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CD44s Expression is Associated with Improved Survival in Soft Tissue Sarcoma

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Abstract. *Expression of CD44 has been identified as a prognostic factor in several malignant diseases. Few studies have correlated CD44 expression in soft tissue sarcoma with subsequent tumor progression or recurrence. We sought to investigate the clinical significance of CD44s (standard) in adult soft tissue sarcoma (STS). Tumor specimens of 62 patients with STS were evaluated by immunohistochemistry for CD44s expression. The primary outcome measures were survival and local recurrence. Of 62 analyzed specimens, 49 tumors were CD44s-positive compared to 13 CD44s-negative tumors. Kaplan-Meier survival analysis indicated significantly better survival among patients whose tumor was CD44s-positive (p=0.015). CD44s expression (hazard ratio, 3.1; 95% confidence interval, 1.5 to 7.0), tumor size (hazard ratio, 11.7; 95% confidence interval, 1.8 to 32.2) and resection quality (R1 vs. R0: hazard ratio, 8.7; 95% confidence interval, 3.1 to 24.5) were independent predictors of survival in multivariate analysis. CD44s expression correlates with prognosis of soft tissue sarcomas and therefore may have a pathogenetic role in tumor progression. Our results suggest that expression of CD44s in primary STS provides value regarding the progression of STS and, therefore, could be useful in selecting patients for adjuvant treatment.*

Soft tissue sarcomas (STS) form a clinically and pathologically heterogeneous group of tumors, leading to therapeutic difficulties. Prognostic favorable outcome depends on early diagnosis and aggressive, but limb-sparing, treatment (1). The most important prognostic factor identified so far is the quality of surgical treatment at initial diagnosis (2). Meanwhile, new prognostic markers are being searched for, especially to determine the value of adjuvant and/or palliative treatment.

Cell-to-cell and cell-to-matrix interactions are essential for normal cell growth and differentiation, though their precise function remains unclear. Only a few studies have investigated CD44 expression by STS. The aim of this study was to evaluate the expression of CD44s (standard) in STS of the adult and to determine any prognostic correlation.

Patients and Methods

Between January 1993 and December 1999, tissue of histologically proven STS was collected prospectively at the time of definite tumor excision after histological biopsy evaluation at the Department of Surgery, University Hospital Hamburg, Germany. Tumor samples from 62 patients older than 18 years were studied. Tumor staging was performed according to the UICC TNM-System (1997). Immunohistochemical analysis of CD44s was carried out using a standard streptavidin-biotin-horseradish peroxidase immuno-histochemistry protocol. The tumor was cut at the largest plane and divided between several slides. Two to three slides with the most viable tumor cells were selected for each case. Tumor samples of all cases were snap- frozen and stored until investigation. Tumor samples were 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 minutes at 121˚C.

Monoclonal antibodies against CD44s were used as primary antibodies (CD44std, Bender MedSystem, Vienna, Austria). After blocking with diluted normal blocking serum, the sections were incubated for 1 hour with a 1:50 dilution of primary antibodies at 37˚C. After rinsing in phosphate-buffered saline (PBS), the sections were sequentially incubated for 30 minutes with diluted biotinylated secondary antibody solution. The slides were washed for 5 minutes in PBS and the sections were incubated for 30 minutes with biotinylated horse anti-mouse antibody. Antigen-antibody binding was revealed by immersing sections in diaminobenzidine hydrochloride with 0.6% hydrogen peroxide as a substrate. The sections were finally counterstained with Mayers Hematoxylin to make the nuclei visible, then rinsed in tap water for 10 minutes, hydrated in a graded alcohol series (70% - absolute methanol) and through xylene, and covered.

The positive control slide was prepared from breast cancer known to contain the antigen. In the positive control tissue, all monoclonal

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Table I. *Histological types according to tumor grade.*

Histology	G1	G2	G3	Total
MFH	0	4	9	13
Liposarcoma	4	3	1	8
MPNST	0	4	4	8
Leiomyosarcoma	2	6	9	17
Others	1	7	8	16
Total	7	24	31	62

antibodies were applied under the same conditions as the STS samples. The negative control slide was prepared from the same tissue block as the specimen. Instead of the primary antibody, we used nonimmune mouse monoclonals of the same isotype as the specific antibodies.

Immunoreactivity was evaluated by light microscopy without knowledge of the treatment outcome or other data of the patients. A tumor was considered positive if staining was confined to the cell membrane or the cytoplasm in the absence of significant background staining. A minimum of 1000 tumor cells were counted and all stained cells were considered positive, regardless of intensity of the stain. The number of positive cells was expressed as a percentage of all counted cells. When the percentage of positive cells was <20%, the specimen was diagnosed as negative and when >20% the specimen was diagnosed as positive. A consensus was reached by a minimum of two of the authors in all cases.

Survival curves were determined using the Kaplan-Meier product-limit method and compared by the log-rank test with 95% confidence intervals based on Greenwood's formula (3). Prognostic risk factors of patient survival were evaluated by univariate and multivariate analysis. The Cox proportional-hazards regression model was used to determine independent multivariate risk factors and hazard ratios and 95% CI were calculated (4). Two-tailed values of $p<0.05$ were considered significant. Statistical analysis was performed with the SAS software package (version 6.12, SAS Institute, Cary, NC, USA).

Results

Between 1993 and 1999, 62 patients were operated on with STS in which a tumor specimen could be obtained. The patient and tumor characteristics are summarized in Tables I and II. There were 31 females and 31 males with a mean age of 56 years (range 32-79). The mean duration of symptoms was 16±5 months. In pathological diagnosis, there were 13 malignant fibrous histiocytomas (MFH), 17 leiomyosarcomas, 8 liposarcomas and 8 malignant peripheral nerve sheet tumors (MPNST). Sixteen patients had 8 other histological types (Table I). Seven patients (11%) had low-grade tumors, while 24 patients (39%) had intermediate grade tumors and 31 patients (50%) had high-grade tumors. Complete histological evaluation of the tumor specimens revealed 10 T1 tumors (16%), while 52 patients had a tumor of >5 cm in diameter (T2, 84%). In 61 patients, regional lymph nodes were either clinically or histologically without metastases (97%), while positive regional lymph nodes were present in one patient. Three patients presented with synchronous distant metastases (5%), while staging procedures revealed M0 in 59 patients (95%). In all patients, a wide resection was planned, resulting in 50 R0 resections (81%) and 10 R1 (16%) resections. Two patients were resected R2. At the end of follow-up, mean survival was 44±35 months. Thirty-three patients (53%) were without evidence of disease; 8 patients (13%) were alive with tumor while 20 patients (32%) died due to tumor disease.

Table II. *Variables associated with patient survival: univariate analysis.*

Variable	Patients	Deaths (%)	Median survival time (months)	Log-rank test	*P* value
CD44s				5.92	0.015*
Positive	49	13 (26)	120		
Negative	13	7 (54)	44		
Grade				1.69	0.43
G1	7	3 (43)	120		
G2	24	6 (25)	110		
G3	31	11 (36)	56		
Tumor size				1.99	0.16
<5cm	10	1 (10)	85		
> 5cm	52	19 (37)	11		
Depth of tumor				4.07	0.25
Subcutaneous	14	2 (14)	120		
Subfascial	22	8 (36)	90		
Retroperitoneal	10	3 (30)	120		
Parenchymatous	16	7 (44)	80		
Lymph nodes				1.70	0.19
Negative	61	19 (31)	110		
Positive	1	1 (100)	42		
Distant metastases				1.74	0.18
No	59	19 (32)	110		
Yes	3	1 (33)	22		
Resection quality				25.58	<0.001*
R0	50	10 (20)	160		
R1	10	8 (80)	15		
R2	2	2 (100)	12		
Histology				0.35	0.99
MFH	13	5 (39)	82		
Liposarcoma	8	2 (25)	120		
MPNST	8	3 (38)	110		
Leiomyosarcoma	17	5 (29)	73		
Other	16	5 (31)	150		
Sex				0.17	0.68
Male	31	10 (50)	120		
Female	31	10 (50)	110		

MFH=malignant fibrous histiocytoma; MPNST=malignant peripheral nerve sheet tumor.

* Significant univariate risk factors.

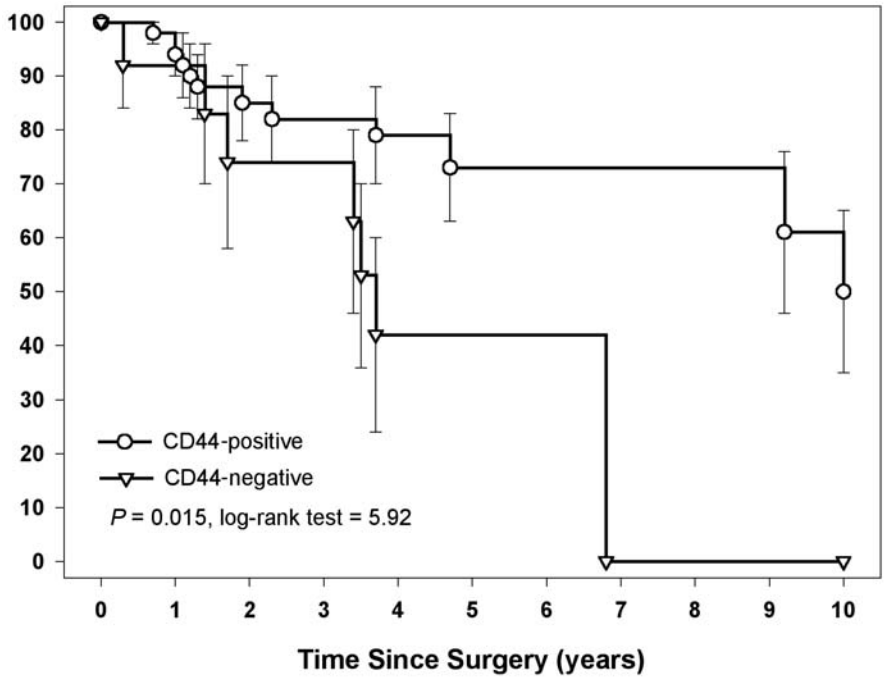

Figure 1. *Patient survival according to positive or negative CD44s. Kaplan-Meier curves indicate significantly better survival in the subgroup of patients who are CD44s- positive ($p=0.015$, log-rank test). Error bars denote 95% confidence intervals.*

In 50 patients (79%) CD44s reactivity was seen, while 12 samples (21%) were CD44s-negative. The number of patients with positive immunostaining for CD44 was 49 (81%) and with negative staining was 12 (19%). Regarding tumor grade, 71% of the G1 tumors were CD44-positive, while 79% of G2 and 80% of G3 tumors were CD44-positive (n.s.).

Kaplan-Meier survival analysis indicated significantly improved survival among patients who were CD44s-positive (log-rank test=5.92, $p=0.015$, Figure 1). Resection quality was found to be the only other predictor of survival by univariate analysis (log-rank test=25.58, $p<0.0001$, Table II). Multivariate prognostic risk factors based on the Cox proportional-hazards regression model included three variables: CD44s ($p=0.01$), tumor size ($p=0.02$), resection quality ($p< 0.01$) were all significant parameters of survival (Table III). Depth of tumor ($p=0.07$) and presence of distant metastases ($p=0.06$) almost reached significance. Patients who have an R1 or R2 resection have an annual risk of death approximately 3 times higher than those who have an R0 resection (Table III)

In this study, CD44s was found to be an independent, multivariate prognostic risk factor and thus provides important prognostic information regarding patient survival, independent of other patient and tumor characteristics. The hazard ratio indicates that patients who are negative for CD44s have an annual risk of death three times higher than those who are positive for CD44s (hazard ratio, 3.1; 95% confidence interval, 1.5 to 7.0). The actuarial analysis based on CD44 expression revealed that the median survival time for patients who are CD44s-positive is 10 years (95% confidence interval, 7.8 to 12.2 years), whereas median survival time for patients who are CD44s-negative is 3.7 years (95% confidence interval, 3.3 to 4.0 years). Therefore, it can be expected that half of the patients whose tumors are CD44s-positive will continue to be alive 10 years after surgery, while half of CD44s-negative patients are expected to die in less than 4 years after initial diagnosis.

Discussion

The transmembrane glycoprotein CD44 is expressed on virtually all cell types acting as a receptor for hyaluronate (5). The encoding gene is located on chromosome 11p3 and consists of at least 21 exons (6). The CD44 molecule has three cope epitopes encoded by ten exons with alternative mRNA splicing of the remaining exons generating multiple isoforms. The standard form of CD44 (CD44s) is expressed on almost all cells and is heavily glycosylated, while variant isoform expression is expressed in a cell- and tissue-specific manner (7).

Qualitative and quantitative changes in expression of CD44 have been demonstrated for several tumor models revealing prognostic significance for neuroblastoma (8), breast cancer (9), squamous cell esophageal cancer (10), osteosarcoma (11) and gastric cancer (12). Only a few reports exist about CD44s expression in STS. CD44 expression was found to be a prognostic factor in rhabdomyosarcoma (7). A recent study examined CD44v expression in a variety of STS (13) and noted a correlation with metastases-free survival in these patients, although the overall patient number was small. Maula observed similar results in a recently published observation of limb or superficial STS in Finland (14).

We found significant differences between the CD44-positive

Table III. *Risk factors of survival: Cox proportional-hazards regression model.*

Variable	Hazard ratio	95% Confidence interval	*P* value
CD44			
Negative *vs*. Positive	3.1	1.5 - 7.0	0.01*
Grade			0.71
Tumor size			
>5cm *vs.* <5cm	11.7	1.8 - 32.2	0.02*
Depth of tumor			0.07
Lymph nodes			0.27
Distant metastases			0.06
Resection quality			
R1 vs. R0	8.7	3.1 - 24.5	<0.01*
R2 vs. R0	17.5	3.5 - 58.3	<0.01*
R1 vs. R2			0.50
Histology			0.66
Sex			0.15

* Significant multivariate prognostic risk factors.

and CD44-negative groups regarding overall survival. Though the role of CD44 in metastasis remains unclear, CD44 may be important during invasion of the target organs, perhaps by interaction of the molecules with special ligands (15). In an earlier series, no correlation between CD44s and metastases-free survival could be seen (13), maybe due to the smaller series being reported. The strong correlation between CD44 expression and actuarial survival revealed CD44 as the strongest prognostic marker, while none of the other variables examined were significant predictors of survival. CD44 expression is not the only independent prognosticator for STS; the quality of resection and tumor size were independent prognostic indicators as well. Tumor depth almost reached significance, confirming results by others. Other well-described factors such as tumor grade did not reach significance level in our series. Future studies with larger cohorts are needed to define the precise role of this cell adhesion molecule in invasion and metastases of STS. Nevertheless, the association between CD44s expression and progression of STS is important, as it suggests that CD44s may play a pathogenetic role in tumor progression.

CD44s immunoreactive sites in STS were dominated by either the cell membrane or the cytoplasm. In an earlier series, cytoplasmatic staining was found as a predictor of worse outcome in gastric carcinoma (16). We did not find any correlation between cytoplasmatic staining and prognosis, thus confirming the results of others (13).

In conclusion, our study suggests that low expression of CD44s correlates with poor outcome. To confirm whether this relationship is independent of STS histological subtype, further investigations need to be performed using a larger number of patients in respective subgroups. Thus, the identification of subgroups with a high risk for tumor relapse, who might benefit from adjuvant treatment strategies, as seen in osteosarcoma (11) might be possible.

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