

Description of a Novel Fusion Transcript between *HMGI-C*, a Gene Encoding for a Member of the High Mobility Group Proteins, and the Mitochondrial Aldehyde Dehydrogenase Gene

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Abstract

Aberrations involving the chromosomal region 12q24 are a nonrandom cytogenetic abnormality in frequent benign tumors mainly of mesenchymal origin, e.g., uterine leiomyomas, pleomorphic adenomas of the salivary gland, lipomas, or hamartomas of the lung. Mostly, these 12q24 abnormalities occur as a result of inversions also affecting chromosomal region 12q14–15. In addition to the frequent tumors mentioned above, these abnormalities have also been found in rare mesenchymal tumors, e.g., hemangiopericytomas. Although recently the molecular basis of the aberrations of chromosomal region 12q14–15, i.e., a rearrangement of the *HMGI-C* gene has been identified, the molecular roots of the 12q24 changes still remain to be elucidated. Herein we report on 3' rapid amplification of cDNA ends PCR results on cDNA from a primary uterine leiomyoma. As an ectopic sequence fused to exon 3 of the *HMGI-C* gene, we have identified a cDNA sequence that revealed 100% homology to exon 13 of the human mitochondrial aldehyde dehydrogenase gene (*ALDH 2*). Because *ALDH 2* maps to 12q24.1, this fusion transcript is a good candidate underlying the chromosomal rearrangements involving 12q24.

Introduction

A huge variety of human benign tumors mainly of mesenchymal origin, e.g., pleomorphic adenomas of the salivary gland (1, 2), uterine leiomyomas (3–5), lipomas (6–8), or pulmonary chondroid hamartomas (9–11), show specific chromosomal abnormalities involving the region 12q14–15 leading to rearrangements of *HMGI-C*,² a gene encoding for the high mobility group family of DNA binding proteins (12–13). *HMGI-C* proteins contain three DNA binding domains binding to the minor grooves of AT-rich DNA (14). Another chromosomal band non-randomly involved in these chromosomal rearrangements is 12q24, often as inversions also involving 12q14–15 (15, 16). Thus, in addition to the known *HMGI-C* rearrangements, band 12q24 represents another interesting target for investigations at the molecular level.

To find a candidate gene in 12q24 rearranged by the chromosomal translocations, we used a primary uterine leiomyoma to characterize a fusion transcript between *HMGI-C* and an ectopic sequence belonging to a the mitochondrial aldehyde dehydrogenase 2 (*ALDH 2*) gene mapping to 12q24.1 (17).

Materials and Methods

Tumor Material. A primary uterine leiomyoma from a 46-year-old woman was surgically removed. Immediately after surgery, one-third of the

tumor was frozen in liquid nitrogen for RNA isolation, another one-third was used for cytogenetic analysis, and the remaining one-third was histologically examined. As a control for the RACE-PCR, the human hepatocellular cell line HEP 3B was used.

Cytogenetic Analysis. Chromosome preparation followed routine methods described earlier (2). Chromosomes were GTG banded.

FISH. To identify the chromosomes unambiguously, FISH analysis was performed after GTG banding of the same metaphase spreads. Treatment of metaphase spreads, subsequent FISH experiments, and cosmid labeling (with biotin-14-dATP) was performed according to the protocol of Kievits *et al.* (18). As DNA probes, a pool of cosmids, 27E12 and 142H1, flanking the third exon of the *HMGI-C* gene (12), was used. The slides were analyzed on a Zeiss (Oberkochen, Germany) Axioplan fluorescence microscope. Results of GTG banding and FISH were processed and recorded with a Power Gene karyotyping system (PSI, Halladale, Great Britain).

3'RACE-PCR. One hundred mg of tumor tissue were homogenized, and RNA was isolated using the trizol reagent (BRL, Gaithersburg, MD) containing phenol and isothiocyanate. For cDNA synthesis, we used the adapter primer (AP2) AAG GAT CCG TCG ACA TC (T)17. For first and secondary rounds of PCR, the universal amplification primer (UAP2) CUA CUA CUA CUA CUA AAG GAT CCG TGG ACA TC was used as a "reversed primer." In the first round of PCR, we used the specific forward primer 5'-CTT CAG CCC AGG GAC AAC-3' (exon 1), and in the second round of PCR, the following nested primer was used: 5'-CAU CAU CAU CAU CGC CTC AGA AGA GAG GAC-3' (exon 1). PCR [thermal cycling: 94°C, 3 min; (94°C, 1 min; 55°C, 45 s; 72°C, 3 min) × 30, 72°C, 10 min] was performed with the AmpliTaq polymerase (Perkin Elmer, Weiterstadt, Germany). The PCR products were separated in 0.8% agarose. Cloning of the gel-extracted fragments was performed using the CloneAmp cloning system (BRL). Therefore, the nested specific primers and the universal amplification primer were CUA/CAU tailed. Sequencing was performed on a 373 DNA sequencer (Applied Biosystem, Weiterstadt, Germany).

Results

A total of 11 GTG-banded metaphases of the primary tumor was cytogenetically studied. All showed an apparently normal karyotype 46, XX, but the band resolution was rather poor. In addition, FISH studies using a pool of cosmids belonging to the third exon of *HMGI-C* were performed. The results revealed a rearrangement of this region in all metaphases analyzed. Using the cosmid pool, we observed a signal on a normal chromosome 12, a split signal on 12q15 and 12q24 on an obviously inverted chromosome 12, and a weak signal on a cytogenetically normal chromosome 14. To characterize possible aberrant fusion transcripts between *HMGI-C* and ectopic sequences, 3'RACE PCR on the cDNA of the uterine leiomyoma was performed, resulting in the occurrence of bands of normal sizes (identical to the control) and one additional band of about 650 bp. The amplified DNA of this band was extracted from the gel, cloned, and sequenced. The analyzed sequence contained ectopic sequences fused to exon 3 of *HMGI-C*. A homology search revealed a 100% identity of the ectopic sequence to the thirteenth exon of the mitochondrial

Received 10/10/95; accepted 11/2/95.

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² The abbreviations used are: *HMGI-C*, high mobility group I-C; *ALDH*, aldehyde dehydrogenase; *FISH*, fluorescence *in situ* hybridization; *RACE*, rapid amplification of cDNA ends.

Fig. 1. Sequenced part of the fusion transcript observed in the uterine leiomyoma. The sequence starts with the specific forward primer (second round) sequence located within the first exon of *HMGI-C*. The gray frame indicates the part of the transcript belonging to *HMGI-C*; the 3' borders of exon's 1 and 2 are given by gray arrows. The ectopic sequence starts with nucleotide 1 of the thirteenth exon of *ALDH 2*. The border to the noncoding part of that exon is indicated by a black arrow.

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.....CGCCTCAGAAGAGAGGACGCGGCCGCCAGGAAGCAGCAGCAAGAACC
AACCGGTGAGCCCTCTCTAAGAGACCCAGGGGAAGACCCAAAGGCAGCAAAA
ACAAGAGTCCCTCTAAAGCAGCTCAAAGAAAGCAGAAGCCACTGGAGAAAAAC
GGCCAAGAGGCAGACCTAGGAAATGGTGCACAGTCAAAGTGCCTCAGAAGAA
CTCAATAAGAATCATGCAAGCTTCCTCCCTCAGCCATTGATGGAAGTTTCAGCAA
GATCAGCAACAAAACCAAGAAAAATGATCCTTGCGTGCTGAATATCTGAAAAGA
GAAATTTTTCTACAAAATCTCTGGGTCAAGAAAGTTCTAGAATTTGAATTGATA
AACATGGTGGGTTGGYTGAGGGTAAGAGTATATGAGGA.....

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aldehyde dehydrogenase gene (*ALDH 2*). The whole aberrant transcript contains the complete exons 1–3 of *HMGI-C* and the ectopic sequence, which starts with nucleotide 1 of exon 13 of the *ALDH 2* gene, contains its whole translated part, and runs into the 3' noncoding region of that gene (Fig. 1).

Discussion

Aberrations involving the chromosomal region 12q14–15 are a nonrandom cytogenetic abnormality in frequent benign tumors mainly of mesenchymal origin, *e.g.*, uterine leiomyomas, pleomorphic adenomas of the salivary gland, lipomas, or hamartomas of the lung. Recently, we were able to show that the breakpoints of these tumors are mainly clustered within intron 3 of *HMGI-C*, making it a good candidate gene for tumorigenesis (12). Nevertheless, abnormalities of other chromosomal bands, as a rule along with 12q14–15 abnormalities, have been described frequently. One such band is 12q24, often affected by inversions or complex chromosomal rearrangements. Herein we have identified a fusion transcript between *HMGI-C* and *ALDH 2* in a uterine leiomyoma with an apparently complex chromosomal rearrangement, as revealed by the results of the FISH experiments. Although we can only hypothesize that this type of transcript also accounts for the other tumors with 12q24.1 abnormalities reported in the literature, the findings presented here are of high interest *per se* because they provide another type of molecular rearrangement related to or even underlying the genesis of these frequent benign tumors. As for the molecular structure of the transcript described herein, it seems noteworthy that *ALDH 2* has contributed no more than 10 codons to the translated part. Thus, it may well be that the truncation of *HMGI-C* rather than its fusion to ectopic coding sequences contributes to tumorigenesis. Alternatively or additionally, the novel noncoding 3' part of the transcript may be of importance by altering its stability. The investigation of additional fusion transcripts will help to resolve this interesting question.

Acknowledgments

We thank Dr. R. Söller and R. Nimzyk for sequencing support. Furthermore, we thank Dr. U. Deichert for providing us with the tumor material.

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The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

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Cancer Res 1995;55:6038-6039.

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