RESEARCH ARTICLE

Chromosome Imbalances and Alterations in the p53 Gene in Uterine Myomas from the Same Family Members: Familial Leiomyomatosis in Turkey

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Abstract

Uterine leiomyomas (UL) are extremely common neoplasms in women of reproductive age, and are associated with a variety of characteristic choromosomal aberrations (CAs). The p53 gene has been reported to play a crucial role in suppressing the growth of a variety of cancer cells. Therefore, the present study investigated the effects of CAs and the p53 gene on ULs. We performed cytogenetic analysis by G-banding in 10 cases undergoing myomectomy or hysterectomy. Fluorescence in situ hybridization (FISH) with a p53 gene probe was also used on interphase nuclei to screen for deletions. In patients, CAs were found in 23.4% of 500 cells analysed, significantly more frequent than in the control group ($p<0.001$). In the patients, 76% of the abnormalities were structural aberrations (deletions, translocations and breaks), and only 24% were numerical. Deletions were the most common structural aberration observed in CAs. Among these CAs, specific changes in five loci 1q11, 1q42, 2p23, 5q31 and Xp22 have been found in our patients and these changes were not reported previously in UL. The chromosome breaks were more frequent in cases, from high to low, 1, 2, 6, 9, 3, 5, 10 and 12. Chromosome 22, X, 3, 17 and 18 aneuploidy was observed to be the most frequent among all numerical aberrations. We observed a low frequency of p53 losses (2-11%) in our cases. The increased incidence of autosomal deletions, translocations, chromatid breaks and aneuploidy, could contribute to the progression of the disease along with other chromosomal alterations.

Keywords: Uterine myomas - chromosomal aberrations - FISH - p53 gene - familial leiomyomatosis - Turkey

Asian Pacific J Cancer Prev, **14 (2)**, 651-658

Introduction

UL is the most common tumor type in women during the reproductive years yet little is known about their etiology. UL is also known as myomas, uterine fibroids or fibromas, which represents the most common neoplasm of the female genital tract, and most frequently affect the uterine myometrium. From a study of serial sections of uteri it has been estimated that up to 77% of women of reproductive age have one or more fibroids (Cramer et al., 1990). Avarage estimates of familial risk are ~25% in first-degree relatives of affected probands, the recurrence rate in siblings, estimates the heritability of UL (Kurbanova et al., 1989; Snieder et al., 1998; Luoto et al., 2000). Myomas are associated with a variety of characteristic cytogenetic abnormalities. However, cytogenetic analyses of multiple fibroids from a single uterus have demonstrated that the tumors can harbour different chromosomal changes, and have suggested that each fibroid develops independently. The significance of these CAs in the pathobiology of myomas remains to be determined. Approximately, 40% of cytogenetically investigated cases show abnormal karyotypes, usually with single or few changes. Rarely, they may show complex karyotypes. Cytogenetic studies on large series of leiomyomata have shown that 50-80% of these tumors have a normal karyotype and 20-50% show clonal CAs (Heim et al., 1988; Vanni et al., 1991; Stern et al., 1992; Hennig et al., 1996; Brosens et al., 1998). Cytogenetic aberrations involving chromosomes 6, 7, 12 and 14 constitute the major CAs seen in leiomyomata. About 40-50% of fibroids show karyotypically detectable CAs that are both non-random and tumor specific (Mark et al., 1990; Nilbert et al., 1990; Rein et al., 1991). These CAs have been classifed into several cytogenetic categories; t(12;14)(q14-q15;q23-24), del(7)(q22q32), rearrangements involving 6p21, 10q, trisomy 12 and deletions of 3q.

p53 gene is a tumor suppressor gene, and its protein is a nuclear phosphoprotein and is capable of suppressing the growth of a variety of cancer cells (Chen et al., 1990;

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Malkin et al., 1990; Yin et al., 1992). An alteration of p53 is a fundamentally important step in genomic instability and susceptibility to neoplastic state transformation (Oren et al., 1992; Jung et al., 2001). However, little information is currently available regarding the content of p53 protein in human leiomyomas. Our study may provide valuable clues to elucidate the CAs and p53 gene content in UL.

Materials and Methods

Patients

A total of 10 uterine myomas from the same family members diagnosed at Department of Obstetrics and Gynecology, Tayfur Ata Sökmen Faculty of Medicine, Mustafa Kemal University, Antakya-Turkey (Figure 1). These patients were referred to Department of Medical Biology and Genetics, Faculty of Medicine, Çukurova University for analyzing cytogenetically and molecular cytogenetically (interphase FISH). The ages of patients are in the order of (from younger to elder) 28, 30, 35, 38, 41, 42, 44, 45, 50, 73 years old (Table 1).

Cytogenetic analysis

Peripheral blood was taken from each subject for culture. Each sample was examined for CA expression in the Genetics Laboratory of the Department of Medical Biology and Genetics, Faculty of Medicine, Çukurova University. A 0.3-ml blood sample was incubated at 37°C for 72 h in RPMI-1640 (Sigma R6767) supplemented with 4% fetal calf serum, phytohemagglutinin, L-glutamin, streptomycin, and penicillin. Standard cytogenetic techniques were used for harvesting and slide preparation. Three slides were prepared for each subject. The slides were prepared by trypsin G-banding and 50 metaphases/individuals were analyzed on coded slides for structural CA, such as chromatid and chromosome breaks, deletions, acentric fragments, di-centric chromosomes, tetraploids, quadriradial exchange figures, and chromosomal exchanges. The classification of CAs was done according to the nomenclature established in human gene mapping HGM 11 (McAlpine et al., 1991).

Slide preparation and flourescence in situ hybridization

A 2-ml venous blood was taken from 7 patients to determine p53 gene deletion. Standard techniques were used for harvesting and slide preparation without incubation. After incubating slides at room temperature overnight, FISH was performed with LSI p53, 17p13.1, Spectrum Orange Probe (Vysis). Initially, slides were pretreated with 2xSSC for 5 min at room temperature and then immersed into a solution containing HCl (1N), water and pepsin A (2:200:2 v/v/v) for 30 min at 37°C. Immediately after the incubation time, slides were washed with water. Then, they were washed with PBS, PBS/MgCl2.6H2O, and PBS/MgCl2.6H2O with paraformaldehyte for 2 min, 2 min, and 10 min, respectively, and later passed through a dehydration series of 70, 85 and 100% ethanol for 3 min each. The slides were then air dried. Simultaneously, 10μl of each probe mixtures were applied on slides instantaneously and a coverslip was sealed onto the slides with rubber cement. The slides were put in ThermoBrite Denaturation/Hybridization System and denaturized for 5 min at 95°C and hybridized overnight at 37°C. For posthybridization process, slides were washed with 0.4xSSC/0.3% tween 20 for 2 min at 73°C and 2xSSC/0.1% tween 20 for 1 min at room temperature. After this process slides were incubated for drying in a dark room. In the next step, DAPI tube was vortexed and slides were counterstained with 10μl of it, and then waited 30 min at -20°C. Later on, slides were analyzed at flourescent microscopy using red, green and DAPI filters. Interphase cells were analyzed using a BX51 Olympus fluorescence microscope equipped with Cytovision Probe Software (Applied Imaging, Santa Clara, CA). For each case and probe, a minimum of 100 interphase cells were evaluated for the signal patterns.

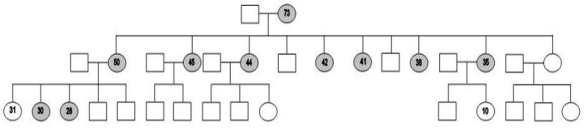

Figure 1. Family Pedigree Showing in Patients with Uterine Myomas

Results

A total of 10 uterine myomas from the same family members were analyzed, cytogenetically. CAs data of the patients were presented in Table 1. There was at least one or more structural and/or numerical CAs in 1 of 50 metaphases of each myoma case. The 76.6% of cells in 10 patients revealed a predominantly normal karyotype. However, numerical, structural aberrations and polymorphic variants were found in 23.4% of 500 cells analyzed. Structural aberrations predominated by deletions of various chromosomes in the patients. In the control group, the aberrations were found in 1.9% of cells among 324 analyzed cells. There was a significant difference in the total abnormalities of chromosomes between patients and the control group determined by the χ^2 test ($p<0.001$). In the patients, the 76% of abnormalities were structural aberrations, and only 24% were numerical. Structural aberrations were the most comman and usually consisted of deletions, breaks and fragilities in various chromosomes. Among these CAs, there was a higher frequency of five abnormalities at the regions 1q42, 1q11, 2p23, 5q31, 6q21 and Xp22. The distribution of structural aberrations on chromosomes included del(1)(q41-qter), del(1)(q42-qter)[2]x2, del(1)(q43-qter), del(2)(p23-pter)x2, del(2)(q31-qter), del(3)(p13-p14), del(3)(p23-pter), del(3)(p25-pter), del(5)(p15.1-p15.3)(q35), del(5)(q13-q15), del(5)(q31-qter), del(7)(q11.22-q11.23), del(7)(p15-pter), del(8)(q11-qter), del(9)(q11.1-qter), del(9)(q11-qter), del(9)(q11-qter)x2, del(9)(q12-qter), del(9)(q13-qter:q21-qter), del(12)(q13.1-q13.2), del(12)(q24-qter), del(13)(q11-q13), del(16)(q25-qter), 17qx3, del(17)(q11-qter), del(17)(q21-qter), del(X)(p22.1-pter), del(X)(q26-qter), del(X)(q27-qter), izo(8q), t(14;22)(q32;q11.2), t(17;X)(q24;q24), inv(6)(p11;p22) and 16q+. Translocations [t(12;15)(q24.3;p11.2), t(11;13)(p13;qq34), t(14;22)(q32;q11.2), t(17;X)(q24;q24)] and

inversion [inv(6)(p11;p22)] were seen in five metaphases (Table 1) (Figure 2). One isochromosome, isochromosome 8q was found. The chromosome breaks were more frequent in the chromosomes, from highest to lowest, 1, 2, 6, 9, 3, 5, 10 and 12. These breaks observed at the breakpoints of chtb(1q32), chtb(1q11), cenbr(1q11), chrb(2p11.2), chrb(2q31), chtb(3q26.2), chtb(5q15), chtb(6q21)x2, chtb(9q12), chbr(9q32), chtb(10q24-qter), chbr(11p13), and chtb(12q13) (Table 1). Aneuploidies (monosomies and trisomies) were observed as common findings in 26 cells. Specifically chromosome Xx6, 22x4, 3x2, 17x2 and 18x2 aneuploidies were observed to be the most frequent in our patients consecutively (Table 1). Numerical aberrations included; 48,XX,+3p,+ace, 47,XX,+7(q11.2-qter), 47,XX,+18, 45,XX,-3, 45,XX,-8, 45,XX,-10, 45,XX,-14, 45,XX,-15, 45,XX,-16, 45,XX,-17x2, 46,XX,+17,-20,9qh+, 45,XX,-18, 45,XX,-19, 45,XX,-21, 45,XX,-22x5, 45,Xx3, 47,XXXx3, 47,XX,+izo(Xq) and 47,XX+ace (Table 1). One of the significant results was numerical sex chromosome abnormalities [45,Xx3, 47,XXYx3 and iso(Xq)].

The distribution of FSs according to each chromosome is shown in Table 1. The distribution of FSs on eight chromosomes display; fra(1p36.1)x2, fra(1q11-q23), fra(1q12-q21), fra(1q21), fra(1q31.2), fra(2q33), fra(3p25), fra(6p25), fra(11p24), fra(17q21), fra(18p23), fra(21q22.1), fra(Xp22.1)x2, gap(2p23), gap(2p25), gap(3p13;p23), gap(3p21), gap(5q31)x2, gap(6q15) and gap(6q21). Among these FS, there was a significantly higher frequency of eleven FSs on chromosomes of 1x5, 2x3 and 3x3 sequentially (Table 1, Figure 2). The 1p36.1x2, 1q11-q23, 1q12-q21, 1q21, 1q31.2 2q33, 2p23, 2p25, 3p25, 3p13-p23, 3p21, 5q31x2 and Xp22.1x2 regions were expressed most frequently in our patients (Figure 2). Chromosome polymorphisms were observed at the hsr(2)(p12;p21), hsr(2)(q13-q21), hsr(3)(q11-q13), 1qh+, 9hsr+, 9qh+ and 15ps+ regions (Table 1). A total of 7 patients however, were screened for p53 gene loss.

Table 1. Cytogenetics and FISH Results in Patients with Uterine Myomas

Patient no	Age	Karyotypes	FISH (p53)
P1	42	46,XX,15ps+, 16qh+ (50/50)	11%
		46,XX,+17,-20, 9qh+ (1/50)	
		46,XX,9qh+,gap(6)(q15) (1/50)	
		46,XX,gap(531)[2] (1/50)	
		46,XX,del(3)(p23-pter),del(7)(p15-pter) (1/50)	
		46,XX,del(2)(q31-qter) (1/50)	
		46,XX,del(5)(p15.1-p15.3)(q35) (1/50)	
		46,XX,fra(17q21) (1/50)	
		46,XX,+3p,+ace (1/50)	
		45,XX,-14 (1/50)	
		46,XX,far(2q33) (1/50)	
		46,XX,del(5)(q31-qter) (1/50)	
		46,XX,gap(2p25),gap(3p13;p23) (1/50)	
		47,XXX,inv(6)(p11;p22) (1/50)	
		45,XX,hsr(2)(p12;p21),-15 (1/50)	
P2	28	46,XX,9hsr+, 16q+ (50/50)	10%
		45,XX,-16 (1/50)	
		45,XX,-8 (1/50)	
		46,XX,del(2)(p23-pter) (1/50)	
		46,XX,chtb(1q11) (1/50)	
		47,XX,+18,chtb(10q24-qter) (1/50)	
		45,X0 (1/50)	
		46,XX,chrb(2q31) (1/50)	
		46,XX,del (9)(q11-qter) (1/50)	
		46,XX,del(17)(q11-qter) (1/50)	
		45,XX,-22 (1/50)	
		46,XX,chrb(2p11.2),+7(q11.2-qter) (1/50)	
		47,XX,+izo(Xq) (1/50)	
P3	44	46,XX,del(9)(q11-qter),hsr(2)(q13-q21) (1/50)	
		46,XX,gap(3p21) (1/50)	
		46,XX,gap(2p23),gap(6q21),del(13)(q11-q13) (1/50)	
		46,XX,chtb(1q32) (1/50)	
		46,XX,del(9)(q13-qter:q21-qter) (1/50)	
		46,XX,far(1q12-q21) (1/50)	
		46,XX,9qh+ (1/50)	
		46,XX,cenbr(1q11) (1/50)	
P4	38	46,XX,9hsr+,16q+ (50/50)	2%
		46,XX,chbr(6q21) (1/50)	
		46,XX,chtb(9q12) (1/50)	
		45,X0 (1/50)	
		46,XX,del(8)(q11-qter) (1/50)	
		46,XX,del(X)(q26-qter) (1/50)	
		45,X0 (1/50)	
		45,XX,-10	
		46,XX,del(9)(q12-qter) (1/50)	
		46,XX,del(7)(q11.22-q11.23) (1/50)	
		46,XX,1qh+,del(3)(p25-pter) (1/50)	
		46,XX,del(1)(q42-qter)[2] (1/50)	
		46,XX,del(5)(q13-q15) (1/50)	
		45,XX,-22 (2/50)	
		46,XX,chtb(3q26.2) (1/50)	
		46,XX,chtb(12q13) (1/50)	
P5	41	46,XX,9qh+,16q+ (50/50)	3%
		46,XX,fra(1q11-q23) (1/50)	
		46,XX,fra(1p36)(q21)(q31.2) (1/50)	
		46,XX,del(1)(q41-qter) (1/50)	
		45,XX,-17 (1/50)	
		46,XX,hsr(3)(q11-q13) (1/50)	
		47,XXX, del(12)(q13.1-q13.2) (1/50)	
		46,XX,fra(3p25) (1/50)	
		45,XX,-3 (1/50)	
		46,XX,far(Xp22.1) (1/50)	
		46,XX,t(14;22)(q32;q11.2) (1/50)	
P6	45	46,XX, 9qh+,16q+ (25/25)	7%
		47,XXX, (1/25)	
		47,XX,+ace (1/25)	
		46,XX,izo(8q) (1/25)	
P7	35	46,XX,9qh+,15ps+ (50/50)	
		45,XX,del(1)(q43-qter),chtb(5q15),-18 (1/50)	
		46,XX,del(1)(q42-qter)[2],del(12)(q24-qter) (1/50)	
		46,XX,t(17;X)(q24;q24) (1/50)	
		46,XX,del(X)(p22.1-pter) (1/50)	
		46,XX,fra(Xp22.1) (1/50)	
		46,XX,del(2)(p23-pter) (1/50)	
		46,XX,far(21q22.1) (1/50)	
P8	50	46,XX,9qh+ (50/50)	5%
		46,XX,far(1p36.1) (1/50)	
		45,XX,-22 (1/50)	
		46,XX,del(9)(q11.1-qter) (1/50)	
		47,XXX (1/50)	
		46,XX,chbr(9q32) (1/50)	
		46,XX,del(3)(p13-p14) (1/50)	
		45,XX,-21 (1/50)	
		46,XX,chtb(6q21) (1/50)	
P9	73	45,XX,t(12;15)(q24.3;p11.2) (1/50)	
		46,XX,fra(6p25) (1/50)	
		46,XX,fra(18q23) (1/50)	
		46,XX,t(11;13)(p13;q34) (1/50)	
P10	31	46,XX,del(X)(q27-qter) (1/50)	4%
		46,XX,chtb(11p13) (1/50)	
		45,XX,-22,del(16)(q25-qter) (1/50)	
		45,XX,-17,fra(11q24) (1/50)	

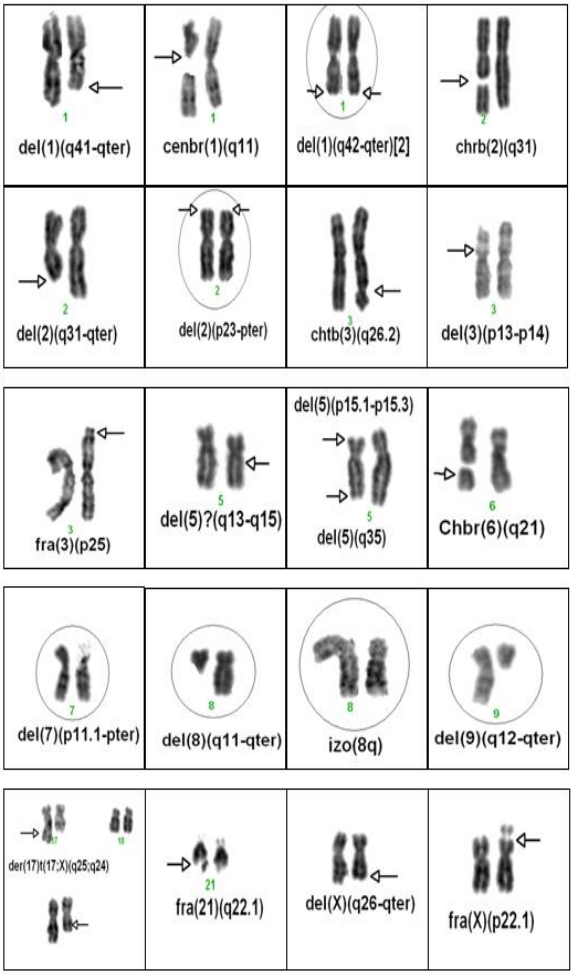

Figure 2. Partial Metaphase Figures Showing Some Chromosomal Abnormalities

We have demonstrated that loss in p53 tumor suppressor gene was not remarkably increased and there was a low frequency of p53 loss in 2-11% of our cases (Table 1).

Discussion

Hereditary leiomyomatosis is particularly relevant to clinicians and patients due to the resulting increased risk of malignant disease for both the affected woman and her family. Little is known about pathobiology of UL, and it would be reasonable to speculate that either: CAs occur as secondary events that facilitate growth of existing tumors, or submicroscopic mutations in genes occur as primary events that initiate tumor growth.

We have identified 8 sisters with UL from the same family, and observed that there is a significant relationship between CAs and myomas. The numerical and structural CAs and polymorphic variants were found in 23.4% of our patients. This ratio was a significant difference between patients and the control group ($p<0.001$) in terms total CAs (Table 1). This significant increase may increase the risk for myoma growth. In general, complex numerical and structural aberrations have been observed in leiomyosarcomas (Nilbert et al., 1990). However, about 40-50% of fibroids show karyotypically detectable CAs that are both non-random and tumor-specific (Nilbert et al., 1990; Rein et al., 1991). In a study, the authors determined that uterine fibroids were diagnosed with a frequency 2.2 fold higher in families (daughters, sisters and mothers) with two or more patients who had fibroids (Vikhlyaeva et al., 1995). An earlier study estimated the risk for sisters of affected probands to develop fibroids as 26%, compared with ~10% for the general population (Kieehle-Schwartz et al., 1991). It showed that the existence of an inherited factor in the etiology of leiomyomata is also suggested in our family.

Cytogenetic analyses of multiple fibroids from a single uterus have demonstrated that the tumors can harbor different chromosomal changes, and have suggested that each fibroid develops independently. Yet these changes may simply be representative of neoplastic smooth muscle. Our study suggests that nine chromosomal regions were important which are promising for localizing susceptibility genes for UL: 1p36, 1q11, 1q42, 2p23, 3p, 5q31, 6q21, 9q and Xp22, and monosomi 22. In some studies, a variety of random and nonrandom cytogenetic abnormalities; rearrangements of chromosome regions 12q14-15 and 7q22 are the most frequently observed in UL (Mark et al., 1990; Nilbert et al., 1990; Rein et al., 1991). In addition, others have reported UL with abnormalities involving chromosome 1, 19, 10q, 6p, 22 and X (Brosens et al., 1998; Nilbert et al., 1990; Kieehle-Schwartz et al., 1991). However, there was a higher frequency of five abnormalities at the regions 1q42, 1q11, 2p23, 5q31, 6q21 and Xp22 in our patients. These regions were not reported previously in UL. These different chromosomal changes may be new hot spots for myomas, or is associated with the tumor formation. At the same time, no relationship between patients' age and the type of CAs has been identified. A recent study showed a positive correlation between the presence of a cytogenetic abnormality and the anatomic location of the uterine fibroid (Heim et al., 1987).

Chromosome 1 abnormalities are often seen as a secondary change in the number of tumor types (Milelman, 1988), including atypical lipomas and well-differentiated liposarcomas (Heim et al., 1987; Ture-Carel et al., 1986). It was marked that consistent breaks and deletions involving specific oncogenes/tumor suppressor genes were present in 1p36 and other regions of chromosome 1, such as 1p22-q21 (Thompson et al., 1995; Smedley et al., 2000). Other structural aberrations of chromosome 1 were seen in leiomyomata including t(1;6) (q23;p21) and t(1;2) (p36;p24) (Havel et al., 1989; Mark et al., 1990). We also found seventeen abnormalities (especially deletions) at bands p36, q11, q12, q21, q31, q32, q41, q42 and q43 on chromosome 1 that were significantly overexpressed in our patients (Table 1) (Figure 2). The fragility of 1p36 in four metaphases and del(1q42-qter, q43-qter and q41-qter) in our patients is remarkable since the chromosome 1p-q could play a role in the pathogenesis of UL. As, del(lp) has been observed in 50% of cytogenetically studied leiomyosarcomas, it is possible that a tumor suppressor gene(s), located on chromosome 1p or 1q, might be critical to the genesis and/or progression of certain types of these tumors. Those findings suggest that del(lq) might be associated with prognosis of UL. As yet, no candidate genes on chromosome 1 have been identified attributed a role in leiomyoma formation.

However, del(lp) is associated strikingly with poor prognosis in neuroblastoma (Hayashi et al., 1989), and it would be worthwhile to prospectively evaluate the prognostic significance of this event in leiomyosarcoma. One of the main result in our study was the changes of chromosome 2; deletions and chromotid breaks at bands p11.2, p23, q31 were significantly overexpressed (Table 1). It is noted that in these loci of chrosome 2 expressed in our patients were not reported previously in UL. It is remarkable that the chromosome 2p-q could play a role in the pathogenesis of UL.

We reported six deletions, fragilities and gaps at bands p13, p23, p25, and two aneuploidies of chromosome 3 (Table 1) (Figure 2). A number of rearrangements of chromosome 3 have been found in leiomyomata, both as sole abnormalities as well as those accompanying other rearrangements, and include the following: ins(2;3)(q31;p12p25), del(3)(p14), del(3)(q24), t(3;7)(p11;p11. Rearrangements at 3p14.2 occur frequently in most human cancers (Sozzi et al., 1997; Corbin et al., 2002). Losses of three distinct regions on chromosome 3 have also been identified at 3p21.3, 3p14 and 3p25, suggesting the presence of multiple tumor suppressor genes (Hibi et al., 1992). Some tumor suppressor genes on 5q31 are important in hematological transformation (Dubourg et al., 2002; Le Beau et al., 1993). In our study, deletions of distinct regions on chromosome 5 have also been identified at 5q13, 5q15, 5q31 and 5q35 regions. Together, losses of three distinct regions p13, p23 and p25 on chromosome 3 and the 5q31 region could play an important role in the pathogenesis of UL in our patients.

Rearrangements of band 6p21 have been observed frequently in the group of previously mentioned mesenchymal tumors, including lipomas, pulmonary chondroid hamartomas, endometrial polyps and UL (Talini et al., 1997; Williams et al., 1997; Xiao et al., 1997). Just as, we also identified four chromatid break and gap at band 6q21 that were significantly overexpressed in our patients. Complex rearrangements [t(1;6)(q23;p21), t(6;14)(p21;q24) and t(6;10)(p21;q22)] of 6p21 have also been observed with a frequency of <5% in leiomyomas (Nilbert et al., 1989; Kiechle-Schwarz et al., 1991, Ozisik et al., 1995). A pericentric inversion of chromosome 6 involving band p21 was observed in a UL case. We were also observed a paracentric inversion of chromosome 6 (bands p11-p21) in one cell (Table 1)(Figure 1). It is remarkable that we were able confirmed an association between the UL and this chromosomal site.

Loss of genetic material from 7q and rearrangements specifically involving band q22 have been found more consistently in UL than in any other solid tumors. In some studies, deletion of chromosome 7q is the most common cytogenetic abnormality in leiomyoma, and is critical for tumor development (Ozisik et al. 1993; Sreekantaiah et al., 1991). Both interstitial deletions and translocations involving chromosome 7q have been reported in lipomas and endometrial polyps (Dal Cin et al., 1995; Hennig et al., 1999). Hennig et al. showed that the most frequent clonal abnormalities were structural rearrangements involving deletions of 7q [del(7q21) and del(7)(q22:q32)] (Ozisik et al., 1993). We have also detected two deletions at q11.22-q11.23 and p15-ter regions of chromosome 7. An interstitial deletion of chromosome 7 involving bands q22-q32, is a common CA of UL, with an observed frequency of ~17% in karyotypical abnormal fibroids (Sargent et al., 1994; Ishwad et al., 1995). We also found an interstitial deletion at 7q11.22-q11.23 regions in one cell. This finding is also supported by reports in the literature. Together, these results suggest that del(7q) may have different effects on myoma growth. The deletions on 9q11, 9q12, 9q13 and 9q32 were particularly interesting in our patients as these regions of chromosome 9 have not been previously reported in UL. But, fragilities and breaks of 9q22 and 9q13 were found in patients with lung cancer (Viegas-Pequignot et al., 1990; Flüry-Herard et al., 1992). Our findings suggest that losses of chromosome 9q play a role in the pathogenesis of UL.

The fundamental importance of the 12q14-15 regions in benign neoplasia is supported by the occurrence of consistent rearrangements in numerous other solid benign tumors (lipoma, pleomorphic adenoma of the salivary gland, pulmonary chondroid hamartoma, endometrial polyps and epithelial breast tumors) (Bullerdiek et al., 1987; Mandahl et al., 1993). It may be observed as the sole cytogenetic abnormality, or together with other changes, and is often associated with t(12;14) or alterations of the chromosome segment 12q. The t(12;14)(q14-15;q22-24) translocation is the first chromosome alteration reported in UL, and found in approximately 20% of the abnormal cases. Deletions and chromatid breaks in bands q13.1-q13.2, q13 and q24-qter of chromosome 12 were observed in our patients. In some instances of this tumor type, the breakpoint have been found to involve the bands 12q13-15 (Turc-Carel et al., 1986; Turc-Carel et al., 1986).

We reported aneuploidies of chromosomes 3, 17, 18 and 22 (Table 1). In colorectal cancers, numerical aberrations of chromosomes 1, 7, 11, 17 and 18 were reported (Huang et al., 2002). FISH studies in cancer have identified non-random chromosomal gains and losses affecting chromosome 17, most often 17q gains and 17p losses (Squire et al., 2002; Veiga et al., 2003). However, we observed a high frequency (1% of all cells) of deletions, translocation, fragility, and aneuploidies for chromosome 17 in the patients (Table 1). Veiga et al. also showed a high frequency of monosomy for chromosome 17 by interphase FISH in three tumors (Nanashima et al., 1997). Our cytogenetic results as well as other previously reported findings suggest that losses of chromosome 17 play a role in the pathogenesis of UL. Several studies have reported LOH of chromosome 18q in colorectal cancers and inactivation of tumor suppressor genes, such as it was deleted in colorectal cancer and in pancreatic carcinoma (Fearon et al., 1990; Pandis et al., 1995; Hahn et al., 1996; Lefter et al., 2002). Trisomy 18 was frequently observed in breast cancers and neurofibrosarcomas (Nanashima et al., 1997). Furthermore, gain of chromosome 18 was found in patients with B-cell non-Hodgkin's lymphomas (Galteland et al., 2005). In accordance with these studies, our findings confirmed that loss and/or gain of chromosome 18 is important in development of UL.

The monosomy 22 in our patients was often observed. In other studies, two of the three UL cases have partial

or complete monosomy 22 (Quade et al., 2002; Hu et al., 1991), and all five specimens with intravenous leiomyomatosis also have monosomy 22 (Rein et al., 1991). But, it has not been previously reported that monosomy 22 contributes to the pathogenesis of fibroid tumors. However, chromosome 22 contains a considerable number of uncharacterized disease genes, e.g. familial schizophrenia susceptibility, glioblastoma and other types of astrocytoma, ependymoma, meningioma, schwannomatosis, pheochromocytoma, breast and colon cancer (Dumanski, 1996).

The X chromosome abnormalities have been reported with lower frequency in leiomyomata. These includes: del(X(p11.2), (X;12)(p22.3q15), -X, der(5)t(X;5)(p11;p15), del(X)(q12), der(X)t(X;3)(p22.3;q11.2) and inv(X)(p22q13) [6,11,23,31]. But, one of the main results in our study was sex chromosome changes (Table 1). The X chromosome losses and gains, iso(Xq), del(X)(p22.1-pter), del(X)(q26-qter), del(X)(q27-qter) and fra(Xp22.1) were seen in our patients, and the FS at band Xp22.1 were significantly overexpressed. It appears that the region of Xp11-p22 may be preferentially involved and important for detecting cancer development. Undoubtedly, further studies are necessary to understand the role of X chromosome changes in UL.

The results obtained in the present study indicate that myomas had a higher incidence of fragile sites (FSs). The fragility of the chromosome may be related to abnormalities in replication, resulting in single-strand DNA gaps, which, if not repaired, may lead to chromosome damage such as deletions within the FS, or translocations or other rearrangements involving breakage at a FS (Stein et al., 2002). Therefore, it may be considered that the expression of FS could be an indicator of chromosomal instability within the genome of myoma individuals. At the same time, myomas increase the potential for chromosome breakage at cancer sites in the genome, and it also may increase the risk for breakage or deletion in individuals. The form of heterochromatic segments, enlarged long arm, and secondary constrictions were observed at hsr(2)(p12;p21), hsr(2)(q13-q21), hsr(3)(q11-q13),1qh+, 9hsr+, 9qh+ and 15ps+ loci (Table 1). These are usually considered as polymorphisms, but their clinical consequences remain unclear.

It is now widely recognized that p53 may be the most frequently mutated protein in human cancer, implying that an alteration of p53 is a fundamentally important step in genomic instability and susceptibility to neoplastic state transformation (De Vos et al., 1994; Blom et al., 1998). Abnormalities in p53 in the form of missense mutation and/or loss of heterozygosity are common in UL (Hall et al., 1997; Niemann et al., 1995). However, little information is currently available regarding the content of p53 protein in human leiomyomas. Some of studies have shown a positive correlation between high levels of immunohistochemically demonstrable p53 gene protein product and the presence of leiomyosarcoma (De Vos et al., 1994; Amada et al., 1995, Jeffers et al., 1995). In general, leiomyomas do not contain immunohistochemically detectable p53 protein product, when many leiomyosarcomas do. Smooth muscle tumors of undetermined malignant potential have less consistently expressed p53 protein product. The results of p53 analysis have been variable, with the preponderance of data supporting a diagnostic utility for this marker. The present study also demonstrates that there are no apparent differences in p53 gene in UL. A similar observation was reported in another sex steroid-dependent tumor, uterine endometrioid carcinoma, in which there were no changes in p53 level between endometrioid carcinoma and the adjacent tissue (Li et al., 1996). Ours and other some studies have confirmed that there has been no significant association between p53 loss and UL.

In conclusion, we have demonstrated that loss in p53 tumor suppressor gene was not remarkably increased in UL patients. However, nine chromosomal regions can be arbitrarily used relevance criteria which appear promising for localizing susceptibility genes for UL: 1p36, 1q11, 1q42, 2p23, 3p, 5q31, 6q21, 9q, Xp22 and monosomi 22. The loci 1q11, 1q42, 2p23, 5q31, 6p21 and Xp22 expressed in our patients were not reported previously in UL. These abnormalities may be primer genetic lesions predisposing cells to tumorogenesis, and growth of myomas. The recurrence rate for UL in siblings support the familial heritability of this tumor. Accordingly, it is possible that cytogenetic instability will prove to have diagnostic and prognostic utility among uterine smooth muscle tumors.

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