Genomics of uterine leiomyomas: insights from high-throughput sequencing

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Uterine leiomyomas are benign smooth-muscle tumors of extremely low malignant potential. Early work utilizing classical cytogenetics revealed that a subset of uterine leiomyomas harbor recurrent chromosomal rearrangements, such as translocations affecting the *HMGA2* gene. Our understanding of the genetics of many tumor types has deepened remarkably with the emergence of next-generation sequencing technologies. Exome sequencing identified that the majority of leiomyomas display highly specific *MED12* mutations. Further studies suggest that these *MED12* hotspot mutations are also frequent in breast fibroadenomas, but not in other human tumors. Whole-genome sequencing showed that a subset of leiomyomas display complex chromosomal rearrangements resembling chromothripsis. These were formed in a single event of chromosomal breakage and random reassembly involving one or a limited number of chromosomes. Although most leiomyomas have been shown to arise independently, these studies also revealed that distinct nodules within a uterus may display identical genetic changes indicating a common clonal origin. A minority of leiomyomas were also found to display deletions within the *COL4A5-COL4A6* genes, leading to upregulation of the adjacent gene *IRS4*. The findings derived from high-throughput sequencing combined with previous knowledge have led to an emerging molecular classification of leiomyomas, suggesting that there are several distinct pathogenic pathways involved in leiomyoma formation. The evidence points to at least 4 molecular subclasses: leiomyomas with *MED12* mutation, *FH* inactivation, *HMGA2* overexpression, and *COL4A6-COL4A5* deletion. Elucidating the molecular pathogenesis of leiomyomas should be relevant for developing treatments for this very common disease. (Fertil Steril® 2014;102:621–9. ©2014 by American Society for Reproductive Medicine.)

Key Words: Uterine leiomyoma, *MED12*, chromothripsis, *CUX1*, *COL4A5-COL4A6*, *HMGA2*

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Uterine leiomyomas, or fibroids, are benign tumors composed of disordered smooth-muscle cells surrounded by an abundant extracellular collagenous matrix. These tumors represent a major public health problem and are the leading cause of hysterectomy worldwide (1). By age 50 years, the incidence of leiomyomas is nearly 70% for Caucasian women and more than 80% for African-American women (2). Approximately 15%–30% of these women present severe symptoms, such as heavy menstrual bleeding, pelvic pressure and pain, pregnancy complications, and even infertility (3, 4). Leiomyomas can be classified into submucous, subserous, and intramural according to their location relative to the layers of the uterus (3). An average symptomatic uterus displays 6–7 individual tumor nodules, and the spectrum of symptoms depends highly on their size, number, and location (3, 5). Estrogen and progesterone promote leiomyoma growth; hence, they appear in women during their reproductive years. Growth factors, cytokines, and chemokines are potential effectors of these hormones (6).

Familial, epidemiological, and cytogenetic studies all indicate that genetic factors play an important role in the initiation and development of uterine leiomyomas (7–9). Further support is provided by the observed ethnic disparities in the incidence and clinical presentation of leiomyomas, as African-American women have larger and more numerous tumors, present with more-severe symptoms, and are diagnosed at an earlier age than Caucasian women (2, 10, 11).

Uterine leiomyomas may on rare occasions be associated with familial tumor susceptibility syndromes, in

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particular hereditary leiomyomatosis and renal cell cancer (HLRCC; 12). Dominantly inherited HLRCC is caused by heterozygous loss-of-function mutations in the *fumarate hydratase* (*FH*) gene, which encodes the tricarboxylic cycle enzyme fumarase (13). Tumors from these patients typically harbor biallelic loss of *FH*, which has been reported in only 1.3% of sporadic leiomyomas (14). A recent genome-wide association study (GWAS) identified polymorphisms at 3 chromosomal loci (10q24.33, 22q13.1, and 11p15.5) to be associated with increased susceptibility to uterine leiomyomas among Japanese women (15). A genome-wide linkage and association study of Caucasian cohorts implicated *fatty acid synthase* (*FASN*) on chromosome 17q25.3 as being involved in predisposition to leiomyomas (16).

Decades of work using classical cytogenetics have revealed that approximately 40% of uterine leiomyomas harbor chromosomal aberrations, a subset of which are recurrent and tumor-specific (9). These include rearrangements of 12q15 and 6q21, and deletions within 7q, which occur in approximately 20%, 5%, and 17% of the karyotypically abnormal lesions, respectively. Other recurrent abnormalities, although less frequent, are monosomy 22 and rearrangements involving 1p36 and 10q22 (9, 17–21). *HMGA2* and *HMGA1* (*High-mobility group AT-hook*) are key target genes of tumors carrying 12q15 and 6q21 rearrangements (22), typically resulting in their upregulation (23). These *HMGA* genes encode small nonhistone chromatin-binding proteins linked to a variety of tumor-promoting functions, including inactivation of p53-induced apoptosis, impairment of DNA repair, and modulation of epithelial–mesenchymal transition (24). *DNA repair protein RAD51 homolog 2* (*RAD51B*), located on chromosome 14q24, is the most frequent translocation partner of *HMGA* rearrangements and encodes for a protein involved in DNA double-strand break repair by homologous recombination (25). Deletions within the long arm of chromosome 7 have been detected both as the only aberration present and as a secondary alteration among tumor cell subpopulations (26). *Cut-like homeobox 1* (*CUX1*), the most likely target of 7q alterations, is located within one of the minimally deleted regions and has been found to be disrupted by inversions (27).

Next-generation sequencing technologies have made it possible to generate high-resolution sequencing data in less time and at lower cost than ever before (28). This technology has revolutionized the study of genetic variation in the human genome. Below, we summarize how discoveries from high-throughput sequencing of uterine leiomyomas have improved our understanding of this very common disease.

HIGH-THROUGHPUT SEQUENCING

Exome Sequencing

***MED12* hotspot mutations in uterine leiomyomas.** Exome sequencing is a powerful tool for identifying mutations located in the protein-encoding parts of the genome, called the exons (29). In 2011, recurrent somatic mutations in *MED12* were identified through exome sequencing of 18 uterine leiomyomas from Finnish (Caucasian) patients (30). Further analysis of a total of 225 tumors from 80 patients revealed that a striking 70% of uterine leiomyomas display *MED12* mutations, making it the most frequently altered gene in leiomyomas. All the identified mutations clustered in an evolutionary conserved region of the gene, in exon 2 and the intron 1–exon 2 junction, suggesting that aberrant functioning of the encoded region contributes to tumorigenesis. Both missense and in-frame insertion–deletion mutations (ranging in size from 1 to 13 amino acids) were observed with a notable predominance of single base substitutions in codon 44. Of note, none of the mutations resulted in a truncated protein product.

This finding has since been validated and replicated in several studies representing various ethnic groups, reporting *MED12* mutation frequencies between 48% and 92% (Table 1). In addition to previous reports, *MED12* exon 1 mutations—affecting the same conserved region of the protein as

TABLE 1

Frequency of *MED12* mutations in uterine leiomyomas, histopathological leiomyoma variants, and uterine leiomyosarcomas.

Ethnicity/nationality	Common leiomyomas	Histopathological uterine leiomyoma variants	Uterine leiomyosarcomas	Reference
Caucasian	159/225 (70)			(30)
	47/80 (58.8)			(31)
	6/9 (66.6)	0/5 (0)	2/10 (20)	(32)
			3/41 (7)	(33)
	10/21 (47.5)		1/14 (7.1)	(34)
	11/19 (57.9)		1/7 (14.3)	(35)
	41/69 (59.4)	18/103 (17.5)		(36)
	11/12 (91.7)			(37)
	138/164 (84.1)			(38)
South African	14/28 (50)			(39)
Korean	35/67 (52.2)		0/5 (0)	(40)
Japanese	36/45 (80)	3/14 (21.4)	2/12 (17)	(41)
North American	100/148 (67)			(42)
	9/12 (75)	0/1 (0)	3/13 (23.1)	(43)
	15/28 (54)		6/20 (30)	(44)
	133/178 (74.7)		3/32 (9.4)	(45)

Note: Values are proportion (%).

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