

Original

**Expression of Standard CD44 in Advanced Gastric Cancer :
Relationship with Metastasis to Lymph Nodes**

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Abstract : Standard CD44 (CD44s) is reported to play an important role in determining the malignant potential of various carcinomas. The aim of the present study was to evaluate CD44s expression in T2-T3 gastric cancer (Japanese Classification of Gastric Cancer stages MP, SS, SE) and the relationship between CD44s expression and clinicopathological parameters. CD44s expression was measured using immunohistochemistry in tumors from 98 patients with primary gastric cancer. Cases were categorized into two groups based on CD44s staining; the CD44s positive group had >10% positively stained tumor cells and the CD44s negative group had <10%. CD44s positivity was demonstrated in 59.1% (58/98) of tumors. CD44s expression showed no significant relationship with patient age or gender, or tumor location, size or macroscopic / microscopic classification. However, CD44s expression showed a significantly negative relationship with metastasis to lymph nodes ($p < 0.0001$). Thus, in T2-T3 gastric cancer, loss of CD44s expression suggests that metastasis of the tumor to lymph nodes is likely.

Key words : CD44s, advanced gastric cancer, lymph node metastasis

Introduction

Gastric cancer is the second most common cause of cancer related mortality worldwide¹⁾. Complete surgical resection, the only potentially curative treatment for advanced gastric cancer, can only be performed on a small subgroup of patients. In 30% to 50% of patients undergoing surgical exploration, the intent of the surgery is to cure the patient. However, almost 60% of patients who undergo an R0 curative resection relapse and die of their disease^{2,3)}.

Clinical staging is determined using TNM classification: T indicating depth of invasion of the primary tumor, N nodal metastasis, and M distant metastasis. This classification system is useful to estimate patient prognosis. Currently, endoscopic ultrasound (EUS) and computed tomography (CT) are widely used for preoperative tumor staging⁴⁾. The accuracy of

staging tumors by determining the T value has been much improved with EUS having an accuracy of between 78%–92%⁵⁻¹¹⁾, and CT between 69%–89%^{9,12-18)}.

However, the accuracy of establishing the N category is still poor; 63%–78% using EUS⁵⁻¹¹⁾ and 51%–78% using CT^{9,12-18)}. The N category estimates 5-year survival rate in patients with N0 gastric cancer as 89.4%, but 68.3% in those with N1 disease¹⁹⁾. Thus, improving the accuracy of the N value in patients with gastric cancer, could improve the accuracy of the preoperative prognosis.

The transmembrane glycoprotein molecule standard CD44 (CD44s) is a cell surface hyaluronate receptor, and is expressed in most epithelial and nonepithelial tissues. The clinical significance of CD44s expression in gastric cancer, however, remains unclear. Various studies have reported an association of CD44s expression with an advanced stages of gastric cancer and a poor prognosis²⁰⁻²³⁾, with one study reporting adverse correlation between CD44s expression and tumor progression²⁴⁻²⁹⁾.

The aim of the present study was to evaluate CD44s expression in T2-T3 gastric cancer (Japanese Classification of Gastric Cancer stages; muscularis propria MP, subserosa SS, serosa exposed SE) and the relationship between CD44s expression and clinicopathological parameters. We thought that it would be possible to improve preoperative prognostic precision by using CD44s, if a relationship would be present between CD44s and various clinicopathological factors (especially N value) in gastric cancer.

Materials and Methods

Tissue samples from 98 patients who had T2-T3 primary gastric cancer surgically resected at Showa University Hospital from 2004 through 2009 were collected. Samples were preserved in 10% formalin for 24 h and the nonnecrotic portion of the tumors was routinely processed and paraffin embedded. Representative sections were stained with hematoxylin and eosin. Various clinicopathological parameters were evaluated based on the Japanese Classification of Gastric Carcinoma (The 13th Edition, web site: <http://www.jgca.jp/PDFfiles/JCGC-2E.PDF>).

Immunohistochemistry

Three micron sections from representative blocks of each case were stained immunohistochemically using monoclonal antibody clone 2C5 (anti-CD44s; R&D Systems, Minneapolis, MN, USA) at a dilution of 1:500. The labeled streptavidin-biotin-peroxidase labeling technique was used. Sections were deparaffinized in xylene, dehydrated in descending grades (100–50%) of ethanol, and subjected to antigen retrieval (microwave, citrate pH 6.0, 25 min). After cooling to room temperature, slides were incubated to quench endogenous peroxidase activity with 1% hydrogen peroxide in ethanol for 30 min. Nonspecific immunoreactivity was blocked by incubation with normal donkey serum for 30 min each. The sections were then incubated with primary antibody at 4°C overnight. After three 5 minute

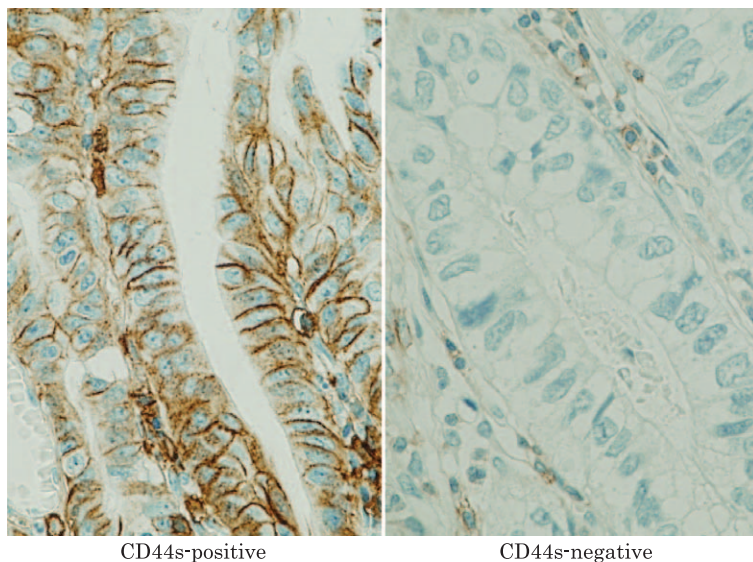


Fig. 1. Sections of gastric tumors stained using CD44s immunohistochemistry demonstrating the tumor grading system

A: Section showing more than 10% of the tumor cells with positive staining for CD44s, and so recorded as a tumor with positive CD44s expression. $\times 400$

B: Section showing lack of CD44s staining of tumor cells, and so recorded as a tumor with negative CD44s expression. $\times 400$

washes with phosphate-buffered saline (PBS), sections were incubated for 60 min with the multilink biotinylated anti-immunoglobulin. Three 5-minute washes with PBS preceded and followed treatment with streptavidin-peroxidase reagent for 30 min. The reactions were visualized with diaminobenzidine (DAKO A-S, Copenhagen, Denmark) as a chromogen. Finally, sections were counterstained with hematoxylin, dehydrated and mounted.

CD44s expression was seen as brown, fine to coarse granular staining on the cytoplasmic membrane. Our study aimed to estimate the averaged CD44s expression in the entire tumor area, and defined CD44s-positivity to be when more than 10% of the tumor cells was stained (Fig. 1). This definition was chosen as it is the most commonly used in the literature^{20, 24, 25}. And cases were categorized into two groups based on CD44s staining; the CD44s positive group had $>10\%$ positively stained tumor cells and the CD44s negative group had $<10\%$. All histological slides were examined by two experienced pathologists blinded to clinical data or disease outcome.

Statistical analysis

Association between CD44s expression and clinicopathological parameters was tested using the chi-square test, with significance set at $P < 0.05$.

Table 1 CD44s expression and clinicopathological parameters

	n = 98	CD44s expression, n (%)		p
		positive = 58 (59.2)	negative = 40 (40.8)	
Age (years)				
< 60	20	14 (70.0)	6 (30.0)	0.396
≥ 60	78	44 (56.4)	34 (43.6)	
Gender				
Male	70	40 (57.1)	30 (42.9)	0.673
Female	28	18 (64.3)	10 (35.7)	
Tumor location				
UM	56	32 (57.1)	24 (42.9)	0.789
L	42	26 (61.9)	16 (38.1)	
Tumor size (cm)				
< 5.0	50	34 (68.0)	16 (32.0)	0.108
≥ 5.0	48	24 (50.0)	24 (50.0)	
Microscopic classification				
tub	56	33 (58.9)	23 (41.1)	1.00
por, sig	42	25 (59.5)	17 (40.5)	
Invasion depth				
T2 (MP)	72	43 (59.7)	29 (40.3)	1.00
T3 (SS, SE)	26	15 (57.7)	11 (42.3)	
Lymph node metastasis (N)				
N0	38	33 (86.8)	5 (13.2)	< 0.0001
N1 / 2	60	25 (41.7)	35 (58.3)	

CD44s ; standard CD44 MP ; muscularis propria
 tub ; well differentiated type SS ; subserosa
 por, sig ; poorly differentiated type SE ; serosa exposed

Results

The characteristics of the study population are shown in Table 1. The subjects consisted of 70 men (71.4%) and 28 women (28.6%), with a mean age of 69.4 years (range, 45–91 years) and a mean gastric tumor size of 5.2 cm (range, 1.5–13.8 cm).

CD44s expression in the primary tumor was demonstrated in 59.1% (58/98) of the tumors. Associations between tumor CD44s expression and the clinicopathological parameters of patients and gastric tumors are summarized in Table 1. No statistically significant differences were found between positive CD44s expression in gastric tumors in patients aged less than 60 years (70%) compared with those 60 years or older (56.4%). No statistically significant differences were found between positive CD44s expression in gastric tumors in male (57.1%) or female patients (64.3%). No statistically significant differences were found between positive CD44s expression in gastric tumors occurring in either the lower stomach (61.9%) or the middle or upper stomach (57.1%). Similarly for tumor size, no statistically significant differences were found between positive CD44s expression in gastric tumors less

than 5.0 cm in diameter, compared with tumors 5.0 cm in diameter or greater. For microscopic tumor type, no statistically significant differences were found between positive CD44s expression in gastric tumors tub1 / tub2 (58.9%) or in por / sig (59.5%). However, strong statistical significance was found in the association between positive CD44 expression and lymph node metastases; positive expression was present in 86.8% of gastric tumors from patients with no detectable lymph node metastasis (classification N0), but in only 41.6% of patients with lymph node metastasis (classification N1 / 2) ($P < 0.0001$). Thus, CD44s expression in gastric tumors had a significantly negative relationship with the presence of lymph node metastasis in the patient.

Discussion

CD44 is a large family of cell surface transmembrane glycoproteins whose members have different extracellular domains as a result of alternative gene splicing^{30,31}. CD44s is a receptor for hyaluronate and is highly expressed on human lymphocytes. In malignant tumors originating from various organs, there is some controversy as to whether CD44s acts as a growth / invasion-promoting molecule or tumor suppression cofactor. While a number of studies have shown that CD44s expression is associated with metastasis and a poor patient prognosis²¹, others have shown that down-regulation of CD44s expression is correlated with an adverse patient outcome. For example, reduced CD44s expression has been shown to be associated with a high incidence of lymphatic or vascular infiltration in endometrial carcinoma²⁶ and breast carcinoma²⁴, and a poor prognosis in patients with pancreatic carcinoma, thyroid carcinoma, and squamous cell carcinoma of the head and neck^{27,28}. These differences in study findings may be due to the use of different antibodies, immunohistochemistry methods, patient material, and duration of patient follow-up.

Similarly, the clinical significance of CD44s expression in the progression and metastasis of malignant gastric tumor is also controversial. However, Montgomery *et al* reported that loss of CD44s expression in gastric stromal tumors was consistent with tumor aggressiveness²⁹, and Joo *et al* found a significant relationship between CD44s expression and lymph node metastasis in gastric cancer²². Further, Mayer *et al* demonstrated expression of CD44s was significantly positively associated with recurrence of gastric adenocarcinoma, distant metastasis and patient mortality²³. These findings have been speculated to be due to CD44s activation of p185 tyrosine kinase and c-Src kinase, interactions which are important in tumor progression²⁹.

Our study found an adverse relationship between CD44s expression and lymph node metastasis in T2-T3 gastric cancer. This finding may be due to the loss of CD44s expression and hence, reduced intercellular adhesion and adhesion between cells and basement membranes. This could facilitate detachment of tumor cells from their primary sites and allow infiltration of lymphatic or vascular spaces³².

The results of this study support a role for CD44s in the inhibition of metastasis of

gastric tumor cells to lymph nodes. This possible suppression of gastric tumor metastasis by CD44s may result from its role in binding gastric tumor cells to the extracellular matrix in the gastric muscularis propria and subserosal layers and thus restricting cancer cell spread.

CD44s expression may thus be a useful prognostic indicator in cases with gastric cancer, and may facilitate patient-specific tailoring of treatment options, such as the use of chemotherapy.

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