Med12 gain-of-function mutation causes leiomyomas and genomic instability

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Uterine leiomyomas are benign tumors that can cause pain, bleeding, and infertility in some women. Mediator complex subunit 12 (MED12) exon 2 variants are associated with uterine leiomyomas; however, the causality of MED12 variants, their genetic mode of action, and their role in genomic instability have not been established. Here, we generated a mouse model that conditionally expresses a Med12 missense variant (c.131G>A) in the uterus and demonstrated that this alteration alone promotes uterine leiomyoma formation and hyperplasia in both WT mice and animals harboring a uterine mesenchymal cell–specific Med12 deletion. Compared with WT animals, expression of Med12 c.131G>A in conditional Med12-KO mice resulted in earlier onset of leiomyoma lesions that were also greater in size. Moreover, leiomyomatous, Med12 c.131G>A variant–expressing uteri developed chromosomal rearrangements. Together, our results show that the common human leiomyoma–associated MED12 variant can cause leiomyomas in mice via a gain of function that drives genomic instability, which is frequently observed in human leiomyomas.

Introduction

Uterine leiomyomas, or fibroids, are benign tumors arising from smooth muscle cells of the uterus. They are clinically diagnosed in 25% of women of reproductive age and are often associated with dysmenorrhea, dyspareunia, menorrhagia, infertility, and miscarriages (1, 2) and are the single largest cause of hysterectomy. Leiomyomas are monoclonal in origin, and 40% of the tumors have karyotypic abnormalities, including deletions in chromosome 7, trisomy of chromosome 12, and rearrangements involving the HMGA1 (6p21) and HMGA2 (12q14) genes (3–5). Whole-exome approaches have identified heterozygous mutations in the mediator complex subunit 12 (MED12, Online Mendelian Inheritance in Man [OMIM] 300188) in approximately 70% of leiomyomas in patients from various ethnic and racial groups (6, 7). The majority of identified mutations occur in exon 2 of MED12.

MED12 is located on the X chromosome and encodes a 250-kDa protein that is a subunit of the large mediator complex and is involved in transcriptional regulation of the RNA polymerase II complex. The MED12 protein is highly conserved among eukaryotes (8) and plays an important role during embryogenesis, as Med12-null mouse embryos arrest at E7.5 due to impaired mesoderm formation (9). Despite the high prevalence of MED12 mutations within human uterine leiomyomas, their causality and mode of action are not well understood. Here, we show that the common Med12 variant associated with human leiomyomas, Med12 c.131G>A, can drive tumor formation alone in a gain-of-function manner and causes genomic instability.

Results and Discussion

Conditional loss of function of Med12 does not lead to uterine hyperplasia or leiomyomas. We first determined whether the conditional inactivation of Med12 causes leiomyomas. Since Med12 is expressed from the X chromosome, random X chromosome inactivation will lead to random expression of either the paternal or maternal Med12 locus in uterine myometrial cells. We crossed anti-Mullerian hormone receptor type II–driven Cre (Amhr2-Cre) (10) with Med12flo/² animals (9) to generate Med12flo/² Amhr2-Cre animals and studied the effects of Med12 deficiency in a subpopulation of uterine mesenchymal cells. The use of Med12flo/² animals, in which 1 allele is floxed and the other is WT, allowed us, in the presence of Amhr2-Cre recombinase, to generate a mosaic population of cells that either express or lack Med12.

Since Amhr2-Cre acts well after X chromosome inactivation is established (E6.5) (11), loss of Med12 function will not lead to skewed X inactivation in mouse uteri. To assess the Cre recombination in our hands, we crossed Amhr2-Cre mice with double-fluorescent Cre-reporter mT/mG mice (12), which express red fluorescence in all tissues and green fluorescence upon Cre recombination. Given our results (Supplemental Figure 1, A and B; supplemental material available online with this article; doi:10.1172/JCI81534DS1), we determined that approximately 60% of uterine mesenchymal cells underwent Cre-mediated excision. Recombination of the Med12flo allele and reduction of Med12 mRNA transcripts were confirmed in Med12flo/² Amhr2-Cre uteri (Supplemental Figure 1, C–E). Neither leiomyoma formation nor hyperplasia were observed in adult uteri of Med12flo/² Amhr2-Cre mice (Supplemental Figure 1, G and I). These results indicate that Med12 loss of function is not a mechanism of leiomyoma formation.

Expression of the Med12 c.131G>A variant on a background of conditional Med12 KO causes leiomyomas. The most common MED12 mutation in leiomyomas among American women is a
c.131G>A variant can cause uterine leiomyomas in mice on a WT background. We investigated whether leiomyoma-like lesions were also present when the Med12R wt/+ female mice were crossed with Med12R mt/+ females to conditionally excise at 1 locus, or silenced by X chromosome inactivation at the other locus. Transcription from the mutant X chromosome (X<sup>mut</sup>) is indicated with an arrow, and the promoter region is depicted in green. The blue star indicates the floxed Med12 allele on the X chromosome (X<sup>fl</sup>), which, in the presence of Amhr2-Cre, will lose exons 1–7. In the cells in which the Med12 floxed allele is inactivated, WT Med12 will be expressed. In cells with an active Med12 floxed allele, only mutant Med12 will be expressed. The red chromosome indicates the inactivated X chromosome. (C) Eighteen-week-old multiparous Med12<sup>fl/fl</sup> Med12R<sup>mt/mt</sup> Amhr2-Cre uterus revealed a 4-mm tumorous lesion (outlined by white dotted lines). (D) Histological examination confirmed the presence of a large leiomyoma nodule growing from the smooth muscle layer of the uterus. A higher-magnification image of the black-boxed neoplastic area appears in E and shows the presence of fascicles with plump spindle cells, eosinophilic cytoplasm, and ECM deposits. (F) Twenty-four-week-old Med12<sup>fl/fl</sup> Med12R<sup>mt/mt</sup> Amhr2-Cre multiparous female uterus showing multiple nodules (white arrows). (G) Multiple leiomyoma nodules are outlined by black dotted lines, and the black box, shown at higher magnification in H, highlights fibrosis and ECM deposition. Approximately 80% (16 of 20 females) of the uteri exhibited leiomyoma-like lesions and hyperplasia. LM, leiomyoma; ES, endometrial stroma; MV, myometrium. Scale bars: 2,000 μm (C and F), 1,000 μm (D), 500 μm (G), 100 μm (H), 50 μm (E).
In 8-week-old Med12Rmt/+ Amhr2-Cre mice, no leiomyoma-like lesions were observed (Supplemental Figure 5B). Fifty percent of the uteri from Med12Rmt/+ Amhr2-Cre–mutant mice that were over 12 weeks of age showed hyperplasia and leiomyomas, characterized by ECM deposition and a disorganized pattern of smooth muscle fiber arrangement (Supplemental Figure 5D). Uteri that expressed mutant Med12 weighed 20% to 30% more than did control uteri (P < 0.05) (Supplemental Figure 5E).

Examination of uteri from mice that were beyond 12 weeks of age revealed nodules that histologically resembled human leiomyomas due to deposition of ECM, whorl formation, and fewer nuclei (Figure 2, C and E). Our results show that the Med12 missense variant c.131G>A causes uterine hyperplasia and leiomyomas in both Med12 WT (Med12Rmt/+ Amhr2-Cre) and conditional KO (Med12fl/+ Med12Rmt/+ Amhr2-Cre) mice. Med12 c.131G>A variant penetrance was 47% in mice on a WT background, while it reached 80% in mice on the conditional KO background. In mice on the conditional Med12 deletion background, leiomyoma-like lesions tended to have earlier onset and achieve greater size. The Med12 missense c.131G>A variant, therefore, acts as a gain-of-function mutation.

Med12 mouse mutations and genomic instability. Chromosomal rearrangements occur in 40% of human leiomyomas, and our data indicate that over 60% of uterine leiomyomas with an abnormal karyotype harbor Med12 mutations (7). To assess the genomic profiles of the Med12-mutated mouse tumors, we conducted array comparative genomic hybridization (aCGH) on 4 uteri with leiomyoma-like lesions (Figure 1) and compared the profiles with those of uteri from littermate controls without Cre (Med12Rmt/+ Med12Rmt/+ Amhr2-Cre). All 4 tumors showed genic copy number gains and losses (40 per tumor), with mouse chromosomes 2, 7, 14, and 17 being most frequently affected. The affected regions often consisted of genes targeting cell cycle checkpoints or tumor pathways such as Ras, Wnt/β-catenin, Tp53/Rb, NF-κB, and TGF-β signaling. The complete list of aberrations in the uteri of Med12fl/+ Med12Rmt/+ Amhr2-Cre females is shown in Supplemental Table 1. Microarray analysis of Med12fl/+ Med12Rmt/+ Amhr2-Cre model 2 (Med12Rmt/+ Amhr2-Cre). A subset of cells that express Amhr2-Cre will express the Med12 c.131G>A variant from the autosomal ROSA locus in the presence of X chromosome WT Med12. Transcription from a mutant autosome (Awt) is shown with an arrow, and the promoter region is depicted in green. The Med12 c.131G>A variant is depicted with a blue star. The red chromosome indicates the inactivated X chromosome.

Figure 2. Med12Rmt/+ Amhr2-Cre uteri develop prominent leiomyomas. (A) Mouse model 2 (Med12Rmt/+ Amhr2-Cre). A subset of cells that express Amhr2-Cre will express the Med12 c.131G>A variant from the autosomal ROSA locus in the presence of X chromosome WT Med12. Transcription from a mutant autosome (Awt) is shown with an arrow, and the promoter region is depicted in green. The Med12 c.131G>A variant is depicted with a blue star. The red chromosome indicates the inactivated X chromosome. (B and D) Uteri from Med12Rmt/+ control mice that, in the absence of Amhr2-Cre, did not express the Med12 c.131G>A variant and showed normal cross-sectional histology. (C and E) Uteri from Med12Rmt/+ Amhr2-Cre mice that expressed the Med12 c.131G>A variant and revealed leiomyoma-like lesions in approximately 47% (8 of 17) of the females, with a typically sparse nuclear arrangement, a nodular pattern of cellular growth, and ECM deposition (black dotted lines). EM, endometrium. Scale bars: 500 μm (B and C), 100 μm (D and E).

Table 1. Med12fl/+ Med12Rmt/+ Amhr2-Cre uteri chromosomal aberrations and corresponding human syntenic regions implicated in human leiomyomas

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gain/loss</th>
<th>Size (kb)</th>
<th>Genes of interest in region</th>
<th>Human syntenic loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>1qH5</td>
<td>Mosaic gain</td>
<td>104</td>
<td>Rbabg2 – TGF-β signaling; Ian2 – cell cycle checkpoint network; Bnpil1 – estrogen metabolism</td>
<td>1q41</td>
</tr>
<tr>
<td>1qD</td>
<td>Mosaic loss</td>
<td>108</td>
<td>Hjurp – maintenance of genomic stability</td>
<td>2q37</td>
</tr>
<tr>
<td>4qD2.3</td>
<td>Mosaic loss</td>
<td>137</td>
<td>Sts54, Map3k6 – MAPK/c-Jun signaling</td>
<td>1p36.1-p35</td>
</tr>
<tr>
<td>6qB1</td>
<td>Mosaic gain</td>
<td>105</td>
<td>Pslg1 – ERM receptors; Pss3 – cell division</td>
<td>7q34</td>
</tr>
<tr>
<td>4qD2</td>
<td>Gain</td>
<td>40</td>
<td>Adam28 – fibronectin receptor; Adam7 – collagen receptors</td>
<td>8p21.2</td>
</tr>
<tr>
<td>14qD3</td>
<td>Gain</td>
<td>133</td>
<td>Pcdh17 – tumor suppression</td>
<td>13q21</td>
</tr>
<tr>
<td>7qA3.3</td>
<td>Mosaic gain</td>
<td>450</td>
<td>Btd9 – Tp53 network; Glot – NF-xB network; Dlpfr – cAMP signaling</td>
<td>6p21.3-p21.3</td>
</tr>
<tr>
<td>18qA1</td>
<td>Mosaic gain</td>
<td>133</td>
<td>Fzd8 – Wnt/β-catenin network; Ccny – cell cycle regulator; Ccat1 – chromosome segregation</td>
<td>18p11.21</td>
</tr>
<tr>
<td>18qA1</td>
<td>Loss</td>
<td>212</td>
<td>Thocl – G2/M cell cycle checkpoint activator/apoptotic pathway</td>
<td>18p11.32</td>
</tr>
</tbody>
</table>

Chr, chromosome.
Amhr2-Cre uteri also showed a few genomic regions with a pattern consistent with focal chromothripsis-like alterations (ref. 14 and Supplemental Figure 6A).

Approximately 50% of the mouse aberrations had syntonic counterparts on human chromosomes (Supplemental Table 2), and a number of these regions are known to be rearranged in human leiomyomas (Table 1). For example, mouse chromosome 17qA3.3, duplicated in Med12fl/+ Med12Rmt/+ Amhr2-Cre female mice on human chromosomal loci. (A) Genomic duplication observed on mouse chromosomal locus 17qA3.3 is syntenic to the human 6p21 locus (shown in blue). A representative array profile of the 17qA3.3 region, highlighting the 450-kb duplication (chr17: 30586287–31049473), is also shown. (B) Genomic deletion observed on the mouse 4qD2.3 locus is syntenic to the human chromosomal locus 1p36.1–p35. The mouse deletion encompasses 137 kb and is shown in the respective array profile (chr4: 132799884–132936192). Positions are displayed approximately to scale according to the hg19 and mm9 physical maps, respectively.

Tests of significance were performed using a 2-tailed Student’s t test (GraphPad Software). Statistical significance was defined at a P value of less than 0.05.
**Study approval.** All procedures were approved by the IACUC of the University of Pittsburgh and conducted in accordance with NIH guidelines for the care and use of laboratory animals.

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