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6p21 rearrangements in uterine leiomyomas targeting *HMGA1*

Maliheh Hashemi Nezhad[a], Norbert Drieschner[a], Sabrina Helms[a], Anke Meyer[a], Mahboobeh Tadayyon[a], Markus Klemke[a], Gazanfer Belge[a], Sabine Bartnitzke[a], Käte Burchardt[b], Christiane Frantzen[c], Ernst Heinrich Schmidt[d], Jörn Bullerdiek[a,e,*]

[a]*Center for Human Genetics, University of Bremen, Leobener Str. ZHG, D-28359 Bremen, Germany*
[b]*Department of Pathology, General Hospital Bremen-Mitte, St.-Jürgen-Str. 1, D-28177 Bremen, Germany*
[c]*Women's Clinic, St. Joseph-Stift Hospital, Schwachhauser Heerstrasse 54, D-28209 Bremen, Germany*
[d]*Department of Obstetrics and Gynecology, Evang. Diakonie Hospital, Gröpelinger Heerstrasse 406-408, D-28239 Bremen, Germany*
[e]*Clinic for Small Animals, University of Veterinary Medicine, Bischofsholer Damm 15, D-30137 Hanover, Germany*

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Abstract To quantify the expression of *HMGA1* mRNA in uterine leiomyomas, the expression of *HMGA1* was analyzed in a series including tumors with aberrations of chromosome 6 ($n = 7$) and cytogenetically normal tumors ($n = 8$) as a control group by quantitative reverse transcriptase–polymerase chain reaction. The average expression level in the 6p21 group was found to be 5.6 times higher than that in the control group, and with one exception, all cases with 6p21 alteration revealed a high expression of *HMGA1* mRNA than cytogenetically normal tumors. Nevertheless, compared to fibroids with a normal karyotype, the upregulation of the *HMGA1* mRNA in these cases was much less strong than that of *HMGA2* mRNA in case of 12q14~15 aberrations identified in previous studies.

1. Introduction

Uterine leiomyomas (ULs) belong to the cytogenetically best investigated human tumors. The cytogenetic analyses have revealed several subtypes, with a frequent group showing rearrangements of chromosomal region 12q14~15, which apparently targets the gene encoding the high-mobility group AT-hook 2 (*HMGA2)* [1,2]. Accordingly, tumors of this type show significantly higher expression of *HMGA2* than fibroids with an apparently normal karyotype [3]. HMGA2 is a protein abundantly expressed in stem cells and casually linked to their self-renewal ability. A decrease of HMGA2 has recently seen linked to the group of hematopoietic as well as neural stem cells [4]. Accordingly, it is tempting to speculate that in terms of pathogenesis, smooth muscle cells continuously expressing HMGA2 are maintaining a self-renewing program that occasionally also display multilineage potential as witnessed by variants as, for example, lipoleiomyomas or leiomyomas with cartilaginous differentiation [5,6].

Of note, a smaller subgroup of ULs shows rearrangements of 6p21 (i.e., the locus where *HMGA1,* the other gene encoding proteins of the HMGA type, has been mapped), suggesting that *HMGA1* is the relevant target gene in that subgroup of ULs [7]. In small series of ULs, it was shown that this rearrangement leads to an overexpression of *HMGA1* [8,9]. However, to our knowledge, no study quantifying the expression of *HMGA1* mRNA in ULs of this subtype has been performed. Thus, we analyzed the *HMGA1* expression in seven ULs with aberrations of chromosome 6 in comparison to myomas with normal karyotype and to the matching myometrial tissues.

2. Materials and methods

2.1. Tissue samples and chromosome analysis

For RNA isolation, samples of ULs and myometrium were snap frozen in liquid nitrogen immediately after surgery and stored at −80°C. For cell culture, samples of primary tumors were transferred to Hank's solution with antibiotics (200 IU/mL penicillin, 200 µg/mL streptomycin) after surgery. Cell culture and chromosome analyses were performed as described previously [3].

2.2. RNA isolation, reverse transcription, and quantitative reverse transcriptase–polymerase chain reaction

Total RNA was isolated from tissue samples with the RNeasy Mini Kit (Qiagen, Hilden, Germany) including

* Corresponding author. Tel.: +49-421-2184239; fax: +49-421-2184239.
E-mail address: bullerd@uni-bremen.de (J. Bullerdiek).

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DNase 1 treatment according to the manufacturer's instructions, and quantitated by spectrophotometry. Reverse transcription of 250 ng RNA was carried out with M-MLV reverse transcriptase, RNaseOUT, and random hexamers (Invitrogen, Karlsruhe, Germany) according to the manufacturer's recommendations. Controls without reverse transcriptase were included for each sample to ensure the absence of DNA contaminations, which, as a result of the high number of *HMGA1*-related retropseudogenes, could lead to false-positive results.

Quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR) was performed on a real-time

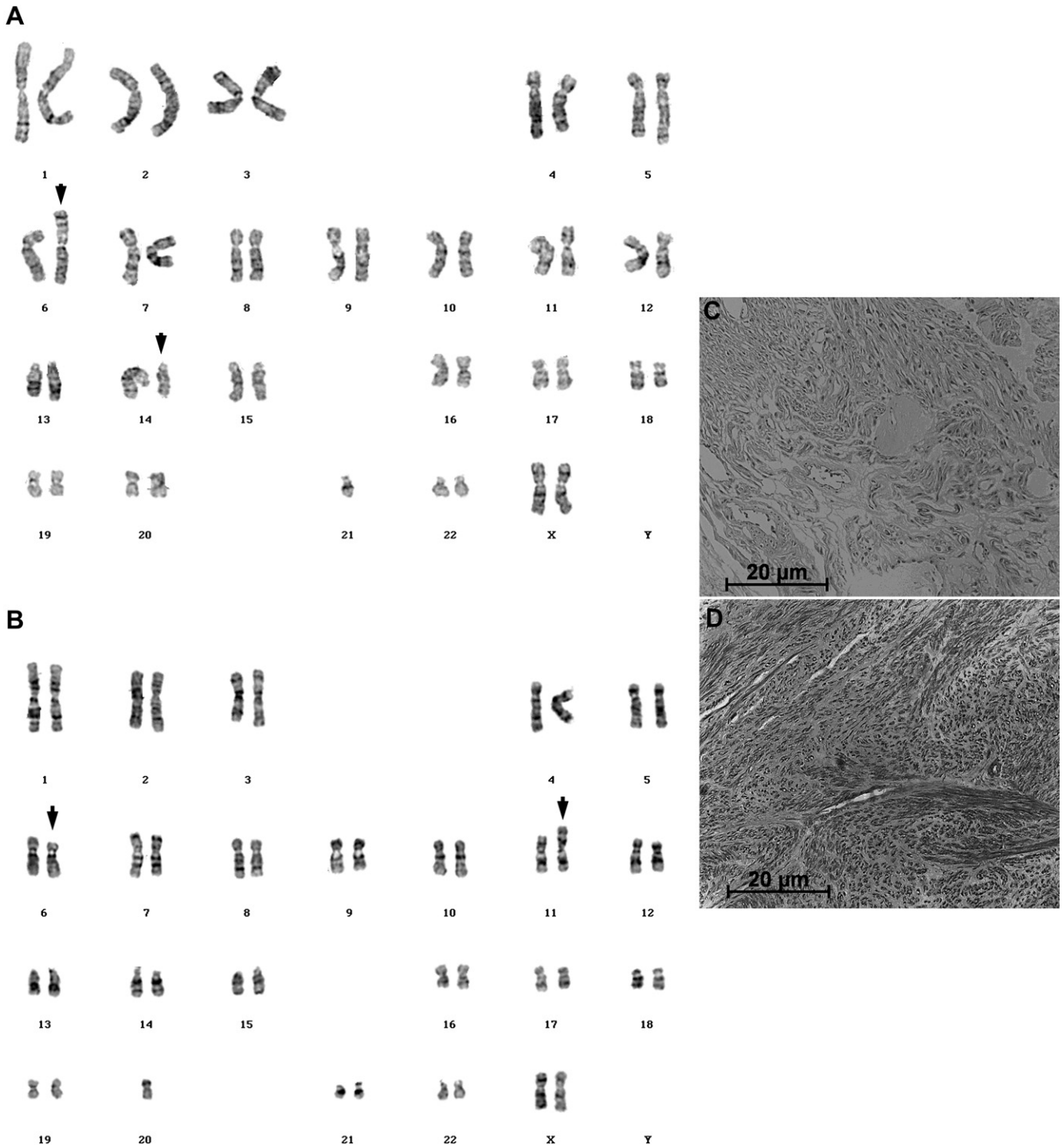

Fig. 1. Karyotypes of two ULs with 6p21 rearrangements with different levels of *HMGA1* expression and the histologic appearance of these ULs. (A) Representative G-banded karyotype of myoma 87: 46,XX,t(6;14)(p23;q24), tas(14;21)(pter;qter). (B) Representative G-banded karyotype of myoma 125A: 46,XX, t(6;11)(p21;p15), chromosomes participating in the 6p21 rearrangements are indicated by arrows. Histologic appearance of myoma 87 (C) and myoma 125A (D).