

SHORT COMMUNICATION

Naohiko Seki · Atsushi Hattori · Sumio Sugano
Yutaka Suzuki · Akira Nakagawara
Miki Ohhira · Masa-aki Muramatsu · Tada-aki Hori
Toshiyuki Saito

Isolation, tissue expression, and chromosomal assignment of a novel human gene which encodes a protein with RING finger motif

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Abstract We identified a novel gene encoding a RING finger (C3HC4-type zinc finger) protein from a human neuroblastoma full-length enriched cDNA library. This cDNA clone consists of 1919 nucleotides with an open reading frame of a 485-amino acid protein. From reverse transcription (RT)-polymerase chain reaction (PCR) analysis, the messenger RNA was ubiquitously expressed in various human adult tissues. The chromosomal location of the gene was determined on the chromosome 6p21.3 region by PCR-based analyses with both a human/rodent monochromosomal hybrid cell panel and a radiation hybrid mapping panel.

Key words RING finger · Full-Length enriched cDNA library · Chromosome 6p21.3 · RH mapping

Introduction

Zinc finger motifs consist of several subfamilies based on their different types of fingers, categorized by the nature

and spacing of their zinc-chelating residues (Schwabe and Klug 1994; Mackay and Crossley 1998). It is estimated that there are 500 zinc-finger proteins encoded in the yeast genome and that approximately 1% of all mammalian genes encode zinc fingers (Mackay and Crossley 1998). The RING finger is a C3HC4-type zinc finger motif, and members of the RING finger family are mostly nuclear proteins; this motif is involved in both protein-DNA and protein-protein interactions (Freemont et al. 1991, Saurin et al. 1996).

Isolation of cDNA clone for novel RING finger protein

A cDNA clone for a novel RING finger protein was isolated from a full-length enriched cDNA library constructed from a neuroblastoma (NB) sample, using the oligo-capping method, as described previously (Maruyama and Sugano 1994; Suzuki et al. 1997). Here we report the sequence features, tissue expression, and chromosomal assignment of the gene.

From the one-pass sequencing and database search, this cDNA clone did not match any human gene. The entire sequence of this clone was determined by a shotgun strategy (Ohara et al. 1997). The isolated cDNA clone was 1919 bp in length and had an open reading frame of 485 amino acids (Fig. 1). The predicted protein of 485 amino acids had a calculated molecular weight of approximately 56 kDa. Our sequence analysis of the cDNA clone revealed a highly conserved motif consistent with a RING finger (C3HC4-type zinc finger) domain encompassing amino acid residues 403–440 (CIICSEYFIEAVTLNCAHSFCSYCI~~NEW~~MKRKIECPIC).

N. Seki · A. Hattori · T. Hori · T. Saito (✉)
Genome Research Group, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan
Tel. 81-43-206-3135; Fax 81-43-251-9818
e-mail: t_saito@nirs.go.jp

S. Sugano · Y. Suzuki
Department of Virology, Institute of Medical Science, the University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

A. Nakagawara · M. Ohira
Division of Biochemistry, Chiba Cancer Center, 666-2, Nitona, Chuoh-ku, Chiba 260-8717, Japan

M. Muramatsu
Helix Research Institute, 1532-3 Yana, Kisarazu, Chiba 292-0812, Japan and Department of Bioregulation, Medical Research Institute, Tokyo Medical Dental University, Bunkyo-ku, Tokyo 113-8510, Japan

A. Hattori
Biotechnology and Medical Engineering Field, Aisin Cosmos R&D Co., Ltd., 5-2-11 Sotokanda, Chiyoda-ku, Tokyo 101-0021, Japan

Tissue expression of the novel human gene

We examined the tissue distribution of the transcript in various human adult tissues by reverse transcription-

ATGGGGAGCCCGGCTTCTTCGTACAGGAGACCCGCGCGTGGCCGGAGCTGGTGCCTG 60
M G E P G F F V T G D R A G G R S W C L
CGCGGGTGGGGATGAGCCCGGGTGGCTGCTGGAAGATGGGTGCCAGGTGACTGTA 120
R R V G M S A G W L L L E D G C E V T V
GGACGAGGATTTGGTGTACATACCAACTGGTATCAAAAATCTGCCCCCTGATGATTTCT 180
G R G F G V T Y Q L V S K I C P L M I S
CGAAACCACTGTGTTTTGAAGCAGAATCCTGAGGGCAATGGACAATATGGACAACAAG 240
R N H C V L K Q N P E G Q W T I M D N K
AGTCTAAATGGTGTGGTGAACAGAGCGGTCTGGAACCTTTAAGGGTCTAATCCATT 300
S L N G V W L N R A R L E P L R V Y S I
CATCAGGGAGACTACATCCAACCTGGAGTGCCTCTGGAAAATAAGGAGAATGGGAGTAT 360
H Q G D Y I Q L G V P L E N K E N A E Y
GAATATGAAGTACTGAAGAAGACTGGGAGACAATATATCTTGTCTTTCCCAAGAAT 420
E Y E V T E E D W E T I Y P C L S P K N
GACCAAAATGATAGAAAAAATAAGGAATGAGAAGTAAAAGGAAATTCAGTTTGGATGAA 480
D Q M I E K N K E L R T T K R K F S L D E
TTAGCAGGTCTGGAGCTGAAGGCCCTCAAATTTGAAATCCAAAATAAATAAGTGTCT 540
L A G P G A E G P S N L K S K I N K V S
TGTAATCTGGTCAAGCAGTGAATACAGGGGAAAGGTGAAGTGGCCAGTACACCTCT 600
C E S G Q P V K S Q G K G E V A S T P S
GACAATTTGGATCTTAAGTTGACTGCCCTTGAGCCCAAGTAAGACCAGGGGCTCCCAT 660
D N L D P K L T A L E P S K T T G A P I
TACCCTGGCTTCCCAAAAGTACAGAGGTTTCATCATGAGCAGAAAGCCTCAAACCTTCA 720
Y P G F P K V T E V H H E Q K A S N S S
GCATCTCAGAGAAGCTTACAGATGTTTAAAGTGACCATGTCAGGATTTCCAGGCTCAA 780
A S Q R S L Q M F K V T M S R I L R L K
ATACAGATGCAGGAAAAACATGAAGCCGTTATGAATGTGAAAAGCAGACCCAAAAGGGG 840
I Q M Q E K H E A V M N V K K Q T Q K G
AACTCAAAGAAAGTGTGCAATGGAGCAGAACTTCCAGACTTACAGTCCCAGCTGTGT 900
N S K K V V Q M E F Q E L Q D L Q S Q L C
GCAGAGCAGGCTCAGCAGCAGGCAAGAGTGGAGCACTAGAGAAGACTTCCAGGAAGAG 960
A E Q A Q Q Q A R V E Q L E K T F Q E E
GAACAGCATCTCAGGGTGGAGATGCCAAGGAAAAGGACCTGAAGCAACAGCTG 1020
E Q H L Q G L E I A Q G E K D L K Q L K
GCCAGGCTCTGCAGGAGCATTTGGCTCTAATGGAAGAGCTAAATCGCAGCAAGAAGGAC 1080
A Q A L Q E H W A L M E E L N R S K K D
TTTGAAGCAATCATTCAAGCAAGAACAAGAATAGAGCAGACCAAGGAAGAGAAGGAG 1140
F E A I I Q A K N K E L E Q T K E E K E
AAGATGCAAGCAGAGAAGGAAGTCTTATGCCACATGAATGATGTCTAGAGAATGAG 1200
K M Q A Q K E E V L S H M N D V L E N E
CTCCAATGTATATTTGTTGAGAATACTTCATGAGGCTGTACCTTGAACCTGTGCCAC 1260
L Q C I I C S E Y F I E A V T L N C A H
AGTTTCTGCTCCTACTGTATCAATGAATGGATGAAGCGGAAGATAGAATGCCCATTTGT 1320
S F C S Y C I N E W M K R K I E C P I C
CGGAAGGACATTAAGTCCAAAACGACTCTTTGGTCTGGACAATGCAITTAATAAGATG 1380
R K D I K S K T Y S L V L D N C I N K M
GTAATAATCTGAGCTCAGAAGTGAAGAAGCAGCAATTTGTTCTCATTAGGGAACGAAA 1440
V N N L S S E V K E R R I V L I R E R K
GCAAAGAGATTTGTTCTGAagaccgtgctctaaagccatttgaagactgccaggtagtgc 1500
A K R L F *
gagcctgagatggtctggaggatctctctagccgtgactccgctgctctgaaggtcaac 1560
tgagaagctctgtgggacagagacttgagttaggaagccctcagtcacttgccttcacag 1620
gtggccagccctgctgccaatctggctgaagcaccaccaggttcacggcacccecaactg 1680
cttcagggtacttcgtagactctgacctactacatgtcgaagagttatttgagttctct 1740
ctctgttttttaatttgttgttgttactgttttgataacctcggaaacacctccgt 1800
tgacagttgttttgataggttgggtgtaccceatggctgacctgaagggcagtgctctat 1860
tttgagaggtggcttacctctctttgtgaaaatactactctcttctctggaataaa 1919

Fig. 1 Nucleotide sequence and deduced amino acid sequence of the human RING finger cDNA. Asterisk denotes the termination codon. The RING finger motif is underlined. The nucleotide sequence reported here will appear in DDBJ, EMBL, and GenBank nucleotide sequence databases under accession number AB012770

coupled polymerase chain reaction (RT-PCR). Primers used for RT-PCR were to amplify the 1.7-kb cDNA of the transcript. Considering its ubiquitous expression in a wide variety of human tissues, this gene could play an essential role in common cellular processes. It is notable that the gene was poorly expressed in spleen (Fig. 2).

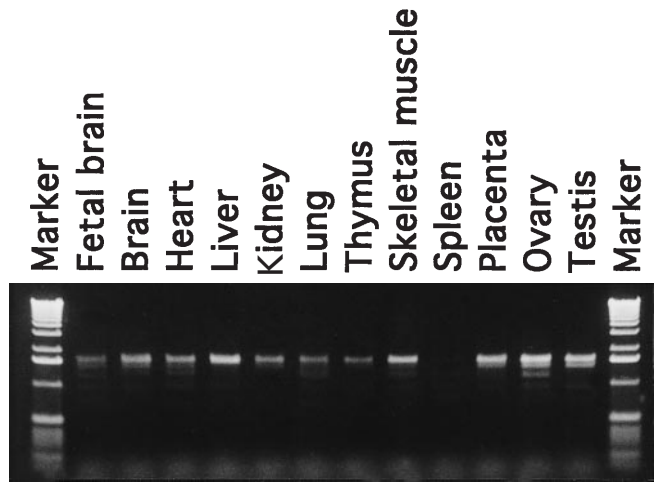


Fig. 2 Primers used for reverse transcription (RT)-polymerase chain reaction (PCR) were to amplify the 1.7-kb cDNA of the transcript. RT-PCR amplification primers were (5'-GGACAATTATGGACAACAAGAG') and (5'-CAAAGAAGAGGTAAGCCATCC-3'). The templates of the human tissues of poly(A)⁺ RNAs were purchased from Clontech (Palo Alto, CA, USA). The cDNA templates for RT-PCR were synthesized from 2μg of poly(A)⁺, using excess amounts of Superscript II reverse transcriptase (GIBCO BRL, Gaithersburg, MD, USA) and random hexamer primers. PCR was carried out in a final volume of 10μl containing 1 × LA-PCR buffer (Takara, Kyoto, Japan), 2μM each primer, 200μM each dNTP, 50ng template DNA, and 0.01 units of LA-Taq DNA polymerase (Takara). Temperature and time schedules were: 30 cycles at 95°C for 20s and 62°C for 1min. PCR products were separated on 1% agarose gel with a 1-kb ladder DNA marker (GIBCO BRL)

Chromosomal assignment of the gene

Chromosomal assignment of the gene was done by PCR analysis of a human/rodent somatic cell hybrid panel and a radiation hybrid panel, as described previously (Saito et al. 1995, 1997, Seki et al. 1997). Primers used for PCR amplification correspond to the 3'-untranslated region (UTR) of the gene (5'-CAAAGAAGAGGTAAGCCATCC-3') and (5'-GTTACTGTTTTGATACC-TCGG-3') (121-bp PCR product). The specific amplified PCR product for human was detected only from the hybrid cell containing human chromosome 6 (data not shown). We performed further mapping analysis, using a PCR-based radiation hybrid panel (Genebridge 4; Research Genetics, ■■) with the same primers as those used in the assay for the human/rodent somatic cell hybrid panel. Statistical analysis of the radiation hybrid data was performed using the RHMAPPOR software package (<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>). The data vector for the gene was 1000000100 0010000100 1100100010 1111010100 0110010010 1011010001 0110100010 0011100110 0121000000 001 and the consequent report indicated that the gene was mapped between markers WI-4186 and WI-7940, both of which have been cytogenetically mapped to 6p21.3. The position of the gene is 6.29 cR proximal to WI-4186.

Discussion

The RING finger domain was first identified in the human RING1 protein, the gene of which was mapped on chromosome 6p21.3 (Freemont et al. 1991). Recently, two other RING finger protein genes were mapped on the 6p21.3 region (Kyushiki et al. 1997; Henry et al. 1997), and in the present study the fourth RING finger protein gene on chromosome 6p21.3 is described. The RING finger protein gene family may exist as a cluster in this region. Some members of the RING finger family have been implicated in carcinogenesis and cell transformation. For example, *PML*, *rpf*, and *TIF1* have transforming capabilities when associated with chromosomal translocations (Takahashi et al. 1988, Goddard et al. 1991; Miki et al. 1991; LeDouarin et al. 1995). The major histocompatibility complex (MHC) exists in the human chromosome 6p21.3 region, and a relatively high gene density has been observed. Since a large number of disorders are associated with the MHC region (Moller 1983; Trent and Ziegler 1992), our precise chromosomal positioning data for the novel zinc finger protein gene could contribute toward ongoing positional candidate approaches for the above-mentioned disease genes linked to this genomic locus.

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