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Original contribution

Recurrent chromosomal aberrations in intravenous leiomyomatosis of the uterus: high-resolution array comparative genomic hybridization study☆

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Summary Uterine intravenous leiomyomatosis (IVL) is a distinct smooth muscle neoplasm with a potential of clinical aggressiveness due to its ability to extend into intrauterine and extrauterine vasculature. In this study, chromosomal alterations analyzed by oligonucleotide array comparative genomic hybridization were performed in 9 cases of IVL. The analysis was informative in all cases with multiple copy number losses and/or gains observed in each tumor. The most frequent recurrent loss of 22q12.3-q13.1 was observed in 6 tumors (66.7%), followed by losses of 22q11.23-q13.31, 1p36.13-p33, 2p25.3-p23.3, and 2q24.2-q32.2 and gains of 6p22.2, 2q37.3 and 10q22.2-q22.3, in decreasing order of frequency. Copy number variants were identified at 14q11.2, 15q11.1-q11.2, and 15q26.2. Genes mapping to the regions of loss include *CHEK2, EWS, NF2, PDGFB*, and *MAP3K7IP1* on chromosome 22q, *HEI10* on chromosome 14q, and *succinate dehydrogenase subunit B, E2F2, ARID1A KPNA6, EIF3S2 , PTCH2*, and *PIK3R3* on chromosome 1p. Regional losses on chromosomes 22q and 1p and gains on chromosomes 12q showed overlaps with those previously observed in uterine leiomyosarcomas. In addition, presence of multiple chromosomal aberrations implies a higher level of genetic instability. Follow-up polymerase chain reaction (PCR) sequencing analysis of MED12 gene revealed absence of G> A transition at nucleotides c.130 or c.131 in all 9 cases, a frequent mutation found in uterine leiomyoma and its variants. In conclusion, this is the first report of high-resolution, genome-wide investigation of IVL by oligonucleotide array comparative genomic hybridization. The presence of high frequencies of recurrent regional loss involving several chromosomes is an important finding and likely related to the pathogenesis of the disease.

1. Introduction

Intravenous leiomyomatosis (IVL) is an uncommon yet distinct uterine smooth muscle neoplasm with intravascular extension and growth, despite its benign histologic appearance [1-3]. The intravenous component may involve uterine or pelvic—broad ligament—vessels but occasionally can also

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extend to the inferior vena cava, to the right side of the heart, and even to pulmonary vasculature leading to potentially life-threatening cardiac sequelae. Pelvic recurrence has also been reported in up to 16.7% of patients [1,4,5].

The pathogenesis of IVL is unclear, with 2 main theories proposed in the literature: origin from a preexisting leiomyoma and from venous vessel walls [1,6]. Although rare cases presented without a distinct uterine leiomyoma, supporting vascular origin, most tumors arise in association with leiomyomas [1,6]. Other entities, that is, leiomyoma with vascular invasion and benign metastasizing leiomyoma have also been linked pathogenetically to IVL, and they may represent different stages of disease progression [7,8].

Although the molecular genetic alterations of uterine leiomyomas and leiomyosarcomas have already been characterized in a relatively large number of cases, comprehensive molecular genetic studies of IVL are essentially lacking. In uterine leiomyomas, the most common recurrent chromosomal abnormalities include rearrangements of 12q14-15 and 6p21, deletions of 7q and 3q, and trisomy 12 [9-13]. Recent studies have also identified frequent mutations (G> A transitions of nucleotides c.130 or c.131 at codon 44) affecting the mediator complex subunit 12 (*MED12*) gene on chromosome Xq13.1 in conventional uterine leiomyomas as well as in leiomyoma variants [14-16]. Leiomyosarcomas, on the other hand, have been found to have much more complex, nonrecurring aberrations, reflecting their high level of genomic instability. Losses of 1p, 2p, 6p, 9p, 10q, 13q, 14q, and 22q and gains of chromosomes 8, 10, 12q 17, and X have been reported with variable frequencies [17,18]. So far, only 2 cases of IVL have been characterized at the cytogenetic and molecular level, and an unbalanced translocation between chromosomes 12 and 14 was observed in both cases, suggesting a pathogenetic relationship between leiomyomas and IVL [19,20].

In the current study, we investigated the genome-wide copy number aberrations in 9 cases of IVL by oligonucleotide array comparative genomic hybridization (aCGH) analysis.

2. Materials and methods

2.1. Case selection and slide review

The study was approved by our institutional review board. Nine cases of uterine IVL were selected from the archives of the Department of Pathology. All available hematoxylin and eosin (H&E)–stained slides were reviewed by 3 pathologists (N. B., R. J. C., and P. H.) to confirm the diagnosis. Patient demographics and clinical follow-up information were retrieved from the pathology reports and the patients' medical records.

2.2. DNA isolation

For DNA extraction, 5-μm-thick unstained sections were generated from formalin-fixed, paraffin-embedded tissue blocks. In the presence of corresponding H&E-stained section, the areas of target tissue with at least 90% of tumor cells were manually dissected into 1.5-mL centrifuge tubes. DNA was extracted from the tissue sections using the Qiagen tissue kit according to the manufacturer's instruction (Qiagen tissue kit; Qiagen, Chatsworth, CA). DNA was quantified by spectrophotometric absorbance at 260 nm using the NanoDrop apparatus (Thermo Scientific, Wilmington, DE).

2.3. Oligonucleotide aCGH assay and data interpretation

The labeling of patient and control DNAs, hybridization onto a oligonucleotide array slide (G4449A SurePrint G3 Human CGH 4 × 180K Oligo Microarray Kit, each array contains 173 341 60-mer oligonucleotide probes; Agilent Technologies, Santa Clara, CA), posthybridization wash, image capture, and signal feature extraction were performed as previously described [21]. Genome-wide copy number variation was analyzed using Agilent's Cytogenomics 2.5 with the built-in ADM-2 algorithm set at threshold value of 6, a cut-off value of 0.25, and a filter of 6 continuous probes. All base pair positions for detected genomic imbalances were designated according to the February 2009 Assembly (GRCh37/hg19) in the University of California Santa Cruz (UCSC) Human Genome browser (http://genome.ucsc.edu/). Known copy number variants were identified using the Database of Genomic Variants (http://dgv.tcag.ca/dgv) [22].To evaluate the genome-wide distribution and relative frequency of chromosomal and genomic abnormalities, raw data of all detected copy number alterations were loaded onto the online software at www.progenetix.org [23]. Lists of tumor-related genes mapped to the affected regions were selected using databases at www.omim.org and www.genecards.org.

2.4. *MED12* mutation analysis by direct polymerase chain reaction sequencing

The polymerase chain reaction (PCR) amplification of the *MED12* gene targeting codon 44 was performed as previously described [19]. Briefly, 20 to 60 ng of genomic DNA extracted from paraffin-embedded tissue was added into a 30-μL reaction mixture containing 0.3 mmol/L dNTP, 1 mmol/L MgSO4, 0.3μmol/L of each primer, and 0.5 U Platinum Pfx polymerase (Invitrogen, Carlsbad, CA). The cycling profile was 35 cycles of denaturation at 94°C for 15 seconds, reannealing at 52.5°C for 30 seconds, and extension at 72°C for 40 seconds, followed by a final extension at 72°C for 5 minutes. The PCR products were examined and extracted from agarose gel by QIAquick Gel Extraction Kit (Qiagen). Sanger sequencing of the purified PCR products using the forward primer was performed. Sequence analysis was carried out by Vector NTI software (Invitrogen).

3. Results

Nine cases with uterine IVL were included in the study. The clinicopathological characteristics are summarized in