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Gene fusion involving *HMGIC* is a frequent aberration in uterine leiomyomas

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Abstract *HMGIC*, a high-mobility-group protein gene encoding an architectural transcription factor, was recently identified as the target of gene fusion in a variety of human benign mesenchymal tumors; some of these events were chromosomal translocations involving 12q13–15. *HMGIC* consists of three DNA-binding domains (encoded by exons 1–3), a spacer, and an acidic carboxyl-terminal regulatory domain (exons 4–5). To determine the spectrum and nature of the aberrations in uterine myomas in Japanese patients, we systematically examined the tumors of 45 patients for all possible types of gene fusions involving *HMGIC*, by means of 3′-rapid amplification of cDNA ends (RACE) and reverse transcriptase-polymerase chain reaction (RT-PCR) experiments. *HMGIC* gene fusions were found in 16 (36%) of the tumors; aberrant splicings to five cryptic sequences located in introns of the *HMGIC* gene were found in 11 of these cases, and translocations causing juxtaposition to other genes, such as *COX6C* and *RAD51B*, were found in 5. In all fusion transcripts, the first two or three exons of *HMGIC* were fused to ectopic sequences. Our results suggest that a fusion event, resulting in the separation of the DNA-binding domains of *HMGIC* from the spacer and the acidic carboxyl-terminal regulatory domain, is a common tumorigenic mechanism in the development of uterine myomas.

Key words *HMGIC* · Uterine leiomyoma · Rearrangement · Aberrant splicing

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Introduction

Uterine leiomyomas are among the most frequent benign smooth-muscle tumors in women of reproductive age. Cytogenetic studies have revealed a close association between these tumors and specific aberrations, especially those involving chromosome 12q15 (Sandors et al. 1990; Sreeckantaiah et al. 1991; Dal Cin et al. 1993). Recent advances in molecular genetics have yielded further insight, in that these cytogenetic changes occur mostly within a region encoding *HMGIC*, a member of the HMG (high-mobility-group) family of proteins, and usually involve rearrangements of the *HMGIC* gene itself (Ashar et al. 1995; Schoenmakers et al. 1995). Two molecular mechanisms underlying such rearrangements have been identified in benign tumors; one is intragenic translocation involving *HMGIC* and a fusion partner, with the formation of a chimeric gene. The other is aberrant splicing that yields variant products (Ashar et al. 1996; Tallini and Dal Cin 1999).

HMG proteins are nonhistone, chromatin-associated molecules, known as "architectural elements," whose selective binding to specific structural conformations of DNA leads to replication, transcription, and repair (Chau et al. 1995; Ashar et al. 1996). The expression of *HMGIC* is developmentally regulated, and is generally not observed in normal adult tissues (Manfioletti et al. 1991; Hirning-Holz et al. 1998), but the gene is frequently expressed in transformed malignant cells and in some human cancers (Lund et al. 1983). Because *HMGIC* protein is presumed to function as a transcription factor, the gene could play some role in transformation and carcinogenesis (Tallini and Dal Cin 1999).

Although many reports describe relationships between 12q15 chromosomal aberrations and benign mesenchymal tumors, the prevalence of *HMGIC* rearrangements in such tumors is unknown. To determine the prevalence and the spectrum of *HMGIC* rearrangements in Japanese women with uterine leiomyomas, we systematically examined 45 patients for all possible types of gene-fusion events involving *HMGIC*, by means of experiments involving 3′-rapid

amplification of cDNA ends (RACE), reverse transcriptase-polymerase chain reactions (RT-PCR), radiation-hybrid mapping, and DNA cloning and sequencing.

Materials and methods

Tumor samples and RNA extraction. Uterine leiomyomas were obtained from 45 patients at the time of surgery at the Nippon Medical School Hospital. The tumors were frozen immediately, and stored at −70°C until use. From approximately 100 mg of each leiomyoma tissue, total RNA was extracted by the guanidine thiocyanate method (Chomczynski and Sacchi 1987), using an Isogen RNA extraction kit (Nippon Gene, Tokyo, Japan).

PCR primers and hybridization probes. Sequences of all oligonucleotide primers used in the experiments (AP1, AP2, H1-H3, and hybridization probe "A") were reported previously (Kurose et al. 2000).

3′ Rapid amplification of cDNA ends (3′RACE). To screen for *HMGIC* fusion genes, 5 mg of each sample of total RNA was reverse-transcribed with superscript II (Life Technologies, Rockville, MD, USA) for 50 min at 42°C, in a 40-μl reaction mixture containing 50 mM Tris-HCl (ph 8.3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, 0.5 mM dNTP, and 50 pmol of adapter primer (AP1). The RNA was then digested with 6 U of RnaseH (Takara, Tokyo, Japan) at 37°C for 30 min. For the first, second, and third rounds of PCR we used adapter primer 2 (AP2) as the reverse primer. As the forward primers, in the first round we used *HMGIC*-specific primer H1 (*HMGIC* exon 1); in the second round, nested primer H2 (*HMGIC* exons 1 and 2); and in the third round, nested primer H3 (*HMGIC* exon 2; see Fig. 1A). First-strand cDNA was the template for first-round PCRs, which took place in total volumes of 10 μl, containing 1X KlenTaq PCR reaction buffer (Clontech, Palo Alto, CA), 0.2 mM of each dNTP, 4 pmol of each primer, and 1X Advantage KlenTaq Polymerase Mix (Clontech). Cycle conditions were 94°C for 2 min, then 30 cycles of 94°C for 10 s, 60°C for 30 s, and 68°C for 4 min, in a Gene Amp PCR 9600 System (Perkin Elmer Cetus, Foster City, CA, USA). In the second- and third-round PCRs, a 1/50 portion of each PCR product was used as template for the nested PCR. Nested PCR conditions were as described above, except that the DNA was amplified for 25 cycles.

Oligonucleotide hybridization. Nested PCR products from the 3′ RACE experiments were electrophoresed on 3% NuSieve agarose gels (FMC, Rockland, ME, USA) and transferred to nylon membranes. An *HMGIC* gene-specific oligonucleotide probe A (Fig. 1A; exon 2) was end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase and hybridized to blotted membranes, according to a procedure we described previously (Tsukamoto et al. 1998).

Cloning and sequencing of fusion transcripts. To characterize the structure of the *HMGIC* fusion transcripts, the 3′ RACE products positive for hybridization were subcloned into the plasmid vector pBlueScript II (SK+) and sequenced on a 377 DNA sequencer (Applied Biosystems, Weiterstadt, Germany).

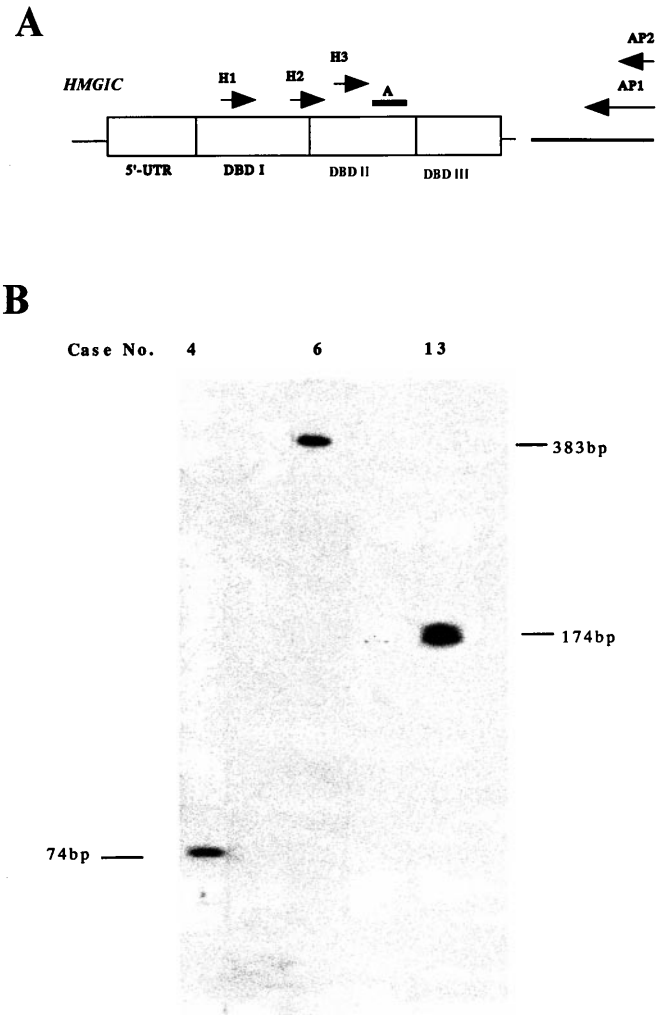

Fig. 1. A Positions of oligonucleotide primers and hybridization probes for 3′-rapid amplification of cDNA ends (3′ RACE) analysis. *Arrows* indicate orientation of primers H1-H3, AP1, and AP2. *The short horizontal bar* represents the position of probe "*A*" within the gene sequence. *DBD*, DNA-binding domain; *UTR*, untranslated region. **B** Representative autoradiograms from 3′ RACE examination of uterine leiomyomas. The 3′ RACE products of all three tumor cDNAs shown here hybridized to the *HMGIC*-specific probe

Results

We first examined RNAs from the 45 uterine leiomyomas by 3′ RACE experiments, using *HMGIC* cDNA sequences as primers (Fig. 1A). We found that 16 of the tumors had *HMGIC* probe-positive bands of unexpected molecular size (Fig. 1B). To identify the nature of these bands, we extracted the relevant PCR products from the gels for cloning and sequencing. Sequence data indicated that all of the aberrant bands contained partial, but not the whole,

HMGIC sequence, and other sequences as well. These findings supported previous indications that chimeric *HGMIC*-containing sequences can originate from rearrangements in which truncated *HMGIC* is fused with ectopic sequences. The results of RT-PCR analysis, demonstrated in Fig. 1B, clearly show that each aberrant fused transcript was the one and only, major component of the *HMGIC*-related transcript detected in each tumor in multiple instances. Rearrangements of the *HMGIC* gene mostly occur within the huge intron 3, which spans over 100 kb in size. Any rearrangements that occur within this vast region could not be detected by regular Southern blotting or genomic PCR, using *HMGIC* exonic sequences, because the rearranged structures are out of the size range of detection by these methods. Cytological analysis depends on successful chromosome preparation after the establishment of a primary culture from each solid tumors, however, the establishment of a primary culture is usually unsuccessful in uterine leiomyoma.

A BLAST search involving all 16 aberrant genes showed that the ectopic sequences in 11 of them (cases 4, 6, 7, 10, 13, 24, 37, 38, 40, 42, and 44) had been reported already in other *HMGIC* rearrangements. The other 5 (cases 8, 11, 12, 39, and 47) contained previously unknown sequences. Sequencing data showed that four of these (in cases 8, 11, 12, and 39) were the result of novel aberrant splicing (Kurose et al. 2001). In the remaining tumor (in case 47), a novel type of *HMGIC* rearrangement was identified in the 3′ RACE product. The nucleotide and amino acid sequence of this novel fusion 3′ RACE product is shown in Fig. 2. In this tumor, the 3′ novel sequence 5′-GTACATCAGGAACGTCTCTAT-3′ was fused in-frame to the *HMGIC* amino acid sequence starting from exon 2 of the *HMGIC* gene. We recently identified this novel ectopic fusion sequence to be the *HEI10* (homo sapiens enhancer of invasion 10) gene as a translocation partner of *HMGIC* (Mine et al. 2001).

Table 1 summarizes the characteristics of the aberrant sequences identified here. All the rearrangements we observed could be assigned to one of two groups on the basis of their underlying mechanisms; i.e., translocation with formation of chimeric genes (5 cases), or aberrant splicing (11 cases). With regard to their breakpoints, the rearrangements could be divided into three groups: group 1, breakpoint in intron 2 (case 47; 1/16 [6%]); group 2, breakpoint in intron 3 (cases 4, 6, 7, 8, 10, 11, 12, 37, 38, and 39; 10/16 [63%]) and group 3, breakpoint in intron 4 (cases 13, 24, 40, 42, and 44; 5/16 [31%]).

Discussion

Many investigators have observed a close relationship between specific cytogenetic changes, especially those involving 12q13–15 and 6p21, and the development of benign mesenchymal tumors such as leiomyomas, pulmonary chondroid harmatomas, and lipomas. Two *HMGI* genes, *HMGIC* and *HMGI(Y)* are located within 12q15 and 6p21, respectively, and rearrangements have been reported in both genes (Schoenmakers et al. 1995; Tallini and Dal Cin 1999). HMGIC and HMGI(Y) have similar structures, with approximately 50% homology in amino-acid sequence. Neither of these proteins has an HMG box domain, but both bind DNA by means of three separate "AT-rich" motifs (Ashar et al. 1995). HMGI(Y) activates transcription by interacting with the promoter regions of a variety of genes. HMGI proteins, as a class, are believed to be involved in regulating transcription and influencing chromatin structure; they also encode C-terminal acidic domains, which are

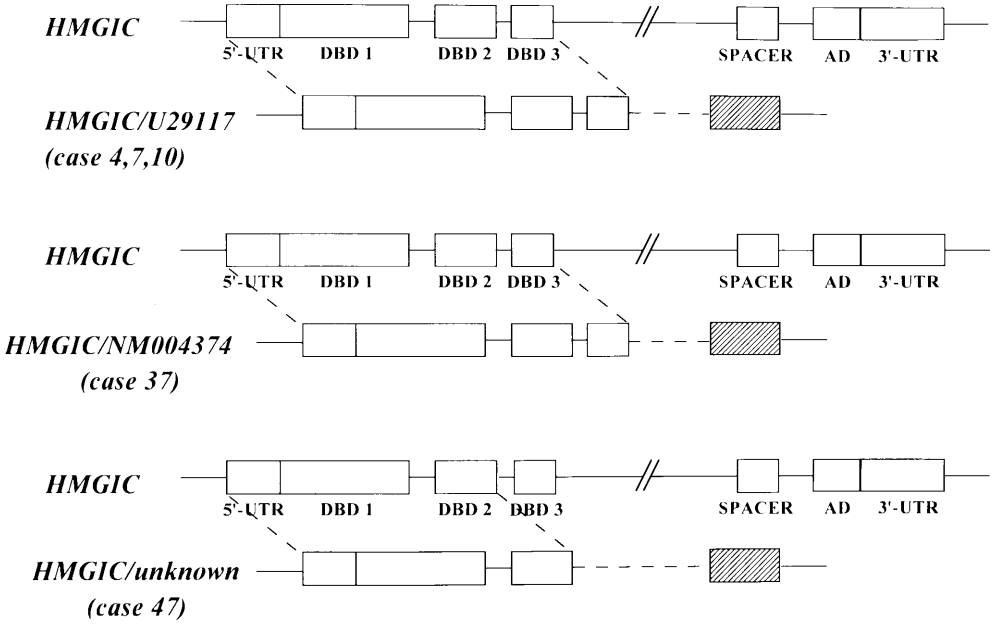

Fig. 2. Schematic representation of three rearrangements involving *HMGIC*. The U29117, NM004374, and unknown (case 47) sequences are fused to *HMGIC* between its DNA-binding domains and the spacer, separating the DNA-binding domains from the spacer and the acidic carboxyl-terminal domain (*AD*). *White boxes* indicate *HMGIC* sequence; *hatched boxes* indicate ectopic sequences. *DBD*, DNA-binding domain; *UTR*, untranslated region

Table 1. Molecular characterization of fusion points

3′ Terminal sequence	Frequency	Case no.	5′ Terminal sequence
HLES-1[a]	3/45	4, 7, 10	3′ End of exon 3 of *HMGIC*
3′ UTR of *COX6C*	1/45	37	3′ End of exon 3 of *HMGIC*
Unknown	1/45	47	3′ End of exon 2 of *HMGIC*
HLES-2[a]	2/45	6, 38	3′ End of exon 3 of *HMGIC*
HLES-3[a]	5/45	13, 24, 40, 42, 44	3′ End of exon 4 of *HMGIC*
Exon a[b]	2/45	8, 39	3′ End of exon 3 of *HMGIC*
Exon b[b]	1/45	11	3′ End of exon 3 of *HMGIC*
Exon c[b]	1/45	12	3′ End of exon 3 of *HMGIC*
Total	16/45 (36%)		

UTR, Untranslated region

[a] HLES, Human lipoma ectopic sequences (Schoenmakers et al. 1995). HLES-1, U29117 from human lipoma cell line Li-538/SV40 ectopic sequence from *HMGIC* fusion mRNA, 3′ sequence; HLES-2, U29112 from human lipoma cell line LM-30.1/SV40 ectopic sequence from *HMGIC* fusion mRNA, 3′ sequence clone pCH109; HLES-3, U29115 from human lipoma cell line Li-192 ectopic sequence from *HMGI-C* fusion mRNA, 3′ sequence, clone pCH148

[b] Exons a, b, and c (see Kurose et al. 2001)

believed to interact with other transcription factors (Chau et al. 1995). On the basis of these observations, we would expect rearrangements of *HMGIC* and the consequent expression of abnormal proteins to have had some role in the development of the benign tumors that harbor them (Wim and Van de Ven 1998).

Some investigators have reported the prevalence of cytogenetic changes believed to be associated with rearrangements of *HMGI* genes in benign tumors. For example, Sandors et al. (1990) reported a 40% prevalence of 12q13–15 abnormalities in pleomorphic adenomas of the salivary gland. In uterine leiomyomas and lipomas, reported rates have been 30% and 60%, respectively (Heim et al. 1986; Vanni et al. 1993). In breast fibroadenomas, the frequency reported was 8% (Calabrese et al. 1991). However, none of these studies provided any direct information about the prevalence of aberrations in the *HMGI* genes themselves.

Two kinds of mechanism are known to underlie the aberrations at 12q13 seen in benign tumors, in terms of the translocation and abnormal splicing of the *HMGIC* gene. To clarify the putative relationship between *HMGIC* rearrangements and the development of uterine leiomyomas, we attempted to identify the prevalence and the spectrum of *HMGIC* changes in Japanese patients. To this end, we examined uterine leiomyomas from 45 women, and found an overall prevalence of 36% (16/45). This rate is close to that reported for 12q13–15 abnormalities in cytogenetic studies. Recently, Tallini et al. (2000) reported a relationship between *HMGIC* immunoreactivity and cytogenetic abnormalities in some benign tumors; in their studies, most of the tumors (85.6%–95%) presenting cytogenetic abnormalities at 12q15 or 6p21 expressed abnormal HMGI proteins. Based on this observation, almost all patients showing 12q15 aberration are likely to have rearrangements of *HMGIC*. If this were to be true, there would be no difference in the general prevalence between Japanese and Western women.

Aberrant splicing occurred about twice as often as translocations in our clinical sample (24% vs 11%). Most of the aberrant splices we observed (7/11; 63.6%) had been reported previously. Five of these seven were the same as U29115, from the human lipoma cell line LM-30.1 (cases 13, 24, 40, 42, and 44) and the other two were identical to U29112, from the human lipoma cell line Li-538 (cases 6 and 38). Of the four novel aberrant splices, two were the same. Because the *HMGIC* aberrations found in several independent cases have been the same (Schoenmakers et al. 1999; Kurose et al. 2001), we suggest that specific sites or patterns of aberrant splicing are common features of *HMGIC* rearrangements.

Fifteen of the 16 rearrangements we observed in our 45 patients had occurred in intron 3 (10/16) or intron 4 (5/16); the single exception had a breakpoint in intron 2. This means that all rearrangements, except for one, retained all three DNA-binding domains, and the one exception retained two of the three DNA-binding domains; all 16 fusion proteins may, therefore, retain the ability to bind DNA. Our sequence data also showed that all 16 fusion genes had lost the sequence encoding the 3′ acidic carboxyl-terminal regulatory domain of *HMGIC*. With respect to the ectopic genes, their fused sequences were all short, and we saw no common feature among them. On the basis of these findings, we speculate that because the *HMGIC* domains characteristic of the fusion proteins may confer unregulated DNA-binding capacity, the fusion events may well participate in the pathogenesis of uterine leiomyomas.

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