Immunohistochemical analysis of p16 expression in uterine smooth muscle tumors

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Summary

The expression of p16 as a tumor suppressor protein was evaluated in a retrospective analysis of paraffin-embedded tissue specimens of leiomyosarcoma (LMS), leiomyoma (LM) and normal myometrium. In this study, we investigated by immunohistochemistry p16 expression in 15 LMSs, 15 LMs and ten normal myometrium. Strong expression of p16 was found in 12 of the 15 LMSs and in three cases weak expression; three LMs had focal and weak p16 staining but none of the normal myometrium. A statistically significant difference regarding the frequency of p16 protein expression was observed between LMS and LM (*p*: 0.0001). We concluded that the results of this study confirm the overexpression of p16 in LMS. Therefore, the present study suggests that p16 might be a useful immunohistochemical marker which could help in distinguishing uterine LMS from LM and its benign variants.

Key words: p16 expression; Uterus; Leiomyoma; Leiomyosarcoma.

Introduction

Uterine smooth muscle tumors (USMTs) are histologically categorized as leiomyoma (LM) or leiomyosarcoma (LMS) based on a combination of mitoses, cytologic atypia, and coagulative tumor cell necrosis. Uterine leiomyomas are the most common benign smooth muscle tumors in women of reproductive age and occur in nearly 40% of women older than 35 years [1, 2]. Uterine leiomyosarcomas are rare tumors, usually exhibiting diffuse moderate-to-severe atypia, a mitotic count of ≥ 10 MFs/10HPFs, and tumor cell necrosis. However, uncommon variants of leiomyoma, such as symplastic (atypical, bizzare or pleomorphic) LM, mitotically active LM, and cellular LM, may result in consideration of a LMS because of the presence of nuclear atypia, high mitotic index and high cellularity, respectively. These features are commonly present in LMS [3]. The smooth muscle tumor of uncertain malignant potential (STUMP) is a smooth muscle tumor that cannot be classified as benign or malignant based on established histopathologic criteria [4].

Immunohistochemistry has been used to evaluate uterine smooth muscle neoplasms for both pathologic classification and clinical correlations [3, 5-7]. The p16 protein has been identified as a tumor suppressor protein, which binds specifically to cyline-dependent kinase CDK-4, inhibiting the catalytic activity of the CDK4-cylin D complex, and thereby acting as a negative cell cycle regulator [8]. p16 is probably important in cell senescence, and recent studies have identified a role for p16 in cell spreading and angiogenesis [9, 10].

In the present study, we have investigated by immunohistochemical analysis, the tissue disturibution of p16 protein in patients with uterine LMs, LM variants, LMSs and normal myometrium.

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Materials and Methods

Specimens of tissues were obtained from 31 patients with smooth muscle tumors who had undergone hysterectomies and ten healthy myometrium samples that had hysterectomies for nonneoplastic reasons from January 2004 to December 2009 at the Department of the Pathology, Mustafa Kemal University Hospital, Antakya, Turkey. All routine hematoxylin and eosin (H&E)-stained slides were reviewed. Microscopic characteristics of all the smooth muscle tumors were analyzed and recorded, such as cellularity, mitotic activity, nuclear atypia, and necrosis. Pathologic diagnosis of the tumors was performed using criteria in the literature [1, 11]. According to this criteria, of the 31 cases of smooth muscle tumors of the uterus, 15 were diagnosed as LMs, 15 as LMSs (Figure 1), one as STUMP, and this case was excluded from the study.

Immunohistochemical study

One or two blocks from each tumor and normal myometrium were stained for immunohistochemical analysis using the avidin biotin and immunoperoxidase methods. Formalin-fixed paraffin-embedded tissues were cut into 4 μm sections and dried on capillary-cap glass slides. The sections were deparaffinized with standard xylene and hydrated through graded alcohol into water. An antigen retrival procedure was performed using citrate buffer and heating for 10 min in a pressure cooker. Slides were placed for 15 min into a 3% hydrogen peroxide blocking medium and then allowed to react with the primer antibody, anti-p16 antibody (DAKO North America; dilution 1:20). Immunoperoxidase detection was employed using AEC substrate. Counter staining was performed with hematoxylin.

Evaluation of immunohistochemical staining

All immunostained sections were analyzed by two different pathologists who had no knowledge of the patient's clinical and pathological status. The interpretation of immunohistochemical staining was expressed as follows: both nuclear or/and cytoplasmic staining was regarded as a positive reaction. p16 expression was scored as negative, focal (fewer than 33% of cells) moderate (33% to 66% of cells), or diffuse (greater than 66% of cells). This cutoff is similar to the study by Bodner-Adler *et al*. [5].

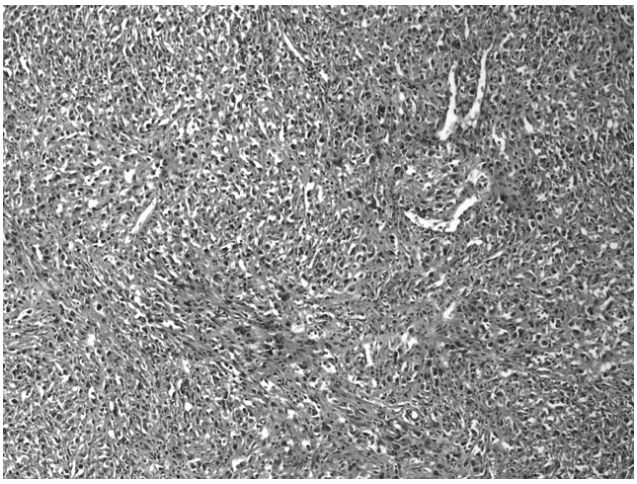

Figure 1. — Histopathology of leiomyosarcoma (H&E x40).

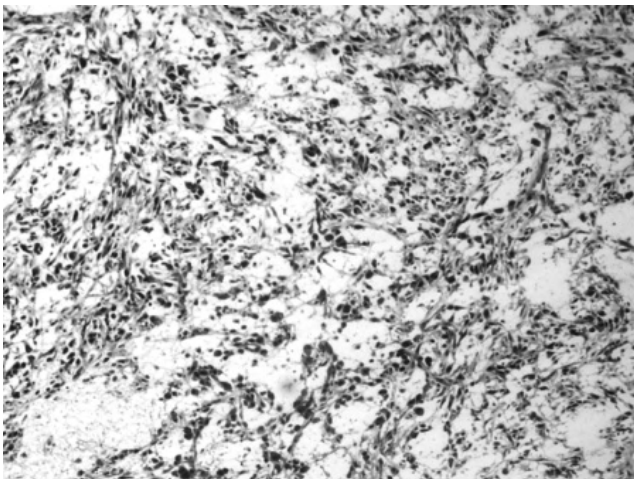

Figure 2. — Strong expression of p16 in leiomyosarcoma (40x).

The chi-square test was used to compare freguency distribution of p16 protein expression between the analyzed groups (LM and LMS); p values of less than 0.05 were considered statistically significant. The SPSS system (Chicago, IL, USA) was used for the calculations.

Results

Clinical findings in patients with LM and LMS

The median age of patients with LM was 45 years (range: 32-48). Hysterectomy was the standard surgical procedure in all cases of LM. All 15 patients with LM were alive and in good health during a median follow-up time of 43 months (range: 15-60 months).

The median age of patients with LMS was 50 years (range: 38-74). Four patients had Stage I, six patients Stage II, three patients Stage III, and two patients Stage IV. All patients with LMS had a hysterectomy and bilateral salpingo-oopherectomy as surcical therapy.

Expression of p16 protein in leiomyoma and LMS

The distribution and immunostaining intensity for p16 expression in uterine smooth muscle neoplasms are summarized in Table 1. p16 protein was expressed in 3/15 (20%) LM, and in 15/15 (100%) LMS. The intensity of p16 staining varied from weak to strong. Of the 15 patients with LMS, three (20%), seven (46.6%) and five (33.3%) were found weak, moderate and strong, respectively (Figure 2). Of 15 patients with LM, only three (20%) were found to have weak positivity. p16 did not stain healthy myometrium. A statistically significiant difference regarding the frequncy of p16 expression was observed between LMS and LM ($p < 0.0001$).

Table 1. — *Immunohistochemical results with p16.*

		Immunohistochemical staining intensity for p16			
	No. of cases	0 (0%)	+ (< 33%)	++ (33-66%)	+++ (> 66%)
Myometrium	10	10	0	0	0
LMS	15	0	3	7	5
LM					
Nos	6	5	1	0	0
Cellular	3	2	1	0	0
Epitheloid	2	2	0	0	0
Bizarre	1	0	1		
Myxoid	3	3	0	0	0

LM: leiomyomas; LMS: leiomyosarcomas.

Discussion

p16 is a cyclin-dependent kinase inhibitor which is expressed in a limited range of normal tissue and tumors. p16 is integral to the retinoblastoma (Rb) gene-mediated control of the G1-S phase transition of the cell cycle [12]. Aberrant expression of p16 protein has been studied in a variety of human neoplasms, including uterine cervical and gastric cancer. Although it is clear that elevated p16 expression in cervical squamous cell carcinoma with its precursors is the surrogate marker for HPV infection, overexpression of p16 in other neoplasms is largely unknown but is not HPV-related [13, 14].

Uterine LM is distinguished from LMS using a combination of morphological criteria, including cellularity, the presence or absence of coagulative tumor cell necrosis, mitotic index and the degree of nuclear pleomorphism. Typically, LMS is characterized by high cellularity, marked nuclear pleomorphism, the presence of coagulative tumor cell necrosis and high mitotic activity. However, in a particular LMS, one or more of these features may be absent. Conversely any one of these features may be present in a LM that is entirely benign and, due to this, these may be classified as LM variants [15, 16].

Accurately diagnosing malignant from benign uterine smooth muscle neoplasms is important clinically for patient management. However, due to the overlapping features between malignant and benign smooth muscle tumors, it can be challenging depending on morphological criteria alone. This is particulary true between bizarre

and cellular LM. Therefore, efforts in searcing for biomarkers that can differentiate benign and malignant smooth muscle tumors have important clinical implications [17].

In a study of LM and LMS, the data of Bodner-Adler *et al*. [5]. showed that there was p16 expression in 12% and 57% of cases, respectively. There was a statistically significant difference in both p16 staining and frequency and intensity between LM and LMS. In a more recent study by O'Neill *et al*. [3], p16 immunoreactivity of LMS was significantly higher than LM and benign LM variants.

We have analyzed the immunohistochemical staining of p16 in normal myometrium, LM and LMS. We found strong expression of p16 in 100% of LMS, but weak expression of p16 in 3/15 (20%) of LM and LM variants, and there was no expression of p16 in normal myometrium.

Conclusion

We have shown statistically higher levels of p16 in LMS compared to LM and normal myometrium. It has been suggested that p16 is a particularly useful marker in the differential diagnosis between LMS and difficult LM variants. The reason for the higher expression is, however, unclear. Although our results were statistically significant, our study was limited by its small sample size. The use of p16 in diagnostic settings should be explored further by a large-scale study.

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