

## The Genetic Bases of Uterine Fibroids; A Review

Veronica Medikare<sup>1</sup>, Lakshmi Rao Kandukuri<sup>2</sup>, Venkateshwari Ananthapur<sup>3</sup>, Mamata Deenadayal<sup>4</sup>, Pratibha Nallari<sup>1\*</sup>

1- Department of Genetics, Osmania University, Hyderabad, India

2- Center for Cellular and Molecular Biology, Habsiguda, Hyderabad, India

3- Institute of Genetics and Hospital for Genetic Diseases, Begumpet, Hyderabad, India

4- Infertility Institute and research Center, Secunderabad, India

### Abstract

Uterine leiomyomas/fibroids are the most common pelvic tumors of the female genital tract. The initiators remaining unknown, estrogens and progesterone are considered as promoters of fibroid growth. Fibroids are monoclonal tumors showing 40-50% karyotypically detectable chromosomal abnormalities. Cytogenetic aberrations involving chromosomes 6, 7, 12 and 14 constitute the major chromosome abnormalities seen in leiomyomata. This has led to the discovery that disruptions or dysregulations of *HMGIC* and *HMGII* genes contribute to the development of these tumors. Genes such as *RAD51L1* act as translocation partners to *HMGIC* and lead to disruption of gene structure leading to the pathogenesis of uterine fibroids. The mechanism underlying this disease is yet to be identified. The occurrence of *PCOLCE* amid a cluster of at least eight Alu sequences is potentially relevant to the possible involvement of *PCOLCE* in the 7q22 rearrangements that occur in many leiomyomata. *PCOLCE* is implicated in cell growth processes. Involvement of Alu sequences in rearrangements can lead to the disruption of this gene and, hence, loss of control for gene expression leading to uncontrolled cell growth. This can also lead to the formation of fibroids. Though, cytogenetics provides a broad perspective on uterine fibroid formation, further molecular analysis is required to understand the etiopathogenesis of uterine fibroids.

\* Corresponding Author:

Pratibha Nallari,  
Department of Genetics,  
Osmania University,  
Hyderabad, 500 007,  
India

E-mail:

prathinallari@yahoo.com

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### Introduction

Uterine leiomyomas (UL), commonly known as fibroids, are non-cancerous tumors arising from the myometrium (smooth muscle layer) of the uterus. In addition to smooth muscle, leiomyomas are also composed of extracellular matrix (i.e., collagen, proteoglycan, fibronectin). Leiomyomas are the most common solid pelvic tumor in women, causing symptoms in approximately 25% of women of reproductive age. However, with careful pathologic inspection of the uterus, the overall prevalence of leiomyomas increases to over 70%, because leiomyomas can be present without symptoms in many women. Leiomyomas

are usually detected in women in their 30's and 40's and shrink after menopause in the absence of post-menopausal estrogen replacement therapy (1).

Leiomyomas arise from the overgrowth of smooth muscle and connective tissue in the uterus (2). There are two components to myoma development; first the transformation of normal myocytes to abnormal myocytes and their growth into clinically apparent tumors. Apart from their tumorigenic potential, they are morphologically similar at the cellular level to normal myometrial smooth-muscle cells (MSMCs). Leiomyomas may

have single or multiple mutated smooth-muscle tumor nodules of varying size attached and/or within the myometrium that are encircled by varying amounts of extracellular fibrous connective tissue. Microscopic determinations reveal they have interlacing bundles of spindle-shaped or stellate smooth-muscle cells with little cellular pleomorphism or mitotic activity (3).

The identity of the factor(s) and molecular mechanisms involved in the cellular transformation of myometrial cells into leiomyoma remains unknown. Evidence also exists supporting the involvement of genomic instability influencing genes such as estrogen and progesterone receptors. Several genomic and proteomic studies have also provided evidence for altered molecular environment of leiomyomas compared to the normal myometrium, as a possible biomarker in their proliferation and regression (4).

Global gene expression profiling of uterine leiomyomas (ULMs) revealed that hundreds of genes were dysregulated including those with functional roles in cell proliferation, differentiation and extracellular matrix production. So far, only a few specific genes or cytogenetic aberrations have been identified to be associated with ULMs. While many of the dysregulated genes may function as either effectors or promoters of ULMs growth, they are likely to be secondarily induced and indirectly responsible for tumor growth into morbid and symptomatic ULMs (5).

**Classification of leiomyomas:** Leiomyomas are classified by their location in the uterus. Subserosal leiomyomas are located just under the uterine serosa and may be pedunculated (attached to the corpus by a narrow stalk) or sessile (broad-based). Intramural leiomyomas are found predominantly within the thickness of myometrium but may distort the uterine cavity or cause an irregular external uterine contour. Submucous leiomyomas are located just under the uterine mucosa (endometrium) and, like subserosal leiomyomas, may be either pedunculated or sessile. Tumors in subserosal and intramural locations comprise the majority (95%) of all leiomyomas; submucous leiomyomas make up the remaining 5% (1).

The clinical sequelae of leiomyomas, which may depend on their location within the uterus, may be associated with a spectrum of symptoms, including excessive menorrhagia, severe abdominal

pain, urinary incontinence, and constipation. Leiomyomas may lead to infertility, spontaneous abortions, premature labor, or dystocia. Progression of leiomyoma to malignant leiomyosarcoma (LMS) is very rare (6).

The present review focusses on the cytogenetic and molecular aspects which contribute to the pathogenesis of uterine leiomyomas. Here the genetics of leiomyomata and the current understanding of cytogenetic aberrations and gene expression with respect to their contributions to the development of these tumors is summarized.

**Risk factors:** Risk factors are characteristics associated with a condition generally identified by epidemiological studies. There is a suggestion of slightly increased risk of fibroids associated with early menarche (7 - 9). The early onset of menstrual cycles may increase the number of cell divisions that the myometrium undergoes during the reproductive years, resulting in an increased risk for mutations in gene/s controlling myometrial proliferation (10, 11).

An increase in age has been demonstrated to increase the prevalence of fibroids during the reproductive years (12). Hormonal factors associated with pre-menopause may be important modulators for the development of fibroids during late reproductive years; alternatively this may also be due to the cumulative culmination of 20-30 years of stimulation by estrogen and progesterone. The other factors involved are menopause, obesity, diet, exercise, racial differences, geographic differences, oral contraceptives, hormone replacement theory, etc (13).

Apart from these, hyperinsulinemia is considered as a risk factor since insulin may influence UL development through direct promotion of myometrial smooth muscle cell proliferation or by increasing circulating levels of ovarian hormones (14).

**Theories of fibroid formation:** Despite the major public health impact of leiomyomas, little is known about their cause. The most important aspect of the etiology of fibroids - the initiator(s) - remains unknown. Several theories have been advanced. One hypothesis states that increased levels of estrogen and progesterone result in an increased mitotic rate that may contribute to myoma formation by increasing the likelihood of somatic mutations (15). Another favors an inher-

ent abnormality in the myometrium of individuals who develop fibroids, based upon the finding of significantly increased levels of estrogen receptors (ER) in the myometrium of fibroid uteri (13).

More recently, growth factors have been shown to mediate the growth-promoting effects of estrogen and play an important role in the development of fibroid tumors (1). Growth factors, proteins/polypeptides produced locally by smooth muscle cells and fibroblasts control the proliferation of cells and appear to stimulate myoma growth, primarily by increasing the extracellular matrix. Some of the identified myoma-related growth factors are transforming growth factor- $\beta$  (TGF- $\beta$ ), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and prolactin (16).

Growth factors affect cells in complex ways, and the response to combinations of growth factors may be different from the response to an individual factor. Many of these growth factors are overexpressed in myomas and either increase smooth muscle proliferation (TGF $\beta$ , bFGF), increase DNA synthesis (EGF, PDGF), stimulate synthesis of extracellular matrix (TGF- $\beta$ ), promote mitogenesis (TGF- $\beta$ , EGF, IGF, prolactin), or promote angiogenesis (bFGF, VEGF) (16).

The steroid hormones, estrogen and progesterone, are considered the most important regulators of leiomyoma growth.

**Estrogen:** There is abundant evidence that estrogen promotes fibroid growth, including the clinical observations that fibroids grow in the presence of high levels of estrogen, such as during the reproductive years, or they regress in the presence of low levels of estrogen, such as following menopause or during gonadotropin releasing hormone (GnRH) agonist therapy (1).

Furthermore, because the risk of fibroids is greater in nulliparous women who might be subject to a higher frequency of anovulatory cycles and obese women with greater aromatization of androgens to estrone in the adipose tissue, the concept of unopposed estrogens, as an underlying cause of uterine fibroids, has been proposed in the literature (17, 18). Increased growth of myomas among women taking tamoxifen or receiving transdermal or injectable estrogen replacement

therapy further supports the importance of estrogen. The estrogen hypothesis has also been supported by clinical trials evaluating the medical treatment of myomas with GnRH agonists, the effective result of which is hypoestrogenism accompanied by regression of the fibroids (19).

**Progesterone:** Progesterone is also thought to play a role in fibroid growth which is supported by clinical studies. For example, fibroid size increases during treatment with synthetic progestones. In contrast to GnRH agonist therapy alone which has been shown to reduce uterine volume, combination of GnRH agonist and progesterone therapy has been shown to have no effect on uterine volume. The observation that fibroids regress with the administration of anti-progesterone agent, RU-486, further supports the role of progesterone as a promoter of fibroid growth. Histologically, fibroids from patients treated with progesterone show more cellular growth than those from patients without progesterone therapy. Altogether, these data suggest that progesterone also enhances fibroid growth (1).

**Estrogen and progesterone receptors:** In the majority of the reviewed studies, the concentrations of both the estrogen receptors (ERs) (at 6q25.1 and 14q) and progesterone receptors (PRs) (at 11q22) were greater in leiomyomata than the myometrium. In addition, researchers found the ER and PR to be elevated in fibroids during all phases of the menstrual cycle when compared with matched myometria. Interestingly, in one study the ER and PR levels were significantly higher in submucous than subserosal leiomyomas, leading the authors to speculate about different etiologies and types of leiomyomas (13).

**Leiomyomas as clonal tumors: X-inactivation studies:** Analyses of multiple leiomyomas from a single uterus have demonstrated that the tumors can harbor different chromosomal changes and suggest that each tumor can develop independently. X-inactivation studies, based on the phenomenon of lyonization, i.e., inactivation of one X-chromosome in normal female cells, have demonstrated that leiomyomas develop as clonal lesions. Initially, glucose-6-phosphate dehydrogenase (G6PD) isoenzyme analysis was used to demonstrate the independent clonal origin of multiple tumors in a single uterus (6).

Another, more informative, approach based on

the CAG repeat polymorphism in the X-linked androgen receptor gene has been used to examine clonality and the results confirmed the monoclonal nature of leiomyoma. A study of a patient with 2 independent leiomyomas, each showing a different pattern of X-chromosome inactivation but with identical del(7)(q21.2q31.2) derivative chromosomes, supports the view that identical cytogenetic changes in multiple leiomyomas from the same patient may represent recurrent chromosomal aberrations in smooth muscles or they may be coincidental. Cytogenetically mosaic tumors were also reported to be clonal (6).

Studies on the inactivation of X-chromosome-linked phosphoglycerokinase (PGK) showed that all studied leiomyomas had a single type of inactive allele and were of unicellular origin but independently generated in the uterus (6). Based on the monoclonal pattern of X-inactivation in the karyotypically normal cells in leiomyomas, Mashal *et al.* (20) suggested that these cells were part of the tumor clone and that clonal expansion of tumor cells might precede the development of cytogenetic changes in some leiomyomas.

The discovery of heterogeneity of chromosomal aberrations is consistent with the multistep hypothesis of tumor development, in which the function (or dysfunction) of several genes at multiple loci results in fibroid growth. Abnormalities at several loci have been documented in individual tumors, and this heterogeneity may explain clinicopathologic differences seen in fibroids, including variation in size or response to hormonal treatments. (21).

**Cytogenetic studies:** Standard karyotyping has been used to detect chromosomal aberrations such as deletions, duplications and translocations which require the culture of leiomyoma cells to obtain metaphase preparations.

An alternative method that has been employed in a few studies (22, 23) is comparative genome hybridization, which permits the recognition of cytogenetic changes such as deletions and amplifications without the need for cell cultures, although it does not allow detection of balanced rearrangements. Neither standard karyotyping nor comparative genomic hybridization permits the detection of small submicroscopic chromosomal abnormalities such as point mutations or epigenetic changes such as methylations (13).

Approximately 40% of UL have non-random and tumor-specific chromosome abnormalities. 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 (24). A tendency for karyotypically abnormal leiomyomas to be more cellular and to have a higher mitotic index than do chromosomally normal tumors, has been reported (6).

Although no relationship between the patient age or parity and the type of chromosomal abnormality has been identified, a few studies have found a positive correlation between the presence of a cytogenetic abnormality and the anatomic location of uterine leiomyomas, i.e., intramural (35%) and subserous (29%) leiomyomas are more likely to have abnormal karyotypes than the submucous (12%) type. Another study, showed a relationship between karyotype and leiomyoma size, with the largest tumors carrying t(12;14) abnormalities. In contrast, tumors with del(7) were found to be smaller and those with mosaic karyotypes were intermediate in size (13).

The abnormal karyotypes in leiomyoma were frequently accompanied by 46, XX, i.e., cytogenetically normal female cells. Although this may represent concomitant growth of normal cells, it is possible that these apparently normal cells may have undergone neoplastic transformation, with the chromosome changes appearing subsequent to this transformation (6).

**The t(12;14)(q15;q23~q24) in leiomyoma:** The most common chromosomal aberration in leiomyoma, seen in approximately 20% of karyotypically abnormal leiomyomas, is the characteristic translocation, t(12;14) (q15;q24), specifically associated with leiomyoma. Other rearrangements involving 12q14~q15, such as paracentric inversions, have also been reported. Rearrangements involving the same region of 12q in leiomyoma and other benign solid tumors (e.g., angiomyxomas, breast fibroadenomas, endometrial polyps, hemangiopericytomas, lipomas, pulmonary chon-

droid hamartomas, and salivary gland adenomas) support the notion that critical genes reside within 12q14~q15 (6).

Besides involvement of chromosome 12 in the t(12;14), trisomy 12 is not an uncommon cytogenetic change in leiomyomas. Leiomyomas with normal karyotypes may have cryptic inversions of 12q. Though 12q15 is often part of t(12;14) in leiomyoma and a der(14)t(12;14)(q15;q23~q24) is seen in most leiomyomas, rearrangements of chromosomes 1, 5, 8, and 10 do not infrequently accompany those of 12q15 (6).

**Chromosome 7 changes in leiomyoma:** Several karyotyping studies have identified the region 7q22~q32, comprising about 30 Mbp, as being commonly deleted (25). Early studies reported deletions of chromosome 7 from band q11.23 to q36. (21). Although interstitial deletions and translocations involving chromosome 7q have also been reported in lipomas and endometrial polyps, the deletion is more commonly observed in fibroids than in any other solid tumor (13). Previous reports of inversions and translocations involving 7q22 suggest that a region within this cytogenetic band is critical for the pathobiology of uterine leiomyomas (21). In addition, del(7) may be associated with t(12;14) or t(1;6), suggesting involvement of del(7) in the karyotypic evolution of leiomyoma (6).

An interstitial deletion of chromosome 7, del(7)(q22q32) is observed with a frequency of about 17% in karyotypically abnormal leiomyomas. Leiomyomas with chromosome 7 deletions or translocations are usually found in the mosaic state with 46, XX cells. Apparent negative selection for these aberrant cells in tissue culture results in frequent loss of the del(7)(q22q32) cells. Cells with the del(7)(q22q32) are more likely to persist in cultures when the t(12;14) is also present (6).

The instability of del(7)(q22q32) cells without chromosome 12 aberrations is intriguing in light of the observation that fibroids with chromosome 12 abnormalities or rearrangements are often larger than tumors with chromosome 7 abnormalities, and larger fibroids are more likely to be chromosomally abnormal than smaller ones. Observations of tumor instability in culture and decreased tumor size in association with the del(7)(q22q32) suggest that a gene regulating cellular growth may reside in 7q22. Dissection of 7q22 to

study leiomyoma-specific sequences is complicated by the fact that this is a gene-dense region including genes involved in developmental processes (*DLX5*, *DLX6*) and collagen metabolism (collagen type 1, procollagen C-endopeptidase enhancer), as well as those that encode acetylcholinesterase, plasminogen activator inhibitor type 1, and mucin (21).

The occurrence of deletions in both chromosome homologues in the same region strongly suggests the involvement of a tumor suppressor gene in the development or progression of uterine leiomyomas (26).

**Rearrangements of 6p21 in leiomyoma:** Rearrangements of 6p21 have been observed in benign mesenchymal tumors, including lipomas, pulmonary chondroid hamartomas, endometrial polyps, and leiomyomas. In leiomyomas, these rearrangements occur with a frequency of 5% and include t(1;6)(q23;p21), t(6;14)(p21;q24), and t(6;10)(p21;q22), as well as inversions and translocations with other chromosomes (6).

**Other cytogenetic abnormalities:** Other cytogenetic abnormalities of lower frequency than those above have been reported in leiomyomas. These include changes of the X-chromosome, including del(X)(p11.2), t(X;12)(p22.3;q15), -X, der(5)t(X;5)(p11;p15), del(X)(q12), der(X)t(X;3)(p22.3;q11.2), and inv(X)(p22q13). Although either the short or long arm of the X chromosome is rearranged, the region Xp11~p22 may be preferentially involved. Structural rearrangements of chromosome 1 in leiomyoma, particularly in the form of ring chromosomes usually occur concomitantly with other chromosomal changes and are, therefore, thought to represent secondary abnormalities. Other structural aberrations of chromosome 1 detected in leiomyoma include t(1;6)(q23;p21) and t(1;2)(p36;p24). Monosomy 10 and deletions of 10q (especially band q22) have been detected in leiomyoma. A number of rearrangements of chromosome 3 have been observed in leiomyoma, both as sole abnormalities or accompanying other rearrangements including: ins(2;3)(q31;p12p25), del(3)(p14), del(3)(q24), and t(3;7)(p11;p11). A subgroup of leiomyomas demonstrating aberrations of 13q has been described with deletions in that arm possibly playing a primary role in the development of some leiomyomas (6).

**Molecular studies in uterine leiomyoma:** In studies on the molecular genetic changes in leiomyomas, much attention has been given to the possible involvement of genes located in chromosomal regions affected by recurrent changes, which are discussed below.

**HMGA genes:** High-mobility group I (HMGI) proteins, including those coded by the *HMGA2* (*HMGIC*) and *HMGAI* (*HMGII*) genes, are expressed predominantly during embryonic development and are generally silenced in adult tissues. The *HMGA2* and *HMGAI* genes encode closely related, low-molecular-mass proteins; HMGA proteins which are capable of binding to the minor groove of AT rich DNA with three DNA-binding domains (the so called AT-hooks), thus inducing conformational changes in chromatin structure and enabling the regulation of the expression of various target genes. In addition, they can interact with other proteins by means of their acidic domain (27).

*HMGAI* and *HMGA2* map to chromosomal bands that are targeted by non-random structural chromosomal abnormalities found in uterine leiomyomas, i.e., 6p21 for *HMGAI* (28) and 12q14-15 for *HMGA2* (29, 30). Usually, these regions are affected by chromosomal translocations but inversions can occur as well with structural chromosomal aberrations affecting 12q14-15, being much more frequent than those affecting 6p21. The molecular alterations resulting from the cytogenetic deviations generally seem to include an upregulation of the genes (31, 32).

*HMGA2* and *HMGAI* are both closely related as well as highly conserved (96% conservation between mouse and human *HMGA2* homologues) (21).

**HMGA2:** The entire *HMGA2* gene spans approximately 160 kb, including a 327 bp coding region, an 854 bp 5' untranslated region (UTR) and a 2966 bp 3' UTR, which together encode a 4.1 kb mRNA. The first three exons of *HMGA2* are clustered within 13 kb of each other and together they encode a DNA-binding domain comprising the AT hook motif. A large intron (approximately 140 kb) between exons 3 and 4 separates the DNA-binding domains from the rest of the protein. Exon 4 encodes an 11-amino acid spacer domain that is absent in *HMGAI* and

precedes a 13 kb intron that separates it from the acidic domain encoded by exon 5 (21).

Chromosomal breakpoints have been assigned to regions outside the open reading frame of *HMGA2* gene, thus primarily affecting its expression rather than its protein sequence (33). Previous studies have shown that *HMGA2* is abnormally overexpressed in many benign and malignant neoplasms, particularly in uterine leiomyomas. Although its role in tumorigenesis is not well understood, overexpression of *HMGA2* is found to be associated with large tumor size in leiomyomas, suggesting its role in promoting tumor growth (34). Some leiomyomas overexpress the *HMGA2* structural transcription factor, which has been shown to cause dysregulation of several genes in leiomyomas. However, no correlations have been found between the levels of *EGR-1* and *HMGA2* in leiomyomas. Furthermore, *HMGA2* transcripts may be truncated in the COOH-terminal in leiomyomas due to chromosome 12 changes and still be pathogenetic. A comparison of *HMGA2* expression in leiomyomas versus LMS of various origins revealed a higher percentage of the former (70% vs. 58%). Relevant observation is trisomy 12 in leiomyomas, which would potentially upregulate *HMGA2* expression by a gene dosage mechanism (6).

MicroRNAs such as let-7 family are important small molecules known to participate in the fine-tuning of functional gene activity (34). *HMGA2* is also predicted as one of the genes targeted by the let-7 family, and overexpression of let-7b in leiomyoma cells resulted in the suppression of *HMGA2* expression (35). *HMGA2* is one of the major targets of Let-7 microRNAs, and large leiomyomas have high levels of *HMGA2*, hence, it is speculated that dysregulation of the Let-7:*HMGA2* regulatory mechanism may be one of key genetic events promoting leiomyoma growth. Characterization of Let-7 repression of *HMGA2* in leiomyomas can provide a better understanding of *HMGA2* function. *HMGA2* overexpression and a loss of Let-7 microRNA expression are more common in large leiomyomas. Peng et al. proposed that disrupting the pairing between Let-7 and *HMGA2* was an important molecular mechanism in promoting leiomyoma growth (34).

**HMGA1:** The smaller (10 kb) *HMGAI* gene con-

sists of eight exons: exons 1 to 4 are noncoding, exons 5 to 7 contain the AT hooks corresponding to exons 1 to 3 of *HMGA2*, and exon 8 encodes the acidic C-terminus of the protein. Alternate splicing of this gene results in two products, one of which is a variant lacking a 33 bp segment between exons 5 and 6, which is also absent from *HMGA2*. Despite the great structural similarity between *HMGA2* and *HMGAI*, expression patterns of the two proteins are strikingly dissimilar, perhaps suggesting the existence of distinct regulatory elements as well as different functional roles (21).

The *HMGAI* gene has been mapped to 6p21, a site of recurrent chromosomal rearrangements in benign mesenchymal tumors, e.g. lipoma, hamartoma, and leiomyoma. HMGAI protein is known to bind to specific AT-rich domains and promoters of a number of diverse genes. A single genomic clone including *HMG1Y* was found to span the breakpoint in a uterine leiomyoma with a complex rearrangement involving chromosome region 6p21.3 (36). Subsequent studies demonstrated rearrangement of the *HMG1Y* locus in hamartoma of the breast (37), a pericentric inversion of chromosome 6 involving band p21 in a uterine leiomyoma (38), and a translocation leading to an intergenic fusion with the *LAMA4* gene (laminin a4) in a pulmonary chondroid hamartoma (39).

**RAD 51L1:** *RAD51L1*, a member of recombination family mapping to 14q23-24, is a strong candidate as a translocation partner for *HMGA2* in leiomyoma. *RAD51L1/HMGIC* chimeric transcripts were first identified in SV-40 transformed cell lines containing this translocation (40). Ingraham *et al.* (41) found the premature truncation of the *RAD51L1* major transcript in the primary culture of a leiomyoma. The RAD51L1 protein is physically associated with other RAD51-like proteins and contains motifs for nucleotide binding, which may play a role in cell cycle regulation and apoptosis. *RAD51L1* plays a role in DNA repair recombination. *RAD51L1* phosphorylates a number of proteins, including TP53, cyclin E, and CDK2, suggesting that *RAD51L1* affects cell cycle progression (6).

Ingraham *et al* 1999 speculated that disruption of the *RAD51L1* gene involved in the t(12;14) (q15;q24), results in the loss of the exon encoding the 3' end of the predominant exon of that gene

which might be an important step in the early pathogenesis of leiomyoma. They were unable to detect expression of a fusion gene resulting from the t(12;14) and speculated that alterations of the relative expression levels of the various RAD51L1 isoforms may be a significant effect of the translocation. Although, studies showed that RAD51L1 is directly interrupted by a translocation breakpoint in only one case, translocation breakpoints previously characterized in 2 other leiomyomas map within the BCR that contains this gene, and could affect its expression through alteration of the translocated chromosome (41).

The majority of 12q15 breakpoints in leiomyomas were found to be located 5' in the *HMGA2* locus, a minority at the 3' region (usually outside the *HMGA2* coding region) or with 5' and 3' breakpoints flanking *HMGA2*. Fusion transcripts of 5' region of *HMGA2* with the 3' region of *RAD51L1* were detected with a breakpoint 3' to *HMGA2*; the derived sequences indicated that the breakpoints resided within the 3' untranslated region of *HMGA2* with the fusion transcripts containing the full coding sequence of *HMGA2* (6).

Though fusion transcripts involving *HMGA2* and *RAD51L1* were seen in a small percentage of leiomyomas with 5' breakpoints, *HMGA2* transcripts as well as those of *RAD51L1* were present in all tumors with t(12;14). With the breakpoint 5' (centromeric) relative to *HMGA2*, the entire gene should be translocated to the der(14), and all of the sequence telomeric to *HMGA2* should be derived from chromosome 12. Exons 8–11 of *RAD51L1* included in a potential *HMGA2/RAD51L1* fusion product would be located either upstream (i.e., centromeric) to *HMGA2* on the der(14) or on the der(12) remote from *HMGA2*, depending on the position of the chromosome 14 breakpoint (6).

Partner genes for *HMGA2* other than *RAD51L1* are: the *COX6C* gene at 8q22~q23, the *ALDH2* gene at 12q24.1, the enhancer of invasion (*HEI10*) gene at 14q11, and the *RTVL-H 3'* gene on chromosome 12, respectively. It is possible that these leiomyomas were associated with either variant translocations involving chromosome 12 with a chromosome other than 14, or with a complex translocation with a chromosome additional to chromosomes 12 and 14 or a chromosomal band other than 12q14~q15 (6).

**PCOLCE:** The human *PCPE* gene (*PCOLCE*) has been mapped to 7q22 (42), an area frequently disrupted in uterine leiomyomata (43). It is a small gene measuring 6.0 kb with a conserved intron/exon structure comprising 9 exons. Wherein the NH<sub>2</sub>-terminal CUB domain (CUB1) is encoded by exons 2 and 3, whereas the second CUB domain (CUB2) is encoded by exons 4 and 5 and by part of exon 6. The remainder of exon 6 encodes a region that shows low sequence homology between human and mouse *PCPEs*. This region probably represents a “linker” region that connects the two conserved and functionally distinct portions of *PCPE*: the NH<sub>2</sub>-terminal CUB domains that provide full C-proteinase enhancing activity and the COOH-terminal domain that appears capable of inhibiting matrix metalloproteases. The COOH-terminal domain is encoded by the last three exons of *PCOLCE*. In *PCOLCE* the 3' exon contains 164 bp of coding sequence and 71 bp of 3' untranslated region (42).

The occurrence of *PCOLCE* amid a cluster of at least eight Alu sequences is potentially relevant to the possible involvement of *PCOLCE* in the 7q22 rearrangements that occur in many leiomyomata, as recombination events involving Alu sequences have previously been found to be involved in generating various gross rearrangements of genes and chromosomes (44). The *PCPE* gene has been implicated in the control of cell growth and differentiation, and the chromosomal location of *PCOLCE* at 7q22 places it at a site where chromosomal rearrangements are associated with leiomyomata (43).

**Transforming growth factor-β:** The TGF-β family comprises five dimeric polypeptides encoded by distinct but closely related genes. TGF-βs are multifunctional growth factors that regulate many aspects of cellular function including proliferation, differentiation, ECM production, and chemotaxis (45). Expression of all the three types of TGF-β, as well as TGF-β receptors I–III, has been detected in human myometrial tissue (13).

TGF-β<sub>3</sub> has been shown to cause increased proliferation of both leiomyoma and myometrial cells, and adherence of a neutralizing antibody to this peptide causes a decrease in proliferation. Leiomyomas express up to six fold higher levels of mRNA for TGF-β<sub>3</sub> than do normal myometrial cells at all stages of the menstrual cycle (45). The

expression of transforming growth factor β (TGF-β) receptor type II is the target of miR-21 in leiomyoma smooth muscle cells (LSMCs). TGF-β is a key profibrotic cytokine that mediates its biological activities by binding to TGF-β receptor (TGF-βR) types I to III, of which types I and II are transmembrane proteins with a cytoplasmic serine/threonine kinase domain. TGF-β and TGF-β receptors as well as their intracellular signaling pathways are overexpressed in leiomyoma compared with that in myometrium. The consequence of a lower expression of miR-21 in leiomyoma and LSMCs compared with that in myometrium might represent the loss of one of the regulatory mechanisms resulting in unregulated expression of TGF-β receptor and increased TGF-β activities (4).

Thus, TGF-β<sub>3</sub> may contribute to the enhanced growth of leiomyomas by increasing cell proliferation as well as potentially increasing ECM production (45). In view of the probable role of this growth factor in fibroid pathophysiology, it is of particular interest that the gene coding for TGF-β<sub>3</sub> is located near the 14q23-24 breakpoints, one of the most common translocation sites identified in cytogenetic studies of fibroids (13).

### Conclusion

Uterine fibroids are the most common benign tumors of the female genital tract, with the etiology of leiomyomas still remaining unknown. Cytogenetic abnormalities indicate different genetic pathways by which fibroids grow and develop. Although most of uterine leiomyomas have a normal karyotype, reports suggest that 50% of these tumors bear specific chromosomal aberrations that include chromosome 3, 6, 7, 13, trisomy 12, reciprocal translocation between chromosomes 12 and 14 and monosomy 22. Such chromosomal rearrangements may be responsible for initiation as well as the growth of these tumors.

The cytogenetic abnormalities indicated to date shows genetic heterogeneity among these tumors. Further molecular analyses would help to identify putative candidate genes in uterine leiomyomata formation. Identification of genes involved may aid in the genetic diagnosis of the condition, prediction of genetic risks and management of the condition by appropriate therapeutic measures.

Alu elements have been associated with genetic diseases, mainly by providing sites for homologous recombination which result in deletions or other more complex genomic rearrangements. This phenomenon can lead to evolution of novel karyotypes. Since Alu sequence influences rearrangement, the candidate genes disrupted by these rearrangements in UL can be mapped using BAC clones in the form of cytochips. With the advent of microarray technology, the development of cytochips can help to detect abnormalities with a much greater resolution. In addition to identifying the genomic changes, the use of cytochips would give a better understanding of genes which are involved in the pathogenesis of UL. This information would help in identifying genotype-phenotype correlations.

An important avenue of research involves studying affected women and their first-degree relatives who also have uterine fibroids. The availability and examination of such individuals not only will hasten cytogenetic and molecular studies, but will also be crucial to dissecting and defining the genetic loci that undoubtedly contribute to the development of uterine leiomyomata. The development of uterine fibroids involves complex interaction among genes and environment; further understanding and uncovering of the genetic and environmental mechanisms responsible for uterine leiomyoma will aid in designing rational gene-therapy protocols.

On the basis of our current state of knowledge, one can only speculate upon the initiators of this common condition. Future research efforts may provide a better understanding of the causes and mechanisms of uterine fibroid tumorigenesis. Insights resulting from elucidation of the basic biology of these tumors might then be successfully translated into preventative strategies that will reduce the incidence and/or morbidity of this disease.

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