifferentiated stomach adenocarcinomas.

Some other extracellular matrix proteins, such as osteopontin [11], laminin, collagen IV and fibronectin [12] are also overexpressed in human stomach cancer. Laminin and fibronectin were highly expressed in differentiated adenocarcinoma while other matrix proteins were moderately expressed. These proteins were hypothesized to be involved in the progression and invasion of cancer cells. However, our results showed that osteonectin-mAb-stained cells were present ubiquitously in the stroma, and suggest that these cells play a role not only in the progression and invasion of cancer cells but also in the development of cancer.

In conclusion, the present study demonstrated that osteonectin is highly expressed in reactive stromal cells associated with malignant cells in differentiated human stomach adenocarcinomas. It is proposed therefore that osteonectin is a potential clinical diagnostic marker for stomach cancer. Further studies should elucidate the importance of osteonectin in malignant transformation and clarify its molecular targets.

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References