Human Pathology (2018) **73**, 122–127

Human
PATHOLOGY

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Original contribution

MVP immunohistochemistry is a useful adjunct in distinguishing leiomyosarcoma from leiomyoma and leiomyoma with bizarre nuclei☆,☆☆

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Received 10 September 2017; revised 14 December 2017; accepted 20 December 2017

Keywords:
Leiomyosarcoma;
Leiomyoma;
Bizarre leiomyoma;
Major vault protein (MVP);
Mass Spectrometry

Summary Morphologically, distinguishing between leiomyoma (LM) and leiomyosarcoma (LMS) is not always straightforward, especially with benign variants such as bizarre leiomyoma (BLM). To identify potential markers of malignancy in uterine smooth muscle tumors, proteomic studies were performed followed by assessment of protein expression by immunohistochemistry. Archival formalin-fixed, paraffin-embedded tissues from tumors (n = 23) diagnosed as LM, BLM, and LMS (using published criteria) were selected for the study. Sequential window acquisition of all theoretical fragment ion spectra mass spectrometry was applied to pooled samples of formalin-fixed, paraffin-embedded LM and LMS tumor tissue to assay the relative protein quantities and look for expression patterns differentiating the 2 tumor types. A total of 592 proteins were quantified, and 10 proteins were differentially expressed between LM and LMS. Select proteins were chosen for evaluation by immunohistochemistry (IHC) based on antibody availability and biologic relevance in the literature. IHC was performed on a tissue microarray, and intensity was evaluated using imaging software. Major vault protein (MVP) and catechol *O*-methyltransferase had 3.05 and 13.94 times higher expression in LMS relative to LM by sequential window acquisition of all theoretical fragment ion spectra mass spectrometry, respectively. By IHC, MVP (clone 1014; Santa Cruz Biotechnology, Dallas, TX) was found to be 50% sensitive and 100% specific when comparing LMS to LM. Catechol *O*-methyltransferase (clone FL-271; Santa Cruz Biotechnology) had a sensitivity of 38% and a specificity of 88%. Six of 7 BLM had expression of MVP similar to LM. Immunohistochemical staining for MVP is a useful adjunct in distinguishing LMS from LM and BLM in difficult cases.

1. Introduction

Uterine smooth muscle tumors can be a diagnostic challenge due to their wide array of morphologies and differing criteria for malignancy based on the morphological subtype. There are benign variants that have a component of the malignant features such as high cellularity, atypia, high proliferative rates, or necrosis. Such variants include cellular, epithelioid, bizarre (symplastic), lipoleiomyoma, mitotically active

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☆ Competing interests: We have no conflicts of interest to report.

☆☆ Funding/Support: Funding for this project was provided by an intradepartmental research fund.

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https://doi.org/10.1016/j.humpath.2017.12.020
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leiomyoma (LM), benign metastasizing, and disseminated peritoneal leiomyometosis. Because of the overlap, extensive sampling is often done. Currently, no genetics or immunohistochemistry assays are required for making diagnosis of leiomyosarcoma (LMS), and it is largely based on criteria established in 1994 [1]. Adding complexity, LMS has 2 main variants, epithelioid and myxoid, which alter the criteria for malignancy. For tumors that do not fall into 1 of these categories, they are classified as smooth muscle tumors of undetermined malignant potential.

Because of the extensive histologic overlap and many morphologic variants, many attempts have been made to find a diagnostic marker. A 2014 review of the panels that have been used for uterine mesenchymal tumors found h-caldesmon to be the most specific for smooth muscle but less sensitive than desmin, which also stains skeletal muscle. These markers tend to be positive in both benign and malignant tumors, but to a lesser degree in malignant ones. Most (86.5%) bizarre leiomyomas (BLMs) are positive for p16, and many (60%) are positive for p53; also, more than 10% of the cells in nearly half the cases are positive for Ki-67 [2]. This staining pattern has shown overlap with LMSs.

Ki-67 has been shown to have higher levels of staining in LMSs compared to benign counterparts; however, it has failed to perform better than counting a mitotic index [3]. Using p16 in conjunction with p53 has been useful in discriminating benign versus malignant for all variants except the atypical LM [2,3].

Distinguishing between atypical LM and LMS appears to be the most challenging. A study from 2013 constructed a tissue microarray of 70 cases, including 9 atypical LMs and 16 LMSs, as well as performed immunohistochemistry (IHC) using 14 different markers. Their conclusion was that none of the markers separately could clearly distinguish the 2 entities. However, PgRA >15% in combination with p16 >90% could ($P < .001$) [4].

Additional studies looking at IHC stains to distinguish atypical LM versus LMS have shown more mixed results. The results on the usefulness of various IHC stains tend to be mixed. A study separate study from 2013 looked at IHC of PTEN and p16 in 40 LMSs and 12 atypical LMs and found that there was no statistical significance in their staining pattern. There was significant difference, however, in comparing other variants of LM to LMS and in comparing other variants of LM to atypical LM [5]. In 2015, another group looked at the staining pattern of p16 in LM with infarct-type necrosis and found significant staining around the infarction. Their conclusion is that the utility of p16 staining to evaluate types of necrosis is low [6].

As can been seen by the above, distinguishing between the spectrum of uterine smooth muscle tumors is not always clear cut, and many attempts have been made to find definitive markers. So far, it appears that all the markers are suggestive but not definitive. To identify potential markers of malignancy in uterine smooth muscle tumors, we performed proteomic studies followed by assessment of protein expression via immunohistochemistry on LMS, BLM, and LM.

2. Materials and methods

2.1. Patient tissue samples

This research was reviewed and approved by the University of Nebraska Medical Center's Institutional Review Board. Archived formalin-fixed, paraffin-embedded (FFPE) tissue from uterine tumors (n = 10) diagnosed as LM (n = 5) and LMS (n = 5) were selected for proteomic analysis. Archival FFPE tissues from uterine tumors (n = 23) diagnosed as LM (n = 8), BLM (n = 7), and LMS (n = 8) were selected for construction of a tissue microarray. Uterine tumor subtypes were defined following published criteria [7]. The patient demographics can be seen in Table 1. For conventional spindle cell LMS, 2 of the following 3 features must have been present: diffuse moderate to marked cytologic atypia, mitotic rate ≥10 mitoses per 10 high-power fields, and tumor cell necrosis. For BLM, there needed to be minimal or no cytologic atypia in background, average mitotic rate of 1-2 mitoses per 10 high-power fields, and absence of tumor cell necrosis. The cases were all reviewed by a subspecialized pathologist to confirm their diagnosis.

2.2. Protein preparation for mass spectrometry

Slide-mounted FFPE tissue sections were placed in a glass slide bath and washed with the following: xylene for 5 minutes, 100% (vol/vol) ethanol twice for 5 minutes, 85% (vol/vol) ethanol for 1 minute, 70% (vol/vol) ethanol for 1 minute, and distilled water for 1 minute. Protein was prepared as previously described [8]. Deparaffinized tumor sections from each slide were macrodissected and the scrapped tissue was placed into a buffer containing 7 mol/L urea, 2 mol/L thiourea, and 1 mol/L ammonium bicarbonate. Samples were heated for 30 minutes at 95°C followed by 2 hours at 60°C. Proteins were reduced with 10 mmol/L dithiothreitol and alkylated with 25 mmol/L iodoacetamide. Proteins were digested with trypsin (1:20 enzyme/substrate wt/wt) (Sigma-Aldrich, St Louis, MO). Peptide extracts were desalted using C18 Peptide Cleanup Tubes according to the manufacturer's instructions (Agilent Technologies Inc, Wilmington, DE). Peptides were dried in a speed vacuum for 1 hour at room temperature and suspended in 0.1% (vol/vol) trifluoroacetic acid (Sigma-Aldrich). Peptide concentration was determined using the reducing agent compatible bicinchoninic acid protein assay

Table 1 Patient data

	Patient demographics					
	Age (y)			Size (cm)		
	Range	Mean	SD	Range	Mean	SD
LMS	33-89	57.4	14.6	8.6-33.2	13.2	7.9
BLM	27-54	43.2	8.5	0.5-14.5	6.8	4.9
LM	34-50	41.4	7.0	2.6-12	7.2	3.0