Alterations of Cell Cycle Regulators in Localized Synovial Sarcoma

A Multifactorial Study with Prognostic Implications

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Genetic alterations of cell cycle regulators are thought to represent uncommon and possible secondary events in sarcomas characterized by recurrent chromosomal translocations. The present study investigates this hypothesis on synovial sarcoma (SS), assessing the frequency of expression and possible clinical implications of detecting alterations in critical cell cycle regulatory proteins.

A homogeneous cohort of 49 patients with localized SS, restricted to the extremity and with available long-term follow-up information, was selected from our files. We focused our study on molecules involved in the G1 checkpoint and G1-S transition, including cyclins D1 and E, p21^{WAF1}, p27^{Kip1}, mdm2, p53, and Ki67. A cutoff point of 10% immunoreactive tumor cell nuclei was selected to define a positive phenotype for any given marker, except for Ki67. High Ki67 proliferative index was considered when $\geq 20\%$ tumor cells displayed nuclear immunoreactivity. Biphasic SS were analyzed, taking into account separately the expression of these proteins in the spindle and glandular components. Disease specific survival was modeled using the Kaplan-Meier method with log rank test and Cox regression.

The cohort of patients analyzed included 23 females and 26 males, and the histological type distribution was 35 monophasic and 14 biphasic SS. The median follow-up for survivors was 53 months, with a 5-year disease-specific survival of 63% and a metastatic disease-free survival of 40%. The positive phenotypes identified for the different markers studied were as follows: cyclin D1, 59%; cyclin E, 29%; p21, 51%; p27, 69%; mdm2, 59%; p53, 16%; and Ki67, 59%. We observed that positive p53, cyclin E, and high Ki67 proliferative index were correlated with survival, but only Ki67 and p53 were independent variables for prognostication. The present study sug-

gests that alterations of cell cycle regulators are more common events in SS than originally thought. p53 overexpression could be of use as a marker together with a high Ki67 proliferative index, in identifying a subset of SS patients with increased risk of tumor relapse. (Am J Pathol 2000, 156:977-983)

Alterations affecting certain cell-cycle regulators have been implicated in the pathogenesis and tumor progression of sarcomas, mainly those lacking other specific genetic alterations.¹ Growth control in mammalian cells is accomplished largely by the action of the RB protein (pRB), regulating exit from the G1 phase, and the p53 protein, triggering growth arrest or apoptotic processes in response to cellular stress.^{2,3} In tumorigenesis, pRB and p53 serve collaborative roles, as evidenced by their frequent alterations in human tumors, and the many tumor types that exhibit mutations in both RB and p53 genes. The mechanistic basis for this dual requirement stems, in part, from the deactivation of a p53-dependent cell suicide program that would normally be brought about as a response to unchecked cellular proliferation resulting from RB deficiency. However, direct genetic alterations of cell cycle regulators are postulated to be less common in translocation-associated sarcomas, in which they are considered secondary events.⁴

Synovial sarcomas (SS) are characterized by a recurrent chromosomal translocation, t(X;18), identified in over 90% of both biphasic and monophasic tumors. In previous studies, a relationship between the transcript fusion type and the biological behavior of the disease was reported.⁵ Nevertheless, little is known regarding the frequency and potential clinical relevance of detecting alterations of cell cycle regulators in SS.

During the G1-to-S transition, multiple target genes are affected by the action of p53 and RB pathways. p53 expression has been extensively investigated in most human malignancies, as p53 overexpression in tumors can be attributed to an extended half-life exerted by most mutant p53 products.⁶ p53 activity is regulated by mdm2,

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which binds to p53, inhibits its transcriptional activity, and targets its degradation.⁷ The MDM2 gene is transactivated by p53, creating an autoregulatory feedback loop.⁷ In addition, p53 transactivates other genes involved in cell cycle arrest, such as p21^{WAF1},⁸ as well as others involved in DNA-damage and apoptotic programs.⁹ p21^{WAF1} was the first member identified of the family of cyclin-dependent kinase (Cdk) inhibitors termed KIP, which also includes the p27^{KIP1} gene. Both p21 and p27 have a strong affinity for cyclin D1-Cdk4 complexes, an activity that is reduced for complexes formed by cyclin E and Cdk2.¹⁰

Most of the pre-existing studies analyzing prognostic markers in sarcomas combined multiple histopathological entities and different tumor grades. Reported results of associations between tumor markers and clinicopathological parameters may be due to the differences in the incidence of various molecular alterations among distinct histological tumor subtypes and grades. Therefore, the goal of our study was to analyze the role of specific molecular alterations affecting certain cell cycle regulators in a highly selected subset of synovial sarcomas. Furthermore, we also extended our study to the analysis of potential clinical associations between these alterations and clinicopathological variables of poor outcome, including patient survival.

Materials and Methods

Tissue and Patient Characteristics

Forty-nine consecutive patients with high grade SS, with localized disease at presentation that was restricted to the extremities, were selected from the files of the Department of Pathology at Memorial Sloan-Kettering Cancer Center (MSKCC). The availability of follow-up information on these patients and adequate tumor tissue for the analysis were among the inclusion criteria for the study. The microscopic slides were reviewed and the histological diagnosis was reconfirmed in all cases. A paraffin-embedded, formalin-fixed representative tumor tissue block from each case was selected for conducting immunohistochemical analyses. Clinical and follow-up information was obtained by reviewing all medical charts.

There were 23 females and 26 males, with a mean age of 37 years (range, 13–69 years). Fourteen tumors (29%) were smaller than 5 cm in greatest dimension and 35 tumors (71%) were larger than 5 cm. The histological type distribution was 35 monophasic (71%) and 14 biphasic (29%) SS. None of our 49 cases was considered to fulfill the histological criteria for the so-called “poorly differentiated variant” of SS. Forty-one patients (84%) underwent a limb-sparing surgical procedure (*en bloc* resection) and only 8 patients (16%) had an amputation. The surgical margins were microscopically positive in 11 cases (22%) and negative in 38 cases (78%).

The median follow-up among survivors in this cohort of 49 patients was 53 months (range, 5–303 months). The actuarial 5-year failure rates were 35% for local recurrence, 60% for distant recurrence, and 37% for death of

disease. The number of endpoints included 14 local recurrences, 28 metastases, and 20 deaths of disease.

Monoclonal Antibodies and Immunohistochemistry

A panel of well characterized antibodies was used, which included mouse monoclonal antibodies to cyclin D1 (Ab-3; Calbiochem, San Diego, CA; 1 μ g/ml), p21 (Calbiochem; 5 μ g/ml), p27 (Calbiochem; 1 μ g/ml), mdm2 (clone 2A10, to the human MDM2 product, kindly supplied by Dr. A. Levine, Rockefeller University, New York, NY; 1:500; no concentration could be given because it was supplied as a tissue culture supernatant), and Ki67 (clone MIB1, Immunotech, Westbrook, ME; 1:100; 2 μ g/ml). In addition a purified rabbit antiserum to cyclin E was also used (supplied by Dr. A. Koff, MSKCC; 1:500; also supplied as a tissue culture supernatant). Two mouse monoclonal antibodies detecting different epitopes on p53 were used: PAb1801 and DO7. Clone PAb1801 (Calbiochem; 1:500; 0.2 μ g/ml) recognizes an epitope located between amino acids 32 and 79 of both wild-type and mutant human p53 proteins,¹¹ whereas the p53 epitope recognized by clone DO7 (Dako, Carpinteria, CA; 1:500; 0.2 μ g/ml) is located between amino acids 19 and 26 of wild-type and mutant human p53 proteins. Deparaffinized sections were treated with 3% H₂O₂ to block endogenous peroxidase activity. Sections were subsequently immersed in boiling 0.01% citric acid, pH 6.0, in a microwave oven for 15 minutes to enhance antigen retrieval, allowed to cool, and incubated with 10% normal horse serum (mouse monoclonal antibodies) or 10% normal goat serum (rabbit antiserum) to block nonspecific tissue immunoreactivities. Primary antibodies were then incubated overnight at 4°C. Biotinylated horse anti-mouse IgG antibodies (Vector Laboratories, Burlingame, CA; 1:500 dilution) or goat anti-rabbit IgG antibodies (Vector Laboratories, 1:800 dilution) were applied for 1 hour, followed by avidin-biotin-peroxidase complexes that were applied for 30 minutes (Vector Laboratories, 1:25 dilution). Diaminobenzidine was used as the final chromogen and hematoxylin was used as the nuclear counterstain. Nuclear immunoreactivities were scored on a continuous scale with values that ranged from undetectable levels or 0% to homogeneous staining or 100%. The staining profile in tumor cells was compared to the negative internal tissues, as well as with both negative and positive control samples. Because the antigens under study are nuclear proteins when functional, we scored those tumor cells displaying nuclear immunoreactivities. We noted cytoplasmic staining in few cases with certain antibodies, but did not use this finding as criteria for determining “positive phenotype.” The scoring was performed by a single pathologist (C. R. A.) without knowledge of the clinical characteristics. The stained slides were reviewed by other two pathologists (J. M. W. and C. C.-C.), and there was agreement among these investigators regarding positive *versus* negative cases. The immunoreactivity scoring was counted as percentage of nuclear staining per 10 high-power fields, in sev-

eral areas, regardless of staining intensity. Based on other reports, a 10% cutoff value for detection of positive nuclear reactivities was selected for all antibodies.¹² An additional cutoff point of 20% positive cells was also recorded for Ki67, as this percentage is also cited as correlating best with survival.^{13,14} We caution the reader that these are preliminary, exploratory studies, and that these findings need to be validated using an independent cohort of patients.

In all biphasic synovial sarcoma (BSS) cases immunoreactivities were scored separately for both spindle and glandular cell components, using the same cutoff values stated above. Immunohistochemical results for each individual cell component were analyzed in relation to clinicopathological parameters of poor outcome, including survival.

Statistical Analyses

The endpoints studied were rates of developing first local recurrence, rate of developing first metastasis, and rate of any recurrence- and disease-related mortality (DSS). Local recurrence-free survival (LFS), metastatic disease-free survival (MDFS), disease-free survival (DFS), and disease-specific survival (DSS) were modeled using the Kaplan-Meier method and analyzed by the log rank test and Cox regression multivariable analysis. The results of Cox model analysis were reported with relative risks (RR) and 95% confidence intervals (CIs).

In addition to the cell cycle regulators and Ki67 proliferative index, the following clinicopathological adverse factors were analyzed for their relationship with DSS: age (<15 and ≥15 years), gender, tumor size (<5 and ≥5 cm), histological type (monophasic and biphasic), surgical procedure (*en bloc* resection and amputation), and status of microscopic margins of resection. Kendall's Tau b was used to measure associations between factors and results used to explain the possible roles of different factors in determining oncological outcomes.

Results

Tables 1 and 2 summarize immunohistochemical data in relationship to disease-specific survival using the univariate statistical analyses. Figures 1 and 2 illustrate representative immunophenotypic profiles of the cell cycle regulators under study and the Kaplan-Meier survival curve.

Cyclin D1 was overexpressed in the nuclei of tumor cells on 29 of 49 cases (59%). The intensity of nuclear staining was found to be strong in most of the cases that overexpressed cyclin D1 (Figure 1A). Eleven of the 14 cases (78%) with biphasic morphology were part of the 29 tumors found to overexpress cyclin D1. We observed that the immunoreactivity had a distinct predilection to the epithelial component (Figure 1A). Furthermore, in 10 of these 11 positive BSS, the spindle component displayed either undetectable cyclin D1 levels or <10% nuclear staining (Figure 1A). Only 1 of the 11 cyclin

Table 1. Immunohistochemical Results of the Analyzed Cell Cycle Regulators in Relation to Disease-Related Mortality

Antibody	Result	N (%)	Alive	DOD	P value
Ki67 (>10%)	+	42 (86)	24	18	0.15
	−	7 (14)	6	1	
Ki67 (>20%)	+	29 (59)	12	17	0.002
	−	20 (41)	18	2	
P53 (1801)	+	8 (16)	2	6	0.001
	−	41 (84)	28	13	
P53 (DO7)	+	8 (16)	3	5	0.01
	−	41 (84)	27	14	
Cyclin D1	+	29 (59)	19	10	0.7
	−	20 (41)	11	9	
Cyclin E	+	14 (29)	8	6	0.03
	−	35 (71)	22	13	
p21 ^{WAF1}	+	25 (51)	16	9	0.5
	−	24 (49)	14	10	
p27	+	34 (69)	22	12	0.6
	−	15 (31)	8	7	
MDM2	+	29 (59)	17	12	0.6
	−	20 (41)	13	7	

DOD, died of disease.

D1-positive BSS cases showed >10% tumor cells with intense labeling in both components.

Cyclin E immunoreactivity was noted in the nuclei of tumor cells in 14 of 49 cases (29%). The intensity of nuclear staining was weaker than that observed for cyclin D1 (Figure 1B). Immunoreactivities for cyclin E were found in 3 of 14 BSS cases (21%), the pattern of staining being also restricted to the glandular component.

p21^{WAF1} nuclear overexpression was detected in 25 of 49 cases (51%). p21^{WAF1} had also a distinctive labeling pattern, being usually identified in the glandular component of the BSS analyzed. Nine of the 14 BSS cases (64%) showed immunostaining in >10% of the glandular component, and in 8 of these 9 cases p21 overexpression was restricted to this cell type (Figure 1C).

p27^{Kip1} was found to be positive in 34 of 49 cases (69%; Figure 1D). There was no predilection for one or the other cellular component in the BSS cases studied. Similarly, we observed no difference between monophasic and biphasic histology and MDM2 nuclear overexpression. MDM2 was overexpressed in the nuclei of tumor cells in 29 of 49 cases (59%; Figure 1E). We observed a cytoplasmic pattern of staining with the anti-MDM2 antibody used (clone 2A10) in approximately half of the BSS cases. This cytoplasmic staining was found in cases with or without MDM2 nuclear immunoreactivities (Figure 1E).

Table 2. Clinicopathological Parameters and Molecular Markers Found to Be Adverse Factors of Outcome, Including Survival, by Univariate Analysis

Adverse factor	Recurrence-free survival	Metastasis-free survival	Disease-free survival
Tumor size (>5 cm)	0.07	0.007	0.02
Ki67 (>20%)	0.22	0.001	0.0001
P53 (1801)	0.26	0.02	0.05
Cyclin E	0.03	0.12	0.007

Figure 1. **A:** Cyclin D1 strong nuclear immunostaining, predominantly restricted to the glandular component of a biphasic synovial sarcoma. The spindle cell component displayed undetectable cyclin D1 levels. **B:** Cyclin E immunoreactivity in a monophasic synovial sarcoma case. Of note that the nuclear staining is more focal comparing with Cyclin D1. **C:** p21^{WAF1} nuclear overexpression in a biphasic synovial sarcoma is detected mainly in the glandular component. **D:** p27^{kip1} nuclear immunostaining in the spindle cell component of a monophasic synovial sarcoma case. **E:** MDM2 overexpression in a biphasic synovial sarcoma is noted mainly in the epithelial component, but scattered positive cells are also noted in the spindle cells. Nuclear as well as cytoplasmic staining is noted in this case. **F:** Strong and diffuse p53 immunoreactivity (clone DO7) in a monophasic synovial sarcoma. This case was associated with undetectable p21 expression.

Although p53 immunoreactivities were identified in only a minority, 8 of 49, cases (16%), there was a significant concordance regarding the staining patterns and final results by the two different antibodies used in this study (clones PAb1801 and DO7). Seven tumors were found to be immunoreactive with both reagents (Figure 1F). However, two additional lesions were found to be positive for only one of the two antibodies.

Ki67 nuclear immunoreactivities were found in 42 of 49 cases (86%) when using >10% as cutoff to define high proliferative index. When using >20% positive tumor

cells, only 29 of 49 cases (59%) were considered to have a high proliferation rate. The reactivity of Ki67 in the BSS cases was not restricted to either of the two cellular components.

Univariate analyses were performed for each biological marker and endpoints under study (Table 1). The following were found to be adverse factors for MDFS, DFS, and DSS: Ki67 proliferation rate using >20% cutoff (but not when using >10%), p53 (both PAb1801 and DO7 clones), and Cyclin E (Table 2). We conducted a separate statistical analysis to further define the potential

Figure 2. Disease-specific survival in patients with localized synovial sarcoma of the extremities ($n = 49$), stratified by p53 (clone PAb1801). Kaplan-Meier survival curve depicting the disease-related mortality stratified by p53 expression.

impact of the immunoreactivities occurring in each spindle or glandular components of the BSS cases. We found no correlation between patterns of expression of the biological markers and either outcome or other adverse factors for these two different morphologies. We found that of all clinicopathological parameters evaluated, only tumor size ≥ 5 cm was significantly associated with a poor outcome.

A multivariable analysis was also conducted to identify the independent prognostic factors. The following were found to be independent adverse factors for DSS: Ki67 $> 20\%$ (RR = 8.3; CI: 2.7–25; $P = 0.0015$) and p53 (antibody clone PAb1801; RR = 7.8; CI: 1.8–35; $P = 0.0001$). The following were found to be independent adverse factors for DFS and MDFS: Ki67 $> 20\%$ and size ≥ 5 cm.

Kendall Tau b method identified a correlation between the expression of cyclin E and p21^{WAF1} (1.5; $P = 0.0002$), DO7 (0.3; $P = 0.02$), PAb1801 (0.5; $P = 0.0017$), and Ki67 $> 20\%$ (0.3; $P = 0.01$) staining. The strong association between cyclin E reactivity and independent prognostic factors, namely Ki67 and p53, may possibly explain its clinical impact as found by univariate analysis.

Discussion

Synovial sarcomas are characterized by a t(X;18)(p11;q11) translocation, which is identified in over 90% of both biphasic and monophasic tumors. Genetic alterations of cell cycle regulators are thought to represent uncommon secondary events in sarcomas characterized by recurrent chromosomal translocations. However, in the present study we found that, at least at the protein level, alterations affecting some critical regulators involved in the G1 checkpoint were frequent events, and that they were associated with poor outcome.

Cell cycle transitions are controlled by the enzymatic activity of complexes formed by cyclins and Cdks. Cyclin D1 and Cdk4 complexes phosphorylate pRB, allowing entry into the cycle and exerting the early regulation

imposed during the G1 phase.¹⁵ Cyclin D1 overexpression, with or without gene amplification, has been reported to be associated with unfavorable outcome in numerous epithelial malignancies, including pancreatic, colorectal, and head and neck cancer.^{16–18} Cyclin D1 overexpression has also been observed in bone¹⁹ and soft tissue sarcomas.²⁰ In the present study, we detected cyclin D1 overexpression in 59% of the cases studied. However, identification of cyclin D1-positive phenotype was not associated with proliferation index, and it had no impact on survival. The lack of correlation with proliferation index can be explained by an unscheduled pattern of cyclin D1 expression, based on the high frequency of identifying a positive phenotype for cyclin D1 in the cohort of patients studied. The effect of the ectopic expression of cyclin D1 in shortening the G1 interval relative to the expression of any single cyclin has been reported.²¹ Thus, there is a good correlation in experimental systems and primary tumor data, including SS, to support such a postulate.

Binding of cyclin E to Cdk2 also leads to pRB phosphorylation and progression from the G1 to the S phases of the cell cycle. In this study, we found cyclin E overexpression in 29% of the cases, and also found that this positive phenotype was associated with high proliferation index. In addition, cyclin E overexpression was significantly correlated with poor outcome by univariate analysis, but not by multivariate analysis. This lack of significance could be due to the strong association between cyclin E and Ki67 proliferation index. In a large study of high-grade osteogenic sarcomas, cyclin E overexpression was found in 47% of cases.²² Moreover, a similar significant correlation between cyclin E and Ki67 was also noted in that study.²² Cyclin E overexpression has also been reported in breast, gastrointestinal, and bladder cancers.^{23–25}

Several lines of investigation suggest that p53 controls a cell cycle checkpoint responsible for maintaining the fidelity of the genetic information, either mediating cell cycle arrest in response to DNA damage or activating pathways of apoptosis if repair cannot be achieved.^{9,10} Although alterations in p53 expression are not uncommon in human sarcomas, their incidence varies with the histological type.^{7,26} The incidence of p53 nuclear overexpression in the present study (16% of cases) closely approximates the percentage found in a homogeneous group of leiomyosarcomas.²⁷ As overexpression of p53 measured by immunohistochemical techniques does not always correlate with gene mutations at the molecular level,²⁸ conclusions should be drawn cautiously regarding the biological interpretation of this finding.⁶ Nevertheless, the clinical impact of detecting p53 nuclear overexpression in SS is evidenced by its significant association with an unfavorable outcome, and it is also in concordance with previous studies on soft tissue sarcomas.¹²

A significant difference between the frequency of p53 nuclear overexpression in large (≥ 5 cm in diameter) versus small (< 5 cm) soft tissue sarcomas was previously reported.¹² In our study, all 8 patients with p53 positive phenotype had tumors > 5 cm. This phenomenon could be explained by the growth advantage and lack of apo-

ptotic response that tumors with p53 alterations suffer. In this context, tumors with enhanced proliferation and lacking cell death will tend to produce larger tumor masses.

p21^{WAF1} is a cyclin-dependent kinase inhibitor that is transactivated by p53 in response to cellular stress and DNA damage.^{29,30} It has been shown that a p21-positive phenotype usually can be related to the presence of a wild-type p53.³¹ However, it is known that p21 can also be induced through a p53-independent pathway, mainly activated by specific growth factors such as the epidermal growth factor receptor.³² In our study, only one p53-positive case was associated with lack of p21 expression, consistent with the presence of an inactive p53. In the remaining p53-positive cases in which p21 was identified, its induction could be related to this alternative pathway. In the setting of soft tissue sarcomas, the alternative mechanism could be due to mitogenic stimuli via growth factor signaling. There is abundant evidence regarding the up-regulation of growth factor receptor/ligand activity in soft tissue sarcomas.^{33,34}

Alterations in the expression of p27^{Kip1}, another member of the p21 family of Cdk inhibitors, were found in approximately 30% of the SS cases analyzed. p27 alterations have been reported to occur at the expression level, and mutations of the p27^{Kip1} gene are rarely found in human malignancies, including soft tissue sarcomas.³⁵ As has been reported for most cyclins, p27 is a target of degradation mediated by the ubiquitin system.³⁶ It appears that this event takes place in the proteasome, which shows increased activity associated with decreased p27 levels.³⁶

In this study, we also observed that approximately 60% of the cases displayed mdm2 overexpression. Although a correlation between the overexpression of p53 and mdm2 proteins within the same tumor and unfavorable outcome was shown in previous studies of adult soft tissue sarcomas,^{28,37,38} our results could not reproduce this finding in this restricted group of SS cases.

Transcriptional regulation of the MDM2 gene is under the control of p53. The MDM2 protein binds to the transactivation domain of p53 and abolishes its function. In addition, MDM2 binding to p53 achieves its degradation by presenting p53 to the ubiquitin system. We and others have previously reported that MDM2 amplification is not always associated with MDM2 overexpression. The mechanisms accounting for MDM2 overexpression are not well understood. It appears that MDM2 also responds to other growth signaling pathways, including those originated in the membrane by certain growth factors, such as bFGF and FGF receptors.

Regarding proliferative index, as measured by Ki67 expression, the present results support previous reports that demonstrate its prognostic significance in soft tissue sarcoma.^{13,14,39} Because high proliferation rate proved to be an independent predictor of survival in SS, it may be of use, together with p53 overexpression and tumor size, to identify patients who require vigilant postoperative surveillance and aggressive adjuvant systemic therapy.

The presence of molecular alterations restricted to the epithelial component of SS, as seen with cyclin D1, p21 and cyclin E expression, questions the possibility of its

potential active role in tumor progression. Although this mechanism alone cannot be implicated in all SS tumors, as the incidence of monophasic SS exceeds the biphasic SS type, other lines of evidence pointed out similar observations. A strong correlation between SYT-SSX fusion type and morphology was recently identified,⁵ possibly related to the 13 amino acids difference of the carboxy-terminal of the SSX proteins included in the fusion transcript. Hypothetically, the SYT-SSX1 fusion protein, although not sufficient by itself to induce architectural epithelial differentiation, may be more permissive than the SYT-SSX2 fusion product. Along these lines, Lopes et al describe a significantly higher proliferation rate within the glandular component of BSS,⁴⁰ and found a significant association between P-glycoprotein and glutathione-S-transferase-pi expression in the epithelial areas of BSS.⁴¹ Other relevant questions that remain unsettled in SS include its putative histogenesis, the relationship between cell differentiation and cell proliferation, and the connection between the two components in the bimorphic histological type.

In sum, p53 overexpression (>10%) appears to be an uncommon but clinically relevant event in this group of selected SS, in that it identifies a subset of patients with increased risk of tumor relapse. This prognostic indicator may be useful in assigning a more aggressive intervention. It should be noted that high Ki67 proliferation index (>20%) proved to be an independent prognostic marker in localized SS. The role of cyclin E and its clinical impact in SS requires further study, but appears to be related to its significant association with Ki67 and p53. Finally, the significance of the epithelial tropism observed for cyclin D1 and p21^{WAF1} expression remains unknown. Further studies including p53 sequence analysis, microdissection techniques, and morphology-related molecular markers, like SYT-SSX fusion type⁵ or Met oncoprotein expression,⁴² may be helpful in elucidating this process.

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