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ARTICLES

Characterization of Tissue-Specific LIM Domain Protein (FHL1C) Which is an Alternatively Spliced Isoform of a Human LIM-Only Protein (FHL1)

Enders Kai On Ng,[1] Simon M. Y. Lee,[1] Hoi-Yeung Li,[1] Sai-Ming Ngai,[1,2] Stephen K. W. Tsui,[1,2] Mary M. Y. Waye,[1,2] Cheuk-Yu Lee,[1,2] and Kwok-Pui Fung[1,2]*

[1]Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong
[2]Hong Kong Bioinformatics Center, Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong

Abstract We have cloned and characterized another alternatively spliced isoform of the human four-and-a-half LIM domain protein 1 (FHL1), designated FHL1C. FHL1C contains a single zinc finger and two tandem repeats of LIM domains at the N-terminus followed by a putative RBP-J binding region at the C-terminus. FHL1C shares the same N-terminal two-and-a-half LIM domains with FHL1 but different C-terminal protein sequences. Due to the absence of the exon 4 in FHL1C, there is a frame-shift in the 3′ coding region. Sequence analysis indicated that FHL1C is the human homolog of murine KyoT2. The Northern blot and RT-PCR results revealed that FHL1 is widely expressed in human tissues, including skeletal muscle and heart at a high level, albeit as a relatively low abundance transcript in brain, placenta, lung, liver, kidney, pancreas, and testis. In contrast, FHL1C is specifically expressed in testis, skeletal muscle, and heart at a relatively low level compared with FHL1. The expression of FHL1C transcripts was also seen in aorta, left atrium, left, and right ventricles of human heart at low level. Immunoblot analysis using affinity-purified anti-FHL1C antipeptide antibodies confirmed a 20 kDa protein of FHL1C in human skeletal muscle and heart. Unlike FHL1B, which is another FHL1 isoform recently reported by our group and localized predominantly in the nucleus [Lee et al., 1999], FHL1C is localized both in the nucleus and cytoplasm of mammalian cell. J. Cell. Biochem. 82: 1–10, 2001.

Key words: zinc finger protein; FHL1C; FHL1; KyoT2; SLIM1; RBP-J

LIM is an acronym of three homeodomain-containing transcription factors, Lin-11, Isl-1, and Mec-3, in which the motif was first identified [Freyd et al., 1990]. LIM domain contains 50–60 amino acids and possesses a highly conserved double zinc finger domain constituted from a C_2HC motif and a C_4 motif. LIM domain proteins can be classified into four subclasses: (1) LIM-homeodomain (LIM-HD) proteins, (2) LIM-functional domain proteins, e.g., LIM kinase [Mizuno et al., 1994], (3) C-terminal LIM proteins, and (4) LIM-only proteins. There is no evidence that LIM domains interact with DNA directly. Instead, studies implicate that LIM domain protein involved in the regulation of development, cellular differentiation, and cytoskeleton [Schmeichel and Beckerle, 1994; Arber and Caroni, 1996]. Among the LIM-only proteins, LIM-only proteins MLP (muscle LIM protein) are found to be required in myogenic differentiation [Arber et al., 1994]. Besides, the LIM-only proteins *RBTN1* (rhombotin-1) and *RBTN2* (rhombotin-2) were identified as proto-oncogenes in T-cells [Boehm et al., 1991]. Therefore, LIM-only proteins are related to cellular differentiation and development and likely human malignant states.

To study the relationship between LIM proteins and the differentiation regulation of heart, we have cloned and characterized several novel

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*Correspondence to: Prof. Kwok-Pui Fung, Department of Biochemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong. E-mail: kpfung@cuhk.edu.hk

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human LIM-only proteins [Tsui et al., 1994, 1996; Chan et al., 1998, 2000; Garcia-Barcelo et al., 1998; Kotaka et al., 1999; Lee et al., 1998, 1999]. Four-and-a-half LIM domain proteins 1, 2, and 3 (FHL1, FHL2, and FHL3) (NGMW-approved nomenclature) possess a zinc finger followed by four LIM domains. Among the FHL family proteins, FHL1 is very similar to the skeletal muscle LIM protein 1, SLIM1, which is a developmentally regulated muscle LIM protein [Morgan et al., 1995; Morgan and Madgwick, 1996]. FHL1 was located to human chromosome at Xq27 by fluorescent in situ hybridization (FISH) and radiation hybrid mapping [Lee et al., 1998]. The exact functions of FHL1 remain unclear. Recently, studies revealed that the expression patterns of FHL1 and FHL3 appeared to be coordinated with important factors such as MyoD, MRF4, and myogenin [Morgan and Madgwick, 1999]. In our previous large-scale random sequencing of cDNA clones from human heart cDNA libraries, gene expression profiles in various developmental stages of the heart, and in cardiac hypertrophy were compared [Liew et al., 1994; Hwang et al., 1995, 1997]. We found that FHL1 is a strong candidate being up-regulated in familial hypertrophic cardiomyopathy [Hwang et al., 1997]. Moreover, FHL1 appeared to be up-regulated by ectopic expression of HOX11, that is the T-cell oncoprotein, in murine NIH3T3 fibroblasts. The result suggested that FHL1 might be a target gene of HOX11 [Greene et al., 1998]. The mouse homolog of FHL1, named KyoT1, has been identified and found to encode an alternatively spliced isoform, named KyoT2. It has been shown that KyoT2 interacts with RBP-J transcription factor, involved in the Notch signalling pathway, to repress transcription [Taniguchi et al., 1998]. In this study, we report the sequence analyses, partial genomic structures, tissue distribution, and subcellular localization of full-length cDNA sequence encoding an alternatively spliced isoform of FHL1, which possesses two-and-a-half LIM domains followed by a putative RBP-J binding region. This alternatively spliced isoform is shown to be the human homolog of murine KyoT2.

MATERIALS AND METHODS

cDNA Cloning and Sequence Analysis of FHL1C

BLAST searches of the Expressed Sequence Tagged (EST) database identified a human EST clone (GenBank accession No. AI209195) which exhibited highly homology to FHL1 (GenBank accession no. U29538) but with some aberrance as the EST cDNA sequence has deletion of a DNA fragment. PCR was performed on a human testis cDNA library (Marathon-Ready cDNA, Clontech) using PCR primers (5′-CAACCATATATCCAAGCCTT-3′ and 5′-ATGCAGAAGAGTCAATCTAC -3′ which were designed from the 5′- and 3′- end untranslated region (UTR) of FHL1, respectively). Advantage cDNA polymerase mix (Clontech) was used for the PCR reaction with the following cycling parameters: 1 min denaturation at 94°C, followed by 30 cycles of 94°C (1 min), 58°C (1 min), 68°C (3 min) and a final 68°C extension for 7 min. PCR fragment was purified and cloned into pT-Adv vector by T/A cloning method (Clontech). The cDNA fragment was completely sequenced by primer walking. Sequence comparisons against the GenBank and EMBL nucleotide and protein databases were performed using the BLAST Web server in NCBI.

Northern Blot Analysis of Tissue Distribution of FHL1C

The probe used was the coding region of FHL1C which was made from PCR products of 585 base pairs (bp). A radioactive random-primed probe was made using the purified PCR products as the template. The human multiple-tissue Northern blots were purchased from Clontech (Palo Alto, CA). The integrity of the ploy(A)$^+$ RNAs of the blots were examined by denaturing gel electrophoresis and normalized with a radioactively labeled human β-actin cDNA control probe (unpublished data, quality control sheet supplied by Clontech). The blots were prehybridized for 1 h and hybridized for 2 h at 65°C, using ExpressHyb hybridization solution (Clontech). Membranes were washed with 2X SSC/0.05% SDS followed by 0.1X SSC/0.1% SDS at room temperature to remove any nonspecific signals. Autoradiography was performed at −70°C for 2 days.

RT-PCR Analysis of Tissue Distribution of FHL1C

PCR primers (5′-ATGGCGGAGAAGTTTGACTGCCACTACT-3′ and 5′-TCACGGAGCATTTTTTGCAGTGGAAGCA-3′ on the 5′- and 3′- end of FHL1 coding region, respectively; Fig. 2A) were used to amplify Multiple Tissue cDNA library panels (Clontech). The panels are sets of normalized first-strand cDNA generated

using poly-$(A)^+$ RNA from various human tissues. PCR was performed using Advantage cDNA polymerase mix PCR system (Clontech). Samples were subjected to 1 min denaturation at 94°C, followed by 30 cycles of 94°C (1 min), 63°C (1 min), 68°C (2 min), and a final 68°C extension for 5 min. As an internal standard, PCR primers (5′-ACCACAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′) were used to amplify glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in a parallel reaction.

Subcellular Localization Study

GFP-FHL1C hybrid (GFP-FHL1C) was constructed by cloning into pEGFP-C1 vector (Clontech). PCR primers used to amplify the constructs are shown as follow: (GFP-FHL1C: 5′-TAGGGCGAATTCTGCGGAGAAGTTTGACTGCCA-3′ and 5′-TAGGGCGTCGACCGGAGCATTTTTTGCAGTGGAA -3′). An *Eco*RI site and a *Sal*I site are present in the forward and reverse primers, respectively. These GFP hybrid constructs were used to transiently transfect C2C12 myoblasts with lipofectamine. After 24 h transfection, the subcellular distribution of the GFP hybrid proteins was examined using a fluorescent microscope.

To determine the synthesis and stability of GFP-FHL1C hybrid proteins, transfected C2C12 cells were lysed by 200 μl of 25 mM Tris, pH 7.0, 5 mM EDTA, 0.5% NP-40. Proteins were separated by 12.5% SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, MA). GFP polyclonal antibody (Clontech) with dilution of 1:1000 was utilized in immunoblotting. The secondary antibody was horseradish peroxidase-conjugated sheep anti-rabbit IgG (Amersham Pharmacia Biotech) diluted to 1:2000. Immunoblot was developed using Enhanced Chemiluminescence (Amersham Pharmacia Biotech).

Antipeptide Antibodies

Anti-FHL1C antibodies were raised against an acetylated FHL1C peptide, amino acids 168–194 (Ac-GLVKAPVWWPMKDNPGTTTASTAKNAP); 0.1 mg of the peptides were conjugated with KLH solution and were purified with G-50 Sephadex desalting column (Amersham Pharmacia Biotech). The conjugated peptide was injected subcutaneously into a New Zealand White rabbits. Affinity-purified antibody was obtained by chromatography of immune sera on HiTrap Protein G column (Amersham Pharmacia Biotech). Antibodies were eluted from the column with 0.1 M glycine-HCl, pH 2.7.

Immunoblot Analysis

Protein lysates of human tissues were purchased from Clontech (Palo Alto, CA). Proteins were separated by 14% SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, MA). The PVDF membrane was probed with affinity-purified FHL1C specific antibodies (1:1000). HRP-conjugated sheep anti-rabbit IgG was used as the secondary antibodies (1:2000). Immunoblot was developed using Enhanced Chemiluminescence (ECL) detection reagents (Amersham Pharmacia Biotech).

RESULTS

cDNA Cloning and Sequence Analysis of FHL1C

BLAST searches of the EST Database in NCBI revealed the presence of an EST clone isolated from a human testis cDNA library that exhibited a high degree of homology to FHL1 but has a deletion of a cDNA fragment in the EST clone. To examine whether FHL1 might have a different isoform expressed in testis, PCR was performed on a human testis cDNA library using PCR primers which were designed from the 5′- and 3′- end UTR of FHL1. Two different sized PCR fragments were amplified (data not shown). The larger size PCR fragment corresponded to the expected size of FHL1 transcript, while a smaller PCR fragment (1.0 kbp) was isolated and completely sequenced. The novel cDNA sequence was shown in Figure 1. Upon alignment with FHL1 sequence, the novel sequence contains the same FHL1 sequences except for the deletion of 258 bp sequence found in the putative isoform of FHL1 at nucleotide position number 586 (Fig. 1). We previously reported that an alternatively spliced isoform of FHL1, designated FHL1B, was isolated and characterized [Lee et al., 1999]. The genomic organization revealed that FHL1 transcript contained exons 1, 2, 3, 4, and 5 while FHL1B transcript contained exons 1, 2, 3, 4, 4b, and 5 (Fig. 2A). In contrast, the new putative isoform of FHL1 was different from both FHL1 and FHL1B transcripts in which it only contains exons 1, 2, 3, and 5 (Fig. 2A). Therefore, another alternatively spliced isoform of FHL1 gene is described here and it is named FHL1C.

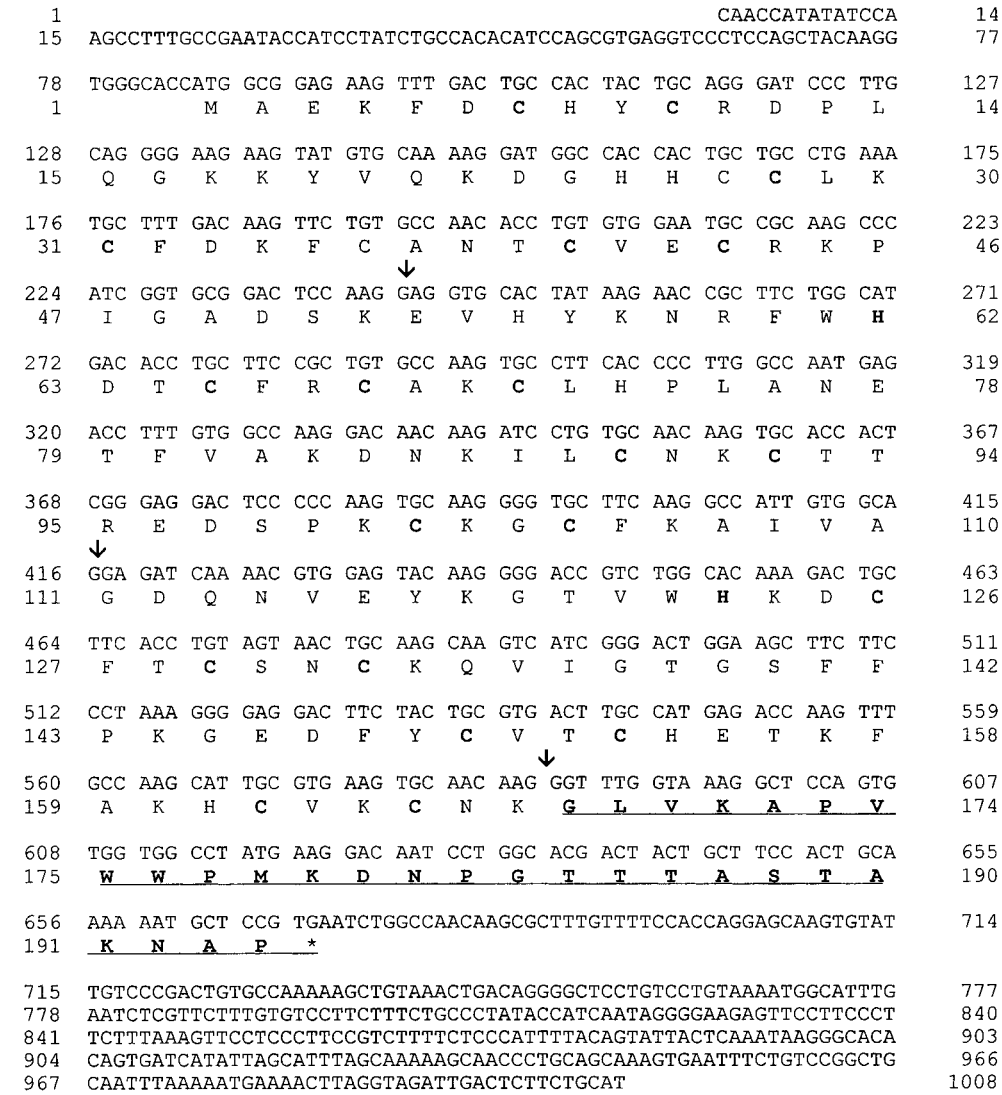

Fig. 1. The cDNA and predicted amino acid sequences of FHL1C. Sequence data of FHL1C have been deposited with GenBank/EMBL Data Libraries under the Accession Number AF220153. The positions of introns are indicated with arrows. The stop codon is indicated with an asterisk. Consensus amino acid residues of LIM domain are in bold type. Putative RBP-J binding region is in bold type and underlined.

The cDNA of FHL1C has a predicted ORF of 585 bp, encoding a 195-amino acid protein (Fig. 1). The nucleotide sequence data have been submitted to the GenBank/EMBL Data Libraies under the accession number AF220153. The protein sequence as predicted from FHL1C sequence possesses a C_4 zinc finger and two tandem repeats of LIM domains at the N-terminus followed by a putative RBP-J binding region at the C-terminus (Figs. 1 and 2B). FHL1C and FHL1 share the same N-terminal two-and-a-half LIM domains but have different predicted C-terminal protein sequences. The absence of exon 4 in FHL1C transcript results in a different translation reading frame in exon 5 compared with that of FHL1; thus, the frame-shifted exon 5 produces a putative RBP-J binding region in FHL1C. On the other hand, FHL1C is different from FHL1B in which FHL1C lacks the third LIM domain encoded by exon 4 and three potential bipartite nuclear localization signals (NLS) and a putative nuclear export sequence (NES) encoded by exon 4b in FHL1B (Fig. 2B). Upon ORF sequence alignment of FHL1C and KyoT2, a murine isoform of FHL1, the nucleotide sequences showed 91% homology (data not shown) while the amino-acid sequences showed 96% homol-

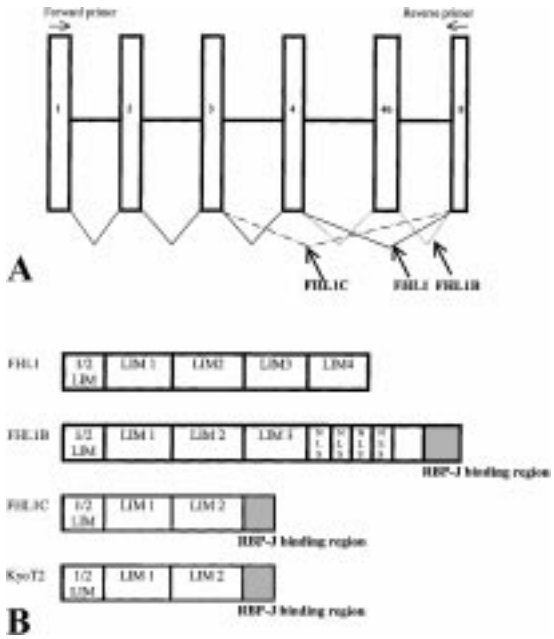

Fig. 2. **Panel A:** Partial genomic organization FHL1C. Exons are represented by boxes with the exon number given inside. Splicing of the predominately expressed *FHL1* transcript is indicated by a solid line, splicing of alternative transcript *FHL1B* containing exon 4b is indicated by a broken line, and splicing of alternative transcript *FHL1C* is indicated by dash line. **Panels B:** Schematic representation of the structures of FHL1, FHL1B, FHL1C, and KyoT2 translation products. NLS represents the consensus bipartite nuclear localization signal motif. NES represents the nuclear export sequence. Shaded boxes represent the RBP-J binding regions.

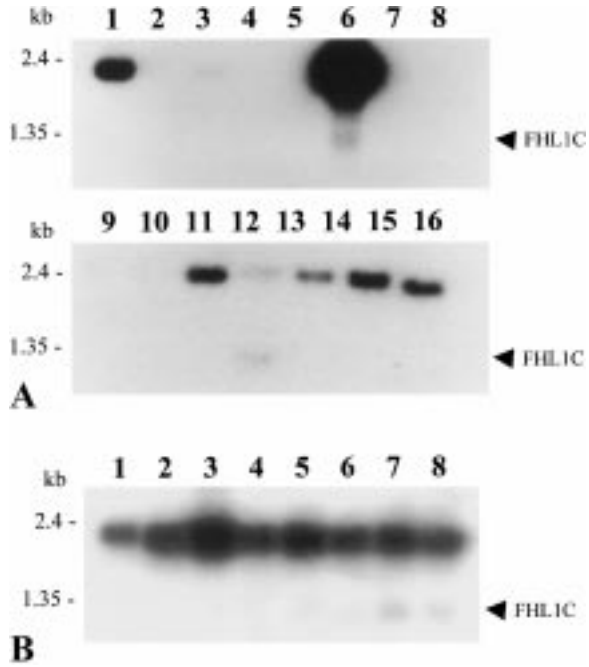

Fig. 3. **Panel A:** Northern hybridization of FHL1C in human tissues. Two human multiple-tissue Northern blots (Clontech) with 2 μg of total poly(A)+ RNA of each of the tissues were hybridized with the coding region of FHL1C cDNA. **Lane 1**, heart; **Lane 2**, brain; **Lane 3**, placenta; **Lane 4**, lung; **Lane 5**, liver; **Lane 6**, skeletal muscle; **Lane 7**, kidney; **Lane 8**, pancreas; **Lane 9**, spleen; **Lane 10**, thymus; **Lane 11**, prostate; **Lane 12**, testis; **Lane 13**, ovary; **Lane 14**, small intestine; **Lane 15**, colon; **Lane 16**, peripheral blood leukocytes. **Panel B:** Northern hybridization of FHL1C in human cardiovascular system. The Northern blot (Clontech) with 2 μg of total poly(A)+ RNA of each of the tissues were hybridized with the coding region of FHL1C cDNA. **Lane 1**, fetal heart; **Lane 2**, heart; **Lane 3**, aorta; **Lane 4**, apex of heart; **Lane 5**, left atrium; **Lane 6**, right atrium; **Lane 7**, left ventricle; **Lane 8**, right ventricle.

ogy. The C-terminal putative RBP-J binding region encoded by the frameshift exon 5 in FHL1C exactly match that of the RBP-J binding region found in murine KyoT2 (Fig. 2B). Since no human homolog of KyoT2 has been identified so far, we hereby report that FHL1C is the human homolog of murine KyoT2.

Tissue Distribution of FHL1 and FHL1C by Northern Blot Analysis

Northern blot analysis utilizing the coding region of FHL1C as a probe detected both FHL1 and FHL1C but they are distinguishable by their different sizes (FHL1 is 2.4 kbp while FHL1C is 1.3 kbp). FHL1C mRNA transcript was specifically expressed in human testis and skeletal muscle albeit at lower levels when compared with FHL1 while FHL1 transcript was highly expressed in skeletal muscle and heart (Fig. 3A). Fewer transcripts of FHL1 were seen in variety of tissues, including prostate, small intestine, colon, ovary, and testis. From the Northern blot result of the human cardiovascular system, FHL1C transcript was expressed in left and right ventricles at low level (Fig. 3B, lane 7 and 8). Few FHL1C transcripts were detectable in aorta and left atrium (seen with a longer exposure of the film; data not shown). Interestingly, higher expression of FHL1 transcripts was found in adult heart than in fetal heart. Moreover, stronger expression of FHL1 was seen in aorta and left atrium (Fig. 3B, lane 3 and 5).

Tissue Distribution of FHL1, FHL1B and FHL1C by RT-PCR

In order to confirm that FHL1C are indeed transcribed, PCR primers designed on 5′ and 3′ end of coding region in FHL1 (Fig. 2A shown where the pair of primers located), were used to amplify two Multiple tissue cDNA library

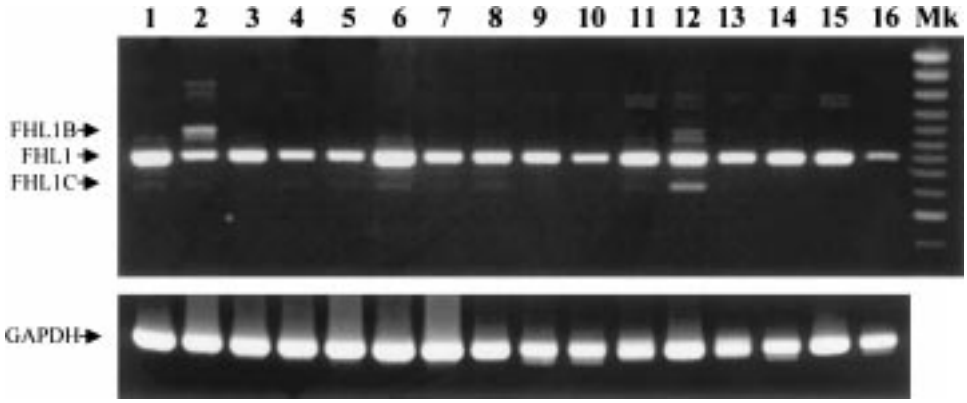

Fig. 4. PCR on a Multiple Tissue cDNA library panel (Clontech). **Lane 1**, heart; **Lane 2**, brain; **Lane 3**, placenta; **Lane 4**, lung; **Lane 5**, liver; **Lane 6**, skeletal muscle; **Lane 7**, kidney; **Lane 8**, pancreas; **Lane 9**, spleen; **Lane 10**, thymus; **Lane 11**, prostate; **Lane 12**, testis; **Lane 13**, ovary; **Lane 14**, small intestine; **Lane 15**, colon; **Lane 16**, peripheral blood leukocytes.

panels (Clontech). The pair of PCR primers was designed to distinguish the three alternatively spliced transcripts by different sized PCR products. The results of RT-PCR generally agreed with our findings from Northern blot analysis. Two expected 769 and 1025 bp band correspond to the FHL1 and FHL1B respectively. FHL1 is shown to be widely expressed in many tissues, including skeletal muscle and heart (Fig. 4). In contrast, FHL1B was only detectable in brain, testis, and skeletal muscle at relatively low level. A 585 bp band present in testis, skeletal muscle, and heart was sequenced and proved to be the third alternatively spliced transcript, FHL1C. Although this study does not show an accurate quantitation of each transcript, it does indicate that FHL1 seems to be most abundant in all tissues. FHL1C is expressed at low abundance in several tissues such as testis, skeletal muscle and heart.

Tissue Distribution of FHL1C by Western Blot Analysis

To study the tissue distribution of FHL1C protein, anti-FHL1C antibodies were derived from the amino acid sequence in FHL1C. From the result of immunoblot analysis, our anti-FHL1C antibody is shown to be detected a 20-kDa band, which corresponds to the FHL1C intact protein without any obvious degradation product. This result revealed that FHL1C protein was expressed in heart and skeletal muscle (Fig. 5) and this also agreed with the finding from Northern blot and RT-PCR analysis. There is another band at about 40 kDa in skeletal muscle. This band may be corresponded to another isoform of FHL1 because cDNA fragments other than the three isoforms were isolated but not characterized further in this study.

Subcellular Localization of FHL1C

To examine the subcellular distribution of FHL1C, GFP-FHL1C hybrid (GFP-FHL1C) was constructed by cloning into pEGFP-C1 vector (Clontech). This GFP hybrid constructs were used to transiently transfect C2C12 myoblasts and the GFP hybrid protein in the transfected cells was examined using a fluorescent microscope. The results show that GFP-FHL1C was distributed diffusely in the cytoplasm and nucleus (Fig. 6B). Besides C2C12 myoblasts,

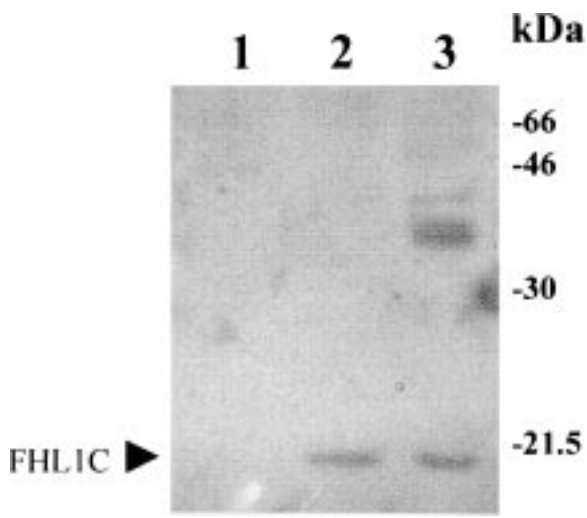

Fig. 5. Western Immunoblotting of FHL1C in human tissue using affinity-purified anti-FHL1C antibody. Human tissue lysates were separated by 14% SDS-PAGE and immunoblotted with affinity-purified anti-FHL1C antibody. **Lane 1**, brain; **Lane 2**, heart; **Lane 3**, skeletal muscle.

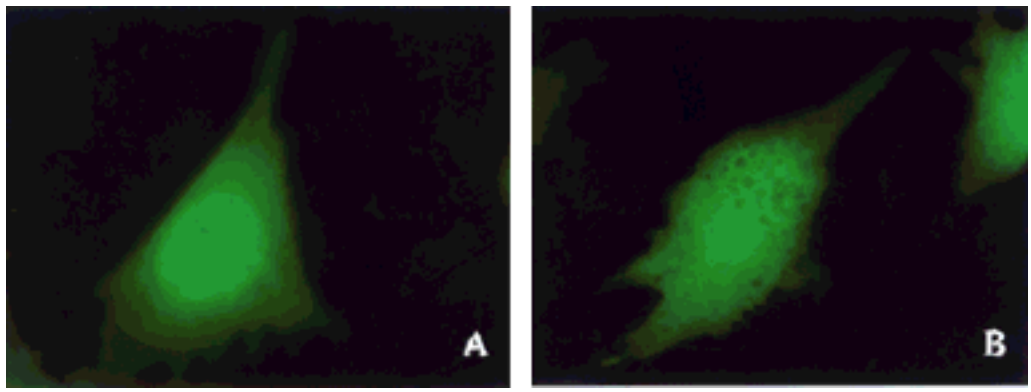

Fig. 6. Subcellular localization of GFP-FHL1C hybrid constructs were used to transiently transfect C2C12 myoblasts. The subcellular distribution of the GFP hybrid proteins was examined using a fluorescent microscope. A, GFP only; B, GFP-FHL1C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

HepG2 cells were also used in the subcellular localization study and it gave the same result as in C2C12 myoblasts (data not shown). To determine the stability of the GFP-FHL1C hybrid proteins, transfected cells were lysed and analyzed by immunoblotting using polyclonal antibodies to GFP. Transfected GFP and GFP-FHL1C migrated at the predicted molecular mass of 27 and 47 kDa (27 kDa + 20 kDa) respectively. The result showed that no proteolysis of GFP-FHL1C was observed (Fig. 7). Our previous study reported that GFP-FHL1 was localized in both the cytoplasm and nucleus

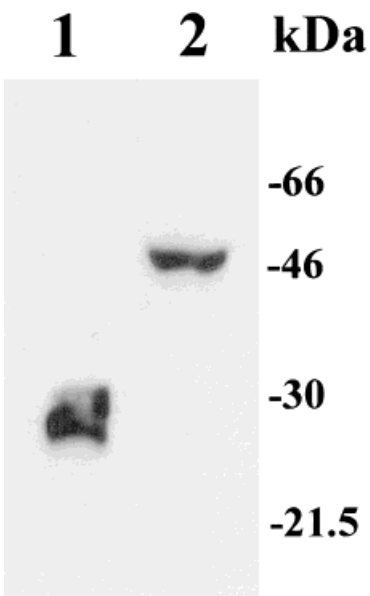

Fig. 7. Western Immunobloting of GFP-FHL1C in transfected C2C12 cells. GFP-FHL1C and GFP were transiently transfected into C2C12 cells, and cell lysates were prepared and separated by 12.5% SDS-PAGE. Immunoblot was performed using GFP.

while GFP-FHL1B was located predominantly in the nuclei of the cells [Lee et al., 1999]. Since FHL1C lacks NLS and NES encoded by exons 4b in FHL1B, it is not surprising that FHL1C is localized in both cytoplasm and nucleus while FHL1B is located predominantly in the nucleus.

DISCUSSION

This report describes the cloning and characterization of an alternatively spliced isoform of the human four-and-a-half LIM protein (FHL1), designated FHL1C, which unlike FHL1, contains N-terminal two-and-a-half LIM domains followed by C-terminal RBP-J binding region. The amino-acid sequence of FHL1C shows high homology to that of murine KyoT2, suggesting FHL1C is the human homolog of KyoT2.

There are several lines of evidence supporting that FHL1C is an isoform of FHL1, not simply a cloning artifact. First, the presence of an EST clone isolated from a human testis cDNA library that encoded partial FHL1C sequence without the 5′ untranslated region. Second, anti-peptide antibodies derived from the amino acid sequence in FHL1C detected a 20-kDa protein, consistent with the predicted molecular weight of FHL1C. Finally, the nucleotide sequence of FHL1C shows high homology with murine KyoT2 which is the isoform of murine FHL1. Accordingly, it is strongly supported that FHL1C is an alternatively spliced isoform of FHL1.

When the tissue distribution of human FHL1C was compared to that of murine KyoT2 [Taniguchi et al., 1998], interesting results were

seen. Murine KyoT2 was shown to express higher level in skeletal muscle, lung, and kidney, with lower level of expression in brain, ovary, and testis, while the strongest expression of FHL1C was shown to be in testis and skeletal muscle, with lower expression in the heart. This difference in expression pattern may be due to the difference in function of FHL1C and KyoT2 in human and mouse, or it could be due to differences in the age and sex of the pool of the subjects chosen for the organs.

The Green Fluorescent Protein (GFP)-tagged fusion protein was used to examine the subcellular localization of FHL1C. The finding revealed that FHL1C was localized in both the nuclei and cytoplasm of transfected C2C12 myoblasts, whereas murine KyoT2 was found to localize in the nuclei of transfected F9 embryonal carcinoma [Taniguchi et al., 1998]. Since there is no typical nuclear localization signal in FHL1C, it might be translocated to the nucleus by modification and/or interaction with other proteins.

Recently, the genomic structure of the mouse homolog of human FHL1, named KyoT1, was reported [Taniguchi et al., 1998]. The positions of introns in mouse and human FHL1 genomic sequences are essentially conserved. The alternatively spliced isoform of KyoT1, named KyoT2, was characterized. The C-terminal 27 amino acids of KyoT2 codes for a motif that was identified to interact with J-recombination signal protein (RBP-J) and, thus, this C-terminal unique sequence is defined as a RBP-J binding region [Taniguchi et al., 1998]. KyoT2 was shown to be a negative regulatory molecule for RBP-J-mediated transcription in mammalian system [Taniguchi et al., 1998]. RBP-J protein is a transcription factor that recognizes the core sequence C(T)GTGGGGA [Tun et al., 1994]. The homozygous RBP-J null mutant mice show embryonic lethality before 10.5 days post-coitum (dpc) and developmental abnormalities, including growth retardation, defective neurogenesis and somitogenesis are observed [Oka et al., 1995]. Moreover, mouse RBP-J interacts with intercellular region (RAMIC) of mouse Notch transmembrane receptor [Tamura et al., 1995] which is involved in cell fate determination of various lineages including germ cell, nerve and muscle [Artavanis-Tsakonas et al., 1995; de la Pompa et al., 1997]. It is suggested that upon ligand binding, Notch receptor undergoes a proteolytic cleavage, resulting in the release of RAMIC that translocate into the nucleus and bind to RBP-J to activate genes involved in differentiation suppression. LIM-only proteins are also involved in cell fate determination and differentiation. Downregulation of transcription can be achieved by the binding of LIM-only proteins to transcription factors. Mouse KyoT2 is one such example that it suppresses the function of RBP-J in vivo and in vitro. KyoT2 was shown to counteract Notch by dissociation of RBP-J from DNA and competition for binding to RBP-J [Taniguchi et al., 1998]. Since FHL1C is the human homolog of KyoT2 and contains the same RBP-J binding region in KyoT2, it is possible that FHL1C may substitute the function of KyoT2 in human, in which it interacts with RBP-J and represses the transcription activity of RBP-J and thus blocks the differentiation suppression.

Our previous study has reported that FHL1 was mapped at human chromosome X, region q27 by FISH chromosomal mapping and radiation hybrid mapping [Lee et al., 1998]. Interestingly, many X-linked recessive diseases are linked to the distal end of the long arm of chromosome X. Previous studies have reported that a phenotypically atypical case of fragile X syndrome with deletions of Xq26.3–Xq27.3 was observed. In that particular case, the patient exhibits a more severe phenotype than typical fragile X patient with the profound mental and growth retardation, small testes, and lower limb skeletal defects [Wolff et al., 1997]. Therefore, important genes for brain, muscle, and testis development may reside in Xq26.3–q27.3. As mentioned above, FHL1C is predicted to interact with RBP-J DNA binding protein as murine KyoT2 does. It is possible that the loss of FHL1C gene, together with FHL1 and FHL1B, may fail to inhibit RBP-J activity and thus somitogenesis and neurogenesis will be suppressed in certain extent. Therefore, since FHL1 and its isoforms located in this chromosomal deletion, it suggests that FHL1, FHL1B, and FHL1C may associate with certain cases of fragile X syndrome. Moreover, it is noteworthy that in Turner's syndrome (45, XO), in which one of the two X chromosomes is partially or completely missing in females, the most common cardiovascular defects are coarctation of the arota and biscuspid aortic valve [Lin et al., 1998]. Studies revealed that in early development the most common fetal cardiac defect

associated with Turner's syndrome is a hypoplastic aortic arch with hypoplasia of the left ventricular outflow tract and left ventricle [Gembruch et al., 1997]. Since our finding of FHL1 and FHL1C expressions localized to the aorta and left ventricle, together with the location of FHL1/FHL1C to the X chromosome, it would lead to the speculation that FHL1/FHL1C may be a potential candidate which associated with the cardiac defects of Turner's syndrome.

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