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ScienceDirect
Biochemical and Biophysical Research Communications 351 (2006) 33–39
BBRC
www.elsevier.com/locate/ybbrc

A novel human gene ZNF415 with five isoforms inhibits AP-1- and p53-mediated transcriptional activity ☆

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Received 25 September 2006
Available online 9 October 2006

Abstract

The zinc finger proteins are the single largest class of transcription factors in human genome. Previous studies revealed that zinc finger proteins are involved in transcriptional activation and regulation of apoptosis, etc. Alternative splicing emerges as a major mechanism of generating protein diversity and many zinc finger proteins reported have isoforms. In this article, we identify and characterize five isoforms of a novel zinc finger gene named ZNF415; these five isoforms were named ZNF415-1 to ZNF415-5. The five isoforms display different subcellular localization and are expressed at different levels in both embryonic and adult tissues. Furthermore, the splicing variants of ZNF415 display different transcriptional activity. Except for ZNF415-1, overexpression of the other ZNF415 isoforms in COS-7 cells inhibits the transcriptional activities of AP-1 and p53, suggesting that the ZNF415 protein may be involved in AP-1- and p53-mediated transcriptional activity.
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Keywords: Zinc finger protein; KRAB A box; Isoform; Expression pattern

Transcription factors regulate important cellular processes, such as cell-lineage determination, cell growth, and differentiation via the temporal or spatial gene expression of specific cell type genes [1–3]. Zinc finger proteins probably constitute the largest individual family of such nucleic acid-binding proteins [4]. A zinc finger is a conserved motif of 28 amino acids, which is often repeated within a protein [5] and may be involved in DNA–protein or protein–protein interactions [6]. Zinc finger proteins (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 [7]. In addition to zinc finger regions, most of these proteins also contain a regulatory domain, the Krüppel-associated box (KRAB). The KRAB domain consists of an A box and a B box encoded by two separate exons [8]. Alternative splicing of A box and B box has been reported [8,9]. Alternative splicing has emerged as a major mechanism for expanding and regulating the repertoire of gene function. Alternative domain splicing in KRAB domain disrupts the protein interaction domain [10]. This kind of domain disruption has apparently been employed in evolution in addition to create useful new functions [11].

In this study, we identified a novel zinc finger protein named ZNF415, from human fetal cDNA library. Five isoforms of ZNF415 have been cloned. ZNF415-1 encodes 11 different C2H2 type zinc fingers, the other isoforms contain a linker region that normally joins the KRAB box to the

☆ *Abbreviations:* AP-1, activation protein 1; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; KRAB, Krüppel-associated box; MAPK, mitogen-activated protein kinase; ZFPs, zinc finger proteins.

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0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.09.161

region coding for the 11 zinc fingers, and finally ZNF415-5 also contains a KRAB A box. These five isoforms display different expression levels in 6-month-old embryos and in adult tissues. ZNF415-1 and ZNF415 have the highest expression in both embryo and adult, while ZNF415-3 has the lowest level in the tissues examined. ZNF415-2 and ZNF415-4 show intermediate levels. The ZNF415-1 protein is localized in the nucleus while the other isoforms are localized in both the nucleus and cytoplasm. Five isoforms fused to the GAL4 DNA-binding display different transcription activity suggesting that splicing may influence the function of ZNF415 isoforms.

All the isoforms except ZNF415-1 can inhibit AP-1 and p53 activity. AP-1 is a dimeric transcription factor which is mainly composed of either a Jun-Jun homodimer or a Jun-Fos heterodimer. AP-1 regulates the expression of multiple genes essential for cell proliferation, cell cycle control, apoptosis, differentiation, and tumorigenesis [12]. The p53 pathway is composed of hundreds of genes and their products that respond to a wide variety of stress signals. These responses to stress include apoptosis, cellular senescence or cell cycle arrest [13]. Thus, by regulating activity of these transcription factors, ZNF415 might also play a role in cell cycle control or apoptosis.

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. 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 [14].

Full-length ZNF415 cDNA cloning and bioinformatics analysis. A search of the human EST database with the conserved Cys2/His2 type zinc finger motifs was performed through a combined BLAST search as previously described [15]. The sequence obtained was subjected to human homology searching against expressed sequence tag (EST) database using Blastn (http://www.ncbi.nlm.nih.gov/blast). We searched the ZNF415 consensus sequence and identified six overlapping human expressed sequence tags (ESTs) (BX414947, AL565661, BX414948, BX411247, BX431463, and AL534796) corresponding to a novel gene. PCR was performed with one pair of degenerate oligonucleotide primers in order to identify whether these ESTs belong to the same novel gene. ZNF415 sequence of open-reading frame (ORF) was confirmed by PCR amplification with a pair of primers (ORF1 and ORF2, Table 1) based on CA423205 and BX484548. The PCR products were cloned into pMD18-T-vector (Takara) and sequenced with 3770 DNA Sequencer (ABI PRISM). Jellyfish 1.4 was used to find ORF and the deduced translated product. 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. BLASTn and Pfam 9.0 were used to analyze the genomic structure and the protein domain, respectively. The homologues of ZNF415 were found with BLASTp. 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 Krüppel family of C2H2-type zinc finger protein and novel C2H2 type zinc finger gene sequences used for this analysis are: NP_060770 (ZNF83); NP_115973 (ZNF347); NP_009080 (ZNF184); NP_003420 (ZNF85); NP_003416 (ZNF45); NP_003421 (ZNF91); NP_666016 (ZNF23); NP_009084 (ZNF208); NP_008889 (ZNF16); NP_057528 (ZNF226); NP_033559 (ZFP29, mouse); NP_076478 (AJ18 protein, *Rattus*); and AAP35086 (ZNF415).

Table 1
Oligonucleotide primers

Name	Sequence
ORF1	5′-CGGCGTCGAGCCATTGACTTCCAA-3′
ORF2	5′-AGCCTCTGCCACACAGTTAGGTGTA-3′
Rs	5′-CCTGGGTTACCTTCCTGTTG-3′
Ra	5′-GAAGTCCTGTTGCTGTTGCTG-3′
Zs	5′-GTGGCAGAGGCTTCATTTAGG-3′
Za	5′-GAGGCAGGAGAATCGTTTGAA-3′
SPs	5′-ATGAGGAAGAAACCCAGAA-3′
SPa	5′-TCCCTGAAGCAAAACTCT-3′
1S	5′-GAAAGGATCCAAATGTGATCTGTG-3′
2-3S	5′-GAGGATCCGAAATGCCTGAACTCTAC-3′
4S	5′-AGGGATCCCCTATGTGGGAGCAC-3′
5S	5′-AGGGATCCGGAATGGCTTTTACTCAG-3′
415A	5′-ACGGATCCATATTAATTTCTTTTATAAGG-3′
Gs	5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′
Ga	5′-CATGTGGGCCATGAGGTCCACCAC-3′

RACE analysis. A rapid amplification of cDNA ends (RACE) technique was performed using mRNA from human fetal heart. The 5′ upstream sequence and 3′ downstream sequence of ZNF415 were amplified by RACE PCR using the SMART cDNA Amplification Kit (TaKaRa Biotechnology) according to manufacturer's protocols [16]. The gene-specific primers used for 5′ RACE and 3′ RACE PCR were Rs /Ra and Zs/Za (Table 1). The heart cDNA was used as template. All the PCR products were then cloned into pMD18-T vector and sequenced.

RNA isolation and RT-PCR. Human tissues from therapeutically aborted fetuses 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 (heart, brain, skeletal muscle, liver, spleen, small intestine, lung, and kidney of early human 6-month-old embryos and heart, atria, cerebra, cerebel, skeletal muscle, liver, and lung of adult). Total RNA (5 μg), primed with oligo(dT), was reverse-transcribed into cDNA using the first-strand cDNA synthesis kit (Ferments) according to the manufacturer's instructions. cDNA amplification reactions were performed with SPs and SPa, and TaKaRa Ex Taq, GAPDH was used as a control with Gs and Ga. In order to distinguish the isoforms exactly, the sample was run in a 6% polyacrylamide gel with 1× TBE buffer on a vertical electrophoresis system [17], The results were visualized by silver staining and the PCR products were purified and sequenced.

Plasmid construction. The following plasmids were constructed and used for mammalian cell transfections. To generate a fusion protein between the five isoforms of ZNF415 and Red fluorescent protein (DsRed), pDsRed-monomer-c1, the DNA fragment containing the ZNF415 isoform coding sequence was amplified from pMD18-T-ZNF415 (1, 2, 3, 4, 5) with primers 415A and 1S, 2–3S, 4S, 5S (ZNF415-2 and ZNF415-3 share the same start site, Table 1), respectively. All the primers contain *Bam*HI site. The amplified DNA fragments were subcloned to pDsRed-monomer-c1 (Clontech) with *Bam*HI site. The same DNA fragments were subcloned to pCMV-BD (Clontech) for GAL4-ZNF415 fusion protein and pCMV-Tag2B (Stratagene) including the *Bam*HI site.

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. Cells were transfected with pDsRed-monomer-c1-ZNF415 (1, 2, 3, 4, 5) using Lipofect-AMINE (Invitrogen) according to described methods [18]. Forty-eight

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