Progressive Loss Of Myogenic Differentiation In Leiomyosarcoma Has Prognostic Value

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Abstract

Aims—Well-differentiated leiomyosarcoma show morphologically recognizable smooth muscle differentiation, while poorly differentiated tumors may form a spectrum with a subset of undifferentiated pleomorphic sarcomas. Expression of certain muscle markers has been reported to have prognostic impact. We investigated the correlation between morphologic spectrum and muscle-marker expression profile of leiomyosarcoma and the impact of these factors on patient outcomes.

Methods and Results—Tissue microarrays including 203 non-uterine and 181 uterine leiomyosarcoma with a spectrum of tumor morphologies were evaluated for expression of immunohistochemical markers of muscle differentiation. Poorly differentiated tumors frequently lost one or more conventional smooth muscle markers (smooth muscle actin, desmin, h-caldesmon and smooth muscle myosin (p<0.0001)), as well as more recently described markers SLMAP, MYLK and ACTG2 (p<0.0001). In primary tumors, both desmin and CFL2 expression predicted improved overall survival in multivariate analyses (p=0.0111 and p=0.043, respectively). Muscle-marker enriched tumors (expressing all 4 conventional markers or any 3 of ACTG2, CFL2, CASQ2, MYLK, and SLMAP, had improved overall survival (p<0.05) in univariate analyses.

Conclusions—Morphologically and immunohistochemically, poorly differentiated leiomyosarcoma can masquerade as undifferentiated pleomorphic sarcoma with progressive loss

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of muscle markers. Expression of muscle markers has prognostic significance in primary leiomyosarcoma independent of tumor morphology.

Keywords

Leiomyosarcoma; differentiation; immunohistochemistry; prognosis; desmin

Introduction

Leiomyosarcomas account for up to 20% of all adult soft tissue sarcomas,[1–7] and are characterized by an aggressive clinical course and relative insensitivity to cytotoxic chemotherapy.[4, 5] Leiomyosarcomas demonstrate smooth muscle differentiation, and are typically subclassified for diagnostic and therapeutic purposes as uterine and non-uterine (somatic) origin. Uterine leiomyosarcoma is distinguished from the more common myometrial leiomyomas, by a combination of mitotic activity at least 5/10 high power fields in the presence of necrosis and cellular atypia, or mitotic activity >10/10 high power fields in the absence of necrosis or atypia.[8–11] In somatic tissues outside of the dermis or gastrointestinal tract, leiomyomas are extremely rare. Thus, smooth muscle tumors with any mitotic activity are generally considered to be leiomyosarcoma, and commonly graded using the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) criteria.[6]

Well-differentiated leiomyosarcomas demonstrate a growth pattern of perpendicularly-intersecting cellular fascicles composed of elongated spindle-shaped tumor cells with abundant, brightly eosinophilic, fibrillary cytoplasm. Nuclei generally have an elongated, blunt-ended oval "cigar-like" shape. Variable numbers of markedly pleomorphic "monster" cells may be seen. In poorly differentiated tumors, classic features may be focal or difficult to appreciate as the proportion of pleomorphic cells increases. Additional morphologic variants include epithelioid and myxoid types, which are more commonly associated with uterine origin. While the majority of cases can be diagnosed on biopsy based on classic histologic features on H&E stained sections, morphologic variants, poorly differentiated tumors, and those with unusual features may be difficult to distinguish from other sarcomas and may require additional immunohistochemical evaluation. In anaplastic variants, or extremely poorly differentiated tumors, myogenic markers may be lost.[12]

Commonly used markers of muscle differentiation in clinical practice include desmin, seen in all types of muscle, smooth muscle actin (SMA), most commonly present in smooth muscle or myofibroblasts, h-caldesmon and smooth muscle myosin (SMMS), both of which are relatively specific for smooth muscle. More recently, expression profiling studies identified an additional set of muscle markers, including smooth muscle gamma actin (ACTG2, typically expressed in enteric smooth muscle), calsequestrin 2 (CASQ2, cardiac and skeletal muscle), human muscle cofilin 2 (CFL2, skeletal muscle), myosin light chain kinase (MYLK, smooth muscle) and sarcolemmal membrane associated protein (SLMAP, all muscle types) as being associated with improved outcomes when 3 or more were co-expressed – so called "muscle-enriched" leiomyosarcomas.[13]

Few studies have investigated the utility of muscle marker expression as a more objective measure of differentiation status compared to histologic appearance on H&E sections. We

therefore queried how well muscle marker expression correlated with histologic assessment of tumor differentiation, and if muscle marker expression could independently predict tumor behavior and survival outcomes in primary leiomyosarcoma.

Materials and Methods

Patents and tumor tissues

Acquisition of tissue specimens and clinical information and subsequent analyses were approved by the Institutional Review Board (IRB) of The University of Texas M. D. Anderson Cancer Center (UTMDACC).

Tissue microarray construction

All available formalin-fixed, paraffin-embedded leiomyosarcoma specimens collected between 1993 and 2010 were retrieved from UTMDACC pathology archives. Hematoxylin and eosin (H&E)-stained sections were reviewed to confirm the diagnosis, define areas of viable tumor, and select one or more areas (if there was morphologic variability) for inclusion in tissue microarrays. Extremely poorly differentiated tumors or those that had heterologous elements were only included in the study if they had a prior documented history leiomyosarcoma with at least focal typical immunohistochemical and/or morphologic features.

An automated tissue microarray apparatus (ATA-27, Beecher Instruments, Sun Prairie, WI) was used to obtain and format paired 1.2 mm punch samples from each case into recipient blocks.[14] H&E staining of 4 μm tissue microarray sections was used to verify all samples. Individual cores on the tissue microarray were screened by an experienced soft tissue pathologist and classified by differentiation score as 1) well, 2) moderately or 3) poorly differentiated (Figure 1).[6] Tumors with myxoid or epithelioid morphology were considered to be moderately and poorly differentiated, respectively. Only the most poorly differentiated pair of cores from each case was considered in data analysis.

Clinicopathologic Data Collection

Patient and tumor variables, including age, gender, tumor site, size, and disease status were recorded. Sites of primary tumors were categorized thusly: uterine; vascular (apparently arising from or extensively invading a large caliber vein); retroperitoneal/pelvic (not clearly arising in association with a specific intra-abdominal organ or vascular structure); extremity (leg or arm, including dermal tumors); trunk (chest wall, superficial abdominal wall, back, paraspinal); uterine; or other miscellaneous sites (including viscera, bone and head and neck).

Outcome data including survival and disease recurrence were tabulated for primary tumors only. Complete FNCLCC grading criteria (mitotic figures / 10 high power fields, percent necrosis) were not tabulated because these were not equivalently applicable in primary uterine leiomyosarcoma.

Immunohistochemical analysis

Immunohistochemical staining was performed on 4-μm-thick tissue microarray sections using an automated stainer (Dako, Carpinteria, CA) following the manufacturer's instructions, and commercially available antibodies (Table 1). Positive and negative controls were run in parallel. Sections were counterstained with hematoxylin. SMA, desmin, SMMS, and h-caldesmon were scored as absent (0), focal (<10%) or diffuse (10%). ACTG2, CASQ2, CFL2, MYLK and SLMAP were scored as completely absent (0), or by intensity if any staining present as weak (1) or strong (2). For the purposes of outcome analysis, only diffuse/strong staining was considered positive. Muscle enriched tumors were defined as those expressing 3 or more of: ACTG2, CASQ2, CFL2, MYLK and SLMAP.

Statistical methods

Associations between histopathological features and immunophenotype were examined using Fisher's exact test or χ^2 test, with an alpha of p 0.05 (adjusted to p 0.025 for multiple comparisons) considered significant. For primary tumors only, the method of Kaplan and Meier was used to assess outcomes. Univariable and multivariable Cox proportional hazards regression models were used to estimate the association between histopathologic features or biomarker expression with overall survival (OS), disease-specific survival (DSS) and time to first metastasis, with alpha of 0.05 considered as significant.

Results

Leiomyosarcoma characteristics

Tumors included 384 leiomyosarcoma from 257 patients, including 198 women and 59 men. Primary sites of origin included the uterus (41%), retroperitoneum/pelvis (19%), large vessels (16%), extremities (11%), trunk (3%), and miscellaneous sites (9%). Vascular leiomyosarcoma predominately arose in the inferior vena cava and renal vessels, with a few cases originating from other deep veins. Tumors in the head and neck arose in the nasal cavity, sinuses and mandible, while organs giving rise to leiomyosarcoma included lung/bronchus, bowel, bladder, liver and penis. Extremity tumors included 4 leiomyosarcomas of dermal origin. There were 98 primary leiomyosarcomas, 93 local recurrences (including both those recurring within a prior surgical site and intra-abdominal spread) and 192 distant metastases. The median size of primary leiomyosarcomas was 8 cm (range 0.7 to 30 cm).

When tumors were classified by their microarray core with the least-differentiated histology, there were 130 (34%) well-differentiated leiomyosarcomas, 170 (45%) moderately differentiated and 82 (21%) poorly differentiated. Loss of differentiation correlated with disease progression (p<0.0001), with primary tumors being more frequently well differentiated (53%) than either recurrent (30%, p=0.0013) or metastatic tumors (26%, p<0.0001) (Figure 2). There was no significant further loss of differentiation between recurrent and metastatic tumors.

Muscle marker expression

Smooth muscle markers SMA and ACTG2 were the most frequently expressed (91% and 90% of cases, respectively), while striated muscle marker CASQ2 was least prevalent (18%

of cases) (Table 2). In sum, 235/377 (62%) tumors expressed all 4 conventional muscle markers, and 292 (77%) expressed 3 or more, while 56 (15%) expressed only one or no markers. Similarly, muscle-enriched phenotype was seen in 298/357 cases (83%).

Expression of muscle markers correlated with morphologic differentiation (Figure 3). Expression of all four conventional muscle markers decreased from 82% (106/129) in well to 60% (100/167) in moderate to 36% (24/81) in poorly differentiated tumors (p<0.0001). The most dramatic loss of expression was seen in SMMS, which was expressed in 118/130 (91%) well differentiated tumors, 133/169 (79%) moderately differentiated tumors and only 38/81 (47%) poorly differentiated, while SMA was retained at the highest levels, being present in 129/130 (99%) of well-differentiated, 158/170 (93%) moderately differentiated and 60/82 (73%) poorly differentiated tumors. Statistically significant loss of expression of SLMAP, ACTG2, MYLK, and COFL2 was also seen as tumors became more poorly differentiated.

Prognostic utility of differentiation markers in primary leiomyosarcoma

Clinical follow-up was available for 97 primary tumors, and ranged from 2.6-258 months (median 50). Five- and 10-year OS were 54% and 33%, respectively, while 5-and 10-year DSS were 60 % and 41% (Figure 4a). Four patients had distant metastases at the time of diagnosis, while 52/97 patients developed metastases during the course of follow-up. Of these 52 patients, median time to development of first metastasis after primary resection was 15 months (range 8–167 months).

There was no difference in outcome by sex or site (uterine vs. somatic). Primary tumor size >5 cm was associated with poor OS and DSS but not time to first metastasis (Tables 3–5). Histopathological differentiation status alone was a modest predictor of clinical outcomes (Figure 4b). Poor differentiation predicted a worse OS compared to well-differentiated tumors (HR, 3.281 [1.311–8.212], p=0.0112), and trended to shorter time to first metastasis (p=0.0587), but was not predictive of DSS (p=0.0897). There was no significant difference in outcome between well- and moderately-differentiated tumors.

Muscle enriched primary leiomyosarcoma predicted improved OS in univariate analysis (HR 0.4177 [0.1763–0.9897], p=0.0473), as did expression of all 4 conventional smooth muscle (HR 0.4887 [0.2711–0.8809], p=0.0172). As individual markers, CFL2, desmin, and h-caldesmon all associated with improved survival (p=0.0049, p=0.0003, and p-0.0177, respectively) (Table 3). The small sample size limited multivariate analyses of OS, and only the 2 known risk factors (tumor size, differentiation score) and 2 muscle markers (desmin and CFL2) were included in the model. All 4 retained independent prognostic significance.

Significant prognostic biomarkers for DSS were similar to those of overall survival (Table 4). Loss of any one of the conventional smooth muscle markers increased risk (HR 2.5031 [1.3078–4.7916]; p=0.0056), while muscle-enriched tumors trended to improved DSS (p=0.0535). Both CFL2 and desmin were associated with decreased risk (p=0.0076 and p<0.0001), while expression of h-caldesmon trended to be protective (p=0.061) in univariate analyses. In multivariate analysis (including size, expression of all 4 conventional muscle

markers, CFL2 and desmin expression in the model), only size >5 cm retained prognostic significance.

Desmin was the only significant predictor of time to first metastasis in univariate analysis (HR 0.46343 [0.2407–0.8957]; p=0.0221).

Discussion

Although immunohistochemistry is frequently used to support a diagnosis of leiomyosarcoma, few studies have specifically addressed the correlation between morphologic evidence of smooth muscle differentiation and immunohistochemical markers. We examined muscle marker expression in a diverse array of leiomyosarcomas to better understand the patterns of marker loss over the course of disease progression (from well- to poorly differentiated tumors, and from primary to metastatic disease). Of particular interest was whether immunohistochemical markers of muscle differentiation could provide a more objective measure of tumor differentiation than morphology in predicting patient outcomes.

When we reviewed the leiomyosarcoma tissue cored on our tissue microarray, we identified a number of poorly differentiated cores which showed few, if any, classic features of leiomyosarcoma and which could easily have been mistaken for undifferentiated pleomorphic sarcoma if examined out of context. These cores demonstrated a significant loss of markers of smooth muscle differentiation compared to well and moderately differentiated cores. While Carvalho *et al.*, reported no correlation of muscle marker expression (SMA, desmin, caldesmon, calponin, and myosin) with histologic differentiation in a series of 78 cases,[15] other studies report similar finding as ours, including retention of diffuse SMA in poorly differentiated tumors, with loss of both desmin and caldesmon (59% vs. 83% and 19% vs. 68%, respectively), compared with well-to-moderately differentiated tumors,[16–18] and loss of smooth muscle marker expression in the less-differentiated areas of pleomorphic and "dedifferentiated" variants of leiomyosarcoma.[12, 19, 20]

Not only were individual muscle markers frequently lost in poorly differentiated tumors in our series, but the overall total number of muscle markers was reduced, with less than half of poorly differentiated tumors expressing 3 or more of SMA, desmin, h-caldesmon and SMMS, compared to over 90% of well differentiated tumors. This is consistent with reports that myosin and caldesmon expression are frequently co-expressed.[15] Similarly to Mills *et al.*,[21] we found that poorly differentiated "undifferentiated pleomorphic sarcoma-like" leiomyosarcoma less frequently expressed a muscle-enriched phenotype—that is, positivity for 3 or more of CASQ2, SLMAP, CFL2, MYLK, and ACTG2, with only 62% of poorly differentiated tumors falling into this category, compared to 98% of well-differentiated.

A significant proportion of so-called undifferentiated pleomorphic sarcomas (UPS, formerly termed malignant fibrous histiocytoma) are likely related to leiomyosarcoma and indeed may represent anaplastic ("dedifferentiated") leiomyosarcoma. In multiple studies using protein expression analyses,[22–26] gene expression analysis[27–30] and/or comparative genomic hybridization,[31] a subset of UPS consistently cluster with leiomyosarcoma, while up to 5% of UPS demonstrate a muscle enriched phenotype.[21] These studies suggest that a subset of

UPS represent a form of tumor progression from leiomyosarcoma.[26, 27, 31, 32] Effectively differentiating between the two classes may have implications for prognostication and therapeutic selection as we more fully understand disease biology.

In our series, desmin and CFL2 were associated with improved OS independent of histologic differentiation or tumor size. Further analysis of desmin expression demonstrated that the impact of this marker in predicting outcome appeared to rest mainly in its ability to segregate moderately differentiated tumors into 2 groups- those that behaved like well-differentiated tumors and those which behaved like poorly differentiated tumors (Figure 4c and d). We also confirmed, in univariate analyses, the reported association of muscle-enriched phenotype with improved outcome. Of note, our study was underpowered for robust analysis of DSS, due to limited available clinical follow-up. However, in general, prognosticators of OS trended to predict DSS, implying that tumor-related deaths may have been more frequent in our cohort than we were able to confirm.

While we found that increased muscle marker expression predicted improved outcomes in leiomyosarcomas, other studies have found that UPS expressing muscle markers behave more aggressively than those without.[22, 33–35] Thus, we are left with the seeming paradox that evidence of myoid differentiation is a positive predictor in leiomyosarcomas, and negative one in true UPS. This contradiction merits further study and reinforces the need to carefully evaluate biomarkers only in context with morphology.

In summary, we assessed the diagnostic and prognostic utility of an array of muscle markers in one of the largest cohorts of uterine and non-uterine leiomyosarcoma to date. We demonstrated that morphologically less-well-differentiated leiomyosarcoma are associated with loss of markers of muscle differentiation, thereby reducing the diagnostic value of immunohistochemistry in biopsies of such cases. Moreover, our findings are congruent with the theory that a subset of UPS represent pleomorphic or very poorly differentiated variants of leiomyosarcoma. Lastly, we identified desmin as an independent predictor of survival, which seems to be of most value in moderately differentiated tumors. Taken together our findings help to more clearly delineate patterns of muscle expression in leiomyosarcoma, which may help to improve diagnostic algorithms, and provide evidence for overlooked prognostic significance of everyday immunohistochemical assays.

Acknowledgments

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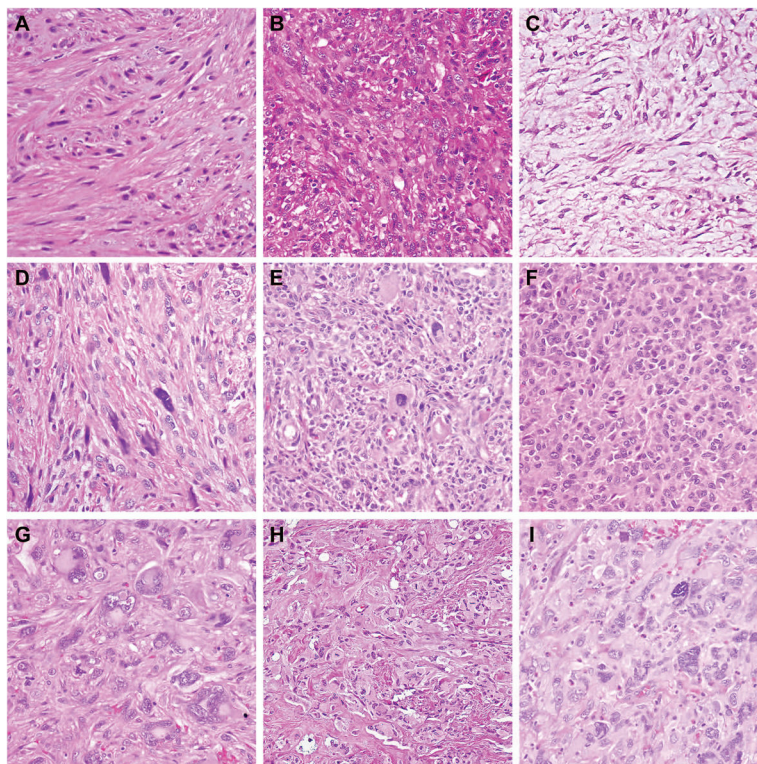
Figure 1. Morphologic Variation in Leiomyosarcoma

A) Well differentiated tumor demonstrating intersecting fascicles of slightly atypical eosinophilic spindle cells. B) Moderately differentiated tumor with nuclear variability and increased disorganization of fascicles. C) Moderately differentiated myxoid tumor with bland spindle cells in a storiform to fascicular arrangement. D) Moderately differentiated tumor with scattered "monster cells." E) Poorly differentiated tumor with loss of fascicular architecture, and increased rounded to epithelioid cells. F) Poorly differentiated tumor with epithelioid features. G) Poorly differentiated tumor showing therapy effect and marked nuclear pleomorphism. H) Poorly differentiated tumor with pleomorphism, loss of architecture, and numerous mitoses. I) Poorly differentiated "undifferentiated pleomorphic sarcoma-like" tumor showing storiform growth, marked pleomorphism and scattered inflammatory infiltrate. (All panels at 200X).
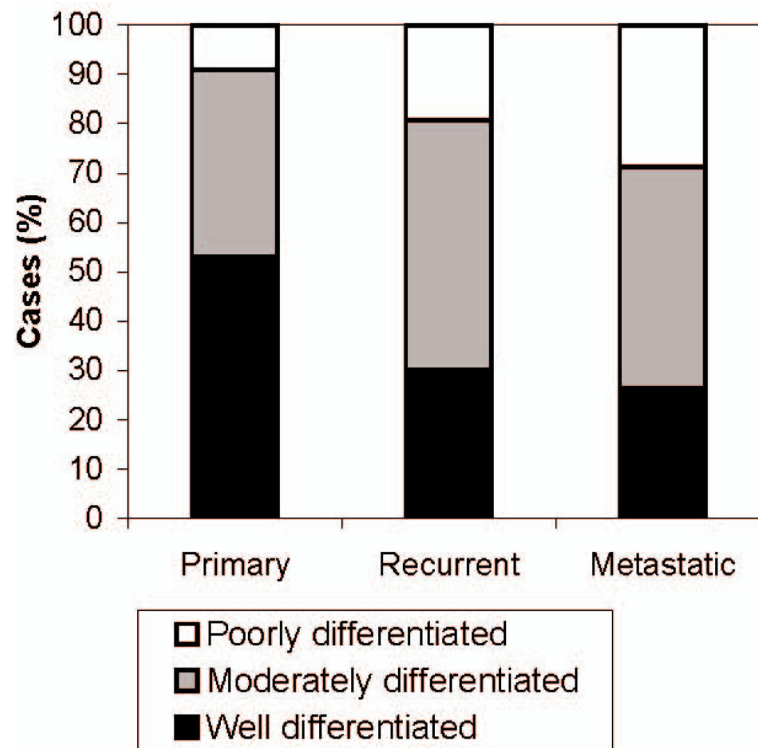
Figure 2. Histologic Features of Leiomyosarcoma by Site and Stage

Tumor status correlates with differentiation. Primary tumors are most frequently well-differentiated (53%) compared to recurrence or metastases (30%, p=0.0013 and 26%, p<0.0001 respectively). Poorly differentiated tumors are most commonly metastases (29%) compared to 19% recurrences (p=0.087), and 9% of primaries (p=0.0001).
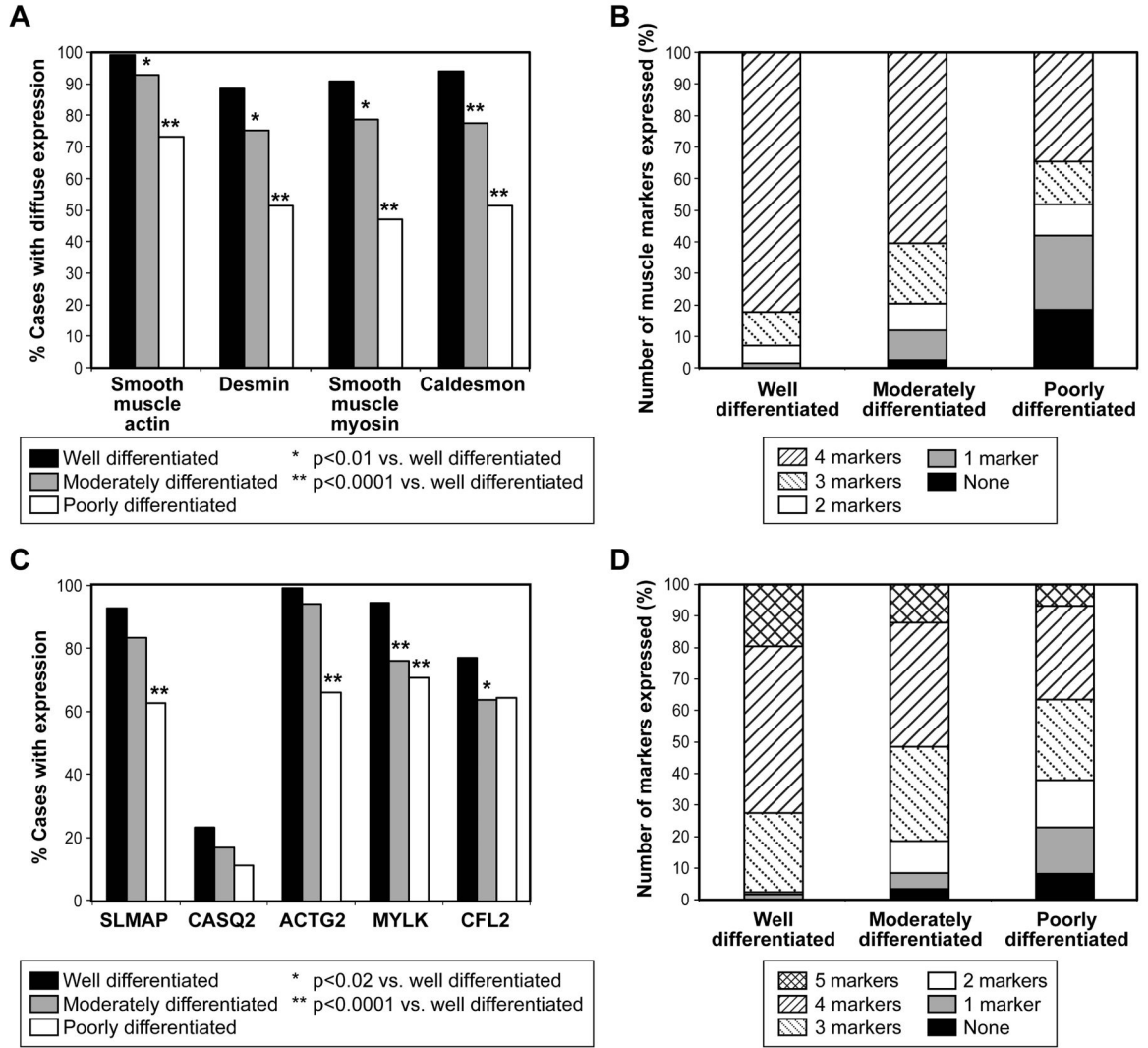
Figure 3. Expression of Markers of Smooth Muscle Differentiation in Leiomyosarcoma
A) Diffuse expression of smooth muscle actin, desmin, smooth muscle myosin, and caldesmon are reduced in less-well-differentiated tumors. B) Total number of conventional muscle markers with diffuse expression is reduced in more poorly differentiated tumors, p<0.0001. C) Expression of SLMAP, CASQ2, ACTG2, MYLK and CFL2 is less frequent in poorly differentiated tumors. D) Muscle-enriched phenotype (3 or more of SLMAP, CASQ2, ACTG2, MYLK, CFL2) is less frequent in more poorly differentiated tumors (p=0.0089).
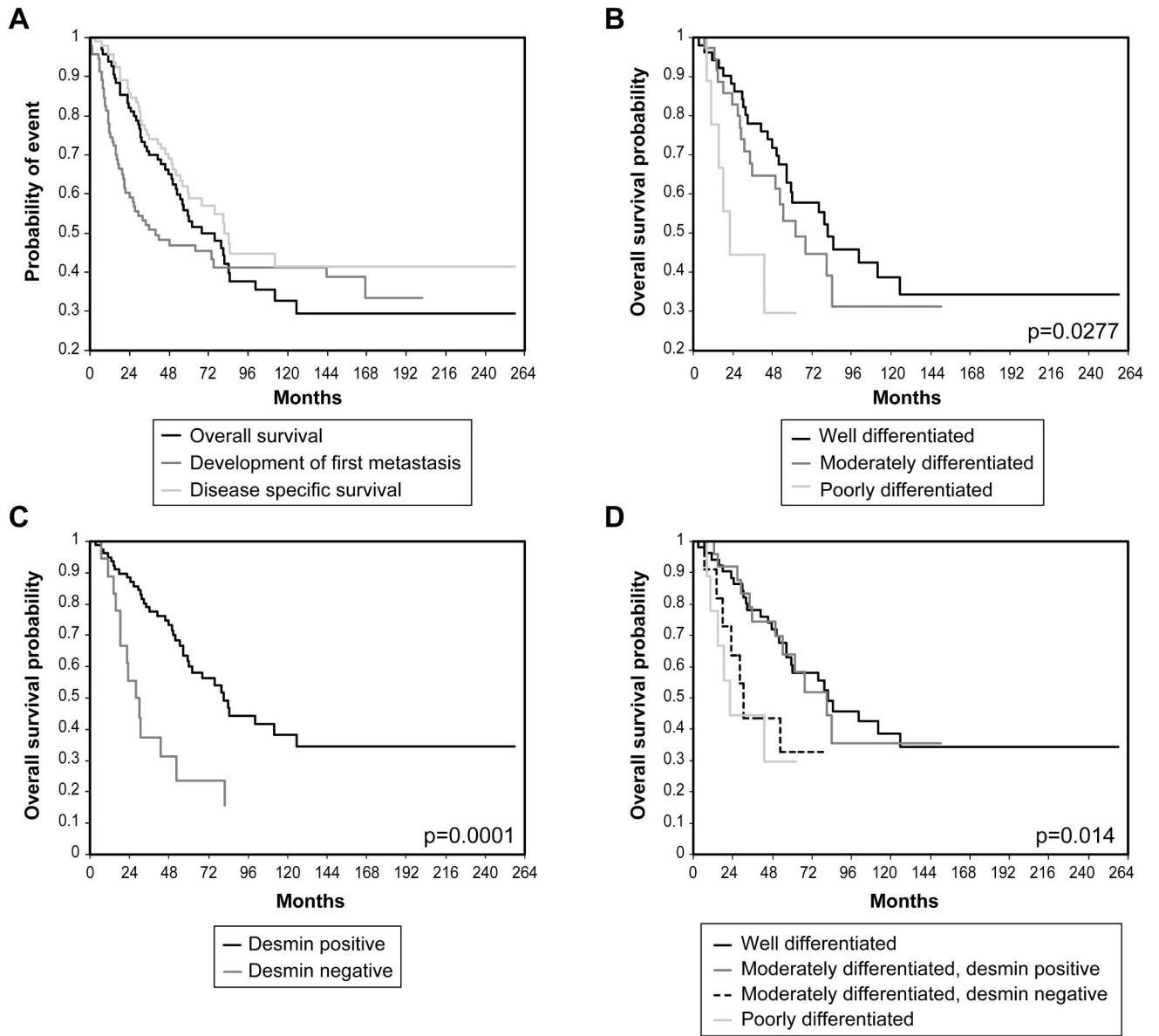
Figure 4. Survival Outcomes in Primary Leiomyosarcoma
A) Overall survival, disease-specific survival and time to development of first metastasis for entire cohort. B) Patients with well-differentiated leiomyosarcoma have improved overall survival than those with poorly differentiated tumors. C) Expression of desmin correlates with improved overall survival. D) Moderately differentiated tumors expressing desmin have identical survival to well differentiated tumors, while moderately differentiated tumors without desmin behave like poorly differentiated tumors.

Table 1

Antibodies used for immunohistochemistry

Marker	Catalog #	Company	dilution
Smooth muscle actin	A2547	Sigma, St Louis, MO	1:80,000
Desmin	M0760	Sigma	1:200
Caldesmon	M3557	Dako, Carpenteria, CA	1:50
Smooth muscle myosin	IR066	Dako	1:200
Smooth muscle gamma actin (AC TG2)	H00000072-A01	Novus Biologicals, Littleton CO	1:2,000
Calsequestrin 2 (CASQ2)	GTX90833	GeneTex Inc, Irvine, CA	1:100
Human muscle cofilin 2 (CFL2)	GTX92818	GeneTex Inc	1:100
Myosin light chain kinase (MYLK)	GTX91044	GeneTex Inc	1:25
Sarcolemmal associated protein (SLMAP)	GTX94163	GeneTex Inc	1:2,500

Table 2

Muscle marker expression by differentiation status.

	Total (n=384)	Well differentiated (n=131)	Moderately differentiated (n=170)	Moderately vs. well-differentiated (p value)	Poorly differentiated (n=82)	Poorly vs. well-differentiated (p value)	Poorly vs. moderately differentiated (p value)
SMA	91%	99%	93%	0.0082	73%	<0.0001	<0.0001
Desmin	74%	89%	75%	0.0045	50%	<0.0001	<0.0001
SMMS	76%	91%	79%	0.0065	47%	<0.0001	<0.0001
Caldesmon	78%	94%	78%	0.00013	51%	<0.0001	<0.0001
SLMAP	83%	93%	84%	0.028	63%	<0.0001	0.003
CASQ2	18%	23%	17%	0.24	12%	0.044	0.34
ACTG2	90%	99%	94%	0.047	66%	<0.0001	<0.0001
CFL2	69%	77%	64%	0.0196	64%	0.0537	1
MYLK	82%	95%	77%	<0.0001	71%	<0.0001	0.42

Table 3

Overall survival, univariate and multivariate

Variable	Level	N	# all cause deaths	Hazard Ratio	95% CI Lower	95% CI upper	P- Value
Univariate analyses							
Actin, smooth muscle	>10% pos	96	51	1.2191	0.1673	8.8841	0.845
Caldesmon	**>10% pos**	**97**	**52**	**0.4154**	**0.2011**	**0.8583**	**0.0177**
Desmin	**>10% pos**	**97**	**52**	**0.3058**	**0.1617**	**0.5783**	**0.0003**
Myosin, smooth muscle	>10% pos	95	51	0.7951	0.3867	1.635	0.5331
Standard muscle markers	**all 4 markers expressed**	**94**	**50**	**0.4721**	**0.2576**	**0.865**	**0.0151**
ACTG2	strong, diffuse	96	51	1.8255	0.2507	13.2914	0.5524
CASQ2	strong, diffuse	96	51	0.8104	0.4433	1.4816	0.4946
CFL2	**strong, diffuse**	**91**	**48**	**0.4191**	**0.2288**	**0.7676**	**0.0049**
MYLK	strong, diffuse	95	50	0.5227	0.2046	1.3356	0.1753
SLMAP	strong, diffuse	97	52	0.6123	0.2981	1.2576	0.1816
Muscle enriched phenotype	**any 3/5**	**94**	**49**	**0.4177**	**0.1763**	**0.9897**	**0.0473**
differentiation score	moderate / **poor**	**97**	**52**	1.4259 / **3.2807**	0.7843 / **1.3107**	2.5923 / **8.2119**	0.2447 / **0.0112**
gender (M/F)	M	97	52	1.068	0.6086	1.8742	0.8186
site (U/S)	Uterine vs all other	97	52	1.2194	0.5489	2.709	0.6262
Size	**>5 cm**	**97**	**52**	**2.7263**	**1.1624**	**6.3944**	**0.0211**
Multivariate analyses							
Differentiation	Moderate / **poor**	91	48	0.9084 / 3.5215	0.4373 / 1.2365	1.8869 / 10.0286	0.7967 / 0.0184
Desmin	**>10% pos**	**91**	**48**	**0.3077**	**0.1216**	**0.7788**	**0.0129**
CFL2	**strong, diffuse**	**91**	**48**	**0.4943**	**0.2615**	**0.9343**	**0.0301**
Size	**>5 cm**	**91**	**48**	**4.5457**	**1.7251**	**11.9782**	**0.0022**

Table 4

Disease specific survival, univariate and multivariate

Variable	Level	N	# all cause deaths	Hazard Ratio	95% CI Lower	95% CI upper	P-Value
Univariate analyses							
Actin, smooth muscle	>10% pos	96	40	0.9083	0.1239	6.658	0.9246
Caldesmon	>10% pos	97	41	0.4042	0.1567	1.0426	0.061
Desmin	**>10% pos**	**97**	**41**	**0.2398**	**0.121**	**0.4753**	**<0.0001**
Myosin, smooth muscle	>10% pos	95	40	0.8018	0.3544	1.8145	0.5961
Standard muscle markers	**all 4 markers expressed**	**94**	**39**	**0.3995**	**0.2087**	**0.7646**	**0.0056**
ACTG2	strong, diffuse	96	40	1.457	0.199	10.6664	0.711
CASQ2	strong, diffuse	96	40	0.6548	0.3087	1.3887	0.2696
CFL2	**strong, diffuse**	**91**	**37**	**0.3978**	**0.2022**	**0.7829**	**0.0076**
MYLK	strong, diffuse	95	39	0.503	0.1761	1.4368	0.1994
SLMAP	strong, diffuse	97	41	0.5184	0.2391	1.1239	0.0961
Type-1 leiomyosarcoma markers	muscle enriched (any 3/5)	94	38	0.3928	0.1521	1.0141	0.0535
differentiation score	moderate poor	97	41	1.3427 2.5765	0.6902 0.8634	2.6119 7.6884	0.3854 0.0897
gender (M/F)	M	97	41	1.2493	0.6692	2.3325	0.4847
site (U/S)	Uterine vs all other	97	41	0.8502	0.3025	2.3895	0.7583
Size	**>5 cm**	**97**	**41**	**2.561**	**1.0028**	**6.5405**	**0.0493**
Multivariate analyses							
Standard muscle markers	all 4 markers expressed	91	37	0.6245	0.139	2.8055	0.5391
Size	**>5cm**	**91**	**37**	**4.2788**	**1.4224**	**12.8719**	**0.0097**
Desmin	>10% pos	91	37	0.2378	0.0474	1.1923	0.0707
CFL2	strong, diffuse	91	37	0.5996	0.2769	1.2986	0.1946

Table 5

Time to first metastasis, univariate analyses

Variable	Level	N	# with metastases	Hazard Ratio	95% CI Lower	95% CI upper	P- Value
Univariate analyses							
Actin, smooth muscle	>10% pos	92	51	0.9286	0.1276	6.7582	0.9417
Caldesmon	>10% pos	93	52	0.6418	0.288	1.4304	0.2781
Desmin	**>10% pos**	**93**	**52**	**0.4643**	**0.2407**	**0.8957**	**0.0221**
Myosin, smooth muscle	>10% pos	91	50	0.8576	0.4156	1.7696	0.6777
Standard muscle markers	all 4 markers expressed	90	49	0.5986	0.3255	1.101	0.0988
ACTG2	strong, diffuse	92	51	1.789	0.2462	13.0021	0.5654
CASQ2	strong, diffuse	92	51	0.6913	0.367	1.302	0.253
CFL2	strong, diffuse	87	46	0.559	0.2876	1.0868	0.0864
MYLK	strong, diffuse	91	50	0.9674	0.3463	2.7026	0.9496
SLMAP	strong, diffuse	93	52	0.8091	0.3794	1.7254	0.5835
Type-1 leiomyosarcoma markers	muscle enriched (any 3/5)	90	49	0.8023	0.2872	2.2412	0.6743
differentiation score	moderate / poor	93	52	1.104 / 2.4072	0.6043 / 0.9684	2.0171 / 5.9836	0.7476 / 0.0587
gender (M/F)	M	93	52	1.324	0.766	2.2886	0.3148
site (U/S) / Size	Uterine vs all other / >5 cm	93 / 93	52 / 52	0.5359 / 1.7609	0.1927 / 0.881	1.4903 / 3.5196	0.2319 / 0.1093