The prognosis in spindle-cell sarcoma depends on the expression of cyclin-dependent kinase inhibitor $p27^{Kip1}$ and cyclin E

Yoshinari Goto,[1,2] Shigeto Kawauchi,[1] Koichiro Ihara,[2] Kenzo Ikemoto,[1] Ritsuko Ohi,[2] Shinya Kawai[2] and Kohsuke Sasaki[1]

[1]The Department of Pathology and [2]Orthopedic Surgery, Yamaguchi University School of Medicine, 1-1-1 Minamikogushi, Ube, Yamaguchi 755-8505

(Received January 6, 2003/Revised March 5, 2003/Accepted March 5, 2003)

The aim of the present study was to examine the prognostic significance of $p27^{Kip1}$ and cyclin E expression in patients with spindle-cell soft tissue sarcomas. In 46 cases of spindle-cell sarcoma including 17 pre-operative biopsy materials, the expression of $p27^{Kip1}$ and cyclin E was immunohistochemically examined. The expression of $p27^{Kip1}$ decreased in the nuclei of metastatic primary tumor cells (stage IV), whereas the expression of cyclin E increased in those lesions. On univariate analysis, when the expression of $p27^{Kip1}$ and cyclin E was analyzed together, patients with spindle-cell sarcoma exhibiting low expression of $p27^{Kip1}$ and high expression of cyclin E showed lower distant-metastasis-free survival (DMFS) and overall survival (OS) than those with other combinations of the two parameters (both $P<0.0001$). Multivariate analysis revealed that patients with low $p27^{Kip1}$ and high cyclin E expression also showed a decrease in DMFS ($P=0.0007$, relative risk=21.3) and OS ($P=0.005$, relative risk=20.8). These results suggest that the combination analysis of $p27^{Kip1}$ and cyclin E expression even in biopsy specimens allows the prediction of the clinical behavior of spindle-cell sarcoma. (Cancer Sci 2003; 94: 412–417)

It is well known that spindle-cell sarcomas have an aggressive nature. The 5-year survival rates of patients with malignant fibrous histiocytoma (MFH), synovial sarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumor (MPNST), and fibrosarcoma have been reported to be 70%,[1] 60%,[2] 64%,[3] 52%,[4] and 39%,[5] respectively.

Recently, several cell cycle-related markers have been examined to predict the clinical behavior of various kinds of carcinomas and soft tissue sarcomas, e.g., Ki-67,[6–9] $p27^{Kip1}$, and cyclin E proteins.[9–12] Cyclins such as D-type cyclins and cyclin E are primarily involved in the G_1/S transition and their expression promotes the progression of the cell cycle.[13–15] In contrast, the $p27^{Kip1}$ protein is a cyclin-dependent protein kinase (CDK) inhibitor that blocks the G_1/S transition of the cell cycle by inhibiting the action of the cyclin E-CDK2 complex.[13, 14] Interestingly, $p27^{Kip1}$ can both an inhibitor and a substrate of cyclin E-CDK2.[14, 16, 17]

A decrease of $p27^{Kip1}$ protein and an increase of cyclin E protein have been shown to be associated with aggressive growth of breast,[18] lung,[19] colorectal,[20] gastric,[21] and prostate tumors.[22] However, there have been a few studies examining the expression of both $p27^{Kip1}$ and cyclin E in mesenchymal neoplasms.[9, 10] The relationship between the expression of these molecules and biological behavior remains unclarified in spindle-cell sarcomas. The purpose of the present study was to investigate the relationship between these cell cycle regulators and clinicopathologic parameters, and to analyze their prognostic significance.

Materials and Methods

Clinical data. Forty-six patients with spindle-cell sarcoma, primarily treated at Yamaguchi University Hospital and related hospitals between 1985 and 1998, were selected for the study. The distribution of clinical, pathological, and therapeutic data for the entire patient population is listed in Table 1. There were 25 males and 21 females with a median age of 57 years. The median tumor size was 6.7 cm (range: 1.5–24.0 cm). The histopathologic type, grade, and the clinical stage of the sarcomas were determined according to the World Health Organization classification and the American Joint Committee on Cancer (AJCC) staging system.[23] The histopathologic grading followed the proposal by AJCC.[23] In addition, grades 1 (well differentiated) and 2 (moderately differentiated) were grouped as low-grade and grades 3 (poorly differentiated) and 4 (undifferentiated) as high-grade.[23] Thirty of the patients had had chemotherapy and/or radiotherapy for the primary tumor with a good response in only two cases. When pre-operation adjuvant therapy was administered (17 cases), contrast enhanced computed tomography and/or magnetic resonance imaging followed by sampling under incisional biopsy was performed.

Distant-metastasis-free survival (DMFS) and overall survival (OS) of the 41 patients (I, II, III, and IVA disease) without distant metastasis (IVB disease) at the time of diagnosis were analyzed. No patients were lost to follow-up. The median follow-up period was 44 months (range: 1.5–176 months). There were 14 patients with distant metastasis, of whom 13 had died of the disease at final follow-up.

Immunohistochemistry. Representative tissue blocks (from 3 to 10 pieces) of each resected primary tumor were selected for immunohistochemical evaluation. When pre-operation adjuvant therapy was administered, immunostaining was performed on biopsy specimens obtained before adjuvant therapy. For immunostaining, 3-μm sections were cut from paraffin-embedded tissue blocks and mounted on silane-coated slides. Sections were then heated in a microwave oven (650 W for 30 min) with 10 mmol/liter sodium citrate buffer (pH 6.0) for antigen unmasking. Endogenous peroxidase activity was blocked by incubation with 0.03% H_2O_2 in absolute methanol at room temperature for 30 min. Low cellularity areas and/or tumor necrosis on tissue sections were removed based on hematoxylin eosin staining. Sections were then encircled by hydrophobic DAKO PEN (DAKO, Carpinteria, CA). The slides were then incubated overnight at 4°C with murine monoclonal antibodies for $p27^{Kip1}$ (clone 1B4; Novocastra, Newcastle upon Tyne, UK) and cyclin E (clone 13A3; Novocastra), which were diluted 1:200 and 1:50 with 1% bovine serum albumin/phosphate-buffered saline, pH 7.6 (BSA/PBS), respectively. The subsequent reaction was based on the streptavidin-biotin complex/horseradish peroxidase method, using a HISTOFINE SAB-PO (M) Immunohistochemical Staining Kit (Nichirei, Tokyo) according to the manufacturer's protocol. Sections were covered uniformly with

E-mail: goto123@hyper.ocn.ne.jp

the thin film during antigen-antibody reaction. Reaction products were visualized with hydrogen peroxide containing 3,3-diaminobenzidine/PBS, and hematoxylin was used for counterstaining. Positive controls included tonsillar tissues for $p27^{Kip1}$ and placental tissues for cyclin E. Negative controls were created by substituting normal serum for the primary antibody, which resulted in no staining of the tissues.

Evaluation. Positive staining for $p27^{Kip1}$ and cyclin E proteins was initially assessed at a low magnification (×40), and areas containing the highest number of sarcoma cells with clear nuclear staining were selected. At a high magnification (×400), the labeling indices (LI) for $p27^{Kip1}$ and cyclin E were calculated as a percentage of sarcoma cells with positive nuclei among all sarcoma cells counted. In each tumor, at least 2000 sarcoma cells were counted. On 23 large tumor specimens (>2 cm in length), 2 or more areas were selected at low magnification, and then several LIs were calculated on each selected area. To evaluate the results of immunohistochemical staining, we scored tumor cells displaying nuclear immunoreactivity, and the intensity of staining was not taken into account, as previously described.[12)]

For examination of the relation between prognosis (DMFS and OS) and immunoreactivity, we selected the cut-off values of $p27^{Kip1}$ LI and cyclin E LI by setting tentative cut-off values and testing the most significant one against DMFS ($p27^{Kip1}$ LI, $P<0.0001$; cyclin E LI, $P=0.0002$). The cut-off values selected were as follows: $p27^{Kip1}$ LI, 55%; and cyclin E LI, 45%. The LIs of $p27^{Kip1}$ and cyclin E were also graded by a two-category system as low- or high-grade groups. Two observers (Y.G. and S.K.) without previous knowledge of the patient outcome evaluated the immunohistochemical results.

Statistical analysis. The results were analyzed using the StatView 4.5 software package (Abacus Concepts, Inc., Berkeley, CA). Differences in LIs between the selected areas on large tumor

Table 1. Clinical and pathological data

Parameter	Category	No.	Median $p27^{Kip1}$ LI (%)	Median cyclin E LI (%)
Gender				
	Males	25	60.2	36.2
	Females	21	61.5	34.5
Age				
	<50	28	60.8	35.9
	≥50	18	62.3	34.9
Size (cm)				
	<5	13	64.0	30.5
	5–10	12	60.8	34
	≥10	21	55.7	44.1
Site				
	Extremities	28	66.8[1)]	33.9
	Trunk	18	53.2[1)]	44.6
Histology				
	MFH	23	61.4	34.5
	Storiform pleomorphic	20	58.6	34.2
	Myxoid	3	67.5	36.2
	Synovial sarcoma	15	70.9	35.3
	Monophasic fibrous	7	61.5	35.3
	Biphasic	6	72.3	41.0
	Poorly differentiated	2	76.6	44.2
	Leiomyosarcoma	4	51.9	38.5
	MPNST	3	60.2	30.1
	Fibrosarcoma	1	30.6	60.0
Grade				
	High	39	60.2[2)]	35.6
	Low	7	73.4[2)]	30.1
Tumor depth				
	Superficial	12	63.5	28.5[3)]
	Deep-seated	34	58.0	39.4[3)]
AJCC stage				
	I	3	64.0[4)]	37.8[5)]
	II	18	71.0[4)]	30.1[5)]
	III	16	60.9[4)]	35.5[5)]
	IV	9	40.2[4)]	55.8[5)]
Surgical margin				
	Intralesional or marginal	23		
	Wide	23		
Adjuvant therapy				
	Chemotherapy	21		
	Radiotherapy	5		
	Chemotherapy+radiotherapy	4		

LI, labeling index; AJCC, American Joint Committee on Cancer; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor.
1) $P=0.04$, *2*) $P=0.04$, *3*) $P=0.02$ (Mann-Whitney U test), *4*) $P=0.002$, *5*) $P=0.001$ (Kruskal-Wallis test).

specimens and categorized values between the sarcoma groups were analyzed by the Mann-Whitney *U* test, or the Kruskal-Wallis test. Correlations among the $p27^{Kip1}$ LI and cyclin E LI were estimated using Spearman's rank correlation coefficients. DMFS and OS were modeled by the Kaplan-Meier method and analyzed by the log rank test. In addition to the $p27^{Kip1}$ and cyclin E LIs, the following clinicopathological adverse factors were analyzed for their relationship with DMFS and OS; tumor location (extremities vs. trunk), AJCC stage (I and II vs. III and IVA), surgical margin (intralesional and marginal vs. wide), and method of therapy (operation only vs. operation and adjuvant therapy). Cox's proportional hazards regression model analysis was performed with relative risk (RR) and a 95% confidence interval (CI). A two-tailed *P* value of <0.05 was considered significant.

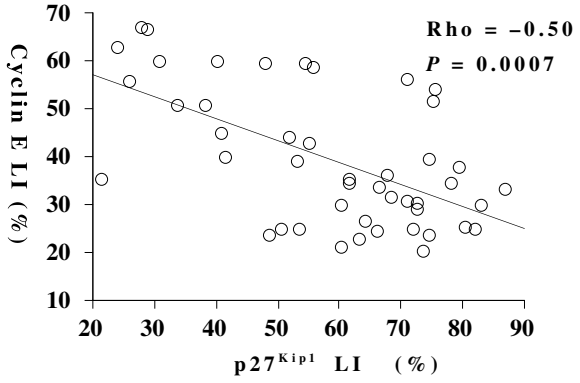

Fig. 1. Correlation between $p27^{Kip1}$ LI and cyclin E LI in 46 patients with spindle-cell sarcoma. Note the significant correlation between $p27^{Kip1}$ LI and cyclin E LI (Rho=-0.50, *P*=0.0007). LI, labeling index.

Results

Immunohistochemistry and clinicopathologic parameters. Immunohistochemical data are summarized in Table 1. $p27^{Kip1}$ and cyclin E were expressed exclusively in nuclei in all cases ranging from 21% to 87% (median: 61%) and 21% to 67% (median: 35%), respectively. There were no significant differences in LIs between the selected areas at low magnification on large tumor specimens (all $P>0.2$). There were no significant differences in either $p27^{Kip1}$ LI ($P=0.6$) or cyclin E LI ($P=0.5$) among MFH,

Table 2. Distant-metastasis-free survival, as determined with univariate analysis

Factor	All lesions (*n*=41)			MFH (*n*=19)			Synovial sarcoma (*n*=15)		
	No.	5-year DMFS (%)	*P* value	No.	5-year DMFS (%)	*P* value	No.	5-year DMFS (%)	*P* value
$p27^{Kip1}$ LI			<0.0001			0.01			0.0001
<55%	14	10		6	22		4	0	
≥55%	27	89		13	92		11	91	
Cyclin E LI			0.0002			0.0001			0.045
<45%	29	76		17	75		8	88	
≥45%	12	18		2	0		7	29	

MFH, malignant fibrous histiocytoma; DMFS, distant-metastasis-free survival; LI, labeling index.

Table 3. Overall survival, as determined with univariate analysis

Factor	All lesions (*n*=41)			MFH (*n*=19)			Synovial sarcoma (*n*=15)		
	No.	5-year OS (%)	*P* value	No.	5-year OS (%)	*P* value	No.	5-year OS (%)	*P* value
$p27^{Kip1}$ LI			0.0001			0.07			0.0002
<55%	14	33		6	63		4	0	
≥55%	27	88		13	92		11	90	
Cyclin E LI			0.0006			0.0002			0.05
<45%	29	85		17	94		8	86	
≥45%	12	25		2	0		7	29	

MFH, malignant fibrous histiocytoma; OS, overall survival; LI, labeling index.

Table 4. Distant-metastasis-free survival and overall survival, as determined with univariate analysis

Factor	High-grade lesions (*n*=34)					Stage III, IVA lesions (*n*=20)				
	No.	5-year DMFS (%)	*P* value	5-year OS (%)	*P* value	No.	5-year DMFS (%)	*P* value	5-year OS (%)	*P* value
$p27^{Kip1}$ LI			<0.0001		0.003			0.001		0.03
<55%	14	10		33		11	0		27	
≥55%	20	85		84		9	76		74	
Cyclin E LI			0.003		0.007			0.01		0.03
<45%	22	66		80		10	49		77	
≥45%	12	18		25		10	11		20	
$p27^{Kip1}$ LI+cyclin E LI			<0.0001		<0.0001			0.0002		0.004
Low $p27^{Kip1}$ LI+high cyclin E LI	9	0		11		9	0		11	
Others	25	70		83		11	55		80	

DMFS, distant-metastasis-free survival; OS, overall survival; LI, labeling index.

Table 5. Factors associated with DMFS and OS, as determined with univariate and Cox multivariate analysis (*n*=41)

Factor	No.	DMFS				OS			
		Univariate	Multivariate			Univariate	Multivariate		
		P value	*P* value	RR	95% CI	*P* value	*P* value	RR	95% CI
Low $p27^{Kip1}$ LI+high cyclin E LI	9	<0.0001	0.0007	21.3	3.6–125	<0.0001	0.005	20.8	2.6–169
Location: trunk	18	0.006	0.4			0.0002	0.1		
AJCC stage: III and IVA	20	0.0003	0.7			0.002	0.7		
Intralesional or marginal excision	24	0.02	0.02	7.1	1.4–36	0.002	0.008	15.2	2.1–111
Adjuvant therapy (+)	26	0.09	0.8			0.4	0.2		

DMFS, distant-metastasis-free survival; OS, overall survival; RR, relative risk; CI, confidence interval; LI, labeling index; AJCC, American Joint Committee on Cancer.

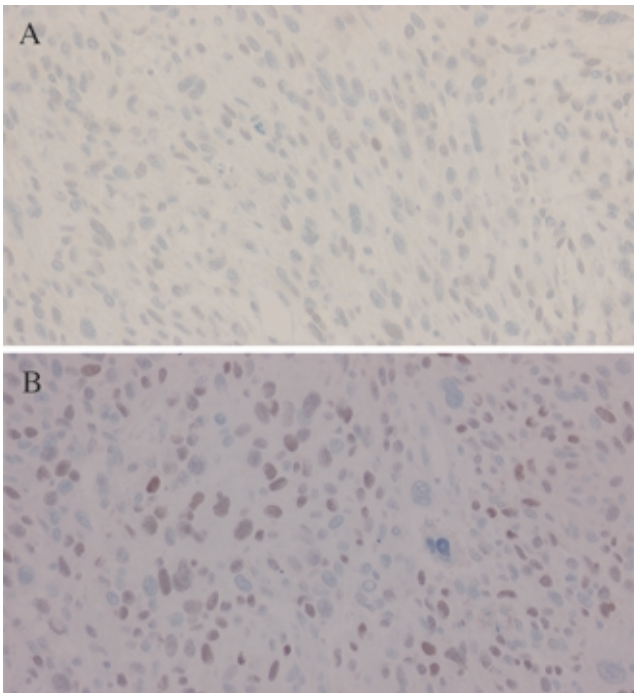

Fig. 2. Low nuclear expression of $p27^{Kip1}$ (a) and high expression of cyclin E (b) in a malignant fibrous histiocytoma patient with AJCC stage III disease. Three months after operation the patient developed lung metastasis and died after 6 months (original magnification,×200). AJCC, American Joint Committee on Cancer.

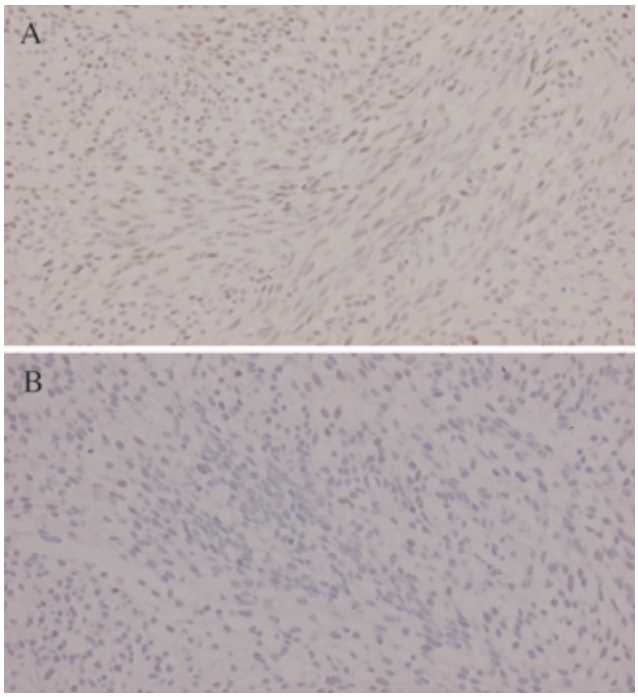

Fig. 3. High nuclear expression of $p27^{Kip1}$ (a) and low expression of cyclin E (b) in a synovial sarcoma patient with AJCC stage III disease. Twelve years after operation the patient was still disease-free (original magnification,×200). AJCC, American Joint Committee on Cancer.

synovial sarcoma, leiomyosarcoma, MPNST, and fibrosarcoma. In MFH and synovial sarcoma, $p27^{Kip1}$ LI (MFH, P=0.7; synovial sarcoma, P=0.1) and cyclin E LI (MFH, P=0.7; synovial sarcoma, P=0.8) showed no significant differences between histologic subtypes. The $p27^{Kip1}$ LI of spindle-cell sarcomas located in the trunk and high-grade histologic findings were significantly lower than those of sarcomas located in the extremities and low-grade histologic findings. The cyclin E LI of spindle-cell sarcomas with a deep location was significantly higher than those of sarcomas with a superficial location. The $p27^{Kip1}$ LI was significantly lower in the primary lesion of tumors with metastasis (stage IV) than in tumors with no metastasis (stage I, II, and III). On the contrary, a significant increase in cyclin E LI was observed in primary tumors with metastasis (stage IV). Neither $p27^{Kip1}$ LI nor cyclin E LI was correlated with patients' gender, age, or tumor size.

There was a significant inverse correlation between $p27^{Kip1}$ LI and cyclin E LI (Rho=−0.50, P=0.0007) (Fig. 1).

Univariate survival analysis. The results of univariate survival analysis are presented in Tables 2, 3, 4, and 5. Each low $p27^{Kip1}$ LI and high cyclin E LI was associated with a decrease in DMFS ($p27^{Kip1}$ LI, P<0.0001; cyclin E LI, P=0.0002) and OS ($p27^{Kip1}$ LI, P=0.0001; cyclin E LI, P=0.0006). In the patients with high-grade histologic findings or high-stage (III and IVA) lesions, each low $p27^{Kip1}$ LI and high cyclin E LI was associated significantly with short DMFS and OS. In the patients with low-grade histologic findings or low-stage (I and II) lesions, each LI was not associated with DMFS or OS. Tumor site, AJCC stage, and surgical margin were prognostic factors in DMFS and OS. In the 19 patients with MFH, each low $p27^{Kip1}$ LI and high cyclin E LI was associated significantly with short DMFS ($p27^{Kip1}$ LI, P=0.01; cyclin E LI, P=0.0001) and OS (cyclin E LI, P=0.0002). In the 15 patients with synovial sarcoma, each low $p27^{Kip1}$ LI and high cyclin E LI was associated significantly with short DMFS ($p27^{Kip1}$ LI, P=0.0001; cyclin E LI, P=0.045) and OS ($p27^{Kip1}$ LI, P=0.0002).

The patients were then divided into two groups based on the expression of $p27^{Kip1}$ and cyclin E; 9 patients with low $p27^{Kip1}$ LI and high cyclin E LI in one group, and 32 other patients in the other. Fig. 2 shows the results for one patient with low $p27^{Kip1}$ LI and high cyclin E LI and Fig. 3 shows the results for a patient from the other group. There were significant differences in DMFS (Fig. 4) and OS (Fig. 5) between the patients

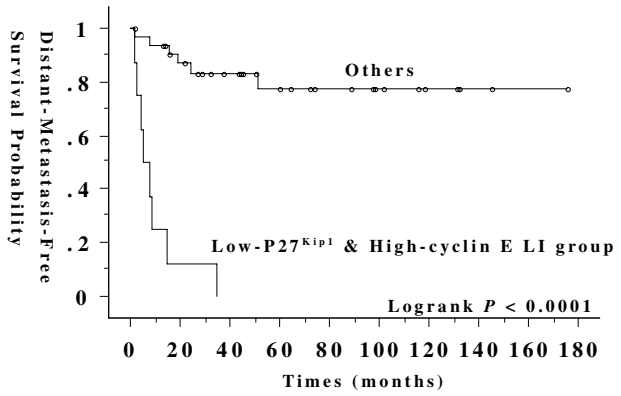

Fig. 4. Kaplan-Meier curves for distant-metastasis-free survival stratified according to combined p27 and cyclin E expression.

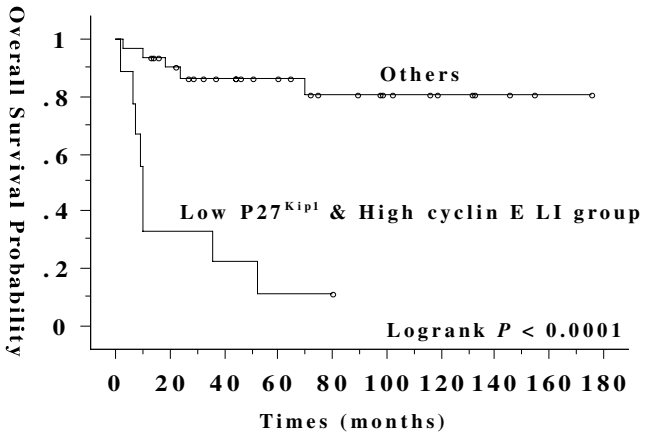

Fig. 5. Kaplan-Meier curves for overall survival stratified according to combined p27 and cyclin E expression.

with low $p27^{Kip1}$ LI and high cyclin E LI and the others (both $P<0.0001$). In the patients with high-grade histologic findings or high stage (III and IVA) lesions, low $p27^{Kip1}$ LI and high cyclin E LI was associated significantly with short DMFS and OS.

Multivariate survival analysis. Multivariate analysis was also performed to identify independent prognostic factors. The results are presented in Table 5. Independent adverse factors for DMFS and OS were low $p27^{Kip1}$ LI and high cyclin E LI (DMFS, $P=0.0007$; OS, $P=0.005$) and intralesional or marginal excision (DMFS, $P=0.02$; OS, $P=0.008$).

Discussion

The cell cycle transition depends mainly on the functional status of CDKs, e.g., the cyclin E-CDK2 complex, which is subject to regulation by CDK inhibitors, e.g., $p27^{Kip1}$, in G_1/S transition.[13, 14] CDK inhibitors are classified into two groups, the INK 4 family and the Cip/Kip family.[13, 14] The Cip/Kip family, including $p21^{Waf1/Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$, inhibits cyclin E-CDK2.[13, 14] $p27^{Kip1}$ can serve as a substrate for cyclin E-CDK2,[14, 16] and the ubiquitination and subsequent degradation of $p27^{Kip1}$ depend on its phosphorylation by cyclin E-CDK2.[14, 17, 24] Since $p27^{Kip1}$ has a close relation with cyclin E-CDK2, we have investigated the expression of $p27^{Kip1}$ and cyclin E in patients with spindle-cell sarcoma.

Numerous studies have demonstrated the clinical usefulness of $p27^{Kip1}$ and cyclin E as markers of aggressive tumor growth in various types of human cancers.[14, 18–22] However, their prognostic significance has not been fully elucidated in sarcomas, except for a few studies examining the expression of both $p27^{Kip1}$ and cyclin E in synovial sarcoma[9] and neurogenic tumors.[10]

In the present study, inverse correlations were found between $p27^{Kip1}$ LI and cyclin E LI in spindle-cell sarcomas. Since $p27^{Kip1}$ can be both an inhibitor and a substrate of cyclin E-CDK2, leading to its phosphorylation and proteolysis,[14, 16, 17, 24] the present study results reflect the interaction between $p27^{Kip1}$ and cyclin E-CDK2. $p27^{Kip1}$ LI values decreased in primary lesions of tumors with metastasis (stage IV). In contrast, cyclin E LI values increased in those tumors. These results may explain why decreased $p27^{Kip1}$ expression and increased cyclin E expression are associated with each other, and contribute to the progression of spindle-cell sarcomas.

Recently, $p27^{Kip1}$ expression was reported to be an independent marker predictive of DMFS and OS in myxoid/round cell liposarcoma[11] and synovial sarcoma.[12] Kourea *et al.*[10] reported that the nuclear expression of cyclin E was more pronounced in MPNST than in neurofibroma. In a study of localized synovial sarcomas, Antonescu *et al.*[9] showed that high expression of cyclin E was an adverse factor for patient survival.

In the present study, low $p27^{Kip1}$ LI (<55%) and high cyclin E LI (≥45%) showed independent adverse effects on DMFS and OS. Because decreased $p27^{Kip1}$ expression and increased cyclin E-CDK2 expression both favor G_1 to S progression,[13–17, 24, 25] we investigated DMFS and OS in relation to $p27^{Kip1}$ and cyclin E expression. Patients with low $p27^{Kip1}$ LI and high cyclin E LI showed worse DMFS and OS than those with other combinations of the two parameters (both $P<0.0001$). Low $p27^{Kip1}$ LI and high cyclin E LI were correlated with the patient's prognosis within the same histologic grade (high) or same AJCC stage (III and IVA). In multivariate analysis, low $p27^{Kip1}$ LI and high cyclin E LI were linked with an increased RR for DMFS (RR=21.3, $P=0.0007$) and OS (RR=20.8, $P=0.005$).

These observations suggest that the measurement of $p27^{Kip1}$ and cyclin E protein expression would be helpful for the estimation of risk stratification and the design of treatment protocols for patients with spindle-cell sarcoma, though the prognosis in the case of low-grade or low-stage (I and II) disease requires further study.

A large post-operative tissue sample may make it easy for a pathologist to diagnose the tumor accurately. However, often only a small sample is available, especially at the time of biopsy. Furthermore, the degenerated samples after pre-operation adjuvant therapy may not be representative of the entire tumor, leading to an underestimatation of the grade.

Immunohistochemical staining for $p27^{Kip1}$ and cyclin E in biopsy specimens after strict radiologic evaluation would make it possible to discriminate between patients with aggressive sarcomas that should be treated with adjuvant systemic therapy and those with low-risk sarcomas that should be managed by local excision at the time of diagnosis.

In conclusion, the present study showed that the decrease of $p27^{Kip1}$ expression and the increase of cyclin E expression are linked with tumor progression in spindle-cell sarcoma. It is suggested that the combined analysis of $p27^{Kip1}$ and cyclin E expression status would be useful for estimating the prognosis of patients with spindle-cell sarcomas.

1. Doussal VL, Coindre JM, Leroux A, Hacene K, Terrier P, Bui NB, Bonichon F, Collin F, Mandard AM, Contesso G. Prognostic factors for patients with localized primary malignant fibrous histiocytoma: a malticenter study of 216 patients with multivariate analysis. *Cancer* 1996; **77**: 1823–30.

2. Bergh P, Meis-Kindblom JM, Gherlinzoni F, Berlin O, Bacchini P, Bertoni F, Gunterberg B, Kindblom LG. Synovial sarcoma: identification of low and high risk groups. *Cancer* 1999; **85**: 2596–607.
3. Gustafan P, Willen H, Baldetorp B, Ferno M, Akerman M, Rydholm A. Soft tissue leiomyosarcoma: a population-based epidemiologic and prognostic study of 48 patients, including cellular DNA content. *Cancer* 1992; **70**: 114–9.
4. Wong WW, Hirose T, Scheithauer BW, Schild SE, Gunderson LL. Malignant peripheral nerve sheath tumor: analysis of treatment outcome. *Int J Radiat Oncol Biol Phys* 1998; **42**: 351–60.
5. Scott SM, Reiman HM, Pritchard DJ, Ilstrup DM. Soft tissue fibrosarcoma: a clinicopathologic study of 132 cases. *Cancer* 1989; **64**: 925–31.
6. Ueda T, Aozasa K, Tsujimoto M, Ohsawa M, Uchida A, Aoki Y, Ono K, Matsumoto K. Prognostic significance of Ki-67 reactivity in soft tissue sarcomas. *Cancer* 1989; **63**: 1607–11.
7. Choong PFM, Akerman M, Willen H, Andersson C, Gustafson P, Baldetorp B, Ferno M, Alvegard T, Rydholm A. Prognostic value of Ki-67 expression in 182 soft tissue sarcomas. Proliferation—a marker of metastasis? *APMIS* 1994; **102**: 915–24.
8. Lopes JM, Bjerkehagen B, Holm R, Bruland O, Sobrinho-Simoes M, Nesland, JM. Proliferation activity of synovial sarcoma: an immunohistochemical evaluation of Ki-67 labeling indices of 52 primary and recurrent tumors. *Ultrastruct Pathol* 1995; **19**: 101–6.
9. Antonescu CR, Leung DH, Dudas M, Ladanyi M, Brennan M, Woodruff JM, Cordon-Cardo, C. Alterations of cell cycle regulators in localized synovial sarcoma: a multifactorial study with prognostic implications. *Am J Pathol* 2000; **156**: 977–83.
10. Kourea HP, Cordon-Cardo C, Dudas M, Leung D, Woodruff JM. Expression of $p27^{kip}$ and other cell cycle regulators in malignant peripheral nerve sheath tumors and neurofibromas: the emerging role of $p27^{kip}$ in malignant transformation of neurofibroma. *Am J Pathol* 1999; **155**: 1885–91.
11. Oliveira AM, Nascimento AG, Okuno SH, Lloyd RV. $p27^{Kip1}$ protein expression correlates with survival in myxoid and round-cell liposarcoma. *J Clin Oncol* 2000; **18**: 2888–93.
12. Kawauchi S, Goto Y, Liu XP, Furuya T, Oga A, Oda Y, Tsuneyoshi M, Ihara K, Sasaki, K. Low expression of $p27^{Kip1}$, a cyclin-dependent kinase inhibitor, is a marker of poor prognosis in synovial sarcoma. *Cancer* 2001; **91**: 1005–12.
13. Sherr CJ. Cancer cell cycle. *Science* 1996; **274**: 1672–7.
14. Lloyd RV, Erickson LA, Jin L, Kulig E, Qian X, Cheville JC, Scheithauer BW. p27: a multifunctional cyclin-dependent kinase inhibitor with prognostic significance in human cancers. *Am J Pathol* 1999; **154**: 313–23.
15. Ohtsubo M, Theodoras A, Schumacher J, Roberts J, Pagano, M. Human cyclin E, a nuclear protein essential for the G1-to-S phase transition. *Mol Cell Biol* 1995; **15**: 2612–24.
16. Coats S, Flanagan WM, Nourse J, Roberts JM. Requirement of $p27^{Kip1}$ for restriction point control of the fibroblast cell cycle. *Science* 1996; **10**: 877–80.
17. Sheaff RJ, Groudine M, Gordon M, Roberts JM, Clurman BE. Cyclin E-CDK2 is a regulator of p27Kip1. *Genes Dev* 1997; **11**: 1464–78.
18. Keyomarsi K, O'Leary N, Molnar G, Lees E, Fingert HJ, Pardee AB. Cyclin E, a potential prognostic marker for breast cancer. *Cancer Res* 1994; **54**: 380–5.
19. Kawana H, Tamaru J, Tanaka T, Hirai A, Saito Y, Kitagawa M, Mikata A, Harigaya K, Kuriyama, T. Role of $p27^{Kip1}$ and cyclin-dependent kinase 2 in the proliferation of non-small cell lung cancer. *Am J Pathol* 1998; **153**: 505–13.
20. Loda M, Cukor B, Tam SW, Lavin P, Fiorentino M, Draetta G, Jessup JM, Pagano, M. Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. *Nat Med* 1997; **3**: 231–4.
21. Ohtani M, Isozaki H, Fujii K, Nomura E, Niki M, Mabuchi H, Nishiguchi K, Toyoda M, Ishibashi T, Tanigawa, N. Impact of the expression of cyclin-dependent kinase inhibitor $p27^{Kip1}$ and apoptosis in tumor cells on the overall survival of patients with non-early stage gastric carcinama. *Cancer* 1999; **85**: 1711–18.
22. Tsihlias J, Kapusta LR, DeBoer G, Morava-Protzner I, Zbieranowski I, Bhattacharya N, Catzavelos GC, Klotz LH, Slingerland JM. Loss of cyclin-dependent kinase inhibitor $p27^{Kip1}$ is a novel prognostic factor in localized human prostate adenocarcinoma. *Cancer Res* 1998; **58**: 542–8.
23. AJCC cancer staging handbook. 5th ed. Philadelphia: Lippincott-Raven; 1998.
24. Carrano AC, Eytan E, Hershko A, Pagano, M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat Cell Biol* 1999; **1**: 193–9.
25. Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, Polyak K, Tsai LH, Broudy V, Perlmutter RM, Kaushansky K, Roberts JM. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis and female sterility in $p27^{Kip1}$-deficient mice. *Cell* 1996; **85**: 733–44.