

Fusion of a Sequence from *HEI10* (14q11) to the *HMGIC* Gene at 12q15 in a Uterine Leiomyoma

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Uterine leiomyoma, a benign smooth-muscle tumor of the myometrium, is the most commonly encountered neoplasm in women of reproductive age. Band q15 of chromosome 12 is often rearranged in benign mesenchymal tumors such as uterine leiomyomas, and the *HMGIC* gene, encoding a protein of the high-mobility-group (HMG), is present in that region. Using 3' rapid amplification of cDNA ends (3'RACE) experiments, we isolated an ectopic sequence that was fused to *HMGIC* in a uterine leiomyoma. Cloning of the fusion cDNA identified a gene termed "homo sapiens enhancer of invasion 10" (*HEI10*) as the fusion partner. Radiation hybrid mapping revealed that the normal location of *HEI10* is at 14q11. In the fusion transcript the first two exons of the *HMGIC* gene, which encode DNA-binding domains, were fused to the 3' portion of the *HEI10* gene. This rearrangement implicates *HMGIC* in the tumorigenesis of uterine leiomyoma, and suggests that its fusion *HMGIC* product may play a role in mesenchymal differentiation.

Key words: Uterine leiomyoma — *HMGIC* — *HEI10* — Gene fusion

Uterine leiomyoma is the most commonly encountered benign muscle tumor in women of reproductive age,¹⁾ but its pathogenesis remains unresolved although cytogenetic studies have described several tumor-specific chromosomal aberrations in benign tumors including leiomyomas.

One of these, chromosomal translocation t(12:14) (q13–15;q23–24), is typically associated with benign tumors; this aberration sometimes includes rearrangement of *HMGIC*, a gene of the high-mobility-group (HMG) family, that may involve novel fusions.^{2,3)} HMG proteins are nonhistone nuclear proteins that play an important role in the regulation of chromatin structure and function. *HMGIC* and *HMG1(Y)* are members of the *HMG1* family of HMG proteins. *HMGIC* and *HMG1(Y)* dysregulation as a result of specific rearrangements involving 12q15⁴⁾ and 6q21,⁵⁾ the respective chromosomal sites in which the *HMGIC* and *HMG1(Y)* genes are located, is also found in a variety of common benign mesenchymal tumors. The *HMGIC* gene consists of five exons, encoding three DNA-binding domains, one spacer domain, and one acidic regulatory domain. We would expect a rearrangement and/or disruption of *HMGIC* to be a critical factor in cellular proliferation and thus in development of neoplasms.

Until now, only a few genes, e.g. *ALDH2*,⁶⁾ *RAD51B*,⁷⁾ and *COX6C*⁸⁾ were known to be fused with *HMGIC* in uterine leiomyomas, but we suspected that other genes might also have this property. Using 3' rapid amplification

of cDNA ends (3'RACE) and reverse transcriptase-polymerase chain reactions (RT-PCRs), we screened 16 uterine leiomyomas and identified a novel fusion partner of *HMGIC* in one of those tumors.

MATERIALS AND METHODS

Tumor samples and extraction of RNA and DNA Uterine leiomyomas were obtained from 16 patients at the time of surgery in the Nippon Medical School Hospital, frozen immediately, and stored at -70°C until extraction. From approximately 100 mg of each leiomyoma tissue, total RNA was extracted by the guanidine thiocyanate method,⁹⁾ using an Isogen RNA extraction kit (Nippon Gene, Tokyo). DNA was isolated by procedures described previously.¹⁰⁾

PCR primers and hybridization probe The sequences of the oligonucleotide primers (AP1, AP2, H1–H5, C1–C3) and the hybridization probe (A) are listed in Table I.

3' rapid amplification of cDNA ends (3'RACE) To screen for fusions involving *HMGIC*, we reverse-transcribed 5 μg of total RNA from each of the 16 tumors with Superscript II (Life Technologies, Rockville, MD) 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 MgCl_2 , 10 mM dithiothreitol (DTT), 0.5 mM dNTP and 50 pmol of adapter primer 1 (AP1). The RNA was then digested with 6 U of RNaseH (TaKaRa, Tokyo) at 37°C for 30 min. For the first, second and third rounds of PCR we used the adapter primer 2 (AP2) as reverse primer. As forward

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Table I. Oligonucleotide Primers and Probe Used in This Study

Primers	
AP1	5'-GACTCGAGTCGACATCG(T) ₁₇ -3'
AP2	5'-GACTCGAGTCGACATCG-3'
H1	5'-CTTCAGCCCAGGGACAACC-3'
H2	5'-GGAAGCAGCAGCAAGAACC-3'
H3	5'-AACCAACCGGTGAGCCCTCT-3'
H4	5'-ATGAGCGCACGCGGTGAGGG-3'
H5	5'-ATTTCTAGGTCTGCCTCTT-3'
C1	5'-CCTTGGAGCTTTTGATACTGACGA-3'
C2	5'-TCATGGTTAGCAATAGTGATGTTTCG-3'
C3	5'-CTCCAGCCTCAACCTCCTA-3'
Probe	
Probe A	5'-CTTTGAGCTGCTTTAGAGGGACT-3'

primers we used the *HMGIC*-specific sequence H1 (*HMGIC* exon 1) in the first round, nested primer H2 (*HMGIC* exons 1 and 2) in the second round, and nested primer H3 (*HMGIC* exon 2) in the third round. First-strand cDNA was used as the template for the first PCR in a total volume of 10- μ l containing 1 \times Klen*Taq* PCR reaction buffer (Clontech, Palo Alto, CA), 0.2 mM of each dNTP, 4 pmol of each primer, and 1 \times Advantage Klen*Taq* Polymerase Mix (Clontech). Cycle conditions were 94°C for 2 min, then 30 cycles of 90°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). In the second and third rounds, a 1/50 portion of the first-round PCR product was used as the template. Nested PCR conditions were as described above except that amplification proceeded for 25 cycles.

Hybridization of oligonucleotide probe The nested PCR products from the 3'RACE experiments were electrophoresed on 3% NuSieve agarose gels (FMC, Rockland, ME) and transferred to nylon membranes. A gene-specific oligonucleotide probe (A) from exon 2 of *HMGIC* was end-labeled with [γ -³²P]ATP by T4 polynucleotide kinase. This probe was hybridized to the blotted membranes according to procedures described previously.¹¹⁾

Cloning and sequencing of a fusion transcript 3'RACE products that were positive for hybridization were subcloned into the plasmid vector pBlueScript II (SK+) and sequenced on a 377 DNA sequencer (Applied Biosystems, Weiterstadt, Germany).

Radiation-hybrid mapping Radiation-hybrid (RH) mapping was performed with the Stanford G3 RH panel available from Research Genetics. DNA from each RH clone was amplified with primers specific to the fused sequence, *HEI10*. PCR products were electrophoresed on 3% agarose gels (3:1 NuSieve: Seakem; FMC) and visualized with ethidium bromide. The gels were scored for the presence of amplified products, and the results were submitted

to the World Wide Web server of the Stanford Human Genome Center (<http://www-shgc.stanford.edu>).

RT-PCR RT-PCRs were performed to detect expression of chimeric *HMGIC/HEI10* mRNA. Each PCR was carried out with a sense primer specific for *HMGIC* sequence and an anti-sense primer specific for *HEI10* sequence, with the primer pairs chosen to flank the breakpoints. The PCR conditions were as described previously.¹²⁾

RESULTS

To identify transcripts representing possible fusions between *HMGIC* and ectopic sequences we screened RNAs from 16 uterine leiomyomas by 3'RACE experiments, using *HMGIC* cDNA sequences as primers. In one tumor (#47), hybridization of a 3'RACE product to *HMGIC* probe A revealed an aberrant band of 873 bp in myoma #47, but, no signal at 3211 bp that would be expected from the native structure of *HMGIC* gene was detected in the lane where the 3'RACE product of normal uterine tissue was loaded (Fig. 1A). This is in accord with the fact that *HMGIC* gene is transcribed only in fetal tissue but not in any adult tissue. When this aberrant band from myoma #47 was extracted from the gel, cloned, and sequenced we detected an ectopic sequence downstream of *HMGIC* exon 2, indicating that this rearrangement had occurred within intron 2 of the *HMGIC* gene.

The fused sequence was mapped by means of the G3 RH mapping panel of 83 hybrid cell lines from the Stanford Human Genome Center (<http://www-shgc.stanford>).

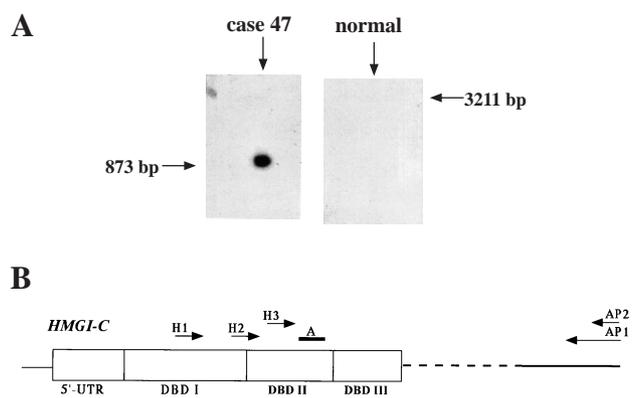


Fig. 1. A. Autoradiogram from a 3'RACE experiment whose product hybridized to the *HMGIC*-specific probe. 3'RACE was performed using primers H1–H3, AP1 and AP2. PCR products were separated on 3% NuSieve agarose gels and transferred to nylon membranes. The gene-specific oligonucleotide probe (A) was hybridized to the blotted membranes. B. Positions and orientation (arrows) of oligonucleotide primers H1–H3, AP1, and AP2 for 3'RACE analysis. The location of probe A is indicated with a short horizontal bar. DBD, DNA-binding domain; UTR, untranslated region.

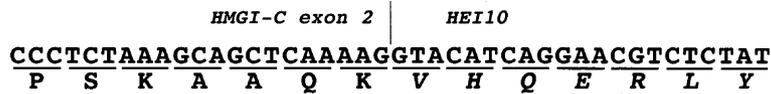


Fig. 2. Nucleotide and predicted amino acid sequences around the respective breakpoints in uterine leiomyoma #47.

edu), through linkage to markers that had been cytogenetically mapped to 14q11. Thus parts of the *HMGIC* gene at 12q15 and the gene at 14q11 were juxtaposed in tumor 47, the most likely mechanism of this event being a chromosomal translocation between 12q15 and 14p11. The breakpoint of each chromosomal band would have occurred within the two genes in question.

A search for homologies with the BLAST program revealed that the novel fusion sequence was identical to a human sequence that had been submitted directly to Genbank under the name "Homo sapiens enhancer of invasion 10" (*HEI10*) mRNA (Fig. 2). Analysis of the aberrant transcript revealed that the first two exons of the *HMGIC* gene, which encode DNA-binding domains, were fused to the 3' portion of the *HEI10* sequence. Fig. 3 shows sequences surrounding the breakpoint between the two genes. The fusion product consists of the N-terminal 66 amino acids of *HMGIC* protein and the 178 C-terminal residues of *HEI10* protein.

To confirm that *HMGIC-HEI10* fusion mRNA was indeed produced in this tumor, we performed RT-PCR experiments with three *HEI10*-specific primers (C1, C2, and C3) and *HMGIC*-specific primer H2. As expected, *HMGIC-HEI10* fusion products of 330, 378 and 694 bp were amplified, respectively, by primer pairs H2-C1, H2-C2 and H2-C3 when RNA from this tumor was used as the template, but not when the RNA was from normal myometrium of the same patient (Fig. 3A).

DISCUSSION

Translocations involving chromosomes 12 and 14, especially region 12q13–15, are observed in a variety of benign tumors, including lipomas, pleomorphic adenomas, and uterine leiomyomas. Rearrangements of *HMGIC*, a gene located on 12q13–14, are often associated with these chromosomal translocations, and fusion with other genes is a typical feature of these events. Since *HMGIC* protein binds to the minor groove of AT-rich DNA¹³⁾ and modifies DNA binding/conformation, we believe that *HMGIC* acts as an architectural transcriptional complex.^{14, 15)} There has been no report of translocation or gene-fusion involving *HEI10* located at 14q11 in any tumor. This chromosomal location is far centromeric from the breakpoint, 14q23–24, observed in some uterine myomas.

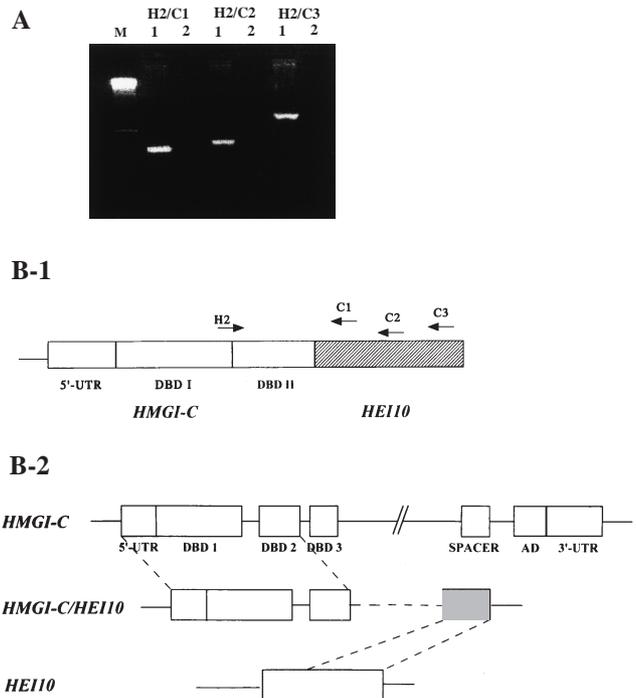


Fig. 3. A. RT-PCR analysis showing specific expression of the *HMGIC-HEI10* fusion transcript in one uterine leiomyoma. PCR products were electrophoresed on 3% NuSieve agarose gels and stained with ethidium bromide after amplification with the three sets of primers shown. M, molecular size standard (50-bp ladder); lane 1, tumor (case 47); lane 2, normal myometrium (case 47). B-1. Positions and orientation (arrows) of oligonucleotide primers (H2, C1–C3) for RT-PCR analysis. DBD, DNA-binding domain; UTR, untranslated region. B-2. Schematic representation of the rearrangement involving *HMGIC* and *HEI10*. The 3' portion of the *HEI10* gene is fused to the second intron of *HMGIC*, within the DNA-binding domains and preceding a spacer and the acidic carboxy-terminal domain. White boxes indicate *HMGIC* sequence; hatched boxes, *HEI10* sequence. AD, acidic domain; DBD, DNA-binding domain; UTR, untranslated region.

Using 3'RACE to analyze 16 primary uterine leiomyomas, we identified a sequence termed *HEI10* as a novel fusion partner of *HMGIC* in one of the tumors. Its mRNA sequence, which had been submitted directly to Genbank by others, was the sole information available for *HEI10* before we mapped it to chromosome 14. Although we

have no clues to the functional significance of the fusion event between *HMGIC* and *HEI10* in tumor 47 with regard to cellular proliferation, we speculate that the rearrangement involving *HEI10* that occurred within DNA-binding domains of the *HMGIC* gene would disrupt the normal structure of the *HMGIC* product and might confer its DNA-binding capacity on the novel fusion product.

Recently a rearrangement of *HMGIC* in a pleomorphic adenoma of the parotid gland was reported in the 3'UTR of *HMGIC*, leaving the complete coding region intact but removing eight AUUUA motifs.¹⁶ That motif has been linked to rapid deadenylation and subsequent decay of mRNAs.¹⁷ A transcript lacking this motif could yield a more stable *HMGIC* mRNA, which might affect levels of HMGIC protein in the cells and ultimately, control of cell proliferation. Therefore, it may well be that separation of the DNA binding domains of *HMGIC* from its 3'UTR sequence, especially the eight AUUUA motifs, is the key mechanism of a variety of *HMGIC*-related gene fusion events that may contribute to development of uterine leiomyomas and other benign neoplasms.

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