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QUARTERLY

A novel isoform of human lymphoid enhancer-binding factor-1 (*LEF-1*) gene transcript encodes a protein devoid of HMG domain and nuclear localization signal✪☼

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Lymphoid enhancer-binding factor-1 (LEF-1), a member of the high mobility group (HMG) family of proteins, regulates expression of T-cell receptor-α gene and is one of the key regulatory molecules in the epithelial-mesenchymal interactions during embryonic development. Among others, LEF-1 regulates expression of cytokeratin genes involved in formation of hair follicles and the gene encoding the cell-adhesion molecule E-cadherin. Transcription factor LEF-1, which acts as a dimer, binds β-catenin and is involved in signal transduction by the wnt pathway.

We have cloned and sequenced a novel isoform of human *LEF-1* gene transcript. This isoform encodes a truncated protein devoid of HMG domain and nuclear localization signal but retaining β-catenin binding domain. This isoform might either act in a dominant-negative manner by interfering with native LEF-1, or might bind β-catenin in the cytosol, which would result in attenuation of the signals transmitted by theLEF-β-catenin pathway.

Lymphoid enhancer-binding factor-1 (LEF-1) belongs to the high mobility group (HMG) of proteins recognizing core consensus sequence 5′-CTTTGA/TA/T-3′ located in the enhancer regions of a number of genes involved in the early stages of T-cell development [1, 2]. It has been demonstrated that upon binding to the minor groove of DNA, LEF-1 induces formation of a

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☼The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number: **AF294627.**

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Abbreviations: HMG, high mobility group; HK-1, transcription factor LEF-1 binding motif; *Lef-1*, gene encoding murine lymphoid enhancer-binding factor-1 (Lef-1); *LEF-1*, gene encoding human (LEF-1); RT-PCR, reverse transcription followed by amplification by polymerase chain reaction (PCR); wnt, signal transduction pathway involving LEF-1 and β-catenin.

sharp bend in the double helix, thus facilitating the binding of other transcription factors to the adjacent DNA sequences in order to modulate transcription [3]. It has been shown that LEF-1 plays a critical role in organogenesis requiring concerted interaction between cells of epithelial and mesenchymal origin [4] and is expressed in neural crest, mesencephalon, tooth buds, hair follicles and eccrine sweat glands [2]. It has been evidenced that LEF-1 interacts with β-catenin to form a ternary complex with DNA, suggesting that this interaction might provide a molecular mechanism of signal transduction by cell-adhesion molecules or components of the wnt pathway [5]. Zhou *et al.* [6] have demonstrated that genes involved in hair development in mice contain Lef-1 binding motif and that Lef-1 can bind to these sequences when expressed in hair follicles. The studies on Lef-1-deficient mice have revealed that normal development of tooth buds requires transient expression of *Lef-1* gene in the dental epithelium [7]. These observations have implicated a functional importance of Lef-1 in hair and tooth development.

Kere *et al.* [8] discovered LEF-1 binding motif (HK-1) within the regulatory region of *EDA* gene, whose protein product is involved in the development of tooth buds, hair follicles, and sweat glands [8, 9]. Localization of the HK-1 motif suggested that this gene is an important target for the regulatory signals carried by LEF-1 during epithelial morphogenesis [8].

In order to investigate a potential role of LEF-1 in the regulation of transcription of *EDA* gene, we have cloned and sequenced cDNA encoding a long form of the human LEF-1 (AF198532) corresponding to the protein described by Waterman *et al.* [10]. In this study we describe a novel isoform of *LEF-1* gene transcript which encodes a protein devoid of the HMG domain but retaining the β-catenin binding domain.

MATERIALS AND METHODS

Lymphocytes of a healthy individual who did not exhibit any abnormalities of teeth, hair and sweat glands, were isolated from peripheral blood by centrifugation in a Ficol density gradient and were washed twice in phosphate-buffered saline. The lymphocytes were cultured in RPMI 1640 medium supplemented with L-glutamine, 10% foetal calf serum and 1% antibiotic antimycotic mixture, and were stimulated with lectin-phytohemagglutinin (10 μg/ml). Three days later the medium was changed and the cells were treated with interleukin-2 (100 U/ml) from that time onwards. All reagents used for cell culture were obtained from Sigma (U.S.A.). After 11 days of culture, total RNA was isolated from samples containing approximately 1×10^6 cells [11]. LEF-1 cDNA was obtained by reverse transcription of polyA$^+$ RNA followed by nested PCR (RT-PCR) with the use of the forward primer F: 5′-TAC TTA GGT ACC TGC CCC AAC TTT CCG GAG-3′. This primer corresponded to nucleotides 991–1008 of the mouse Lef-1 mRNA and contained a *Kpn*I restriction site (underlined). The reverse primer: R: 5′-GGG GTT TCA ACA AGC TTC CAT CTC CAG AAG-3′, whose sequence corresponded to nucleotides 2246–2275 of mouse Lef-1 mRNA, contained a restriction site for *Hin*dIII (underlined).

Total RNA was also extracted from hair follicles of the same individual, as well as from human peripheral blood lymphocytes, chondrocytes, keratinocytes, umbilical cord and ovary [11]. The isolated polyA$^+$ RNA was reverse-transcribed and the specific cDNA fragments were amplified (RT-PCR). The following primers were used for amplification: forward (CAC TGT CAG TCG ACA CTT), complementary to exon 6 and reverse (TGC TCC TTT CTC TGT TCA), complementary to exon 9 of *LEF-1* gene.

The amplification was conducted for 30 cycles (94°C, 45 s; 65°C, 45 s; 72°C, 60 s; final extension 72°C, 5 min) in the reaction mixture containing 1 mM dNTPs, 1.5 mM $MgCl_2$, 500 nM of each primer, and 0.5 unit of Taq polymerase (Qiagen, U.S.A.). A fragment of expected length (Fig. 1) was cloned in *Kpn*I and in *Hin*dIII sites of an expression vector pQE32 (Qiagen, U.S.A.). The selected clone was sequenced using fluorochrome Cy5-labeled primers: Vex F: 5′-CGG ATA ACA ATT TCA CAC AG-3′, Lef-1F: 5′-AAC GAG TCT GAA ATC ATC CC-3′ and Vex R: 5′-GGT CAT TAC TGG AGT CTT G-3′. The sequence was estab-

lished with the use of ALFexpress DNA sequencer (Pharmacia-LKB, Sweden).

RESULTS AND DISCUSSION

The full length cDNA encoding the isoform of human *LEF-1* gene transcript was amplified with the use of primers designed for its murine counterpart, since at the time of the initial experiments the sequence of introns of human gene was unknown. During selection of the clones, in addition to the long isoform of *LEF-1* gene transcript, we identified a novel isoform which differed from the short and the long forms, as well as from transcript isoforms described by Hovanes *et al.* [12] but corresponded to proteins described earlier by Waterman *et al.* [10]. The nucleotide sequence of cDNA, corresponding to the novel isoform of *LEF-1* gene transcript (Fig. 2) was almost identical to cDNA sequence of the long form of human LEF-1, except an 80 nt insertion, and contained in-frame stop codon.

As shown in Fig. 3A, nucleotide changes did not result in any amino-acid substitutions in the protein product, which was devoid of the nuclear lo-

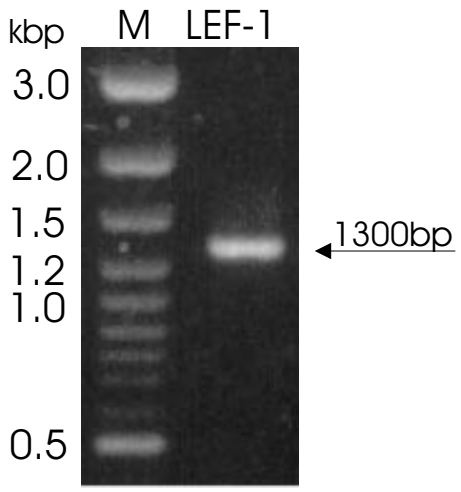

Figure 1. Identification of the lymphoid enhancer-binding factor-1 (LEF-1) cDNA cloned from human cultured lymphocytes.

The cDNA was amplified by PCR using primers specific for mouse Lef-1. The amplification product was resolved by electrophoresis in 1% agarose gel and visualized by ethidium bromide staining.

```
1 ATGCCCCAAC TTTCCGGAGG AGGTGGCGGC GGCGGGGGGG ACCCGGAACT CTGCGCCACG GACGAGATGA TCCCCTTCAA GGACGAGGGC
1 ATGCCCCAAC TTTCCGGAGG AGGTGGCGGC GGCGGGGGGG ACCCGGAACT CTGCGCCACG GACGAGATGA TCCCCTTCAA GGACGAGGGC

  GATCCTCAGA AGGAAAAGAT CTTCGCCGAG ATCAGTCATC CCGAAGAGGA AGGCGATTTA GCTGACATCA AGTCTTCCTT GGTGAACGAG
  GATCCTCAGA AGGAAAAGAT CTTCGCCGAG ATCAGTCATC CCGAAGAGGA AGGCGATTTA GCTGACATCA AGTCTTCCTT GGTGAACGAG

  TCTGAAATCA TCCCGGCCAG CAACGGACAC GAGGTGGCCA GACAAGCACA AACCTCTCAG GAGCCCTACC ACGACAAGGC CAGAGAACAC
  TCTGAAATCA TCCCGGCCAG CAACGGACAC GAGGTGGCCA GACAAGCACA AACCTCTCAG GAGCCCTACC ACGACAAGGC CAGAGAACAC

  CCCGATGACG GAAAGCATCC AGATGGAGGC CTCTACAACA AGGGACCCTC CTACTCGAGT TATTCCGGGT ACATAATGAT GCCAAATATG
  CCCGATGACG GAAAGCATCC AGATGGAGGC CTCTACAACA AGGGACCCTC CTACTCGAGT TATTCCGGGT ACATAATGAT GCCAAATATG

  AATAACGACC CATACATGTC AAATGGATCT CTTTCTCCAC CCATCCCGAG AACATCAAAT AAAGTGCCCG TGGTGCAGCC ATCCCATGCG
  AATAACGACC CATACATGTC AAATGGATCT CTTTCTCCAC CCATCCCGAG AACATCAAAT AAAGTGCCCG TGGTGCAGCC ATCCCATGCG

  GTCCATCCTC TCACCCCCCT CATCACTTAC AGTGACGAGC ACTTTTCTCC AGGGTCACAC CCGTCACACA TCCCATCAGA TGTCAACTCC
  GTCCATCCTC TCACCCCCCT CATCACTTAC AGTGACGAGC ACTTTTCTCC AGGGTCACAC CCGTCACACA TCCCATCAGA TGTCAACTCC

  AAACAAGGCA TGTCCAGACA TCCTCCAGCT CCTGATATCC CTACTTTCTA TCCCTTGTCT CCGGGTGGTG TTGGACAGAT CACCCCACCT
  AAACAAGGCA TGTCCAGACA TCCTCCAGCT CCTGATATCC CTACTTTCTA TCCCTTGTCT CCGGGTGGTG TTGGACAGAT CACCCCACCT

  CTTGGTTGGC AAGGTCAGCC TGTATATCCC ATCACGGGTG GATTCAGGCA ACCCTACCCA TCCTCACTGT CAGTCGACAC TTCCATGTCC
  CTTGGTTGGC AAGGTCAGCC TGTATATCCC ATCACGGGTG GATTCAGGCA ACCCTACCCA TCCTCACTGT CAGTCGACAC TTCCATGTCC

  AGGTTTTCCC ATCATATGAT TCCCGGTCCT CCTGGTCCCC ACACAACTGG CATCCCTCAT CCAGCTATTG TAACACCTCA GGTCAAACAG
  AGGTTTTCCC ATCATATGAT TCCCGGTCCT CCTGGGCCCC ACACAACTGG GATCCCTCAT CCAGCTATTG TAACACCTCA GGTCAAACAG

  GAACATCCCC ACACTGACAG TGACCTAATG CACGTGTGCT CTGCTTTTCT CCTCCCCCAT CCCTTCCTCA TTCCTTCAAC CCCTTCCCCT
  GAACACCCCC ACACTGACAG TGACCTAATG CACGTG---- ---------- ---------- ---------- ---------- ----------

  AACCACCACC ACCACCACCT TTTAGGAAGC CTCAGCATGA ACAGAGAAAG GAGCAGGAGC CAAAAAGACC TCACATTAAG AAGCCTCTGA
  ---------- ---------- ------AAGC CTCAGCATGA ACAGAGAAAG GAGCAGGAGC CAAAAAGACC TCACATTAAG AAGCCTCTGA

  ATGCTTTTAT GTTATACATG AAAGAAATGA GAGCGAATGT CGTTGCTGAG TGTACTCTAA AAGAAAGTGC AGCTATCAAC CAGATTCTTG
  ATGCTTTTAT GTTATACATG AAAGAAATGA GAGCGAATGT CGTTGCTGAG TGTACTCTAA AGGAAAGTGC AGCTATCAAC CAGATTCTTG

  GCAGAAGGTG GCATGCCCTC TCCCGTGAAG AGCAGGCTAA ATATTATGAA TTAGCACGGA AAGAAAGACA GCTACATATG CAGCTTTATC
  GCAGAAGGTG GCATGCCCTC TCCCGTGAAG AGCAGGCTAA ATATTATGAA TTAGCACGGA AAGAAAGACA GCTACATATG CAGCTTTATC

  CAGGCTGGTC TGCAAGAGAC AATTATGGTA AGAAAAAGAA GAGGAAGAGA GAGAAACTAC AGGAATCTGC ATCAGGTACA GGTCCAAGAA
  CAGGCTGGTC TGCAAGAGAC AATTATGGTA AGAAAAAGAA GAGGAAGAGA GAGAAACTAC AGGAATCTGC ATCAGGTACA GGTCCAAGAA

  TGACAGCTGC CTACATCTGA 1280
  TGACAGCTGC CTACATCTGA 1200
```

Figure 2. Sequence alignment of cDNA corresponding to the novel isoform of *LEF-1* gene transcript.

The upper sequence represents the novel isoform, whereas the lower sequence the long isoform of *LEF-1* gene transcript. The 80 nt insertion is underlined. The sequence corresponding to stop codon is printed in bold.

A

```
LEF-1 long-     1 mpqlsggggg gggdpelcat demipfkdeg dpqkekifae ishpeeegdl adiksslvne seiipasngh
LEF-1 novel-    1 mpqlsggggg gggdpelcat demipfkdeg dpqkekifae ishpeeegdl adiksslvne seiipasngh

LEF-1 long-    71 evarqaqtsq epyhdkareh pddgkhpdgg lynkgpsyss ysgyimmpnm nndpymsngs lsppiprtsn
LEF-1 novel-   71 evarqaqtsq epyhdkareh pddgkhpdgg lynkgpsyss ysgyimmpnm nndpymsngs lsppiprtsn

LEF-1 long-   141 kvpvvqpsha vhpltplity sdehfspgsh pshipsdvns kqgmsrhppa pdiptfypls pggvgqitpp
LEF-1 novel-  141 kvpvvqpsha vhpltplity sdehfspgsh pshipsdvns kqgmsrhppa pdiptfypls pggvgqitpp

LEF-1 long-   211 lgwqgqpvyp itggfrqpyp sslsvdtsms rfshhmipgp pgphttgiph paivtpqvkq ehphtdsdlm
LEF-1 novel-  211 lgwqgqpvyp itggfrqpyp sslsvdtsms rfshhmipgp pgphttgiph paivtpqvkq ehphtdsdlm

LEF-1 long-   281 hvkpqheqrk eqepkrphik kplnafmlym kemranvvae ctlkesaain qilgrrwhal sreegakyye
LEF-1 novel-  281 hvCSAFLLPH PFLIPSTPSP NHHHHHLLGS LSMNRERSRS QKDLTLRSL

LEF-1 long-   351 larkerqlhm qlypgwsard nygkkkkrkr eklqesasgt gprmtaayi
```

B

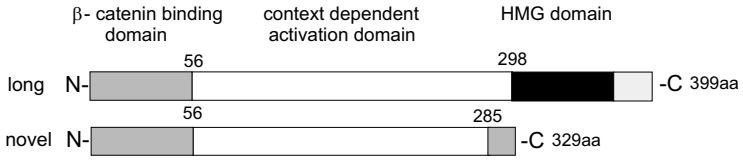

Figure 3. Comparison of the amino-acid sequence (A) and organization of functional domains (B) of the isoforms of LEF-1 proteins.

The sequence of HMG domain is underlined, and the fragment encoded by the additional 80 nt is printed in capitals.

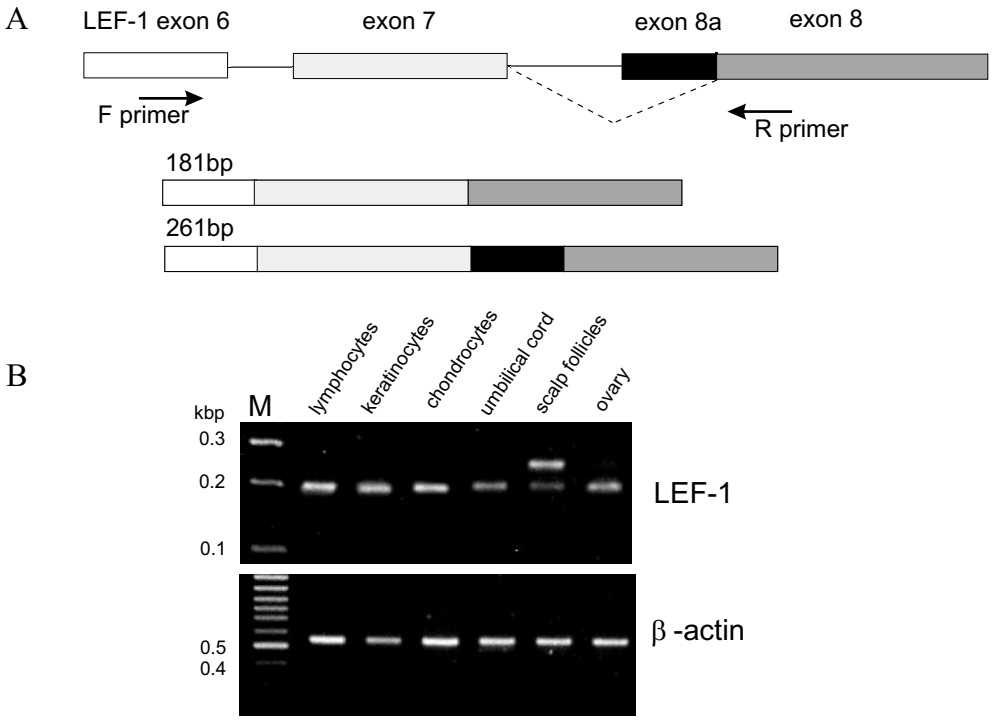

Figure 4. Amplification of LEF-1 cDNA (A) and identification of isoforms of *LEF-1* transcript in human cells and tissue specimens (B).

Reverse transcription-PCR was conducted with the use of specific primers flanking the additional 80 bp sequence and complementary to exon 6 and 8. Amplification products were resolved by electrophoresis in 1% agarose gel and were visualized by ethidium bromide staining.

calization signal and the entire HMG domain, but retained β-catenin binding domain (Fig. 3B).

The 80 nt insertion was localized between the sequences corresponding to exons 7 and 8 (Fig. 4A). Comparison of the 80 nt insertion with a partial sequence of human chromosome 4, deposited in GenBank (AC021524), revealed a 100% homology with part of the sequence of intron 7. This suggested that the novel isoform of *LEF-1* gene transcript might arise due to selection of an alternative splice site localized in intron 7 (Fig. 4A).

In order to substantiate this observation, we searched for this isoform in a number of cells and tissues. The novel isoform was detected in hair follicles, but not in freshly isolated lymphocytes, cultured keratinocytes, or chondrocytes, nor in the umbilical cord and ovary (Fig. 4B). Our findings suggest that the novel isoform is specifically expressed in hair follicles. It was inferred from our results that the expression of the novel isoform in cultured lymphocytes was illegitimate, since it was absent from freshly isolated peripheral blood lymphocytes.

Recently, Hovanes *et al.* [12] demonstrated that *LEF-1* gene encodes multiple isoforms of the transcript, which are due to alternative splicing. The isoform described herein encodes a truncated protein devoid of HMG domain and nuclear localization signal, but retaining the region responsible for interaction with β-catenin. The function of this protein is unknown.

We postulate that it might either act in a dominant-negative manner by interfering with native LEF-1 as suggested by Behrens *et al.* [5], or it might bind β-catenin in the cytosol which results in attenuation of the signals transmitted by the wnt pathway in hair follicles.

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