The relationship between the expressions of the cell adhesion molecule CD44H, CD44v3, and CD44v6 and metastases in gastric cancer

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ABSTRACT

Expression of CD44 variants in some tissues appears to relate to tumor progression, and particularly to the metastatic potential of some cancers. The aim of this study was to clarify the relation between the expression of CD44 splice variants and tumor metastasis by using CD44v-specific gastric cancer monoclonal antibodies. A total of 110 patients with primary gastric cancer were studied. Histological samples of 70 of the 110 (63.6%) were stained with three monoclonal antibodies directed against the CD44H and CD44 variants (CD44v3, CD44v6) in gastric cancer. The incidence of lymph node metastasis was higher in the CD44H strongerexpression group than in the weaker expression group. No significant correlation could be found between CD44H, CD44V3, or CD44v6 expression and liver metastasis or histological types (differentiated vs.undifferentiated). Lymph node metastasis correlated with CH44H rather than CD44v3 or CD44v6. These results suggested that CD44H might be a useful marker for lymph node metastasis in resected gastric cancer. The 5-year survival rate was 57.3% in the group positively expressing CD44 and 52.4% in the group with negative expression of CD44. Concerning prognosis, we found here that the expression of CD44 is not significantly associated with increased mortality. Further study of the expression of specific isoforms may help elucidate in more detail the mechanisms of these findings.

Key words: Human gastric cancer, CD44 isoforms, Metastases

INTRODUCTION

Gastric cancer is the most common cancer and one of the most frequent causes of cancer death in the World(1). Therefore, it is crucial to identify its

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presence so that the most appropriate therapy can be selected and the patient's prognosis predicted. Recently, remarkable developments in molecular biology have provided valuable techniques for assessing malignant potential more accurately. The CD44 molecule expressed on lymphocytes is important in the adhesion and homing of lymphocytes to high endothelial venules in lymph nodes(2). CD44 is known to bind hyaluronate, collagen(3), and fibronectin(4). The numerous functions and molecular interactions of CD44 probably relate to its complex structure. Gastric cancer seemed an appropriate malignancy in which to look at CD44 expression and its prognostic value for solid tumors generally because of variety of histomorphological types which differ both in their organotropism to distant metastases and in their prognosis (5).

Human tumors express a variety of CD44 isoforms(6,7). CD44 variant isoforms are also expressed differentially by leukocytes, with CD44v9 expressed at very low levels and CD44v6 and v4 virtually absent. CD44v9 expression in gastric cancer is significantly and positively associated with tumor recurrence and mortality(8). Little is known about the function or regulation of CD44 variants molecules. Expression of CD44 variants in some tissues appears to relate to tumor progression, and particularly to the metastatic potential of some cancers(9). This paper reported on the relation between the expression of CD44 splice variants and tumor metastasis assessed using CD44v-specific monoclonal antibodies.

MATERIALS AND METHODS

Patients

A total of 110 patients with primary gastric cancer were studied. The 73 men and 37 women ranged in age from 31 to 81 years (57.3 ± 10.6, mean ± SD). All patients had undergone gastrectomy at the First Department of Surgery, Sapporo Medical University between January 1975 and December 1989. The surgical procedures for 80 of the patients were considered curative on clinical and pathologic grounds, whereas 30 procedures were regarded as noncurative because metastatic tumor remained in the liver, peritoneum, or both at surgery. All patients were followed until death or until the end of the observation period (December 31, 1997). Clinicopathological features of the gastric cancers were described in accordance with the TNM classification (10) and the Lauren's category(11).

Immunohistochemical Staining

Surgically resected primary gastric cancers and their metastases were fixed in 10% buffered formalin, embedded in paraffin, and then cut into 4 μm

sections. Sections were deparaffinized in xylene, washed with phosphate-buffered saline (PBS) three times for 5 minutes, and immersed in 1% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Sections were then washed with PBS three times for 5 minutes and incubated with 30% normal bovine serum albumin (BSA) at room temperature for 60 minutes to minimize background staining. Slides from each tumor sample then were incubated with secondary biotinylated antibody (DAKO Corp., Santa Barbara, CA) for 30 minutes at room temperature. After incubation with streptavidin-biotin-peroxidase complex (Nichirei Corp., Tokyo, Japan) for 30 minutes, sections were incubated with 0.02% 3,3'-amino-9-ethylcarbazole (Wako, Osaka, Japan) in N, N-dimethylformamide for 5 to 10 minutes. The reaction was stopped in tap water, cells were counterstained with hematoxylin, mounted with glycerol-gelatin, and then viewed by microscopy.

Evaluation of CD44 isoforms Immunoreactivity

Immunoreactivity was evaluated by complete examination of each section and was classified into three grades: -, no expression; +, expression in less than 50% of all the carcinoma cells viewed under the microscope (weaker expression); + +, expression in more than 50% of the cells (stronger expression) (Fig. 1). Evaluations were performed independently by two investiga-

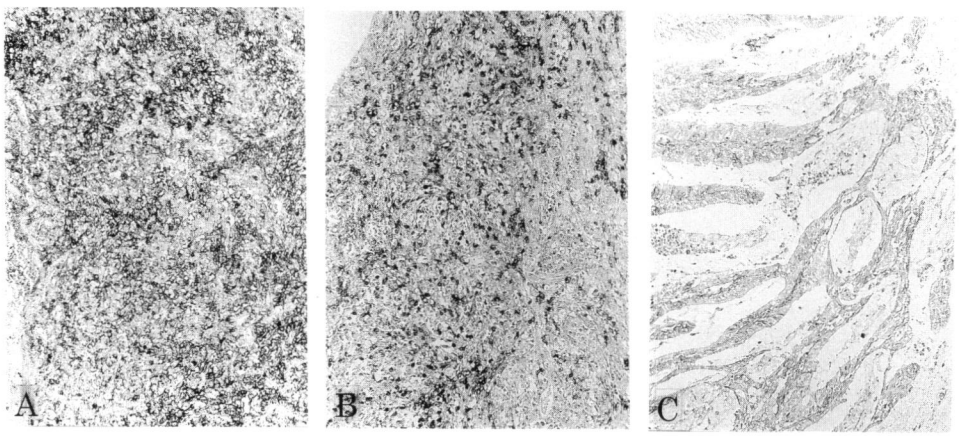

Fig. 1 Photomicrographs demonstrating the grades of CD44H immunoreactivity: stronger expression (A), weaker expression (B), and no expression (C). CD44H, magnification ×118.

tors (H.U. and R.D.). In the event of disagreement, slides were additionally reviewed by a third observer (K.Y.), and a consensus was obtained.

Statistics

General group comparisons were made using the $\chi 2$ test. Cumulative survival was calculated using the Kaplan-Meier approach. Additional comparisons were made by the generalized Wilcoxon test. Results were considered statistically significant when the p value was less than 0.05.

RESULTS

Immunohistochemical staining

41.8%(46/110), 7.3%(8/110), and 0.9%(1/110) of primary gastric cancer was stained in only CD44H, only CD44v3, and only CD44v6 respectively. 63.6% (70/110) of samples was stained in CD44H and /or CD44 variants (Table 1). The proportion of tumors that penetrated the gastric wall or invaded adjacent structures was higher in the CD44H weaker expression group than in the CD44H stronger expression group (p=0.0384). No significant correlation was found between expression of CD44v3, or CD44v6 and depth of tumor invasion (Table 2). The incidence of lymph node metastasis was higher in the CD44H stronger-expression group than in the weaker expression group (p=0.0324). No significant correlation was found between expression of CD44v3, or CD44v6 and nodal metastasis (Table 3). Of the samples from patients, 8 of 66 (34.8%) showed expression of CD44 isoforms in the positive nodes stronger than or the same as in the primary lesion, and 23 of 66 (34.8%) exhibited the same grade in both the positive node and the primary tumor. 6 of 66 (9.1%) showed stronger expression of CD44 isoforms within

Table 1 CD44 isoforms expression of 110 patients with primary gastric cancer.

CD isoforms expression	Positive cases (%)
CD44H only	46 (41.8%)
CD44v3 only	8 (7.3%)
CD44v6 only	1 (0.9%)
CD44H+V3	11 (10.0%)
CD44H+V6	1 (0.9%)
CD44V3+V6	0 (0.0%)
CD44H+V3+V6	3 (2.7%)
in all cases	70 (63.7%)

Table 2 Relationship between depth of tumor invasion and staining intensity of CD44 isoforms.

Isoforms	Depth	Staining intensity of CD44 isoforms			P value
		(-)	(+)	(++)	
CD44H	pT1	15	24	2	0.0384*
	pT2	26	17	9	
	pT3,4	8	9	0	
CD44V3	pT1	35	5	1	0.6157
	pT2	40	9	3	
	pT3,4	13	4	0	
CD44V6	pT1	41	0	0	0.4571
	pT2	48	3	1	
	pT3,4	16	1	0	
CD44	pT1	11	27	3	0.0192*
	pT2	22	19	11	
	pT3,4	7	10	0	

*p<0.05

Table 3 Relationship between nodal metastasis and staining intensity of CD44 isoforms.

Isoforms	Nodal metastasis	Staining intensity of CD44 isoforms			P value
		(-)	(+)	(++)	
CD44H	Negative	20	16	8	0.0324*
	Positive	29	12	25	
CD44v3	Negative	38	4	2	0.1551
	Positive	50	5	11	
CD44v6	Negative	42	1	1	0.9329
	Positive	63	1	2	
CD44	Negative	18	17	9	0.0032**
	Positive	22	11	33	

*p<0.05, **p<0.005

the metastatic tumor despite there being no expression of CD44 isoforms in the primary tumor. The remaining metastatic tumors showed weaker expression of CD44 isoforms (Table 4). No significant correlation could be found between expressions of CD44H, CD44v3, or CD44v6 and liver metastasis or histological types (differentiated vs. undifferentiated) (Table5,6).

Table 4 Comparison of expression of CD44 between primary tumors and nodal metastasis (n=66).

Expression of CD44 isoforms in primary tumor	Expression of CD44 isoforms in lymph node (%)	
Positive (n=44)	Stronger	8 (12.1)
	Same	23 (34.8)
	Weaker	13 (19.7)
Negative (n=22)	Stronger	6 (9.1)
	Same	16 (24.2)
	Total	66 (100)

Table 5 Relationship between liver metastasis and staining intensity of CD44 isoforms.

Isoforms	Liver metastasis	Staining intensity of CD44 isoforms			P value
		(-)	(+)	(++)	
CD44H	Negative	33	23	34	0.2628
	Positive	16	5	9	
CD44v3	Negative	66	7	7	0.2617
	Positive	22	2	6	
CD44v6	Negative	78	1	1	0.2235
	Positive	27	1	2	
CD44	Negative	28	24	28	0.1912
	Positive	12	4	14	

Table 6 Relationship between histological types and staining intensity of CD44 isoforms.

Isoforms	Histological type	Staining intensity of CD44 isoforms			P value
		(-)	(+)	(++)	
CD44H	Differentiated	30	15	14	0.2462
	Undifferentiated	19	13	19	
CD44v3	Differentiated	45	7	7	0.3118
	Undifferentiated	43	2	6	
CD44v6	Differentiated	57	0	2	0.2813
	Undifferentiated	48	2	1	
CD44	Differentiated	23	15	21	0.7931
	Undifferentiated	17	13	21	

Expression of CD44 isoforms and prognosis.

The cumulative 5-year survival rates of the patients undergoing gastrectomy was 57.3% in the positive expression of CD44 isoforms, and 52.4% in the negative expression of CD44 isoforms. There was no statistically significance between the former and latter groups.

DISCUSSION

Cancer invades and destroys adjacent normal tissue, and metastasizes through lymphatic channels or through blood vessels to distant lymph nodes and other tissues. During circulation in the vascular system, tumor cells can undergo a variety of interactions, including aggregation with platelets, lymphocytes, and neutrophils, leading to the capillary bed of a distant organ. The CD44 expressed in tumor cells may facilitate metastasis by mediating tumor cell binding to endothelial cells at the site of metastasis, and /or by mediating tumor cell diapedesis(12).

Hyaluronic acid as the ligand for adhesion molecule CD44H is contained in the peritoneum, and CD44H may play an important role in the dissemination of scirrhous gastric cancer(13,14). On the other hand, expression of CD44 variants (CD44v3 and CD44v6) is associated with clinically aggressive behavior in several types of tumors, such as gastric cancer(8), colonic cancer(7), and breast cancer(15). Therefore, we investigated the relationships between the expression of CD44H, CD44 variants (CD44v3 and CD44v6) and metastases.

Here we immunohistochemically studied the association between CD44H, CD44v3, and CD44v6 expression and the clinicopathological features of primary gastric cancer resected. Expression of CD44H and /or CD44 variants was observed in 70 of 110 (63.6%) gastric cancers, and CD44v6 was present in only 5 of 70 (7.1%) of our cases. But there was a report stating that all of the intestinal type gastric cancers were strongly positive for epitope encoded by variant exons v6(16).

In our clinicopathological evaluation, the expression of CD44H correlated with lymph node metastasis, gastric wall invasion, but it did not significantly correlate with liver metastasis or histological types (differentiated and undifferentiated) of gastric cancer. Nakashio et al.(17) found that CD44H plays important roles in the initial attachment of gastric cancer cells. Our data indicated that CD44H plays important roles in lymph node metastasis in human gastric cancer. On the other hand, serum CD44H concentrations did not correlate with the lymph node involvement in gastric cancer(18), and expression of CD44H was not correlated with clinicopathological incidences(19).

Therefore, the actual mechanism by which CD44H modifies the metastatic properties of cancer cells is unknown. The expression of CD44 variants was not associated with any clinicopathologic factors. Lymph node metastasis correlated with CD44H rather than with CD44v3 or CD44v6. These results suggested that CD44H might be a useful markers for lymph node metastasis in resected gastric cancer. It has been reported that the expression of CD44 variants in gastric cancer was significantly associated with the depth of invasion of the tumor and lymph node metastasis(7). However it has also been reported that CD44v6 was not a predictor of survival time in patients with intestinal type gastric cancers(20) and no correlation was found between tumor type, stage, or patient survival(21). The metastatic process consist of several sequential steps involving multiple host-tumor interactions. In order for metastasis to occur, a tumor cell or group of cells required to leave the primary tumor, invade local tissues and basement membranes, enter the circulation, avoid host immunological responses, attach to a distant vascular bed, extravasate into the interstitium of the target organ, and colonize to form a second tumor(22). It is apparent that intense scrutiny of CD44H gene has led not only to a better understanding of the role played by this molecule in the lymph node metastasis, but also to a realization of its importance in cell-cell interactions mediated via carbohydrate recognition.

There are now many reports concerning the relationship between the expression of the CD44 gene and survival time(23). In the present study, the 5-year survival rate was 57.3% in the positive expression and 52.4% in the negative expression of CD44 and we therefore concluded that the expression of CD44 is not significantly associated with the 5-year survival.

Further studies of the expression of specific isoforms may help elucidate more clearly the above findings.

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