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ZNF411, a novel KRAB-containing zinc-finger protein, suppresses MAP kinase signaling pathway

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Abstract

Cardiac differentiation involves a cascade of coordinated gene expression that regulates cell proliferation and matrix protein formation in a defined temporo-spatial manner. The zinc-finger-containing transcription factor has been implicated as a critical regulator of multiple cardiac-expressed genes as well as a regulator of inducible gene expression in response to hypertrophic stimulation. Mitogen-activated protein kinase (MAPK) signal transduction pathways are among the most widespread mechanisms of eukaryotic cell regulation. The MAPKs function inside the nucleus and target transcription factors that are prebound to DNA. Many transcription factors are probably important MAPK targets. Here, we have cloned a new zinc-finger gene named *ZNF411* using degenerate primers from an early embryo heart cDNA library, which mapped to 19p13.11. The *ZNF411* gene consists of 2360 nucleotides and encodes a protein of 499 amino acids with an amino-terminal KRAB domain and eleven carboxy-terminal C2H2 zinc-finger units. Northern blot analysis indicates that a 2.4 kb transcript specific for *ZNF411* is expressed in heart, skeletal muscle, and placenta at adult stage and is expressed in most of the examined embryonic tissues, especially at a higher level in skeletal muscle, heart, and pancreas. *ZNF411* protein distributes evenly in nuclei when overexpressed in the cells. Reporter gene assays show that *ZNF411* is a transcriptional repressor and overexpression of *ZNF411* in the COS-7 cells inhibits the transcriptional activities of AP-1 and SRE. These results indicate that *ZNF411* is a member of the zinc-finger transcription factor family and may be involved in the heart development, and it probably works as a negative regulator in MAPK signaling pathway.

Keywords: Cardiac differentiation; Transcription factors; ZNF411; MAPK signaling pathway

Nowadays much progress has been made toward a physiological and clinical understanding of cardiac development and heart disease. However, the molecular mechanisms underlying these processes are still not completely understood, and most cardiovascular diseases are still largely recognized and treated phenotypically [1,2]. Transcription factors may play an essential role in altering gene expression involved in the developmental process of the normal heart. One of the largest families of potential transcriptional regulators is the krupple-associated box-zinc-finger proteins (KRAB-ZFPs) [3]. In the human genome, for example, approximately 300–700 different genes encode the Cys2/His2 (C2H2) zinc finger, one-third of which also contain a KRAB domain which is usually encoded by two exons known as KRAB-A and KRAB-B [3–5]. The C2H2 zinc fingers represent one of the most common types of DNA-binding domains, the motif frequently occurs in tandem repeats and is defined by two cysteine and two histidine residues that coordinate a zinc ion and fold the domain into a finger-like projection that can interact with DNA [6]. The krupple-like subclass of mammalian C2H2 zinc-finger proteins, first identified in the zinc-finger transcription factor TF111A, shares a conserved link between the last histidine of the preceding finger motif with the first cysteine of the next finger (H-C link) [7]. The zinc-finger motif carrying a zinc atom tetrahedrally coordinated by four residues participates in the

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formation of a 12-amino-acid loop [8]. Most studied C2H2 zinc-finger proteins were found to bind to specific DNA sequences and to be involved in the transcriptional regulation of gene expression [9–13].

The mitogen-activated protein kinase (MAPK) family is an important mediator of signal transduction and is activated by a variety of stimuli, such as growth factors and cellular stresses [14]. Among the MAPK family, three members in particular, the extracellular signal-regulated kinases (ERKs) [15], c-Jun NH_2-terminal kinases (JNKs, also called SAPKs) [16], and p38 MAPK [17], have been well characterized. 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) [18]. These modules may be activated by STE20 kinases or small GTP-binding proteins [19]. One of the most explored targets of MAPK signaling modules is transcript factors, such as c-jun, Elk-1, which regulate transcription immediate early gene expression through binding to the serum response element (SRE). Although transcription factors are important MAPK targets, only part of the active MAPK pool translocates to the nucleus and much remains in the cytoplasm and other subcellular compartments. One of the most explored functions of MAPK signaling modules is regulation of gene expression in response to extracellular stimuli [20]. MAPKs phosphorylate Ets transcription factors that are involved in induction of fos genes, whose products heterodimerize with Jun proteins to form activation protein 1 (AP-1) complexes [20]. Activation of AP-1 involves both 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. Thus, c-Jun is phosphorylated in resting cells at a region immediately upstream of the COOH-terminal, the DNA-binding domain (Thr-231, Thr-239, Ser-243, and Ser-249). The MAPKs at the end of these signaling cascades phosphorylate their target proteins, many of which are nuclear proteins such as transcription factors. Hence, MAPKs have a key role in the regulation of many genes. The MEK1-ERK1/2 pathway likely occupies a central regulatory position in the signaling hierarchy of a cardiac myocyte given its unique ability to respond to virtually every characterized hypertrophic agonist and stress stimuli examined to date.

The KRAB domain was originally identified as a conserved motif at the NH2 terminus of zinc-finger proteins and was shown to be a potent, DNA-binding-dependent transcriptional repression module [21–23]. KRAB-ZFPs appear to play important regulatory roles during cell differentiation and development. The KRAB-ZFPs ZNF43 and ZNF91 exhibit expression that is mainly restricted to lymphoid cell, suggesting roles as transcriptional regulators specific for lymphoid cell differentiation [24,25]. The KRAB motif can physically interact with human and mouse RING proteins known as KAP1/TIF1b and KRIP-1, respectively, to mediate the silencing function and the repressor effect of KRAB protein may extend over a distance [26]. 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 GAL-4 transcription factor [22]. A number of KRAB-ZFPs are candidate genes for human diseases based on their chromosomal locations [27,28]. Although the functions of most ZFPs are not completely characterized, some have been implicated in the cardiac developmental or pathological process. For example, GATA-4 (C2H2-type) was found to be important in the very early stages of cardiac development and was recently found to play a critical role in calcineurin-dependent hypertrophy of the adult myocardium [29]. HFHZ (KRAB domain-containing C2H2-type ZFP), a gene transcript recently discovered in a human fetal heart cDNA library, was shown to be upregulated in the hypertrophic heart, suggesting it may play an important role in the pathogenesis of cardiac hypertrophy [30]. Given the high possibility of members of the KRAB-ZNF family being involved in cardiac development and the MAPK signaling cascade, it would be valuable to identify and characterize novel members of this family.

In this paper, we isolated a novel zinc finger *ZNF411* by using degenerated oligonucleotide primers based on the highly conserved KRAB domain, bioinformatics, and rapid amplification of cDNA ends (RACE) technique. We described the cloning and characterization of *ZNF411*, and its function as a repressor in MAPK signaling pathway to mediate cellular activities.

Materials and methods

Construction of cDNA library of human embryo heart. The total RNA from 20-week human embryo heart was extracted using standard methods. The RNA was pretreated with DNase I (RNase free) to eliminate DNA contamination. mRNA preparation and reverse transcription reaction were performed using a cDNA PCR Library Kit and cDNA Synthesis kit according to manufacturer's protocol (TaKaRa). Briefly, 5 μg mRNA was purified from 500 μg total 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 cDNA Synthesis kit protocol. After Cassette Adaptor Ligation reactions using cDNA PCR Library Kit, cDNA amplification reactions were performed with RA primer, CA primer, and TaKaRa Ex *Taq*.

Isolation of cDNA of novel zinc-finger gene and bioinformatics analysis. PCR was performed on a PCRSPRINT reactor (Thermo Hybaid) with one pair of degenerated oligonucleotide primers P1s and P1as (Table 1) corresponding to the amino acid sequence of KRAB domain. A PCR was performed using the heart cDNA library as the template according to standard procedures. The amplification products were separated by agarose gel and the bands were cloned into PMD_{18}T-vector (TAKARA). The transformants were randomly cho-

Table 1
Sets of specific oligonucleotide primers

Primer	Orientation	Nucleotide sequence
P1s	Sense	5′-GTNACNTTMMGNGAMGTNGCNGT-3′
P1as	Antisense	5′-CCANGGMTCMTCNCCMTGMTCNAG-3′
P2s	Sense	5′-ATCTCCGGGCAGGAGACACCGG-3′
P2as	Antisense	5′-TTTTGGATATTTGGATATTGTTC-3′
P3 5′-RACE	Antisense	5′-TGTGCAGTGTCCAGGCAATGCCACT-3′
P4 5′-RACE	Antisense	5′-GACAGGTAACCAGGTCTGGCTTAGA-3′
P5 EGFP	Sense	5′-GACTCGAGGAAATGGGACCATTGCAAT-3′
P6 EGFP	Antisense	5′-TCCTCGAGGAATGATCTTATGCTAATTAA-3′
P7 PCMV	Sense	5′-AGCTCGAGAGATGAACCCCGGGACCCCTG-3′
P8 PCMV	Antisense	5′-ACTCGAGATTGTCTTATGCTAATTAAAAG-3′

sen and sequenced with 250 DNA Sequencer (ABI PRISM) according to manufacturer's procedures. The sequence obtained was subjected to human homology searching against expressed sequence tag (EST) database using Blastn. To confirm the cDNA sequence from the database, one pair of gene-specific primers, P2s and P2as (Table 1), was designed based on the sequences of the two ESTs (AV65901, BX09041) for RT-PCRs. The heart cDNA library was used as template. Amplification was carried out at 94 °C, 4 min; 94 °C, 30 s, 55 °C, 1 min, and 72 °C, 1 min for 25 cycles; then 72 °C, 10 min. The band was obviously excised, cloned into PMD_{18}T-vector (TAKARA), and sequenced. 5′-RACE PCR was performed with TAKARA 5′-RACE Core Kit to confirm the 5′-terminus of *ZNF411*. Jellyfish 1.4 was used to find the open reading frame (ORF) and the deduced translated product. Then, the coding sequence was cloned from human heart library. All the primers for these PCRs are listed in Table 1 and these PCR products were confirmed by sequencing. The full-length sequence of *ZNF411* was submitted to GenBank.

RNA isolation and membrane making. Human tissues from therapeutically aborted fetus (23-week) were obtained under the approval of Health Center of Changsha Women and Children Hospital, People's Republic of China, with the consent of the patients and the regulation of university policy. Total RNA was isolated from various tissues of early human embryos at different developing stages using standard methods as described [31]. For Northern blot analysis, 20 μg total RNA of each tissues was electrophoresed in a 1% agarose gel containing 5.4% formaldehyde and 1× Mops buffer (1× Mops buffer = 20 mM Mops, 5 mM sodium acetate, and 1 mM EDTA). Transfer of RNA to Hybond-N + nylon transfer membrane (Amersham, Arlington Heights, IL) was performed by capillary blotting with 20× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate). After transfer, the nucleic acids were cross-linked to the membrane in a UV cross-linker. At the same time, commercially available Northern blot containing mRNA from a variety of adult tissues was purchased from Clontech.

Cell culture, transient transfections, and subcellular localization analysis. COS-7 cells were cultured in DMEM (Dulbecco's modified Eagle's medium; Gibco-BRL) which was supplemented with 10% FCS, and kept in the incubator (37 °C, 5% CO_2). Sixteen to twenty hours prior to transfection, exponentially growing cells were seeded (10^5 cells/ml) on sterile microscope cover-glasses placed in a 35 mm Petri dish. We transfected cos-7 cells using LipofectAMINE (Invitrogen) reagent according to the method described before [32]. For luciferase assays, we harvested cells 48 h after transfection. The total DNA concentration was held constant by adding the GAL4-DBD expression plasmid. The pL8G5-luciferase reporter contains eight LexA-binding sites upstream of five copies of the GAL4-UAS site. To generate a chimeric protein of *ZNF411* with green fluorescent protein (GFP), the coding sequence of *ZNF411* cDNA was inserted into PEGFP-N1 plasmid vector in-frame. Cos-7 cells were transfected with the recombinant plasmid PEGFP-ZNF411, subcellular localization of the EGFP-ZNF411 fusion proteins was detected 48 h later by fluorescence microscopy.

Transient expression reporter gene assays. COS-7 cells were transfected with LipofectAMINE (Invitrogen) according to the method described before [32]. The reporter constructs for SRE-Luc were obtained from Stratagene and reporter constructs for AP-1-Luc were kindly provided by Dr. K.L. Guan from the University of Michigan. Luciferase activity assays were performed according to the protocols of Stratagene. 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.

Northern blot hybridization. Membranes containing mRNA from a variety of adult tissues and membranes containing total RNA from 23-week embryonic tissues were hybridized with cDNA probe specific for *ZNF411*. The *ZNF411* cDNA was labeled with [α-^{32}p]dCTP by using a Random Primer Labeling Kit (TaKaRa). An adult human Multiple Tissue Northern blot (Clontech) and the embryonic blots were hybridized sequentially to the radiolabeled *ZNF411* 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 and twice in 0.1× SSC and 0.1% SDS at 65 °C for 15 min, then subjected to autoradiography at −80 °C. The blots were stripped by incubated 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.

Phylogenetic tree analysis. Phylogenetic tree analysis of amino acid sequences deduced from *ZNF411* DNA sequences was performed using the MegAlign program of DNASTAR. The CLUSTAL method was chosen to correct the distances for multiple substitutions at a single site. Genbank accession numbers of previously known members of the Krupple family of C2H2-type zinc-finger protein and novel C2H2 type zinc-finger gene sequences used for this analysis are P35789 (*ZNF93*), NP_003420 (*ZNF85*), NP_079465 (*ZNF430*), Q8TF32 (*ZNF431*), Q9P255 (*ZNF492*), NP_689839 (*ZNF92*), AAH35579 (*ZNF100*), NP_258429 (*ZNF257*), XP_291202 (*ZNF479*), DAA01864 (TAP), and *ZNF411*.

Results

Identification and sequence analysis of ZNF411

To search novel members containing zinc-finger motif expressed in human heart and in cell signaling pathway, we isolated homologous genes of this family using PCR amplification from a heart cDNA library of human early embryo. Since nucleotide sequences of the

Krupple-like zinc-finger motif are highly conserved, it was feasible to isolate similar genes of this family by PCR amplification with degenerate oligonucleotide primers [33]. The heart cDNA library was constructed from a 20-week-old human heart embryo by using a PCR cDNA library kit (TAKARA Biotechnology). One pair of degenerated oligonucleotide primers (Table 1), P1s/P1as, was designed based on the amino acid sequence of KRAB domain as described under Materials and methods. The PCR was performed using the heart cDNA library as the template and amplification products were cloned into PMD_{18}T-vector and sequenced. The sequences obtained were subjected to human homology searching against expressed sequence tag (EST) database using Blastn. An EST AV651901 was found to match the cDNA clone. A number of ESTs representing the same novel gene were identified in a further search. The partial cDNA sequences of this novel gene were assembled from ESTs BX474263, BG142261, CB988847, BX090541, BU186714, BX107128, AV650072, BQ215037, BG504312, and AA628456. To confirm the cDNA sequence from the database, one pairs of gene-specific primers (Table 1), P2s/P2as, was designed based on the sequences of two ESTs (AV651901, BX090541) for PCR. The heart cDNA library was used as template and the PCR products were cloned into PMD_{18}T-vector and sequenced. 5′-Rapid amplification of cDNA ends (5′-RACE) was performed in order to obtain the full-length cDNA. The procedure yielded a 250 bp DNA for *ZNF411* 5′-RACE fragments. Combining the results of the overlapping EST analysis, Northern blotting, and 5′-RACE, the 2360 bp full-length cDNA of the novel gene was confirmed, which was named zinc-finger protein 411 (*ZNF411*) as approved by HUGO nomenclature committee. The nucleotide sequence data reported here are available in GenBank.

The full-length cDNA was 2360-bp in length and contained a region encoding a KRAB domain and a 105 bp 5′-untranslated terminus and a 3′-untranslated terminus of 859 bp with a consensus polyadenylation signal (AATAAA) (Fig. 1A). The deduced ZNF411 protein is 499 amino acids and its calculated relative molecular mass is 57,602 Da (~58 kDa) (Fig. 1A). It contains a KRAB motif at its amino terminus and 11 tandemly repeated krupple-type zinc fingers at its carboxyl terminus (Fig. 1B). Alignment between the cDNA sequence and human genome indicates that *ZNF411* is identical to the genomic sequence of PAC CTC-559E9 on chromosome 19p13.11, spanning approximately 25 kb on the genome and organized into 4 exons. A summary of the various sizes of the exons and introns and the sequence of the splice junctions is shown in Table 2. The exon–intron boundaries conform to the consensus splicing signals, where there is a gt and an ag dinucleotide at the 5′ donor and 3′ acceptor site, respectively.

Expression of the ZNF411 mRNA at embryo and adult stages

Northern blot analysis was performed to determine whether *ZNF411* has a restricted pattern of expression. We used *ZNF411* cDNA as the probe to examine its expression at adult and fetal multiple tissues. Northern blot analysis indicates that a 2.4 kb transcript specific for *ZNF411* is expressed in heart, skeletal muscle, and placenta at adult stage (Fig. 3B) and is expressed in most of the examined embryonic tissues, especially at a higher level in skeletal muscle, heart, and pancreas (Fig. 3A). The control 2.0 kb β-actin mRNA was present in all tissues. The results indicate that *ZNF411* is expressed in human heart during both early embryonic developmental stage and in adult tissue.

ZNF411 is conserved during evolution

The *ZNF411* gene shares a strongly conserved C2H2 domain between 11 zinc-finger region (Fig. 2B). The KRAB region is homologous with similar elements in several other zinc-finger transcription factors (Fig. 2A). The high similarity of *ZNF411* C2H2 domain and KRAB domain to the other KRAB-ZFPs suggests that *ZNF411* 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 *ZNF411* protein and the other zinc-finger proteins with phylogenetic tree analysis (Fig. 2C). Sequence alignment of these proteins demonstrates that *ZNF411* is one of the conserved proteins during evolution.

Subcellular localization of ZNF411 protein

In order to test the subcellular localization of ZNF411 protein, the EGFP-ZNF411 was transfected into Cos-7 cells, and 48 h after the transfection, the cells were visualized with epifluorence microscope after labeling with FITC rhodamine for actin and DAPI for nuclei. As shown in Fig. 4, ZNF411 protein distributes evenly in nucleus when overexpressed in the cells (Fig. 4A). The combined image shows ZNF411 protein with actin and nucleus in the cell (Fig. 4C). Although we consider ZNF411 as a nuclear protein, we could not rule out the possibility that the protein also exists in the cytoplasm as shown in the fluorescence staining of the protein in the cell.

ZNF411 is a nuclear protein suppressing SRE- and AP-1-mediated transcriptional activation

Bioinformatics analysis indicates that *ZNF411* protein includes a cAMP-dependent protein kinase phosphorylation site and eleven protein kinase C

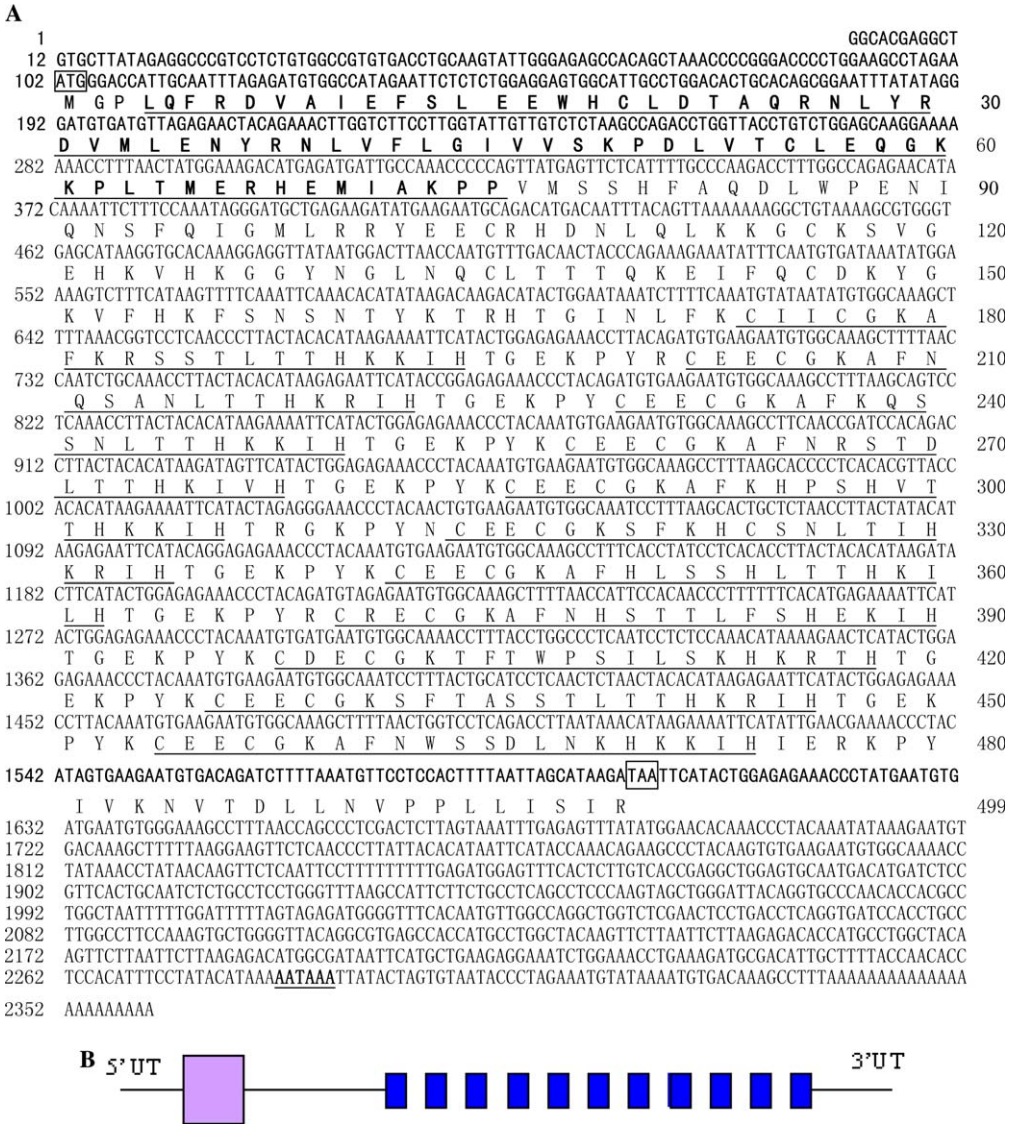

Fig. 1. (A) Nucleotide sequence and deduced protein sequence of the human *ZNF411* gene. *ZNF411* encodes a polypeptide of 499 amino acids. The initiation ATG and termination TAA codons are boxed. Amino acids are identified by their one-letter code. The KRAB domain is in boldface and is underlined. The eleven zinc-finger regions are underlined. Both nucleotides and amino acids are numbered at the left side of each line, respectively. The putative polyadenylation signal sequence **AATAAA** is underlined and is given in bold characters. (B) The structure of *ZNF411* protein deduced from the cDNA open reading frame. The protein sequences contain eleven zinc finger repeats in the C-terminal moiety, and the non-zinc-finger regions of the protein have KRAB domain in the N-terminal moiety. The location of KRAB in the 5′UT is denoted in purple and zinc finger in the 3′UT is implied in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

phosphorylation sites. One of the best-characterized signal pathways that regulate the activation of MAPKs is cAMP. The small G proteins, however, such as Rap1, Rac, and Cdc42 play an inhibition role in the regulation of MAPKs depending on cell and receptor type. It was shown that PKC activation induced dephosphorylation of site in the C-terminal of c-Jun and increased AP-1-binding activity by enhanced phosphatase or inhibited c-Jun protein kinase. In addition, c-Jun is positively regulated by phosphorylation of its N-terminal activation domain by MAPK, resulting in a rapid and significant increase in the activity of AP-1. Although *ZNF411* protein belongs to zinc-finger protein family, the potential role of *ZNF411* is not clear. As a first step in our understanding of *ZNF411* in cell signal transduction, we examined whether *ZNF411* is directly or indirectly involved in the regulation of transcription factors, especially in the MAPK pathway. As an important nuclear effector of MAPK signaling pathway, the c-fos SRE forms a ternary complex together with serum response factor (SRF). As a first step in our understanding of *ZNF411* for transcriptional repressor activity, we used a system in which a luciferase reporter gene was activated by a fusion protein of the LexA

Table 2
Genomic structure of *ZNF411* gene

Exon number	Exon size	Splice acceptor	Splice donor
1	96		TGACCTGCAA**gt**attgggagag
2	139	attgcaattt**ag**AGATGTGGCC	CAGAAACTTG**gt**cttccttggt
3	96	gttgtctcta**ag**CCAGACCTGG	AAGACATGAG**at**gattgccaaa
4	2011	Attttgccca**ag**ACCTTTGGCC	

Exon sizes are given in base pairs and the exonic and intronic sequences at the splice junction are shown in capital and lowercase letters, respectively. The exon–intron splicing signals **gt**, **at**, and **ag** are in bold.

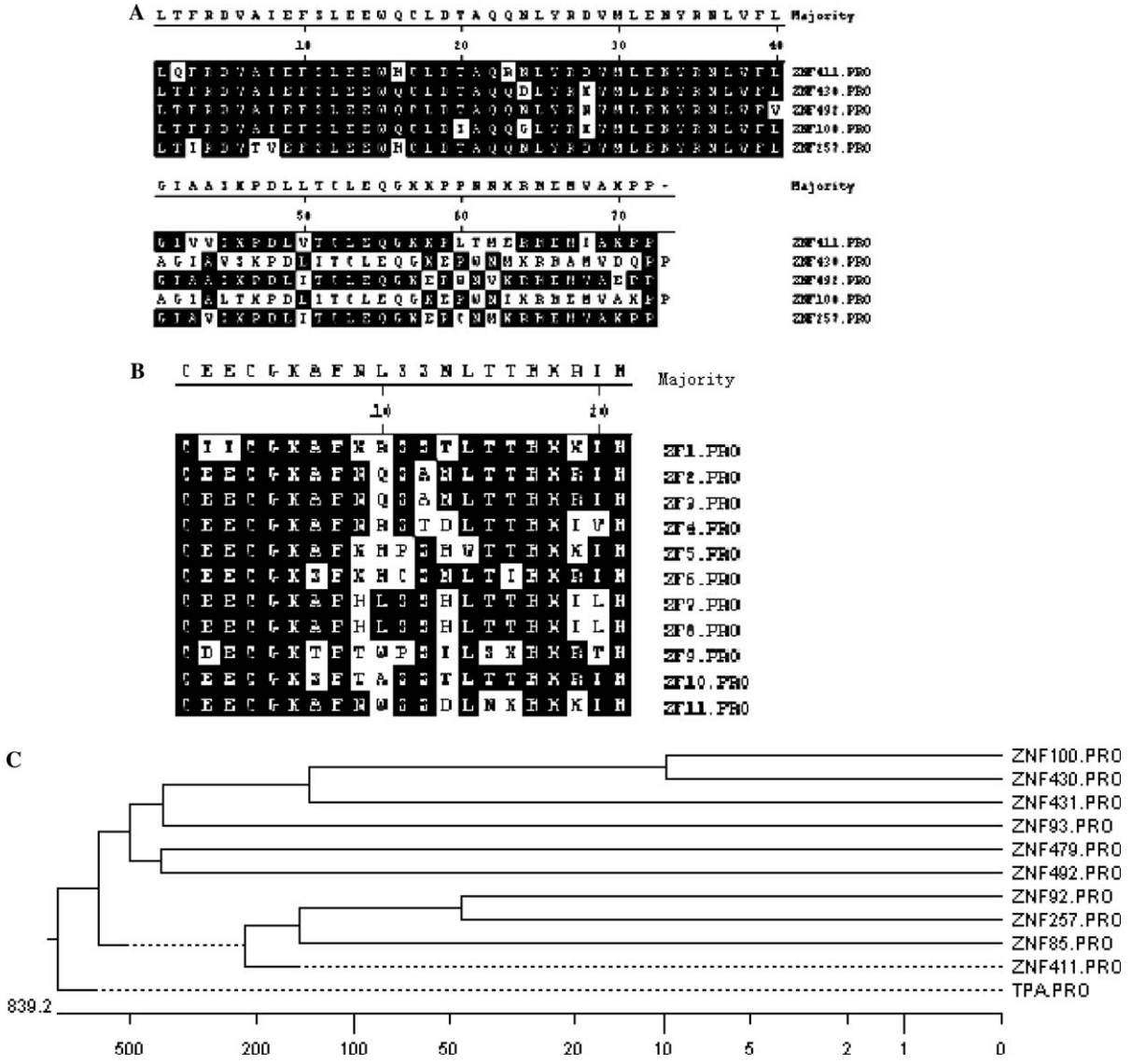

Fig. 2. (A) Amino acid alignment of KRAB domain. The amino acid sequences of the KRAB domains from the following genes are aligned: NP_079465 (*ZNF430*), Q9P255 (*ZNF492*), AAH35579 (*ZNF100*), NP_258429 (*ZNF257*), and *ZNF411*. A consensus sequence for the KRAB domain is presented underneath the alignment. Residues that are highly conserved among these sequences are indicated within the majority sequence in boldface type. Identical residues fitting the KRAB consensus have been boxed and are shaded in dark. (B) Sequence comparisons of zinc-finger domain (ZF1-ZF11) found in *ZNF411* with previously reported nucleotide and amino acid sequences and the C2H2 consensus sequence. Identical amino acid residues of the consensus sequence are shaded. (C) Evolutionary relationship between *ZNF411* and other krupple-like zinc-finger protein. *ZNF411* and *ZNF85* are closely related to each other. Gene names and accession numbers are listed in Materials and methods. All genes originate from *Homo sapiens*, except that TAP originate from mouse.

DNA-binding domain fused to the potent activation domain of the viral co-activator protein, VP16 (LexA-VP16) [34]. Interaction of GAL4-binding sites, located between the LexA-binding sites and the reporter, with a GAL4-ZNF411 fusion protein consistently inhibited luciferase expression in a dose-dependent manner,

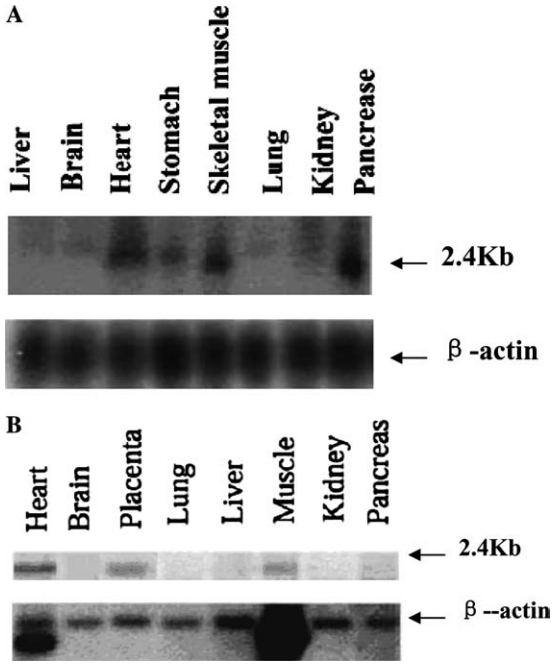

Fig. 3. Expression of *ZNF411* in human embryonic and adult tissues analyzed by Northern blot. Membrane containing 20 μg per lane RNAs from human embryo tissues was hybridized with a ^{32}P random-labeled cDNA probe, which contains coding sequence of *ZNF411*. (A) Twenty-three-week human embryo tissues. (B) Human adult tissues. β-Actin was used as a control for the equivalent amount of loaded mRNA (total RNA) in each other. A band at ~2.4 kilobases (kb) was detected.

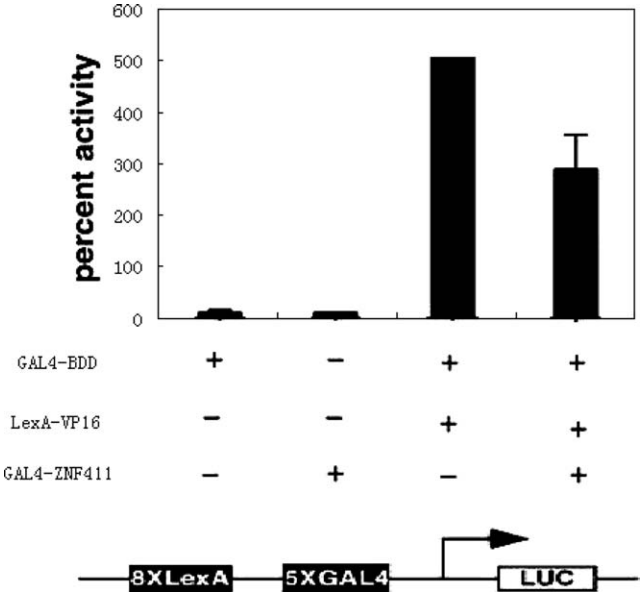

Fig. 5. Cos-7 cells were transiently transfected with the pL8G5-luc reporter along with the indicated LexA or GAL4 mammalian expression vectors. GAL4-ZNF411 was co-transfected with LexA-VP16. Relative luciferase activity was normalized for transfection efficiency by co-transfection with GFP.

compared with LexA-VP16 alone or both LexA-VP16 and GAL4-DBD (Fig. 5).

To examine the effect of *ZNF411* on MAPK pathway, we performed reporter gene assays to measure the modulation of SRE and AP-1 by *ZNF411* protein in the cells. COS-7 cells were co-transfected with the expression plasmids pCMVTag2c-ZNF411, pSRE-Luc, and pAP-1-Luc, which encodes for luciferase controlled by SRE and AP-1, respectively. Expression of *ZNF411* significantly reduced the endogenous SRE-luciferase activity by ~55% (Fig. 6A). We then tested the effect of *ZNF411* on the transcriptional activity of AP-1. As observed in the SRE-luciferase assays, we found that expression of *ZNF411* strongly inhibited the endogenous transcriptional activity of AP-1 by ~60% (Fig. 6B). Taken together, our results suggest that *ZNF411* is a transcriptional repressor that potentially participates in the MAPK signaling pathways in the cell.

Discussion

Although more than one hundred members of the KRAB/C2H2 zinc-finger protein family have been described, little is known of their biological function.

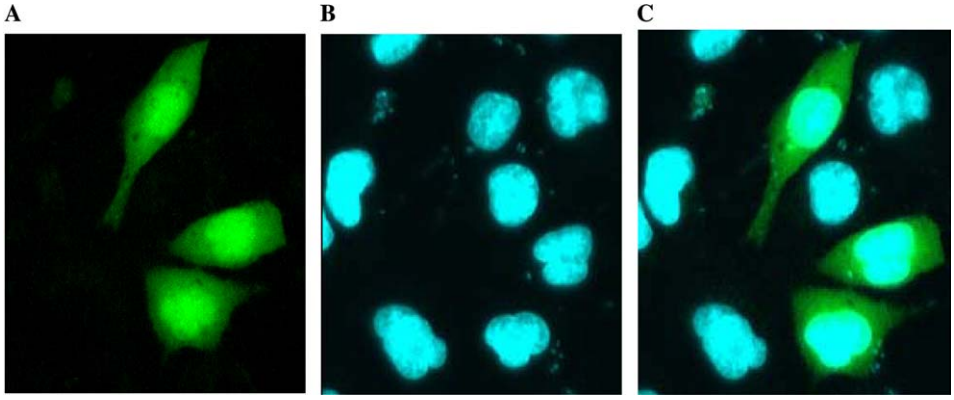

Fig. 4. *ZNF411* is a nuclear protein. (A) EGFP-ZNF411 protein is expressed in Cos-7 cells. (B) The nucleus of cells stained with DAPI. (C) The combined image of (A,B).

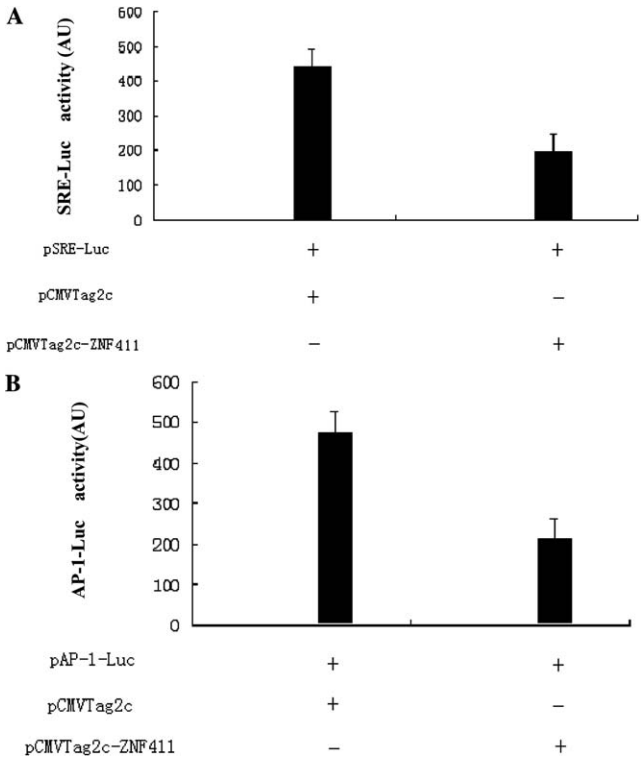

Fig. 6. Overexpression of *ZNF411* suppresses transcriptional activities of SER and AP-1 in COS-7 cells. (A) Co-transfection of pCMVTag2c-ZNF411 and pSRE-Luc suppresses SRE activation in the reporter assay. (B) Inhibition of AP-1 transcriptional activity by expression of *ZNF411*. COS-7 cells transfected with individual reporter plasmid. The data are means of three repeats in a single transfection experiment. Each transfection experiment was performed at least three times.

Moreover, few target DNA sequences or target genes have been identified for KRAB/C2H2 proteins. MAPKs are important signal transducing enzymes that are involved in many facets of cellular regulation. They are transformed into the nucleus and plasma membrane after activation, phosphorylating their targets such as transcription factors and cell surface receptors, so as to regulate gene transcription and other events. In this study, we have isolated and characterized a novel human KRAB-containing krupple-like zinc-finger gene *ZNF411*. Expression of *ZNF411* was found in the tissues tested with the highest in adult heart, skeletal muscle, and placenta, and in fetal heart, skeletal muscle, and other tissues. We showed that *ZNF411* is a transcriptional repressor using a luciferase reporter gene. Overexpression of *ZNF411* in the cell significantly inhibits the activities of SRE and AP-1, which are the targets of ERK, JNK, and p38 [35]. MAPK pathways have also been associated with the development of cardiac failure. For example, samples obtained from human patients with heart failure revealed an increase in all three MAPKs with no changes in samples obtained from hypertrophied human hearts [36]. Levels of activated ERKs were unchanged in heart samples from patients with heart failure secondary to ischemic heart disease, whereas levels of JNK1/2 and p38 activation were significantly increased [37]. MAPK pathways are involved in multiple cellular living processes through phosphorylating their specific endpoint targets, such as ELK-1 and SRE, which compose a ternary complex together with SRF to induce expression of c-fos and other early growth response genes that control the transition from quiescence to proliferation [38]. *ZNF411* is a conserved gene during evolution with high sequence identity among homologues from various species, suggesting a potential role in regulating some essential cellular processes, such as cell growth and proliferation, through the MAPK signaling pathway.

In summary, we have characterized a novel human KRAB/C2H2 zinc-finger transcription factor, designated as *ZNF411*, which is expressed in heart and skeletal muscle at embryonic and adult stages. Sequence analysis reveals that *ZNF411* displays structural homology with other previously described C2H2 zinc-finger proteins. ZNF411 may function as a transcriptional repressor. Overexpression of *ZNF411* in the cell inhibits the transcriptional activities of AP-1 and SRE, suggesting that it may be involved in the heart development and probably works as a negative regulator in MAPK signaling cascade. Further study is needed to elucidate the detailed mechanisms of actions of this novel transcriptional repressor.

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