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ZNF307, a novel zinc finger gene suppresses p53 and p21 pathway

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Abstract

We have cloned a novel KRAB-related zinc finger gene, *ZNF307*, encoding a protein of 545 aa. ZNF307 is conserved across species in evolution and is differentially expressed in human adult and fetal tissues. The fusion protein of EGFP-ZNF307 localizes in the nucleus. Transcriptional activity assays show ZNF307 suppresses transcriptional activity of L8G5-luciferase. Overexpressing ZNF307 in different cell lines also inhibits the transcriptional activities of p53 and p21. Moreover, ZNF307 works by reducing the p53 protein level and p53 protein reduction is achieved by increasing transcription of MDM2 and EP300. ZNF307 might suppress p53–p21 pathway through activating MDM2 and EP300 expression and inducing p53 degradation.

Keywords: KRAB; ZNF307; p53; p21

In the approximately 30,000 genes identified in the human genome, there are at least 2000 loci encoding transcription factor proteins (TFs) [1]. TFs bind DNA to regulate gene transcription and the most common DNA-binding motif is the zinc finger (ZNF) motif. Of various ZNF proteins, the C_2H_2 motif is the most common one. This motif frequently occurs in tandem repeats and is defined by two cysteine and two histidine residues coordinating a zinc ion which folds the motif into a finger-like projection that interacts with DNA [2]. About one-third of mammalian ZNF genes encode effector motifs called the Krüppel-associated box (KRAB) [3]. KRAB domain is a transcriptional repression module, thus KRAB-associated ZNF proteins function as potent transcriptional repressors [4]. SCAN domain is another domain found in some zinc finger transcription factors, sometimes also referred to as a leucine-rich region (LER). The tumor suppressor protein p53 responds to DNA damage and other cellular stresses by regulating many target genes related to cell-cycle arrest and apoptosis [5]. The cyclin-dependent kinase inhibitor 1A (p21) is a direct transcriptional target of p53 and is strongly induced by DNA damage in cells expressing wild-type p53 [6]. p21 is essential for the onset of cell-cycle arrest in cell damage response and cell senescence.

We have cloned a novel zinc finger gene, zinc finger protein, ZNF307, from human embryonic heart cDNA. ZNF307 contains an LER domain, a KRAB domain and seven C_2H_2 zinc finger motifs. Northern blot analysis indicates ZNF307 is specifically expressed in adult heart, brain, placenta, lung, and kidney tissue, and in embryonic (17 weeks) heart, brain, skeletal muscle, small intestine, and liver tissue. ZNF307 protein localizes in nucleus, probably as a result of the presence of ZNF motifs which are known to function as nuclear localization signals and the LRE domain may join with itself by homodimerization and form spots in nucleus. The GAL4-BD-ZNF307 fusion protein

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shows strong transcriptional suppressor activity by luciferase assay, and this activity can be attributed to different functions of its domains. Full-length ZNF307 also inhibits the transcriptional activities of p53 and p21 in different cell lines. In HEK-293 cells, for example, the ZNF307 domain represses the transcriptional activity of p53 and p21. We find ZNF307 suppresses p53 activity at the protein level rather than at the mRNA level. ZNF307 also upregulates mRNA levels of MDM2 (p53-binding protein MDM2) and EP300 (E1A binding protein p300) which is involved in p53 ubiquitination-mediated degradation [7,8]. Consequently we propose that ZNF307 transcription factor might suppress the p53–p21 pathway by increasing MDM2 and EP300-mediated p53 degradation.

Materials and methods

Full-length ZNF307 cDNA cloning and bioinformatics analysis. PCR was performed on a PCRSPRINT reactor (Thermo Hybaid) with one pair of primers P1/P2 (Supplement Table 1), corresponding to highly conserved amino acid consensus sequences of the KRAB domain and ZNF motifs in krüpple-like type zinc finger genes. The sequence obtained was subjected to human homology searching against an expressed sequence tags (ESTs) database using Blastn searching program (http://www.ncbi.nlm.nih.gov). We searched the consensus sequence and identified a 646 bp fragment corresponding to a novel gene. RACE (rapid amplification of cDNA ends) was performed using human fetal heart mRNA. The 5′ upstream sequence and 3′ downstream sequence were amplified by RACE PCR using the SMART cDNA Amplification Kit (TaKaRa Biotechnology). The gene specific primers for 3′ RACE were 3′GSP-UPM 5′-CTGGGAAAGTCA GGGTAGGACGG-3′ and 3′NGSP-NUP 5′-GAAAATACTGAGGCT CCCGTGTC-3′. The gene specific primers for 5′ RACE were 5′GSP-UPM 5′-GGCTTCCTCCTTCTCCACCTTCA-3′ and 5′NGSP-NUP 5′-AGG AGCCCCGTCTGGTCTTCTGC-3′. The 1st and 2nd PCR gene-specific primers for ORF of ZNF307 were P3/P4 and P5/P6, respectively (Supplement Table 1), and the human fetal heart cDNA library was used as template. All the PCR products were sequenced with 377 DNA Sequencer (Abiprism). After sequencing, these cDNA fragments of RACE were assembled to complete the full-length cDNA (the cDNA of ZNF307) and the cDNA was cloned into pMD18-T vector (ZNF307-pMD18-T).

Sequence and protein domains analysis of ZNF307 was performed using BLAST program from NCBI and Pfam 9.0. The homologs of ZNF307 were found with Blastp, and sequence alignment and phylogenetic tree analysis were performed with the Jellyfish program and MegAlign programs, respectively.

RNA isolation and Northern blot hybridization. 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 according to the university policies. A multiple embryonic tissues membrane was prepared as described in previous studies [9]. We also used commercially available Northern blots containing a variety of adult tissues mRNA (Clontech). The adult human multiple tissue Northern blot and the 17 weeks embryo multiple tissue membrane were hybridized with cDNA probe of ZNF307 and β-actin cDNA probe (Clontech). The method of Northern blot was described in previous studies [10].

Plasmid construction. Full-length ZNF307 and truncated fragments were inserted into plasmids pEGFP-N1, pCMV-BD, and pCMV-Tag2B, respectively, to generate fusion proteins (see Figs. 1B and 2B, E, H).

Cell culture and subcellular localization analysis. COS-7, HEK-293, and A549 cells used in all studies were maintained and passaged according to standard methods in DMEM (Gibco) with 10% FCS (fetal calf serum) (Gibco) in a humidified atmosphere of 95% air and 5% CO_2. MCF-7 cells used in all studies were maintained and passaged according to standard methods in RIPM1640 (Gibco) supplemented with 10% FCS in a humidified atmosphere of 95% air and 5% CO_2.

COS-7 cells were transfected with pEGFP-N1-ZNF307, pEGFP-N1-307-D-LER, and pEGFP-N1-307-D-ZNF using So-fast according to the protocol (Sunma Biotechnology). The transfected cells were treated according to the method described previously [10]. Subcellular localization of the EGFP-ZNF307, EGFP-ZNF307-D-ZNF, and EGFP-ZNF307-D-LER fusion proteins was detected using a Nikon fluorescence microscope.

Transcriptional reporter gene assays. COS-7 cells were co-transfected with L8G5-Luciferase, pLexA-VP16, and pCMV-BD-ZNF307 or other truncated GAL4-BD-ZNF307 vectors to investigate the effect of ZNF307 on transcriptional activity. The reporter plasmids were described in previous studies [11]. HEK-293 were co-transfected with pAP-1-Luciferase (or pSRE-Luciferase, pSRF-Luciferase, pNF-κB-Luciferase) and pCMV-Tag2B-ZNF307 to investigate the effect of ZNF307 on transcriptional activity of AP-1 (or SRE, SRF, and NF-κB); COS-7, HEK-293, A549, and MCF-7 cells were co-transfected with p53-Luciferase and pCMV-Tag2B-ZNF307 to investigate the effect of ZNF307 on transcriptional activity of p53; COS-7 and HEK-293 cells were co-transfected with p21-Luciferase and pCMV-Tag2B-ZNF307 to investigate the effect of ZNF307 on transcriptional activity of p21. HEK-293 cells were also co-transfected with p53-Luciferase (or p21-Luciferase) and other truncated FLAG-ZNF307 fusion constructs to investigate the effects of individual ZNF307 domains on transcriptional activity of p53 (or p21). The luciferase activity assay was performed according to established protocols (Promega Luciferase Assay System). Relative luciferase activity was normalized for transfection efficiency through co-transfection with pCMV-LacZ and spectrophotometry analysis. Each experiment was performed in triplicate. Means of the data from three individual transfected wells are presented after normalization for β-galactosidase.

RNAi analysis. A pSUPER.retro.puro vector-based system was used to deliver siRNA into HEK-293 cells. A pair of oligonucleotides was designed by the RNAi program (http://www.openbiosystems.com/RNAi). The sequences were s1: 5′-GATCCCCGCCCTTACACGATAGAGTTTTCT AGAGAAACTCTATCGTGTAAGGGCTTTTTA-3′ and s2: 5′-TCGA TAAAAAGCCCTTACACGATAGAGTTTCTCTAGAAAACTCTATC GTGTAAGGGCGGG-3′. The oligos were annealed and cloned according to the instructions (OligoEngine). Luciferase activity assay was performed in HEK-293 cells transfected with p53-Luciferase (or p21-Luciferase), pCMV-Tag2B-ZNF307, and pSUPER-ZNF307RNAi. Western blot was performed in HEK-293 cells transfected with pCMV-Tag2B, pCMV-Tag2B-ZNF307, pSUPER.retro.puro, or pSUPER-ZNF307RNAi to test p53 protein level. RT-PCR was performed in the same cells to show RNA level of ZNF307.

RT-PCR and Western blot. A549 cells were transfected with pCMV-Tag2B-ZNF307 as described above. Total RNA (4 μg) was reverse-transcribed into cDNA using the first-strand cDNA synthesis kit (Ferments). The resulting cDNA was used as template for PCR. Primers β-actinS/β-actinAS were used to normalize the cDNA concentration (Supplement Table 1). RT-PCR primers shown in Supplement Table 1 were used for detecting the expression of p53, ZNF307, MDM2, and EP300. For Western analysis, HEK-293 and A549 cells transfected with pCMV-Tag2B-ZNF307 and pSUPER-ZNF307RNAi were lyzed in RIPA lysis buffer (150 mmol/L NaCl, 1 mmol/L EDTA, 50 mmol/L Tris–HCl, 1% NP-40, 1 mmol/L PMSF, 1% Sodium deoxycholate, and 0.1% SDS), and the membranes were labeled with HRP (Sigma) specific for p53 antibody (Boster) and β-actin antibody (Lab Vision-Neo Markers).

Result and discussion

Molecular characterization and evolutional conservation of the human ZNF307

We cloned 2428 bp of a novel gene, named ZNF307 (AY781778) as approved by HUGO Nomenclature Committee. The deduced ZNF307 protein has 545 amino acids