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Cloning of an isoform of mouse TGF-β type II receptor gene

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Abstract A variant of transforming growth factor-β type II receptor (TGF-βRII) cDNA was isolated from a mouse brain cDNA library. The predicted receptor is identical to previously reported mouse TGF-βRII except that the isoform has an insertion sequence of 25 amino acids in the predicted ligand-binding domain. By the use of reverse transcription-polymerase chain reaction (RT-PCR), transcripts for both isoforms were detected in all tissues and developing embryos examined. The isoform transiently expressed in COS cells showed a similar ligand-binding specificity to authentic TGF-βRII. These results suggest that the mouse TGF-βRII gene generates multiple isoforms, possibly by alternative splicing, as reported for activin type IIB receptor; and an isoform which has the extra sequence in the ligand-binding domain is also involved in the TGF-β signal transduction.

Key words: Transforming growth factor-β receptor; Alternative splicing; Serine-threonine kinase

1. Introduction

Transforming growth factor-β (TGF-β) is a family of polypeptide growth factors the members of which are involved in the regulation of cell growth and differentiation [1,2]. There are three subtypes of TGF-β, namely TGF-β1, -β2 and -β3, which are closely related to one another in primary structure and their transcripts have been detected in a wide variety of vertebrate tissues and cultured cell lines. The biological effects of TGF-βs are mediated by interaction between the ligand and specific TGF-β receptors, which have been studied mostly with TGF-β1. Three types of receptors with distinct molecular weights were identified in a variety of tissues by affinity cross-linking experiments using ^{125}I-labeled TGF-β1, which are designated as type I, II and III receptors [3]. The type III receptor with the largest molecular weight ranging from 200 to 300 kDa, has been characterized as a proteoglycan and does not appear to be involved in intracellular signaling [4,5]. Conversely, type I and type II receptors, which show molecular weights of 53 and 75 kDa, respectively, play a central role in TGF-β signaling. It was reported that the type II receptor can bind TGF-β1 by itself but requires the type I receptor for signal transduction. In contrast, the type I receptor requires the type II receptor for both signal transduction and ligand binding [6]. Recently, cDNAs that encode type I and II receptors have been cloned and found to encode serine-threonine kinases in their cytoplasmic domain [7]. The mouse TGF-β type II receptor (TGF-βRII) cDNA has previously been reported to encode a 567 amino acid membrane protein with a 159 amino acid extracellular domain, and a high level of transcript was detected in a variety of tissues except brain [8]. Here we report the isolation of an isoform of TGF-βRII cDNA from a mouse brain cDNA library. The isoform encodes the TGF-β type II receptor with a 25 amino acid insertion sequence in the extracellular domain which retains the capacity to bind TGF-β1.

2. Materials and methods

2.1. Cloning of mouse TGF-βRII$_2$ cDNA

Degenerate oligonucleotides (5′-TA(T/C)ATGGC(T/C/A/G)CC-(T/C/A/G)GA(A/G)GT-3′ and 5′-(A/G)TC(A/G)TG(A/G)TCCCA-(A/G)CA(T/C)TC-3′) were designed based on conserved kinase subdomain sequences (domains VIII and XI) and used as primers for reverse transcription-polymerase chain reaction (RT-PCR) to isolate cDNAs encoding serine-threonine kinase receptors from an osteoblastic cell line, MC3T3-E1. The PCR fragments were subcloned into T-overhanged BlueScriptIIKS(−) and sequenced. The PCR fragment that contains the putative coding region with similarity to human TGF-β type II receptor was used to screen a mouse brain cDNA library. Five positive clones were isolated from 1×10^6 plaques. The longest cDNA (3.8 kb) was subcloned into BlueScriptIIKS(−) and sequenced with a BcaBEST dideoxy sequencing kit (TAKARA Biochemicals).

2.2. Detection of mouse TGF-βRII mRNAs

Total RNA was prepared from mouse adult tissues and embryos using the acid guanidinium thiocyanate-phenol/chloroform extraction method [9]. For PCR analysis, cDNA was synthesized from 500 ng aliquots of total RNA as described previously [10]. Samples, which contained 5 ml of the cDNA solution, were cycled 30 times at 94°C for 1 min, 60°C for 1 min, 72°C for 2 min according to the manufacturer's instruction (Perkin-Elemer Cetus). The amplified products were separated on an 1.5% agarose gel and stained with ethidium bromide. Primers used for the PCR analysis were 5′-TGTGGACGCGCA-TCGCCAGC-3′ (nucleotide position 202–221, for 5′) and 5′-ACAC-GGTAGCAGTAGAAGAT-3′ (nucleotide position 783–802, for 3′).

2.3. Cell culture and affinity cross-linking

COS cell culture and affinity cross-linking were carried out using ^{125}I-labeled TGF-β1 as previously described [11]. ^{125}I-Labeled recombinant human TGF-β1 was purchased from Du Pont Inc. Recombinant human TGF-β1 and TGF-β2 were purchased from Austral Biologicals.

3. Results and discussion

In an attempt to isolate new members of receptor serine-threonine kinase receptors, oligonucleotide primers designed in subdomain VIII and XI were used to amplify cDNA synthesized from MC3T3-E1 cells mRNA [12]. Several cDNA frag-

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The sequence reported in this paper has been deposited in the EMBL/Genbank/DDBJ data base (accession number: D32072).

Abbreviations: RT-PCR, reverse transcription-polymerase chain reaction; TGF-β, transforming growth factor-β; TGF-βRII, TGF-β type II receptor.

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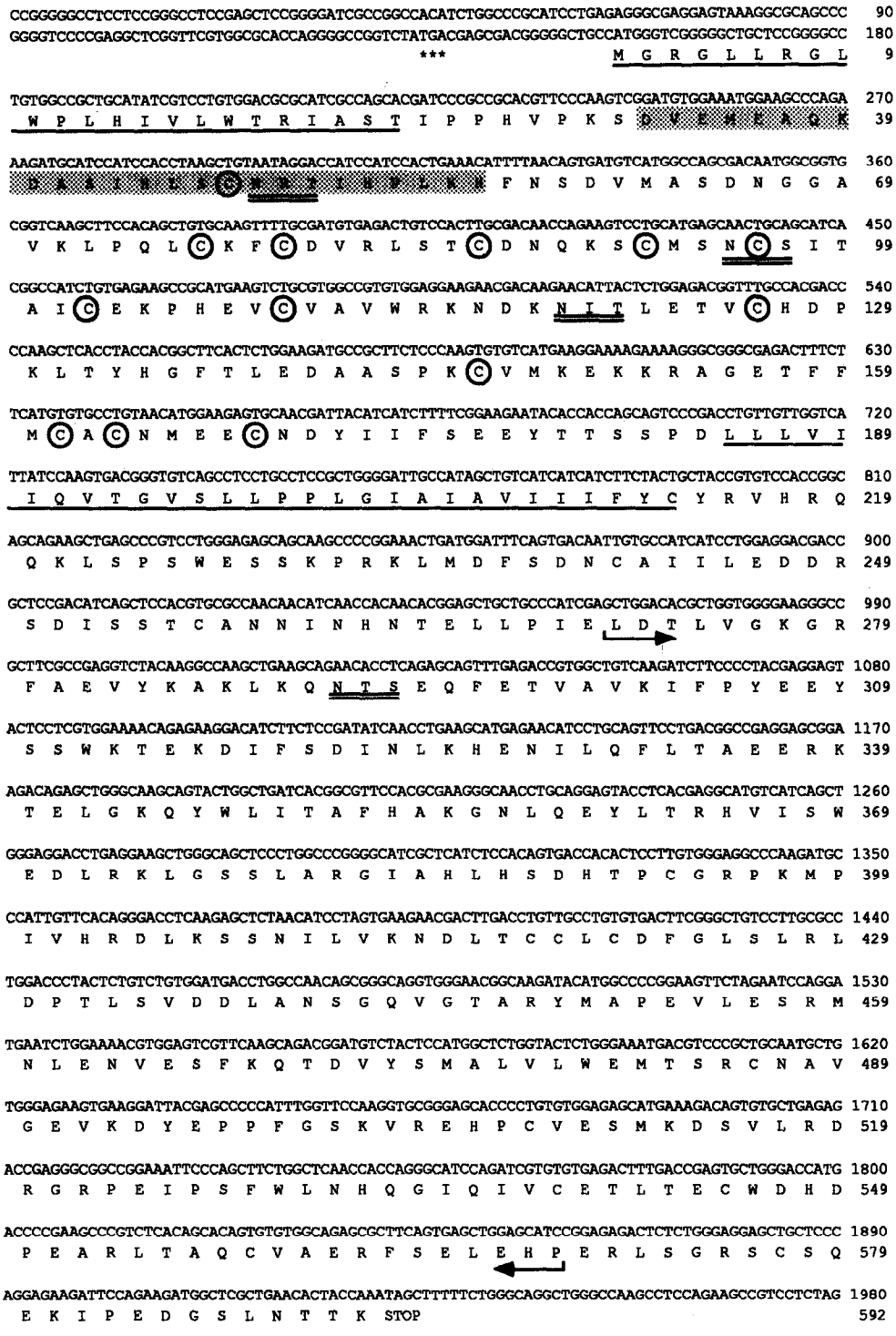

Fig. 1. Nucleotide and deduced amino acid sequence of mTFR17-7 (mTGF-βRII$_2$) cDNA. The upstream in-frame stop codon is indicated by asterisks. The signal peptide and the transmembrane domain are indicated by single underline. Potential N-linked glycosylation sites are indicated by double underline. An insertion sequence of 25 amino acids is shaded. Cysteine residues conserved in the extracellular domain are circled. The ends of the intracellular kinase domain are indicated by arrows.

ments which encode serine-threonine kinase sequences were isolated and then each used to screen a mouse brain cDNA library. Finally, one of the clones, designated as mTFR17-7, was found to be very similar yet distinct from a previously reported mouse TGF-βRII cDNA [8]. Fig. 1 shows the nucleotide and deduced amino acid sequences of the mTFR17-7 cDNA. The major structural difference between mTFR17-7 receptor and the mTGF-βRII (which we designated as mTGF-βRII$_2$ and mTGF-βRII$_1$, respectively) is that the mTFR17-7 receptor has a 25 amino acid insertion sequence in the extracel-

lular domain (shaded in Fig. 1). Furthermore, the sequence contains a potential N-linked glycosylation site and a cysteine residue, suggesting that the mTGF-βRII$_2$ may have different ligand-binding specificity and biological function from those of mTGF-βRII$_1$. Comparison of the cDNA sequences of mTGF-βRII$_1$ and mTGF-βRII$_2$ at the nucleotide level revealed that a 75 nucleotide sequence was inserted at a codon which encodes Val^{32} of mTGF-βRII$_1$, and the insertion generates one amino acid change (Val^{32} to Phe^{57} of mTGF-βRII$_2$) in addition to the 25 amino acid insertion (Fig. 2). There are some minor differences between the two isoforms at the nucleotide level, but these are thought to be due to the diversity of the mouse strains. Therefore, it is most likely that these type II receptor isoforms are generated by the differential splicing of mRNA that is transcribed from the originally identical TGF-β type II receptor gene, as previously reported for mouse activin type IIB receptor gene [13].

In order to examine expression levels of the two distinct forms of the mTGF-βRII in various tissues and developing embryos, RT-PCR was performed using synthetic oligonucleotide primers that discriminate between the two isoforms. In this assay, a set of primers, which were designed so as to cover the insertion site and amplified products corresponding to the isoforms, show different mobility on an agarose gel (mTGF-βRII$_1$ for 526 bp and mTGF-βRII$_2$ for 601 bp in Fig. 3A). As shown in Fig. 3B and C, both RNA species were detected in all tissues and embryos examined; this suggests that mTGF-βRII$_2$ may act as a signaling receptor for TGF-β in these tissues and embryos because we have reported that the TGF-β type I receptor also co-expressed in these sites and the type I receptor could form a functional receptor complex with mTGF-βRII$_2$ ([8,11], and unpublished observations). Although Lawler et al. has shown that mTGF-βRII$_1$ was rarely expressed in brain [8], both forms of mTGF-βRII were detected in brain at a significant level under our experimental conditions. This discrepancy may be due to the improved sensitivity of RT-PCR.

A line of evidence suggests that a specific receptor for TGF-β2 exists in mammals but the TGF-βRII$_1$ isolated from mouse and human could not bind efficiently to TGF-β2 [8,14]. Moreover, TGF-β2 protein is more abundant in brain from which mTGF-βRII$_2$ cDNA was isolated [15]. Therefore, it was expected that the insertion of 25 amino acid might alter the ligand specificity of the mTGF-βRII and make the receptor as for TGF-β2. This possibility was examined by an affinity cross-linking experiment using ^{125}I-labeled TGF-β1. In Fig. 4, transiently expressed mTGF-βRII$_2$ in COS cells bound [^{125}I]TGF-β1 and formed an affinity-labeled complex of 95 kDa under non-reducing condition. After subtracting the molecular weight

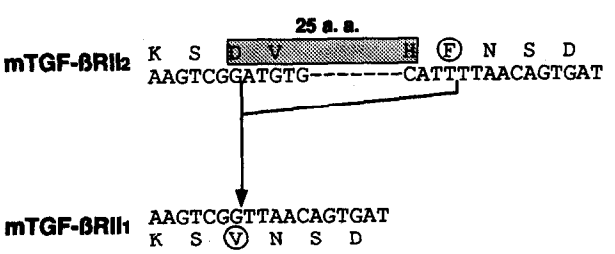

Fig. 2. Generation of mouse TGF-β type II receptor isoforms. The cDNA and translated amino acid sequences near possible alternative splicing site are shown. An insertion sequence of 25 amino acids is shaded. Amino acids that change as a result of the splicing are circled.

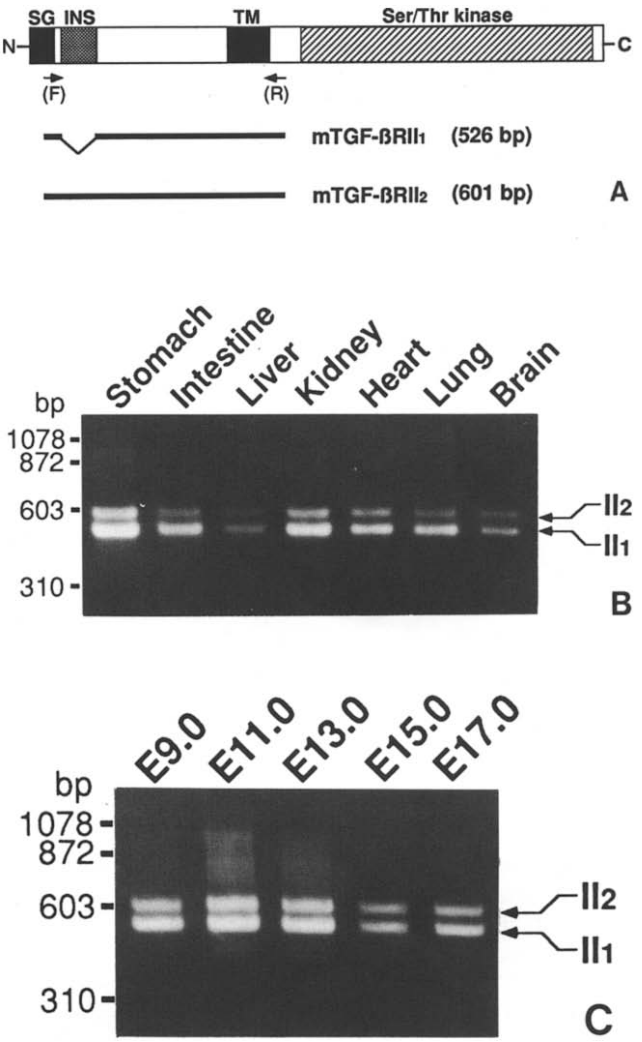

Fig. 3. Expression of mouse TGF-β type II receptor isoforms in adult tissues and embryos. (A) Primer positions used for RT-PCR. Schematic structure of mouse TGF-β type II receptor is represented by a box. Black boxes represent the signal sequence (SG) and the transmembrane (TM) domain. An insertion sequence (INS) within the extracellular domain is represented by a shaded box. The intracellular serine-threonine kinase domain is represented by a hatched box. Forward (F) and reverse (R) primers are indicated by arrows. The amplified products of 526 bp and 601 bp are expected for mTGF-βRII$_1$ and mTGF-βRII$_2$, respectively. (B and C) Detection of transcripts for mTGF-βRII isoforms in adult tissues and developing embryos. Total RNA (500 ng) purified from adult tissues (B) and embryos (C) were subjected to RT-PCR (see section 2). Positions of PCR products for mTGF-βRII$_1$ and mTGF-βRII$_2$ are indicated as II$_1$ and II$_2$, respectively.

of the cross-linked [^{125}I]TGF-β1 (25 kDa), this band corresponds to a protein of 70 kDa. The binding was effectively replaced by an excess amount of unlabeled TGF-β1 but not by that of TGF-β2. The human TGF-βRII$_1$ expressed in COS cells also shows specific binding to TGF-β1, as previously reported. Control cells transfected with an expression vector alone exhibited no cross-linked products. These results indicate that the mTGF-βRII$_2$ has similar ligand-binding specificity to the TGF-βRII$_1$.

In this paper, we identified a new isoform of mTGF-βRII which has an insertion sequence in the extracellular domain.

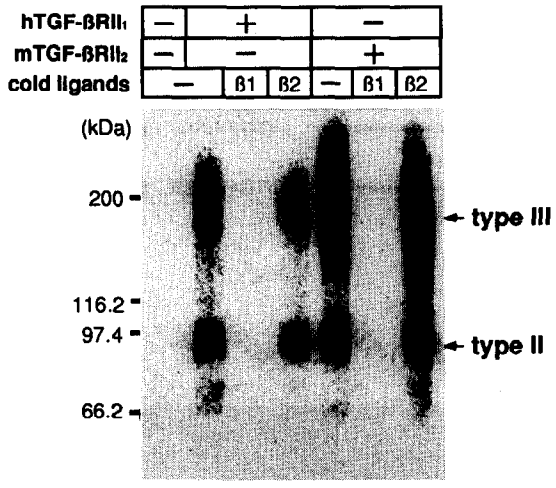

Fig. 4. Affinity cross-linking of mTGF-βRII$_2$. COS cells transfected with the indicated expression plasmids were incubated with [^{125}I]TGF-β1 and subjected to chemical cross-linking. The positions of type II and type III receptors are indicated on the right of the figure. Ligand binding specificity of the receptors was examined by the use of TGF-β1 or -β2 as competitor.

Although the receptor was isolated from adult brain which produces TGF-β2 preferentially, the receptor displayed effective binding of TGF-β1 but not TGF-β2: the regular function of the mTGF-βRII$_2$ is not yet clear. However, the insertion of an extra 25 amino acids into the mTGF-βRII may alter the cell's response to TGF-β1 because Glansbeek et al. reported that a molecular weight shift of the type II receptor expressed in bovine articular chondrocytes is linked to change of the cell response to TGF-β1 [16]. The biological function of the new isoform of TGF-βRII will be investigated in future studies.

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