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SHORT COMMUNICATION

**Nobuhide Ueki · Naohiko Seki · Kazuhiro Yano
Miki Ohira · Toshiyuki Saito · Yasuhiko Masuho
Masa-aki Muramatsu**

Isolation and chromosomal assignment of human genes encoding cofactor of LIM homeodomain proteins, CLIM1 and CLIM2

Abstract Cofactors of LIM homeodomain proteins (CLIM) are transcriptional activators that associate with the LIM homeoproteins and coordinate transcription. LIM homeoproteins and CLIMs are involved in a variety of developmental processes. Two CLIMs, CLIM1 and CLIM2, have been identified in the mouse. Here we report the isolation of human CLIM1 and CLIM2 cDNAs and the determination of their chromosome locations by using a human–rodent monochromosomal hybrid cell panel and a radiation hybrid mapping panel. The proteins deduced from human CLIM1 and CLIM2 cDNAs were composed of 373 and 375 amino acids, respectively, and had 97.3% and 98.7% amino acid identity, respectively, to their mouse counterparts. Human CLIM1 and CLIM2 proteins were 75.5% identical. Human *CLIM1* and *CLIM2* genes were mapped to the chromosome on 4p15.3 and 10q24–q25 regions, respectively. Mapping of a pair of developmentally important genes may provide new clues to the understanding of genetic disorders caused by these chromosome regions.

Key words CLIM · LDB · NLI · LIM domain · RH mapping · 4p15.3 · 10q24–q25 · SHFM3

N. Ueki · K. Yano · Y. Masuho · M. Muramatsu (✉)
Helix Research Institute, 1532-3 Yana, Kisarazu, Chiba 292-0812, Japan
Tel: +81-438-52-3951; Fax +81-438-52-3952
e-mail: mmasaaki©hri.co.jp

M. Muramatsu
Department of Biological Cybernetics, Medical Research Institute, Tokyo Medical Dental University, Tokyo, Japan

N. Seki
Laboratory of Gene Function II, Kazusa DNA Research Institute, Chiba, Japan

N. Seki · T. Saito
Genome Research Group, National Institute of Radiological Sciences, Chiba, Japan

M. Ohira
Department of Biochemistry, Chiba Cancer Center, Chiba, Japan

Introduction

LIM homeoproteins consist of a large family of transcription factors that play essential roles in embryonic development, organogenesis, and pattern formation (Dawid et al. 1998). LIM homeoproteins consist of two LIM domains and a DNA-binding homeodomain. Recently, a family of nuclear proteins that bind to the LIM domains of LIM homeoproteins was isolated from the mouse (Jurata et al. 1996; Bach et al. 1997). This protein family currently consists of two members, CLIM1 and CLIM2 (cofactor of LIM homeodomain protein). CLIM2 is also known as LIM-domain binding (LDB1) (Agulnick et al. 1996) and nuclear LIM interactor (NLI) (Jurata et al. 1996). CLIM1 and CLIM2 can synergize the transcriptional activity of LIM homeoproteins (Bach et al. 1997). They can also form complexes with other transcription factors, indicating that CLIMs may be involved in a wide spectrum of gene regulation (Bach et al. 1997; Visvader et al. 1997; Wadman et al. 1997). However, the precise role of CLIM1 and CLIM2 in vivo still needs to be determined.

Transcription factors in embryonic development are occasionally involved in tumorigenesis and congenital abnormalities. For example, LIM homeoproteins hLH-2 and LMX1B are known to be involved in chronic myelogenic leukemia (Wu et al. 1996) and nail-patella syndrome (Vollrath et al. 1998), respectively. To this end, we isolated and determined chromosomal location of human *CLIM1* and *CLIM2* genes. This information should prove valuable in designing studies to evaluate their cellular function and relation to diseases.

Materials and methods

Cloning of human CLIM1 and CLIM2 cDNAs

An initial CLIM1 cDNA fragment was isolated during the course of screening for nuclear proteins. (Ueki et al., in

press). To obtain the 5′ region of the *CLIM1* gene, primers 5′-TGT TTG TTA GCC CCA TCC TGG TG-3′ (according to the sequence of CLIM1) and 5′-CAC ACA GGA AAC AGC TAT GAC CAC TAG-3′ (according to the sequence of the library vector) were designed, and PCR was performed using a human fetal brain cDNA library (Gibco-BRL, Gaithersburg, MD, USA). To obtain human CLIM2 cDNA, we searched with BLAST against the dbEST database for sequences with significant homology to mouse CLIM2 cDNA (GenBank Accession No. U70375) and found two ESTs (AA569020 and AA194121). PCR primers, 5′-TGT TCC TCA AAG TCA TTC AAG CTG TAC TCG-3′ and 5′-AGA TGC TCA GTC TCT TCA TTC TGT CTT CTG C-3′, were designed according to these sequences. A human CLIM2 cDNA was cloned by the RT-PCR method using these primers and the human fetal brain cDNA library as a template. The parameter for PCR was 30 cycles of 94°C for 30s, 61°C for 30s, and 72°C for 2min. PCR products were cloned into pT7Blue(R) vector (Novagen, Madison, WI, USA), and nucleotide sequences were determined by using an ABI377 sequencer.

Mapping of human CLIM1 and CLIM2 genes

Chromosomal assignment of *CLIM1* and *CLIM2* genes was determined by using PCR analysis of NIGMS human–rodent somatic cell hybrid mapping panel 2 (Coriell Cell Repositories, Palo Alto, CA, USA). CLIM1 gene-specific primers were 5′-GTG TGC GTG CGT CTA CTT TGT-3′ and 5′-TGA AAG GAG AAG AAT AGA AGG-3′; *CLIM2* gene-specific primers were 5′-GCA TTT GCC TCC ATC TTC ACC-3′ and 5′-AGC TTT ACC CCT CTA TTA CCA-3′. Human, mouse, and hamster genomic DNA were included as controls. PCR was carried out in a final volume of 10μl containing 1× LA-PCR buffer (Takara, Kyoto, Japan). The parameter for PCR was 30 cycles at 95°C for 20s and 62°C for 1min. Radiation hybrid mapping used a Genebridge 4 mapping panel (Research Genetics, Huntsville, AL, USA) and the same primers as just described. The data vector was submitted to Whitehead Institute/MIT Center for STS mapping server, and statistical analysis was performed using the RHMAPPER software package (*http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl*).

Results

Isolation of human CLIM1 and CLIM2 cDNAs encoding the entire ORFs

Combining the sequences of the initial CLIM1 cDNA fragment and that of the 5′ portion obtained by rescreening, the resultant human CLIM1 cDNA was 2330bp (GenBank Accession No. AF047337) and encoded a protein of 373 amino acids (a.a.) (Fig. 1). The human CLIM1 protein showed 97.3% amino acid identity to the mouse CLIM1 protein. The mouse CLIM1 cDNA has two alternative splicing forms of CLIM1a and CLIM1b (Bach et al. 1997), which encode different ORFs in the carboxyl-terminal domain. The CLIM1 cDNA we cloned was the counterpart of the mouse CLIM1a spliced form.

The human CLIM2 cDNA cloned by RT-PCR was 1240bp (AB016485) and encoded a protein of 375 a.a. (Fig. 1). The human CLIM2 protein showed 98.7% amino acid identity to the mouse CLIM2 protein. Alignment of human CLIM1 and CLIM2 proteins showed that both were 75.5% identical throughout the entire molecule (Fig. 1). Two potential nuclear localizing signals were present in both proteins (underlined in Fig. 1), and no other known domains could be found.

Mapping of human *CLIM1* and *CLIM2* genes

Using *CLIM1*-specific primers, a PCR product of 145bp was detected in human genomic DNA, control DNA, and somatic cell hybrids containing only human chromosome 4. The RH mapping data vector for *CLIM1* was 0100000101 0010000100 2011010010 0010000100 0011111000 1102001010 1110011000 0000000001 0000000002 010. The consequent report indicated that the *CLIM1* gene was mapped (LOD > 3.0) between markers D4S1601 and D4S934 (Fig. 2a, left panel). The position of the gene was 0.90cR proximal to the marker D4S1601. D4S1601 maps 84.57cR from the top of chromosome 4 linkage group. Loci D4S1601 and D4S934 have been approximately mapped to the 4p15.3 region (Riess et al. 1996).

Using CLIM2-specific primers, a PCR product with the expected size of 164bp was detected in human genomic

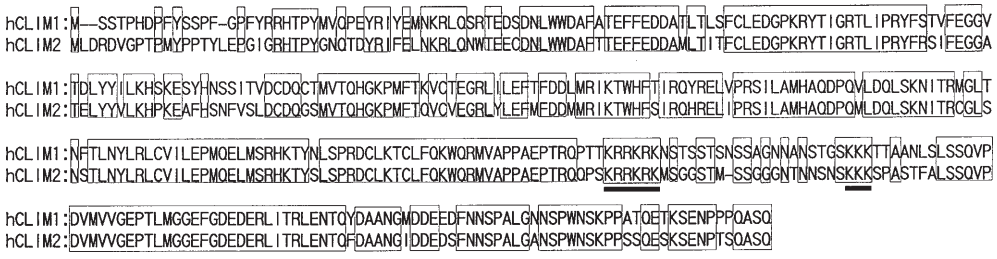
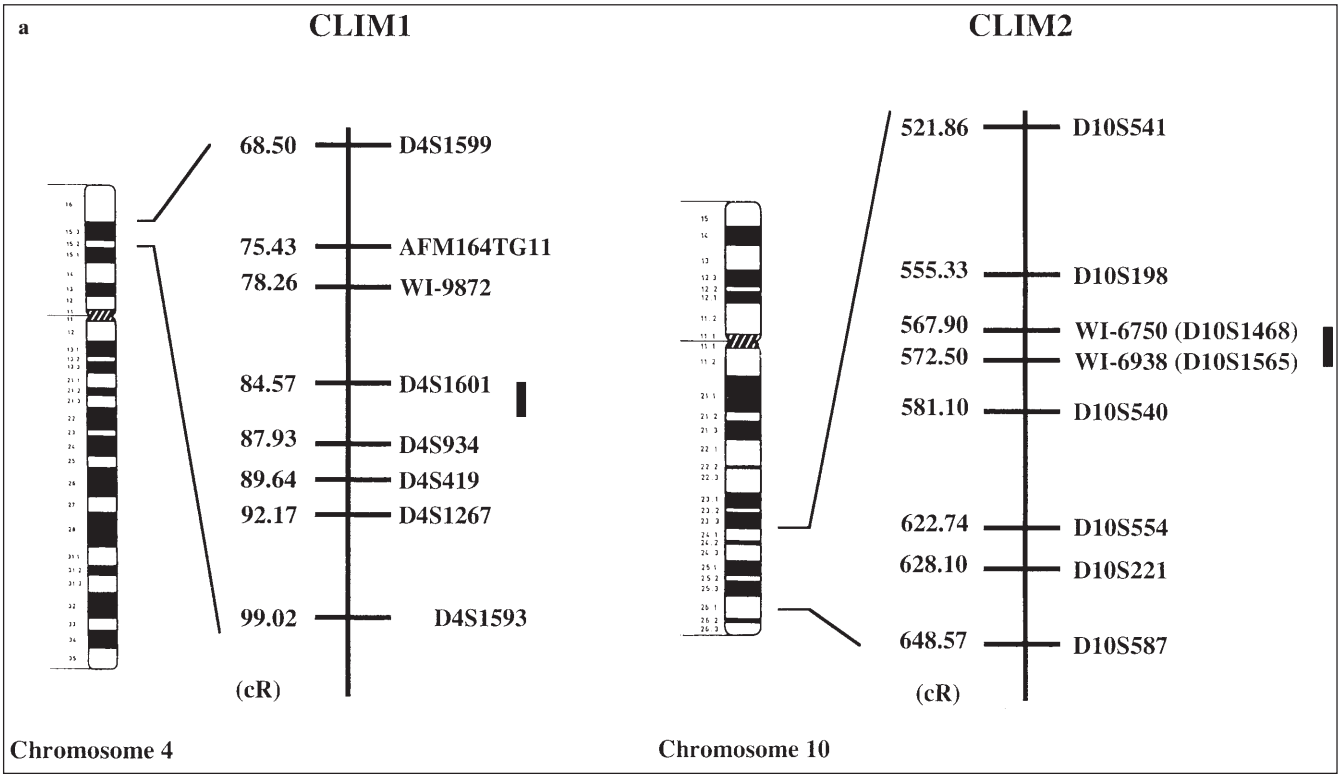


Fig. 1 Amino acid sequence alignment of human CLIM1 and CLIM2 proteins. A *hyphen* indicates that this residue is not present. Identical residues are *boxed*. The putative nuclear localization signals are *underlined*. GenBank accession numbers for human CLIM1 and CLIM2 cDNAs are AF047337, and AB016485, respectively



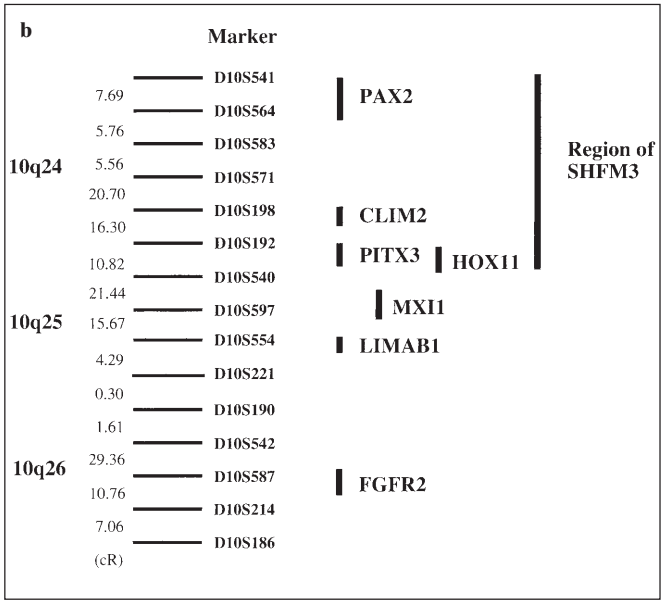


Fig. 2 a, b Schematic representation of human *CLIM1* and *CLIM2* loci. **a** Chromosomal placement of human *CLIM1* and *CLIM2* genes at relative distances to framework markers on the WICGR radiation hybrid map of the human genome. The approximate corresponding cytogenic locations of *CLIM1* and *CLIM2* are indicated by *vertical bars*. Distances are in centirays (*cR*) from the top of chromosome 4 and chromosome 10 linkage groups. **b** Location of *CLIM2* gene and other genes in the 10q24–q25 region. *CLIM2*, *Pax2*, *PITX3*, *Hox11*, *MXI1*, *LIMAB1*, *FGFR2*, and the *SHFM3* region are indicated by *vertical bars*. The locations were determined by OMIM Gene Map and references in the text. The distances between the markers are in centirays (*cR*).

DNA, control DNA, and somatic cell hybrids containing only human chromosome 10. The RH mapping data vector for the *CLIM2* gene was 1000110010 00001000010 0010100110 0000101010 0000110000 0010010010 0000100000 0110100011 1010001001 001. The consequent report indicated that the *CLIM2* gene was mapped (LOD $>$ 3.0) between markers WI-6750 and WI-6938 (Fig. 2a, right panel). The position of the gene was 1.92 cR distal to the marker WI-6750. The marker WI-6750 maps 567.90 cR from the top of chromosome 10 linkage group. Loci WI-6750 and WI-6938 have been approximately mapped to the 10q24–q25 region (Meitinger et al. 1997).

Discussion

In the present study, we isolated human CLIM1 and CLIM2 cDNAs and mapped their chromosomal locations. CLIMs are highly conserved among species, and homologs are found in *Xenopus laevis* (GenBank Accession No. U74360), in *Danio rerio* (AF031375), and also in *Drosophila* (AF010328). The *Drosphila* CLIM, Chip, is present at numerous sites along the salivary gland polytene chromosomes, suggesting a novel function of the gene product in chromosome regulation (Morcillo et al. 1997). Whether or

not mammalian CLIMs have similar function needs to be determined.

Human CLIM1 and CLIM2 were mapped to the 4p15.3 and 10q24–q25 regions, respectively. No information is available for the chromosome location of the mouse *CLIM1* gene at present. The mouse *CLIM2* gene (alias *LDB1*) was recently mapped to the distal region of mouse chromosome 19, which is syntenic with human chromosome 10q (Yamashita et al. 1998). Our mapping result indicates that human *CLIM2* resides in the syntenic region.

The 10q24–q25 region is rich in genes involved in development, such as *Pax2*, *Hox11*, *PITX3*, *HMX2*, *MXI1*, *LIMAB1*, and *FGFR2* (Kim et al. 1997; Meitinger et al. 1997; Semina et al. 1998). The location of *CLIM2* relative to these genes is shown in Fig. 2b. With regard to diseases, 10q24–q26 is a region of frequent loss of heterozygosity in human tumors, including glioblastoma (Rasheed et al. 1995), prostate cancers (Komiya et al. 1996), endometrial carcinomas (Nagase et al. 1996), and thyroid tumors (Zedenius et al. 1996). Thus, CLIM2 can be included as a positional candidate for one of the several tumor suppresser genes in this region.

Other chromosomal gains or losses of 10q24–q25 without identification of a specific gene has also been reported in cases of congenital abnormalities. One such disease is the autosomal dominant split hand/split foot malformation (SHFM) 3. The SHFM3 region as determined by Nunes et al. (1995) and Gurrieri et al. (1996) includes *CLIM2* (see Fig. 2b). Given the role of homeotic genes in pattern formation, its cofactor CLIM2 might be considered as a positional candidate of the SHFM3 gene in this region.

Our precise chromosomal positioning of the *CLIM1* and *CLIM2* genes may contribute toward ongoing positional candidate approaches for the aforementioned disease genes linked to these chromosomal loci.

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