# Prognostic Value of Alternative Lengthening of Telomeres–Associated Biomarkers in Uterine Sarcoma and Uterine Carcinosarcoma

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**Objective:** A subset of cancer cells maintains telomere lengths in a telomerase-independent manner known as the alternative lengthening of telomeres (ALT). The goal of this study was to evaluate the frequency of ALT in uterine sarcoma and carcinosarcoma and to assess its association with clinical parameters.

**Methods:** Retrospectively collected paraffin blocks from 41 patients with uterine sarcomas and carcinosarcomas were analyzed for ALT-associated promyelocytic leukemia bodies (APBs), which are a significant feature of ALT cells, using combined immunofluorescence and telomere fluorescence in situ hybridization. In addition, a C-circle assay and human telomerase reverse transcriptase immunohistochemistry were performed to support these results.

**Results:** The APB assay and C-circle assay indicated that 46.3% (19/41 cases) and 36.4% (8/22 cases) of sarcomas of the uterus, respectively, were positive for ALT. Alternative lengthening of telomerase positivity was correlated with high-grade uterine sarcoma and parameters indicative of an aggressive tumor, such as tumor size ($P = 0.033$) and mitotic index ($P = 0.001$); ALT positivity was negatively correlated with human telomerase reverse transcriptase reactivity ($P = 0.036$). In a survival analysis, the presence of APBs was found to be a poor prognostic factor for disease-free survival ($P = 0.018$) and overall survival ($P = 0.021$).

**Conclusions:** Alternative lengthening of telomeres is a prevalent mechanism in uterine sarcomas and carcinosarcomas and is associated with the aggressiveness of the tumor and tumor progression. Importantly, ALT positivity is an indicator of poor prognosis for patients with uterine sarcoma and carcinosarcoma.

**Key Words:** Alternative lengthening of telomeres, ALT-associated promyelocytic leukemia body, Uterine sarcoma, Uterine carcinosarcoma, Prognostic factor

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The ends of human chromosomes, called telomeres, are composed of regions containing 4 to 15 kb of a repetitive DNA sequence, TTAGGG.[1] Because the synthesis of the lagging strands of chromosomal DNA requires upstream priming, linear eukaryotic chromosomes experience end-replication problems and are at risk for the shortening of telomeres during each cell division. If not protected, telomeres shorten to a critical point that triggers the entry of cells into senescence and then crisis.[2] It is firmly established that cells must escape replicative senescence and retain their telomeres to achieve immortalization before neoplastic transformation. Therefore, tumors can arise only if tumor cells are able to compensate for the loss of telomeres.

In undifferentiated cells (eg, embryonic stem cells), telomeres are maintained by the action of the enzyme telomerase, which is composed of the catalytic subunit human telomerase reverse transcriptase (hTERT) and the RNA component TERC.[1] Normal differentiated human cells do not have hTERT activity. It is known that one mechanism by which cancer cells retain telomeres is by expressing hTERT. In most epithelial tumors, hTERT is the primary enzyme responsible for telomere maintenance.[3] However, some tumors, such as glioblastomas, astrocytomas, and soft tissue sarcomas, maintain telomere length through a telomerase-independent mechanism, termed *alternative lengthening of telomeres* (ALT).[4,5]

The prevalence of ALT and hTERT positivity differs among different tumor types. Whereas ALT is rare in adenocarcinomas, the incidence of ALT is relatively high in sarcomas—from 25% to 50%.[6–8] The reason for the existence of at least 2 different telomere length-maintenance mechanisms associated with different cancer types is not clear. However, it has been suggested that ALT predominates in cancer tissues of mesenchymal origin.[9,10]

Sarcomas of the uterus are uncommon; they account for 3% to 7% of uterine cancers that develop from the uterine body.[11] Recently, carcinosarcoma, traditionally considered a uterine sarcoma, has been reclassified as a dedifferentiated endometrial carcinoma according to the World Health Organization classification. Nonetheless, the tumor behavior of carcinosarcoma resembles that of uterine sarcoma.[12] No matter which available treatment options are used to treat uterine sarcoma or carcinosarcoma, the response to therapy is poor, and a large proportion of patients with sarcoma of the uterus die within 5 years.[12–14] The tumorigenesis of uterine sarcoma is not well understood; in particular, the molecular mechanisms responsible for the proliferative potential of this cancer have not yet been elucidated.

To understand the basis of tumorigenesis in uterine sarcoma and to apply this information clinically, we investigated the incidence of ALT positivity in tumors from uterine sarcoma patients. Alternative lengthening of telomerase–positive cells are characterized by heterogeneity in telomere lengths; the existence of extrachromosomal telomeric DNA, including t-circles; extratelomeric C-circles and G-circles; an increase in the number of telomeric sister chromatid exchanges; and the presence of ALT-associated promyelocytic leukemia bodies (APBs).[15–19] Among these characteristics, we examined the occurrence of APBs and C-circles. We found that APB and C-circle assays are robust methods for use with paraffin-embedded tissues. Using these assays, we determined the clinical value of ALT in uterine sarcomas and carcinosarcomas.

## MATERIALS AND METHODS

### Cell Culture of Cancer Cell Lines

Alternative lengthening of telomeres–positive human sarcoma (U2OS), uterine carcinosarcoma (SNU539 and SNU685), and epithelial ovarian cancer (OVCAR3 and SK-OV-3) cell lines were obtained from the Korean Cell Line Bank (Seoul, Korea) and were grown in monolayer cultures in Dulbecco modified Eagle medium (MEM) or RPMI 1640 supplemented with fetal bovine serum (10% vol/vol). Human genital tract sarcoma cell lines (SK-LMS-1 and SK-UT-1) were obtained from CLS-Cell Lines Service (Eppelheim, Germany) and were cultured in Dulbecco MEM or MEM supplemented with fetal bovine serum (10% vol/vol).

### Patients and Tissue Samples

Uterine sarcoma samples were collected from patients undergoing cytoreductive surgery at Seoul National University Hospital (SNUH). Of the 93 patients with a diagnosis of uterine sarcoma between March 2000 and March 2009, 41 who were followed up after primary surgical treatment were included in this study. Eighty-two epithelial ovarian carcinoma patients who received the diagnosis between 2000 and 2005 were included in the study to compare sarcomas and epithelial carcinomas with 1:2 matching. All samples were taken from primary uterine or ovarian lesions in each patient. Formalin-fixed, paraffin-embedded tissues were used in the analyses. The study was approved by the institutional review board (H-1005-082-320) of SNUH.

### Immunofluorescence In Situ Hybridization Analysis for APB Detection

All tissue sections were evaluated histologically with hematoxylin-eosin (HE) staining by a pathologist (M.K.). Five-micrometer-thick sections adjacent to those used for HE staining were cut from paraffin-embedded uterine sarcoma samples for use in immuno–fluorescence in situ hybridization (FISH) assays. Samples were deparaffinized in xylene, rehydrated with graded ethanol, and washed in phosphate-buffered saline (PBS). Samples were then placed in 10 mM citrate buffer (pH 6.0) and boiled in a microwave. Endogenous peroxidase activity was quenched by incubating tissue sections in 3% $H_2O_2$ for 10 minutes. Sections were incubated overnight at 4°C in a humidified chamber with a mouse monoclonal antibody against promyelocytic leukemia (PML) antigen (Santa Cruz Biotechnology, Santa Cruz, CA; 1:100 dilution). After washing in PBS, the sections were cross-linked with 4% formaldehyde and dehydrated. Telomere FISH was performed by mixing 1 μg/mL of 3′ Cy3-labeled telomere peptide nucleic acid probe (Cy3-CCCTAA) in 70% formamide containing 0.25% blocking reagent (Roche, Penzberg, Germany), 10 mmol/L Tris pH 7.5, and 5% $MgCl_2$ buffer and incubating the tissue samples in this solution for 3 minutes at 80°C. The slides were then hybridized for 3 hours at room temperature. After hybridization, slides were first washed with a 70% formamide/0.01 mol/L Tris pH 7.2 solution and

then with 0.05 mol/L Tris (pH 7.5). Sections were counterstained with DAPI (4′,6-diamino-2-phenylindole). Genital tract sarcoma cell lines and epithelial ovarian carcinoma cell lines were also stained according to the immuno-FISH protocol.

Three-dimensional images were acquired using a CoolSNAP HQ-cooled CCD camera on a DeltaVision Restoration microscope built around an Olympus IX70 stand, with a 100×/1.35 NA lens (Applied Precision Life Sciences, Issaquah, WA). Images were acquired as a series of 0.3-μm-thick sections, and the entire 3-dimensional image was reconstructed and deconvoluted using an iterative algorithm implemented in softWoRx software (Applied Precision).

Alternative lengthening of telomeres–associated promyelocytic leukemia bodies were determined to be present if staining of the telomeric probe was localized within a PML body in the nucleus, and the slide was scored APB positive if both equal to or greater than 0.5% cells in the section contained APBs and equal to or greater than 10 APB-containing cells were present.[5] The U2OS and HeLa cell lines were analyzed as positive and negative controls, respectively.

## C-Circle Assay

Twenty-micrometer-thick sections adjacent to those used for HE staining were cut from paraffin-embedded uterine sarcoma samples and used in C-circle assays. The samples were deparaffinized in xylene, rehydrated with graded ethanol, and washed in distilled water. They were then incubated at 52°C overnight in 500 μL of lysis buffer (40 mM Tris, 1 mM EDTA, 0.5% Tween-20, 0.5 μg/μL proteinase K, pH 8.0) until all tissue fragments were completely dissolved. DNA was extracted using the phenol-chloroform method and dissolved in 10 mM Tris (pH 7.6).

Genomic DNA samples from cancer cell lines and uterine sarcoma tissues were digested with 4 U/μg *Hin*fI and *Rsa*I restriction enzymes (NEB) in the presence of 25 ng/μg DNase-free RNase (Roche). Each DNA sample (30 ng) was combined with 0.2 mg/mL bovine serum albumin; 0.1% Tween; 1 mM each of dATP, dGTP, and dTTP; 1× 29 buffer; and 7.5 U 29 DNA polymerase (NEB) and incubated at 30°C for 8 hours, followed by incubation at 65°C for 20 minutes. The reacted samples were UV cross-linked onto the membrane and then hybridized at 37°C with end-labeled $^{32}$P-$(CCCTAA)_3$.[20] U2OS (both cell culture and paraffin-embedded cell block) and HeLa cells were used as positive and negative controls, respectively. The images were acquired using Bio-Imaging Analyzer Systems (Fujifilm Corporation).

## Immunohistochemical Analysis for hTERT Detection

Paraffin blocks from uterine sarcomas were cut at 4 μm adjacent to HE sections. Samples were deparaffinized in xylene, rehydrated with graded ethanol, and washed in PBS. The sections were then placed in 10 mM citrate buffer (pH 6.0) and boiled in a microwave for epitope retrieval. Endogenous peroxidase activity was quenched by incubating tissue sections in 3% $H_2O_2$ for 10 minutes. Prepared sections were incubated with a primary rabbit anti-hTERT antibody (Rockland, Gilbertsville, PA) at a dilution of 1:200 in a humidifying chamber at 4°C overnight. Then, the sections were washed in PBS for 5 minutes at room temperature and subsequently stained by the labeled streptavidin biotin (LSAB) method using a Dako LSAB kit (Dako) and visualized using 3,3′-diaminobenzidine. The sections were then counterstained with hematoxylin.

The negative controls were stained using the same method, except that the primary antibody incubation step was omitted. Microscopic fields from each stained section were randomly sampled. For immunohistochemistry (IHC) scoring for hTERT, only nuclear staining for telomerase was considered positive. The IHC scores were as follows (percentage of cells with positive staining): 0 = no staining (negative), +1 = 1% to 10% of the cells were positive, +2 = 11% to 30% of the cells were positive, and +3 = more than 30% of the cells were positive.

## Statistical Analysis

The relationships between categorical variables were assessed using $\chi^2$ tests and analysis of variance, and continuous variables were evaluated using a Spearman test. Disease-free survival (DFS) and overall survival (OS) were estimated using the Kaplan-Meier method, and the differences in survival were compared using the log-rank test. Multivariate analysis was performed using the Cox regression method. $P < 0.05$ was considered statistically significant. The data were analyzed using SPSS software, version 12.0 (SPSS Inc, Chicago, IL).

# RESULTS

## Clinicopathologic Features of Cancer Patients

The clinicopathologic characteristics of the 41 uterine sarcoma patients included in the current study are presented in Table 1. The age of the uterine sarcoma patients ranged from 21 to 84 years (median, 51.0 years). Histologically, tumors were classified into endometrial stromal sarcomas (ESSs), carcinosarcomas, and leiomyosarcomas. Endometrial stromal sarcomas and carcinosarcomas each accounted for approximately 40% of the cases, and leiomyosarcomas accounted for 20% of the cases. After primary debulking surgery, 30 patients underwent adjuvant treatment, including chemotherapy (18 patients), radiation therapy (7 patients), and concurrent chemoradiation therapy (5 patients). Of the 41 patients, 19 experienced a relapse, 14 of whom died of uterine sarcoma.

For evaluation of epithelial carcinoma, we selected 82 ovarian cancer patients (median age, 49.0 years; range 15–79 years). Two thirds of these patients belonged to the advanced stage (stages III–IV) group, and 54% of the patients showed serous histology.

## Correlation Between APB Positivity and Tumor Behavior

An immuno-FISH–based APB assay was used to investigate the frequency of ALT positivity among the 41 uterine sarcoma and 82 epithelial ovarian carcinoma samples. The presence of APBs was correlated with patient data to determine whether ALT positivity was associated with the characteristics of uterine sarcomas (Table 2). Whereas 8 samples (9.8%) were positive for APBs in the epithelial ovarian

 

**TABLE 1.** Clinicopathologic characteristics of uterine sarcoma and carcinosarcoma patients

| | No. Patients (%) or Median (Range) |
|---|---|
| Age, y | 51.0 (21–84) |
| Histology | |
| ESS, low grade | 11 (26.8) |
| ESS, high grade | 6 (14.6) |
| Leiomyosarcoma | 8 (19.5) |
| Carcinosarcoma | 16 (39.0) |
| Size | |
| Tumor <5.0 cm | 12 (29.3) |
| 5.0 ≤ tumor < 10.0 cm | 11 (26.8) |
| 10.0 ≤ tumor < 15.0 cm | 10 (24.4) |
| Tumor ≥ 15.0 cm | 8 (19.5) |
| Mitotic index (MI) | |
| MI < 5 | 10 (24.4) |
| 5 ≤ MI < 10 | 1 (2.4) |
| 10 ≤ MI < 15 | 4 (9.8) |
| MI ≥ 15 | 26 (63.4) |

carcinoma group, 19 (46.3%) were positive for APBs in the uterine sarcoma group (Fig. 1). Alternative lengthening of telomeres–associated promyelocytic leukemia bodies were a more prominent characteristic among sarcomatous tumors than among epithelial carcinomas in gynecologic cancers ($P < 0.001$). In a carcinosarcoma patient sample, APB positivity was stronger in the sarcoma, compared with carcinoma components.

The proportion of APB-positive high-grade leiomyosarcomas was remarkably high (88%) compared with that of low-grade ESSs (9%; $P = 0.001$). Tumor size and mitotic index (mitotic figures per 10 random high-power fields), 2 important measures of uterine sarcoma aggressiveness, were associated with APB positivity ($P = 0.033$ and $P = 0.001$, respectively). However, the stage of the uterine sarcoma was not correlated with the presence of APBs ($P = 0.508$). In addition, in our screening of known human cancer cell lines of female genital tract origin, none of them turned out to be APB positive.

## C-Circle Assay Confirmed the Identification of ALT

The presence of telomeric C-circles is thought to represent ALT.[20] Therefore, we investigated whether the C-circle assay could be applied to paraffin-embedded tissue samples. Among 22 sarcoma cases, 8 (36.4%) showed C-circle–positive results (Fig. 2B). Because of limitation in the sufficient amount of samples, we could obtain only 22 samples of genomic DNA from the paraffin blocks of the 41 sarcoma cases. Nonetheless, the C-circle assay results correlated very well with the immuno-FISH–based APB assay results, despite the small sample size ($P = 0.012$, Table 3). We confirmed that C-circle assays support the result from APB assays: both the APB and C-circle assays were effective and robust in measuring the characteristics of ALT from paraffin-embedded tumor tissue. According to the results of the C-circle assay, there was no ALT-positive sarcoma cell line originating from the female genital tract (Fig. 2A).

## Telomerase Activity Does Not Overlap With ALT Positivity in Uterine Sarcoma

To compare ALT cancers with telomerase-positive cancers, we evaluated hTERT-expressing tumors. For this experiment, IHC against hTERT was performed on uterine sarcoma tissue.[21] Immunohistochemistry scores for hTERT of +2 and +3 were considered to be positive reactivity; other scores were considered to be negative (scores of 0 and +1). Eighteen cases (43.9%) of uterine sarcoma presented positive hTERT expression, and hTERT reactivity determined by IHC was negatively correlated with APB expression ($P = 0.036$, Table 3). Representative images are presented in Supplemental Figure 1 (http://links.lww.com/IGC/A82).

## Prognostic Significance of APBs and ALT

Survival analyses showed that the presence of APBs was a significant prognostic factor for poor DFS ($P = 0.018$) and OS ($P = 0.021$). A Kaplan-Meier analysis showed that the median DFS for patients with APB-positive tumors was 3 months (range, 1–43 months) compared with a mean DFS of 9 months (range, 1–90 months) for patients with APB-negative tumors (Fig. 3). Overall survival was similarly associated with APB status; patients with APB-positive tumors

**TABLE 2.** Correlation between APB expression and clinicopathologic parameters

| | No. Patients | APBs | | *P* |
|---|---|---|---|---|
| | | Negative | Positive | |
| Age | | | | |
| <50 y | 19 | 12 | 7 | 0.257 |
| ≥50 y | 22 | 10 | 12 | |
| Histology | | | | |
| ESS, low grade | 11 | 10 | 1 | 0.030* |
| ESS, high grade | 6 | 3 | 3 | |
| Leiomyosarcoma | 8 | 1 | 7 | |
| Carcinosarcoma | 16 | 8 | 8 | |
| Tumor size | | | | |
| <10 cm | 23 | 15 | 8 | 0.033† |
| ≥10 cm | 18 | 7 | 11 | |
| Mitotic index | | | | |
| <10 | 11 | 10 | 1 | 0.001† |
| ≥10 | 30 | 12 | 18 | |

*Analysis of variance test.

†Spearman test for continuous variables of tumor size and mitotic index.

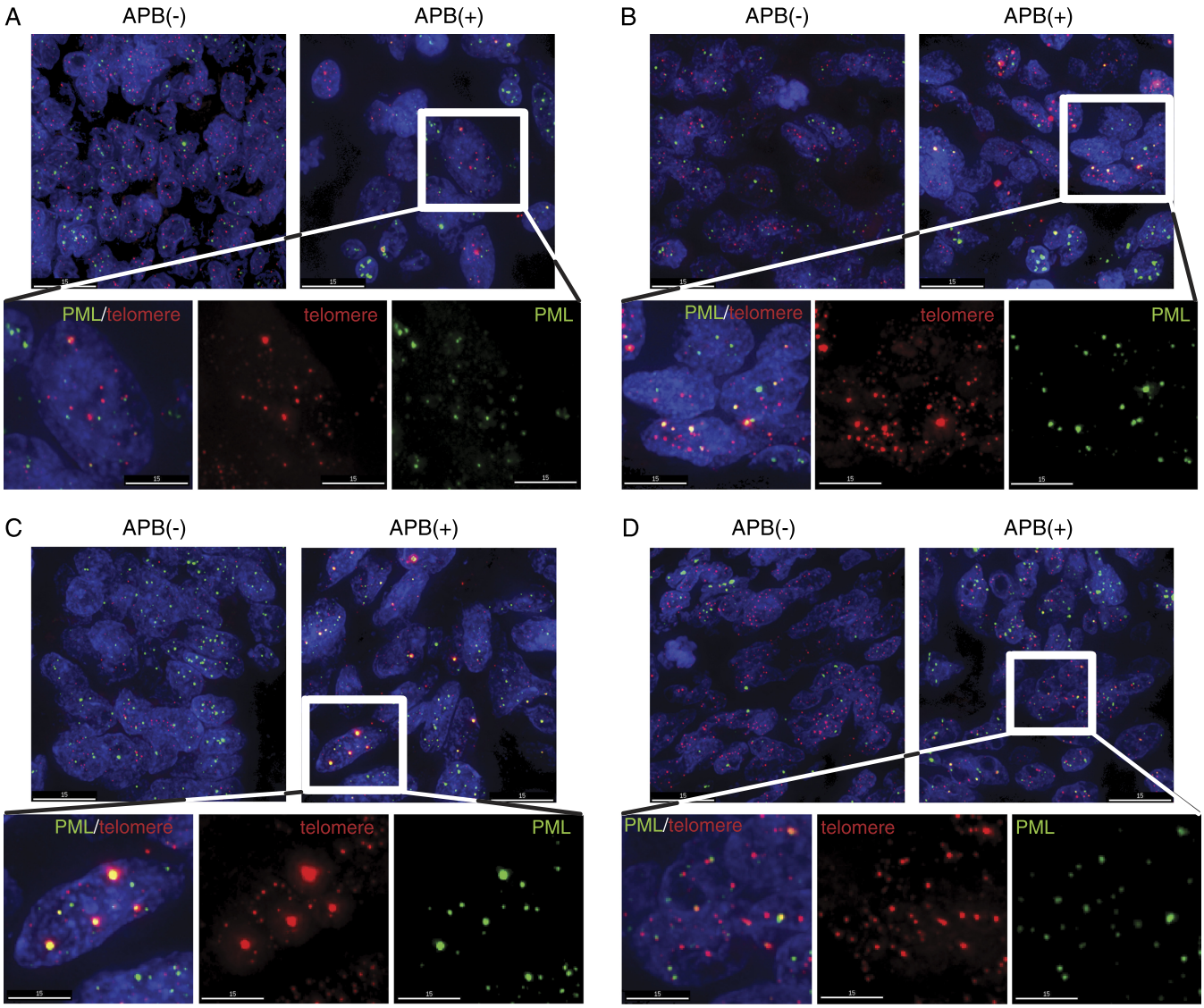


**FIGURE 1.** Detection of APBs in paraffin-embedded sections of uterine sarcoma by immuno-FISH. Representative images of negative and positive results of APBs in (A) low-grade ESS, (B) high-grade ESS, (C) leiomyosarcoma, and (D) carcinosarcoma. Paraffin-embedded tumor tissue sections were analyzed using an anti-PML immunofluorescence (IF) assay (green) combined with telomere FISH (red). Nuclei were counterstained with DAPI (blue). Enlarged IF images of APB (+) cells are shown in the lower panel. Note that the intensity and the size of the telomere fluorescence, detected by the Cy3-conjugated telomere probes in APBs (red), are high and large, respectively, in most of the APB-positive cells. This result indicates that telomeres from different chromosomes may be clustered in APBs. Scale bar, 15 μm.

had a median OS of 6 months (range, 3–46 months), whereas those with APB-negative tumors had a median OS of 12 months (range, 1–92 months). However, the presence of APBs was not an independent prognostic factor in the patients with uterine sarcomas in the multivariate analysis.

## DISCUSSION

Uterine sarcomas exhibit aggressive features, but there is little consensus on how to clinically diagnose or manage these cancers because of their rarity and histological diversity.[14] Carcinosarcomas develop from epithelial components but differ from endometrial carcinomas in that their behavior is highly aggressive compared with that of high-grade endometrial carcinomas.[22] Therefore, we evaluated the characteristics of uterine tumors with sarcoma components, including ESSs, leiomyosarcomas, and carcinosarcomas, which are collectively referred to as uterine sarcomas in this study. To develop a strategy for the clinical management of uterine sarcomas, it is crucial to understand the mechanism of

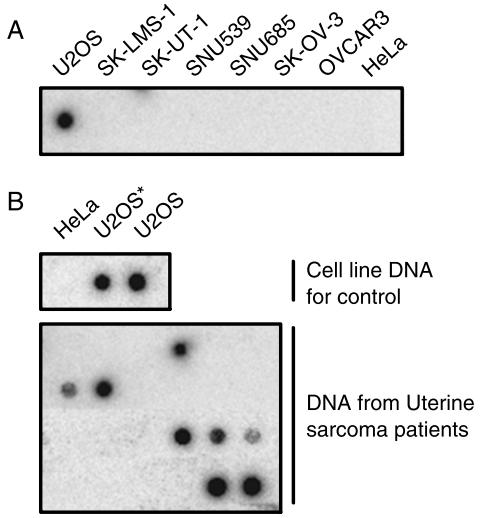


**FIGURE 2.** Detection of extratelomeric C-circles (ECTR) in cell lines and tumor tissues. Thirty nanograms of genomic DNA was used. A, All of the evaluated gynecologic cancer cell lines were negative for C-circles. The cell lines were as follows: female genital tract sarcoma cell lines (SK-LMS-1 and SK-UT-1), carcinosarcoma cell lines (SNU539 and SNU685), and epithelial ovarian carcinoma cell lines (SK-OV-3 and OVCAR3). U2OS and HeLa cells were used as positive and negative controls, respectively. B, Among 22 uterine sarcoma tissue samples, 8 tissue samples were positive for the presence of ECTR. To evaluate the robustness of the C-circle assay in paraffin-embedded samples, DNA extracted from a paraffin-embedded U2OS cell block (U2OS*) and that from fresh cell culture (U2OS) were analyzed at the same time.

tumorigenesis and to identify prognostic factors. Here, we determined whether ALT is a prognostic factor. We successfully applied an immuno-FISH technique and a C-circle assay to detect APBs and C-circles, respectively, the 2 characteristics of ALT, in paraffin-embedded tumor samples, which are stable and easily accessed.[5,20,23]

Telomerase reactivity showed a negative correlation with ALT status, suggesting that the clinical treatment should differ for the 2 different types of cancer. The frequency of ALT in uterine sarcoma samples was 46%, similar to the values of 24% and 33% reported for liposarcomas[5,6] and of 35% and 47% reported for osteosarcomas.[5,24] Meanwhile, only 9.8% of epithelial ovarian carcinomas presented ALT characteristics. Notably, ALT was found at remarkably high frequencies in leiomyosarcomas (62%) and malignant fibrous histiocytomas (77%).[5] The reasons for the prevalence of ALT in sarcomas, in general, and the unusually high prevalence of ALT in leiomyosarcomas and histiocytomas, in particular, are not understood yet.

Although the prevalence of ALT in low-grade ESSs was relatively low (8%), 50% of high-grade ESSs exhibited the signature of ALT (Table 2). It is not easy to grade carcinosarcomas and leiomyosarcomas because of the aggressiveness of these tumors. Nonetheless, both carcinosarcomas and leiomyosarcomas featured a high frequency of ALT (50% and 87%, respectively). Thus, with the exception of low-grade ESSs, most uterine sarcomas displayed a high frequency of ALT. Notably, ALT positivity correlated with other clinicopathologic parameters correlated with aggression, such as mitotic index and tumor size (Table 2), which are considered to be significant prognostic factors for uterine sarcoma.[13,25] Taken together, these data suggest that the induction of ALT has significant implications for the progression of uterine sarcoma.

With respect to patient survival, uterine sarcomas positive for ALT appeared to be clinically aggressive and were associated with poor outcomes. Clinical factors that determine survival outcomes are critical in the management of patients because they provide helpful clues in the choice of treatment options. Consistent with our results, ALT positivity has been shown to be a negative prognostic factor in osteosarcoma; osteosarcomas positive for ALT exhibit aggressive behavior and are associated with high mortality.[14] Similar results have been reported for liposarcomas.[6] In particular, ALT positivity was suggested to be a more potent indicator of poor prognosis compared with telomerase positivity, although both telomere-maintenance mechanisms were associated with short survival in liposarcoma patients.[6] In comparison, ALT positivity is considered to be a good prognostic indicator in glioblastoma multiforme.[4,5] The reasons for these different prognostic indications are unclear, but it has been suggested that patient survival depends on the histology of tumors. We were not able to define the value of APB for the subgroup of uterine sarcomas because of the limited sample size. Nonetheless, this study has revealed the clinical significance of APBs and ALT in sarcomas of the uterus for the first time. Moreover, it should be emphasized that the results of the C-circle assay using paraffin-embedded tissues corroborated the results of the APB immuno-FISH assay. Thus, the association of APB assays with clinicopathologic parameters is related to the nature of ALT.

In this study, we demonstrated that the APB immuno-FISH and C-circle assays are feasible and reliable methods for assaying ALT status in paraffin-embedded sections, and we further showed that ALT is associated with high-grade, aggressive tumors and poor prognosis in patients with uterine sarcomas and carcinosarcomas. In conclusion, ALT is a

**TABLE 3.** Correlation between APB expression and C-circle assay results or hTERT expression

| | No. Patients (%) | APBs | | *P* |
|---|---|---|---|---|
| | | Negative | Positive | |
| C-circle assay | | | | |
| Negative | 14 (63.6) | 10 | 4 | 0.012 |
| Positive | 8 (36.4) | 1 | 7 | |
| hTERT expression | | | | |
| Negative | 23 (56.1) | 9 | 14 | 0.036 |
| Positive | 18 (43.9) | 13 | 5 | |

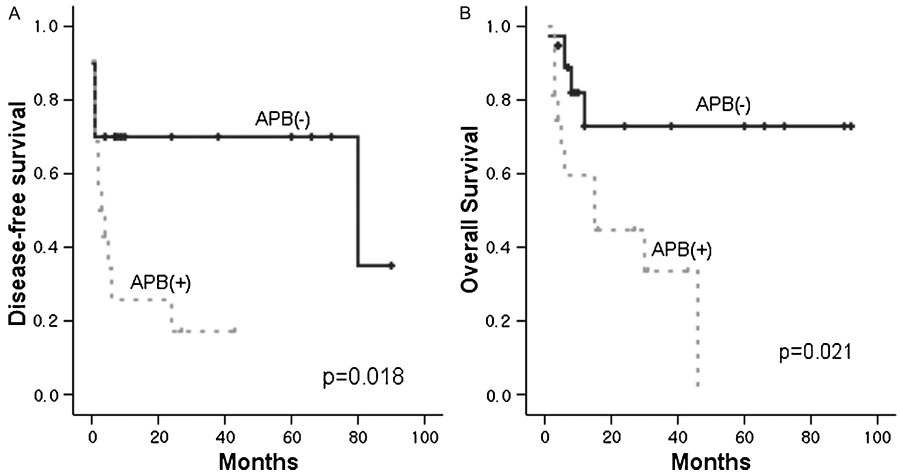


**FIGURE 3.** Disease-free survival and OS ratio in relation to ALT status. Kaplan-Meier analysis showing that positive ALT status is a prognostic factor for poor DFS ($P = 0.018$) (A) and poor OS ($P = 0.021$) (B).

prognostic indicator of poor clinical outcome in uterine sarcomas and carcinosarcomas, and this result suggests that determining the ALT mechanism in uterine sarcoma may provide a promising therapeutic target.

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