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REVIEW

Advanced studies on human gene *ZNF322*

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Abstract The human novel gene of *ZNF322* is cloned from human fetal cDNA library using the primers on the basis of the *ZNF322* sequence analyzed with computer. The gene is located on Chromosome 6p22.1, and encodes a protein consisting of 402 amino acid residues and containing nine tandem C_2H_2-type zinc-finger motifs. Northern blot result shows that the gene is expressed in all examined adult tissues. Subcellular location study indicates that ZNF322–EGFP fusion protein is distributed in the nucleus and cytoplasm. Reporter gene assays show that ZNF322 is a potential transcriptional activator.

Keywords *ZNF322* gene, zinc finger protein, gene expression, transcriptional activator

1 Introduction

It is estimated that 1% of genes in human genome encodes zinc finger proteins (ZFPs), in which Cys_2/His_2 (C_2H_2) type zinc finger genes consist of the largest subfamily, and these genes are over 700 (Tucker et al., 2001). Studies on the C_2H_2 ZFPs have suggested that their unique involvements in the regulation of normal cell growth, differentiation, embryogenesis and tumorigenesis (Postigo et al., 1997; Brewster et al., 1998; Postigo and Dean, 2000). Using the cDNA sequence of zfh-1 in drosophila and method analyzed with computer, a novel homlogous gene of zfh-1 in human Chromosome 9 has been found, and named *ZNF322* as approved by the Human Gene Nomenclature Committee (Li et al., 2001). In this paper, the full cDNA sequence of the gene is obtained from a human heart library by polymerase chain reaction (PCR), using the primers designed on the basis of *ZNF322* sequence analyzed with computer. Moreover, Northern blot, subcellular location and reporter assays are performed. The results are suggested that ZNF322 may be a transcriptional activator.

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2 Materials and methods

2.1 Construction of cDNA library

Total RNA (1 mg) from 20-week human embryo heart was extracted using the method of hot phenol (Li et al., 2001; Wang et al., 2001). mRNA preparation and reverse transcription reaction were performed using a cDNA PCR Library Kit and cDNA Synthesis kit according to manufacturer's protocol (TaKaRa). Then, the human heart cDNA library was finished.

2.2 Design of primers and PCR

Based on the entire cDNA sequence, two pairs of nest primers, PORFO and PORF were designed. The sequences of PORFO are 5′-GGTCAAGAAGACCAGCGGAAA-3′ (the left), and 5′-GCACAACTGACACTGAAGGCA-3′ (the right). The sequences of PORF are 5′-GCGCTGCAGTCCCTAGGAGAGAGCCATAGAAA-3′ (the left), and 5′-CTTCTGCAGTGGCCAAGACATCTGTGAGGCTTC-3′ (the right). These primers were used to amplify the Open Reading Frame (ORF) of *ZNF322*. The gene specific primers, GSP1 (5′-GCTTGCATTCAAGGCACT-3′) and GSP2 (5′-CTTCCGAGGGCATACATT-3′) were designed to amplify the 5′-terminal of the gene by 5′ RACE PCR. GSP3 (5′-GCCTTCAGTGTCAGTTGTGCT-3′) and GSP4 (5′-GTGGTACATCAGGGAACTCA-3′) were designed for 3′ RACE PCR. All PCR reactions were performed using the heart cDNA library as template.

2.3 Constructions of recombinant plasmids

Separated by agarose gel, all PCR products were inserted to pUCm-T vector (SANGO), and were transformed into *E.coli* DH-5α, and then were sequenced, respectively. After sequencing, the ORF fragment was inserted into the *Pst* I site of pEGFP-N1 to generate pZNF322-EGFP-N1 plasmid. To construct the plasmid pCMV-BD-ZNF322, the *ZNF322* ORF, which was derived from pZNF322-EGFP-N1 by digested

with *Sac* I and filled in with DNA Blunting Kit (TaKaRa), and then digested with *Apa* I, was inserted into plasmid pCMV-BD that was digested with *Pst* I, filled in and cut again with *Apa* I. The two recombinant plasmids were respectively transformed into *E.coli* DH-5α strain, and the right clones were screened by digestion of restriction endonucleases.

2.4 Northern blot

A commercially available Northern blot containing mRNA from variety of adult tissues was purchased from Clontech Inc. The membrane was hybridized with cDNA probes of *ZNF322* labeled with [α-32p]dCTP according to the standard method (Zhu et al., 2004). All reagents were purchased from Clontech Inc and Sagon Inc.

2.5 Subcellular location of ZNF322 protein

The COS-7 cells used in all studies were cultured according to the standard method. pZNF322-EGFP-N1 plasmid was transfected into COS-7 cells with LipofectAMINE (Invitrogen Inc.). Forty-eight hours after transfection, cells were fixed with 4% paraformaldehyde for 10 to 15 min, and nuclear fractions were stained with 4′,6′-diamidino-2-phenylindole (DAPI). Subcellular localization of the ZNF322-EGFP fusion proteins was detected and photographs were taken using fluorescence microscopy (Wang et al., 2004).

2.6 Transcriptional activity assay on ZNF322

The COS-7 cells were co-transfected with reporter plasmid pL8G5-Luc and effector plasmid pCMV-BD or pCMV-BD-ZNF322 with LipofectAMINE, respectively. Forty-eight hours later, cells were lysed. Cell extracts were then prepared and luciferase assays were done using the Luciferase Assay System (Promega). Luciferase activities were normalized with respect to parallel *β*-gal activities, to correct for differences in transfection efficiency. Each experiment was carried out in triplicate and each assay was at least three times. The means of the data from three individual transfected wells was presented. The transcriptional activities were compared on the basis of the data.

3 Results and discussion

3.1 Identification and sequence analysis of *ZNF322*

The 1,253-bp ORF fragment of *ZNF322* was obtained from human heart library with two pairs of nested primers PORFO and PORF. To obtain the full-length cDNA, 5′ upstream and 3′ downstream sequences of the gene were amplified by RACE PCR. These procedures yielded a 501-bp cDNA for 5′-RACE fragment, and a 426-bp cDNA for 3′-RACE fragment. Analysis of these three cDNAs suggested that they were cDNA fragments from the same novel gene. Therefore, they were assembled into contigs to complete the full-length cDNA. The full-length *ZNF322* cDNA assembled is 2,717-bp, and consists of 429-bp 5′-UTR, 1,209-bp ORF and 1,055-bp 3′-UTR. The ORF encodes a polypeptide of 402 amino acids extending from the first ATG codon present at nucleotide 430, to a termination TGA codon located at nucleotide 1,638. In the 3′ untanslated region, a potential polyadenylation signal, AATAAA, is identified at nucleotide positions 2,669–2,674, 18 nucleotides upstream from the poly (A+) tail. The deduced ZNF322 protein is 402 amino acids and its calculated relative molecular mass is about 44 KDa. Alignment between the cDNA sequence and human genome indicates that *ZNF322* is identical to the genomic sequence of BAC RP11-457M11 (GenBank accession number: AL513548) on chromosome 6p22.1, but not on chromosome 9. The gene spans approximately 23-Kb long and organizes into four exons. A summary of the various sizes of the exons and introns and the sequence of the splice junctions is shown in Table 1. The nucleotide sequence data reported here are available in GenBank with the accession number AY376736.

Table 1 Genomic structure of the *ZNF322* gene from 6p22.1

Intron	Exon		Intron	Size
	Number	Size		
	1	199	CTGCCAGG**gt**aagcgcggcc	796
tttttcctct**ag**GAGAGGCA	2	89	TTGTTACG**gt**aagtggtata	14,829
tgtgttttac**ag**AATGCTAT	3	70	GATTCTAG**gt**aagctgcagt	4,932
tttgtctcac**ag**GGTTGGAA	4	2,791		

Note: exon and intron sizes are given in base pairs; intronic and exonic sequences are shown in lower-case and upper-case characters, respectively; the acceptor-splice site ag and the donor-splice site gt are shown in bold.

Bioinformatics analysis shows that the protein encoded by *ZNF322* gene is a C_2H_2-type zinc finger protein with nine tandem repeated C_2H_2-type zinc finger motifs. Many studies have suggested that C_2H_2-type ZFPs involvements in the regulation of cell growth, differentiation, embryogenesis and tumorigenesis (Rue et al., 2001). For example, macho-1 encodes a transcription factor of the Zic family and was involved in the autonomous differentiation of primary muscle cells in ascidian embryos (Yagi et al., 2004). Zic family genes, which encode proteins with five conserved C_2H_2 zinc fingers, were known to have an effect on neural development and embryonic patterning (Purandare et al., 2002). The expression of ZZaPK (zinc finger and ZAK associated protein) in cells promoted cell growth by increasing E2F expression and

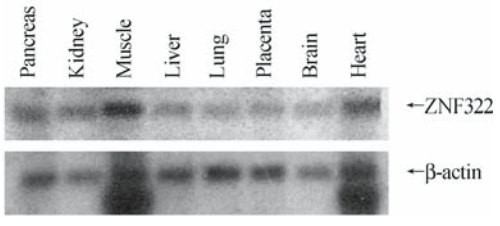

Fig. 1 Results of Northern blot in human adult tissues

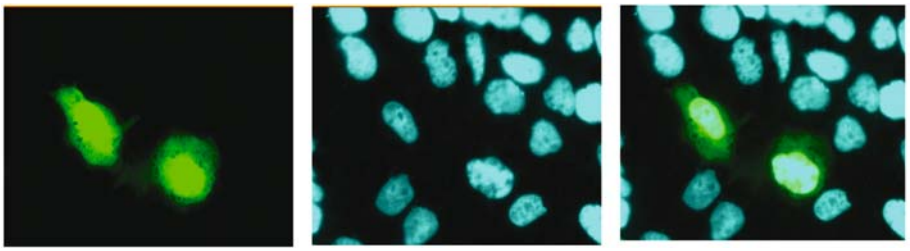

Fig. 2 Subcellular location of ZNF322 protein
(a) ZNF322-EGFP fusion protein expressed in COS-7 cells; (b) the corresponding nuclei of cells stained with DAPI; (c) the combined image of (a) and (b)

cyclinE/CDK2 activity (Yang, 2003). On the other hand, another C_2H_2-type ZFP, Lot1, has been demonstrated to concurrently regulate apoptosis and cell cycle arrest (Ciani et al., 2003). Based on the molecular structure of ZNF322 protein, it is speculated that it may be a transcription factor.

3.2 *ZNF322* gene is ubiquitously expressed

To characterize the transcript size and expression pattern of *ZNF322* gene in various tissues, *ZNF322* cDNA was used as the probe to hybridize mRNAs from a variety of human adult tissues (Clonetech Inc). The result indicated that an mRNA of 2.7 kb for *ZNF322* was widely expressed in various adult tissues with a higher level in the heart and skeletal muscle (Fig. 1). The expression pattern of *ZNF322* gene suggests that it may involve in important biologic processes.

3.3 ZNF322 protein is a nuclear and cytoplasmic protein

To determine the subcellular localization of ZNF322, pZNF322-EGFP-N1 plasmid, which could express that an EGFP-ZNF322 fused protein in eukaryotic cells was constructed. Forty-eight hours after the transient transfection, ZNF322-EGFP fused protein was examined using fluorescence microscopy. The fusion protein distributes strongly in the nucleus and weakly in the cytoplasm (Fig. 2), indicating that ZNF322 protein is a nuclear and cytoplasmic protein. The distribution of ZNF322 protein corresponds with that of transcription factors.

3.4 Effect of ZNF322 protein on transcriptional activities

To identity the effect of ZNF322 on transcription, transcriptional activity analysis was performed using a luciferase reporter gene (L8G5-Luc). The luciferase reporter exhibited a basal level of transcriptional activity when transfected with the control plasmid pCMV-BD encoding the GAL4-DBD alone. Cotransfection of GAL4-ZNF322 activated the transcriptional activity by 109% (Fig. 3), suggesting that ZNF322 can function as a positive transcriptional regulator.

In this paper, *ZNF322* gene has been cloned from a human embryonic heart library. The deduced protein is a C_2H_2-type ZFP, composed of 402 amino acids with nine tandems repeated

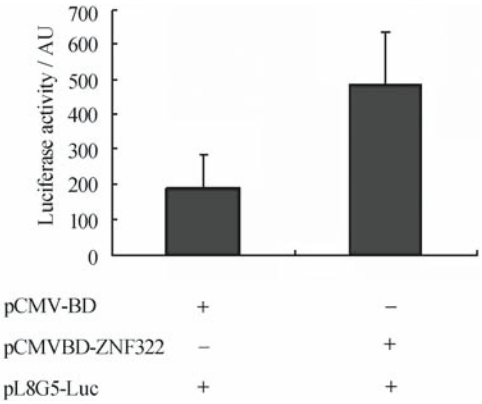

Fig. 3 Overexpression of ZNF322 activates transcriptional activities of L8G5-luc reporter gene

C_2H_2 zinc fingers. The result of Northern blot indicated that *ZNF322* was expressed in every adult tissue, with a higher level in the heart and skeletal muscle, suggesting that the gene may involve in heart development and function. Transcriptional activity assay with L8G5-Luc reporter system demonstrated that overexpression of ZNF322 activated the transcription of reporter gene, indicating that ZNF322 protein possesses of positive effect on transcription, and is a potent transcription activator. The accurate function of *ZNF322* and its regulation passway remain to be further studied.

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