PTEN, tau-AP-3, thymidylate synthase immunohistochemistry scoring expression in patients with uterine leiomyomas, uterine smooth muscle tumors of uncertain malignancy potential and uterine leiomyosarcomas

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Summary

Uterine smooth muscle tumors are frequently classified as benign and malignant. However, an assortment of mitotic counts and nuclear atypia can be indecisive between uncertain malignant potential, and malignant uterine smooth muscle tumors. We applied three immunohistochemical parameters to distinguish between cases of benign, malignant, and those with uncertain malignant histology.

Key words: Allred immunohistochemistry scoring; Immunohistochemistry; Uterine smooth muscle tumors.

Introduction

Neoplasms of uterine smooth muscle can be classified into benign, malignant, and tumors of uncertain malignant potential (STUMP). The diagnosis of benign, uncertain malignant potential, and malignant uterine smooth muscle tumors depends on mitotic counts, nuclear atypia, and other morphologic features [1]. However, differentiating between STUMP and uterine leiomyosarcoma as well as assessing malignant potential can be problematic, especially when difficulty in recognizing mitotic figures occurs or when clumped and degenerative nuclei are misinterpreted as mitotic figures [2]. In this study, we used three immunohistochemical parameters to distinguish between cases of benign, malignant and those with uncertain malignant histology.

Immunohistochemistry (IHC) is a technique used to identify specific types of cells within a given sample and has been used since the 1940s [3]. Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as in cancerous tumors.

The principle behind IHC is the detection of a particular protein or antigen on or within a cell with the use of a commercially available antibody. This antibody antigen complex is then magnified and tagged with a stain that is visible under a light microscope. The ability to use antibodies on formalin-fixed, paraffin-embedded tissue has improved the applicability of IHC [4]. IHC has found numerous applications in medicine, especially in cancer diagnosis. The aims of this study were to systematically evaluate immunohistochemical staining patterns of the expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), tau-AP-3, thymidylate synthase (TS) in benign and malignant uterine smooth muscle neoplasms; to study whether the expression patterns of PTEN, tau-AP-3, and TS correlate with uterine smooth muscle neoplasms; and, to evaluate whether the expression patterns of PTEN, tau-AP-3, and TS are helpful in differentiating benign tumors from malignant uterine tumors and tumors with uncertain malignant potential.

Materials and Method

Formalin-fixed and paraffin-embedded surgical specimens of uterine leiomyoma (n = 10), leiomyosarcoma (n = 5), and tumors of uncertain malignant potential (n = 3) were subjected to the following procedures.

After cutting the wax-embedded tissues into 2 mm pieces, the wax was melted at 56°C in distilled water and the tissue samples were removed. The tissue sample was heated at 65~75°C for 20 min, triple soaked in xylene solution for 10 min, followed by rinsing in 100% alcohol, 95% alcohol, 75% alcohol, 60% alcohol, 30% alcohol distilled water and PBS buffer individually for 10 min. The tissue sample was boiled in citrate buffer (pH6, > 95°C) for 20 min, cooled at room temperature and rinsed in PBS buffer for 5 min. A section was circled on the tissue sample and covered with H_2O_2 for 10 min. After washing twice in TBST buffer for 5 min, 10% horse serum was used to block the background of tissue sample. The primary antibodies PTEN-TS (Novocastra, Vision Biosystem, Norwell, MA) and Tau AP-3 (Neomarker, CA, USA) were applied, followed by TBST buffer washing for another 5 min, and HRP polymer conjugate (Zymed Laboratories, San Francisco, CA) was applied as a secondary antibody. Finally, DAB Chromogen (Zymed Laboratories) was added to stain the protein component of the circled sections. Immunostaining of protein expression and protein display were visualized under a light microscope after the tissue sample had been washed in hematoxylin and flowing water.

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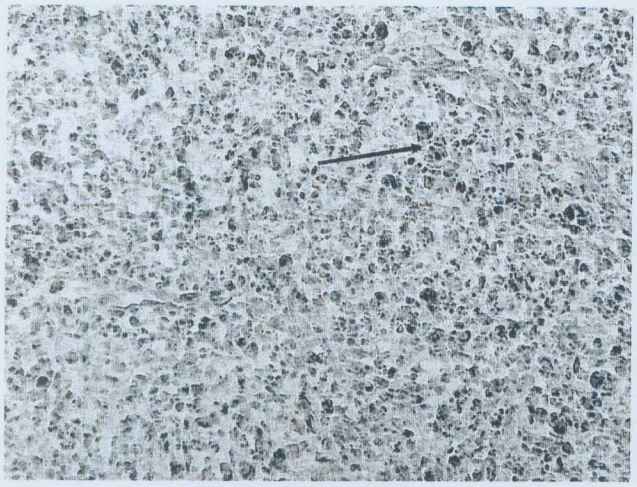
Fig. 1



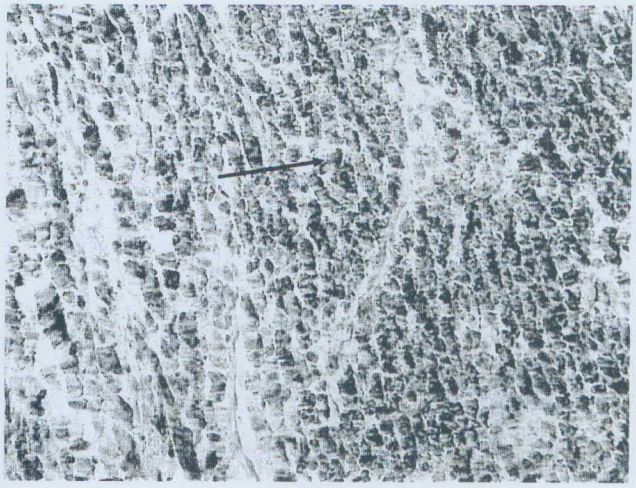


Fig. 2

Fig. 3

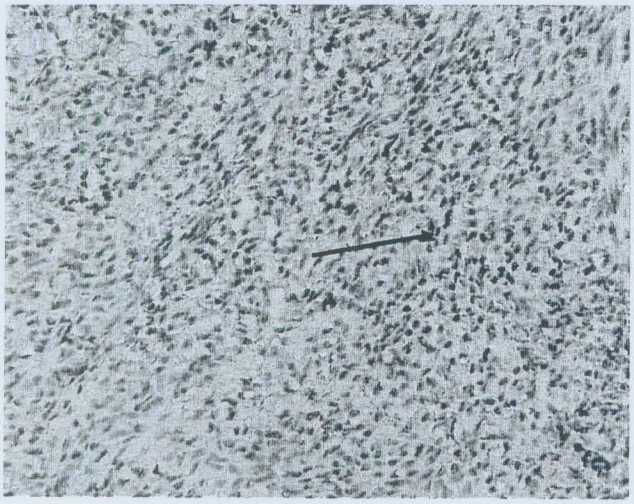


Figure 1. —

Figure 2. —

Figure 3. —

legends?

Results

In this study, two pathologists used the Allred immunohistochemistry scoring system to analyze (a) the proportion score (PS), (b) the intensity score (IS), and (c) the total score (TS) in leiomyoma specimens, leiomyosarcoma specimens, and STUMP specimens. The proportion score represents the estimated proportion of positively stained tumor cells (0 = none; 1 < 1/100; 2 = 1/100 to < 1/10; 3 = 1/10 to < 1/3; 4 = 1/3–2/3; 5 ≥ 2/3). The intensity score represents the average estimated intensity of positively stained cells (0 = none; 1 = weak; 2 = intermediate; 3 = strong). The total score was obtained by adding the PS and IS (range 0-8). One-way ANOVA and the paired t-test were used to evaluate significant differences.

Table 1. — *One-Way ANOVA to measure differences in PS/IS/TS among the three biomarkers.*

Treatment	PTEN			TAU			THYM		
Measure	PS	IS	TS	PS	IS	TS	PS	IS	TS
F*	5.43	3.74	7.06	6.12	16.01	10.40	43.64	18.55	35.60
p value	0.017	0.048	0.007	0.011	0.000	0.001	0.000	0.000	0.000

Note: Numbers of observation in myoma, leiomyosarcoma, and STUMP are 10, 5, and 3, respectively.

All three IHC scores showed statistical significance among the three groups of tumor specimens (paired t-test).

Myoma vs leiomyosarcoma

In our analysis, all three of the immunostaining scores differed significantly between the myoma group and the leiomyosarcoma group. All three scores showed that the level of PTEN expression (Figure 1) was higher in the myoma group than in the leiomyosarcoma group and that the levels of TAU (Figure 2) and TS expression were lower in the myoma group than in the leiomyosarcoma group (Table 2).

Leiomyosarcoma vs STUMP

The PS and the IS for TS expression were both higher in the leiomyosarcoma group (Figure 3) than in the STUMP group (Table 2).

Myoma vs STUMP

All three immunostaining scores for TS were significantly higher in the STUMP group than in the myoma group, indicating that the expression of TS was markedly

Table 2. — *Mean difference ~~between~~ among groups.*

Treatment		PTEN			TAU			THYM		
Measure		PS	IS	TS	PS	IS	TS	PS	IS	TS
PS	(1)	1.10	0.95	(1) > (2)**	1.20	0.92	(1) < (2)**	0.60	0.70	(1) < (2)**
	(2)	0.00	0.00		3.20	0.84		3.80	0.45	(3) > (1)*
	(3)	0.33	0.58		2.00	1.74		1.67	0.57	
IS	(1)	1.30	0.68	(1) > (2)**	0.80	0.42	(1) < (2)**	0.50	0.52	(1) < (2)**
	(2)	0.40	0.55		2.20	0.45		2.20	0.45	(3) > (1)*
	(3)	0.67	0.58		1.33	0.58		1.33	0.57	
TS	(1)	2.40	1.17	(1) > (2)**	2.00	1.25	(1) < (2)**	1.10	1.20	(1) < (2)**
	(2)	0.40	0.55		5.40	0.90		6.00	0.71	(2) > (3)**
	(3)	1.00	1.00		3.33	2.31		3.00	1.00	(3) > (1)*

Note: Group: (1) Myoma, (2) Leiomyosarcoma, and (3) STUMP.
* significant at $\alpha = 0.05$ level; ** significant at $\alpha = 0.01$ level.

higher in specimens classified as uterine smooth muscle tumors of uncertain malignant potential (Table 2).

In summary, TS expression differed significantly among all three tumor types. The proportion scores and the total scores differed uniformly between the three groups. Total scores showed leiomyosarcoma > STUMP > myoma.

Discussion

Uterine smooth muscle tumors are the most frequent neoplasms in the female genital tract [2]. The majority of the uterine smooth muscle tumors are readily classifiable as benign or malignant based on the gross and microscopic appearance [5]. However, there is substantial overlap in morphological features among leiomyomas, smooth muscle tumors of uncertain malignant potential (STUMP), and leiomyosarcomas, making it difficult to establish a definitive diagnosis. In this study, we used immunohistochemical staining to evaluate the potential of three cancer biomarkers, namely PTEN, tau AP-3 and thymidylate synthase, for differentiating between leiomyoma, STUMP and leiomyosarcomas.

The tumor suppressor gene PTEN (also known as MMAC or TEP1) is located on human chromosome 10q23. Mutation of PTEN is common in advanced stages of many human cancers and is one of the most commonly lost tumor suppressors in human cancer. In tumor development, mutations and deletions of PTEN inactivate its enzymatic activity leading to increased cell proliferation and reduced cell death [6]. In our study, PTEN showed higher immunostaining scores in leiomyoma than in leiomyosarcoma and STUMP.

Thymidylate synthetase is an enzyme that generates thymidine monophosphate (dTMP), which is subsequently phosphorylated to thymidine triphosphate for use in DNA synthesis and repair. Expression levels are often higher in malignant tumors than in normal cells. As an anti-cancer chemotherapy target, thymidylate synthetase can be inhibited by thymidylate synthase inhibitors such as fluorinated pyrimidine fluorouracil, or certain folate analogues [7]. In this study, the proportion score and the intensity score for TS were higher in the leiomyosarcoma group (malignant) than in the STUMP group. In addition, all three scores (proportion, intensity and total score) for TS revealed that the expression of thymidylate synthetase was significantly higher in the STUMP group than the myoma group (benign).

Tau protein is a highly soluble microtubule-associated protein. In humans, these proteins are mostly found in neuronal cells. One of the main functions of tau is to modulate the stability of axonal microtubules [8]. The usage of this protein as a cancer biomarker in malignant tumors of non central nervous system origin has not been reported; however, we found that the expression of tau was highest in the leiomyosarcoma group.

Uterine leiomyoma is the most common benign tumor in women of reproductive age, but uncommon variants of leiomyoma, such as symplastic (atypical, bizarre, or pleomorphic) leiomyoma, mitotically active leiomyoma and cellular or highly cellular leiomyoma, may result in a diagnosis of leiomyosarcoma because of the presence of nuclear atypia, high mitotic index and high cellularity [9]. On the other hand, uterine sarcoma is rare, accounting for approximately 1% of female genital tract malignancies and 3-7% of uterine cancers [10]. The clinical diagnosis of leiomyosarcoma is based on the presence of abnormal vaginal bleeding, rapid growth of a palpable pelvic mass and pelvic pain. Histopathologic diagnosis of uterine leiomyosarcoma is usually straightforward because most clinically malignant smooth muscle tumors of the uterus show the microscopic constellation of hypercellularity, severe nuclear atypia, and mitotic rate that generally exceeds 15 mitotic figures per 10 high-power-fields (MF/10HPF) [10].

Another uterine tumor that frequently cannot be classified as benign or malignant is the uterine smooth muscle tumor of uncertain malignant potential. STUMP tumors represent a poorly defined subcategory of uterine smooth muscle tumors. One way to define STUMP is by exclusion, i.e., tumors that do not fit the definition for any of the other categories of uterine smooth muscle tumors are classified as STUMP [11]. Applying conventional morphological criteria to distinguish leiomyoma from STUMP and uterine leiomyosarcoma is problematic. Thus, additional parameters to distinguish between these tumors are needed.

Conclusion

Immunohistochemical staining for PTEN, tau-AP-3 and TS expression is helpful in differentiating benign tumors from malignant tumors and tumors with uncertain malignant potential.

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