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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 HMGAI (6p21) and HMG2 (12q14) genes (3–5). Whole-exome approaches have identified heterozygous somatic 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 Med12fl/fl animals (9) to generate Med12fl/fl Amhr2-Cre animals and studied the effects of Med12 deficiency in a subpopulation of uterine mesenchymal cells. The use of Med12fl+/− 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 Med12fl allele and reduction of Med12 mRNA transcripts were confirmed in Med12fl/− Amhr2-Cre uteri (Supplemental Figure 1, C–E). Neither leiomyoma formation nor hyperplasia were observed in adult uteri of Med12fl/− 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
nonsynonymous variant, c.131G>A, predicted to substitute a highly conserved glycine with aspartic acid (p.Gly44Asp) (7). We investigated whether this Med12 mutation causes leiomyoma formation by generating a floxed Med12-mutant knockin mouse model (Supplemental Figure 2, A and B). We engineered the c.131G>A variant into the mouse Med12 cDNA (Med12m) fused with a FLAG tag, subcloned it into the pROSA26-DV1 vector, and integrated it into the autosomal ROSA26 genomic locus. The presence of the FLAG reporter allowed us to distinguish the expression of mutant Med12 from WT Med12 (Supplemental Figure 2, D and F). The mice generated were heterozygous for mutant Med12 cDNA at the ROSA26 locus (Med12Rm/mt). We mated Med12Rm/mt with Amhr2-Cre mice to conditionally express the mutated Med12 (c.131G>A) as early as E13.5 in the mouse uterine mesenchyme (10).

We investigated whether uterine leiomyomas will form in mice that express the Med12 c.131G>A variant on a conditional KO background (Figure 1A). In this model, Med12m/ Amhr2-Cre males were bred with Med12Rm/females to generate Med12m/ Med12Rm/ Amhr2-Cre females. A subset of uterine cells will express Med12 c.131G>A on an X chromosome Med12-null background (Figure 1B). We analyzed the Med12m/ Med12Rm/ Amhr2-Cre female reproductive tracts at 8 weeks and beyond 12 weeks of age. Nulliparous Med12m/ Med12Rm/ Amhr2-Cre female mice presented with pathological changes associated with leiomyoma formation as early as 8 weeks of age (Supplemental Figure 3, B and D). Histological evaluation revealed that, beyond 12 weeks of age, 80% of the uteri contained lesions consistent with leiomyomas. These lesions consisted of extracellular matrix (ECM) deposits, accompanied by infiltration of fibroblasts and macrophages, hyperplasia, and disorganized muscle fiber arrangement, leading to complete destruction of myometrial architecture. Tumors formed in Med12m/ Med12Rm/ Amhr2-Cre–mutant uteri expressed mutant Med12, as shown by the expression of FLAG, which was fused to mutant Med12 in our ROSA construct (Supplemental Figure 2F).

It has been noticed that estrogen and progesterone promote leiomyomatous growth, and 30% of leiomyomas in human pregnancies increase in volume (13). To corroborate these observations, we studied the effects of mouse parturition on leiomyomatous growth. Multiparous Med12m/ Med12Rm/ Amhr2-Cre females often had either grossly visible large leiomyomas (Figure 1C) or multiple small leiomyoma-like nodules (Figure 1F). Histology confirmed that these tumors arose from the smooth muscle layer and consisted of whorled fascicles of fusiform smooth muscle cells with an abundance of eosinophilic cytoplasm and ECM deposits (Figure 1, D, E, G, and H), consistent with the pathology seen in human uterine leiomyomas. Large tumors were often necrotic, hemorrhagic, and fibrotic. In addition, characteristic of leiomyomas, all tumors stained positive for smooth muscle actin and showed an abundance of collagen deposits when stained with Masson’s trichrome stain (Supplemental Figure 4, A–C). Eighty percent of multiparous Med12m/ Med12Rm/ Amhr2-Cre females had leiomyoma-like lesions. These results indicate that the Med12 c.131G>A variant causes leiomyoma-like lesions in mice.

The Med12 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 Med12 c.131G>A variant was expressed in mice on a WT background (Figure 2A). We generated animals coexpressing mutant Med12 from the autosome and a WT Med12 from the X chromosome (Med12Rm/ Amhr2-Cre) by crossing Med12Rm/male and Amhr2-Cre mice. Uteri from nulliparous Med12Rm/ Amhr2-Cre and control mice (Med12Rm/mt) were examined at 8 weeks of age and after 12 weeks of age.
In 8-week-old \( Med12^{\text{Rmt/+}} \) \( Amhr2\)-Cre mice, no leiomyoma-like lesions were observed (Supplemental Figure 5B). Fifty percent of the uteri from \( Med12^{\text{Rmt/+}} \) \( Amhr2\)-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 in both \( Med12 \) WT (\( Med12^{\text{Rmt/+}} \) \( Amhr2\)-Cre) and conditional KO (\( Med12^{\text{Gcr/+}} \) \( Med12^{\text{Rmt/+}} \) \( Amhr2\)-Cre) mice. \( Med12^{\text{Gcr/+}} \) \( c.131G>A \) 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 (\( Med12^{\text{Gcr/+}} \) \( Med12^{\text{Rnt/+}} \)). All 4 tumors showed genomic 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 \( Med12^{\text{Gcr/+}} \) \( Med12^{\text{Rnt/+}} \) \( Amhr2\)-Cre females is shown in Supplemental Table 1. Microarray analysis of \( Med12^{\text{Gcr/+}} \) \( Med12^{\text{Rnt/+}} \)

**Figure 2.** \( Med12^{\text{Rnt/+}} \) \( Amhr2\)-Cre uteri develop prominent leiomyomas. (A) Mouse model 2 (\( Med12^{\text{Rnt/+}} \) \( Amhr2\)-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 \((A^{\text{Rnt/+}})\) 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 \( Med12^{\text{Rnt/+}} \)–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 \( Med12^{\text{Rnt/+}} \) \( Amhr2\)-Cre mice that expressed the \( Med12 \) c.131G>A variant and revealed leiomyoma-like lesions in approximately 47% \((8 \text{ of } 17)\) 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 \( \mu \text{m} \) (B and C), 100 \( \mu \text{m} \) (D and E).

**Table 1.** \( Med12^{\text{Gcr/+}} \) \( Med12^{\text{Rnt/+}} \) \( Amhr2\)-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>Rbab3gap2 – TGF-β signaling; iars2 – cell cycle checkpoint network; Bppt1 – 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>S cyc4, Mapk6 – MAPK/c-Jun signaling</td>
<td>1p36.1-p35</td>
</tr>
<tr>
<td>6qB1</td>
<td>Mosaic gain</td>
<td>105</td>
<td>Prst1 – ECM receptors; Prss3 – cell division</td>
<td>7q24</td>
</tr>
<tr>
<td>4qD2</td>
<td>Gain</td>
<td>40</td>
<td>Adam28 – fibronectin receptor; Adam7 – collagen receptors</td>
<td>8p12.1</td>
</tr>
<tr>
<td>14qD3</td>
<td>Gain</td>
<td>133</td>
<td>Pdcd17 – tumor suppression</td>
<td>19q21</td>
</tr>
<tr>
<td>7qA3.3</td>
<td>Mosaic gain</td>
<td>450</td>
<td>Btd9 – Tp53 network; Glc1 – NF-xB network; Gpifr – cAMP signaling</td>
<td>6p21.1-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; Crt1 – chromosome segregation</td>
<td>10p11.21</td>
</tr>
<tr>
<td>18qA1</td>
<td>Loss</td>
<td>212</td>
<td>Thoc1 – 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 syntenic 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.

Contrast, Amhr2-Cre–driven expression of the gain-of-function mutant form of β-catenin causes tumors in both the mouse myometrium and the stroma (18). These results indicate that Med12 exon 2 mutations have specific tumorigenic effects in smooth muscle cells.

aCGH of Med12 c.131G>A mouse uteri not only revealed genome-wide aberrations, but also showed complex chromosomal alterations such as chromothripsis. Recently, chromothripsis was reported in human leiomyomas and proposed as a possible mechanism of tumor progression (19). Chromosomal aberrations in mice also occur in regions that are syntenic to human 1p, 1q, 2q, 6p21, and 18p regions, also rearranged in human leiomyomas. It was previously shown that 60% of human leiomyomas with 6p21 rearrangements harbored MED12 exon 2 mutations (20). These data suggest that Med12 exon 2 mutations are precursors to genomic rearrangements and, hence, can cause genomic instability and drive tumor progression.

The limitations of our model include regulatory differences that may exist in the expression of Med12 on the X chromosome versus on an autosome. Autosomal Med12 is under the control of the ROSA promoter, which probably differs from the native Med12 promoter. Nonetheless, our model mimics the human condition and shows that Med12 variants can act through a gain-of-function mechanism. In the future, such models will provide a valuable tool for studying the role of MED12 in the genesis of uterine leiomyomas and the specificity of its effects on smooth muscle cells.

Methods

Further information can be found in the Supplemental Methods.

Materials. Med12fl/fl mice were a gift of Heinrich Schreve (Max-Planck Institute, Berlin, Germany), and the Amhr2-Cre mice were a gift of Richard Behringer (The University of Texas, Houston, Texas, USA).

Statistics. A 2-tailed Student’s t test was applied to determine the difference of means among groups using GraphPad Prism 4.0 (GraphPad Software). Statistical significance was defined at a P value of less than 0.05.
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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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