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ZNF649, a novel Kruppel type zinc-finger protein, functions as a transcriptional suppressor ☆

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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. Many of the KRAB-ZFPs are involved in cardiac development or cardiovascular diseases. Here we report the identification and characterization of a novel human zinc-finger gene named *ZNF649*. The cDNA of *ZNF649* is 3176 bp, encoding a protein of 505 amino acids in the nuclei. Northern blot analysis indicates that *ZNF649* is expressed in most of the examined human adult and embryonic tissues. *ZNF649* is a transcription suppressor when fused to GAL-4 DNA-binding domain and cotransfected with VP-16. Overexpression of *ZNF649* in COS-7 cells inhibits the transcriptional activities of SRE and AP-1. Deletion analysis with a series of truncated fusion proteins indicates that the KRAB motif is a basal repression domain when the truncated fusion proteins were assayed for the transcriptional activities of SRE and AP-1. These results suggest that ZNF649 protein may act as a transcriptional repressor in mitogen-activated protein kinase signaling pathway to mediate cellular functions.

Keywords: *ZNF649*; MAPK signaling pathway; SRE; AP-1; Transcriptional suppressor; KRAB motif; ZNF motif

Mitogen-activated protein kinase (MAPK) pathways are major signaling systems by which the cells transduce extracellular signals into intracellular responses, such as proliferation, differentiation, damage repair mechanisms, and cell death. There are three kinase cascades consisting of sequential phosphorylation and activation of MAPK kinase kinases (MAPKKK), which phosphorylates and activates MAPK kinases (MAPKK), and in turn phosphorylates MAPKs [1–3]. In mammals, there are at least four distinct groups of MAPKs: extracellular signal-related kinases (ERK)-1/2, Jun amino-terminal kinases (JNK1/2/3), p38 proteins (p38α/β/γ), and ERK5. One of the most studied targets of MAPK signaling pathways is transcription factors, which regulate transcription immediate-early gene expression [4,5]. The MAPKs at the end of these signaling cascades phosphorylate their target proteins, such as transcription factors (C-jun, Elk-1), which regulate transcription immediate-early gene expression through binding to the serum response element (SRE) [4]. MAPKs phosphorylate its transcription factors that are involved in induction of fos genes, whose products heterodimerize with Jun proteins to form activation protein 1 (AP-1) complexes. Activation of AP-1 involves the direct phosphorylation/dephosphorylation of AP-1 components as well

☆ *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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as the phosphorylation and activation of transcription factors that induce elevated expression of c-jun or c-fos [6–8].

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 coordinates a Zn^{2+} and causes the intervening amino acids to loop out and form the secondary structure [9,10]. Zinc-finger motif may be involved in the interaction of DNA and protein or protein and protein [11]. 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 [12]. More than one hundred ZFP genes have been found in 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) [13]. The C_2H_2 type is the primary type of ZFP genes present in cardiovascular system. The zinc-finger nucleic acid-binding motif (C_2H_2) occurs in hundreds to thousands of vertebrate proteins and is traceable to all eukaryotes [14]. One family of zinc-finger genes, termed the Kruppel type family, encodes C_2H_2 fingers where the zinc-finger motifs are located in tandem arrays of various sizes. KRAB is enriched in charged amino acids and can be divided into subregions A and B, which are predicted to fold into two amphipathic α helices [17]. The KRAB domain functions as a transcriptional repressor when tethered to the template DNA by a DNA-binding domain and is also involved in protein–protein interactions [15,16]. Transcription factors play an essential role in altering gene expression. It has been estimated that 10% of proteins within a cell are transcription factors that regulate important cellular processes, including cell lineage determination, differentiation, cell growth, and temporal or cell type-specific gene expression [18].

In this report, we identified a novel KRAB-containing zinc-finger protein, named ZNF649, from human fetal cDNA library. Overexpression of *ZNF649* in cells inhibits the transcriptional activities of SRE and AP-1. Deletion analysis with truncated fusion proteins indicates that the KRAB motif is a basal repression domain when cotransfected with VP-16 and suppresses the transcriptional activities of SRE and AP-1. The results suggest *ZNF649* may act as a new repressor in growth factor-mediated signaling pathway.

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 [19].

Full-length cDNA cloning and bioinformatics analysis. The consensus sequence of KRAB region was used to search human EST database with BLASTx algorithm [http://www.ncbi.nlm.nih.gov/BLAST]. 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 (SANGON). The transformants were randomly chosen and sequenced with 250 DNA Sequencer (ABI PRISM) according to manufacturer's procedures. The sequence obtained was subjected to human homology search against expressed sequence tag (EST) database using BLASTn. To confirm the cDNA sequence from the database, one pair of gene-specific primers, S1 and S2 (Table 1), were designed based on the sequences of the six ESTs (CB151734.1, CN392794.1, AI284154.1, CA395211.1, BI89355.1, and BX097174.1) for RT-PCRs. 5′-Gene-specific reverse primers (R1 and R2) were designed for 5′-RACE reactions according to the previous method [20]. 5′-RACE were performed using SMART RACE cDNA Amplification Kit (Clonetech). All the PCR products were then cloned into pMD18T-vector (Sagon) and sequenced with 377 DNA Sequencer (ABI PRISM). 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 *ZNF649* sequences was performed by DNAstar software. BLASTn and Pfam 9.0 were used to analyze the genomic structure and the protein domain, respectively. The homologues of

Table 1
Sets of specific oligonucleotide primers

Primers	Nucleotide sequence	Programs	Cycles
S1	5′ AGACGAAGCATCCCAGTT 3′	94 °C, 30 s	30
S2	5′ CATTCCGCTGGAGGCTAT 3′	56 °C, 30 s	
		72 °C, 1 min	
S3	5′ GACTCGAGATCCCAGTTCTTGGGTAT 3′	94 °C, 30 s	28
S4	5′ ATGTCGACTGTGGGCAAACTCACACT 3′	58 °C, 30 s	
		72 °C, 1 min	
R1	5′ GCCGCCATAGGCCAGTGCCGGGGTT 3′	94 °C, 30 s	32
R2	5′ GCCGCCATAGGCCAGTGCCGGGGTT 3′	53 °C, 30 s	
		72 °C, 1 min	

ZNF649 were found with BLASTp, and the sequence alignment and phylogenetic tree analysis were performed with MegAlign program (DNAstar).

Northern blot analysis of ZNF649 expression in human fetal and adult tissues. 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 *ZNF649*. The *ZNF649* 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 blot were hybridized sequentially to the radiolabeled *ZNF649* cDNA probe and β-actin cDNA probe (Clontech) [10]. 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, 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 [21–26].

Cell culture, transient transfection, and subcellular localization analysis. COS-7 cells used in all studies were maintained according to standard methods in DMEM (Dulbecco's modified Eagle's medium; Gibco BRL) supplemented with 10% fetal calf serum (FCS) in an humidified atmosphere of 95% air and 5% CO_2. The *ZNF649* ORF was subcloned into the *Xho*I and *Sal*I sites of pEGFP-N1 vector with the TGG codon instead of the TAG stop codon. Cells were transfected with pEGFP-N1-ZNF649 using LipofectAMINE (Invitrogen) according to the method described previously [21]. 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-ZNF649 fusion protein was detected using a Nikon inverted fluorescence microscope.

Transient expression reporter gene assay. ZNF649 ORF was subcloned into the *Bam*HI and *Sal*I sites of the pCMV-BD vector in-frame to construct pCMV-BD-ZNF649 for GAL-4-ZNF649 fusion protein. Then pCMV-BD-ZNF649 or pCMV-BD is transiently co-transfected into COS-7 cells along with the pL8G5-Luc reporter and pLexA-VP-16 using LipofectAMINE as described above. Forty-eight hours later, the luciferase activity assay was performed according to the protocols of Stratagene [22]. Relative luciferase activity was normalized for transfection efficiency by co-transfection with pCMV-lacZ and spectrophotometric 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 after normalization for β-galactosidase activity.

The *ZNF649* ORF was then subcloned into the *Bam*HI and *Sal*I sites of the pCMV-Tag2B vector in-frame. The luciferase reporter plasmids were described in previous studies. COS-7 cells were co-transfected with pFR-Luc, pFA2-SRE, pFC-MEK1, and pCMV-Tag2B-ZNF649 or pCMV-Tag2B vector to investigate the effect of ZNF649 on the transcriptional activity of SRE. To examine the effect of ZNF649 on the transcriptional activity of AP-1, cells were co-transfected with pAP-1-Luc and pCMV-Tag2B-ZNF649 or pCMV-Tag2B vector. Forty-eight hours later, the luciferase activity assay was performed according to the protocols of Stratagene [23]. Relative luciferase activity was normalized for transfection efficiency by co-transfection with pCMV-lacZ and spectrophotometric 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 after normalization for β-galactosidase activity.

Transient expression reporter gene assays of KRAB motif and zinc-finger motif in ZNF649. ZNF649 sequence was separated into five segments as described above and transcriptional reporter assays as described above. 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. ZNF649 sequence was separated into four segments: segment KRAB involving KRAB domain; zinc-finger domain and C-telopeptide and transcriptional reporter assays as described above. 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 after normalization for β-galactosidase activity.

Results and discussion

Molecular cloning and domain structure of ZNF649

With the aim of identifying genes involved in heart development and in cell signaling pathway, we have performed a screen of heart cDNA library that was constructed from a 20-week-old human heart embryo. Since nucleotide sequences of the Kruppel-like zinc-finger motif are highly conserved, it was feasible to isolate similar genes of this family by PCR amplification with degenerate oligonucleotide primers [22]. The PCR was performed using the heart cDNA library as the template and the sequences obtained were subjected to human homology search against expressed sequence tag (EST) database using BLASTn. A number of ESTs representing the same novel gene were identified in a further search. The partial cDNA sequence of this novel gene was assembled from ESTs BX954697, BX327729, CR739576, BX089598, and BM724141. To confirm the cDNA sequence from the database, two pairs of gene-specific primers (Table 1), the first forward primer in BX954697(S1) and the reverse primer in BX327729(S2), and the second forward primer in BX954697(S3) and the reverse primer in CR739576(S4) were designed using Primer Premier 5.0 to perform standard PCR. The heart cDNA library was used as template and a 1556 bp PCR product was obtained and confirmed to be the cDNA sequence of this new gene. Combining the results of the overlapping EST analysis, Northern blotting, and 5′-RACE, the 3176 bp full-length cDNA of the novel gene was confirmed, which was named zinc-finger protein 649 (*ZNF649*) as approved by HUGO Nomenclature Committee. The nucleotide sequence data reported here are available in GenBank.

The *ZNF649* gene consists of an open reading frame (ORF) of 1518 bp extending from the first ATG codon at nucleotide 269 to a termination TGA at 1786, a 268 bp 5′-untranslated region (UTR), and a 1390 bp 3′-UTR with a consensus polyadenylation signal (aataaa) [27] (Fig. 1). The deduced ZNF649 protein has 505 amino acids (Fig. 1A) with a calculated molecular mass of 59 kDa. *ZNF649* is assigned to human chromosome 19q13.41 according to the mapping information in NCBI, and spans approximately 17 on the genome. *ZNF649* gene contains five exons and four introns on the genome (Fig. 1). 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

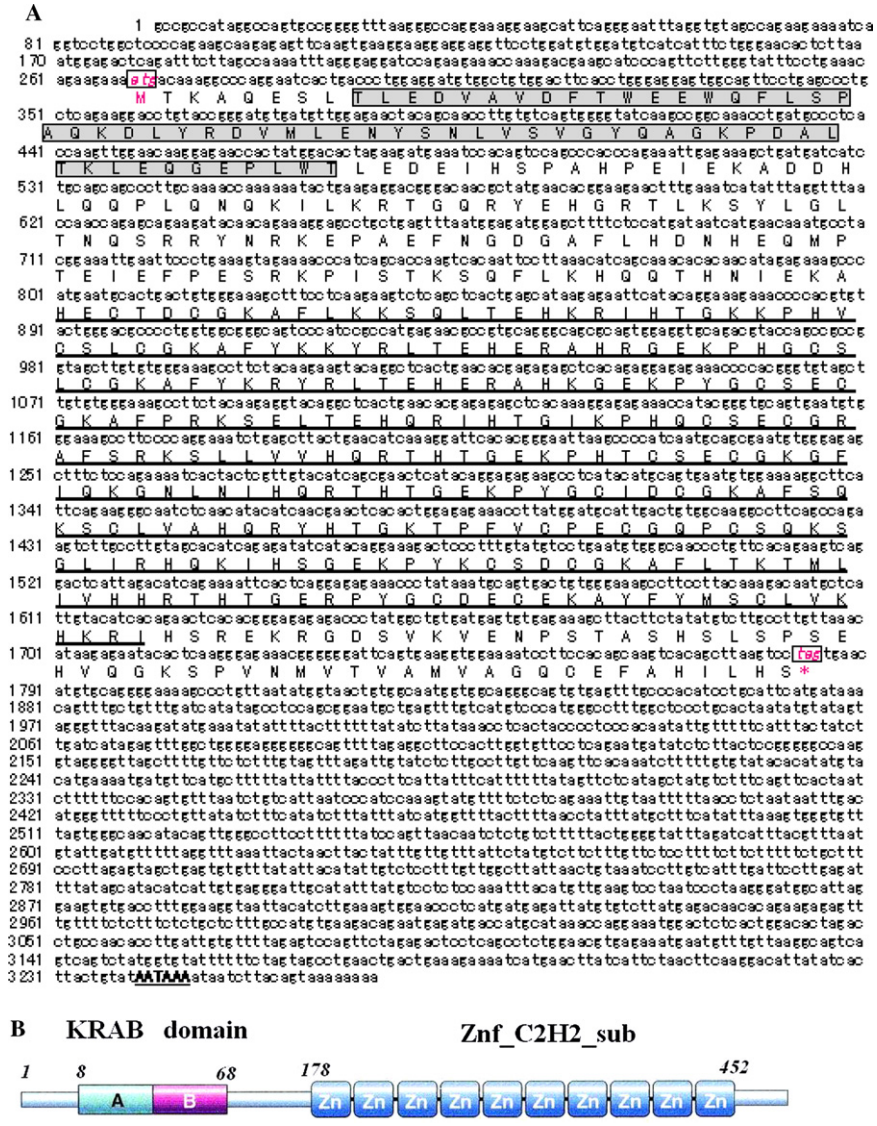

Fig. 1. Nucleotide sequence and deduced protein sequence of ZNF649. (A) The nucleotide sequence and predicted protein sequence of ZNF649. The nucleotides are numbered in the left and the amino acid sequence is shown in capital letters. The transcriptional starting and stop codons are marked with red italics. The poly(A)$^+$ signal **AATAAA** is underlined. The KRAB box is boxed and shaded in gray. The zinc-finger motifs are underlined. (B) The domain structure of ZNF649. KRAB box is at N-terminal of the sequence and 10 zinc fingers in the C-terminal of the sequence.

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. Analysis of ZNF649 protein using the SMART program indicates that amino acid sequence of ZNF649 contains an N-terminal Kruppel-associated box (KRAB) domain (amino acids 8–68) and 10 C_2H_2 zinc-finger motifs that extend to the end of the protein sequence (Fig. 1B).

ZNF649 is conserved during evolution

A search of published DNA databases for sequences similar to that of *ZNF649* demonstrated that they had varying degrees of similarity to a number of previously identified C_2H_2 type zinc-finger proteins. The alignment of amino acid sequences between the N-terminus (KRAB domain) of ZNF649 indicates that KRAB box of ZNF649 belongs to the classical KRAB-AB box (Fig. 1B). Sequence analysis and database comparison indicate that the predicted protein contains 10 different C_2H_2 zinc-finger domains in tandem arrays, characteristic of transcription factor proteins of this family. The amino acid sequence of this region was aligned with similar domains in several other zinc-finger proteins of *Homo sapiens*, such as ZNF613, ZNF615, and ZNF350. By comparing some sequences and structure of zinc-finger family proteins existing in different species, the putative evolutionary relationship of these genes is depicted by a phylogenetic tree using a multiple sequence alignment program (Fig. 2).

Table 2
Genomic structure of the *ZNF649* gene

Exon number	Exon size (bp)	Splice donor site	Intron size (bp)	Splice acceptor size
I	81	***GT***GAGTGTTTGGGT	4619	TTTTTACTCCAT***AG***
II	202	***GT***AACTTTTGGTTT	3114	CTGTGTTATTAC***AG***
III	127	***GT***GAGGACAGCTGC	284	TTTCCCACAAAC***AG***
IV	96	***GT***AAGTGAGAGAGA	4574	GTTCTCTCTCTT***AG***
V	2662			

Note. Summary of the sizes of the exons and introns of the *ZNF649* gene based on the comparison of the cDNA and the genomic sequence. Invariant nucleotides (GT/AG) are in bold italics.

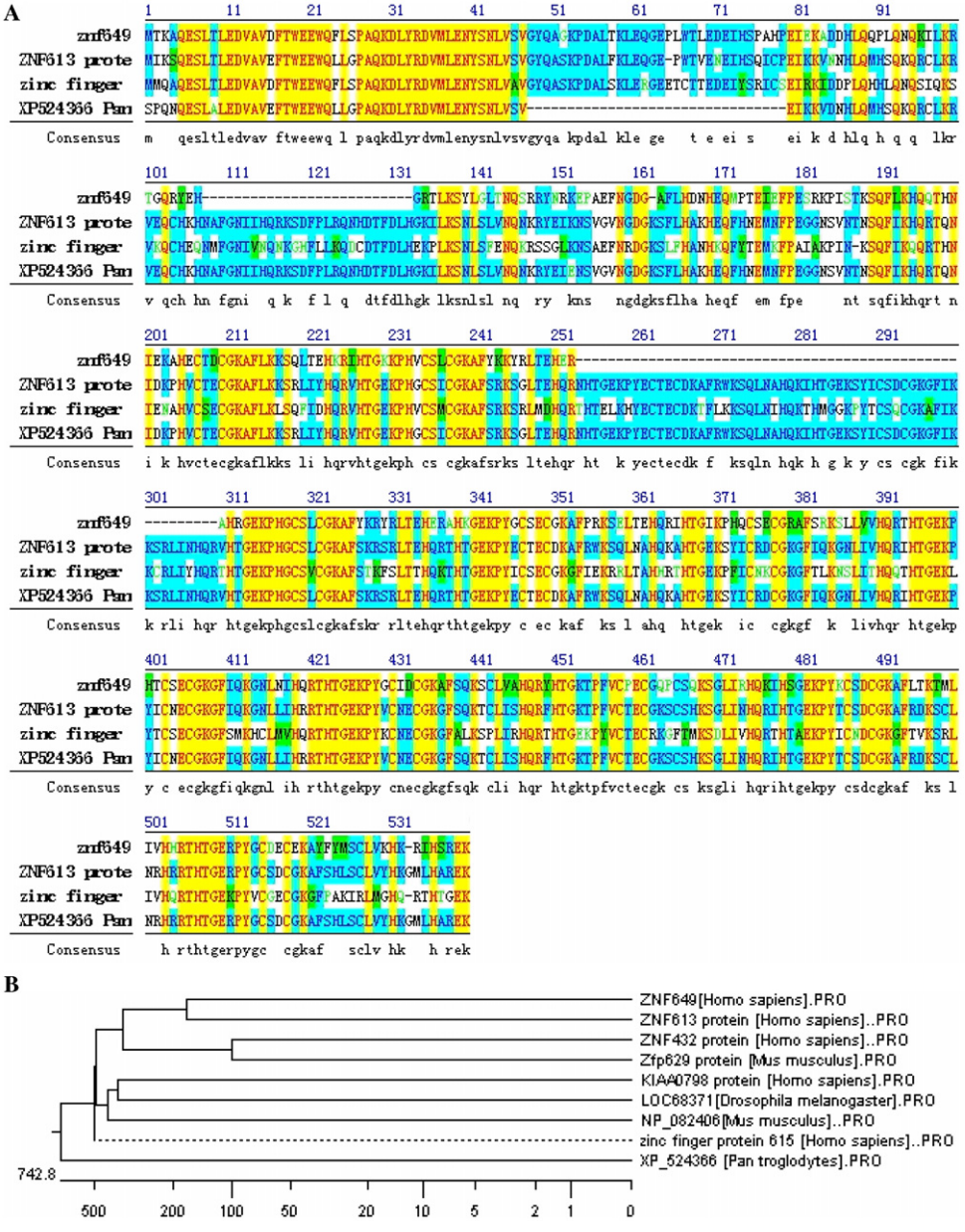

Fig. 2. *ZNF649* is conserved during evolution. (A) Amino acid sequence alignment of *ZNF649*. Comparison of the amino acid sequences of *ZNF649* and its homologues (*ZNF613*, *ZNF615*, and XP524366 *Pan troglodytes*) indicates that *ZNF649* is conserved during evolution. (B) The proposed evolutionary tree of the zinc-finger family of proteins.

ZNF649 is expressed in most of the human adult and embryonic tissues

Northern blot analysis was performed to determine whether *ZNF649* has a restricted pattern of expression. We used *ZNF649* cDNA as the probe to examine its expression at adult and fetal multiple tissues. Northern blot analysis detects an expected transcript of about 3.2 kb in the 20-week embryo tissues, including heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas (Fig. 3A). The expression of *ZNF649* at adult is predominant in heart, skeletal muscle, and brain, but weaker in other tissues (Fig. 3B). The control 2.0 kb β-actin mRNA was present in all tissues.

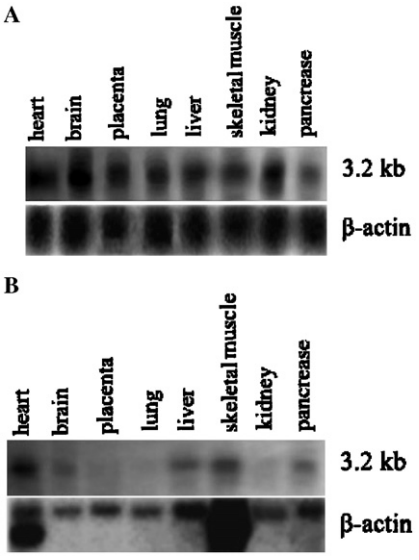

Fig. 3. Northern blot analysis of *ZNF649* in human adult and embryonic tissues. (A) Twenty-week fetal tissues. (B) Human adult tissues. The cDNA of *ZNF649* was labeled with [α-^{32}P]dCTP and used as the probe.

Subcellular localization of the ZNF649 protein

We examined the subcellular localization of *ZNF649* to determine whether it could function as a transcriptional regulator. The pEGFP-N1-ZNF649 was transfected into COS-7 cells, and 48 h after the transfection, the cells were visualized with fluorescence microscope after labeling with DAPI for the nucleus. As shown in Fig. 4, ZNF649 protein distributes evenly in the nucleus when overexpressed in cells. The combined image shows that ZNF649 protein exists primarily in the cell nucleus (Fig. 4C), although 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.

ZNF649 is a transcription repressor

To examine a potential function for ZNF649 in transcriptional activity, we constructed a fusion protein of ZNF649 with the DNA-binding domain (BD) of yeast transcription factor GAL-4 under the driving of a CMV promoter, the pCMV-BD-ZNF649. We examined the transcriptional activity of ZNF649 by co-transfecting of the COS-7 cells with pCMV-BD-ZNF649 and the reporter, pL8G5-Luc. The pL8G5-Luc reporter contains eight copies of the LexA DNA-binding sites and five copies of the GAL-4 DNA-binding sites linked to the luciferase reporter gene. In this system, transcriptional activity of ZNF649 could affect luciferase gene expression and luciferase activity. When co-transfecting with pL8G5-Luc plasmid, the GAL-4-ZNF649 fusion protein inhibited luciferase activity by 1%. While co-transfecting with pLexA-VP-16, the GAL-4-ZNF649 fusion protein inhibited the VP-16-activated luciferase activity by 65% (Fig. 5). Therefore, ZNF649 is able to suppress transcriptional activity of reporter gene when targeted to the template via the GAL-4 DNA-binding domain, which suggests that ZNF649 may play direct roles in transcriptional repression.

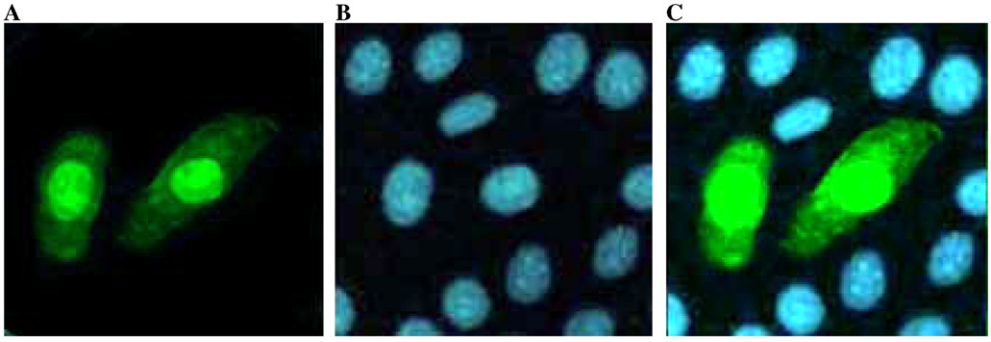

Fig. 4. Cellular localization of the ZNF649 protein in COS-7 cells. (A) EGFP- ZNF649 is localized both in the nucleus and the cytoplasm when transfected into the COS-7 cells. (B) The nucleus of the cells was stained with DAPI. (C) The combined image of (A) and (B), showing subcellular localization of ZNF649 when overexpressed in COS-7 cells.

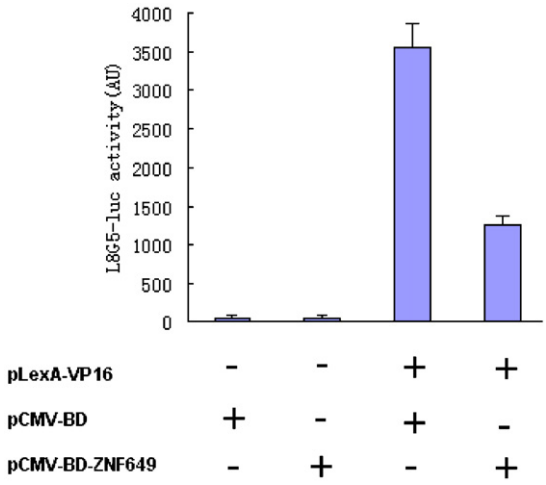

Fig. 5. *ZNF649* is a transcriptional suppressor. Forty-eight hours after transient transfection, the luciferase activity assay was performed. The data are means of three repeats in a single transfection experiment after normalization for β-galactosidase activity. Each experiment was repeated at least three times. pCMV-BD-ZNF649 or pCMV-BD is transiently co-transfected into COS-7 cells along with the pL8G5-Luc reporter and pLexA-VP16 as indicated in the figure.

ZNF649 suppresses SRE- and AP-1-mediated transcriptional activation

KRAB type proteins play important roles in a variety of cellular functions from cell proliferation, cell apoptosis, to different cell signal transduction pathways. We performed pathway-specific reporter gene assays to measure the modulation of SRE and AP-1 by ZNF649 in the COS-7 cells. As shown in Fig. 6A, full-length ZNF649 reduced SRE transcriptional activity by 74.5%. Furthermore, using AP-1-Luc designed for monitoring the regulation of the activator protein 1 (AP-1), ZNF649 was found to reduce AP-1 transcriptional activity by ~65% (Fig. 6B). Taken together, our results suggest that ZNF649 is a transcriptional repressor, which suppresses transcriptional activities of SRE and AP-1 transcriptional factors.

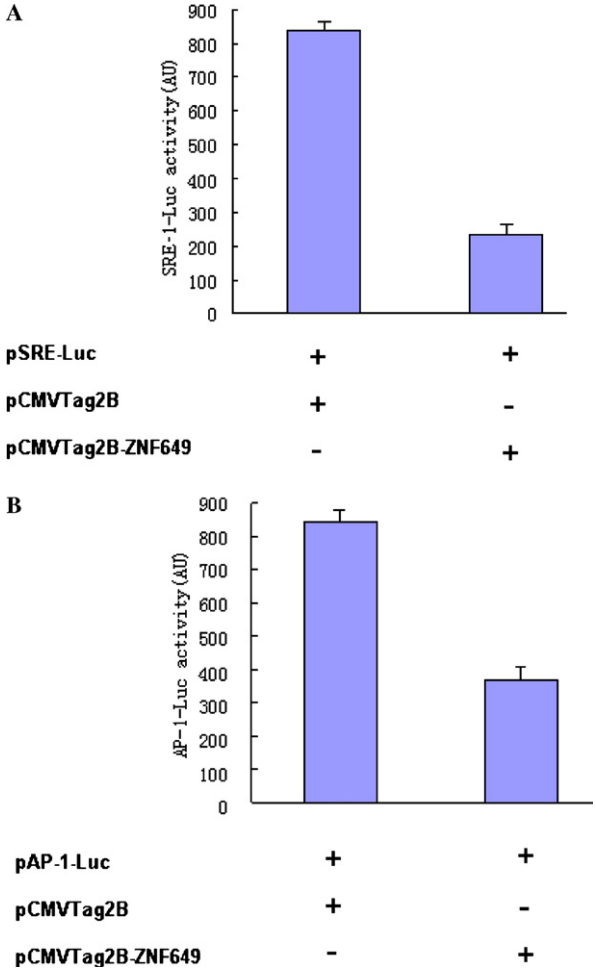

Fig. 6. Overexpression of ZNF649 suppresses transcriptional activities of SRE and AP-1. (A) Inhibition of SRE-Luc transcriptional activity by the overexpression of ZNF649. (B) Inhibition of AP-1-Luc transcriptional activity by the overexpression of ZNF649. COS-7 cells transfected with reporter plasmid and the corresponding plasmids are shown in the figures. Forty-eight hours later, the luciferase activity assays were performed. The data are means of three repeats in a single transfection experiment after normalization for β-galactosidase activity. Each experiment was performed at least three times.

KRAB motif represents potent repression domain

To examine whether the KRAB motif and ZNF motif in ZNF649 function as a potent repression domain in transcriptional activity, a series of truncated pCMV-Tag2B-ZNF649 fusion proteins were examined for their transcriptional suppression activity. We construct some fusion proteins of pCMV-Tag2B-KRAB, pCMV-Tag2B-C_2H_2, and pCMV-Tag2B-c and then performed pathway-specific reporter gene assays to measure the modulation of SRE and AP-1 by 10 ZF and KRAB in the COS-7 cells. As shown in Fig. 7A, expression of pCMV-Tag2B-KRAB significantly reduced the endogenous SRE-luciferase activity by 72.3%. The C_2H_2 motif and the C-telopeptide region exhibited a slightly repressive activity. We then tested the effect of pCMV-Tag2B-KRAB on the transcriptional activity of AP-1 and found that expression of pCMV-Tag2B-KRAB strongly inhibited the endogenous transcriptional activity of AP-1 and no obvious repressive activity for the C_2H_2 motif and the link region (Fig. 7B). Taken together, our results suggest that the KRAB domain of ZNF649 represents a basal repressive domain in transcriptional regulation.

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,

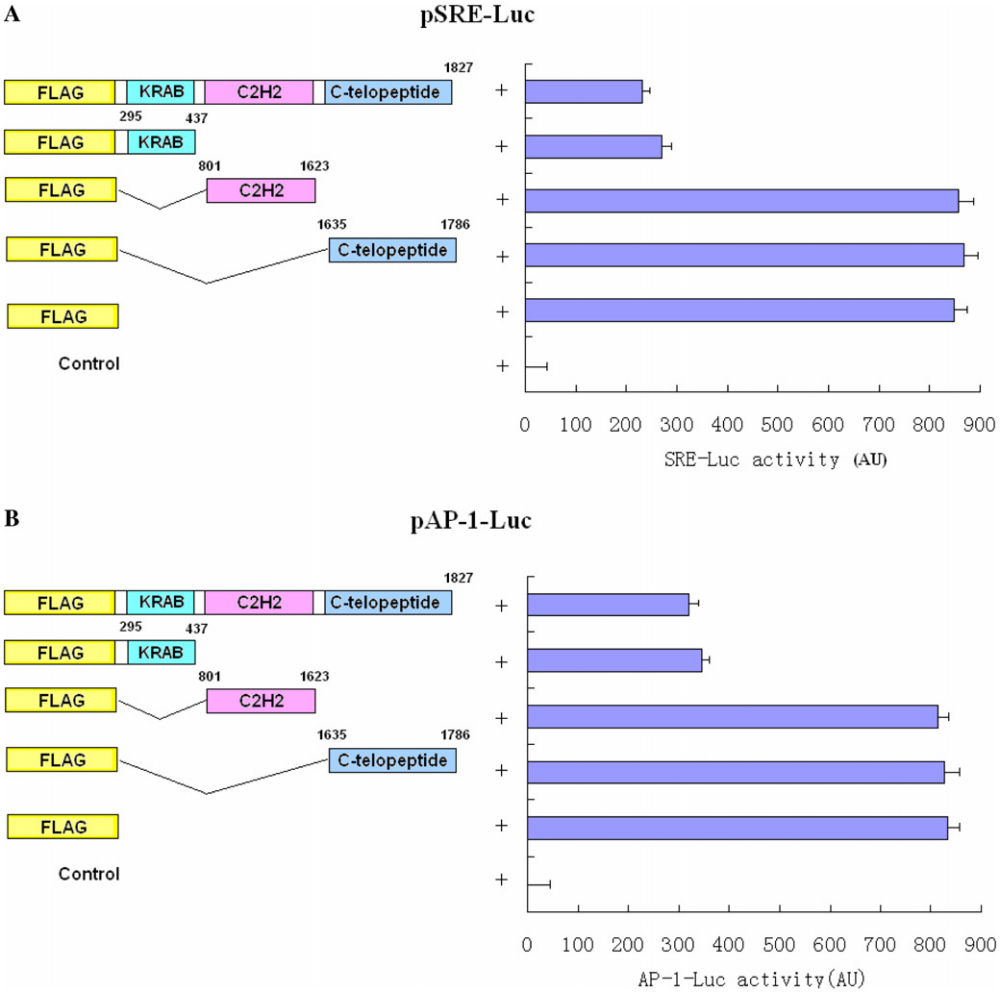

Fig. 7. The KRAB domain of ZNF649 is required for the transcriptional repression activity of the protein. (A) Inhibition of SRE-Luc transcriptional activity by the overexpression of the truncated fusion proteins of ZNF649. (B) Inhibition of AP-1-Luc transcriptional activity by the overexpression of the truncated fusion proteins of ZNF649. The full-length and different truncated forms of ZNF649 were generated and transiently transfected into COS-7 cells along with the reporter to identify the repression domain of the protein. Each experiment was performed in triplicate and each assay was repeated at least three times.

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 ZNF649 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.

Kruppel-associated box (KRAB) is an evolutionarily conserved protein domain with respect to the finger repeats which represents the distributed transcriptional repressor motif when tethered to the template DNA by a DNA-binding domain. The potential α-helical structure of KRAB domain may mediate protein–protein interaction [28–33]. Analysis of the KRAB domain sequence of ZNF649 reveals that the KRAB domain shows high homology to other zinc-finger proteins,

including two conserved motifs DV (at position 12–13) and MLE (at position 37–39) which have been shown to be important for repression and interaction with TIF1-β. The zinc-finger domain is found in a large number of eukaryotic proteins and represents a nucleic acid-binding motif. Additional studies also support that KRAB domain is likely to form a protein–protein complex. Therefore, it is possible that, via the KRAB domain and zinc-finger domain, ZNF649 protein binds to other transcription factors and regulators to form the mammalian Mediator complex needed for basal-level and specific transcription.

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