Human Pathology (2013) **44**, 1597–1604

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Mediator complex subunit 12 exon 2 mutation analysis in different subtypes of smooth muscle tumors confirms genetic heterogeneity☆

Marieke A. de Graaff MD[a], Anne-Marie Cleton-Jansen PhD[a], Károly Szuhai MD, PhD[b], Judith V. M. G. Bovée MD, PhD[a,*]

[a]*Department of Pathology, Leiden University Medical Center, 2300 RC, Leiden, The Netherlands*
[b]*Department of Molecular Cell Biology, Leiden University Medical Center, 2300 RC, Leiden, The Netherlands*

Received 31 October 2012; revised 20 December 2012; accepted 2 January 2013

Keywords:
β-catenin;
Leiomyoma;
Leiomyosarcoma;
MED12 mutation;
Wnt-pathway

Summary Recently, heterozygous mutations in exon 2 of the *mediator complex subunit 12* gene have been described in 50% to 70% of uterine leiomyomas; the recurrent nature of these mutations suggests an important role in their pathogenesis. Mediator complex subunit 12 is involved in regulation of transcription and Wnt signaling. So far, little is known about the pathogenesis of the different subtypes of extrauterine leiomyomas and leiomyosarcomas. We performed mutation analysis of *mediator complex subunit 12* and immunohistochemistry for β-catenin, using 69 tumors of 64 patients including 19 uterine leiomyomas, 6 abdominal leiomyomas, 9 angioleiomyomas, 5 piloleiomyomas, and 7 uterine and 23 soft tissue leiomyosarcomas. In line with previous observations, 58% of uterine leiomyomas carried a *mediator complex subunit 12* mutation. However, all other extrauterine leiomyomas were negative with the exception of 1 abdominal leiomyoma with a likely primary uterine origin. Of the 30 leiomyosarcomas, only 1 uterine tumor harbored a mutation. A new observation is the identification of 3 tumors with a homozygous mutation; a monosomy X or interstitial deletion was excluded. β-Catenin immunohistochemistry showed nuclear positivity in only 55% of the *mediator complex subunit 12*–mutated uterine leiomyomas, suggesting the involvement of pathways other than canonical Wnt signaling in tumorigenesis. Interestingly, 80% of *mediator complex subunit 12* wild-type sporadic piloleiomyomas displayed nuclear β-catenin positivity, indicating its involvement in this leiomyoma subtype. The lack of *mediator complex subunit 12* mutations in extrauterine leiomyomas and leiomyosarcomas indicates that these tumors arise through a different pathway, emphasizing the genetic heterogeneity of smooth muscle tumors.

1. Introduction

Leiomyomas are benign tumors (fibroids) consisting of a proliferation of smooth muscle cells. Most of these tumors arise in the uterus, and by age 45 years, 60% of women have at least 1 leiomyoma [1]. The tumors can reach a substantial size, and multiple tumors are often present, leading to a considerable morbidity. Surgery is the mainstay of treatment

☆ Duality of interest: The authors declare that they have no conflicts of interest.

* Corresponding author.
E-mail address: j.v.m.g.bovee@lumc.nl (J. V. M. G. Bovée).

0046-8177/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2013.01.006

to relieve symptoms [1,2]. At histologic examination, the lesions are composed of interlacing fascicles of spindle-shaped cells with eosinophilic cytoplasm and blunt-ended, cigar-shaped nuclei [3].

An identical histology can be seen in smooth muscle tumors in the abdomen and in the dermis (piloleiomyoma), although these leiomyomas are far less frequent compared with the uterine lesions. In angioleiomyomas, smooth muscle cells spin off from vessel walls, presenting as a small and painful mass located in the subcutis of the lower extremities. Simple surgical excision is an adequate treatment for symptomatic cases [4-6].

Uterine leiomyomas are genetically heterogeneous because approximately 30% to 50% of the tumors display chromosomal aberrations involving (balanced) translocations of chromosomes t(6;14) or t(12;14) and deletions of chromosome 7 [7-9]. The *HMGA1* and *HMGA2* (*high mobility group A1 and A2*) genes mapping to chromosome 6p21.31 and 12q14.3, respectively, are frequently involved in the breakpoints, and as no partner gene on chromosome 14 has been identified so far, the deregulation of *HMGA1/2* is believed to be important for tumor development [10-12]. Mäkinen et al [13] recently identified *mediator complex subunit 12* (*MED12*) mutations in 70% (159/225) of uterine leiomyomas of 80 different patients in a study using next-generation sequencing. All mutations were heterozygous and resided in exon 2, and each tumor only contained 1 mutation, suggesting that these alterations contribute to tumorigenesis. *MED12* changes and 12q14 rearrangements turned out to be mutually exclusive [14]. In 5 consecutive studies, mutation rates of 52.2% (35/67), 58.8% (47/80), 66.6% (6/9), 67% (100/148), and 50% (14/28) were identified, including a study with African patients [14-18]. The occurrence of *MED12* mutations in other leiomyoma subtypes has not been investigated.

MED12 consists of 45 exons and is located at chromosome Xq13.1. It is a subunit and activator of the CDK8 module, which includes in addition to MED12 also MED13, CCNC (Cyclin C), and CDK8. The CDK8 module associates with 25 other complexes in the highly conserved Mediator complex, which is responsible for the transcription of all RNA polymerase II–dependent genes and functions as a transcription factor [19-21]. The CDK8 module controls global and gene-specific transcription and regulates specific transcription of members of the Wnt pathway. MED12 is involved in the binding of transcription factors to the Mediator complex, and it is crucial for activation of the CDK8 histone kinase [20-22]. Moreover, MED12 interacts directly with β-catenin, a member of the Wnt pathway, and mediates β-catenin–activated transcription [23].

Leiomyosarcomas are malignant tumors originating from smooth muscle cells. They account for 10% to 15% of the soft tissue sarcomas, and predilection sites are the retroperitoneum, the large blood vessels, and the soft tissues of the lower extremities [6,24]. They can also arise in the uterus with an estimated incidence of 0.40 per 100 000 women per year [25]. Although leiomyomas of the uterus are quite common, uterine leiomyosarcoma is very rare. There is not much known about the pathogenesis of this group of highly malignant tumors. Cytogenetic analysis reveals often complex karyotypes with multiple nonrecurrent genetic aberrations, probably resulting from genetic instability [6,26,27]. A tumor progression model has been postulated for leiomyoma and leiomyosarcoma as both originate from smooth muscle cells, with an estimated frequency of less than 0.1%; however, such a relation is still controversial, as no convincing evidence is present [26]. In a recent study, mutations in *MED12* have been found in 2 of 10 uterine leiomyosarcomas [17], whereas the occurrence in leiomyosarcoma of soft tissue has, so far, not been investigated.

To determine a possible role for *MED12* mutations both in benign and malignant extrauterine smooth muscle tumors, we evaluated the presence of mutations in different smooth muscle tumor subtypes occurring in the soft tissues. As MED12 has both direct and indirect interactions with β-catenin, we performed immunohistochemistry for β-catenin to study activity of canonical Wnt signaling and correlate expression to mutation status.

2. Materials and methods

2.1. Tissue samples

A total of 69 formalin-fixed, paraffin-embedded (FFPE) tissue samples of smooth muscle tumors from 64 different patients were selected for DNA extraction and mutation analysis and were retrieved from the archives of the Department of Pathology at Leiden University Medical Center (Leiden, The Netherlands), including 6 abdominal leiomyomas, 9 angioleiomyomas, 5 piloleiomyomas, 19 uterine leiomyomas, 7 leiomyosarcomas of the uterus, and 23 leiomyosarcomas of soft tissue (Table 1). Of 1 patient, 3 different uterine leiomyomas were included as well as a leiomyosarcoma of the femoral artery; another patient underwent surgical removal of an abdominal leiomyoma and, shortly thereafter, leiomyoma-related hysterectomy; both tumors were included.

Tissue microarrays (TMAs) were constructed from a panel of FFPE tumors including 7 uterine leiomyomas, 53 leiomyosarcomas (30 of which were also used for mutation analysis), 14 myxofibrosarcomas, 4 pleomorphic liposarcomas, 2 rhabdomyosarcomas, 6 undifferentiated spindle cell sarcomas, and 24 undifferentiated pleomorphic sarcomas. The TMAs were constructed using a semiautomated TMA apparatus (TMA Master; 3DHistech, Budapest, Hungary) to transfer tumor punches to the recipient block. A biopsy needle of 1.5 mm was used resulting in a surface area of 1.767 mm^2 per core; all tumor samples are present in triplicates. Cores from colon, liver, placenta, prostate, skin, and tonsil were included for control and orientation purposes. Using a tape-transfer system (Instrumedics, Hackensack, NJ), 4-μm sections were transferred to coated glass slides. For 12 of the