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Expression of osteopontin and CDX2: Indications of phenotypes and prognosis in advanced gastric cancer

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Abstract. We have investigated the expression of osteopontin (OPN) and CDX2 in advanced gastric cancers, and analyzed correlations with clinicopathological features to assess their prognostic potential. One-hundred and nine patients suffering from gastric cancer were recruited. Expression of OPN and CDX2 and other molecular markers was determined by immunohistochemistry. The total positive rate for OPN expression was 46.8%, with a relation to depth of cancer invasion and down regulation of intestinal markers (P<0.001), but not age, gender, or histological type. OPN was more frequently expressed in CDX2-negative (39/109=35.7%) as compared with positive lesions (12/109=11.0%) and a significant reverse correlation was noted between the two factors (P<0.001). Patients with positive OPN tumors had worse 5-year survival than those with OPN-negative cancer (P<0.001). Further analysis revealed the $OPN^-/CDX2^+$ group to have better 5-year survival than all the other three groups: $OPN^+/CDX2^-$, $OPN^-/CDX2^-$ and $OPN^+/CDX2^+$. With multivariate analysis for 5-year survival, OPN was the most significant predictor of a poor prognosis of advanced gastric cancer (P=0.0043), with tumor depth of invasion as another independent indicator (P=0.0315). Osteopontin is a useful prognostic marker in gastric cancer, and combined with CDX2, may have particular advantage for predicting survival of advanced gastric cancer patients. Furthermore the present results provide a clue that in gastric cancer, CDX2 may be a transcription factor modulating the expression of osteopontin.

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Introduction

Gastric cancer (GC) is one of the most aggressive tumors and the second leading cause of cancer mortality worldwide (1). Finding a useful molecular marker to predict malignant potential is therefore of great importance. Although many molecular indicators have been reported (2,3), there are still problems with accurate prediction. Osteopontin (OPN), a 34 kDa extracellular matrix glycophosphoprotein with a cell-binding domain, plays multifunctional roles in cell adhesion, chemotaxis, macrophage-dependent angiogenesis, prevention of apoptosis, and anchorage-independent growth of tumor cells. Its activity regulates cell-matrix interactions and cellular signaling through binding to integrin and CD44 receptors (4-6). It has been widely reported to demonstrate altered expression in relation to tumorigenesis, invasion, metastasis and its expression may have prognostic potential in colon (7), lung (8), prostate (9), and breast cancers (10). Overexpression correlated with poor prognosis in gastric cancer has also been reported (11,12).

In previous studies, others and our group have demonstrated that expression of the caudal-related homeobox gene (CDX) 2 is strongly associated with an intestinal phenotype in gastric cancer (13,14), providing a useful prognostic marker for intestinal and gastrointestinal phenotypic gastric tumors with good outcomes (15,16). To further probe useful indicators for gastric cancer survival, in the present retrospective study we examined the expression of OPN and CDX2 in 109 advanced gastric cancer surgical specimens, and analyzed correlations with clinicopathological factors. A particular focus was on links between OPN and CDX2 expression and the different phenotypes of gastric cancer.

Materials and methods

Patients and tumor specimens. All 109 cases of primary advanced gastric cancer were surgically resected at Aichi Cancer Center Hospital, Nagoya, Japan, between 1994 and 1996 after obtaining informed consent. The patients were 63 males and 46 females and the mean age was 62.43±10.12 years.

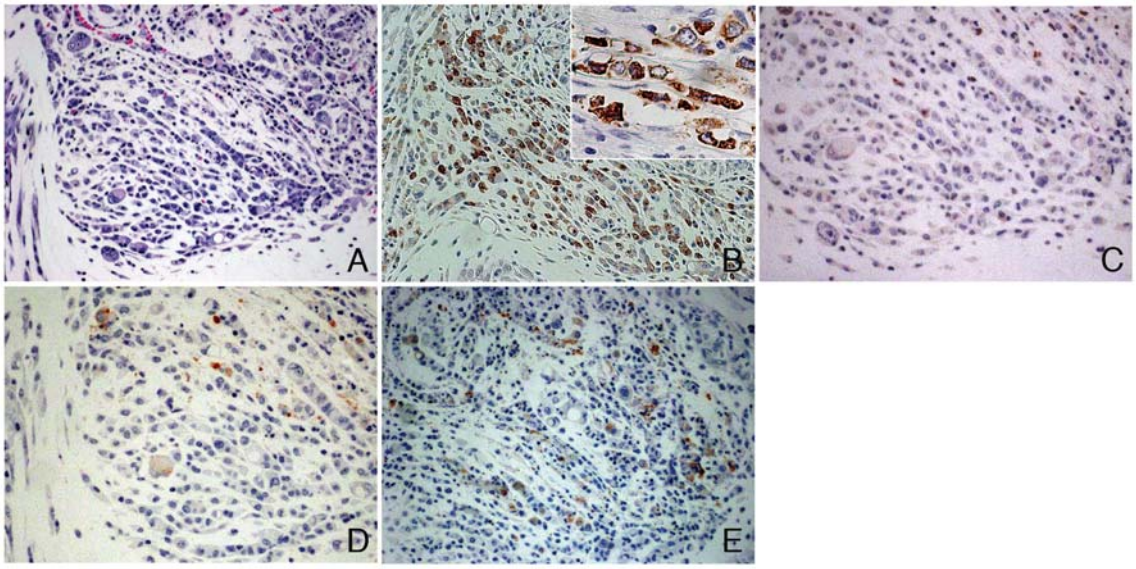

Figure 1. Poorly differentiated gastric adenocarcinomas. (A) H&E staining. (B-E) Immunohistochemical analysis. Osteopontin (B) is present in the cytoplasm, CDX2 (C) and MUC2 (D) being barely detected in the same area. MUC5AC (E) is weakly positive. Original magnification, x200. Inset of B, x400.

None had received preoperative chemotherapy or radiotherapy before surgery. All specimens were fixed in 10% buffered formalin. Carcinomas with adjacent mucosa tissue were serially cut into 3-mm slices and embedded in paraffin, and then thin-sectioned and stained with hematoxylin and eosin for histological examination.

Immunohistochemistry. We examined expression of MUC5AC, MUC6, MUC2, and villin in carcinoma cells by immunohistochemistry, as previously described in detail (17,18). Briefly, 3 μm-thick consecutive sections were deparaffinized and hydrated through a graded series of ethanols. After inhibition of endogenous peroxidase activity by immersion in 3% H_2O_2/methanol solution, antigen retrieval was carried out with 10 mM citrate buffer (pH 6.0) in a microwave oven for 10 min at 98˚C. Then, sections were incubated with the primary antibodies. After thorough washing in phosphate-buffered saline (PBS), incubation with biotinylated secondary antibody was performed, followed by exposure to avidin-biotinylated horseradish peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA, USA). Finally, immune-complexes were visualized by incubation with 0.01% H_2O_2 and 0.05% 3,3'-diaminobenzidine tetrachloride (DAB). Nuclear counterstaining was accomplished with Mayer's hematoxylin. We also examined expression of CDX2 using an anti-CDX2 monoclonal antibody (BioGenex, San Ramon, CA, USA) and expression of osteopontin with anti-osteopontin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) using the same immunohistochemical approach. The results of antibody staining were evaluated with reference to the percentage of positively stained cancer cells. A result was considered positive if at least 10% of the cells were stained. The results were evaluated by two of the authors (X.Z. and T.T.) without any previous knowledge of the clinical information for each patient.

Classification of phenotypes. As we previously reported, MUC5AC and MUC6 are markers of gastric epithelial cells, whereas MUC2 and villin are typical of the intestinal epithelial cell phenotype (19,20). In gastric cancers, if >10% of the section area expresses at least one of the markers specific for gastric or intestinal phenotypes classification is made as gastric (G type) or intestinal (I type), respectively. Those which show both gastric and intestinal phenotypes are classified as gastric-and-intestinal-mixed phenotype (GI type) cancers, while those showing neither expression are grouped as null type (N type).

Tumor staging. Classification of tumor staging was made according to the Japanese Classification of Gastric Carcinomas (21). The cancers had invaded the muscularis propria (T2 for TNM classification), the subserosa (T2), or the serosa and the peritoneal cavity (T3), sometimes including the adjacent organs (T4).

Statistical analysis. The data were analyzed by Fischer's exact test or χ^2-test for differences between groups using StatView statistical software (ver. 5, SAS Institute, Inc., Cary, NC, USA). To determine the relative survival of patients, the Cox's proportional-hazards regression model was used, and survival curves after surgery were drawn using the Kaplan-Meier method. Statistical comparison of survival was performed using the log-rank test. $P<0.05$ were considered statistically significant.

Results

OPN expression in GC tissues and correlation with clinicopathological factors. The follow-up period of the patients ranged from 4 to 96 months. Among all 109 cases, the positive OPN immunohistochemistry expression rate was 46.8% (51/109) (Table I). OPN was frequently expressed in the cytoplasm of gastric tumor cells (Fig. 1) and commonly found to be most intense in the margins of tumor tissue or in tumor cells invading into the muscle layer or serosa. OPN expression was significantly related with tumor depth

)PN expression and clinicopathological factors.

Clinicopathological data	n=109	OPN expression		P-values
		Positive (n=51)	Negative (n=58)	
Gender				
Male	63	21	42	NS
Female	46	17	26	
Age				
Years (means ± SD)		63.34±10.17	61.78±11.96	NS
Histological classification				
Differentiated	58	43	15	NS
Undifferentiated	51	36	15	
Phenotypes				
G	18	16	2	P<0.001
GI	19	7	12	
I	40	7	33	
N	32	21	11	
Cdx2				
Positive	57	12	45	P<0.001
Negative	52	39	13	
Depth of invasion				
T1	31	4	27	P<0.001
T2-T3	52	21	21	
T4	36	26	10	

(P<0.001), positive rates being 12.9, 50.0 and 72.2% for T1, T2-T3, and T4, respectively (P<0.001). However, OPN expression did not correlate with patients' age, gender, or histological status (Table I).

Expression of gastric and intestinal epithelial cell markers in gastric cancers. Expression of MUC5AC, MUC6, MUC2, and villin were judged positive in 54 (49.5%), 29 (26.6%), 51 (48.1%), and 47 (43.1%) cases, respectively. Taking into account the combinations of expression of these four markers, the 109 gastric cancers were divided phenotypically into 18 G, 19 GI, 40 I, and 32 N types, independent of the histological classification (Fig. 1 and Table I).

OPN expression in different phenotypes of GC and its correlation with CDX2. OPN was mainly expressed in G and N type (16 and 21 cases, respectively) and less expressed in GI and I type (7 cases each) (P<0.001) (Table I). Based on these results and our previously published data (15) for advanced GC with intestinal phenotypic expression, CDX2 is a useful marker of a good prognosis. Further analysis of the correlation of OPN and CDX2 expression showed only 12 of 51 OPN-positive to be CDX2 expression-positive. However, of 58 OPN-negative cases, 45 demonstrated binding of the CDX-2 antibody. Thus an inverse correlation was observed (P<0.001).

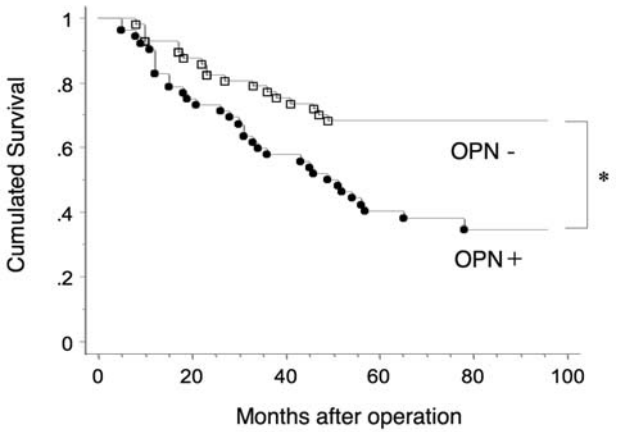

Figure 2. Kaplan-Meier cumulated survival curves for the 109 GC patients with reference to OPN expression. *P<0.001.

In almost all GCs, areas with OPN-positive expression were CDX2-negative (Fig. 1 and Table I).

Postoperative survival analysis of GCs with reference to OPN and CDX2 expression. Among the 109 cases of GC, the 5-year survival rates with OPN-negative and -positive lesions were 77.6 and 25.5%, respectively. From the Kaplan-Meier

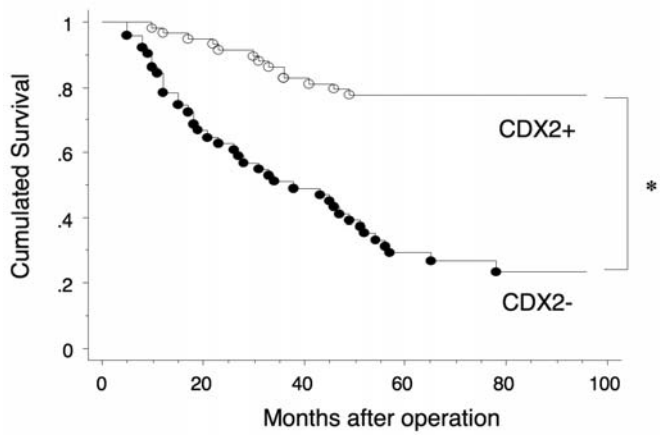

Figure 3. Kaplan-Meier cumulated survival curves for the 109 GC patients with reference to CDX2 expression. *P<0.001.

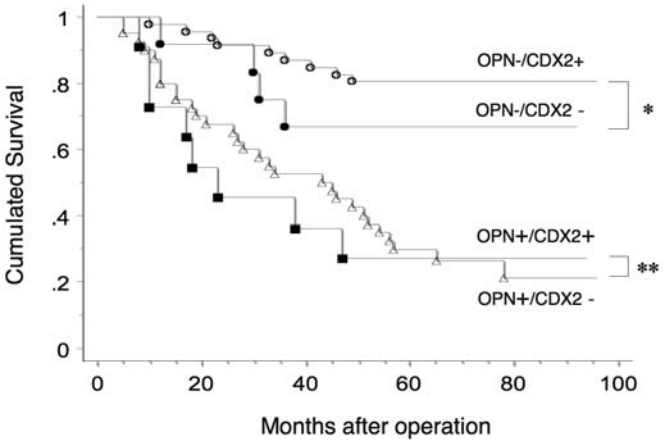

Figure 4. Kaplan-Meier cumulated survival curves for the 109 GC patients with reference to OPN and CDX2 expression. *P=0.013 ($OPN^-/CDX2^+$ vs. $OPN^-/CDX2^-$); **P=0.093 ($OPN^+/CDX2^+$ vs. $OPN^+/CDX2^+$).

survival curve analysis, the patients with OPN-negative expression had the better overall survival (P<0.001) (Fig. 2). Fig. 3 shows that the 5-year survival rates with CDX2-positive and -negative expression were 68.4 and 36.5%, respectively (Kaplan-Meier survival curve analysis, P=0.0025). Furthermore, 5-year survival rates for $OPN^-/CDX2^+$, $OPN^-/CDX2^-$, $OPN^+/CDX2^+$, and $OPN^+/CDX2^-$ were 80.4, 66.7, 27.2 and 25.2%, respectively (Fig. 4). The patients with $OPN^-/CDX2^+$ cancers had a better 5-year survival outcome than the other three groups (P<0.01). The 5-year survival of the $OPN^-/CDX2^-$ group was better than that of the $CDX2^-$ (P=0.024) or $CDX2^+$ group (P=0.028) with OPN^+ expression. However, in $OPN^+/CDX2^-$ and $OPN^+/CDX2^+$ groups, the difference in 5-year survival was not significant (P=0.093) (Fig. 4).

Multivariate analysis for overall survival of GC cases. Using the Cox' proportional hazards regression model, we performed multivariate analysis of clinicopathological variables, including the patient age, gender, tumor histological classification, phenotypic classification, tumor depth, and OPN and CDX2 expression. This revealed OPN to be the most independent factor for 5-year overall survival (P=0.0043). Tumor depth of invasion was also an independent indicator (P=0.0315). CDX2 expression status, patient age, gender, phenotypic type, and histological status were not independent factors for overall survival of gastric cancer cases (P>0.05).

Discussion

OPN is a highly modified integrin-binding extracellular matrix glycophosphoprotein produced by cells of the immune system, epithelial tissue, smooth muscle cells, osteoblasts, and tumor cells. Although extensive research has elucidated pivotal roles of OPN in cell signaling relevant to inflammation, tumor progression and metastasis, and inhibition of apoptosis, the mechanisms by which OPN may enhance malignancy in gastric cancer are still unclear. Clearly, molecular binding to the cell adhesion molecules integrin and CD44 (4,22) and depletion of growth factors and cytokines (23) could be involved. Of prime importance, however, is the link with prognosis revealed by the present study, in line with the association with malignancy reported earlier (5,24,25).

CDX2 is a caudal-related homeobox transcription factor that is expressed specifically in intestine epithelial cells (15,26,27), playing a probable role in regulation of their proliferation. It is well known that both gastric and intestinal phenotypic cell markers are expressed in gastric cancers and CDX2 expression is evident in a high proportion of early intestinal-type cancers, becoming reduced with perineural invasion and lymph node metastasis. Thus CDX2 might be a useful marker in predicting the clinical outcome for patients with gastric cancers (28-30). In Mongolian gerbils, celecoxib, a cyclooxygenase-2 inhibitor, suppressed the expression of CDX2 and prevented intestinal metaplasia and gastric carcinogenesis (31). In our previous study, we also established that in mixed GI and I phenotypes of gastric cancer with CDX2 expression, the prognosis was significantly better than with G or N types harboring little CDX2 (15). We also found that in GI and I phenotypes with high CDX2 expression, the OPN expression was low, whereas in G and N types without CDX2, the OPN expression level was high.

Multivariate analysis revealed OPN as the most independent factor for survival, followed by tumor depth and lymph node metastasis. In contrast, the patient gender, age, tumor histological type, and CDX2 expression status were not independent prognostic factors. In conclusion, OPN is a very useful indicator for predicting the prognosis of gastric cancer, and combined with CDX2, might give a particularly accurate picture.

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