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Biochemical and Biophysical Research Communications 323 (2004) 9–16
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TRIM45, a novel human RBCC/TRIM protein, inhibits transcriptional activities of ElK-1 and AP-1☆

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Received 27 July 2004
Available online 21 August 2004

Abstract

The tripartite motif (TRIM) proteins play important roles in a variety of cellular functions including cell proliferation, differentiation, development, oncogenesis, and apoptosis. In this study, we report the identification and characterization of the human tripartite motif-containing protein 45 (*TRIM45*), a novel member of the TRIM family, from a human embryonic heart cDNA library. TRIM45 has a predicted 580 amino acid open reading frame, encoding a putative 64-kDa protein. The N-terminal region harbors a RING finger, two B-boxes, and a predicted α-helical coiled-coil domain, which together form the RBCC/TRIM motif found in a large family of proteins, whereas the C-terminal region contains a filamin-type immunoglobulin (IG-FLMN) domain. Northern blot analysis indicates that *TRIM45* is expressed in a variety of human adult and embryonic tissues. In the cell, TRIM45 protein is expressed both in cytoplasm and in cell nucleus. Overexpression of TRIM45 in COS-7 cells inhibits the transcriptional activities of ElK-1 and AP-1. These results suggest that TRIM45 may act as a new transcriptional repressor in mitogen-activated protein kinase signaling pathway.

Keywords: *TRIM45*; RBCC/TRIM proteins; Transcription factor repressor; Elk-1; MAPK signaling pathway

The tripartite motif (TRIM) protein family was originally described as the RBCC family because members belonging to this family contain a RING finger (R), one or two B-boxes (B1, B2), followed by a coiled-coil domain (CC) [1,2]. The definitive element of the RBCC/TRIM motif is the RING finger, which is a specialized type of C3HC4 Zn finger of 40–60 residues that binds two atoms of zinc in a unique cross-braced metal ligation scheme, and probably involved in mediating protein–protein interactions [3]. The second signature domain is the B-box, a cysteine and histidine-rich region that binds to zinc. B-box is found essentially in transcription factors, ribonucleoproteins, and protooncoproteins, but no function is clearly assigned to this domain [4]. Two B-box domains are often found immediately COOH-terminal to the RING finger in the RBCC/TRIM motif. The B1 and B2 domains have different lengths and consensus. Both domains bind zinc but differ in spacing of the conserved Cys and His ligands. The B-boxes appear to be critical determinants of the TRIM motif and are found only in this protein family [4]. The third signature sequence is the coiled-coil domain that displays amphipathic helical character and is required for protein–protein interactions mediated by

☆ *Abbreviations:* RBCC, RING-B box-coiled-coil domain; TRIM, tripartite motif; IG-FLMN, filamin-type immunoglobulin domain; MAPK, mitogen-activated protein kinase; MAPKK, MKK or MEK, MAPK kinase; MAPKKK or MEKK, a MAPKK kinase or MEK kinase; AP-1, activation protein 1; DMEM, Dulbecco's modified Eagle's medium; DAPI, 4′,6′-diamidino-2-phenylindole hydrochloride; UTR, untranslated region; ORF, open reading frame.

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doi:10.1016/j.bbrc.2004.08.048

the RBCC/TRIM motif. Inspection of the whole family reveals conserved residues spacing between the RING, B-box(es), and coiled-coil domains [5], suggesting that the overall architecture of the RBCC/TRIM motif is highly conserved throughout evolution, and that the TRIM motif serves as an integrated functional structure in protein–protein interactions [6]. The C-terminal portion of these proteins also contains known protein domains, such as the IG-FLMN, the RFP-like, the NHL, the TSS-PHD-BROMO, and the ARF. IG-FLMN domains form a rod-like structure in the actin-binding cytoskeleton protein, filamin and the C-terminal repeats of filamin bind β1-integrin [5].

The RBCC/TRIM protein was first identified in the *Xenopus* transcriptional regulator XNF7 [7]. An increasing number of TRIM proteins have been identified recently [1,5,8]. Genes belonging to this family are involved in a variety of processes, including cell proliferation, differentiation, development, oncogenesis, apoptosis, and several human diseases. For example, transcriptional intermediary factor 1α (TIF1α)/TRIM24, TIF1β/KAP1/TRIM28, and PML/TRIM19 modulate transcriptional machinery to control specific gene expression during cell proliferation, differentiation, and development [9–11], whereas TIF1, PML, and RFP/TRIM27 acquire oncogenic activity when fused to RARα, RET or B-raf, respectively [12–14]. PML and RFP have also been shown to be pro-apoptotic reagents [15–17]. In addition, TRIM proteins have been implicated in several human diseases. Mutations in Pyrin/TRIM20, MID1/TRIM18, and MUL/TRIM37 have been associated with familial Mediterranean fever, X-linked Opitiz/GBBB syndrome, and mulibrey nanism, respectively [18–20].

With the aim of identifying the genes involved in human heart development and diseases, a novel RBCC/TRIM gene, *TRIM45*, was cloned from human embryonic heart cDNA library. *TRIM45* N-terminal region contains a RING finger, two B-boxes, and a predicted α-helical coiled-coil domain, and the C-terminus contains a filamin-type immunoglobulin domain. Northern blot analysis indicates that *TRIM45* is specifically expressed in skeletal muscle, brain, heart, and pancreas tissues of adult human, and in brain, lung, skeletal muscle, heart, and intestine of the 80-day embryo. Overexpression of *TRIM45* in the cell inhibits the transcriptional activities of ElK-1 and AP-1, suggesting that TRIM45 may act as a new transcriptional repressor in MAPK signaling pathway.

Materials and methods

Construction of cDNA library of human embryonic heart. The 20-week human embryonic heart cDNA library was constructed as reported previously [21]. Briefly, 5 μg mRNA was purified from 500 μg total human embryonic heart 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 (TaKaRa). After cassette adaptor ligation reactions using cDNA PCR Library Kit, cDNA amplification reactions were performed with RA primer, CA primer, and Ex Taq (TaKaRa).

Full-length TRIM45 cDNA cloning and bioinformatics analysis. The consensus sequences of B-box domain were obtained from the SMART database (http://smart.embl-heidelberg.de), and used to search human EST database with the BLAST searching program (http://www.ncbi.nlm.nih.gov). Through combined BLAST search as previously described [22], four overlapping ESTs—BX444521, BQ881123, BX383988, and BU078420—belonging to the same novel gene were identified. The first forward primer in BX444521 and the reverse primer in BX383988 (P1 and P2, Table 1), and the second forward primer in BX383988 and the reverse primer in BU078420 (P3 and P4, Table 1) were designed using Primer Premier 5.0 to perform standard PCR. 5′-gene specific reverse primers (GSP1 and GSP2) and 3′-gene specific primers (GSP3 and GSP4) were designed for 5′-RACE and 3′-RACE reactions according to the previous method [17]. 5′-RACE and 3′-RACE were performed using SMART RACE cDNA Amplification Kit (Clonetech). *TRIM45* sequences of open reading frame (ORF) were confirmed by PCR amplification with a pair of primers (PORF1 and PORF2, Table 1). All the PCR products were then cloned into pMD18T-vector (Sagon) and sequencing 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 *TRIM45* sequences was performed by DNAstar software. BLASTn and Pfam 9.0 were used to analyze genomic structure and the protein domain, respectively. The homologues of *TRIM45* were found with BLASTp, and the sequence alignment and phylogenetic tree analysis were performed with Meg-Align program (DNAstar).

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 the regulation of university policy. Total RNA was isolated from various tissues (liver, intestine, heart, lung, brain, kidney, and skeletal muscle) using standard methods. Twenty microgram samples of each tissue were separated by electrophoresis through formaldehyde–agarose gel. The embryo multiple tissues membrane was prepared as described in previous studies [21]. At the same time, a commercially available Northern blot containing mRNA from a variety of adult tissues was purchased from Clontech. The adult human Multiple Tissue Northern blot (Clontech) and the embryo multiple tissue membrane were hybridized with cDNA probe of *TRIM45*. The *TRIM45* cDNA was labeled with [α-^{32}P]dCTP using a Random Primer Labeling Kit (TaKaRa). This autoradiograph was developed after exposure for several days at −80 °C. The membranes were then stripped and probed with radiolabeled β-actin cDNA (Clontech) as indicator of mRNA loading [23].

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. To generate a fusion protein of TRIM45 with enhanced green fluorescent protein (EGFP), the *TRIM45* ORF was subcloned into the *Eco*RI site of pEGFP-N1 vector in-frame with the TGG codon instead of the TGA stop codon in the TRIM45 coding sequence. Cells were transfected with pEGFP-N1-TRIM45 using LipofectAMINE (Invitrogen) according to the method described previously [24]. Forty-eight hours after transfection, cells were fixed with 4% paraformaldehyde for 15 min and nuclear fractions were stained with 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI). Subcellular localization of the

Table 1
PCR primers, programs, and cycles

Primers	Nucleotide sequences	Programs	Cycles
P1	5′-GCCTTTCGCAGAGGGAAGGAG-3′	94 °C, 30 s;	
P2	5′-GTCCGCATGTCTGCCAGTAAC-3′	55 °C, 30 s;	30
		72 °C, 1.5 min	
P3	5′-TTACTGGCAGACATGCGGACT-3′	94 °C, 30 s;	30
P4	5′-TGGAAATCTCAAGTGGTGCTG-3′	55 °C, 30 s;	
		72 °C, 1 min	
GSP1	5′-AAGGGCAGACGGGAAGACGAG-3′	94 °C, 30 s;	25
		54 °C, 30 s;	
		72 °C, 1 min	
GSP2	5′-TCCTGCTGCCAACAAAGTCAC-3′	94 °C, 30 s;	32
		56 °C, 30 s;	
		72 °C, 1 min	
GSP3	5′-TCTACTTAGGACTGTGGCTCT-3′	94 °C, 30 s;	25
		54 °C, 30 s;	
		72 °C, 1 min	
GSP4	5′-GCAGGCAGCACCACTTGAGAT-3′	94 °C, 30 s;	32
		56 °C, 30 s;	
		72 °C, 1 min	
PORF1	5′-GCGGAATTCAGGAGTATGTCAGAAAACAGA-3′	94 °C, 30 s;	35
PORF2	5′-CAGGAATTCACCAGAGAGCCACAGTCCTAAG-3′	60 °C, 30 s;	
		72 °C, 2 min	

EGFP-TRIM45 fusion proteins was detected using fluorescence microscopy.

Transcriptional reporter gene assays. The *TRIM45* ORF was subcloned into the *Eco*RI site of pCMV-Tag2B vector in-frame. The reporter constructs for Elk-1-Luc and AP-1-Luc were kindly provided by Dr. K.L. Guan at the University of Michigan. pCMV-βgal was constructed and provided by Dr. J. Zhang at Hunan Normal University. COS-7 cells were co-transfected with pFR-Luc, pFA2-Elk-1, pFC-MEK1, and pCMV-Tag2B-TRIM45 or pCMV-Tag2B vector to investigate the effect of TRIM45 on the transcriptional activity of Elk-1. To examine the effect of TRIM45 on the transcriptional activity of AP-1, cells were co-transfected with pAP-1-Luc and pCMV-Tag2B-TRIM45 or pCMV-Tag2B vector with LipofectAMINE as described above. Forty-eight hours later, the luciferase activity assay was performed according to the protocols of Stratagene [25]. 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.

Results and discussion

Identification and domain structure of the human TRIM45

The RBCC/TRIM is found in an increasing number of proteins with important roles in cell differentiation, development, oncogenesis, and apoptosis [9–12]. In an effort to understand the role of TRIM proteins in heart development, a novel human gene, *TRIM45*, of 3584 bp in full-length was isolated through combining the human EST database scanning, standard PCR, 5′- and 3′-RACE, and Northern blotting. Unlike the RING and coiled-coil motifs, the B-box is only found in RBCC/TRIM family members, suggesting that the B-box is a critical determinant of the overall structure and function of this family of proteins [5]. To identify potential B-box containing genes, we screened the human EST database with the conserved B-box1 sequence. A number of ESTs (BX444521, BX383988, BU078420, CN258805, and BQ881123) representing the same novel gene (TRIM45) were identified in our database search. To confirm the cDNA sequences identified from the database, two pairs of primers (P1 and P2, P3 and P4, Table 1) were designed based on the sequences of ESTs, and standard PCR was carried out using the human embryonic heart cDNA library as template. Two PCR product fragments of 1567 and 961 bp, with 157 bp overlap, were obtained and confirmed to be the cDNA sequences of TRIM45. To obtain the full-length of cDNA, 5′- and 3′-RACE were performed, respectively, using 5′-gene specific primers (GSP1 and GSP2) and 3′-gene specific primers (GSP3 and GSP4) as described previously [21]. The procedure yielded a 290 bp DNA fragment for 5′-RACE and a 1225 bp for 3′-RACE. The products were then cloned and sequenced. Finally, a 3584 bp of full-length novel gene, *TRIM45*, was assembled and submitted to GenBank with Accession No. AY669488.

The *TRIM45* gene consists of an open reading frame (ORF) of 1743 bp extending from the first ATG codon at nucleotide 589 to a termination TGA at 2331, a 588 bp 5′-untranslated region (UTR), and a 1253 bp 3′-UTR with a consensus polyadenylation signal (aataaa) (Fig. 1). The deduced TRIM45 protein has 580 amino acids (Fig. 1A) with a calculated molecular mass of 64 kDa.

Comparison of the *TRIM45* sequence with the genomic sequence shows that *TRIM45* gene is mapped to

A

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1    ctcgccggagtagaccgggctgggattggtgtgaggaggggccagagcccgcgggcgtcccgggccgaggcgcgggagaggcggtggaca
91   ccgagaagcccgccggcggcttgcaattccctcacccggcgcgcgccttttcgcagagggaaggagcaagaggggccctacctcatcgtgc
181  gcgggtggggtcggcgcttgtcgcgtgtggcgcgcagtgggcgggcggcgggaggggggtggcagtggaggaagcgagaggtgcagggg
271  tgactttgttggcagcaggactagctggagagctagacctggaagcgcatccgggggaggacttgcgggggcagaggagggcgtgggcgtgt
361  ctggtatgggatgcagtggaaaggaggggggccctcctgagtagatctgtgggtgattccttcgaggacgcctcgtcttcccgtctgccct
451  tttatttgtcagcgaggggagtccccatggtctctgttcaagttctggagactttctctttgggtgggcttaatcacctgctactaaatcg
541  tagaactgcccagggccctttctaatattggtcacaaacgtgaggagtATGTCAGAAAACAGAAAACCGCTGCTGGGCTTTGTAAGCAAA
1                                                    M  S  E  N  R  K  P  L  L  G  F  V  S  K
631  CTCACTAGTGGGACTGCACTTGGGAACTCAGGCAAGACTCACTGCCCCCTGTGCTTGGGGCTTTTCAAAGCCCCCAGGCTCTTGCCTTGT
15   L  T  S  G  T  A  L  G  N  S  G  K  T  H  C  P  L  C  L  G  L  F  K  A  P  R  L  L  P  C
721  TTGCATACAGTTTGCACCACGTGTCTGGAGCAGCTGGAGCCCTTCTCAGTAGTGGACATCCGAGGGGGAGACTCTGACACAAGCTCTGAG
45   L  H  T  V  C  T  T  C  L  E  Q  L  E  P  F  S  V  V  D  I  R  G  G  D  S  D  T  S  S  E
811  GGGTCAATATTCCAGGAACTCAAGCCACGAAGTCTGCAGTCGCAGATCGGCATCCTTTGTCCTGTATGTGATGCTCAGGTGGACCTGCCC
75   G  S  I  F  Q  E  L  K  P  R  S  L  Q  S  Q  I  G  I  L  C  P  V  C  D  A  Q  V  D  L  P
901  ATGGGTGGAGTGAAGGCTTTAACCATAGACCACCTGGCCGTGAATGATGTGATGCTGGAGAGCCTACGTGGGGAAGGCCAGGGCCTGGTG
105  M  G  G  V  K  A  L  T  I  D  H  L  A  V  N  D  V  M  L  E  S  L  R  G  E  G  Q  G  L  V
991  TGTGACCTGTGCAACGACAGGGAAGTAGAGAAGAGGTGTCAGACCTGCAAAGCCAACCTCTGCCACTTCTGCTGCCAGGCTCATAGGCGG
135  C  D  L  C  N  D  R  E  V  E  K  R  C  Q  T  C  K  A  N  L  C  H  F  C  C  Q  A  H  R  R
1081 CAGAAGAAAACGACTTACCACACCATGGTGGACCTAAAAGACTTGAAAGGCTACAGCCGGATTGGGAAGCCCATCCTGTGTCCTGTTCAC
165  Q  K  K  T  T  Y  H  T  M  V  D  L  K  D  L  K  G  Y  S  R  I  G  K  P  I  L  C  P  V  H
1171 CCTGCAGAGGAACTGAGGCTGTTCTGTGAGTTCTGTGACCGGCCCCTGTGCCAGGATTGTGTGGTGGGGGAGCATCGGGAACACCCCTGT
195  P  A  E  E  L  R  L  F  C  E  F  C  D  R  P  V  C  Q  D  C  V  V  G  E  H  R  E  H  P  C
1261 GACTTCACCAGCAATGTCATCCACAAGCATGGGGACTCTGTGTGGGAGCTCCTCAAAGGTACTCAGCCCCACGTGGAGGCCCTGGAGGAA
225  D  F  T  S  N  V  I  H  K  H  G  D  S  V  W  E  L  L  K  G  T  Q  P  H  V  E  A  L  E  E
1351 GCCCTGGCTCAGATCCACATAATAAACAGTGCCCTCCAGAAGCGAGTGGAGGCAGTGGCAGCTGATGTCCGGACATTCTCGGAGGGCTAC
255  A  L  A  Q  I  H  I  I  N  S  A  L  Q  K  R  V  E  A  V  A  A  D  V  R  T  F  S  E  G  Y
1441 ATTAAGGCCATTGAGGAGCATCGGGACAAGCTGCTGAAGCAGCTGGAAGACATACGGGCCCAGAAGGAAAATTCCCTGCAGCTGCAGAAG
285  I  K  A  I  E  E  H  R  D  K  L  L  K  Q  L  E  D  I  R  A  Q  K  E  N  S  L  Q  L  Q  K
1531 GCCCAGCTGGAACAGTTACTGGCAGACATGCGGACTGGAGTGGAGTTCACCGAGCACTTGCTGACCAGCGGCTCAGACTTGGAGATCCTC
315  A  Q  L  E  Q  L  L  A  D  M  R  T  G  V  E  F  T  E  H  L  L  T  S  G  S  D  L  E  I  L
1621 ATCACCAAGAGGGTGGTGGTAGAACGGCTCAGGAAGCTGAACAAAGTTCAATATAGCACCCGTCCTGGAGTAAATGATAAGATACGCTTC
345  I  T  K  R  V  V  V  E  R  L  R  K  L  N  K  V  Q  Y  S  T  R  P  G  V  N  D  K  I  R  F
1711 TGTCCTCAGGAGAAAGCAGGCCAGTGCCGTGGCTATGAAATTTATGGTACGATTAATACCAAAGAGGTTGATCCAGCCAAATGTGTCCTA
375  C  P  Q  E  K  A  G  Q  C  R  G  Y  E  I  Y  G  T  I  N  T  K  E  V  D  P  A  K  C  V  L
1801 CAAGGAGAAGACCTCCACAGAGCCCGGGAGAAACAGACGGCCTCTTTCACCCTGCTTTGTAAGGATGCCGCAGGAGAAATCATGGGCAGG
405  Q  G  E  D  L  H  R  A  R  E  K  Q  T  A  S  F  T  L  L  C  K  D  A  A  G  E  I  M  G  R
1891 GGAGGAGACAACGTTCAAGTTGCCGTTGTCCCTAAAGATAAGAAAGACAGCCCAGTCAGAACAATGGTCCAGGATAACAAGGATGGGACA
435  G  G  D  N  V  Q  V  A  V  V  P  K  D  K  K  D  S  P  V  R  T  M  V  Q  D  N  K  D  G  T
1981 TACTACATTTCCTACACCCCCAAGGAACCTGGCGTCTATACTGTGTGGGTCTGCATCAAAGAACAGCATGTGCAGGGCTCGCCATTCACT
465  Y  Y  I  S  Y  T  P  K  E  P  G  V  Y  T  V  W  V  C  I  K  E  Q  H  V  Q  G  S  P  F  T
2071 GTGACGGTGAGGAGAAAGCACCGCCCACACTCAGGCGTGTTTCACTGCTGCACCTTCTGCTCCAGCGGGGGCCAGAAAACCGCTCGCTGC
495  V  T  V  R  R  K  H  R  P  H  S  G  V  F  H  C  C  T  F  C  S  S  G  G  Q  K  T  A  R  C
2161 GCCTGTGGAGGCACCATGCCAGGTGGGTACCTAGGCTGTGGCCATGGACACAAAGGCCACCCAGGTCATCCCCACTGGTCATGCTGTGGA
525  A  C  G  G  T  M  P  G  G  Y  L  G  C  G  H  G  H  K  G  H  P  G  H  P  H  W  S  C  C  G
2251 AAATTTAATGAGAAATCTGAATGCACATGGACAGGTGGGCAGAGCGCACCGAGGAGTCTACTTAGGACTGTGGCTCTCTGAtgggtttgt
555  K  F  N  E  K  S  E  C  T  W  T  G  G  Q  S  A  P  R  S  L  L  R  T  V  A  L  *
2341 gctcagccttttaaagctgcaggcagcaccacttgagatttccagaggaccagacctcgttcattctaaagagactgatagaattaaa
2431 aacaaagtgccttatcttgcactggagttcaagtgctcttttgttagttattcactgattgtacttgtggatggttgagcacaaaaaacc
2521 acctctacatttctcttaaaggtaggttggcaacgttctttttctaccacaggatactccttttcattttgtacagtatggaggtatcta
2611 ttccattggcactgaaggtctaggtagtttagttcctttctgatataaccctttttttttctttttttttttttaaagacagagtc
2701 tcactctgtcacccaggctggagtgcagtggcatgatctcggctcactgcaacctctgcctcccggttcaaatgattctcgtgccttggt
2791 gtcccgagtagctgggattacaggttccccccacccttccccacacccagctaatttttgtgtttttagtagagacggggtttcaccat
2881 gttggccaggctggtctcaaactcctgacctcgtgatcccacctgcctcggcctcccaaagtgctggggttacaagcatgagccaccacac
2971 ctggcctattatgaccttttcatttttctctgagctacacattatttagatatttcccctagagattagaaacctcctttcatcctgtc
3061 cacttttttgttagcatacttttcattggatttactggtacatgttttgtgatcatgataatgtgatcttgttttggtggtgtccccctact
3151 ccgtctctatgccatgacagcctttctgccccaaagcctggaagattactctttggtcaagggcatacaaatagaaagttaataaagggga
3241 atggtgttgaaatagaacctcatacatttattacagttctacttatacagaaaatctagacaccttcatcctttctcacagctctgttttt
3331 tagtagagtagtctttagagtagtcttcactgttatctatatacctagaaagttaactgggagctttgactcttactgtgccttcactca
3421 tagtgtgccttcttagctgctggagaatttggggagaggatgtacacagcactggtgcctcagccctgccaaggtcttcactgtatatat
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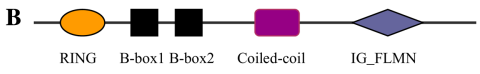

Fig. 1. Sequences and domain structure of human TRIM45. (A) Nucleotide sequence and deduced protein sequence of the human *TRIM45* gene. *TRIM45* encodes a polypeptide of 580 amino acids. Both nucleotides and amino acids are numbered at the left side of each line, respectively. The initiation ATG and termination TGA codons are boxed and in gray. The putative polyadenylation signal sequence aataaa is underlined and in bold. (B) The domain structure of TRIM45. Shown is the schematic diagram of RING (amino acids 29–97), B-box 1 (amino acids 130–176), B-box 2 (amino acids 186–227), coiled-coil (amino acids 282–330), and IG-FLMN (amino acids 398–500) of TRIM45 protein.

chromosome 1p22 and spans approximately 10.7 kb on the genome. *TRIM45* gene consists of six exons and five introns. The exon–intron boundaries conform to the consensus splicing signals, where there are a gt and an ag dinucleotide at the 5′-donor and 3′-acceptor site, respectively (Table 2).

Analysis of TRIM45 protein using the SMART program indicates that TRIM45 protein contains several evolutionary conserved molecular domains, including a RING finger (amino acids 29–97), two B-boxes (amino acids 130–176 and 186–227, respectively), and an α-helical coiled-coil domain (amino acids 282–330). Together, these domains form a tripartite motif near the NH_2-terminus of the protein. An IG-FLMN domain (amino acids 398–500) exists in the COOH terminus of TRIM45 (Fig. 1B).

The amino acid sequence of RING finger and the B-box1 of TRIM45 were aligned, respectively, with similar domains in several other TRIM proteins of *Homo sapiens*, such as TRIM2, TRIM3, TRIM56, TIF1α, and TIF1γ (Figs. 2A and B). The RING domain of TRIM45 is approximately 61% identical to the consensus of RING domains, and the B-box1 is about 51% identical to the consensus of B-box domains. Furthermore, an evolutionary relationship among TRIM proteins was examined using phylogenetic tree analysis. As shown in Fig. 2C, TRIM45 is one of the conserved proteins during evolution. Human TRIM45 shares more than 83% amino acid sequence identity with mouse TRIM45 protein.

Tissue distribution and expression of human TRIM45 transcripts

To characterize the transcript size and the expression pattern of *TRIM45*, an adult human multiple tissue membrane and an embryo multiple tissues membrane were hybridized with α-^{32}P-labeled *TRIM45* cDNA. A 3.6 kb transcript specific for *TRIM45* was detected in skeletal muscle, brain, pancreas, and heart of human adult tissues (Fig. 3A), as well as in brain, lung, skeletal muscle, heart, and intestine of embryonic tissues (Fig. 3B). The amount of transcript appeared to greatly vary among different tissues and a relatively higher level of expression was detected in skeletal muscle of adult (Fig. 3A, lane 6) and in brain of embryo (Fig. 3B, lane 5).

TRIM45 is a nuclear and cytoplasmic protein

To examine the subcellular location of TRIM45, the pEGFP-N1-TRIM45 was transfected into COS-7 cells, and 48 h after the transfection, the cells were visualized with epifluorescence microscope after labeled with DAPI for nucleus. EGFP-TRIM45 protein distributes both in nucleus and in cytoplasm (Fig. 4A) and DAPI binds to DNA (Fig. 4B). The combined image (Fig. 4C) shows that TRIM45 protein exists both in cytoplasm and in nuclei as well.

TRIM45 suppresses ElK1- and AP-1-mediated transcriptional activation

Recent studies have shown that the TRIM family members, TIF1 proteins, function as transcriptional intermediary factors, either as coactivators or corepressors [26,27]. Although TRIM45 shares tripartite motif with the TIF1 family (Figs. 2A–C), the potential roles

Table 2
Genomic structure of the *TRIM45* gene

Exon number	Exon size (bp)	Splice donor site	Intron size (bp)	Splice acceptor size
I	1076	gtaaagatgggaca	1946	ctgtttgccccta**g**
II	734	gtaggaaggcattt	1288	tttatctttttc**ag**
III	130	gtttgtatgacaca	926	ttatctccattc**ag**
IV	115	gtaagactggtgca	2089	tctgaacactgc**ag**
V	127	gtaaggagaaagca	905	tttgtcccatgt**ag**
VI	1389			

Summary of the size of the exons and introns of the *TRIM45* gene based on comparison of the cDNA and the genomic sequence. Invariant nucleotides (ag/gt) are in boldface type.

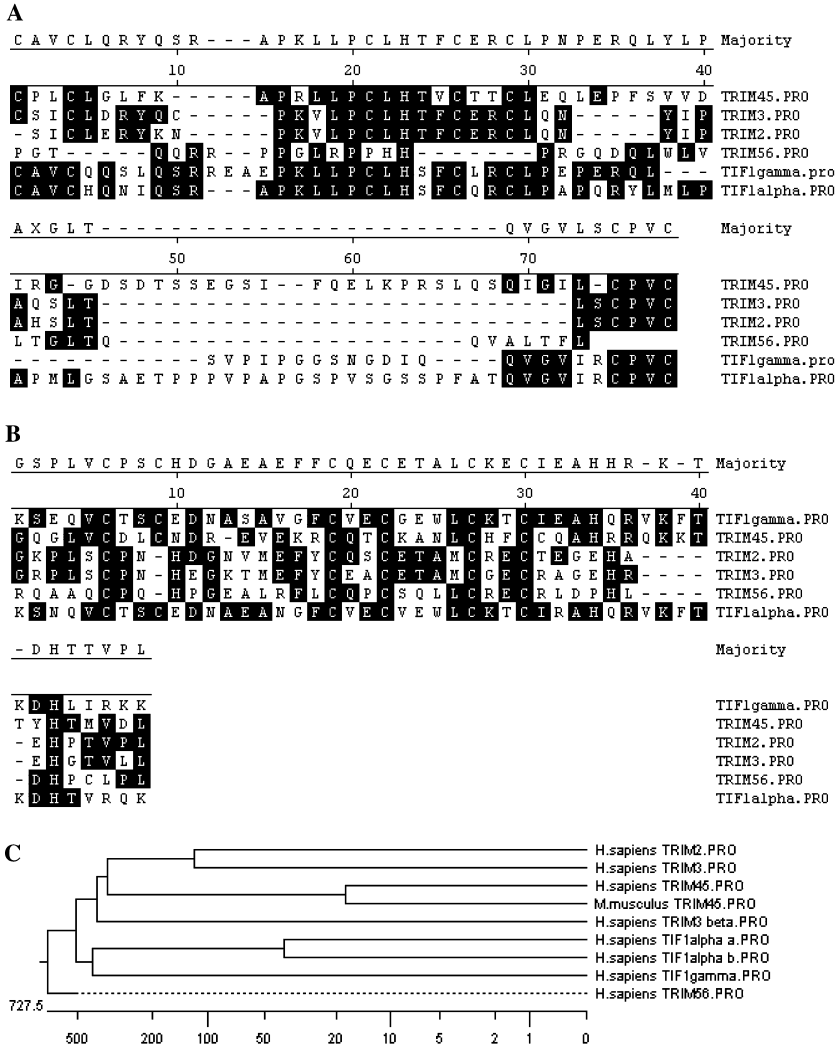

Fig. 2. (A) Comparison of the amino acid sequences of RING domains among TRIM45 and other TRIM domain proteins (*H. sapiens*). The amino acid sequences of the RING domains from TRIM45, TRIM3, TRIM2, TRIM56, TIF1γ, and TIF1α are compared. Residues that are highly conserved among these sequences are indicated in boldface type. The identical amino acid residues are boxed and shaded in dark. (B) Comparison of the amino acid sequences of B-box1 domains among TRIM45 and its homologues (*H. sapiens*). The amino acid sequences of the B-box1 domains from different proteins shown in (A) are compared. Residues that are highly conserved among these sequences are shaded in dark. The identical amino acid residues are boxed and shaded in dark. (C) The phylogenetic tree analysis of TRIM45 protein and its homologues. Besides proteins shown in (A), TRIM3 β (*H. sapiens*), TIF1α b (*H. sapiens*), and TRIM45 (*Mus musculus*) were used in the analysis.

of TRIM45 and other TRIM proteins are not clear. In order to investigate the role of TRIM45 in cell signal transduction, we examined whether TRIM45 is directly or indirectly involved in the regulation of transcription factors, especially in MAPK-mediated transcriptional regulation.

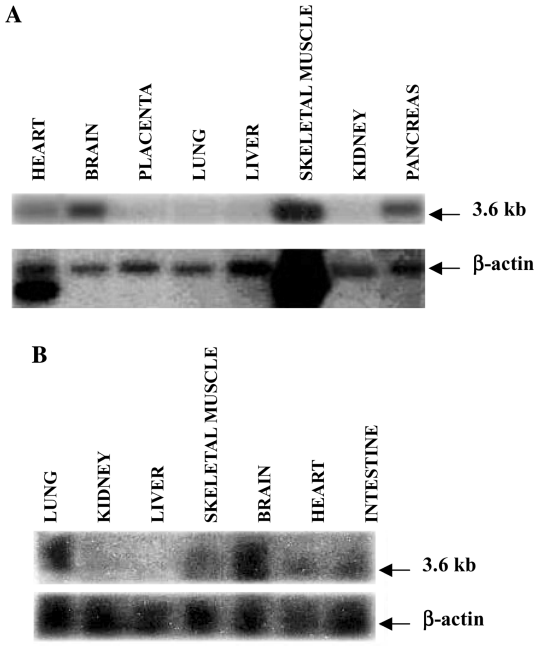

Fig. 3. Northern blot analysis of *TRIM45* in human adult and embryonic tissues. A commercially available Northern blot membrane (Clontech) containing multiple human adult tissues and a membrane containing multiple human embryonic tissues were hybridized with α-^{32}P random-labeled *TRIM45* cDNA fragment probe. This autoradiograph was developed after exposure for several days at −80 °C. A transcript of ~3.6 kb specific for *TRIM45* was detected in both adult and embryo membrane. (A) Hybridization of the Northern blot membrane of human adult tissues. A transcript of ~3.6 kb was detected in skeletal muscle, brain, pancreas, and heart. (B) Hybridization of a Northern blot membrane containing multiple human embryonic tissues. A transcript of ~3.6 kb was detected in brain, lung, skeletal muscle, heart, and intestine. Subsequently, the membranes were stripped and probed with β-actin cDNA probe supplied with the blot (Clontech) to confirm equal loading of mRNA on the blot (bottom of A,B).

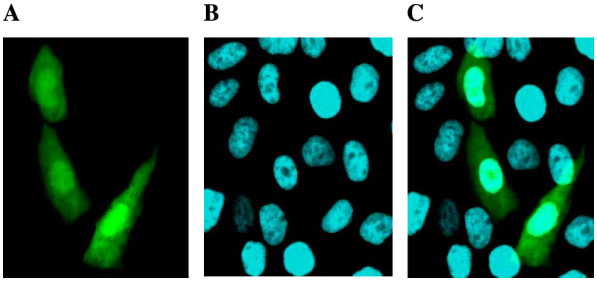

Fig. 4. TRIM45 is expressed in cell nucleus and in cytoplasm. (A) EGFP-N1-TRIM45 was expressed in COS-7 Cells. (B) The nucleus of cells stained with DAPI. (C) The combined image of (A,B).

MAPK family is an important mediator of signal transduction and is activated by a variety of stimuli, such as growth factors and cellular stresses [28]. MAPK controls a wide range of processes including cellular proliferation and differentiation [29]. The MAPK cascades are regulated through three-tiered cascades composed of a MAPK, MAPK kinase (MAPKK, MKK or MEK) and a MAPKK kinase or MEK kinase (MAPKKK or MEKK), which work in series and comprise a module [30]. 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 (p38 α/β/γ), and ERK5, which are activated by specific MAPKKs and phosphorylate specific cellular targets. One of the most studied targets of MAPK signaling pathway is transcription factors, such as c-Jun and Elk-1, which regulate transcription immediate early gene expression through binding to the serum response element (SRE) [30,31]. Most MAPKs phosphorylate ETS transcription factors that are involved in induction of *fos* genes, whose products heterodimerize with Jun proteins to form AP-1 complexes [32]. 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

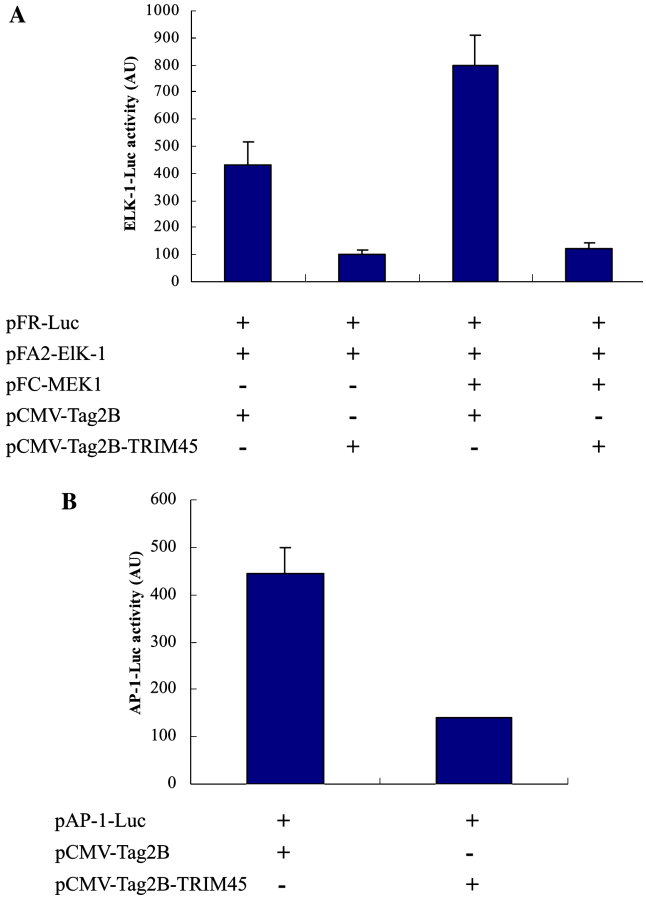

Fig. 5. Overexpression of TRIM45 suppresses transcriptional activities of Elk-1 and AP-1. (A) Inhibition of endogenous and MEK1-activated Elk-1 transcriptional activity by the expression of TRIM45. (B) Inhibition of AP-1-Luc transcriptional activity by the expression of TRIM45. COS-7 cells transfected with individual reporter plasmid and the corresponding plasmids shown in the figure. The data are means of three repeats in a single transfection experiment after normalization for β-galactosidase. Each experiment was performed at least three times.

these signaling cascades, MAPKs phosphorylate their target proteins, including many transcription factors, to regulate the expression of many genes in response to environmental stimuli, such as hypertrophic agonist and stress stimuli in myocytes [29].

To examine the effect of TRIM45 on this specific cell signaling pathway, we performed pathway-specific reporter gene assays to measure the modulation of Elk-1 and AP-1 by TRIM45 in the cell. First, we tested the effect of TRIM45 on the transcriptional activity of Elk-1, a member of the ternary complex. Expression of TRIM45 strongly inhibited the endogenous transcriptional activity of Elk-1 and the MEK1-mediated Elk-1 transcriptional activity by proximately 80%, suggesting a potential role of this protein in MEK1-mediated transcriptional regulation (Fig. 5A). Furthermore, using AP-1-luciferase reporter, we demonstrated that TRIM45 significantly reduced AP-1 transcriptional activity by 69% (Fig. 5B). Taken together, our results suggest that TRIM45 is a new TRIM family protein that suppresses transcriptional activities mediated by MAPK signaling pathways in the cell.

In conclusion, we have identified and characterized a novel human gene, *TRIM45*, from a human embryonic heart cDNA library. Northern blot analysis indicates that *TRIM45* was expressed in various tissues of human with higher expression in skeletal muscles of human adult and in brain of embryo. TRIM45 protein is localized in both cell cytoplasm and nucleus. Overexpression of TRIM45 inhibits the transcriptional activities of ElK-1 and AP-1, suggesting that TRIM45 may act as a negative transcriptional regulator in MAPK-mediated signaling pathways.

Acknowledgments

We are grateful to all members of the Center for Heart Development, College of Life Sciences in Hunan Normal University, for their excellent technical assistance and encouragement. This study was supported by grants from the National Natural Science Foundation of China (Nos. 30170479, 30270722, 30270644, and 30213904), the key project of Education Ministry of China (No. 02A028), and the pre-973 project of Ministry of Science and Technology of China (No. 2002CCC00100).

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