Genomic structure and chromosomal location of the human TGFβ-receptor interacting protein-1 (TRIP-1) gene to 1p34.1

Claudia Galli-Stauber[a], Giovanna Raho[a], Daniela Rossi[a], Davide F.V. Corona[a], Barbara Pirola[b], Maria Clara Bonaglia[b], Orsetta Zuffardi[b,c], Vincenzo Sorrentino[a,d,*]

[a] *Department of Biology and Technology (DIBIT), San Raffaele Scientific Institute, via Olgettina 58, I-20132 Milan, Italy*
[b] *Laboratorio di Citogenetica, San Raffaele Scientific Institute, Milan, Italy*
[c] *Istituto di Biologia Generale e Genetica Medica, Università di Pavia, Pavia, Italy*
[d] *Department of Biomedical Sciences, School of Medicine, University of Siena, Siena, Italy*

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Abstract The human TRIP-1 (transforming growth factor-β (TGBβ)-receptor interacting protein-1) cDNA encodes a protein able to associate specifically with the type II TGFβ receptor. It is phosphorylated on serine and threonine by this receptor kinase which makes it a strong candidate as part of the TGFβ signal transduction pathway. We have isolated the genomic sequence of TRIP-1 and found that the complete coding region is organised into 11 exons ranging from 39 to 397 bp and spanning approximately 9 kb of genomic DNA. The 5′ flanking region lacks a TATA box but is GC-rich, suggesting that it is a constitutively expressed gene which is in agreement with its wide pattern of expression. Fluorescence in situ hybridisation mapped the TRIP-1 gene to chromosome 1p34.1 whereas a pseudogene is located on chromosome 7q32.

Key words: Transforming growth factor-β; TGFβ-receptor interacting protein-1

1. Introduction

The transforming growth factor β (TGFβ) family of growth factors controls a wide range of biological processes affecting cell growth, differentiation and development. Signalling is mediated by two types of serine/threonine kinase membrane receptors. Upon binding of the ligand to the transmembrane receptor complex, probably a heterotetramer, type II receptor kinase phosphorylates the type I receptor through which the signal is then propagated intracellularly [1,2].

Some components of the TGFβ signalling pathway have been recently identified. They form a new protein family, named Mad proteins. Members of this family have been isolated from *Drosophila*, *Caenorhabditis elegans*, *Xenopus*, mouse and human [1,2]. The present model of TGFβ signal transduction proposes that ligand-dependent activation of receptor kinase initiates a cascade of phosphorylation events allowing the translocation of new phosphorylated Mad proteins into the nucleus where they associate with specific DNA binding proteins to bind to the promoter of responsive genes and activate transcription [3,4].

Additional potential candidates for this signal transduction pathway have been identified using the yeast two-hybrid system [5–7]. One of them, named TGFβ-receptor interacting protein-1 (TRIP-1), has been shown to specifically associate only with type II TGFβ receptor having no affinity to type I or activin receptors [5]. TRIP-1 is phosphorylated by the heteromeric TGFβ receptor complex but not by the activin receptors, which strongly suggests that it is implicated in TGFβ signal transduction. The 1.3 kb long mRNA is expressed ubiquitously and appears to be regulated in parallel to type II TGFβ receptor during development. The 325 amino acid long TRIP-1 protein contains five WD domains [8] and a putative ATP/GTP binding site. The family of WD proteins is characterised by at least four highly conserved repeating units usually ending with Trp-Asp (WD). It has been proposed that WD repeats might be involved in protein-protein interactions. Recently, biochemical purification and subsequent cloning of p36, a subunit of translation initiation factor eIF3, has been reported and it was found that human p36 is identical with TRIP-1 [9]. How and whether TRIP-1 is involved in such different processes as signal transduction and translation initiation remains to be determined. The genomic organisation of TRIP-1 was determined, including the exon and intron sizes, the exon-intron boundaries, and the promoter flanking region. The TRIP-1 gene was mapped by fluorescence in situ hybridisation (FISH) to the chromosomal region 1p34.1. A TRIP-1-related pseudogene was also identified and mapped to 7q32.

2. Materials and methods

Conditions for human genomic library screening and for PCR conditions have been described previously [14]. Briefly, RT product was amplified in a 100 μl reaction containing 50 pmol of each oligonucleotide primer, 200 mM of each dNTP, 1.5 mM $MgCl_2$, 10 mM Tris (pH 8.3), 50 mM KCl, and 2 U Taq polymerase. Amplification conditions were 94°C for 2 min followed by 45 cycles of 64°C for 1 min, 72°C for 1 min, and 94°C for 1 min. Amplified fragments were subcloned in pBluescript vector (Stratagene). The identity of the amplified clones was confirmed by nucleotide sequencing.

FISH was performed as previously described [14] using as hybridisation probe the TRIP-1 cDNA and in a separate experiment, DNA from Sp2 and Sp3 phages.

3. Results and discussion

A human genomic library from placenta was screened with a cDNA probe corresponding to the complete TRIP-1 coding sequence. This procedure resulted in the isolation of five independent phages. Restriction and Southern blot analysis showed that two phages (Sp3 and Sp2) contained most of the TRIP-1 genomic sequences although these phages did

*Corresponding author (a). Fax: (39) (2) 2643 4767.
E-mail: sorrenv@dibit.hsr.it

Abbreviations: FISH, Fluorescence in situ hybridisation; TGFβ, Transforming growth factor-β; Trip-1, TGFβ-receptor interacting protein-1

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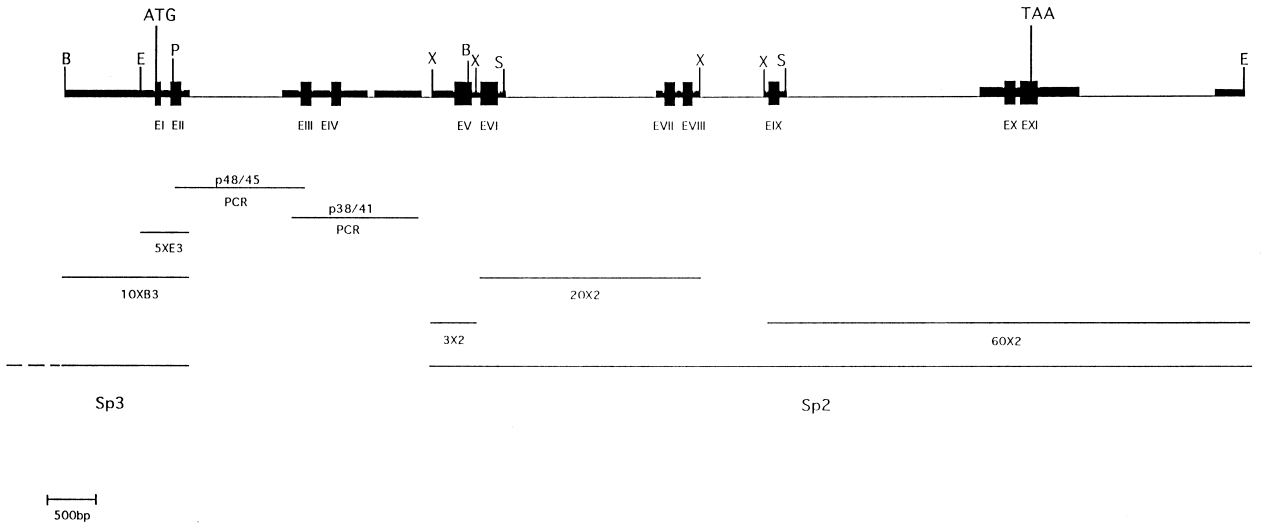

Fig. 1. Map of the TRIP-1 gene with its location of exons and introns and restriction sites. The locations of *Bam*HI (B), *Eco*RI (E), *Pst*I (P), *Xba*I (X), *Sac*I (S) are indicated above the line. Exons are shown as black boxes separated by a line representing introns or flanking regions. The thick line refers to genomic regions that were sequenced. Sp2 and Sp3 indicate the genomic regions covered by the two lambda phages. p48/45 and p38/41 are the two genomic fragments amplified by polymerase chain reaction. The subclones derived from the lambda Sp2 and Sp3 are indicated by their sizes in kilobases followed by the initial restriction enzymes used for subcloning and the number 2 or 3 depending on whether they were cloned from the two lambda phage Sp2 or Sp3.

not overlap (Fig. 1). Suitable DNA restriction fragments were subcloned into the pBluescript vector for further analysis. Coding regions were initially identified by sequence analysis with oligonucleotides designed on the cDNA-derived coding sequence. Exon/intron boundaries were sequenced in both directions. Sequencing analysis indicated that phage Sp3 contained exons I and II and extended in the 5′ flanking region, while phage Sp2 contained exons V–XI (Fig. 1).

The region carrying exons III and IV, which was not represented in the genomic clones isolated, was obtained by PCR of human genomic DNA. A first clone, named p48/45, was amplified using primers designed on the cDNA sequence corresponding to exon II, oligo cs48 (5′-GCCATGAGCGGTCCATTACGCAGATTA-3′), and to exon III, oligo cs45 (5′-ACAGCTCCGGTATGGCCCATGTAGGT-3′). The second clone, named p38/41, was amplified with a second set of oligonucleotides derived from the cDNA sequence corresponding to exon III, cs38 (5′-ATCGTCAATGTATGGTACTCTGTGAATGGT-3′) and from the genomic sequence corre-

Table 1
Exon-intron boundaries of TRIP-1

3′ acceptor sequence		Exon	Position of exon	Exon size (bp)	5′ donor sequence		Intron	Intron size (bp)	Reading frame
Intron	Exon				Exon	Intron			
		I	−37–3	40	GGGATG met	gtgagtttca	**1**	93	0
gtccccacag	AAGCCG lyspro	**II**	4–96	93	GACCCT asppro	gtgagtgttg	**2**	∼1400	0
acaaaaccag	ATCGTC lylysg	**III**	97–184	88	CTGACT laaspt	gtatccttatt	**3**	288	I
aactcttcag	GGGACA rpaspt	**IV**	185–250	66	AAACAG aspcys	gtaagctggg	**4**	∼1000	0
ctgactccag	GAAAGC gluthr	**V**	251–400	150	AGATTG lnilea	gtgagggctg	**5**	82	I
ccccacacag	ACAACA spasna	**VI**	401–528	128	GCCAAG alalys	gtaagaggcc	**6**	∼1600	0
ccttccctag	TCTGGA sergly	**VII**	529–639	111	GCCAAG alalys	gtgagcctgg	**7**	119	0
ctctctccag	CTTTTT leuphe	**VIII**	640–729	90	GACCAT asphis	gtaagagaac	**8**	∼1000	0
ttccattcag	GTGGTC valval	**IX**	730–803	74	GGCCAG ualaar	gtaaagaaga	**9**	∼1950	II
tttcctccag	GTTCTT gpheph	**X**	804–896	93	CAAGAG ylysse	gtagggtacc	**10**	95	II
ctttttccag	CTACAG rtyrse	**XI**	897–1293	397					

Exon sequence is in uppercase letters and intron sequence is in lowercase letters. The position of the exons is given according to the numbering of the previously published cDNA sequence [5]. The reading frame is indicated by 0 if the intron is positioned between codons. Interruption of the codon after the first nucleotide is frame I, and interruption after the second nucleotide is frame II.

sponding to intron 4, cs41 (5′-ACTTCTTACCTAGACTC-TTTCCTCGAC-3′).

Fig. 1 shows the restriction map of the TRIP-1 gene with the subcloned fragments indicated. The TRIP-1 gene is distributed over 9 kb and divided into 11 exons ranging from 39 to 397 bp in size (Table 1). The coding region sequence of the exons is identical with the TRIP-1 cDNA sequence reported by Chen et al. [5].

The first 8 bp in the 5′ untranslated region of the TRIP-1 cDNA reported by Chen et al. [5] are not found in our genomic sequence or in the sequence of five cDNAs found by screening an expression sequence tag (EST) database with the 5′ region of the TRIP-1 cDNA. These clones have homology to our genomic sequence upstream of the ATG in exon I. Comparing the sequence of EST clone 56792, which extends more 5′ than others, against our genomic sequence we detected a match for positions 1–7 of the EST clone with nucleotides at positions 1065–1071 of the genomic sequence. On the basis of this homology we tentatively extend the 5′ untranslated region to 36 nucleotides upstream of the ATG codon. A visual inspection of the genomic sequence (1064 bp) upstream of the 5′ untranslated region does not evidence a consensus sequence with significant similarity to the initiator sequence already described [10,11]. The termination codon in exon 11 is followed by 301 nt of non-coding sequence.

Compared to the published TRIP-1 cDNA sequence we detected some nucleotide divergences in the 3′ non-coding region which are an insertion of T at position 1067, an insertion of a C at position 1134, deletion of the 8 nucleotides AAAAATAC starting from position 1146, and two substitutions from T to G at position 1249 and from A to G at position 1329.

A total of more than 1 kb sequence 5′ to the start of the published cDNA was obtained. Neither a good consensus sequence for a TATA box nor a CCAAT box was evident. Typical features of a TATA-less promoter are a high GC content [12] and the presence of Sp1 binding sites. Indeed, this region has an overall high GC content of 55%, which increases to 61% for the 200 bp immediately upstream of the translation start site which contains several Sp1 binding sites as observed in several TATA-less promoters. These characteristics would agree with TRIP-1 being a housekeeping gene which is in line with the finding that TRIP-1 RNA is ubiquitously expressed [5].

The intron/exon organisation of the TRIP-1 gene is reported in Table 1: the sizes of the introns are between 82 bp and 1950 bp and all exon/intron junction sequences conform to the GT/AG rule [13]. According to Southern blot and sequence analysis (data not shown) there are some repetitive elements within the 5′ untranslated region, in introns 4 and 9 and in the 3′ untranslated region.

The other three isolated phages (Sp1, Sp4, Sp5) share a common restriction pattern. Therefore, only phage Sp1, carrying the largest insert, was analysed further. Sequence analysis of subcloned fragments revealed that the *Eco*RI 4 kb fragment of the SP1 phage carries a sequence with 77% identity to the part of the TRIP-1 cDNA corresponding to exons III–XI. This sequence lacks introns suggesting that it represents a TRIP-1 pseudogene likely originated by a retroposition event (A. Alloni, data not shown). Southern blot analysis of human genomic DNA, hybridised with both TRIP-1 genomic and cDNA probes (not shown), indicates that there is only one TRIP-1 gene and one related pseudogene.

Fig. 2. FISH analysis for chromosomal localisation of the TRIP-1 gene and pseudogene. FISH analysis using a mixture of Sp1, Sp2 and Sp3 probes. Arrows indicate chromosomes with signals: arrowhead points to chromosome 1p34.1. Arrow points to chromosome 7q32.

Chromosomal assignment of the human TRIP-1 gene was performed by FISH [14] using as hybridisation probe the TRIP-1 cDNA and, in a separate experiment, DNA from Sp2 and Sp3 phages. The map position was determined by visual inspection of the fluorescent hybridisation signals on DAPI-stained metaphase chromosomes. Only those chromosomes with signals present on both chromatids at the same band position were taken into consideration. The probe was considered mapped when the same chromosome band showed signals in not less than 20% of 200 metaphases examined. For both probes, analysis of metaphase chromosomes conclusively indicated that the TRIP-1 gene is localised to human chromosome 1p34.1. Hybridisation with total DNA from phage Sp1 unequivocally mapped the TRIP-1 pseudogene to chromosomal region 7q32 (Fig. 2).

The 1p34.1 region has been found to be altered in some human neoplasias [15]. Given the evidence that TGFβ inhibits cell growth and that alterations in genes that encode proteins involved in TGFβ-specific signalling have been identified [16,3,17], whether structural alterations are involved with the TRIP-1 gene in human tumours is under investigation. Preliminary results revealed no gross rearrangement in the TRIP-1 gene on Southern blot analysis of human primary tumours (N. Fracchiolla, A. Neri et al., unpublished observations).

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