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The selected biomarker analysis in 5 types of uterine smooth muscle tumors☆,☆☆,★

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Summary Uterine smooth muscle tumors (USMTs) consist of a group of histologically heterogeneous and clinically diverse diseases ranging from malignant leiomyosarcoma (LMS) to benign leiomyoma (ULM). The genetic alterations in LMS are complex, with some genetic alterations present in both LMS and other atypical histologic variants of USMT. In this study, we reviewed 119 USMTs with a diagnosis of LMS, smooth muscle tumor of uncertain malignant potential, atypical leiomyomas/leiomyoma with bizarre nuclei, and cellular leiomyoma, as well as 46 ULMs and 60 myometrial controls. We selected 17 biomarkers highly relevant to LMS in 4 tumorigenic pathways including steroid hormone receptors (estrogen receptor [ER] and progesterone receptor [PR]), cell cycle/tumor suppressor genes, AKT pathway markers, and associated oncogenes. ER and PR expression was significantly lower in LMS than smooth muscle tumor of uncertain malignant potential, atypical leiomyomas/leiomyoma with bizarre nuclei, cellular leiomyoma, and ULM ($P < .01$). Sixty-five percent of LMSs showed complete loss of ER, and 75% of LMSs showed complete loss of PR. All cell cycle genes were differentially expressed in different types of tumor, but significant overlap was noted. More than 75% of LMSs had Ki-67 index greater than 33%, and only 5% in all other types of USMT. Expression of the selected oncogenes varied widely among different types of USMT. PR positivity and p53 had a borderline association with progression-free survival ($P = .055$ for PR and $P = .0847$ for p53). Furthermore, high PR expression was significantly associated with a longer overall survival ($P = .0163$, hazard ratio 0.198). Cell proliferative indices (Ki-67) and sex steroid hormone receptors were the most valuable markers in differentiating LMS from other USMT variants.

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1. Introduction

Uterine leiomyosarcomas (LMSs) are rare neoplasms representing approximately 1% of all uterine malignancies [1] and 1 in 800 uterine smooth muscle tumors (USMTs). Although the reported 5-year LMS survival rates are variable, these tumors are clinically aggressive with a high risk of recurrence and an overall poor prognosis [2]. The diagnosis and management of LMS can be challenging because we cannot always predict the tumor behavior based on histologic pattern or differentiation and because the definitive diagnosis of LMS from other USMTs with atypical histology (smooth muscle tumor of uncertain malignant potential [STUMP], atypical leiomyoma or leiomyoma with bizarre nuclei [ALM/LM-BN], and cellular leiomyoma [CLM]) can be difficult [3,4]. The new World Health Organization criteria (WHO 2014) provides the current consensus in classifying these tumor variants based on their histology features and clinical behaviors. Therefore, the diagnosis and prognosis for these major tumor types could be significantly facilitated by histologic and molecular biomarker analysis.

Usual-type leiomyoma (ULM) is the most common benign variant of USMT, comprising more than 90% of all USMTs [5]. The cause of this disease entity is thought to be mostly related to single gene mutations/alterations, including *MED12*, *HMGA1/2*, *FH*, and *COL5/6* [6]. Whereas ULMs are more well defined, the molecular features of LMS are less understood and appear to be more complex. Previous studies of the molecular alterations found in LMS have proposed that the common driving pathway may be related to cell cycle, steroid hormones, AKT and RB pathways, and minor metabolites and proteinases [7]. In addition, some oncogenes (c-Kit [8], FASCIN [9], HMGA2 [10], and EGFR [11]) seem to be dysregulated in a portion of LMS. Cross-comparison between LMS and other variants is less clear.

In this study, we collected 119 cases of problematic USMTs, including LMS, STUMP, ALM/LM-BN, and CLM. We selected ULM and myometrium as controls. We then selected 17 immunohistochemical markers thought to be associated with LMS tumorigenesis and evaluated them for each case to determine if any of these markers helps in the diagnosis of difficult USMT cases or provide prognostic significance. The objective of this study is to examine the value of each marker in determining a differential diagnosis and to correlate these findings with clinical outcomes.

2. Materials and methods

2.1. Case selection

We reviewed the pathology database from Northwestern Memorial Hospital at Northwestern University from 1993 to 2013 and identified all patients with a diagnosis of LMS, STUMP, ALM/LM-BN, or CLM. In total, 119 cases were selected for this study (Table 1). In addition, 46 usual-type leiomyoma (ULM) and 60 myometrial samples (20 samples each from LMS, STUMP, and ALM/LM-BN hysterectomy cases) were randomly selected as controls. Each case was reviewed by at least 2 pathologists to confirm the diagnosis based on the Stanford scheme (5) and the 2014 WHO criteria. Each patient medical record was then reviewed, and patient demographics, clinical findings, treatment modalities, tumor recurrence, and patient survival were recorded up to October 2016. Of the 119 cases, 102 cases had additional follow-up data, and the median clinical follow-up was 33 months (range 0-179). The study was approved by the Northwestern University Institutional Review Board.

2.2. Tissue microarrays

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks with the most accurate morphological features were selected for each case, and 2-mm tissue cores were taken to create 4 tissue microarrays. The tissue microarrays were sectioned at 4-μm intervals consecutively for 25 unstained slides. The first 2 and last 2 slides were hematoxylin and eosin stained for quality assurance to confirm the correct tumor types and the presence of viable tumor tissue.

2.3. Immunohistochemistry

Seventeen markers were selected for immunohistochemical (IHC) analysis and included steroid hormone receptors (estrogen receptor [ER] and progesterone receptor [PR]), cell cycle and proliferative gene markers (p16, p53, p21, RB, RBL2, CD24, Ki-67), AKT pathway markers (pAKT, pS6, PTEN, BCL2), and selected oncogenes (c-Kit, FASCIN, HMGA2, and EGFR). (Table 2) [12]. All immunohistochemical staining procedures were performed on a Ventana Nexus automated system. All information regarding selected antibodies is summarized in Supplementary Table 1.

The percent and intensity of each stain were evaluated by 2 pathologists independently. The intensity was scored as negative (0), weak (1+), moderate (2+), or strong (3+), and the percentage of positive tumor cells was scored from 0% to 100%. The results were then semiquantitatively analyzed, with cutoffs at 0%, 1%-9%, 10%-32%, 33%-66%, and greater than 66%. A receiving operator characteristics (ROC) curve and Youden index were then used to identify the best cutoff values between benign (ULM) and malignant (LMS) tumor types. The ROC curve is the true-positive rate (sensitivity) as a function of the false-positive rate (1 − specificity) for the considered range of cutoff values. The Youden index (J) is the difference between the true-positive rate and the false-positive rate. Maximizing this index allows one to find, from the ROC curve, an optimal cutoff point independently from the prevalence. Score multiplied by percent was used as the final semiquantitative score for each case.

2.4. Clinical correlation analysis

A medical record review was performed for the 38 LMS, 8 STUMP, 41 ALM/LM-BN, and 15 CLM cases. Patient and disease characteristics including age, presenting symptoms,