Cluster Analysis of Immunohistochemical Markers in Leiomyosarcoma Delineates Specific Anatomic and Gender Subgroups

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BACKGROUND: Leiomyosarcoma (LMS) can be categorized into uterine, retroperitoneal, nonretroperitoneal soft tissue, cutaneous, visceral, and osseous anatomic subtypes. The differential expression of smooth muscle markers, estrogen receptor (ER), progesterone receptor (PR), and Wilms tumor-1 protein (WT1) by anatomic subtype and gender was explored. **METHODS:** A total of 78 LMS comprised of 30 uterine and 48 nonuterine tumors were studied. Nonuterine tumors were comprised of 17 soft tissue, 16 retroperitoneal, 7 cutaneous, 5 visceral, and 3 osseous subtypes. Immunohistochemical staining intensity on tissue microarray slides was scored as 0, 1+, or 2+, and cluster analysis was performed on the data. **RESULTS:** Smooth muscle actin was the most sensitive antibody (95%), followed by muscle-specific actin (91%), calponin (88%), desmin (73%), caldesmon (66%), and myosin (64%). Caldesmon and myosin were usually coexpressed, and were highest in retroperitoneal tumors (94%). There was no discernable correlation noted between histologic differentiation and smooth muscle marker expression. ER was much more common in women, with the highest frequencies noted in female retroperitoneal (86%) and uterine (63%) tumors. Nuclear WT1 was expressed in 11% of all tumors, and was limited to ER-positive uterine and female retroperitoneal tumors. Cluster analysis segregated 4 groups, most notably 1 driven by ER and PR, with the vast majority being uterine and female retroperitoneal tumors. **CONCLUSIONS:** Smooth muscle markers demonstrated variable sensitivities in LMS, with a tendency for anatomic subtypes to segregate based on expression patterns of these markers. ER defined a subgroup of uterine and female retroperitoneal tumors, and WT1 was limited to such tumors, suggesting a common line of differentiation as well as potential therapeutic targets. **Cancer 2009;115:4186–95. © 2009 American Cancer Society.**

KEY WORDS: leiomyosarcoma, immunohistochemistry, microarrays, cluster analysis, smooth muscle, estrogen receptor, progesterone receptor, Wilms tumor-1 protein (WT1) protein.

Leiomyosarcoma (LMS) is the most common human sarcoma. In addition to being the most common soft tissue[1] and uterine[2] sarcoma, it occurs in visceral organs, arises from muscular veins, and presents as primary skin and bone tumors. The histology, however, varies little among sites of origin. Previous immunohistochemical studies of smooth muscle differentiation in LMS have focused mainly on the sensitivities and specificities of the various antibodies,[3-9] but to our knowledge limited information exists

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regarding the differential distribution of smooth muscle markers by anatomic site. Uterine leiomyomas almost invariably express estrogen and progesterone receptors (ER and PR), yet to our knowledge only a small number of studies published to date have explored ER and PR in uterine LMS and results have been highly variable.[10-13] Even less data exist concerning ER and PR in nonuterine LMS.[14,15] Unlike breast cancer, the prognostic and therapeutic value of ER and PR status in LMS is unproven. Wilms tumor-1 protein (WT1), a transcription factor essential for development of the genitourinary tract,[16] has been detected in both uterine and soft tissue LMS; however, these data are also very limited.[17,18] Preliminary results indicate that WT1 may be an adverse prognostic indicator in soft tissue sarcomas.[17] In addition, WT1 may serve as a potential therapeutic target; clinical trials specifically targeting WT1-positive cancers are currently under development.

Tissue microarray technology coupled with cluster analysis is an efficient method for evaluating large amounts of immunohistochemical data to identify recognizable tumor characteristics.[19,20] Therefore, the objective of the current study was to examine the differential expression of common smooth muscle markers, ER, PR, and WT1 in a large series of uterine and nonuterine LMS using tissue microarrays and cluster analysis to see if discernable expression patterns correlate with anatomic subtype and gender.

MATERIALS AND METHODS

Case Selection

After approval from the University of Michigan Institutional Review Board for human subject research, a search of the pathology database from January 1990 to April 2007 yielded 78 LMS with ample tissue for analysis consisting of 30 uterine and 48 nonuterine tumors. All slides were reviewed and the diagnosis confirmed. The nonuterine LMS were divided into 17 nonretroperitoneal soft tissue, 16 retroperitoneal, 7 cutaneous, 5 visceral (3 colorectal, 1 pulmonary, and 1 laryngeal), and 3 primary osseous tumors. The uterine tumors included 27 primary neoplasms and 3 metastatic tumors from uterine primary tumors. Although the criteria for malignancy in smooth muscle tumors vary depending on uterine versus nonuterine location (in particular, a lower mitotic rate is required in soft tissue primary tumors,[21,22]), all of our cases were unequivocally malignant. Tumors were categorized as well-differentiated, moderately differentiated, or poorly differentiated based on standard criteria.[21] Clinical follow-up data were collected from the University of Michigan Cancer Center registry.

Tissue Microarrays and Immunohistochemistry

Tissue microarray blocks were constructed from 1.0-mm cores of formalin-fixed, paraffin-embedded neoplastic tissue in triplicate with a variety of normal tissues inserted as controls. The slides were stained with a panel of 9 antibodies by standard immunohistochemical technique on an automated Ventana Benchmark XT stainer (Ventana, Phoenix, Ariz). Antibody information with pretreatments and incubation times are listed in Table 1. Intensity of staining was scored as 0, 1+, or 2+. For ER, PR, and WT1, only nuclear reactivity was considered positive.

Cluster Analysis and Outcome Statistics

For cluster analysis, intensity data expressed as plain scores (0, 1, and 2) were arranged in a text delimited file and broadcasted from the DataMatrixViewer (DMV) module of the gaggle software suite (http://gaggle.systemsbiology.org/docs accessed on June 10, 2009) to the MultiExperiment viewer of the TM4 software suite (http://www.tm4.org/mev.html accessed on June 10, 2009). Hierarchical clustering was performed using average linkage analysis with Euclidean distance metric. For outcome data, Kaplan-Meier overall survival plots were constructed comparing ER-positive with ER-negative cases for all tumors as well as specifically for uterine tumors, WT1-positive with WT1-negative tumors, and the 4 major groups (Groups A, B, C, and D) determined by cluster analysis. A P value $\leq .05$ was considered statistically significant.

RESULTS

Demographics

The nonuterine LMS group was comprised of 25 men and 23 women with ages ranging from 32 to 93 years

Table 1. Antibodies and Treatment Conditions

Antibody	Clone	Company	Dilution	Pretreatment
Smooth muscle actin	1A4	Ventana, Tucson, Ariz	Predilute	Buffer at pH 8.0 (60 min)
Muscle-specific actin	HHF35	Dako, Carpinteria, Calif	1:200	Protease
Calponin	CALP	Novocastra, Norwell, Mass	1:100	Buffer at pH 8.0 (60 min)
Desmin	D33	Dako, Carpinteria, Calif	1:10	Buffer at pH 8.0 (30 min)
Caldesmon	hCD	Dako, Carpinteria, Calif	1:100	Buffer at pH 8.0 (60 min)
Myosin	SMMS/1	Ventana, Tucson, Ariz	Predilute	Buffer at pH 8.0 (60 min)
Estrogen receptor	SP1	Ventana, Tucson, Ariz	Predilute	Buffer at pH 8.0 (60 min)
Progesterone receptor	1E2	Ventana, Tucson, Ariz	Predilute	Buffer at pH 8.0 (60 min)
WT1	6F-H2	Dako, Carpinteria, Calif	1:100	Buffer at pH 6.0 (60 min)

WT1 indicates Wilms tumor-1 protein.

(average, 57 years). Twenty-six patients (54%) were still alive after 25 to 304 months (average, 71 months) from the time of the initial diagnosis. Eighteen patients (38%) had died within 6 to 78 months (average, 36 months) from the time of the initial diagnosis. Twenty patients (44%) had documented metastases with an average time to metastasis of 27 months (range, 2 months-62 months). Four patients were lost to follow-up.

The ages of the patients in the uterine LMS group ranged from 34 to 71 years (average, 52 years). Seven patients (23%) were still alive 22 to 93 months (average, 54 months) from the time of the initial diagnosis. Twenty-two patients (73%) had died within 2 to 221 months (average, 52 months) from the time of the initial diagnosis. Fourteen patients (47%) had documented metastases with an average time to metastasis of 33 months (range, 7 months-73 months). One patient was lost to follow-up.

Comparative Outcomes Based on Hormone Receptor and WT1 Status

Overall, ER-positive tumors demonstrated a slight survival advantage over ER-negative tumors ($P = .037$) (Fig. 1A). The median survival was 97 months for ER-positive tumors and 47 months for ER-negative tumors. In addition, when uterine and nonuterine LMS were analyzed separately, ER-positive tumors continued to be associated with a survival advantage ($P = .0002$ for both groups) (Fig. 1B). Among the 9 patients with WT1-positive tumors for whom follow-up was available, 4 patients had died and 5 were still alive, including 3 patients alive with metastases. The median survival was 123 months for WT1-positive tumors and 54 months for WT1-negative tumors. There was no significant difference noted with regard to overall survival (Fig. 1C).

Immunohistochemistry

Smooth muscle markers

Among the muscle markers, smooth muscle actin (SMA) was found to be the most sensitive (95%) in all anatomic subtypes, staining 100% of nonuterine and 87% of uterine tumors. Muscle-specific actin (MSA) and calponin were slightly less sensitive, staining 91% and 88% of all tumors, respectively. Desmin was positive in 73%, caldesmon was positive in 66%, and myosin was positive in 64% of tumors. Although desmin was expressed by all anatomic subtypes, it was found to be most frequent in uterine and retroperitoneal tumors from women (83% and 86%, respectively). Caldesmon and myosin as a pair demonstrated nearly identical staining patterns with the majority of tumors coexpressing both or neither of these markers. Among anatomic subtypes, caldesmon and myosin were most sensitive for retroperitoneal tumors (94%), especially retroperitoneal tumors from women (100%). Nonretroperitoneal soft tissue tumors were least reactive for caldesmon and myosin, with >67% being negative for both markers. There was no correlation noted between histologic differentiation and degree of smooth muscle antigen expression.

ER, PR, and WT1

ER and/or PR were expressed in most categories of tumor except osseous LMS, all 3 cases of which were negative for both receptors. All 7 cutaneous LMS were negative for ER, although 57% demonstrated weak (1+) PR staining. The highest frequencies of ER and PR expression

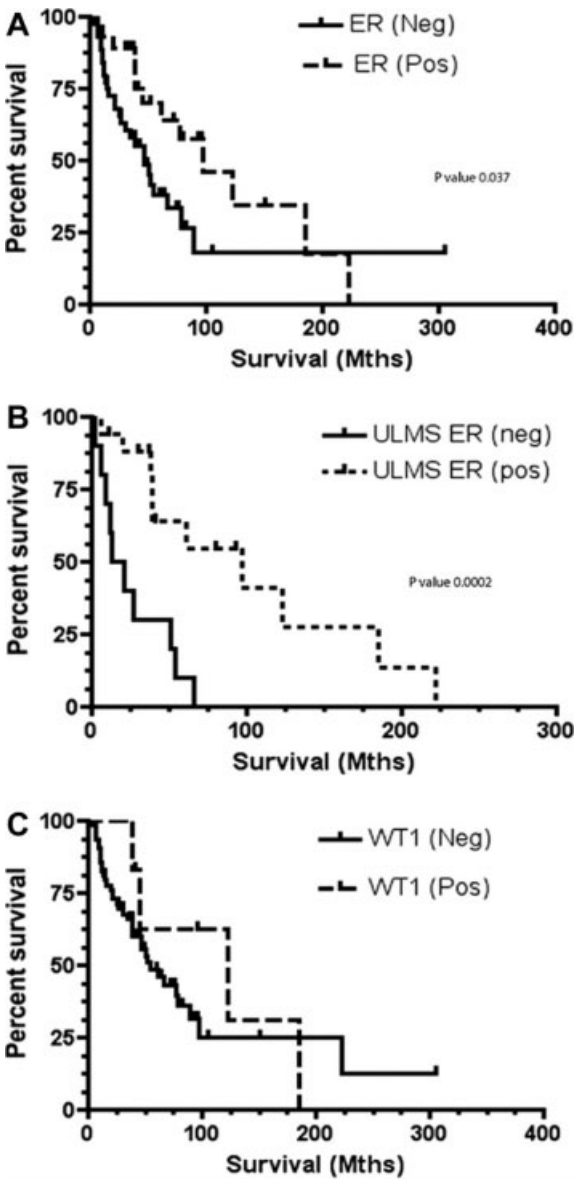

FIGURE 1. Kaplan-Meier overall survival plots are shown comparing estrogen receptor (ER)-positive (Pos) versus ER-negative (Neg) tumors in (A) all cases, as well as specifically in (B) uterine primary cases and in (C) Wilms tumor-1 protein (WT1) -positive versus WT1-negative tumors in all cases. ER-positive leiomyosarcomas (LMS) had a significant overall survival advantage over ER-negative tumors for all tumors ($P = .037$) and for uterine tumors ($P = .0002$). There was no significant difference noted in overall survival when comparing WT1-positive and WT1-negative tumors. ULMS indicates uterine leiomyosarcoma.

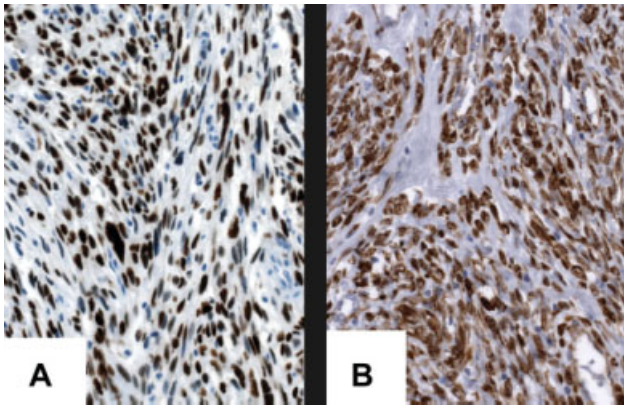

FIGURE 2. (A) Uterine leiomyosarcoma (LMS) with intense (2+) reactivity for estrogen receptor (original magnification × 400) is shown. (B) Uterine LMS with strong nuclear reactivity for Wilms tumor-1 protein (WT1) is shown, a finding that was present exclusively in uterine and retroperitoneal LMS from women (original magnification × 400).

were found in uterine and retroperitoneal tumors from women. Among retroperitoneal tumors from women, 86% were positive for ER and 86% were positive for PR. In uterine LMS, 63% were ER positive (Fig. 2A) and 73% were PR positive. Compared with women, retroperitoneal LMS in men expressed ER and PR only 22% and 33% of the time, respectively, and the staining intensity was always weak. Among nonuterine, nonretroperitoneal LMS, there was also a female predominance in the frequency of ER and PR positivity. For example, 9 of 10 (90%) nonuterine/nonretroperitoneal tumors that expressed ER and 13 of 19 (68%) PR-positive tumors were from women. Overall, among the nonuterine LMS, 15 of 24 (63%) tumors from women expressed ER or PR, whereas only 6 of 24 (25%) tumors from men expressed ER or PR, with the majority (4 of 6) weakly expressing only PR. Only 2 tumors from men, both retroperitoneal tumors, were found to be weakly (1+) positive for ER. WT1 was positive in 11% of all tumors (Fig. 2B). WT1 was noted exclusively in women and was limited to uterine and retroperitoneal tumors (23% and 43%, respectively). In addition, all WT1-positive tumors were found to be positive for ER.

Cluster Analysis

Cluster analysis segregated 4 groups with similar staining patterns (Fig. 3). Group A was a small group

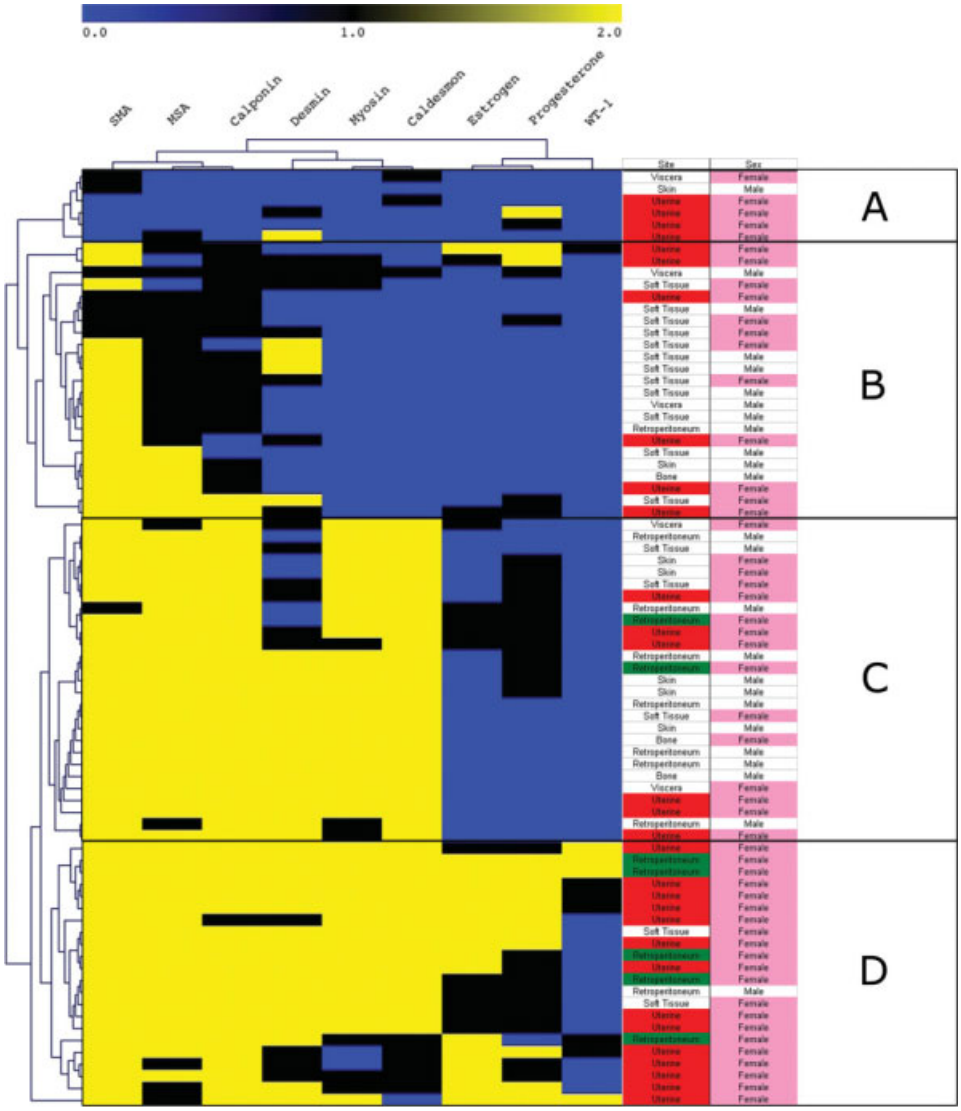

FIGURE 3. A cluster map of immunohistochemical data is shown. Antibodies are arrayed at the top of the map with anatomic subtype and gender set along the right side. Yellow indicates strong (2+) staining intensity, black indicates weak (1+) staining, and blue indicates no staining. Uterine and retroperitoneal leiomyosarcomas (LMS) from women are highlighted in red and green, respectively. Women are highlighted in pink. Four major groupings were identified. Group A was composed of mostly uterine LMS with minimal expression of smooth muscle markers or estrogen receptor (ER)/progesterone receptor (PR). Group B was composed of predominantly nonretroperitoneal soft tissue tumors and others that express the most sensitive smooth muscle markers (smooth muscle actin [SMA], muscle-specific actin [MSA], and calponin), with minimal ER/PR reactivity. Group C was composed of a mixture of uterine and nonuterine LMS that intensely expressed all smooth muscle markers, but very little ER/PR. Group D was composed of nearly all uterine and retroperitoneal LMS from women that intensely expressed most smooth muscle markers and ER/PR. Wilms tumor-1 protein (WT1) was present only in uterine and female retroperitoneal tumors in this group.

(n = 6) comprised of tumors that demonstrated minimal expression of smooth muscle markers. The 4 uterine LMS in this group were negative for SMA, calponin, and myosin. One uterine tumor had only weak caldesmon staining, 1 demonstrated only weak desmin staining, 1 demonstrated only MSA and desmin staining, and 1 was negative for all 6 markers. Two were positive for desmin and negative for SMA, representing the only 2 examples with this immunophenotype in the current study. Two uterine LMS in this group expressed PR, yet all 4 were

negative for ER. One visceral and 1 cutaneous LMS, both with weak SMA staining, also clustered into Group A.

Group B (n = 23) was dominated by nonretroperitoneal soft tissue tumors (52%). There were also 6 uterine tumors (26%). The defining characteristic of this subset of tumors was positive staining for the 3 most sensitive smooth muscle markers (SMA, MSA, and calponin) and very little expression of caldesmon and myosin. In the majority of tumors, SMA staining was intense, whereas MSA and calponin were weakly positive. Desmin was expressed in approximately half of the tumors in this group, most often with weak staining. Three tumors, all uterine LMS, expressed ER. Six tumors were PR positive. A single uterine LMS demonstrated weak reactivity for WT1.

Group C (n = 27) contained various anatomic subtypes, most of which had intense reactivity for all the smooth muscle markers studied. With the exception of 5 tumors that did not stain for desmin, all 6 smooth muscle stains were positive. A defining feature of this group, which distinguished it from Groups A and B, was coexpression of caldesmon and myosin in 100% of tumors. Five tumors (19%) were weakly positive for ER, including 1 tumor from a man. PR was expressed in 12 of 27 tumors (44%), with approximately 75% obtained from women. No WT1 staining was observed. Although all anatomic subtypes were represented, there were a disproportional number of retroperitoneal tumors (33%) in this group.

Group D (n = 22) was comprised almost entirely of women (95%), with the majority of tumors arising from either the uterus or retroperitoneum. Only a single man with a retroperitoneal tumor was included in this cluster. There were 2 nonretroperitoneal soft tissue tumors, both from women. Most tumors demonstrated intense staining for all 6 smooth muscle markers. However, the major defining feature was ER and PR expression (100% and 95%, respectively). WT1 staining was present in 9 of 22 tumors (41%) and was noted exclusively in women and only in uterine and retroperitoneal tumors, a very specific finding that was present in only 1 other tumor outside this group (a uterine LMS in Group B).

The median survival times of these 4 cluster groups were 27 months for Group A, 79 months for Group B, 54 months for Group C, and 97 months for Group D. Comparing the 4 groups in terms of overall survival based on a Kaplan-Meier plot, there were no significant differences noted. However, Group A demonstrated a trend toward a shorter survival compared with Group D ($P = .060$) (Fig. 4).

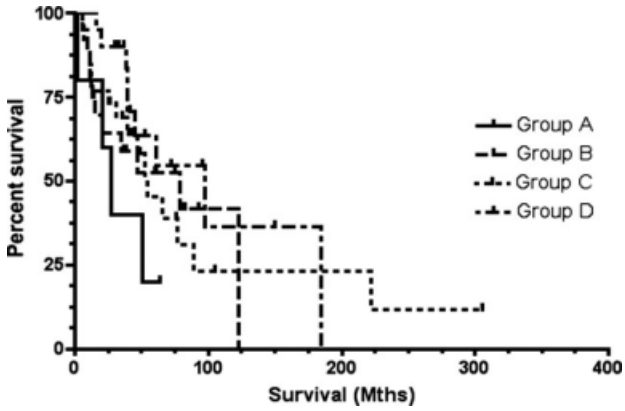

FIGURE 4. (A-D) Kaplan-Meier plot comparing the 4 major groups defined by cluster analysis is shown. There were no statistically significant differences noted in overall survival between any of the groups. However, Group A, the least differentiated by immunohistochemistry, demonstrated a trend toward a worse overall survival compared with Group D, the group with the highest frequency of hormone receptor expression ($P = .060$).

DISCUSSION

Specific tissue substrates have been proposed in the histogenesis of smooth muscle neoplasms such as myometrium for uterine tumors, erector pili apparatus for cutaneous tumors, and muscular veins for retroperitoneal and other deep-seated soft tissue tumors. However, to our knowledge, there is very little information regarding the differential distribution and expression profiles of cellular markers in LMS by anatomic subtype or gender. For example, although a variety of muscle-specific antibodies are routinely used in diagnostic immunohistochemistry, to the best of our knowledge no study published to date has systematically examined the differential distribution of them by site.

Hormone receptor status (ER and PR) is becoming increasingly important in LMS. In addition to serving as a potential therapeutic target, it may have prognostic[11] and diagnostic value as well. For example, it may be helpful for distinguishing uterine and extrauterine LMS in tissue biopsies.[14] However, to our knowledge, only very limited data exist concerning ER and PR expression in either uterine or extrauterine LMS.[10-15] WT1 protein, a

transcription factor essential for the development of the genitourinary system,[16] has been implicated as a marker of müllerian smooth muscle tumors[23] and may have diagnostic utility in this regard. In addition, WT1 is currently under investigation as a target for immunotherapy in hematologic malignancies (http://bethesdatrials.cancer.gov/clinical-research/search_detail.aspx?ProtocolID=NCI-08-C-0051 accessed on June 20, 2009), and thus might prove to have therapeutic value as well. However, to our knowledge, data regarding WT1 in LMS are very limited.[17,18]

Given this background, we evaluated 6 commonly used smooth muscle markers, ER, PR, and WT1 in a large series of LMS from a variety of anatomic sites to determine the spectrum of distribution and whether there are specific expression patterns based on anatomic subtype or gender.

SMA and MSA have been shown to be the most sensitive markers of LMS,[4,5,7,8,24-26] which is consistent with our results. We found 100% of nonuterine LMS to stain for 1 and usually both of these markers, with MSA being slightly less sensitive than SMA. By contrast, not all our uterine tumors stained for SMA and MSA. For example, 4 uterine and 2 nonuterine tumors were negative for both, and this result did not correlate with differentiation. Although lack of staining in these tumors may be explained by limited sampling inherent with tissue microarrays, it certainly reflects that very low levels of actin staining are present in some LMS.

Calponin is another sensitive smooth muscle marker[24-26] and was the third most sensitive marker in the current study, staining 88% of tumors distributed fairly evenly across all anatomic subtypes. Desmin was less sensitive, staining 73% of our tumors. It also stained most anatomic subtypes, but demonstrated the greatest sensitivity for uterine and retroperitoneal tumors. An interesting finding in the current study was coexpression of caldesmon and myosin. Tumors tended to be either positive for both or negative for both, and this result was independent of grade. We found that retroperitoneal tumors almost always coexpressed these markers, whereas nonretroperitoneal soft tissue tumors were least likely. We are aware of only 1 other study of caldesmon and myosin in LMS.[4] In that study, Perez-Montiel et al reported coexpression of caldesmon and myosin in 8 of 8 superficial LMS, which helped distinguish them from myofibroblastic proliferations. We are not aware of any prior study of uterine or retroperitoneal LMS.

To the best of our knowledge, published reports of ER and PR in LMS are limited and have focused primarily on uterine tumors, with only 2 studies including nonuterine tumors.[14,15] In uterine LMS, wide ranges of ER and PR frequencies have been reported, varying from 21% to 87% for ER and 20% to 80% for PR.[11,13-15,27,28] In the current study, uterine tumors were positive for ER in 63% and positive for PR in 73%. In nonuterine LMS, Rao et al found only 2 of 16 tumors to be ER positive, both of which were from women,[14] whereas Kelley et al found ER staining in 4 of 16 tumors, 3 of which from women.[15] Our results in nonuterine tumors demonstrated a similar percentage of cases expressing ER at 23%. Although the rate of PR expression in nonuterine tumors in the current study was higher than that reported by others at 40%, the majority of tumors in the current study were only weakly positive. As seen in Table 2 and Figure 3, ER and PR were highest in uterine and retroperitoneal LMS from women, a defining feature of Group D in the cluster analysis. LMSs from men rarely express ER, as evidenced by only 2 weakly positive tumors in the current study.

The prognostic significance of hormone receptor status in LMS is still indefinite, limited to only a small number of studies with opposing results.[10,11,13] In the current series, we found survival advantage for ER positivity in both uterine and nonuterine tumors. However, we were not able to compare these parameters with age, disease stage, and treatment in all our cases. More conclusive results might be brought out in a prospective study. However, the preliminary results of the current study suggest that ER positivity may indicate a more favorable prognosis in LMS. In addition, it may be a potential therapeutic target for tamoxifen, selective ER modulators, and aromatase inhibitor treatments. As an example, Hardman et al reported a patient with widely metastatic uterine LMS expressing ER and PR who was treated with anastrozole, an aromatase inhibitor, and had a dramatic decrease of her disease after 1 year.[29]

To our knowledge, very little has been reported to date regarding WT1 in LMS. Recently, Coosemans et al examined 38 uterine LMS by immunohistochemistry and found 76% were positive for WT1.[18] However, the

Table 2. Immunohistochemical Profiles of Leiomyosarcoma Subtypes by Percentage of Positive Tumors

	Retroperitoneum						
	Uterine (n=30)	Women (n=7)	Men (n=9)	Soft Tissue (n=17)	Skin (n=7)	Viscera (n=5)	Bone (n=3)
Smooth muscle actin	87	100	100	100	100	100	100
Muscle-specific actin	87	100	100	94	86	80	100
Calponin	83	100	100	88	86	80	100
Desmin	83	86	67	71	57	60	67
Caldesmon	67	100	89	29	71	80	67
Myosin	63	100	89	35	71	60	67
Estrogen receptor	63	86	22	12	0	20	0
Progesterone receptor	73	86	33	29	57	20	0
WT1	23	43	0	0	0	0	0

WT1 indicates Wilms tumor-1 protein.

staining pattern was exclusively cytoplasmic and not nuclear. They also performed Western blot analysis for WT1 protein in 3 tumors and found it in the cytoplasmic fraction. By contrast, in the current study, we observed true nuclear expression of WT1 in 11% of our tumors. Of interest, nuclear WT1 staining was present only in women, and only in ER-positive retroperitoneal and uterine tumors. Thus, nuclear WT1 expression appears to define a very specific subset of tumors from women, which suggests a common line of differentiation, possibly müllerian. With regard to prognosis, Coosemans et al found no correlation between WT1 expression and clinical outcome. Similarly, in our study we were unable to find survival advantage based on WT1 status. However, the number of WT1-positive tumors in the current study was quite limited. In a study of soft tissue sarcomas, Sotobori et al[17] found elevated levels of WT1 transcript in 4 of 5 LMS. Although they suggested WT1 as an adverse prognostic factor in soft tissue sarcomas, they did not analyze LMS separately. Thus, there are not enough data at this time to determine the prognostic value of WT1 in LMS. More importantly, however, with advances in immunotherapy, WT1 might serve as a novel therapeutic target, as we discussed.

The differential distribution of markers by anatomic subtype and gender was highlighted by cluster analysis. For example, 1 group (Group A) was characterized by a small number of tumors that demonstrated low frequency expression of all smooth muscle markers, consisting mostly of uterine LMS. Another group (Group B) was characterized by expression of only the most sensitive smooth muscle markers (SMA, MSA, and calponin) dominated by nonuterine/nonretroperitoneal LMS. Another group (Group C) was driven by coexpression of caldesmon and myosin in addition to the other smooth muscle markers and had the largest proportion of retroperitoneal LMS among the 4 clusters. Finally, 1 group (Group D) was driven by ER and PR expression and contained mostly uterine and retroperitoneal tumors. This group was the most homogenous in terms of anatomic site and gender because all but 2 tumors were from the uterus or retroperitoneum and all but 1 of the patients were women. In addition, WT1 staining was largely limited to this group with only 1 tumor, a uterine LMS, clustering outside this group. There was no discernable correlation noted between antigen expression and histologic differentiation. Finally, although there were no statistically significant differences in the 4 groups with regard to overall survival, there was a trend for the least immunohistochemically differentiated tumors (Group A) to have a lower survival rate compared with the tumors with the highest frequencies of hormone receptor expression (Group D) ($P = .060$).

By examining a large, diverse group of LMS efficiently through the use of tissue microarrays, immunohistochemistry, and cluster analysis, several recognizable patterns emerge. In the current series, cluster analysis defined 4 relatively distinct groups based on varied expression profiles. We observed variable expression patterns for smooth muscle markers that trend toward certain anatomic subtypes. We observed that caldesmon and myosin are usually coexpressed in a given tumor with the highest

rate of expression in retroperitoneal tumors and that non-retroperitoneal soft tissue tumors are least likely to express these markers. We demonstrated that ER and PR expression defines a distinct subgroup of uterine and retroperitoneal LMS almost exclusively in women, which, along with WT1 expression, suggests a common line of differentiation. In conclusion, despite essentially identical histologic features independent of site, LMS appears to represent a heterogeneous group of related neoplasms defined by anatomic subtype, gender, and immunophenotype.

Conflict of Interest Disclosures

The authors made no disclosures.

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