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Molecular and immunohistochemical evidence for the origin of uterine leiomyosarcomas from associated leiomyoma and symplastic leiomyoma-like areas

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It is uncertain whether uterine leiomyosarcoma arises *de novo* or in preexisting leiomyoma. Leiomyoma-like areas can be seen associated with uterine leiomyosarcoma, raising the possibility of precursor lesions for uterine leiomyosarcoma. In this study, we examined cases of uterine leiomyosarcoma associated with leiomyoma-like areas at the histological, immunohistochemical and DNA level to further evaluate if benign-looking leiomyoma-like and uterine leiomyosarcoma areas are related. Cases of uterine leiomyosarcoma observed at the New York University Medical Center from 1994 to 2007 were reviewed for the presence of leiomyoma-like areas. Of the 26 cases of uterine leiomyosarcoma observed during this period, 18 cases had an associated leiomyoma-like area (five cellular leiomyoma, four symplastic leiomyoma, four cellular and symplastic leiomyoma and five usual type leiomyoma). Sixteen of the 18 cases were examined immunohistochemically for Ki-67, for estrogen receptor, progesterone receptor and for p53. Immunohistochemical profiles were as expected for leiomyoma-like (the mean expression of p53, ER, PR and Ki-67 at 0.3, 63, 75 and 0.6%, respectively), symplastic leiomyoma-like areas (the mean expression of p53, ER, PR and Ki-67 at 0.6, 85, 89 and 5.5%, respectively) and uterine leiomyosarcoma areas (the mean expression of p53, ER, PR and Ki-67 at 52, 38, 39 and 61%, respectively). In six cases, the leiomyoma-like and uterine leiomyosarcoma areas from each case were examined using high-density oligonucleotide array-CGH to determine genetic aberrations in the two areas. Nearly all the genetic aberrations found in leiomyoma-like areas were also found in the corresponding uterine leiomyosarcoma areas. In addition, uterine leiomyosarcoma areas had additional genetic aberrations. The immunohistochemical profiles and genetic aberrations of the examined cases suggest that uterine leiomyosarcoma could arise from the preexisting leiomyoma-like areas that often have a symplastic or cellular morphology.
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Although many advances have been made in understanding the pathogenesis of carcinomas at various sites, including the uterus, very little is known with regard to the pathogenesis of uterine leiomyosarcoma. Leiomyosarcoma is the most common uterine sarcoma. It comprises approximately 1% of all uterine malignancies. These are aggressive tumors that may be a diagnostic challenge at times.[1–7] We have previously noted the origin of leiomyosarcoma within a leiomyoma,[8] as well as the presence of leiomyoma-like areas associated with some uterine leiomyosarcomas.[9] In this study, we examined cases of uterine leiomyosarcoma associated with leiomyoma-like areas at the histological, immunohistochemical and DNA level to further evaluate if benign-looking leiomyoma-like and uterine leiomyosarcoma areas are related.

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Materials and methods

Cases of uterine leiomyosarcoma observed at the New York University Medical Center from 1994 to 2007 were reviewed for the presence of leiomyoma-like areas on microscopic examination. These leiomyoma-like areas had to be present in continuity with uterine leiomyosarcoma to qualify. Separate leiomyomas were present in many cases, but these were not considered leiomyoma-like areas for the purposes of this study. On microscopic examination, the leiomyoma-like areas and uterine leiomyosarcoma areas merged with each other. Leiomyoma areas were defined as those areas that showed nuclear atypia of only 1+ to 2+ (mild to moderate), except for symplastic leiomyoma areas that showed very large bizarre nuclei, with <5 mitoses/10 HPF. No coagulative tumor cell necrosis was observed. Leiomyosarcomas were defined as showing 2+ to 3+ (moderate to marked) nuclear atypia, with 10 or more mitoses/10 HPF. Coagulative tumor cell necrosis was present in all cases.

Of the total 26 cases of uterine leiomyosarcoma found during this period, 18 had associated leiomyoma-like areas. Sixteen of these 18 cases were examined immunohistochemically with appropriate controls. Briefly, 5-μm tissue sections on adhesive-coated slides were immunostained by mouse monoclonal antibodies for Ki-67 (MIB-1, Immunotech), p53 (Ventana), estrogen receptor (ER, Ventana) and progesterone receptor (PR, Ventana) using Ventana ES immunostainer. The expression of immunohistochemical markers within the tumor was subjectively assessed to the nearest 5%.

DNA Isolation

The DNA was isolated from leiomyoma-like and uterine leiomyosarcoma areas in six of the 18 cases with leiomyoma-like areas. Five- to 10-μm slices from paraffin-embedded tissue block were washed with HemoDee, HemoDee:Ethanol (1:1) and Ethanol (100%) twice each. The MagnaCompact system (Roche Diagnostics Indianapolis, IN) was used to isolate DNA according to the manufacturer's instructions after proteinase K treatment.

CGH Assay

The leiomyoma-like and uterine leiomyosarcoma areas from six cases were examined using high-density oligonucleotide array-CGH (comparative genomic hybridization) technology[10] (Agilent 44K arrays) to compare genetic aberrations in these two areas.

Agilent's Human Genome CGH Microarray Kit 44B is a customized, high-definition microarray that was designed using 44 000 probes comprised of 60-mer oligonucleotides designed by Agilent and printed using Agilent Sureprint technology. We designed the array with E-array software 4.0 (Agilent, Palo Alto, CA), every third probe was removed and replaced by probes recognizing the subtelomeric region (1 Mb proximal to telomeres) for 41 subtelomeric regions. The average resolution of the array is 5 kb in subtelomeres and 125 kb in the remaining human genome software.

Test and reference DNAs are digested with Alu I and Rsa I (Promega), and purified with the QIAprep Spin Miniprep kit (Qiagen). Test DNA and reference DNA were labeled with either Cy3-dUTP or Cy5-dUTP (Perkin Elmer) using the Bioprime Array CGH Genomic Labeling kit (Invitrogen). After the labeling reaction, individually labeled test and reference samples were combined and concentrated using Microcon YM-30 filters (Millipore). The hybridization mixture contained the labeled DNAs, 2× Hybridization buffer (Agilent, Palo Alto, CA), 10× blocking agent (Agilent,) and Human Cot-1 DNA (Invitrogen). Microarrays were hybridized in an Agilent SureHyb chamber in a rotisserie oven for 24 h at 65°C. Four washing steps were carried out: room temperature with Agilent's Oligo CGH Wash buffer 1 for 5 min, a 37°C wash with Agilent Oligo CGH Wash buffer 2 for 1 min, an acetonitrile rinse at room temperature for 1 min and a 30 s wash in Agilent's Stabilization and Drying Solution at room temperature. All slides were scanned by Agilent Axon 4000B scanner, using the Genepix 4.0 software. Data were obtained by Agilent Feature extraction software 8.0, and then imported into Agilent CGH analytics 3.4 software for analysis.

DNA copy number changes were detected by CGH analytics software 3.4 (Agilent). The statistical algorithm was ADM-1, sensitivity threshold was 6.0 and the moving average window was 1 Mb. To determine that there is a copy number change in a particular locus, three criteria were required to be met. These were positive call by the software, presence of 10 consecutive probes pointing out the same direction and a 1.5-fold average fold difference in the test DNA compared with the reference DNA.

Results

Leiomyoma-like areas were observed to be associated with uterine leiomyosarcoma in 18 of the 26 cases. Of the 18 cases with leiomyoma-like areas, in 5 cases, the morphology of the leiomyoma-like areas was that of a cellular leiomyoma, in 4 cases, the morphology was that of a symplastic leiomyoma (Figure 1a and b), in 4 cases, the morphology was that of a cellular symplastic leiomyoma and in 5 cases, the morphology was that of usual leiomyoma. In all 18 cases, leiomyoma-like and leiomyosarcoma areas merged into each other (Figure 2).

Immunohistochemical profiles were as expected for leiomyoma-like (the mean expression of p53, ER, PR and Ki-67 at 0.3, 63, 75 and 0.6%, respectively), symplastic leiomyoma-like areas (the mean expres-

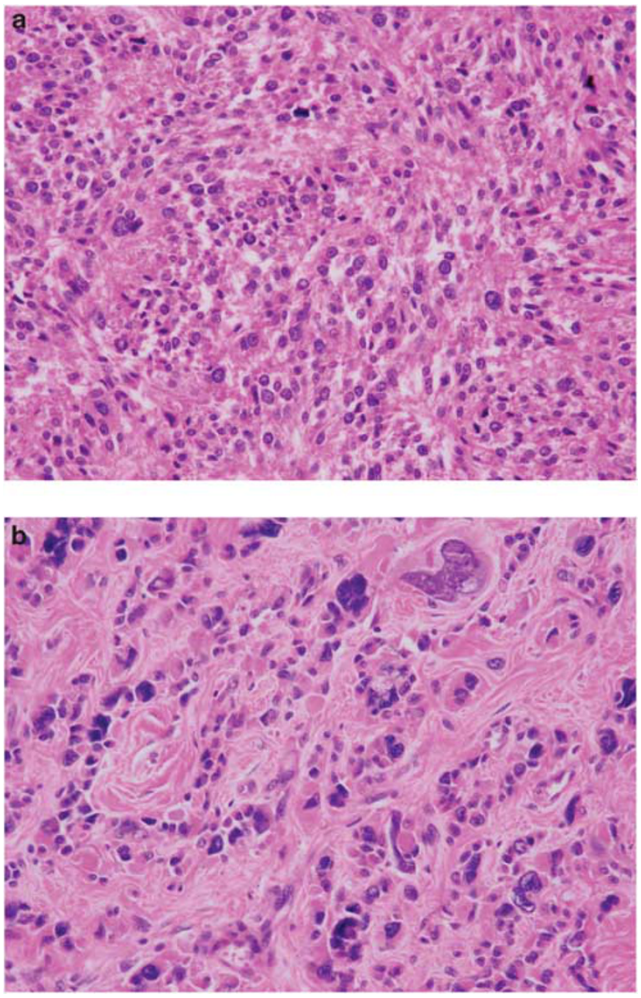

Figure 1 Uterine leiomyosarcoma (**a**) and symplastic leiomyoma (**b**) areas from the same case.

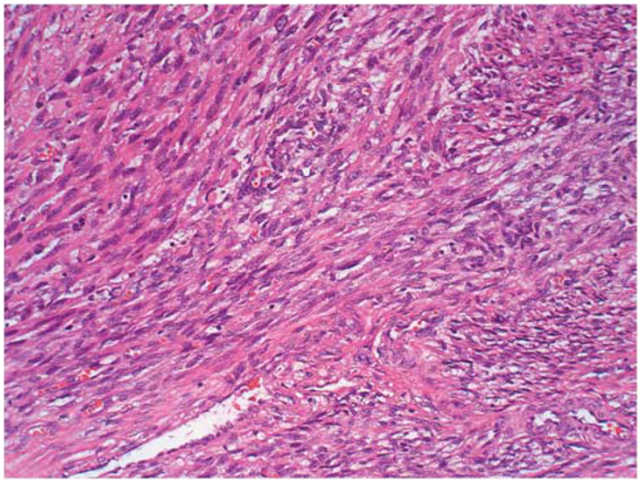

Figure 2 Transition from a cellular leiomyoma-like area (lower half) to a leiomyosarcoma (upper half and to the left).

sion of p53, ER, PR and Ki-67 at 0.6, 85, 89 and 5.5%, respectively) and leiomyosarcoma areas (the mean expression of p53, ER, PR and Ki-67 at 52, 38, 39 and 61%, respectively).

Of the six cases examined using array-CGH, three cases were associated with cellular leiomyomas, two cases were associated with symplastic leiomyoma and in one case, the benign component was cellular as well as symplastic.

Table 1 shows the genetic changes observed in uterine leiomyosarcoma and associated leiomyoma-like areas from all six cases.

Chromosomal Changes in Leiomyoma-like Areas

There were more chromosomal losses than gains in leiomyoma-like areas associated with uterine leiomyosarcoma. On an average, there were 2.3 chromosomal gains and 6.5 chromosomal losses in such areas. No chromosomal gains were observed in two cases of leiomyoma-like areas. The average foci of chromosomal gain were five in symplastic leiomyoma-like areas, but only 1.33 in cellular leiomyoma-like areas. Both symplastic and cellular leiomyoma-like areas had a similar level of chromosomal losses. The smallest number of chromosomal changes was observed in case 6, with two gains and one loss, but notably, the entire chromosome 2 was gained. Some of the aberrations observed are listed below. The number in parenthesis indicates the number of cases with this change, followed by significant genes known to be present in those areas.

Chromosomal gains in leiomyoma-like areas and genes located in those areas are as follows:

- 1p21.3–p31.3 (2/6) *JUN*
- 1q22–q24.3 (2/6) *CSk1*
- 6q16.3 (1/6) *FYN*
- 12p11.22–p13.3 (2/6) *K-Ras*
- 12q21.2–q24.3 (2/6) *ELK3*

Chromosomal losses in leiomyoma-like areas, and genes located in those areas are as follows:

- 2q36.1–q37.2 (2/6)
- 9p21.3 (2/6) *p16, p14ARF*
- 9q33.1–34.2 (2/6) *Endoglin*
- 16q12.2–24.2 (2/6)
- 17p13.2 (1/6) *p53*
- 22q13.1–22q13.3 (4/6) *PDGFB*

Chromosomal Changes in Uterine Leiomyosarcoma

Nearly all the chromosomal changes observed in associated leiomyoma-like areas were also present in the leiomyosarcoma in each of the cases (Table 1).

Additional chromosomal losses were also more frequent than were additional chromosomal gains. There were an average of 3.3 additional foci of chromosomal gain and 5 additional foci of chromosomal losses in uterine leiomyosarcoma areas. The minimal number of such additional changes was one gain and three losses, also observed in case 6, but notably, a whole chromosome 4 was lost. Some of the genetic aberrations observed are listed below.

Table 1 CGH data on all cases

	Patient 1 leiomyoma-like Gain	*Patient 1 leiomyosarcoma Gain*	*Patient 1 leiomyoma-like Loss*	*Patient 1 leiomyosarcoma Loss*	
ch1	27404305–142416881 (p12–p35.3)	28823016–120166662 (p12–p35.3)	None	None	ch1
ch2	None	None	None	29193–66581845 (p14–p25.2)	ch2
ch3	None	None	35699830–87726473 (p12.1–22.3)	8590166–53190621 (p12–p22.3)	ch3
ch3	None	None	None	100002244–101882517 (q13.11–q24)	ch3
ch3	19466746–34291321 (p24.1–p24.3)	19466746–34291321 (p24.1–p24.3)	None	None	ch3
ch4	None	None	None	93211181–110904627 (q22.1–q24)	ch4
ch4	None	None	None	95942529–97957 (p15.31–p16.2)	ch4
ch4	None	None	None	124022188–160713017 (q34.3–q35.2)	ch4
ch6	101843782–109066782 (q16.3)	101843782–109402376 (q16.3)	97634–70932652 (p25.2–q12)	111278–70932652 (p25.2–q12)	ch6
ch7	None	None	130480577–158624081 (q32.2–q36.2)	130169469–158624081 (q32.2–q36.2)	ch7
ch9	5012053–5012112 (p13.2)	5012053–5012112 (p13.2)	19386608–26311944 (p21.3)	19386608–26311944 (p21.3)	ch9
ch9		None	117119997–138301707 (q33.1–q34.2)	68573802–138301707 (q21.11–q34.2)	ch9
ch11	None	None	None	188577–21322975 (p14.3–p15)	ch11
ch12	221757–37052430 (p11.22–p13.32)	221757–34278525 (p11.22–p13.32)	None	None	ch12
ch12	64511097–132323928 (q21.2–q24.32)	64511097–132323928 (q14.3–q24.32)	None	None	ch12
ch14	None	None	None	Whole ch	ch14
ch15	83877936–100128177 (q25.3–q26.2)	83877936–100134228 (q25.3–q26.2)	20531513–83001694 (q12–q25.1)	20531513–83483235 (q12–q25.1)	ch15
ch16	None	None	37133–88643347 (whole ch)	48564–88587406 (whole ch)	ch16
ch18	None	None	17653448–56349885 (q12–q21.32)	14763516–56668586 (q12–q21.32)	ch18
ch19	670897–24132581 (p13)	258717–33198519 (p13)	None	None	ch19
ch22	None	None	40778992–49466331 (q13)	38828357–49466331 (q13)	ch22
chx	8444312–67838628 (p22.2–q12)	8444312–67838628 (p22.2–q12)	None	None	chx
	Patient 2 leiomyoma-like Gain	*Patient 2 leiomyosarcoma Gain*	*Patient 2 leiomyoma-like Loss*	*Patient 2 leiomyosarcoma Loss*	
ch1	None	14389559–203334041 (q21.2–q32.2)	None	None	ch1
ch2	None	None	29193–29852167 (p23.2–p25.2)	29193–30017573 (p23.2–p25.2)	ch2
ch2	None	None	237783694–238967853 (q33.2–q37.2)	237783694–238967853 (q33.2–q37.2)	ch2
ch4	None	32738–363042 (p15.31–p16.2)	None	None	ch4
ch5	None	7679287–986173 (q12.3–p15.32)	None	82396327–180608261(q14.1–q35.2)	ch5
ch6	None	None	None	97634–34323734 (p21.32–p25.2)	ch6
ch6	None	35290540–170839444 (p21.2–q26)	None	None	ch6
ch7	None	18043346–28307829 (p15.2–p21.1)	None	None	ch7
ch8	None	30521361–143623380 (p12–q24.23)	None	90816–29299063 (p21.2–p23.2)	ch8
ch9	None	20943032–28735046 (p21.3)	None	None	ch9
ch10	None	None	103331419–135263084 (q25.1–q26.2)	103907311–134430279 (q25.1–q26.2)	ch10

Table 1 Continued

	Patient 2 leiomyoma-like Gain	*Patient 2 leiomyosarcoma Gain*	*Patient 2 leiomyoma-like Loss*	*Patient 2 leiomyosarcoma Loss*	
ch11	None	None	None	61987224–72180028 (q24.3)	ch11
ch13	None	None	39554773–54441379 (q13.1–q14.2)	39078605–51421842 (q13.3–q14.2)	ch13
ch14	None	18590766–27249760 (q12)	None	None	ch14
ch17	None	None	None	4290896–9093308 (p13.2)	ch17
ch17	None	4636397–4636447 (p12)	None	None	ch17
ch18	None	37434138–42794639 (q12.3)	None	None	ch18
ch19	None	43157301–45815473 (q13.12)	None	None	ch19
chx	None	41293660–48426115 (p11.3)	None	77184568–150974294 (q21.1–q27.3)	chx
	Patient 3 leiomyoma-like Gain	*Patient 3 leiomyosarcoma Gain*	*Patient 3 leiomyoma-like Loss*	*Patient 3 leiomyosarcoma Loss*	
ch1	142614442–169361017 (q21.2–q25)	14261442–172837313 (q21.2–q25)	None	None	ch1
ch4	None	None	None	171384255–191159953 (q34.1–q35.2)	ch4
ch9	None	67121526–137998177 (q21.1–q34.2)	195675–31357372 (p21.1–p24.2)	195675–31357372 (p21.1–p24.2)	ch9
ch11	None	None	118710944–134447221 (q24.1–q24.3)	118715438–134448891 (q24.1–q24.3)	ch11
ch13	None	59622915–84274268 (q21.3–q31.1)	None	None	ch13
ch15	None	39657278–100208004 (q12–q14)	None	None	ch15
ch21	None	None	37515115–14334601 (q21–q22.12)	None	ch21
chx	None	39529943–90931616 (p11.3–q21.31)	None	2838295–19573044 (p22.12–p22.32)	chx
	Patient 4 leiomyoma-like Gain	*Patient 4 leiomyosarcoma Gain*	*Patient 4 leiomyoma-like Loss*	*Patient 4 leiomyosarcoma Loss*	
ch1	None	None	604268–45520709 (p33–p36.2)	792533–46383163 (p33–p36.32)	ch1
ch1	61633481–96915627 (p21.3–p31.3)	61633481–96915627 (p21.3–p31.3)	None	None	ch1
ch1	245071740–245119648 (q21.1–q43)	245071740–245119648 (q21.2–q43)	None	None	ch1
ch2	None	None	169626937–177077585 (q24.3–q31.2)	169626937–177077585 (q24.3–q31.2)	ch2
ch2	190746779–201551690 (q32.3–q33.2)	190746779–201551690 (q32.3–q33.2)	206657960–242768117 (q33.2–q37.2)	206657960–242768117 (q33.2–q37.2)	ch2
ch4	None	None	32738–23502385 (p15.31–p16.2)	32738–23502385 (p15.31–p16.2)	ch4
ch5	14481224–65583743 (p13.2–p15.2)	14731686–41620820 (p13.2–p15.2)	None	None	ch5
ch6	None	None	None	None	ch6
ch6	57101495–170022082 (q12–q26)	57101495–170022082 (q12–q26)	None	None	ch6
ch8	23765726–139224392 (p21.2–q24.23)	23765726–139224392 (p21.2–q24.23)	None	None	ch8
ch9	None	None	None	195675–20768773 (p22.2–p24.2)	ch9
ch9	None	None	73551215–138301707 (q21.3–q34.2)	64168318–138301707 (q21.1–q34.2)	ch9
ch13	94611477–113209399 (q32.2–q33.3)	94611477–113209399 (q32.2–q33.3)	None	None	ch13
ch13	51501779–7138186 (q21.1–q21.33)	51501779–7138186 (q21.1–q21.33)	None	33297902–49884865 (q14.2)	ch13
ch13	None	None	79911534–92406928 (q22.2–q31.3)	78201697–92406928 (q22.2–q31.3)	ch13
ch16	None	None	37133–88643347 (p13.2)	37133–88643347 (p13.2)	ch16

Table 1 Continued

	Patient 4 leiomyoma-like Gain	*Patient 4 leiomyosarcoma Gain*	*Patient 4 leiomyoma-like Loss*	*Patient 4 leiomyosarcoma Loss*	
ch16	None	None	45283842–884643347 (q12.2–q24.2)	45283842–884643347 (q12.2–q24.2)	ch16
ch17	403373–5343563 (p13.3)	403373–5343563 (p13.3)	None	None	ch17
ch17	9263874–10352437 (p12)	9263874–10352437 (p12)	5402766–9093308 (p13.2)	5402766–9093308 (p13.2)	ch17
ch20	None	None	15681–9933305 (p12.3)	8747–10072914 (p12.3)	ch20
ch22	None	None	18568719–49463928 (q11.22–q13.32)	17459097–49463928 (q11.22–q13.32)	ch22
chx	None	None	2838295–154405159 (whole ch)	2838295–154405159 (whole ch)	chx
	Patient 5 leiomyoma-like Gain	*Patient 5 leiomyosarcoma Gain*	*Patient 5 leiomyoma-like Loss*	*Patient 5 leiomyosarcoma Loss*	
ch1	None	None	None	224430231–245433898 (q42.12–q43)	ch1
ch4	None	None	None	174871381–191159953 (q34.1–q35.2)	ch4
ch5	None	None	None	93689–19991517 (p14.3–p15.2)	ch5
ch11	None	None	None	917004–55572888 (p11.12–p15.4)	ch11
ch12	None	None	None	195171–34067482 (p11.22–p13.32)	ch12
ch13	None	None	None	whole ch	ch13
ch14	None	None	None	57529234–63784623 (q13.12–q13.43)	ch14
ch15	None	95432300–100208004 (q26.2)	None	None	ch15
ch17	None	None	49411–20485054 (p12–p13.2)	None	ch17
ch19	None	None	33594470–63784623 (q13.12–q13.43)	33594470–63784623 (q13.12–q13.43)	ch19
ch19	None	None	227281–15220720(p13.2)	227281–15220720 (p13.2)	ch19
ch22	None	None	18644996–48896067 (q11.22–q13.32)	18644996–48896067 (q11.22–q13.32)	ch22
	Patient 6 leiomyoma-like Gain	*Patient 6 leiomyosarcoma Gain*	*Patient 6 leiomyoma-like Loss*	*Patient 6 leiomyosarcoma Loss*	
ch1	142565191–200365015 (q21.2–q31.1)	142565191–200365015 (q21.2–q31.1)	None	None	ch1
ch8	None	92330975–146254382 (q21.3–q24.23)	None	176279–41025189 (p11.22–p23.2)	ch8
ch12	whole ch	Whole ch	None	None	ch12
ch14	None	None	None	whole ch	ch14
ch16	None	None	None	45284566–88151678 (q12.2–q24.2)	ch16
ch17	None	None	None	25018032–33242217 (q12)	ch17
ch22	None	None	22731544–48965505 (q11.22–q13.32)	18179221–48896067 (q11.22–q13.32)	ch22

The gains and losses seen in the leiomyoma-like areas persist in the leiomyosarcoma areas, with additional changes in the leiomyosarcoma.

The additional chromosomal gains observed in uterine leiomyosarcoma and associated genes are the following:

- 8q21.2–q24.23 (2/6) *MYC*
- Xp11.3 (2/6) miRNA-221

The additional chromosomal losses observed in uterine leiomyosarcoma and associated genes are the following:

- 4q34.3–q35.2 (3/6) *ING2*
- 8p21.1–p23.2 (2/6) *LOXL2*
- 11p14.3–p15 (2/6)
- 13q14.2 (2/6) *RB-1*
- 14q13.12–q13.43 (3/6) *TTF-1*
- 17p13.2 (1/6) *p53*

Discussion

The pathogenesis of uterine leiomyosarcoma is poorly understood. It is generally believed that

uterine leiomyosarcomas arise *de novo*, rather than from any precursor lesions. However, cases of uterine leiomyosarcoma arising in leiomyoma have been reported, suggesting that leiomyosarcomas may arise from preexisting leiomyomas.[2,11–13]

In this study, we found benign-looking leiomyoma-like areas in 18 of the 26 cases of uterine leiomyosarcoma. The immunohistochemical profile of these benign-looking areas in this study was similar to that reported for leiomyomas previously.[14] The immunohistochemical profile of the leiomyosarcoma areas was similar to that for leiomyosarcomas reported previously.[14]

We found chromosomal losses and gains in leiomyoma-like areas, with a retention of these losses and gains in the associated leiomyosarcomas. In addition, leiomyosarcomas showed further additional losses and gains of chromosomal areas (Table 1).

Leiomyoma-like areas showed amplification in many oncogene and transcription factor genes, including *C-JUN*, *Cks-1*, *Fyn*, *K-Ras* and *ELK-3*. C-JUN forms AP-1 early response transcription factor in association with c-Fos.[15] *Cks1*, located at 1q21, has been proposed recently as an oncogene for breast cancer.[16] Fyn is a tyrosine-specific phosphotransferase and activates Ras.[17] *K-Ras* is mutated in many carcinomas. The K-Ras protein is a GTPase and acts as a molecular on/off switch in many signal-transduction pathways.[18,19] ELK-3 activates transcription in the presence of Ras.[20]

Losses were observed generally in foci with tumor suppressor genes. p16 and p14ARF are tumor suppressors located on 9p21.[21] *Endoglin* has been identified as a candidate tumor suppressor gene in esophageal squamous cell carcinoma.[22] PDGFB is a mitogenic factor for mesenchymal cells,[23] and its loss is difficult to explain in leiomyoma-like areas. Perhaps other tumor suppressor genes are located in the vicinity of *PDGFB*, which is at 22q13.1. Deletion of 22q13 is common in ovarian, breast and colorectal cancer.[24,25]

Overall, chromosomal losses were more frequent than were chromosomal gains in the leiomyosarcoma area, similar to that which has been reported previously in uterine leiomyosarcomas.[26]

The amplification of additional chromosomal foci, including that for Myc, was observed in leiomyosarcoma areas. The Myc gene encodes for a transcription factor that regulates the expression of approximately 15% of all genes and has an important function in many malignancies.[27] Overexpression of miRNA-221 has been observed in high-grade glioma.[28]

Additional chromosomal foci, many involving tumor suppressor genes, were lost in leiomyosarcomas. These included foci for *ING2*, *LOXL2*, *TTF-1*, *RB-1* and *p53*. *ING2* deletions have been observed in head and neck cancer.[29] *LOXL2* is frequently lost in prostate carcinoma.[30] TTF-1 is a lineage-associated marker for thyroid and lung tissue. It is associated with improved survival, suggesting a potential tumor-suppressing function.[31] *RB-1* is a tumor suppressor gene that is lost or mutated in many malignancies. Loss of chromosome 13, in which *RB-1* is located, is a frequently reported finding in leiomyosarcomas.[32] t(10;17)(q22.1;p13) has been described as the sole genetic change that is observed in a uterine leiomyosarcoma.[33] 17p13 loss involving the *p53* location was observed in two of the six cases of uterine leiomyosarcoma in this study, involving the associated leiomyoma-like area also in one case, and as an additional change in another case. Figure 3 summarizes the changes observed in leiomyoma-like areas and in uterine leiomyosarcoma. The findings of this study show that the gains of oncogenes and loss of tumor suppressor genes underlie the growth of uterine leiomyosarcomas, as has been shown previously.[34]

The presence of benign- and malignant-looking areas in a tumor could be explained in three different ways. The benign- and malignant-looking areas could be different tumors, in which case, they would be expected to show distinct genetic changes. The benign-looking areas could be better differentiated areas of the tumor, in which case, the genetic profile of these two different areas would be the same. Finally, malignant areas could arise from benign-looking areas. In this case, the two areas would share similar changes, but additional changes would be present in malignant areas. The findings of this study showed the last possibility as the underlying explanation for the presence of leiomyoma-like areas associated with uterine leiomyosarcoma areas examined in this study. In all six cases examined, genetic aberrations found in leiomyoma-like areas are also found in the corresponding uterine leiomyosarcoma areas. In addition, uterine leiomyosarcoma areas have additional genetic aberrations.

The large variation of the patterns of DNA changes from one uterine leiomyosarcoma to another suggests that the growth properties needed to develop the malignant phenotype in these tumors may result from a varied collection of DNA changes. Such

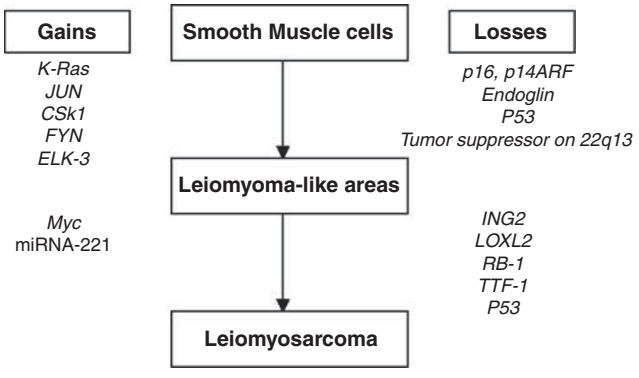

Figure 3 Some of the chromosomal gains and losses that may have an important function in the pathogenesis of uterine leiomyosarcomas and associated leiomyoma-like areas.

varied changes are also indicative of genetic instability.

Although these DNA findings provide potential insight into the origins of uterine leiomyosarcoma, the readers should be aware that there may be additional, and potentially more important, changes that cause uterine leiomyosarcoma. Any balanced genetic alterations (such as a translocation), any mutations or any changes below the threshhold of detection of DNA CGH would not be picked up by this CGH study. Nevertheless, the persistence of DNA changes observed in the leiomyoma-like areas in the associated uterine leiomyosarcoma supports our hypothesis that uterine leiomyosarcoma may arise from associated leiomyoma-like areas.

The findings of this study should not be interpreted to mean that leiomyomas in general are precancerous lesions. As the frequency of leiomyosarcomas is only 0.1–0.3% of the frequency of leiomyomas,[35] only rare leiomyomas progress to leiomyosarcoma. As cellular and symplastic leiomyoma-like areas were overrepresented in uterine leiomyosarcoma-associated leiomyoma-like areas, leiomyomas with this morphology may be more prone to malignant transformation than usual type leiomyomas.

The findings of this study may have implications for the origin of sarcomas in general. Although some sarcomas have been associated with specific genetic changes,[36] very little is currently known about preneoplastic lesions for most sarcomas. Most malignant peripheral nerve sheath tumors are thought to arise by malignant transformation of neurofibromas,[37] but DNA studies to confirm such progression have not been reported.

Disclosure/conflict of interest

The authors have no financial interest in the contents of the manuscript.

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