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A novel human KRAB-containing zinc-finger gene *ZNF446* inhibits transcriptional activities of SRE and AP-1 ☆

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Abstract

Kruppel-related zinc-finger proteins constitute the largest individual family of transcription factors in mammals [C. Looman, L. Hellman, M. Abrink, A novel Kruppel-associated box identified in a panel of mammalian zinc-finger proteins, Mammalian Genome 15 (1) (2004) 35–40. [1]]. Here we identified and characterized a novel zinc-finger gene named *ZNF446*. The predicted protein contains a KRAB and three C_2H_2 zinc fingers. Northern blot analysis shows that *ZNF446* is expressed in a variety of human adult tissues with the highest expression level in muscle. *ZNF446* is a transcription repressor when fused to GAL4 DNA-binding domain and co-transfected with VP-16. Overexpression of *ZNF446* in COS-7 cells inhibits the transcriptional activities of SRE and AP-1, in which the KRAB motif represents the basal transcriptional repressive activity, suggesting that the *ZNF446* protein may act as a transcriptional repressor in mitogen-activated protein kinase (MAPK) signaling pathway to mediate cellular functions.

Keywords: *ZNF446*; Kruppel type zinc finger; KRAB; Transcriptional repressor; Heart development; SRF and AP-1; MAPK signaling pathway

The zinc-finger protein (ZFP) family belongs to one of the largest human gene families and contains many of the currently known transcription factors. The zinc-finger motif contains spatially conserved cysteines (C) and histidines (H), which coordinate a Zn^{2+} and cause the intervening amino acids to loop out and form the secondary structure [2,3]. Zinc-finger motif may be involved in the interaction of DNA and protein or protein and protein [4]. ZFPs are involved in the binding of transcription factors to their cognate DNA recognition site, resulting in the specific activation or repression of gene expression during cell differentiation and development [5]. More than one hundred ZFP genes have been found in the cardiac cardiovascular system. Based on the structure of zinc-finger motifs, these ZFPs can be divided into seven types: C_2H_2-, C_2C_2-, C_2HC-, C_2HC_4C (HD)-, C_3H-, C_3HC_4-, and combination types (containing more than one type of zinc finger) [6]. The zinc-finger proteins are known to interact with nucleic acids through the zinc finger motifs and have diverse molecular functions, including stimulation of transcription [7], transcriptional repression, cell proliferation, and development [8–12]. Many Kruppel-like zinc-finger proteins contain highly conserved amino-terminal motifs such as the Kruppel-associated box (KRAB), the finger-associated box (FAX), the poxvirus and zinc-finger (POZ) domain, and SCAN box or leucine-rich region (LER) [13–15]. These conserved domains play distinct roles in terms of transcription regulation of target genes.

☆ *Abbreviations:* MAPKK, MKK or MEK, MAPK kinase; MAPKKK or MEKK, a MAPKK kinase or MEK kinase; SRE, c-fos serum response element; DMEM, Dulbecco's modified Eagle's medium; DAPI, 4′,6′-diamidino-2-phenylindole hydrochloride; AP-1, activation protein 1; MAPK, mitogen-activated protein kinase.

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The KRAB domain was originally identified as a conserved motif at the NH_2 terminus of zinc-finger proteins and was shown to be a potent, DNA-binding dependent transcriptional repression module [16–18]. In addition, it has been demonstrated that the KRAB domain is able to confer distance-independent transcriptional repression when fused to the DNA-binding domain of the yeast GAL4 transcription factor [17]. KRAB-ZFPs appear to play important regulatory roles during cell differentiation and development [19]. The SCAN domain was originally derived from the first four proteins found to contain this domain (SRE-ZBP, CT-fin-51, AW-1, and number 18 cDNA) [20–23]. The function of the LER domain is not clear although it has been shown to function as a protein interaction domain and mediate both hetero-/homoprotein–protein associations by multi-domain zinc-finger-containing transcription factors [23]. Furthermore, the LER (or SCAN domain) appears to be a vertebrate-specific domain [24].

The mitogen-activated protein kinases (MAPKs) are important signal transducing enzymes in eukaryotes and are involved in many facets of cellular regulation [25]. MAPKs consist of three main protein kinase families: the extracellular signal-regulated protein kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and the p38 family of kinases. Each family of MAPKs has different roles in the regulation of intracellular metabolism, gene expression, cell growth and development, and apoptosis in response to different growth factors and external stresses [26]. MAPK activity is regulated through three-tiered cascades composed of a MAPK, MAPK kinase (MAPKK, MKK or MEK), and a MAPKK kinase or MEK kinase (MAPKKK or MEKK). And most MAPKs phosphorylate its transcription factors that are involved in the induction of *fos* genes, whose products heterodimerize with Jun proteins to form activation protein 1 (AP-1) complexes.

With the aim of identifying genes with transcription regulatory activity involved in human embryonic development and differentiation, a novel human gene named *ZNF446* was cloned from an embryonic heart cDNA library. *ZNF446* encodes a predicted protein of 450 amino acids containing three different C_2H_2 type zinc fingers and a KRAB. Northern blot analysis demonstrated that *ZNF446* is expressed in various human adult tissues. The ZNF446 protein is localized mainly to the nucleus, which supports its function as a transcriptional regulator. ZNF446 fused to the GAL4 DNA binding domain apparently inhibits transcriptional activity of GAL4-dependent reporter in transfected COS-7 cells, suggesting that ZNF446 plays a direct role in transcriptional repression. Overexpression of ZNF446 in the cell inhibits the transcriptional activities of SRE and AP-1, in which the KRAB motif represents the basal transcriptional repressive activity. Together the data suggest that ZNF446 may function as a transcriptional repressor of SRF and AP-1 mediated by the MAPK signaling pathway.

Materials and methods

Construction of cDNA library of human embryonic heart. The 20-week human embryonic heart cDNA library was constructed as reported previously [27]. Briefly, 5 μg mRNA was purified from 500 μg total human embryonic heart RNA using Rapid mRNA Purification Kit (Amresco). Reverse transcription reactions were performed with the purified embryonic heart mRNA and oligo(dT)-RA primer according to the cDNA Synthesis kit protocol (TaKaRa). After cassette adaptor ligation reactions using cDNA PCR Library Kit, cDNA amplification reactions were performed with RA primer, CA primer, and Ex Taq (TaKaRa).

Full-length ZNF446 cDNA cloning and bioinformatics analysis. The amino acid sequence of *ZNF446* (AY280800) was obtained from NCBI (http://www.ncbi.nlm.nih.gov) and used to search human EST database with the BLAST searching program (http://www.ncbi.nlm.nih.gov) through combined BLAST search as previously described [27]. The first forward primer in BX353954 and the reverse primer in BM925632 (P1 and P2, Table 1) and the second forward primer in BU151642 and the reverse primer in BU528534 (P3 and P4, Table 1), were designed using Primer Premier 5.0 to perform standard PCR. Jellyfish 1.4 was used to find the open reading frame (ORF) and the deduced translated product. 5′-Gene-specific reverse primers (R1 and R2) were designed for 5′-RACE reactions according to the previous method [28,29]. 5′-RACE was performed using SMART RACE cDNA Amplification Kit (Clontech). *ZNF446* sequences of open reading frame (ORF) were confirmed by PCR amplification with a pair of primers (S1 and S2, Table 1). All the PCR products were then cloned into pMD18T-vector (Sagon) and sequenced with 377 DNA Sequencer (ABI PRISM). The full-length sequence of *ZNF446* reported here is available in the GenBank. Sequence analysis was performed using the DNASTAR program and BLAST program from NCBI. Blastn program was used to identify the cytological locus of genes and to look for exons and introns. Analysis of *ZNF446* sequences was performed by DNAstar software. BLASTn and Pfam 9.0 were used to analyze the genomic structure and the protein domain,

Table 1
Sets of specific oligonucleotide primers

Primer	Orientation	Nucleotide sequence
S1	Sense	5′ GCCCATCTTGACGATTCCAA 3′
S2	Antisense	5′ GGGGAGGCACAGTTTTTGAA 3′
S3 EGFP	Sense	5′ ATTTGAATTCCACCCCCTTGAGCAAG 3′
S4 EGFP	Antisense	5′ GGTCGACCATGGAACCTCCGGC 3′
R1	Sense	5′ GCAACGGTCCGAACCTCAT 3′
R2	Antisense	5′ CTGGTAGCAGAACCCTCGGA 3′
S5 PCMV	Sense	5′ AGGCCCATCTTGACGGGATCCAGAC 3′
S6 PCMV	Antisense	5′ CGAGGGACTGTGCTGGTCGACTGCT 3′

respectively. The homologues of *ZNF446* were found with BLASTp, and the sequence alignment and phylogenetic tree analysis were performed with MegAlign program (DNAstar).

Northern blot hybridization. The *ZNF446* cDNA was used as a probe which was labeled with [^{32}P]dCTP using a random prime labeling kit (TaKaRa). An adult human Multiple Tissue Northern blot (Clontech) was hybridized to the radiolabeled *ZNF446* cDNA probe and β-actin cDNA probe (Clontech). Hybridization was carried out with 5× SSC, 5× Denhardt's, 10% dextran sulfate, and denatured human DNA, at 65°C overnight. After hybridization, the blots were washed three times at 65 °C in 2× SSC containing 0.1% SDS for 5 min, twice in 0.1× SSC, and 0.1% SDS at 65 °C for 15 min, and then subjected to autoradiography at −80 °C. The blots were stripped by incubating for 10 min in 0.1× SSC and 0.5% SDS at 95 °C. The membranes were reprobed with radiolabeled β-actin cDNA as an indicator of mRNA loading [30].

Cell culture, transient transfection, and subcellular localization analysis. COS-7 cells used in all studies were maintained and passaged according to standard methods in DMEM (Dulbecco's modified Eagle's medium; Gibco-BRL) supplemented with 10% fetal calf serum (FCS) in a humidified atmosphere of 95% air and 5% CO_2. To generate a fusion protein of ZNF446 with enhanced green fluorescent protein (EGFP), the *ZNF446* ORF was subcloned into the *Eco*RI and *Sal*I sites of the pEGFP-N1 vector in-frame with the TGG codon instead of the TGA stop codon in the ZNF446 coding sequence. Cells were transfected with pEGFP-N1-ZNF446 using LipofectAMINE (Invitrogen) according to the method described previously [31]. Forty-eight hours after transfection, cells were fixed with 4% paraformaldehyde for 15 min and washed with PBS three times. Then the nucleus was stained with 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI). Subcellular localization of the EGFP-ZNF446 fusion protein was detected using a Nikon inverted fluorescence microscope.

Transient expression reporter gene assays. The *ZNF446* ORF was subcloned into the *Bam*HI and *Sal*I sites of the pCMV-BD vector in-frame to construct pCMV-BD-ZNF446 for GAL4-ZNF446 fusion protein. COS-7 cells were transfected with lipofectAMINE (Invitrogen) [31]. pCMV-BD-ZNF446 or pCMV-BD was co-transfected into COS-7 cells along with the pL8G5-Luc reporter and pLexA-VP-16. Forty-eight hours later, the luciferase activity assay was performed according to the protocols of Stratagene [32]. Relative luciferase activity was normalized for transfection efficiency by co-transfection with pCMV-lacZ and spectrophotometry analysis. Each experiment was performed in triplicate and each assay was repeated at least three times. The means of the data from three individual transfected wells are presented.

The *ZNF446* ORF was subcloned into the *Bam*HI and *Sal*I sites of the pCMV-Tag2B vector in-frame. COS-7 cells were co-transfected with pFR-Luc, pFA2-SRE, pFC-MEK1, and pCMV-Tag2B-ZNF446 or pCMV-Tag2B vector to investigate the effect of *ZNF446* on the transcriptional activity of SRE. To examine the effect of *ZNF446* on the transcriptional activity of AP-1, cells were co-transfected with pAP-1-Luc and pCMV-Tag2B-ZNF446 or pCMV-Tag2B vector. Forty-eight hours later, the luciferase activity assay was performed according to the protocols of Stratagene [32]. Relative luciferase activity was normalized for transfection efficiency by co-transfection with pCMV-lacZ and spectrophotometry analysis. Each experiment was performed in triplicate and each assay was repeated at least three times. The means of the data from three individual transfected wells are presented.

Transient expression reporter gene assays of LER, KRAB, and ZNF motifs. ZNF446 sequence was separated into three segments: segment KRAB involved KRAB motif; segment C_2H_2 involved three C_2H_2 motifs; segment LER involved LER motif. The segment KRAB (sequence from 721 to 947), segment C_2H_2 (sequence from 1226 to 1494), and segment LER (sequence from 79 to 534) were subcloned into the *Bam*HI and *Sal*I sites of the pCMV-Tag2B vector in-frame. Each experiment was performed in triplicate and each assay was repeated at least three times. The means of the data from three individual transfected wells are presented.

Results and discussion

Identification and sequence analysis of ZNF446

In order to search for novel zinc-finger genes which are involved in human heart development and in the cell signaling pathway, we isolated genes by using PCR amplification from human heart cDNA library. Since nucleotide sequences of the Kruppel-like zinc-finger region are highly conserved, it was feasible to isolate homologous genes of this family using PCR amplification from a cDNA library of human early embryo [33]. We designed specific primers S1/S2, which were based on the amino acid sequence of the open reading frame of *ZNF446* as described under Materials and methods. A novel 1480-bp fragment of a putative Kruppel-like zinc-finger cDNA was isolated.

To obtain the full length of cDNA, 5′-RACE was performed using 5′-gene-specific primers (R1 and R2 in Table 1). The procedure yielded a 240 bp DNA fragment for 5′-RACE. A 2038 bp of full-length novel gene was assembled, which was named *ZNF446* as approved by HUGO (Human Gene Nomenclature Committee). The nucleotide sequence reported here is available in GenBank with Accession No. AY280800. The complete cDNA of *ZNF446* consists of an ORF of 1353 bp from 161 to 1513, a 160 bp 5′-untranslated terminus, and 3′-untranslated terminus of 835 bp with a consensus polyadenylation signal (aataaa) (Fig. 1A). The full-length ZNF446 protein contains 450 amino acids, including a KRAB, a LER, and three C_2H_2 zinc fingers (Fig. 1A).

Comparison of the *ZNF446* sequence with the genomic sequence shows that *ZNF446* gene is mapped to chromosome 19q13.43 and spans approximately 4.7 kb on the genome. *ZNF446* consists of six exons and five introns. The exon–intron boundaries conform to the consensus splicing signals, where there are a gt and an ag dinucleotide at the 5′-donor and 3′-acceptor sites, respectively (Table 2).

ZNF446 is conserved during evolution

The *ZNF446* gene shares a strongly conserved C_2H_2 domain between three zinc-finger region (Fig. 2B). The LER is homologous with similar elements in several other zinc-finger transcription factors (Fig. 2A). The highly similarity of *ZNF446* C_2H_2 domain and LER domain to the other C_2H_2-ZFPs suggests that *ZNF446* is a novel member of this family, and it probably functions as a transcription repression factor.

We then tried to analyze the evolutionary relationship between the *ZNF446* protein and the other zinc-finger proteins with phylogenetic tree analysis (Fig. 2C). Sequence alignment of these proteins demonstrates that *ZNF446* is one of the conserved proteins during evolution.

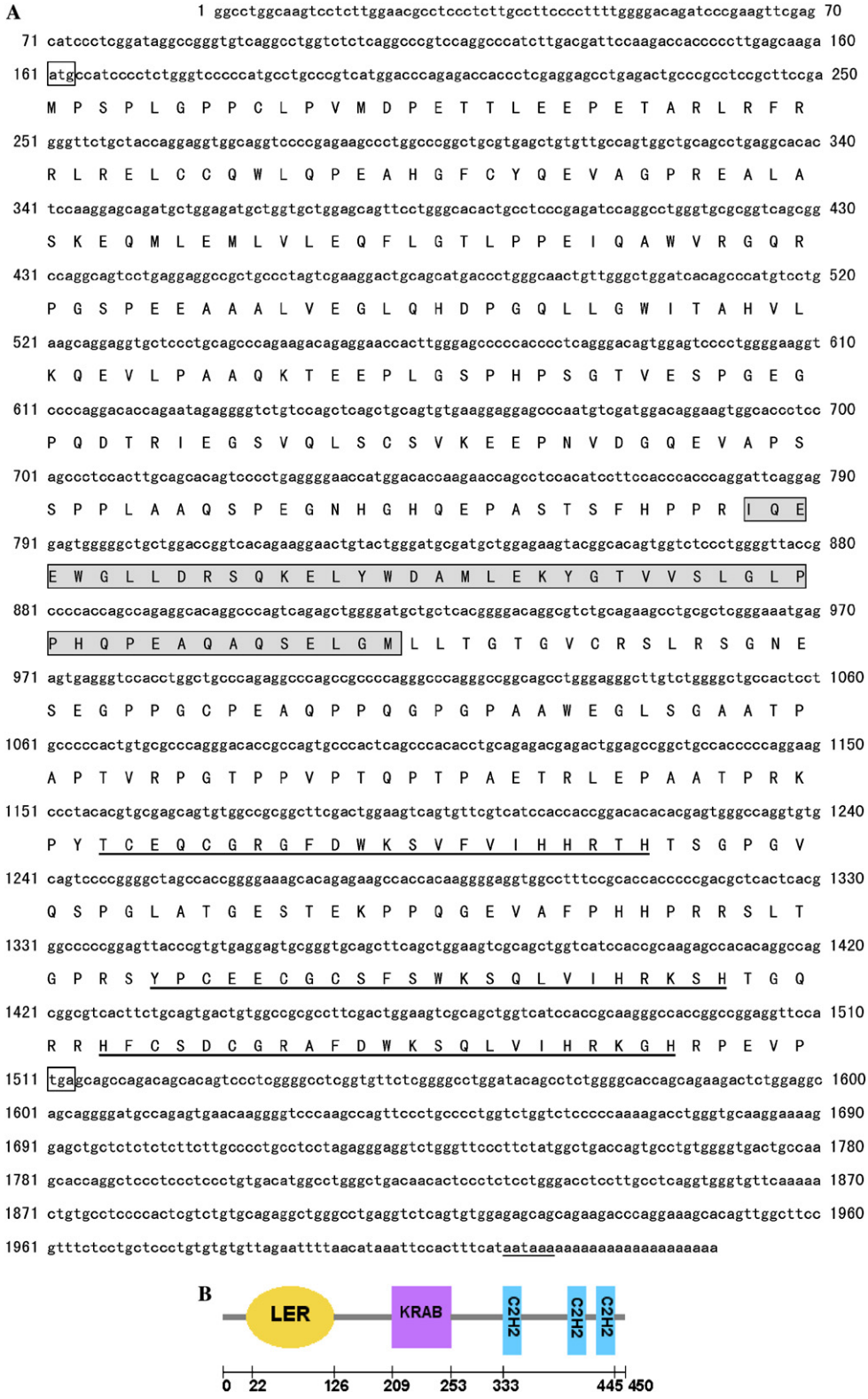

Fig. 1. Nucleotide sequence and deduced protein of *ZNF446*. (A) Nucleotide sequence and deduced protein of the human *ZNF446* gene. *ZNF446* encodes a polypeptide of 450 amino acids. The initiation atg and termination tga codons are boxed. Amino acids are identified by their one-letter code. The KRAB is boxed and shaded in gray, and three C_2H_2 zinc fingers are underlined. Nucleotides and amino acids are numbered at each line. The putative polyadenylation signal sequence **aataaa** is underlined. (B) The domain structure of *ZNF446*. The protein sequence contains three zinc fingers in the C-terminal moiety, and the N-terminal of the sequence contains a KRAB box and a LER box. The red region is of low compositional complexity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Table 2
Genomic structure of the *ZNF446* gene

Exon number	Exon size (bp)	Splice donor site	Intron size (bp)	Splice acceptor size
I	77	**GT**GGGCGTCTTTCA	674	TTCCTTTTCTGT**AG**
II	382	**GT**GAGTGTGGCTGG	76	CCTCTTCTCCTC**AG**
III	190	**GT**GAGGTTGGGGTC	260	GCCCCACTTTTC**AG**
IV	95	**GT**GAGCAGCCCCAA	1461	GGCCACATCCGC**AG**
V	377	**GT**GAGTGCCCCACA	156	GCCCCTGCCCCC**AG**
VI	1049			

Summary of the size of the exons and introns of the *ZNF446* gene based on comparison of the cDNA and the genomic sequence. Invariant nucleotides (GT/AG) are underlined and in boldface type.

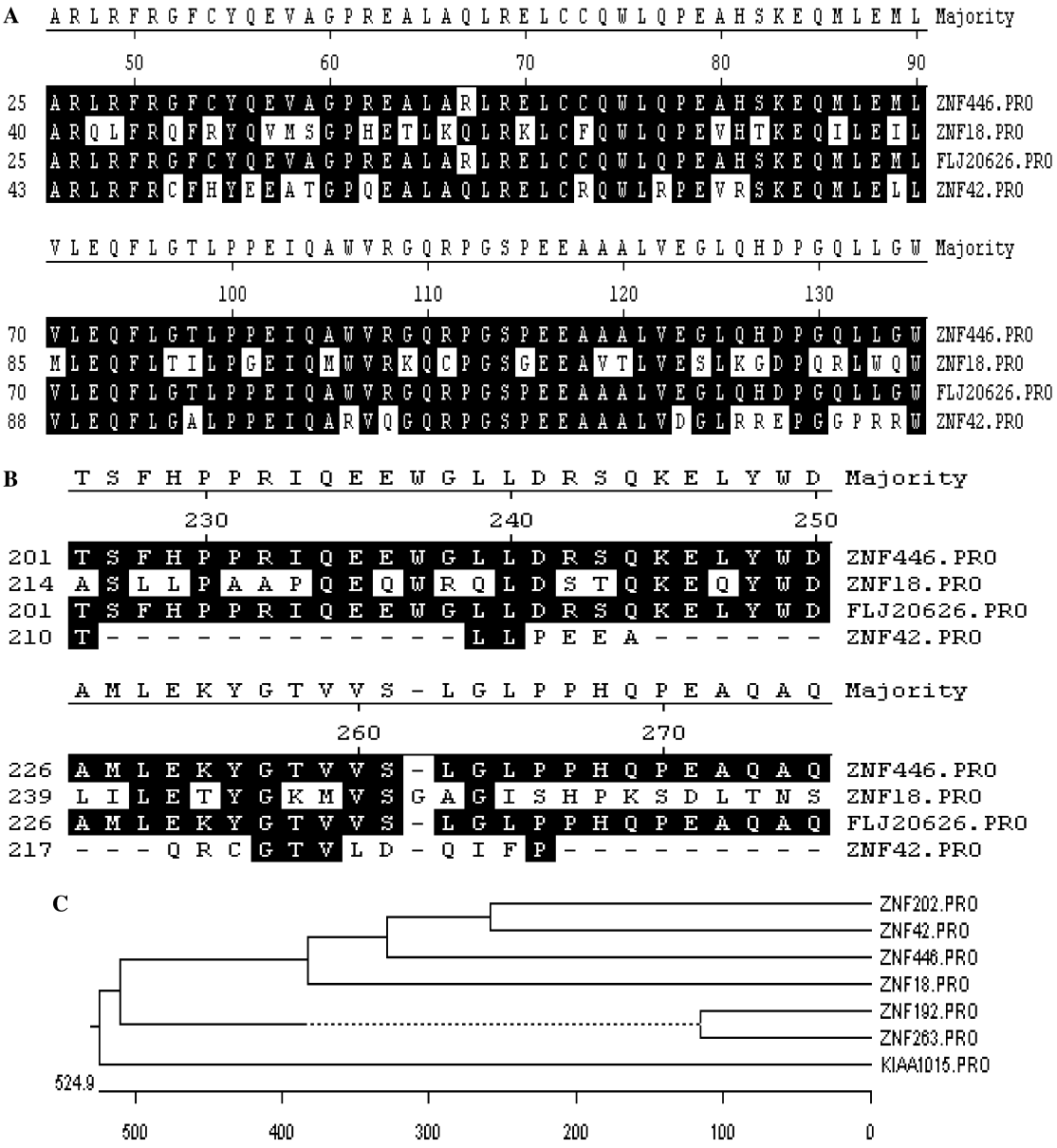

Fig. 2. *ZNF446* is conserved during evolution. Comparison of the amino acid sequences of the LER domains (A) and C_2H_2 finger region (B) between *ZNF446* and its homologues (*ZNF446*, *ZNF18*, FLJ20626, and *ZNF42*) indicates that *ZNF446* is conserved during evolution. The residues that are highly conserved among these sequences are indicated within the majority sequence. Identical residues fitting the LER domain and C_2H_2 finger repeat consensus have been boxed and are shaded in dark. (C) Unrooted phylogenetic tree analysis of *ZNF446* and other zinc-finger proteins. All genes originate from *Homo sapiens*.

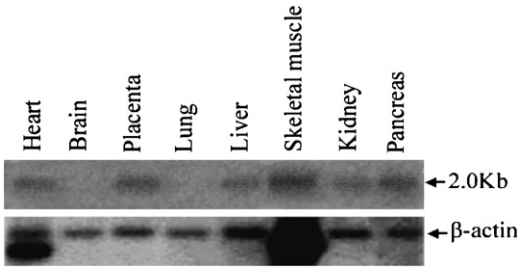

Fig. 3. Expression of *ZNF446* in human adult tissues analyzed by Northern blot. *ZNF446* is expressed in adult heart, placenta, liver, skeletal muscle, kidney, and pancreas. β-Actin was used as a control for the equivalent amount of loaded mRNA in each lane. A band of about 2.0 kilobases (kb) was detected.

Expression of the ZNF446 mRNA in human adult tissues

We used *ZNF446* cDNA as the probe to examine its expression in adult tissues. As shown in Fig. 3, Northern blot analysis detected an expected transcript of about 2.0 kb in different tissues. The gene is expressed in adult heart, skeletal muscle, placenta, liver, kidney, and pancreas with the highest level in skeletal muscle (Fig. 3), suggesting an important role for this protein in different tissues.

Subcellular localization of ZNF446 protein

To examine the subcellular localization of *ZNF446*, the pEGFP-ZNF446 plasmid was transfected into COS-7 cells, and 48 h after the transfection, the cells were visualized with epifluorescence microscope after labeling with DAPI for nuclei. As shown in Fig. 4, *ZNF446* protein distributes evenly in the nucleus. Although we consider *ZNF446* as a nuclear protein, we could not rule out the possibility that small amount of the protein may exist in the cytoplasm as shown in the fluorescence staining of the protein in the cell.

ZNF446 is a transcriptional repressor

To examine the potential function of *ZNF446* in transcriptional regulation, we constructed a fusion protein of *ZNF446* with the DNA binding domain (BD) of the yeast transcription factor GAL4 under the control of a CMV promoter, the pCMV-BD-ZNF446, and then, co-transfected COS-7 cells with pCMV-BD-ZNF446 and the reporter, pL8G5-Luc, and pLexA-VP-16, respectively. As shown in Fig. 5, when co-transfected with pL8G5-Luc plasmid, the GAL4-ZNF446 fusion protein inhibited the endogenous luciferase activity by 12% while co-expression of GAL4-ZNF446 with pLexA-VP-16 significantly inhibited the VP-16-activated luciferase activity by 75% (Fig. 5), suggesting that ZNF446 may function as a transcriptional repressor.

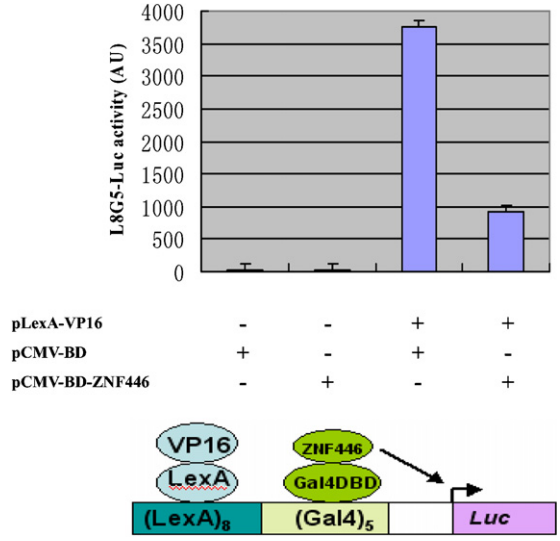

Fig. 5. *ZNF446* is a transcriptional repressor. pCMV-BD-ZNF446 or pCMV-BD is transiently co-transfected into COS-7 cells along with the pL8G5-Luc reporter and pLexA-VP-16 as indicated. Forty-eight hours later, the luciferase activity assay was performed. The data are means of three repeats in a single transfection experiment after normalization for β-galactosidase activity. Each experiment was repeated at least three times.

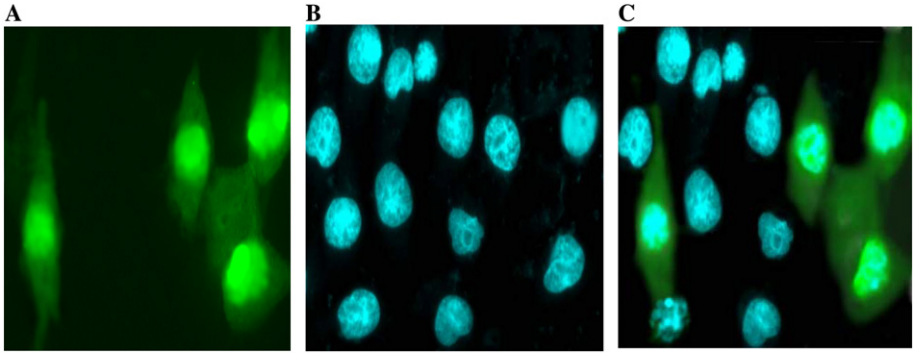

Fig. 4. Nuclear localization of *ZNF446* protein. (A) EGFP-ZNF446 protein is expressed in COS-7 cells. (B) The nucleus of the cells stained with DAPI. (C) The combined image of ZNF446 and DAPI (A,B) showing nuclear staining of the protein.

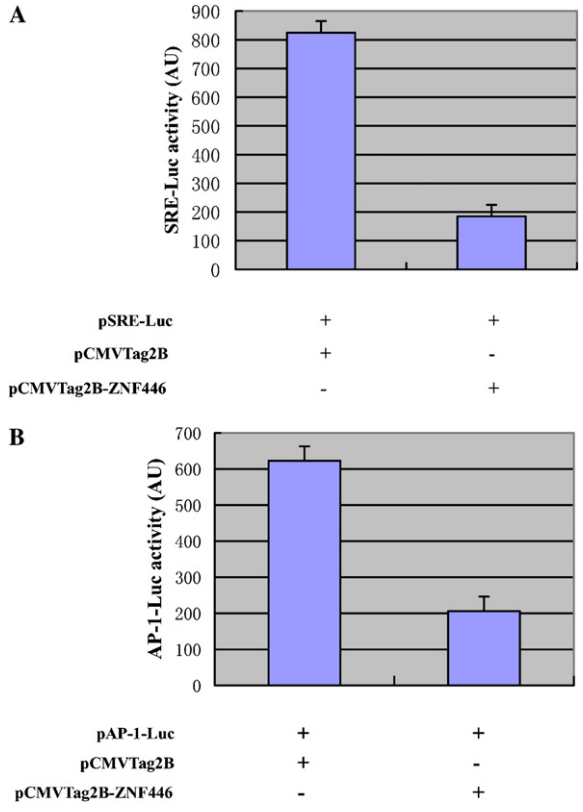

Fig. 6. Overexpression of ZNF446 suppresses transcriptional activities of SRE and AP-1 in COS-7 cells. (A) Co-transfection of pCMV-Tag2B-*ZNF446* and pSRE-Luc suppresses SRE activation in the reporter assay stimulated by 10% serum. (B) Inhibition of AP-1 transcriptional activity by expression of *ZNF446*. COS-7 cells transfected with individual reporter plasmid or combination as indicated. The data are means of three repeats in a single transfection experiment. Each transfection experiment was performed at least three times.

ZNF446 suppresses the transcriptional activities of SRE and AP-1

To further understand the role of *ZNF446* in cell signaling transduction, we examined how ZNF446 regulated the transcriptional activities of SRE and AP-1 in the cells. COS-7 cells were transfected with the plasmids encoding pCMVTaq2B-ZNF446, pSRE-Luc, or pAP-1-Luc, respectively, and stimulated with serum. As shown in Fig. 6, expression of *ZNF446* significantly inhibited the transcriptional activities of AP-1 and SRE by ~66% and 77%, respectively, suggesting that ZNF446 is a potent transcriptional repressor for SRE and AP-1, which is mediated by the MAPK signaling pathway.

The KRAB motif of ZNF446 is a potent repression domain

To establish which motif/domain in ZNF446 plays a role in transcriptional repression, a series of Flag-tagged motif fusion proteins were generated to investigate their potential repressive activity. As shown in Fig. 7, expression of pCMV-Tag2B-KRAB significantly inhibited SRE transcriptional activity by being approximately 78.9% similar to the full-length protein (Fig. 7A). No obvious repressive activity for pCMV-Tag2B-C_2H_2 and pCMV-Tag2B-LER was found. Similar results (78% repression) were obtained when assayed with the AP-1 transcriptional reporter assays (Fig. 7B), suggesting that the KRAB motif represents the basal repression domain in ZNF446.

Mitogen-activated protein kinase (MAPK) signal transduction pathways are among the most widespread mechanisms of eukaryotic cell regulation. All eukaryotic cells possess multiple MAPK pathways, each of which is preferentially recruited by distinct sets of stimuli, thereby allowing the cell to respond coordinately to multiple divergent inputs. Mammalian MAPK pathways can be activated by a wide variety of different stimuli acting through diverse receptor families, including hormones and growth factors. The biological effects of MAPKs are mediated by downstream phosphorylation substrates, which in the nucleus are often transcription factors. MAPK pathways are involved in multiple cellular processes through phosphorylating their specific endpoint targets such as ElK-1 and SRE, which forms a ternary complex together with SRF to induce expression of c-fos and other early response genes. SRE is one of the several cis elements which mediate c-fos induction and is recognized by a dimer of the serum response factor (SRF) that recruits the monomeric ternary complex factors (TCFs). The c-fos products heterodimerize with c-Jun proteins to form AP-1 complexes. Activation of AP-1 involves the direct phosphorylation/dephosphorylation of AP-1 components as well as the phosphorylation and activation of transcription factors that induce elevated expression of c-Jun or c-fos. Therefore, at the end of these signaling cascades, MAPKs phosphorylate their target transcription factors. Our observation indicates that *ZNF446* is a regulator of transcriptional factor complexes and may suppress SRE and AP-1 transcription activities mediated by growth factor signaling pathways in the cell.

In mammals, zinc-finger proteins probably constitute the largest individual family of such nucleic acid-binding proteins [34]. The KRAB domain is present in the amino-terminal regions of more than one-third of Kruppel-class C_2H_2 zinc-finger proteins and is highly conserved from yeast to human [35]. Although more than one hundred members of the KRAB/C_2H_2 zinc-finger protein family have been described, little is known of their biological function [36,37]. Moreover, few target genes have been identified for KRAB/C_2H_2 proteins [38]. In this study, we isolated and characterized a novel human KRAB-containing Kruppel-like zinc-finger gene *ZNF446*. Northern blot analysis shows that the gene is expressed in a variety

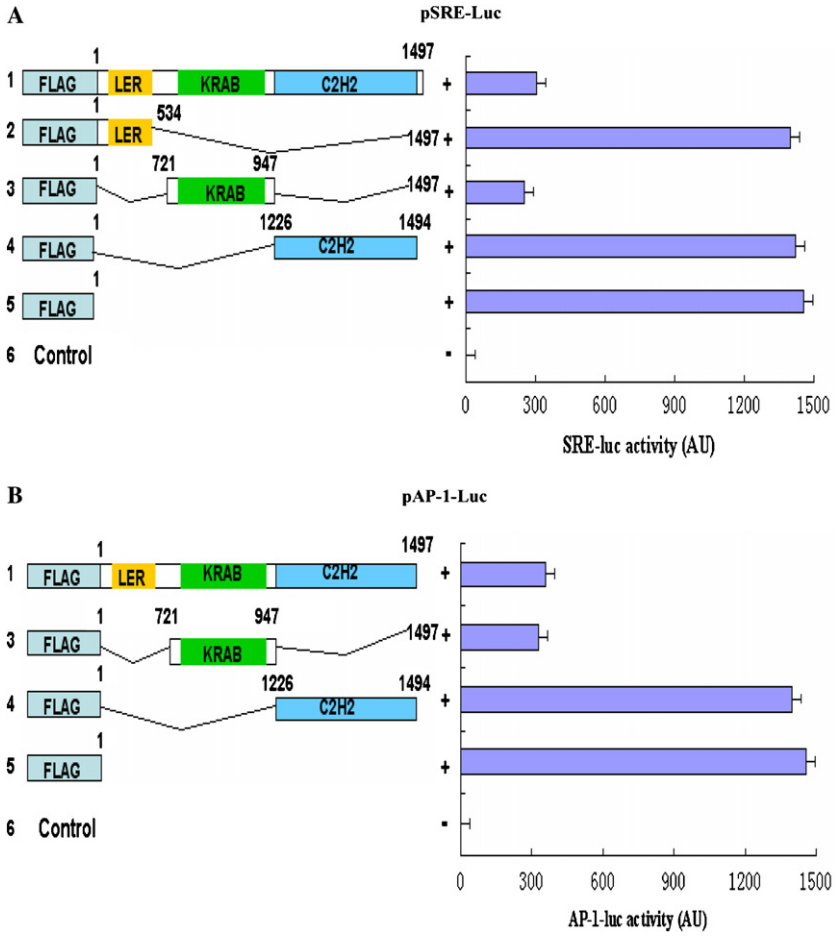

Fig. 7. The KRAB domain is essential for the suppression of es-transcriptional activities of SRE and AP-1. (1) pCMV-Tag2B-ZNF446; (2) pCMV-Tag2B-LER; (3) pCMV-Tag2B-KRAB; (4) pCMV-Tag2B-C_2H_2; (5) pCMV-Tag2B. (A) Inhibition of SRE-Luc transcriptional activity by the overexpression of pCMV-Tag2B-KRAB. (B) Inhibition of AP-1-Luc transcriptional activity by the overexpression of pCMV-Tag2B-KRAB. Each experiment was performed in triplicate and each assay was repeated at least three times.

of adult tissues with high level expression in the skeletal muscle. Furthermore, we have demonstrated that ZNF446 functions as a transcriptional repressor for SRE and AP-1 transcription factors, and may play an important role in cell growth and proliferation signaling pathways.

Acknowledgments

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