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Genetic heterogeneity among uterine leiomyomata: insights into malignant progression

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Uterine leiomyomata (UL), also known as fibroids, are the most common pelvic tumors in women of reproductive age and are the primary indication for hysterectomy in the USA. Many lines of evidence indicate a strong genetic component to the development of these tumors. In fact, ~40% of UL have non-random, tumor-specific chromosome abnormalities which have allowed classification into well-defined subgroups (deletion of portions of 7q, trisomy 12 or rearrangements of 12q15, 6p21 or 10q22) as well as identification of candidate genes for UL predisposition. Although benign, UL have been linked to malignancy through two genomic regions on chromosome 1. Mutation of fumarate hydratase (*FH*) at 1q43 is known to cause the Mendelian syndromes of multiple cutaneous and uterine leiomyomata (MCL) and hereditary leiomyomatosis and renal cell cancer (HLRCC), and recently, *FH* mutations have been detected in some non-syndromic UL. In addition, transcriptional profiling suggests that loss of the short arm of chromosome 1 in cellular leiomyomata, an uncommon histological variant of UL, may account in part for the presumed yet rare malignant transformation of UL to uterine leiomyosarcoma.

INTRODUCTION

Uterine leiomyomata (UL), frequently referred to as fibroids, are the most common pelvic tumors of the female genital tract with peak onset in the third to fourth decades of life. Symptomatic disease occurs in 20–25% of reproductive-age women, although systematic histologic examination of hysterectomy specimens has shown a prevalence of UL as high as 77% with an average of 6.5 tumors per uterus (1). Despite their benign nature, UL have a significant level of morbidity manifesting as a spectrum of clinical symptoms related to the size and anatomic location of the tumors. Symptoms include urinary incontinence, constipation, menorrhagia, abdominal pain, difficult and extended menstrual periods and impairment of fertility (2,3).

Such high morbidity results in UL being the most common indication for hysterectomy, accounting for one-third (more than 200 000) of procedures, approximately one in five visits to a gynecologist, and expenditures of greater than 2.1 billion dollars in direct health care costs in 2000 in the USA (4–6). Alternative treatment options for women who wish to maintain their childbearing potential remain limited, frequently offer only a temporary reprieve and are associated with complications (7–11).

In this review, we summarize the genetics of UL and discuss the two areas of research linking benign UL to cancer.

GENETICS OF UL

The causes of fibroid growth and development remain largely unknown, although genetic liability has been supported by a variety of epidemiological, molecular and cytogenetic studies. A familial aggregation case control analysis determined that first degree relatives of affected probands have a 2.5-fold higher risk for developing UL, and the odds ratio increases to 5.7 after selecting for early onset cases (12). These findings reflect the trend of prior reports (13–15) and that genetically influenced traits generally exhibit an earlier age of onset (16). UL are also reportedly three to nine times more prevalent in black women than in white women (17–19), even after adjustment for known risk factors (18). In addition, monozygotic twins are twice as likely to be concordant for hysterectomy when compared with dizygotic twins (20,21), wholly consistent with the expected rates for a genetically influenced trait. Although multiple conditions may contribute to hysterectomy, UL is the most common

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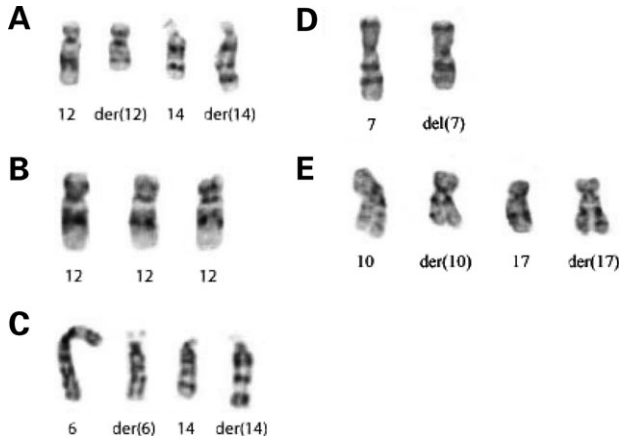

Figure 1. Partial GTG-banded karyotypes illustrating common chromosomal changes in UL. (**A**) t(12;14)(q14–15;q23–24) is the most frequent translocation in UL and usually results in translocation of *HMGA2* to the der(14) and its elevated expression. (**B**) Trisomy 12 provides three copies of *HMGA2*. (**C**) t(6;14)(q21;q24) increases expression of another HMGA family member, *HMGA1*, encoded at 6p21. (**D**) del(7)(q22q32) is the most frequent deletion in UL and results in the loss of a gene-rich region at 7q22. (**E**) t(10;17)(q22;q23) involves a region in 10q22 that disrupts *MORF*, likely creating a fusion gene with a sequence on chromosome 17. Modified version reprinted with permission from Cytogenetics in Reproductive Medicine. Ed: Dagan Wells. UL: Aspects in reproduction, susceptibility and tumor cytogenetics. Landes Bioscience. In press online (to be printed in March, 2007).

indication, making these findings in twins strongly suggestive of a genetic liability for UL.

Chromosomal and molecular analyses have shown that each UL is an independent monoclonal process (22–24) and that ~40% of UL have non-random and tumor-specific chromosome abnormalities (25,26). This has allowed classification of some UL into well-defined subgroups which include deletion of portions of 7q, trisomy 12 or rearrangements of 12q15, 6p21 or 10q22. Additional abnormalities, which appear consistently but not as frequently, include rearrangements of chromosomes X, 1, 3 and 13. The variety of chromosomal rearrangements, including but not limited to translocation, deletion and trisomy, predict different molecular genetic mechanisms for UL formation and growth.

Of karyotypically abnormal UL, ~20% have a rearrangement of 12q14–q15 and the majority are t(12;14)(q15;q23–24) (Fig. 1A), though other translocation partners observed include chromosomes 1, 2, 4, 5, 8, 10, 22 and X (27,28). The gene involved at 12q15 is *HMGA2* (29), a member of the high mobility group (HMG) family of abundant, non-histone components of chromatin which act as architectural factors to influence diverse cellular processes such as differentiation, death, growth and proliferation (30,31). The presence of t(12;14)(q15;q23–24) causes upregulation of *HMGA2* expression and a similar mechanism contributing to UL development may occur with trisomy 12 (Fig. 1B). Another member of the HMGA family, *HMGA1*, is located at 6p21 and represents an additional hotspot of cytogenetic rearrangement in UL (32). Rearrangements of 6p21 arise in <10% of karyotypically abnormal UL and include translocations with 14q23–q24 (Fig. 1C) and other partners as well as inversions (33).

An interstitial deletion of chromosome 7 most frequently involving bands q22–q32 occurs in ~20% of karyotypically abnormal UL (Fig. 1D). The pathogenic region in del(7) was proposed to be q22 based on translocations that disrupted this band (34), and the minimal deletion region has since been narrowed to a gene-dense <500 kb area in 7q22 (35). Although numerous candidate genes have been studied (36,37), none have proven to have a consistent causative role in the genesis of UL.

In ~5% of chromosomally abnormal UL, rearrangements affecting the long arm of chromosome 10 have been found. The majority are balanced translocations between 10q22 and a variety of chromosomal partners including 4, 6, 12 and 17 and less frequently a del(10)(q22q24) (27). Recently, a strong candidate gene emerged as 10q22 breakpoints were identified in four UL within the third intron of *MORF* (monocytic leukemia zinc finger protein-related factor or *MYST4*), a member of the MYST family of histone acetyltransferases; three of the UL had a t(10;17)(q22–q24; q21–q22) (Fig. 1E) (38).

FUMARATE HYDRATASE MUTATIONS IN UL ASSOCIATED WITH CANCER

Other evidence supporting the genetic basis of UL as well as establishing a link between UL and cancer is the discovery of two rare Mendelian syndromes. The first, Reed Syndrome (MIM 150800), also called multiple cutaneous and uterine leiomyomatosis (MCUL1 or MCL), is an autosomal dominant condition with incomplete penetrance first described in two kindreds in which three generations exhibited a combination of UL and cutaneous leiomyomata (39,40). The cutaneous tumors arise from the smooth muscle of erector pili in hair follicles typically in the early teens to fourth decade of life on the trunk or limbs and are often painful to touch or cold temperature.

In women with MCUL1, UL are observed at a higher prevalence, an earlier age of onset and usually are associated with severe symptoms. In fact, in one population of 48 women with cutaneous leiomyomas, 98% also had UL with a mean age of onset of 30 years. Eighty-nine percent of these women had a hysterectomy for UL symptom abatement, 57% of whom had a total hysterectomy at age ≤30 years (41). This is in contrast to non-syndromic UL in the general population which have a prevalence of 77%, with only 20–25% of cases being symptomatic, and the highest rate of hysterectomy occurring in women 40–44 years of age (1,18,42).

Renal cancer has also been shown to cluster with skin and UL initially in two kindreds with genetic linkage to 1q42–44. This finding led to a definition of a second Mendelian inherited syndrome, hereditary leiomyomatosis and renal cell cancer (HLRCC, MIM 605839) (43). Multiple additional studies have confirmed this association with either type II papillary or renal collecting duct carcinoma (44,45).

It is of note that a uterine leiomyosarcoma (ULMS), which may be the malignant counterpart of UL (46), was identified in a single individual in the original report describing MCUL1 (39). Since then, ULMS have been found in two women from one Finnish family with HLRCC (43) and was suspected

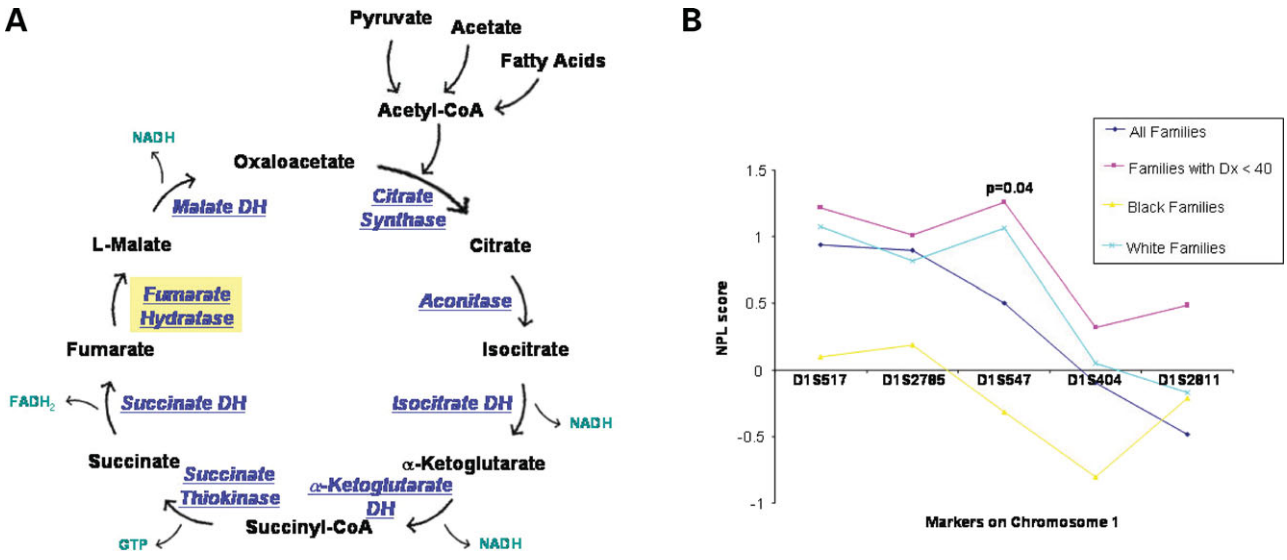

Figure 2. FH and predisposition to non-syndromic UL. (**A**) FH, mutated in MCUL1 and HLRCC and some sporadic tumors, is a component of the tricarboxylic acid (Krebs) cycle. DH, dehydrogenase. (**B**) An affected sister-pair non-parametric two-point linkage analysis of the 1q42–44 region (356 subjects, 123 pedigrees) using five markers (62). Inclusion of all families reached a maximum NPL score of 0.94 ($P = 0.14$) at marker D1S517. Stratification by race suggests linkage of UL to *FH* occurs in white women only. Data analysis of only those sister-pairs with at least one sister diagnosed before age 40 at marker D1S547 showed statistical significance with a NPL score of 1.26 ($P = 0.04$). These results indicate that *FH* could predispose to early onset non-syndromic UL in some white women.

in another woman from an unrelated Finnish kindred (44). As discussed below, biallelic inactivation of fumarate hydratase (*FH*) was also seen in a single non-syndromic ULMS (47). The ages of diagnosis of the latter four tumors were 30, 32, 35 and 39 years, which is earlier than in the general population where ULMS usually occur after the onset of menopause (48,49).

Interestingly, heterozygous germline mutations in *FH* at 1q42.1 have been detected in both HLRCC and MCUL1, confirming the allelic nature of these disorders. *FH*, which encodes an enzyme in the mitochondrial tricarboxylic acid (Krebs) cycle (Fig. 2A), appears to act as a tumor suppressor because its measured activity is very low to absent in tumors from individuals with MCUL1/HLRCC (45,50). These tumors follow the classic Knudson 'two-hit' model as loss of heterozygosity (LOH) was found in skin, uterine and renal tumors of affected individuals (43–45,50). The fact that there is no difference in the mutation spectrum seen in individuals with MCUL1 and those with HLRCC suggests that other genetic or environmental modifying factors play a role in determining susceptibility to renal cancer in addition to mutation of *FH* (51).

At this time, *FH* mutations have been identified in 37 of 46 (80%) UK and three of three Finnish kindreds examined (45,50) as well as in 31 of 35 (89%) North American families (41) and five of five additional MCUL1 families (52). Thus, 76 of 89 total kindreds analyzed worldwide were found to have mutated *FH* (85%). Although this suggests the possibility of genetic heterogeneity, detection of decreased *FH* activity in four of the UK probands who did not have identifiable *FH* mutations argues against this idea (45). In addition, some studies used single-strand conformation polymorphism analysis or conformation-specific gel electrophoresis as mutation detection methods (50), both of which have lower sensitivity than direct sequencing. The type of *FH* gene alterations detected reflect the loss of enzyme activity and include missense, frameshift, nonsense mutations and whole gene deletions (51). These mutations occur throughout the gene and no genotype–phenotype correlations have been observed (41).

It remains unknown how loss of *FH* expression promotes tumorigenesis. It is not however unprecedented that tumors result from disruption of a TCA cycle component as germline mutations in subunits of succinate dehydrogenase were observed in patients with non-syndromic hereditary head and neck paraganglioma and pheochromocytoma (53,54). It has been hypothesized that *FH* mutation could induce cancer through TCA cycle-mediated mitochondrial dysfunction, triggering both increased free radical generation and energy depletion. This in turn could cause oxidative DNA damage, loss of energy-dependent apoptosis and/or activation of hypoxic pathways that induce growth factor transcription (55).

In contrast to MCUL1/HLRCC, which have one germline mutation with subsequent somatic loss of the second allele resulting in tumorigenesis, germline loss of both alleles is known to cause autosomal recessive fumarate hydratase deficiency (FHD, MIM 136850) (56,57). This rare inborn error of metabolism is characterized by gross developmental delay, encephalopathy, hypotonia, seizures, cerebral atrophy and death in the first decade. Skin, uterine or renal tumors have not been reported in this recessive condition, however, most affected individuals survive only a few months and very few survive into the third decade of life. Obligate carrier mothers of individuals with autosomal recessive FHD have been reported to develop UL (50).

Identification of *FH* in inherited, syndromic forms of neoplasms has led to investigations of whether *FH* mutations also play a role in the more common simplex or sporadic presentation of UL and ULMS. Two reports have shown a very low *FH* mutation rate (47,58). In the first analysis, no *FH* mutations were detected in 26 ULMS or 129 UL examined, although LOH for 1q42–43 was found in 54% of ULMS and 5% of UL. ULMS LOH involved the entire region probed, reflecting extensive loss of the long arm of chromosome 1; sequence analysis in this study was only directed to find exonic and splice site mutations (58). In the second report, sequencing of only the exons of *FH* in sporadic tumors including uterine and cutaneous leiomyomata as well as a range of malignant growths found biallelic inactivation in three tumors. These included one ULMS and one cutaneous leiomyomata, each harboring a germline *FH* mutation, and one soft tissue sarcoma of the lower limb for which both mutations were acquired, resulting in this sarcoma representing the only reported neoplasm with biallelic somatic *FH* mutations at that time (47).

Recent studies show a more prominent role for *FH* in non-syndromic UL. A series of 299 malignant tumors (representing 10 different types) were negative for *FH* mutation while LOH at 1q43 was identified in five of 153 UL tested (3.3%). Subsequent sequencing for detection of the putative second alteration in the remaining allele revealed a 'second hit' somatic *FH* mutation in two UL, although defects in regulatory regions of *FH* would not have been uncovered. The somatic nature of these mutations was confirmed through analysis of germline DNA. These data indicate that *FH* is the target of the 1q43 deletions and thus *FH* is specifically involved in a small subset of non-syndromic UL (59). This low but significant rate of LOH for non-syndromic UL was confirmed by both a genome-wide allelotyping study which found LOH for the 1q41–42.13 region in 2% of 102 UL (60), and by an analysis of 24 UL which revealed 4% had LOH of 1q42–q44 markers (44). Chromosomal rearrangements in UL affecting 1q42 have also been reported (61,62), but they are infrequent (63).

We recently performed an affected sib-pair linkage analysis of the 1q42–44 region in 123 families containing at least one sister pair with UL to determine if *FH* mutations predispose women to developing non-syndromic UL (62). Evidence suggestive of genetic linkage to *FH* was found among women less than 40 years of age at diagnosis (Fig. 2B). Fluorescence *in situ* hybridization (FISH) analyses were also used to detect loss of one copy of *FH* in nine of 11 non-syndromic UL with 1q rearrangements. As indicated by Lehtonen *et al*. (59), the presence of LOH is often accompanied by a small mutation on the other *FH* allele, however, the FISH method used in this study could not address this possibility. In addition, mosaicism was detected for the 1q rearrangements which could explain why LOH for *FH* has been infrequently identified in non-syndromic UL. Thus, loss of *FH* may be a significant event in the pathobiology of a subset of non-syndromic UL. In fact, a recent microarray analysis comparing seven UL with *FH* mutations to 15 UL with wild-type *FH* found 297 differentially expressed genes (64).

OTHER DISTINCT DISORDERS WITH OVERLAPPING FINDINGS

It is noteworthy that UL are included as part of a minor criteria (genito-urinary tumors) for the diagnosis of another Mendelian disorder, Cowden syndrome (CS, MIM 158350). This autosomal dominant condition is characterized by multiple hamartomas of the skin, breast, thyroid, oral mucosa and gastrointestinal tract and by a risk of breast, thyroid, endometrial and renal cell carcinomas. Eighty percent of CS cases result from mutations of *PTEN* (65). Although no relationship has been identified between *PTEN* and UL development, it remains to be determined whether CS cases without *PTEN* mutations represent a genetically heterogeneous subgroup that might share a pathogenetic mechanism with UL. Also of interest and in this light, another hamartomatous lesion of mesenchymal origin, pulmonary chondroid hamartoma, frequently involves rearrangements of *HMGA* genes as do benign lipomas which represent another minor criteria for CS diagnosis (66).

LEIOMYOSARCOMA AND 1P DELETIONS IN CELLULAR UL

It has been estimated that a risk of $<0.1\%$ exists for progression of UL to ULMS (46). However, the dichotomy of the extremely rare ULMS being derived from the highly prevalent and numerous benign UL raises some intriguing questions. In fact, UL are present in 77% of reproductive age women with an average of 6.5 tumors per uterus (1), whereas ULMS are very rare with an estimated annual incidence of 0.64 per 100 000 women (67).

UL are characterized histologically by whorled bundles of smooth muscle cells forming well-circumscribed nodules with scant and normal mitoses (46) whereas ULMS are typified by necrosis, high proliferative activity, nuclear and cytologic atypia and hypercellularity. Variant smooth muscle tumors which have one of the key histologic features normally associated with ULMS but a benign clinical course also occur, but no clear genotype has been correlated with any histomorphologic phenotype.

Furthermore, no unifying chromosomal aberrations have been found commonly in UL and ULMS. In fact, ULMS are karyotypically highly complex relative to UL (Fig. 3A), which tend to be genetically stable and either karyotypically normal or have a single simple aberration. Recurrent chromosome abnormalities have been observed in ULMS and one of the most common is 1p13–1pter translocations and deletions (68–75).

The repetitive finding of 1p rearrangements in ULMS is particularly interesting because our examination of more than 800 karyotyped UL led to the identification of a new cytogenetic subgroup when nine tumors were found to have loss of the majority of the short arm of chromosome 1 [i.e. del(1)(p11p36)]. Of eight tumors with 1p- available for histologic analysis, five were diagnosed as cellular UL (hypercellular) and one as an atypical UL (hypercellular and nuclear atypia). Ten additional archival UL specimens with either cellular or atypical histology were selected for LOH

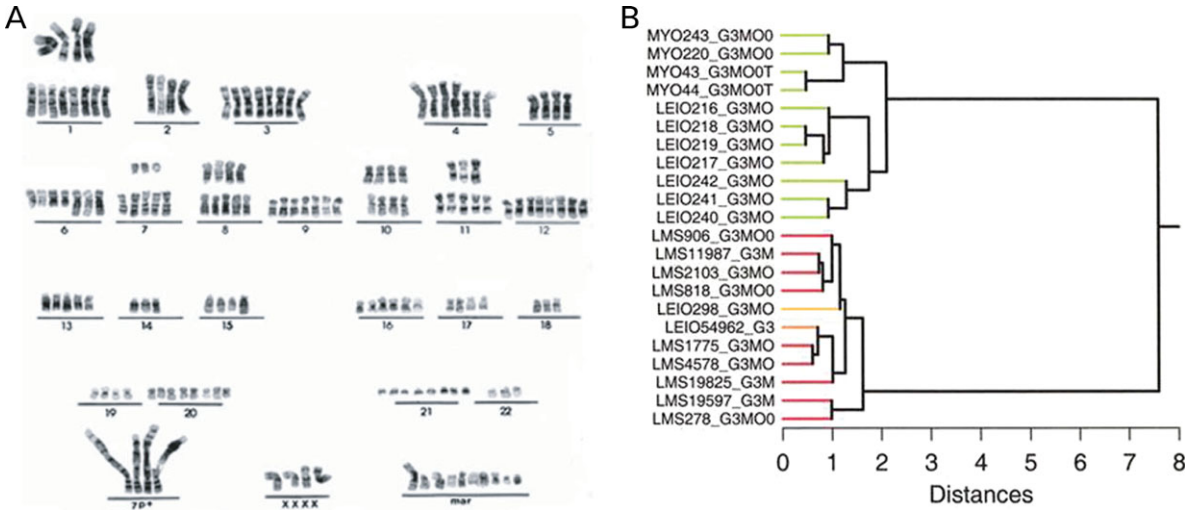

Figure 3. Clustering of 1p- UL with ULMS. (**A**) ULMS are karyotypically complex and genetically unstable relative to benign UL. (**B**) Comparison of the expression profiles of two UL with deletions of 1p (LEIO298_G3MO and LEIO54962_G3) to a previously defined smooth muscle tumor expression profile using hierarchical clustering (76). The horizontal length of each arm reflects the relatedness of clusters. Samples with green terminal segments are myometrium or benign UL without 1p-, those with red terminal segments are malignant leiomyosarcoma and those with yellow terminal segments are UL with 1p-. Sample identifiers are listed as MYO (myometrium), LEIO (typical UL), LMS (leiomyosarcoma). Part A is reprinted from Obstet Gynecol Clin N Am, 33, Lobel MK, Somasundaram P and Morton CM, The Genetic Heterogeneity of UL, 13–39, 2006, with permission from Elsevier.

analysis of 1p36.23 and 1p21.1; half of these cases demonstrated allelic loss for either or both regions indicating that 1p- occurs in a subset of UL with variant histology (76).

A useful tool applied in the above-mentioned study was a recently reported gene signature capable of resolving the spectrum of uterine smooth muscle neoplasms. Specifically, 147 genes in a hierarchical cluster analysis separated samples into benign versus malignant histotypes and further subdivided the myometrium from benign UL (77). This gene list was applied in a cluster analysis of two of the eight 1p- cellular variant UL and revealed that the expression profiles of both 1p- UL were more similar to ULMS than to the profiles of myometrium or UL without 1p- karyotypes (Fig. 3B). This finding is complemented by two cases of UL with cellular histology and ring 1 chromosomes [r(1)(p34q32) and r(1)(p31q44)], the formation of which would result in deletion of portions of the long and short arms of chromosome 1, including *FH* in the former (25).

These data raise the fascinating idea that ULMS do indeed derive from UL and the discrepancy in their frequency lies in the fact that only rare histological and karyotypic variants of UL may be amenable to malignant progression (76). Indeed, the large number of random chromosomal aberrations in ULMS suggests that genomic instability is involved in the pathogenesis of these tumors, and such instability hinders efforts to identify the primary change(s) that may now be discovered through studies of variant UL.

Based on the diversity of chromosomal findings in both UL and ULMS, it is generally accepted that genetic heterogeneity underlies UL formation and may also occur in malignant transformation of UL. For example, two of three UL with t(10;17) involving formation of a fusion gene with a member of the MYST family of histone acetyltransferase called *MORF* (monocytic leukemia zing finger protein-related factor) at 10q22 were of the cellular subtype (38). Further, a t(10;17)(q22.1;p13) has been reported as the sole abnormality in one ULMS (78), and a t(10;17) was identified as part of a complex karyotype in another (75). Expression profiling of UL with t(10;17) would be invaluable in assessing its malignant potential with respect to the earlier described gene signature. In sum, transformation of UL to ULMS may occur via rare UL that have 1p-, t(10;17) and, doubtless, yet to be discovered abnormalities.

CONCLUDING REMARKS

Much remains to be understood about the relationship between benign mesenchymal tumors and their malignant counterparts, which is illustrated well by UL. Basic questions remain about how the extremely common UL may give rise to the exceedingly rare malignant ULMS. What genetic steps are required? Perhaps of greater importance, how is the rate of malignant transformation maintained at such a low frequency for ULMS in relation to UL? Recent exciting data have generated the hypothesis that a small subset of UL with variant histology and/or karyotype may represent a premalignant transitional state while other UL have greatly reduced malignant potential. Perhaps consideration of whether such a mechanism may underlie the relationship between other benign and malignant tumors of mesenchymal origin is warranted.

Cancer has also been associated with UL through the Mendelian syndrome hereditary leiomyomatosis and renal cell cancer, raising concern whether women with UL are being properly evaluated for their risk for renal cancer or ULMS. At this time it would seem judicious for clinicians to inquire of their patients with UL about personal and family history of skin leiomyomas and renal cell cancer, particularly those women with an early onset of multiple UL. Indeed, identification of MCL/HLRCC patients is of

importance not only to screen for the related malignancies but also because these women will have a poor prognosis for their UL with a high likelihood of hysterectomy at a younger age. Nonetheless, relative to the rate of UL formation in the population, the risk for MCL/HLRCC is minimal, necessitating caution to avoid unnecessary testing or alarm.

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Conflict of Interest statement. None declared.

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