Expression of Fatty Acid Synthase as a Prognostic Indicator in Soft Tissue Sarcomas[1]

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ABSTRACT

Purpose: **Fatty acid synthase (FAS) is a key enzyme in the *de novo* biosynthesis of fatty acids. Carcinoma cells are dependent on endogenous fatty acid synthesis for growth *in vitro*. In a subset of human cancers, elevated FAS is associated with poor prognosis; however, the expression of FAS and the relationship between FAS and prognosis in soft tissue sarcomas (STSs) have not been studied. The objective of this study is to examine the expression of FAS in STSs and determine its relationship to clinicopathological features and prognosis.**

Experimental Design: **Sixty-four cases of STS were examined. The clinicopathological features and immunohistochemical expression of FAS and Ki-67 antigen were studied. Survival analysis was performed using the log-rank test and the Cox multivariate regression model.**

Results: **FAS expression was observed in 20 of 64 cases (31.3%) of STS. FAS-positive sarcomas were found in 13 of 23 malignant fibrous histiocytomas, 3 of 17 liposarcomas, 3 of 7 malignant peripheral nerve sheath tumors, and 1 extraskeletal mesenchymal chondrosarcoma. No expression of FAS was seen in the synovial sarcomas, leiomyosarcomas, or rhabdomyosarcomas that were examined. Clinicopathologically, FAS-positive tumors were significantly deep-seated ($P = 0.02$) and large in size ($P = 0.03$). FAS expression correlated with decreased disease-free survival ($P = 0.006$) and decreased overall survival ($P = 0.003$). In a multivariate analysis, expression of FAS was able to predict decreased disease-free survival but did not reach the level of significance for overall survival.**

Conclusions: **FAS expression is one of the predictive indicators for disease prognosis in STS.**

INTRODUCTION

Animals derive fatty acids from two sources, diet and *de novo* biosynthesis. Fatty acid biosynthesis requires a coordinated series of enzymatic reactions. In mammals and birds, these multiple functions for *de novo* biosynthesis of fatty acids are consolidated into a single protein that is the product of a single gene. This multifunctional enzyme, FAS[3] (EC 2.3.1.85), exists in the cytoplasm as a homodimer with each subunit protein approximately M_r 270,000 and contains, in eight separate functional domains, seven different catalytic activities. FAS synthesizes long-chain, saturated fatty acids from acetyl-CoA, malonyl-CoA, and nicotineamide-adenine dinucleotide phosphate. The predominant product of FAS is a 16-carbon-atom fatty acid, palmitate, but FAS may also produce smaller amounts of myristate (C_{14}), laurate (C_{12}), and even shorter-chain fatty acids (1–3). Long-chain fatty acids are essential constituents of all biological membrane lipids and are important substrates for energy metabolism. Under normal conditions, cells generally use circulating fatty acids from dietary lipids. FAS is therefore expressed at low levels in most normal human tissues (4), except for liver, adipose tissue (5), cycling endometrium (6), fetal lung (7, 8), and lactating breast (9–11).

Recent studies have demonstrated that FAS is highly expressed in human neoplasms such as breast (12–18), prostate (19–21), ovarian (22), colorectal (23, 24), and endometrial cancers (25). In breast, prostate, and ovarian carcinomas, high levels of FAS expression are associated with poor prognosis. Human cell lines established from breast cancer tumors showed increased rates of lipogenesis commensurate with elevated levels of FAS (26). Inhibition of the fatty acid synthetic pathway with FAS inhibitor, cerulenin (27–29), leads to cell death by induction of apoptosis (26, 30–33). Moreover, cerulenin treatment was shown to inhibit the growth of a human ovarian carcinoma cell line that increased fatty acid synthetic activity in a xenograft model (34). Thus, FAS is important as a prognostic factor in certain cancers and may represent a potential therapeutic target for chemotherapy.

STSs consist of a highly heterogenous group of malignant neoplasms with different morphological and histological subtypes. STSs constitute less than 1% of all malignant tumors (35). Due to this rarity in occurrence, reliable and reproducible factors to determine patient prognosis are needed. Recent immunohistochemical studies on STSs have shown that overexpression of Ki-67 antigen (36–41), p53 (37, 42–44), proliferating cell nuclear antigen (39), topoisomerase IIα (41), bcl-2 (43), and/or mdm2 (45, 46) was associated with poor prognosis. To our

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[3] The abbreviations used are: FAS, fatty acid synthase; STS, soft tissue sarcoma; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; DFS, disease-free survival; OS, overall survival.

knowledge, the expression of FAS has not been analyzed in STS. Recent immunohistochemical studies at our institute have shown FAS expression not only in fetal epithelial cells but also in proliferating fetal mesenchymal cells (47). These findings suggest that FAS may be expressed in STS, which develops from immature mesenchymal cells. The objective of the present study is to examine the expression of FAS in STSs and determine its relationship to clinicopathological characteristics and patient prognosis.

MATERIALS AND METHODS

Patient Selection. We examined 101 patients with STS who were diagnosed and treated at the Department of Orthopedic Surgery, Fukushima Medical University Hospital (Fukushima, Japan) during the period from 1981 to 1998. Of these, we selected 64 for whom full clinical information was recorded and whose paraffin-embedded biopsy and/or surgically resected tumor specimens were available for immunohistochemical study. Thirty-seven patients were excluded from the study due to inadequate clinical data (23 cases) or distant metastasis or regional lymph node involvement of the disease at the first presentation (14 cases). Clinical data were collected by reviewing patient medical records and included the following: patient's age at presentation; sex; family history and past history of malignancies; duration of symptoms before presentation; type of surgery; pre- and postoperative adjuvant therapy; date and site of the recurrence including regional lymph node metastasis or distant metastasis; and the patient's current status.

Tumors. Pathological data included the location, depth, size of maximum diameter of primary tumors, tumor presentation (primary or recurrent), histological type and grade, number of mitotic figures, and surgical margin (48) at definitive surgery. The histological type and grade of malignancy were evaluated according to the criteria of Enzinger and Weiss (49). The histological grade of malignancy was divided into three grades based on degree of tumor differentiation, tumor necrosis, and mitotic count. For immunohistochemical studies, selection of paraffin-embedded samples was based on the most representative and best-preserved tumor tissues.

Patient Follow-up Techniques. The patients were observed at 6- or 8-week intervals during the 2 years following surgical treatment and then observed every 3 or 4 months for the next 3 years and every 6 months thereafter. Follow-up consisted of chest radiographs and physical examination. DFS was calculated as the time of the initial surgery for the primary tumor to the date of the first documentation of clinical recurrence, metastasis of the disease, or the date of the last follow-up visit. OS was calculated as the date of the initial operation to the date of death or the last follow-up visit. No patients were lost during follow-up.

Antibodies and Immunohistochemistry. We used polyclonal rabbit antibody against human brain FAS (dilution, 1:200; Immuno-Biological Laboratories Co., Fujioka, Japan) and monoclonal mouse antibody for anti-Ki-67 (clone MIB-1; dilution, 1:100; Immunotech Corp., Marseille, France) to label specimens. The method for producing this polyclonal anti-FAS antibody and the specificity have been described previously (47).

Tissue sections (4-μm thick) from archival, formalin-fixed, paraffin-embedded tissue specimens were mounted on poly-L-lysine (Muto Chemicals, Tokyo, Japan)-coated slides. The samples were deparaffinized in xylene for 15 min and rehydrated in a series of graded ethanol. Endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide in 95% methanol for 20 min. Antigen retrieval was performed with citrate buffer (0.01 M, pH 6.0) in an 800-W microwave oven (Toshiba Corp., Tokyo, Japan) for a total of 15 min. The tissues were then cooled and incubated with 5% skim milk (Snow Brand Milk Products Co., Tokyo, Japan) in PBS for 30 min to block nonspecific adsorption of immunoglobulins to the tissues. Primary antibodies were then applied to the tissues and incubated with them overnight at 4°C. After extensive washing in PBS, the products of the immunoreaction were visualized with streptavidin-biotin complex immunoperoxidase kits for mouse IgG or rabbit IgG (Histofine SAB-PO kit; Nichirei Corp., Tokyo, Japan) with 3,3′-diaminobenzidine tetrahydrochloride (Dojindo Laboratory, Kumamoto, Japan) as the chromogen. Cellular nuclei were counterstained with 1% methyl green. The slides were then dehydrated, and coverslips were applied using permount. Negative controls were constructed by omitting the primary antibodies or staining with nonimmune rabbit or mouse IgG. No staining was observed in negative control samples.

Evaluation of Staining. FAS staining was considered positive when the cytoplasm of the tumor cells was stained. For nuclear antigen of Ki-67, tumor cells showing a definite nuclear staining were scored as positive. The intensity of staining was not graded.

Positive staining was evaluated with a KS Image Analysis Systems (Carl Zeiss Vision). The areas containing the largest number of positive tumor cells were selected on a high-power field of a microscope, and the microscopic images of these areas were downloaded to a computer with a color video camera. The images were enhanced to help distinguish the density of the brown diaminobenzidine reaction product from the methyl green-stained nuclei. A minimum of 1000 tumor cells were counted on the computer display with the manual point measure mode of this software. The percentage of positive cells was then calculated.

The tumor tissues were estimated to be positive when Ki-67 or FAS labeling of more than 10% of the tumor cells was seen. A cutoff value of 10% for Ki-67 was chosen arbitrarily for statistical requirements following literature data (37, 38, 41), and a cutoff value of 10% for FAS was pursuant to Ki-67.

Statistical Analysis. The following variables were analyzed for their possible prognostic value: patient age at presentation; sex; duration of symptoms before presentation; location; depth and size of the primary tumor; tumor presentation (primary or recurrent); histological type and grade; number of mitotic figures; surgical margin at definitive surgery; and immunoreactivity of Ki-67 and FAS. Statistical significance between these clinicopathological factors and status of immunoreactivity of FAS was evaluated with Fisher's exact test, a χ^2 test, and Mann-Whitney U test. The correlation of the percentage of FAS and Ki-67 positivity was evaluated using bivariate analysis with calculation of Spearman's rank correlation coefficient. DFS and OS in relation to all potential prognostic variables and immunostaining status were computed by the

Kaplan-Meier method (50) for 64 STS patients. The differences between the survival curves were evaluated by the log-rank test to determine statistical significance levels (51). Univariate and multivariate regression analyses were based on the Cox proportional hazards model (52). In the multivariate analysis, all variables with hazard ratios significantly different from 1.0 in univariate analysis were taken into consideration. The confidence level for statistical significance was set at $P < 0.05$. These statistical analyses were performed using StatView 5.0 software (SAS Institute Inc., Cary, NC).

RESULTS

Clinicopathological Features. A summary of the clinical and pathological data is presented in Table 1. Subjects included 35 males and 29 females ranging in age from 0 to 82 years (median age, 53.0 years). The duration of symptoms fluctuated from 0 to 312 months (median, 7.0 months). Fifty-one patients were initially treated for primary tumors at our hospital, and the other 13 patients were treated for locally recurrent tumors after resection elsewhere. Surgical treatment was as follows: 5 amputations; 33 wide local excisions; 21 marginal excisions; and 5 intracapsular excisions.

The primary tumors were located in the upper extremities in 12 cases, the lower extremities in 36 cases, and the trunk in the remaining 16 cases, including 1 in the neck region. Fifteen tumors were located superficially, and 49 were deep-seated (below the superficial fascia). Tumor size ranged from 1 to 26 cm (median, 10.0 cm), and 54 cases (84.4%) had tumors of >5 cm in diameter. Histological types included 23 MFHs (35.9%), 17 liposarcomas (26.5%), 7 MPNSTs (10.9%), 4 synovial sarcomas (6.2%), 3 leiomyosarcomas (4.7%), 2 each of rhabdomyosarcomas and undifferentiated sarcomas (3.1%), and 1 each of clear cell sarcoma, extraskeletal Ewing's sarcoma, extraskeletal mesenchymal chondrosarcoma, extraskeletal myxoid chondrosarcoma, fibrosarcoma, and malignant hemangiopericytoma (1.6%). The number of mitotic figures revealed a variation from 0 to 28 per 10 high-power fields (median, 1; average, 4.1). The tumors were classified as grade 1 in 13 patients, grade 2 in 23 patients, and grade 3 in 28 patients. The surgical margin was evaluated as wide in 37 patients, marginal in 22 patients, and intracapsular in 5 patients.

The median and mean follow-up periods for patients in this study were 52 and 68 months, respectively. Local recurrence occurred in 15 patients (23.4%), and 19 developed distant metastasis or regional lymph node metastasis (29.7%). At the conclusion of this investigation, 46 patients were alive with no evidence of disease, 1 patient was alive with disease, and 17 had died of their disease. The 5-year survival probability was 70.2%.

Table 1 Clinicopathological characteristics of 64 patients with STS

Characteristics		No. of patients	Percentage
Sex			
Male		35	54.7
Female		29	45.3
Age (yrs)			
Median	53.0		
Range	0–82		
Duration of symptoms (mo)			
Median	7.0		
Range	0–312		
Presentation			
Initial		51	79.7
Recurrent		13	20.3
Location			
Limb		48	75.0
Trunk		16	25.0
Depth			
Superficial		15	23.4
Deep-seated		49	76.6
Size (cm)			
Median	10.0		
Range	1–26		
Histological type			
MFH		23	35.9
Liposarcoma		17	26.6
MPNST		7	10.9
Synovial sarcoma		4	6.3
Leiomyosarcoma		3	4.7
Rhabdomyosarcoma		2	3.1
Others[a]		8	12.5
No. of mitotic figures/10 HPF[b]			
Median	1		
Range	0–28		
Grade			
1		13	20.3
2		23	35.9
3		28	43.8
Surgical margin			
Wide		37	57.8
Marginal		22	34.4
Intracapsular		5	7.8
Recurrence			
Negative		49	76.6
Positive		15	23.4
Metastasis			
Negative		45	70.3
Positive		19	29.7

[a] Others contain two undifferentiated sarcomas, and one each of clear cell sarcoma, extraskeletal Ewing's sarcoma, extraskeletal mesenchymal chondrosarcoma, extraskeletal myxoid chondrosarcoma, fibrosarcoma, and malignant hemangiopericytoma.
[b] HPF, high-power fields.

Immunohistochemical Features of FAS. Immunohistochemical expression of FAS was seen in 20 patients (31.3%). FAS reactivity was located in the cytoplasm and usually displayed a granular pattern. The staining was heterogeneous within the tumor, and the intensity of staining varied from region to region and from cell to cell. The cytoplasmic membranes of normal fat cells in each specimen stained strongly with the FAS antibody and served as an internal positive control. FAS-positive sarcomas included 13 of the 23 MFHs (56.5%; Fig. 1, *A* and *B*), 3 of the 17 liposarcomas (17.6%; Fig. 1, *C* and *D*), 3 of the 7 MPNSTs (42.9%; Fig. 1, *E* and *F*), and the extraskeletal mesenchymal chondrosarcoma. There were no FAS-positive tumors in the patients with synovial sarcomas, leiomyosarcomas, or rhabdomyosarcomas.

Statistical Relationship between FAS and Clinicopathological Variables. FAS expression was associated with depth (Fisher's exact test, $P = 0.02$) and size (Mann-Whitney U test, $P = 0.03$) of the tumors. FAS-positive tumors were significantly deep-seated and large in size. MFHs expressed FAS more significantly than the other STSs (Fisher's exact test, $P = 0.002$). FAS-positive tumors were also significant for Ki-67

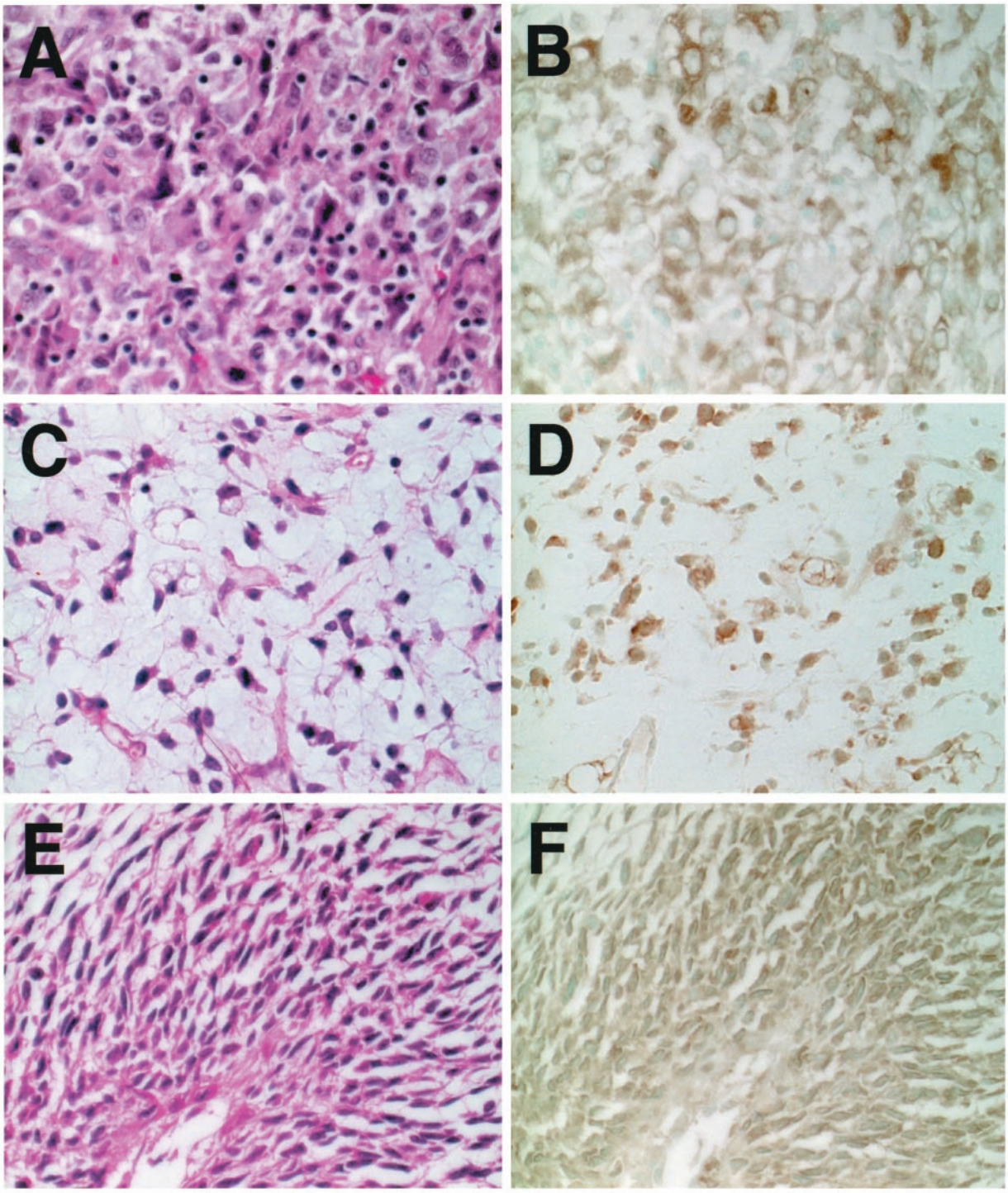

Fig. 1 Representative storiform-pleomorphic-type MFH (*A* and *B*), myxoid-type liposarcoma (*C* and *D*), and a MPNST (*E* and *F*) are shown. Immunohistochemical staining for FAS is detected in the cytoplasm of the tumor cells, displaying a granular pattern in all cases. *A, C,* and *E,* H&E staining; *B, D,* and *F,* immunostaining for anti-FAS antigen; original magnification, ×400.

(Fisher's exact test, $P = 0.02$), and there was a significant correlation between FAS-positive and Ki-67-positive status (Spearman's rank correlation coefficient = 0.26, $P = 0.04$). There was no significant association between FAS and the other variables (sex, age, location, number of mitotic figures, and so forth; Table 2).

Survival Analysis. Survival analysis determined that FAS-positive tumors were significantly associated with a higher

Table 2 Statistical relationship between FAS status and other variables in 64 patients with STS

Parameter	FAS status: Negative (n = 44)	FAS status: Positive (n = 20)	P
Sex			
Male	27	8	0.2[a]
Female	17	12	
Age (yrs)			
Median	52.0	56.0	0.5[b]
Range	0–82	21–71	
Duration of symptoms (mo)			
Median	7.0	7.0	0.9[b]
Range	1–312	1–108	
Presentation			
Initial	35	16	>0.99[a]
Recurrent	9	4	
Location			
Limb	34	14	0.6[a]
Trunk	10	6	
Depth			
Superficial	14	1	0.02[a]
Deep-seated	30	19	
Size (cm)			
Median	9.0	12.0	0.03[b]
Range	1–26	5–20	
Histological type			
MFH	10	13	0.002[a]
Not MFH	34	7	
No. of mitotic figures/10 HPF[c]			
Median	1	1	>0.99[b]
Range	0–28	0–19	
Grade			
1 + 2	27	9	0.3[a]
3	17	11	
Ki-67 status			
Negative	24	4	0.02[a]
Positive	20	16	

[a] Evaluated with Fisher's exact test.
[b] Evaluated with Mann-Whitney U test.
[c] HPF, high-power fields.

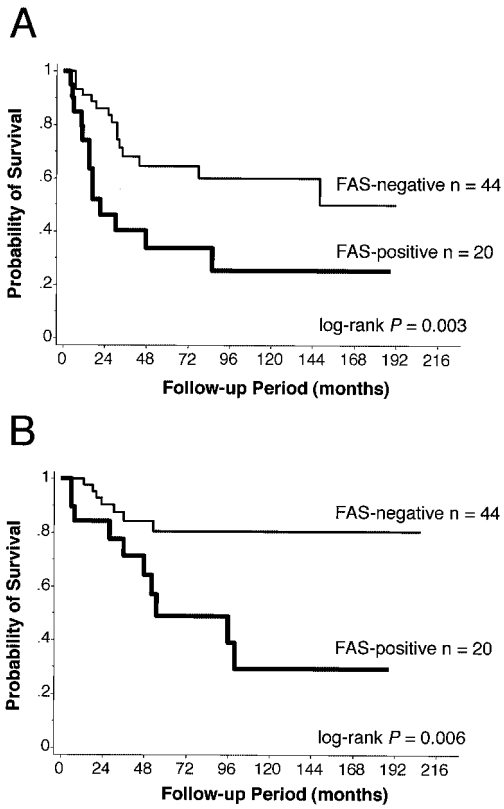

Fig. 2 The probability of DFS (*A*) and OS (*B*) in 64 patients with STSs evaluated for FAS expression. FAS status significantly predicts DFS (P = 0.006) and OS (P = 0.003).

risk of recurrence and metastasis. We found that FAS status also significantly predicted DFS (P = 0.006; Fig. 2*A*) and OS (P = 0.003; Fig. 2*B*). The 5- and 10-year probabilities of DFS of patients with FAS-positive tumors were 33.7% and 25.3%, respectively, whereas those of patients with FAS-negative tumors were 64.5% and 59.9%, respectively. The 5- and 10-year OS rates of patients with FAS-positive tumors (48.9% and 29.3%, respectively) were significantly lower than those of FAS-negative tumors (80.5% each). Other prognostic factors, such as location (P = 0.04), tumor grade (P = 0.002), surgical margin (P = 0.003), and Ki-67 immunoreactivity (P = 0.004), were associated with DFS. Tumor grade, surgical margin, and Ki-67 status were also associated with OS (P = 0.0001, 0.008, and 0.003, respectively; Table 3).

Multivariate analysis showed that FAS status was a predictor for DFS. Patients with tumors expressing FAS were 2.73 times more likely to relapse than those with FAS-negative tumors (95% confidence interval, 1.17–6.41; P = 0.02). Another significant factor for DFS was surgical margin (hazard ratio = 3.51; 95% confidence interval, 1.45–8.50; P = 0.005). The only significant predictor for OS was tumor grade (P = 0.04); FAS status did not reach the level of significance for OS (P = 0.07) in multivariate analysis (Table 4).

DISCUSSION

In the present study, FAS expression was seen in about 30% of 64 STSs by immunohistochemistry. Thirteen of 23 MFHs, 3 of 7 MPNSTs, 3 of 17 liposarcomas, and 1 extraskeletal mesenchymal chondrosarcoma exhibited FAS in the cytoplasm of tumor cells. From these results, expression of FAS seems to be up-regulated mainly in MFHs and MPNSTs. In liposarcomas, FAS expression was a rather rare event, despite FAS-positive results in mature adipocytes as reported previously (47), and was confirmed by the immunostaining present.

Expression of FAS in STSs displayed good correlation with deep-seated localization of tumors. Deep-seated STSs generally show malignant behavior and have a predictably poorer prognosis (53–55). FAS seems to be associated with tumor cell aggressiveness through energy production and supply of membrane constituents through the metabolism of fatty acids. In addition, FAS expression in STSs correlated with the size of the tumor. This indicates that FAS expression is associated with progression of STS, although several human tumors revealed up-regulated FAS expression even in premalignant or precancerous lesion (56).

As described in the "Introduction," there are two sources

Table 3 Log-rank analysis of DFS and OS in 64 patients with STS

Variable	DFS			OS		
	5-year	10-year	*P*	5-year	10-year	*P*
Sex						
Male	52.8	47.5	0.9	65.4	57.3	0.7
Female	57.6	50.4		74.2	68.5	
Age (yrs)						
<53	60.0	50.0	0.5	79.6	68.6	0.3
≥53	49.9	49.9		60.5	60.5	
Duration of symptoms (mo)						
<7	54.9	49.4	0.9	72.6	66.5	0.8
≥7	55.2	48.3		66.5	59.1	
Presentation						
Initial	59.0	49.9	0.7	73.9	64.6	0.9
Recurrent	42.0	42.0		64.2	54.2	
Location						
Limb	60.6	56.3	0.04	75.0	70.3	0.07
Trunk	39.4	29.5		56.3	45.0	
Depth						
Superficial	44.3	44.3	0.8	71.8	71.8	0.7
Deep-seated	57.0	49.8		69.6	61.1	
Size (cm)						
<10	46.3	46.3	0.9	68.0	68.0	0.7
≥10	60.7	49.7		72.3	60.7	
Histological type						
MFH	40.8	40.8	0.3	51.9	41.5	0.046
Not MFH	63.2	54.1		82.6	77.4	
No. of mitotic figures/10 HPF[a]						
<1	68.5	45.6	0.4	84.4	84.4	0.4
≥1	49.3	41.7		66.0	57.8	
Grade						
1 + 2	70.5	64.6	0.002	90.0	90.0	0.0001
3	34.7	28.9		44.7	33.6	
Surgical margin						
Wide	70.5	64.1	0.003	78.6	71.4	0.008
Marginal	46.8	40.1		67.2	67.2	
Intracapsular	0	0		26.7	0	
FAS status						
Negative	64.5	59.9	0.006	80.5	80.5	0.003
Positive	33.7	25.3		48.9	29.3	
Ki-67 status						
Negative	73.6	73.6	0.004	90.0	90.0	0.003
Positive	40.3	30.3		55.6	44.5	

[a] HPF, high-power fields.

Table 4 Univariate and multivariate analysis of DFS and OS in 64 patients with STS

Variable	DFS						OS					
	Univariate[a]			Multivariate[a]			Univariate[a]			Multivariate[a]		
	HR[b]	(95% CI[c])	*P*	HR	(95% CI)	*P*	HR	(95% CI)	*P*	HR	(95% CI)	*P*
Location (Limb *vs.* trunk)	2.21	(1.02–4.79)	0.045	2.00	(0.78–5.16)	0.2						
Grade (1 + 2 *vs.* 3)	3.16	(1.47–6.81)	0.003	2.27	(0.91–5.68)	0.08	8.69	(1.98–38.02)	0.004	4.34	(1.05–17.87)	0.04
Surgical margin (wide *vs.* intracapsular)	5.47	(1.87–15.96)	0.002	3.51	(1.45–8.50)	0.005	5.58	(1.63–19.14)	0.006	2.16	(1.02–12.13)	0.2
FAS status (negative *vs.* positive)	2.74	(1.30–5.78)	0.008	2.73	(1.17–6.41)	0.02	3.93	(1.45–10.07)	0.007	2.54	(0.91–7.07)	0.07
Ki-67 status (negative *vs.* positive)	3.31	(1.40–7.83)	0.006	2.12	(0.80–5.58)	0.1	6.87	(1.57–30.06)	0.01	2.48	(0.46–13.33)	0.3

[a] Evaluated with Cox proportional hazards model.
[b] HR, hazard ratio.
[c] CI, confidence interval.

of fatty acids for animals, diet and *de novo* biosynthesis, of which the latter is regulated by FAS. Increased FAS expression implies that fatty acids from the *de novo* biosynthesis are increased in cells or tissues. However, it is not clear whether increased FAS expression is intrinsic for highly proliferative cells (including tumor cells). It is possible that the increased FAS expression of tumor cells is an indirect, associated phenomenon, which occurs to compensate for an insufficiency of dietary fatty acids due to, *e.g.*, lack of angiogenesis. FAS expression may not be essential even in highly prolif-

erative tumor cells in the presence of enough fatty acids. Pizer *et al.* (30) showed that HL60 human promyelocytic leukemia cells maintained in a fatty acid-free medium died when cerulenin, an inhibitor of FAS, was administered. The cell death by this cerulenin addition was rescued by adding palmitate, which is a product of FAS. They reported (30) that HL60 cells maintained in medium to which serum containing endogenous fatty acid was added retained their sensitivity to cerulenin. These results demonstrate that HL60 cells are dependent on the endogenous fatty acid produced by FAS for their proliferation and survival. However, it remains unclear whether this phenomenon is common to sarcoma cells.

In 20-week-old human fetal tissues, FAS expression was observed not only in epithelial cells but also in growing mesenchymal cells, such as fibroblasts, osteoblasts, chondrocytes, and Schwann cells (47). These findings support the idea that FAS is necessary for proliferation of fetal mesenchymal cells and may also suggest that proliferation of STSs, which develop from immature mesenchymal cells, requires the endogenous fatty acids produced by FAS.

Our study also demonstrates that immunohistochemical detection of FAS provides important prognostic information in patients with STS. Patients with tumors expressing FAS demonstrated a statistically significant lower rate of DFS and OS compared with those whose tumors did not express FAS. Expression of FAS, however, was not an independent prognostic predictor in STS when compared with Ki-67 labeling index.

The prognostic significance of FAS expression levels has been described previously in certain cancers. Kuhajda *et al.* (26) showed that about 30% of patients with early breast cancers expressed haptoglobin-related protein epitopes later proven to be identical with FAS. They concluded that patients expressing haptoglobin-related protein had a significantly increased risk of recurrence (13). In the immunohistochemical studies focusing on node-negative breast carcinomas, FAS was strongly and statistically associated with recurrence and short DFS or OS (15, 16). In prostate cancers, FAS was associated with higher tumor grade, larger tumor volume, advancing clinical stage, and Gleason's score, one of the most powerful predictors (19–21). FAS expression has also been studied in ovarian neoplasia, where it was seen to be associated with histological tumor grade and shorter survival (22).

Although it has not previously been associated with poor prognosis, FAS expression has been detected in various tumors and associated with histological subtype, histopathological grade, and tumor aggressiveness. Such cases include colorectal adenocarcinomas that show an increase of FAS intensity in colloid/mucinous carcinomas and carcinomas with extensive metastatic involvement of lymph nodes (23) and endometrial adenocarcinomas that show FAS expression intensity increasing with tumor grade (25). FAS expression was also correlated with the degree of dysplasia and correlated with late-stage colon carcinogenesis (24).

The association between FAS expression and tumor size or a marker of proliferative activity of tumor cells suggests that fatty acid synthesis is related to growth and proliferation of these malignant tumors. Alò *et al.* (16) have shown that there is a significant association between FAS and the maximum diameters of tumor masses in breast carcinoma. In prostate cancer, FAS expression has also been shown to be significantly associated with tumor volume (19). On the other hand, the immunohistochemical detection of Ki-67 antigen is a suitable method for determination of the proliferative rate of tumors. A close linkage between FAS and Ki-67 expression has been reported previously in breast (15) and endometrial carcinomas (25). Our present data demonstrate that FAS expression is also associated with Ki-67 expression in STS. In the expression of immunohistochemically detectable markers connected with proliferative activity, Ki-67 has been shown to be of prognostic value for a heterogeneous group of STSs (36–39).

Although FAS expression has been correlated with the Ki-67 index, FAS expression was not related to the number of mitotic figures, which formed one of the indices of proliferative activity. A possible reason for this is that the power of the test of the number of mitotic figures was lower than that of Ki-67 expression. The number of mitotic figures counted in this study ranged widely from 0 to 28. However, there were 22 cases in which mitotic figures were not observed, and these accounted for more than one-third of all 64 cases. The number of mitotic figures was 1 in 11 cases. The median number of mitotic figures was therefore 1, and the cases in which the number of mitotic figures was less than 1 occupied the majority. On the other hand, Ki-67 reacts with a nuclear antigen expressed throughout the cell cycle, except in the G_0 phase, and mitotic figures were detected in M-phase tumor cells. Ki-67 expression reflects proliferative potency more precisely than the number of mitotic figures (35, 57). Because the number of mitotic figures did not sufficiently reflect the proliferative activity of STSs examined in this study, it did not significantly correlate with FAS expression.

Würl *et al.* (44) suggest that for immunohistochemical evaluation of p53 overexpression in STS, a panel of at least two NH_2-terminal antibodies should be used. They compared the prognostic relevance of five different p53 antibodies in STS, and only the NH_2-terminal-binding monoclonal antibodies showed a multivariate correlation with survival. With p53 polyclonal antibody, there was a strong association between immunostaining and survival, but it showed no significant correlation with prognosis on multivariate analysis. They attributed this to segments in the NH_2-terminal area of the p53 protein with a regulatory effect on the transcription of a variety of proteins that regulate the cell cycle. Therefore, the preservation of certain NH_2-terminal epitopes and their overexpression (and thus the possibility of detecting this protein segment) have a particular biological and therefore prognostic significance. The FAS antibody that was used in this study was polyclonal, and multivariate analysis was not able to find a correlation between immunoreactivity and survival. As was the case with the p53 monoclonal antibody, if an anti-FAS monoclonal antibody had been used, significant correlation with survival may have been found by multivariate analysis. This will need to be examined in future investigations.

Würl *et al.* (58) reported that tumor type, stage (including mitotic counts and the appearance of necrosis), localization, type of surgery, and p53 and mdm2 protein overexpression were each independent prognostic factors for STS in the Cox model. This is consistent with our findings. In our study, the clinicopathological indicators that correlate with prognosis were tumor type, grade, localization, surgical margin, and FAS and Ki-67

expression. However, our method of analysis was different. They subdivided tumor histology into six types (fibrosarcoma, MFH, MPNST, liposarcoma, leiomyosarcoma, and rhabdomyosarcoma), subdivided localization into four regions (extremity, head and neck, trunk wall, and abdomen and retroperitoneum), and subdivided stage into eight stages (from IA to IVB). These factors were directly used in the Cox model multivariate analysis. On the other hand, we divided each factor, such as tumor type, grade, and localization, into two groups. We performed univariate and multivariate analysis based on the Cox proportional hazards model to determine which of the prognostic factors was significantly different in a log-rank test. We were not able to use the examination method used by Würl *et al.* (58) because there were too few cases. Nevertheless, despite the limited number of cases, our method of analysis was appropriate because the prognostic indicators provided were similar.

In addition to its utility as a prognostic indicator, FAS also shows promise as a chemotherapeutic target. Several studies have shown that cerulenin, a specific noncompetitive inhibitor of the β-ketoacyl synthase activity of FAS (27–29), is selectively cytotoxic to cancer cells that have increased fatty acid synthesis, but not to normal cells (26, 30–33), which have constitutively low levels of FAS (4). Tumors that are found to express FAS at an increased level are the same subsets of STS shown in the present study and might be an excellent therapeutic target for inhibition of fatty acid synthesis.

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