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CD44 expression in soft tissue sarcomas

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Abstract Recent studies have shown that expression of alternatively splicing variants of CD44 is correlated with prognosis for several kinds of malignant tumors. However, little is known about the expression of CD44 standard and variant isoforms in soft tissue sarcomas. In this study 47 cases of soft tissue sarcoma [18 malignant fibrous histiocytomas (MFHs), 13 synovial sarcomas (SSs), 7 malignant schwannomas (MSs), and 9 liposarcomas (LSs)] were examined immunohistochemically. The monoclonal antibodies to the standard form of CD44 (CD44H) and variant exons of CD44v3, 4, 5, 6, 7, 9, and v10 were used. We analyzed the membranous expression pattern of CD44H and CD44 variant exons and assessed the relation between expression of CD44s and metastasis-free survival rates (MFSR) of patients with soft tissue sarcoma. A few sarcomas expressed CD44v3 (2/47) and v7 (2/47), but none of the sarcomas expressed CD44v10. CD44v4 (5/47), v5 (4/47), v6 (10/47), and v9 (9/47) are relatively common types of variant isoforms in soft tissue sarcomas. Expression of CD44v6 is more frequently detected in high-grade than in low-grade tumors. CD44v6 or CD44v9 expression was correlated with metastasis-free survival of patients with soft tissue sarcomas.

Key words CD44 · Adhesion molecules · Prognosis · Soft tissue sarcoma

Introduction

There are successive steps in tumor metastasis. Tumor cells become detached from the primary sites, penetrate the vascular endothelium, circulate in the blood, and colonize distant sites [11]. Adhesion molecules play an important part in the formation of tumor metastasis [11, 13, 15]. CD44 is expressed in a wide variety of cell types, including hematopoietic cells such as lymphocytes, epithelial cells, macrophages, and fibroblasts [1, 6, 9, 16]. The standard form of CD44 (CD44H) is a highly glycosylated cell surface molecule, which appears to be involved in cell–cell and cell–matrix interactions [17]. The human CD44 gene is composed of 19 exons, 9 of which are sometimes expressed due to alternative splicing [2]. In recent years, the relation between tumor metastasis and expression of CD44 variants has sometimes been reported [5, 8, 11, 12, 19, 20]. It has been discovered that one of the CD44 isoforms is expressed more frequently in metastatic adenocarcinoma cells than in nonmetastatic cell lines in the rat [4]. This particular type of CD44 splice variants has been shown to give metastatic potential to nonmetastatic tumor cell lines [4]. Using several malignant tumors, CD44 isoforms were examined for any correlation with the prognosis [5, 8, 11, 19, 20]. This study was performed to analyze the expression pattern of CD44 variant exons and demonstrated the correlation between expression of CD44 variant exons and prognosis in patients with soft tissue sarcomas.

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Materials and methods

Patient selection

The 47 cases of soft tissue sarcoma reviewed were 18 malignant fibrous histiocytomas (MFH), 13 synovial sarcomas (SSs), 7 malignant schwannomas (MSs), and 9 liposarcomas (LSs). Eleven patients had low-grade tumors, and 36 tumors were of a high grade according to the criteria published by Enneking et al. [3]. All cases were surgically treated at Okayama University Hospital in western Japan between 1975 and 1993. The mean patient age was 47 (10–84) years. The mean follow-up period was 82 (24–273) months. Twenty patients were male and 27 were female. The tumors were located in the trunk in 10 patients and in the extremities in 37 patients. Sixteen patients had local recurrences. Preoperative chemotherapy was performed only in 1 patient with synovial sarcoma. Fourteen patients received postoperative chemotherapy. Anticancer drugs were administered in the form of the VAC-A regimen composed of vincristine, actinomycin D, cyclophophamide, and doxorubicin [14]. In all patients receiving chemo-

Table 1 CD44 variants and positive expression (*MFH* malignant fibrous histiocytoma, *SS* synovial sarcoma, *MS* malignant schwannoma, *LS* liposarcoma)

CD44 isoforms (*N*=47)	Positive				
	n (%)	MFH (*N*=18) *n*	SS (*N*=13) *n*	MS (*N*=7) *n*	LS (*N*=9) *n*
CD44v3	2 (4%)	1	0	1	0
CD44v4	5 (11%)	1	4	0	0
CD44v5	4 (9%)	2	1	1	0
CD44v6	10 (21%)	3	4	1	2
CD44v7	2 (4%)	0	2	0	0
CD44v9	9 (19%)	2	4	2	1
CD44v10	0 (0%)	0	0	0	0
CD44H	25(53%)	10	7	4	4

therapy the total dose of doxorubicin was more than 400 mg and chemotherapy according to the initial schedule was possible for at least 4 months. One patient received preoperative radiotherapy (45 Gy).

Immunohistochemical staining

Tumor samples of all cases were fixed in buffered formalin and embedded in paraffin. The paraffin sections were soaked in xylene to remove paraffin and dehydrated in a graded alcohol series (100–50%). Antigen retrieval was performed by autoclaving for 15 min at 121°C. After cooling, endogenous peroxidase activity was quenched with 0.3% H_2O_2 in absolute methanol. After blocking with normal horse serum, monoclonal antibodies against CD44H, v3, v4, v5, v6, v7, v9, and v10 were used as primary antibodies (CD44H clone 2C5, R&D Systems; CD44v3 clone VFF-327v3, Bender MedSystems; CD44v4 clone VFF-11, Bender MedSystems; CD44v5 clone VFF-8, Bender MedSystems; CD44v6 clone 2F10, R&D Systems; CD44v7 clone VFF-9, Bender MedSystems; CD44v9 clone 441V, Seikagaku Corporation; and CD44v10 clone VFF-14, Bender MedSystems) and then incubated for 30 min with biotinylated horse anti-mouse antibody. After rinsing in PBS, they were incubated with ABC (Mouse Vecta Stain Elite Kits; Vector Laboratories, Burlingame, Calif.) and then washed with distilled water. Finally, sections were counterstained with hematoxylin. Stock tissue sections with known CD44 positivity (gastric cancers, colon cancers, breast cancers, and normal skin) served as positive controls.

Immunoreactivity was blindly evaluated by orthopedic surgeons who were interested in the histological diagnosis of soft tissue sarcomas by means of light microscopy. Immunohistochemical reactions were scored for intensity using semi-quantitative gradings (–, no staining; +, weak staining or strong stains in 10–25% of tumor cells; ++, moderate staining or strong stains in 25–75% of tumor cells; +++, strong staining of more than 75% of tumor cells). A tumor was considered positive if staining was confined to the cell membrane with or without the cytoplasm and without significant background staining. A consensus was reached by three of the authors in all cases. We evaluated the expression pattern of CD44 variant exons and the site of the tumor where CD44 variant exons is expressed. In addition, we assessed the relation between membranous expression of CD44H and variant exons (v3, 4, 5, 6, 7, 9, and 10) and metastasis-free survival rates (MFSR). Moreover, we evaluated the survival of cases of cytoplasmic dominant pattern separately. For the analysis of survival data, Kaplan–Meier curves were contracted and the log-rank test for trend was performed. A *Chi*-square test was used to analyze the relation between variant positive group and negative group.

Results

Expression of CD44 isoforms was observed (Table 1). Ten of the 47 cases (3 MFHs, 4 SSs, 1 MS, and 2 LSs) were positive for CD44v6, 9 of the 47 (2 MFHs, 4SSs, 2MSs, and 1LSs) were positive for CD44v9, no cases were positive for CD44v10, and 25 of the 47 cases were positive for CD44H (Fig. 1).

Table 2 Semi-quantitative grading of CD44 variants in soft-tissue sarcoma

N=47	Positive *n* (%)	Semi-quantitative grading[a]			Negative *n* (%)
		(+)	(++)	(+++)	
CD44v3	2 (4%)	2	0	0	45 (96%)
CD44v4	5 (11%)	2	3	0	42 (89%)
CD44v5	4 (9%)	2	2	0	43 (91%)
CD44v6	10 (21%)	7	3	0	31 (66%)
CD44v7	2 (4%)	2	0	0	45 (96%)
CD44v9	9 (19%)	5	3	1	31 (66%)
CD44v10	0 (0%)	0	0	0	47 (100%)
CD44H	25 (53%)	14	9	2	22 (47%)

[a] Scoring of positive cells: –, 0–10%; +, 10–25%; ++, 25–75%; +++, >75%

Of the positive expression samples, moderate or high intensities were detected in 3 of 10 CD44v6-positive cases, 4 of 9 CD44v9-positive cases, and 11 of the 25 CD44H-positive cases (Table 2).

In general, CD44-positive staining was located in pleomorphic-type cells and spindle-shaped cells in MFH. In the former cells staining was dominant in the membrane and in the latter cells it was sometimes also noted in the cytoplasm. In synovial sarcomas staining was localized in the epithelioid cells. In malignant schwannomas, spindle cells were generally stained. In liposarcomas, lipoblast-like cells and round cells tended to react to staining.

We examined the relationship between expression of CD44 and histological grade (Table 3). Expressions of CD44v6 were more frequently detected in high-grade than in low-grade tumors. In other variant forms, however, there was no clear tendency to any connection between tumor grade and expression of variant isoforms.

Twenty-seven patients had metastases. There was no significant relation between expression of CD44 and metastasis rate (Table 4). There were significant differences in metastasis-free survival between the CD44v6-positive and CD44v6-negative groups ($P<0.03$) (Fig. 2). There was also a significant difference between the

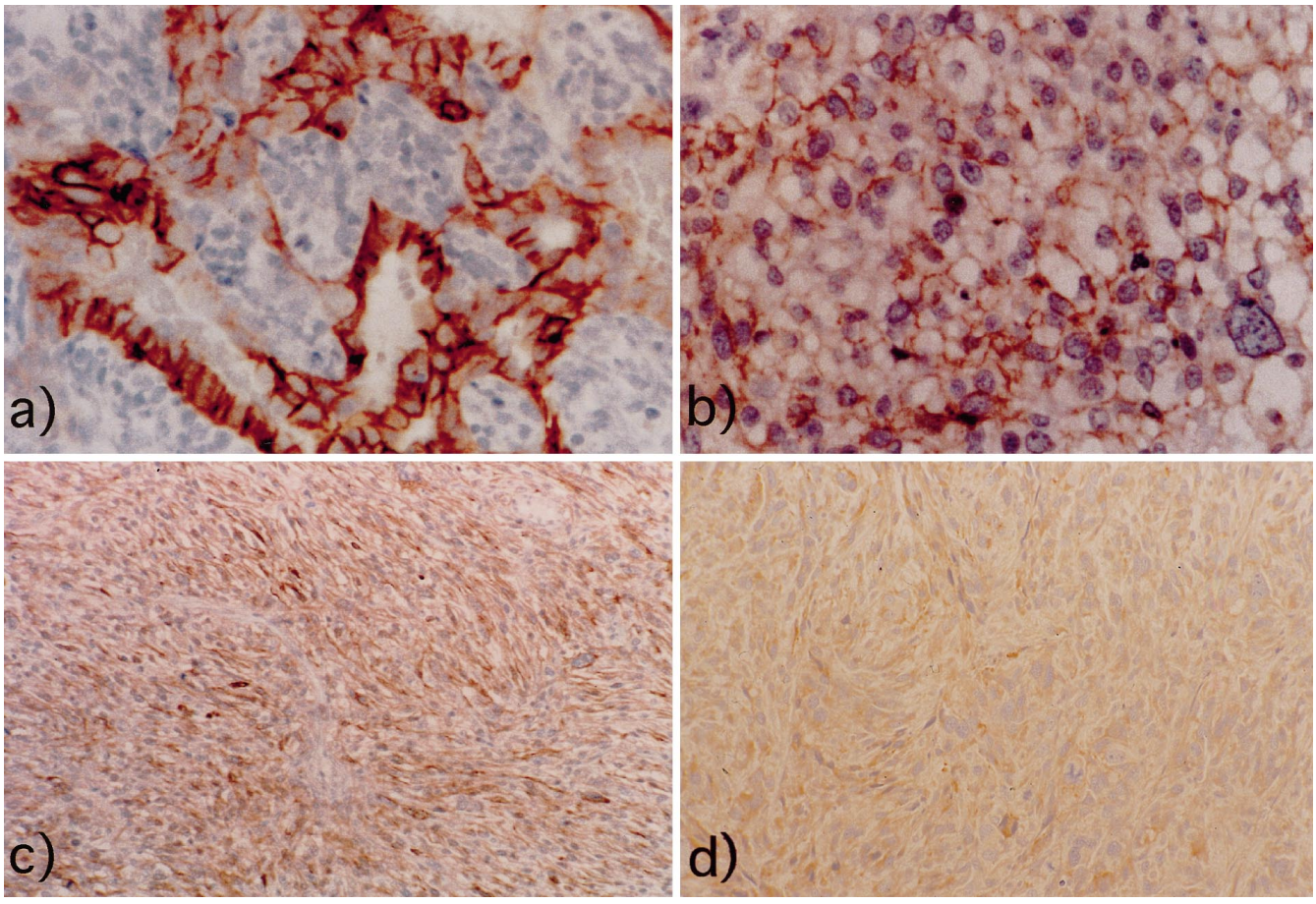

Fig. 1 **a** Biphasic synovial sarcoma expressing CD44v6 on the membrane. **b** Liposarcoma expressing CD44v6 on the membrane. **c** Malignant schwannoma expressing CD44v9 mainly on the membrane. **d** Malignant fibrous histiocytoma expressing CD44H both on the membrane and in the cytoplasm

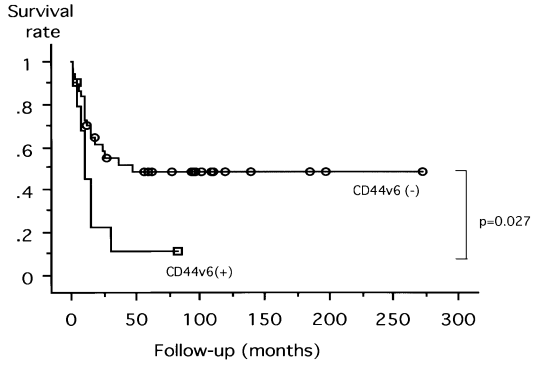

Fig. 2 Expression of CD44v6 and metastasis-free survival rate of patients with soft tissue sarcoma. The patients with CD44v6-negative tumors showed significantly better survival than those with CD44v6-positive tumors

CD44v9-positive and -negative groups in metastasis-free survival ($P<0.04$). However, there were no differences in metastasis-free survival between the groups that were positive and negative in the other variants. On examination of expression of CD44H or the other variants, cases showing a cytoplasmic pattern did not dem-

Table 3 Variants and histological grade (*NS* not significant)

CD44 isoforms	Low grade	High grade	*P*
CD44v3 (+)	1	1	NS
(–)	10	35	
CD44v4 (+)	2	3	NS
(–)	9	33	
CD44v5 (+)	2	2	NS
(–)	9	34	
CD44v6 (+)	0	10	0.08
(–)	11	26	
CD44v7 (+)	0	2	NS
(–)	11	34	
CD44v9 (+)	1	8	NS
(–)	10	28	
CD44v10 (+)	0	0	NS
(–)	11	36	
CD44H (+)	10	15	NS
(–)	1	21	

Table 4 Expression of CD44 variants and metastasis

	Metastasis in positive (%)	Metastasis in negative (%)
CD44v3	1/2 (50%)	26/45 (58%)
CD44v4	2/5 (40%)	25/42 (60%)
CD44v5	1/4 (25%)	26/43 (60%)
CD44v6	7/10 (70%)	20/37 (54%)
CD44v7	1/2 (50%)	26/45 (58%)
CD44v9	6/9 (67%)	21/38 (55%)
CD44v10	0/0 (0%)	27/47 (57%)
CD44vH	13/25 (52%)	14/22 (64%)

onstrate a worse prognosis than cases with a membranous dominant pattern.

Discussion

Previous studies have demonstrated that expression of CD44 variants on tumor cells appears to be correlated with the metastatic potentials and clinical stage of a number of human cancers, including colon carcinoma [16, 20], gastric cancer [10], breast cancer [5, 8], and non-Hodgkin's lymphoma [19]. As for soft tissue sarcomas, there have been a few studies using CD44H, and little is known about the expression of CD44 variant isoforms in soft tissue sarcomas [23]. In this study, we assessed the expression pattern and prognosis of soft tissue sarcomas by immunohistochemical methods.

Although CD44H was frequently present in soft tissue sarcomas, few sarcomas expressed CD44v3 (4%) and CD44v7 (4%), and no sarcomas expressed v10. CD44v4 (11%), v5 (9%), v6 (21%), and v9 (19%) are relatively popular types of variant exons. Other tumors were examined, and CD44v5, CD44v6, and CD44v7–8 were detected in 83%, 63%, and 27%, respectively, of vulvar squamous carcinomas [21]. In squamous cell carcinomas, expression of CD44 seems to be higher than that in soft tissue sarcomas. In non-Hodgkin lymphomas, CD44H was found in 100% of cases, CD44v3 in 28%, CD44v4 in none, CD44v6 in 26%, and CD44v9 in 6% [22]. In the hematopoietic tumors, expression of CD44H is higher than that in the soft tissue sarcomas.

We found significant ($P<0.02$) differences between the CD44v6-positive and CD44v6-negative groups in MFSR, but there were no differences between MFSR of groups positive for other variants and the corresponding negative groups. Although the role of CD44 in metastasis is not clear, CD44v6 may be important during invasion of the target organs, perhaps by interaction of the molecules with special ligands. The exact ligands of CD44s currently remain unknown.

An earlier study indicated that CD44H positivity was 59% in MFHs, 50% in SSs, 73% in MSs and 7% in LSs and that there was no significant association between the clinical stage or tumor grade and CD44H reactivity [23]. Those results were similar to ours. Moreover, our data indicate expression of CD44H was not correlated with prognosis and a correlation between expression of CD44v6 or v9 and an unfavorable high-grade pathological status.

CD44 immunoreactive site in soft tissue sarcomas was dominated by either the cell membrane or the cytoplasm. In our analysis, the cytoplasmic reactive pattern was regarded as a negative pattern. A previous report suggested that cytoplasmic staining may reflect overproduction of CD44 [24]. However, in our study, cases with a cytoplasmic dominant pattern did not demonstrate a worse prognosis than cases with a membranous dominant pattern. Only CD44 with membranous expression may work as an adhesion molecule for the tumor metastasis. Another report indicated that CD44H staining could be used in resolving the differential diagnoses of epithelioid neoplasms [23]. In our study too, the epithelioid component of synovial sarcomas was stained by CD44H.

In a recent study, it was shown that elevated CD44v6 serum levels were significantly correlated with clinical evidence in cervical cancer [7]. However, there has also been a report that in ovarian cancer, the serum level of CD44v6 is not elevated compared with that in healthy controls [18]. The significance of the serum level of CD44v6 remains unknown.

Conclusion

CD44H is expressed in most soft tissue sarcomas. A few sarcomas in this study expressed CD44v3 and v7, but there were no sarcomas expressing v10. CD44v4, v5, v6, and v9 are relatively common types of variant isoforms. CD44v6 is more commonly expressed in high-grade than in low-grade tumors. CD44v6 expression was correlated with metastasis-free survival of patients with soft tissue sarcomas. The results of this study indicate that expression of CD44 variants may be an additional prognostic marker for patients with soft tissue sarcoma.

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