Cloning and characterization of a naturally occurring soluble form of TGF-β type I receptor

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Choi, Mary E. Cloning and characterization of a naturally occurring soluble form of TGF-β type I receptor. Am. J. Physiol. 276 (Renal Physiol. 45): F88–F95, 1999.—Transforming growth factor-β (TGF-β1) has been implicated to play an important role both in the process of normal development and in the pathogenesis of a wide variety of disease processes, including those of the kidney. TGF-β1 regulates diverse cellular functions via a heteromeric signaling complex of two transmembrane serine/threonine kinase receptors (types I and II). Several distinct type I receptors have been described and are thought to determine specificity of the TGF-β response and confer multifunctionality. This report reveals the cloning of a novel, naturally occurring soluble form of TGF-β type I receptor, designated stTβR-I, from a rat kidney cDNA library. In vivo expression of a mRNA transcript encoding the stTβR-I, which lacks the transmembrane and cytoplasmic domains, is confirmed by RT-PCR followed by Southern blot analysis and by RNase protection assay. The stTβR-I mRNA abundance is greater in the neonatal rat kidney compared with the adult rat kidney. Furthermore, stTβR-I is a functional protein capable of binding TGF-β1 ligands in the presence of a TGF-β type II receptor on the cell surface, as determined by affinity cross-linking with 125I-labeled TGF-β1. Studies using pSTP-Lux reporter construct reveal that this novel protein may function as a potentiator of TGF-β signaling. The discovery of a stTβR-I provides an additional level of complexity to the TGF-β receptor system.

variant activin receptor-like kinase-5; alternative splicing; signaling; renal development; transforming growth factor-β1

TRANSFORMING GROWTH factor-β1 (TGF-β1) is a multifunctional cytokine that regulates diverse cellular functions including cell proliferation and differentiation, as well as extracellular matrix protein synthesis. TGF-β1 has been implicated in both the process of normal development and morphogenesis (19) and in the pathogenesis of a wide variety of disease processes, including tissue fibrosis, inflammation, and tumorigenesis (16). In embryonic kidney tissues, both temporally and spatially regulated differential expression patterns of TGF-β and its receptors have been demonstrated, suggesting an important role for TGF-β in renal development (6, 19).

TGF-β1 exerts its multiple biological actions by the interaction with two transmembrane serine/threonine kinases (types I and II), which are coexpressed by most cells (3). Molecular cloning has revealed that a distinct type II receptor exists for TGF-β1 and for members of the TGF-β superfamily, including activin and bone morphogenetic protein (BMP), and that the type II receptor is capable of binding its respective ligands directly and interacting with different type I receptors (2, 10, 15). To date, at least six distinct type I receptors of the TGF-β superfamily, named activin receptor-like kinases (ALK), have been cloned. ALK-5 has been identified as the predominant TGF-β type I receptor (TβR-I) in most cell types and has been shown to mediate TGF-β signaling (12). ALK-1 and ALK-2 are thought to be activin type I receptors, but both have also been demonstrated to bind TGF-β (1, 9). ALK-4 also appears to be an activin type I receptor (21). ALK-3 and ALK-6 are thought to be type I receptors for BMP (22). Features common to all type I receptors include a cysteine-rich extracellular domain and a single transmembrane-spanning domain. Furthermore, besides the characteristic cytoplasmic serine/threonine kinase domain, type I receptors have a region, between the transmembrane and kinase domains, containing a conserved TSGSGSG motif denoted the GS domain. Muta
tional analyses have revealed that phosphorylation of serine and threonine residues in the GS domain of TβR-I by the TGF-β type II receptor (TβR-II) is essential for TGF-β signaling (20, 25).

TβR-I is thought to determine the specificity of the cellular response to TGF-β1, whereas TβR-II determines the ligand specificity. TβR-I alone is unable to bind TGF-β1, on the basis of 125I-labeled TGF-β1 cross-linking studies, and TβR-II is unable to signal without TβR-I (27). Thus interaction of TβR-II with different type I receptors may be a mechanism that confers multifunctionality of TGF-β1. This report shows that there are variant forms of the TβR-I. Three unique cDNA clones encoding multiple receptor forms of ALK-5 have been isolated from a neonatal rat kidney cDNA library. Two of the rat cDNA clones encode membrane-spanning receptors that differ in the COOH-terminal region of the extracellular domain. The third clone encodes a previously undescribed soluble form of TβR-I (stTβR-I). The present studies clearly demonstrate, for the first time, the existence of a naturally occurring stTβR-I mRNA expressed in greater abundance in the neonatal rat kidney compared with the adult rat kidney. Furthermore, the stTβR-I is a functional protein capable of binding TGF-β1 ligands in the presence of TβR-II. This novel protein may function as a potentiator of TGF-β signaling.

METHODS

Cloning and sequencing of rat TβR-I cDNA clones. A 333-bp cDNA probe for ALK-5 (12) was generated by RT-PCR of total
RNA from rat kidneys using sense primer A (nt 88–117 of human ALK-5, 5'-GGGGCGACGCGGTTCAGTTTCTCGCAG-3') and antisense primer B (nt 391–420 of human ALK-5, 5'-TGAGATGCAGACGAACACTGGTCCAGCAGC-3'). Using this PCR-amplified cDNA probe, a rat neonatal kidney cDNA library was screened by methods previously described (6). Three positive clones were identified. Partially overlapping nested deletion clones were prepared from the cDNAs with the Erase-a-Base kit (Promega), and then the sense and antisense strands were sequenced using the dideoxy chain termination method with 35S-dATP and Sequenase version 2.0 (Amersham).

RT-PCR. Total RNA (3 µg) from Sprague-Dawley rat neonatal and adult kidneys (Charles River, Wilmington, MA) was reverse-transcribed using M-MLV reverse transcriptase (GIBCO BRL), and the first-strand cDNA was used as a template for amplification. Each template cDNA was combined with 1 µM of each set of PCR primers, 200 µM dNTPs, and 2 U of AmpliTaq DNA polymerase in 1× PCR buffer (Perkin-Elmer); placed in the GeneAmp PCR System 9600 (Perkin-Elmer), and heated to 95°C for 1 min. This was followed by 30 cycles consisting of denaturing for 15 s at 95°C, annealing for 15 s at 50°C, and extending for 15 s at 72°C. The sequence of primers A and B were as described above; the sequence of primer C (nt 480–505 of rat cDNA) was as described for the kinase-deleted TbR-II and sTbR-I. The amplified probe was hybridized and then digested in the presence of a random primer [32P]dCTP-labeled cDNA probe, as recommended by the manufacturer (GIBCO BRL). The two TbR-I cDNA probes were Hind III/Sph I and Sph I fragments of clone 29-1 cDNA in pSPORT1 (GIBCO BRL), produced by restriction digests with the respective enzyme.

Solution hybridization/RNAse protection. RNAase protection analysis was done using the RPA II kit (Ambion) according to the manufacturer's instructions. The [32P]labeled antisense riboprobe was prepared from the linearized plasmid containing the Hind III/Sma I fragment of clone 29-1 cDNA using Sp6 RNA polymerase, yielding a probe 337 nt long. One, ten, and twenty micrograms of total RNA from neonatal and adult rat kidneys, or 10 µg of total RNA from wild-type and transfected COS-7 cells, were hybridized with the [32P]labeled probe for 16–18 h at 42°C, then digested with RNAase A/T1 and resolved on a 6% acrylamide/7.7 M urea sequencing gel. A sample of [32P]-labeled, 1-kb ladder DNA was loaded in an adjacent lane. The gel was stained with Coomassie brilliant blue (Bio-Rad) to allow visualization of equivalence in protein loading and was destained before autoradiography.

Luciferase assay. Luciferase reporter plasmid, p3TP-Lux (kindly provided by J. Massagué), was used to assay TGF-β1-induced transcription of plasminogen activator inhibitor-1 (PAI-1). The p3TP-Lux contains elements from the PAI-1 promoter and drives the expression of a luciferase reporter gene in response to TGF-β1 (26). COS-7 cells were transiently transfected with p3TP-Lux and the indicated receptor constructs, or with empty vector pcDNA3 (see Fig. 5). A truncated TbR-I construct (TbR-I(M)) that lacks the cytoplasmic GS and kinase domains but contains the transmembrane and extracellular domains was generated by PCR using rat TbR-I cDNA (6) as a template, using a similar strategy as previously described for the kinase-deleted TbR-II construct (TbR-II(M)) (5), and cloned in pcDNA3. Primer sequences used to amplify the TbR-I(M) construct were as follows: sense primer, 5'-ACGGGTACCCATGAGGGCGCGCCTCGGTCTTCT-3', antisense primer, 5'-GGCGCTCTAGAGCGGCTTGGTATGGCGCGG-3'. They contained the sequences for the restriction enzymes Kpn I and Xba I, respectively (underlined), for directional cloning, and a stop codon in the antisense primer. Control reporter vector pRL-CMV (Promega) was included in all transfections as an internal control to normalize transfection efficiency. Forty-eight hours after transfection using Lipofectin, cells were incubated for 20 h in the absence or presence of TGF-β1 (1 ng/ml). Luciferase activities in cell lysates were measured using the dual-Luciferase reporter assay system (Promega) in a Monolight 2010 luminometer (Analytical Luminescence Laboratory, Ann Arbor, MI). All experiments were performed at least three times.

RESULTS

Identification of a soluble TbR-I. A 333-bp rat cDNA fragment obtained by RT-PCR, containing nucleotides
of the extracellular region and homologous to the human ALK-5 cDNA (12), was used as a probe to screen a neonatal rat kidney library to identify cDNAs encoding variant forms of TβR-I that differ in their extracellular ligand binding domains. A schematic representation of the three cDNA clones isolated is shown in Fig. 1A. All three rat cDNA clones were identical in the extracellular ligand binding domain with the exception of the COOH-terminal region. Clone 12-1 was found to be more than 95% homologous with the human ALK-5 at the amino acid level. In clone 21-1, an insert of 12 nt corresponding to amino acid residues GPFS was noted just upstream to the transmembrane domain. The remainder of the sequence was identical to clone 12-1, including the single hydrophobic transmembrane spanning domain. Interestingly, in clone 29-1, the divergent region in the extracellular domain also differed. It contained an insert of 15 nt, corresponding to amino acid residues GKLLY, followed by an in-frame stop codon. The remainder of the sequence downstream from the stop codon contained no homologous regions with the other two clones, and no sequence that would predict a transmembrane or a serine/threonine kinase domain was found in clone 29-1. The nucleotide sequence, including the flanking untranslated regions, and the deduced amino acid sequence are shown in Fig. 1B. Like the membrane-anchored form of ALK-5, clone 29-1 contained an NH₂-terminal signal peptide domain. Based on the nucleotide sequence of clone 29-1, the deduced amino acid sequence predicted a soluble form of TβR-I.

In vivo expression of stTβR-I mRNA. To determine whether mRNA corresponding to the stTβR-I is endogenously expressed in vivo, RT-PCR followed by Southern blot analysis were performed with rat neonatal and adult kidney tissue samples. As illustrated in Fig. 2, A and B (top), primer pairs A and C should only amplify sequences homologous to the soluble form of ALK-5, a

![Diagram](https://example.com/diagram.png)

**Fig. 1. A**: schematic illustration of human activin receptor-like kinase (ALK)-5 and 3 variant forms of rat ALK-5 cDNAs. Human ALK-5 cDNA and rat cDNA clones 12-1 and 21-1 are membrane-anchored forms of transforming growth factor-β type I receptor (TβR-I). Clone 29-1 represents soluble form of TβR-I, lacking transmembrane and cytoplasmic domains. Note divergent region in COOH-terminal region of extracellular domain of 3 cDNA clones. Horizontal arrows denote primers A and B, used to generate a cDNA probe to screen the library, and primer C, the additional primer used to perform RT-PCR. Horizontal solid bars denote 2 cDNA probes (Hind III/Sph I and Sph I fragments of clone 29-1 used for Southern analysis) and the antisense riboprobe containing the Hind III/Sma I segment of clone 29-1 used for RNase protection assay. B: nucleotide and deduced amino acid sequences of soluble form of TβR-I (stTβR-I) cDNA from rat. Nucleotides and amino acids are numbered at beginning and end of each line, respectively. The protein coding sequence is indicated by uppercase letters, with +1 position nucleotide denoting the start codon (ATG). NH₂-terminal hydrophobic signal sequence is enclosed by arrows, and potential N-glycosylation site is underlined. Divergent amino acid residues are in boldface letters, followed by in-frame stop codon (TAG). Extracellular cysteine residues are circled. Flanking untranslated nucleotide sequences are shown by lowercase letters.
predicted product 430 bp in length. Primers A and B are expected to amplify sequences corresponding to the membrane-anchored form of ALK-5, with a predicted product 333 bp long. As controls, 29-1 and 21-1 cDNAs were used as templates. Primers A and C would only amplify the 430-bp product with clone 29-1 and not clone 21-1. Conversely, primers A and B would amplify the 333 bp with clone 21-1 cDNA and not clone 29-1.

To determine specificity of the RT-PCR products, Southern blot analysis was performed after RT-PCR with cDNA probes as indicated by the horizontal bars in Fig. 1A. Note that the 32P-labeled probe from Hind III/Sph I fragment of clone 29-1 cDNA is specific for the soluble form of ALK-5 and would hybridize only with the amplified products 430 bp in length corresponding to the sTβR-I, as illustrated by solid bands in Fig. 2A, top, and not with the 333-bp products (open bands). The second probe generated from Sph I fragment of clone 29-1 cDNA, which overlaps the extracellular domains of both membrane-anchored and soluble forms of ALK-5, would be predicted to hybridize with both the 333-bp and 430-bp amplified products (Fig. 2B, top). As predicted, the probe 29-1 Hind III/Sph I specifically hybridized only with the 430-bp products, corresponding to the sTβR-I (Fig. 2A, bottom), whereas the 29-1 Sph I probe hybridized both 430-bp and 333-bp products (Fig. 2B, bottom).

Further confirmation of in vivo expression of the sTβR-I mRNA was provided by RNase protection assay. The 337-nt riboprobe contained 304 nt of authentic rat sTβR-I sequence and included the extracellular ligand-binding domain that overlapped with 252 nt of the membrane-anchored receptor and a portion of the 3'-untranslated region (Fig. 3, lane 1). The predicted 304-bp-long protected fragments were detected in both the neonatal and adult kidneys, corresponding to sTβR-I (Fig. 3, lanes 3–8). A second, slightly smaller protected fragment was also observed in lanes 3–8 and may represent an alternatively spliced variant. The 252-bp-long protected fragments, corresponding to the membrane-anchored TβR-I, were seen in both the neonatal and adult kidneys. In addition, note that there was greater mRNA abundance of both TβR-I and sTβR-I in the neonatal kidneys compared with the adult kidneys.

Overexpression of sTβR-I in COS-7 cells. COS-7 cells were stably transfected with a construct containing the coding sequence for the rat sTβR-I ligated in pcDNA3, either alone or cotransfected with a full-length, wild-type rat TβR-II cDNA. Wild-type COS-7 cells and cells transfected with empty vector pcDNA3 were used as controls. mRNA expression of the sTβR-I construct was confirmed by RNase protection assay using the same riboprobe complementary to clone 29-1 from Hind III to Sma I sites, which contained 268 nt of the coding sequence of sTβR-I. As expected, no protected fragments were observed in control wild-type COS-7 cells (data not shown) and in cells transfected with empty vector alone (Fig. 3, lane 9). The predicted 268-bp protected fragments were observed in COS-7 cells transfected with sTβR-I construct alone (Fig. 3, lane 10) and in cells cotransfected with sTβR-I and TβR-II constructs (Fig. 3, lane 11).

sTβR-I binds TGF-β1 in the presence of TβR-II. Cell-surface ligand binding is demonstrated by affinity cross-linking with 125I-labeled TGF-β1. Two distinct bands, approximately 97 and 70 kDa in size, corresponding to TβR-II and TβR-I, respectively, are detected in wild-type COS-7 cells (data not shown) and in cells transfected with empty vector (Fig. 4, lane 2). In COS-7 cells transfected with full-length TβR-II cDNA, a more intensely labeled 97-kDa band is observed (Fig. 4, lane 4). Because TβR-I alone did not exhibit significant binding of TGF-β1 ligand when assessed by cross-linking analysis (27), COS-7 cells were cotransfected with full-length TβR-II and sTβR-I cDNAs. In these cells, additional specifically labeled bands of lower molecular weight are seen (Fig. 4, lanes 6 and 8), corresponding to sTβR-I. Preincubation with unlabeled TGF-β1 demonstrates specificity of ligand binding. Based on the deduced amino acid sequence of clone 29-1, the sTβR-I protein has a predicted molecular mass of ~12 kDa. It also contains a potential N-glycosylation site, as indicated in Fig. 1B. Hence, the 45-kDa bands observed by affinity cross-linking are
interpreted to represent glycosylated sTβR-I protein cross-linked to TGF-β dimer. Evidence for glycosylation of the ALK-5 protein, which also contains the same potential site for N-glycosylation in the extracellular domain, has been previously shown by enzymatic deglycosylation of the affinity cross-linked complexes using endoglycosidase F (12).

TGF-β1 signaling. To explore the potential function of sTβR-I, the effects of its expression on TGF-β1 signaling were determined using the luciferase reporter plasmid, p3TP-Lux (Fig. 5). Transient transfection of p3TP-Lux and empty vector pcDNA3 (as controls) into COS-7 cells resulted in the induction of luciferase activity by exogenous TGF-β1, presumably
Fig. 5. Transcriptional activation of p3TP-Lux reporter by TGF-β1. COS-7 cells were transiently transfected with luciferase reporter p3TP-Lux and expression plasmids for indicated receptors, or with parental vector pcDNA3. Forty-eight hours after transfection, cells were treated with (+) or without (−) exogenous TGF-β1 (1 ng/ml) for another 20 h. Luciferase activity in cell lysates was measured in a luminometer, and results are expressed in relative light units. Data represent means ± SD for triplicate determinations from a representative experiment. pcDNA3, empty vector control; sTβR-I, soluble type I receptor; TβR-II, wild-type full-length type II receptor; TβR-IΔK, kinase-deleted dominant negative mutant type I receptor; TβR-IIΔK, kinase-deleted dominant negative mutant type II receptor.

due to endogenous expression of TGF-β receptors in these cells. Remarkably, in cells transfected with sTβR-I and p3TP-Lux, further enhancement of TGF-β1 signaling was observed, with consistently higher levels of TGF-β1-induced luciferase activity observed in cells expressing sTβR-I compared with pcDNA3 controls. In cells transfected with full-length TβR-II and p3TP-Lux, increased basal luciferase activity was observed, which increased little with exogenous TGF-β1 addition. Similarly, in cells cotransfected with full-length TβR-II and sTβR-I, and p3TP-Lux, basal luciferase activity was increased, but in the presence of exogenous TGF-β1, further induction of luciferase activity above basal levels was noted. TGF-β1 responsiveness was completely abrogated when signaling was blocked by transfection of a dominant negative mutant TβR-IΔK or TβR-IIΔK.

**DISCUSSION**

TGF-β1 exerts diverse biological activities through the interaction with a heteromeric signaling complex consisting of TβR-I and TβR-II (28). Given that TβR-II is unable to mediate TGF-β1 signaling without TβR-I, the specificity of cellular response to TGF-β1 is thought to be determined by TβR-I. One plausible mechanism whereby different sets of cellular responses may be elicited by a single regulatory molecule is the interaction of the type II receptor with different type I receptors, or ALKs, to form the heteromeric signaling complex. Although ALK-5, ALK-1, and ALK-2 have all been demonstrated to complex with ligand-bound TβR-II, ALK-5 is the predominant TβR-I in most cell types (12).

Alternatively, diversity of TGF-β actions may be mediated by TβR-II interacting with variant forms of the signaling TβR-I subtype. This study reports the identification of three variant forms of ALK-5. Two of the cDNA clones encoded membrane-anchored forms of TβR-I but differed in the COOH-terminal region of the extracellular ligand-binding domain. One clone contained an in-frame insertion of four amino acid residues, GPFK, not present in the second clone. At precisely the same position in the extracellular domain, where the nucleotide sequences diverged in the two membrane-anchored forms of ALK-5, the third cDNA clone contained an insert of five amino acid residues, GKLKY, followed by an in-frame stop codon, and lacked the entire transmembrane and cytoplasmic serine/threonine domains. Based on the nucleotide sequence of this clone, the deduced amino acid sequence predicted a secreted form of ALK-5, named sTβR-I. In vivo expression of the sTβR-I mRNA was demonstrated in the neonatal and adult rat kidneys by RT-PCR followed by Southern analysis (Fig. 2), and confirmed by RNase protection assay (Fig. 3).

To date, a naturally occurring soluble form of TGF-β receptor had not previously been reported. Curiously, similar variant receptor forms, including a naturally occurring soluble form, have been described for another cytokine, fibroblast growth factor (FGF) (8). The FGF receptor 1 (FGFR-1) gene contains three alternative exons encoding the COOH-terminal portion of the extracellular region (13). One of these alternative exons includes a stop codon and therefore encodes a secreted form of FGFR-1, and the other two exons encode membrane-anchored forms of FGFR-1 with the characteristic intracellular tyrosine kinase domain. As with the FGFR-1, the variant forms of TβR-I described here may arise from three alternatively spliced exons at the position in the extracellular domain where the nucleotide sequences diverge. The published human TβR-I gene consists of nine exons, and alignment with the nucleotide sequences for the three variant forms of ALK-5 described in this report reveals that the point of sequence divergence occurs precisely between exons 2 and 3, located just upstream of the transmembrane domain (24). Thus it is likely that the three variant receptor forms of ALK-5 are produced by major alternative splicing. Moreover, given the high degree of nucleotide sequence homology between the human and rat TβR-I, indicating that the gene is highly conserved during evolution, it is likely that similar alternatively spliced forms of ALK-5 also occur in humans.

The significance for the existence of the variant forms of TβR-I is yet unknown but may represent an endogenous mechanism to carefully and precisely control and modulate the activity of TGF-β. Given that the multiple receptor forms vary in their extracellular ligand-binding domain, a potential physiological function may be to confer specificity for binding TGF-β ligands. Alternative exons conferring ligand-binding specificity by altering ligand affinity have been demonstrated for the FGF receptors. For instance, an alternatively spliced variant of FGFR-2, named keratinocyte growth factor (KGF) receptor, binds acidic FGF with higher affinity than basic FGF (17), in contrast to the bek form of
FGFR-2, which bound both basic and acidic FGF with high affinity (7). Moreover, a secreted form of FGFR-1 protein, when expressed in Chinese hamster ovary cells, oligomerized on ligand binding and preferentially bound basic FGF over acidic FGF (8).

In the case of TGF-β receptors, although TβR-II is required for initial binding of TGF-β1 and thus is said to possess ligand-binding specificity (27), additional ligand specificity may be determined by the particular form of TβR-I involved in the heteromeric signaling complex with TβR-II, perhaps by differing affinities for the three isoforms of TGF-β. During embryonic development, all three isoforms, TGF-β1, -2, and -3, have been shown to be temporally and spatially regulated and differentially expressed in embryonic tissues including the kidneys (19). Both TβR-I and TβR-II have also been shown to be temporally and spatially regulated and differentially expressed in developing kidneys (6). It is possible that each of the multiple forms of TGF-β receptors may possess specific roles and patterns of expression during embryonic development and may mediate different functions. Interestingly, the stTβR-I mRNA expression was observed to be greater in abundance in the rat neonatal kidneys compared with the adult kidneys, similarly to a previously reported (6) developmental study of TβR-I mRNA abundance, thus implicating a role for stTβR-I in renal development.

Soluble receptors may potentially act as either antagonists or potentiators of cytokine activities. Inhibitory actions by soluble receptors, presumably through competition with membrane-anchored receptors for ligand binding, have been demonstrated for soluble receptors of interleukin (IL)-4, platelet-derived growth factor (PDGF)-α, and vascular endothelial cell growth factor (VEGF) (14, 18, 23). In contrast, potentiating action of IL-6 by its soluble receptor, IL-6sR, to markedly induce collagenase 3 expression in osteoblasts has also been demonstrated (11).

In this report, the functional role of stTβR-I in TGF-β signaling was explored using a TGF-β-inducible luciferase reporter assay. First, the ability of stTβR-I to complex with ligand-bound TβR-II on the cell surface was demonstrated by affinity cross-linking with 125I-labeled TGF-β1 in COS-7 cells expressing both the stTβR-I and TβR-II. Next, the effects of its expression on TGF-β1 signaling were determined using the luciferase reporter plasmid, p3TP-Lux, to ascertain TGF-β1-induced transcriptional activation of the PAI-1 promoter. Transient transfection of stTβR-I with p3TP-Lux in COS-7 cells resulted in higher luciferase activity in response to exogenous TGF-β1 compared with control COS-7 cells transfected with empty vector pcDNA3 and p3TP-Lux. This increased TGF-β1 responsiveness demonstrates the ability of stTβR-I to potentiate signaling by the endogenously expressed wild-type TGF-β receptors. To determine whether TGF-β1 responsiveness could be further enhanced by increased levels of TβR-II and ligand binding, the effects on TGF-β1 signaling were assayed on transfection of TβR-II and in combination with stTβR-I. Transient transfection of wild-type TβR-II in COS-7 cells resulted in higher basal luciferase activity, indicating that high-level expression of the receptors can initiate TGF-β signaling, due to either the presence of endogenous TGF-β1 or the inherent affinity of the receptors to interact and cooperate with each other to signal in a ligand-independent manner. Transfection and overexpression of wild-type TβR-I have previously been shown to exhibit ligand-independent activation (4). The inability of exogenous TGF-β1 to further increase luciferase activity in TβR-II-transfected cells suggests probable saturation of the signaling receptors with the ligand. However, exogenous TGF-β1 was able to induce increased luciferase activity upon expression of stTβR-I, by cotransfection of TβR-II with stTβR-I, but not above the levels observed in the stTβR-I-transfected cells. Taken together, these data suggest that the signaling TβR-I may be expressed in limiting levels and that the stTβR-I functions as a potentiator but not as a direct signaling receptor, as it lacks the cytoplasmic domain. Next, the effects of stTβR-I were compared with those of another truncated form, TβRI-M, which is membrane anchored but lacks the cytoplasmic GS and kinase domains and inhibits TGF-β1 signaling. As predicted, complete inhibition of TGF-β1-induced luciferase activity was observed in cells expressing the truncated receptors, TβRI-M. Thus the stTβR-I actions clearly differ from those of the kinase-deleted mutant TβRI-M, which inhibits TGF-β1 signaling, presumably by functioning in a dominant negative fashion to compete with membrane-anchored TβR-I for complexing with the ligand-bound TβR-II. Similarly, transfection of a dominant negative TβRI-M also inhibited TGF-β signaling.

The stimulatory action exhibited by a soluble type I receptor for TGF-β is rather intriguing. In contrast to IL-6sR, which binds IL-6 directly to potentiate its action, stTβR-I and TβR-I require the presence of TβR-II for ligand binding. Thus the soluble form of TβR-I presumably functions as an agonist to augment TGF-β1 actions through an entirely novel mechanism of action not yet understood. The secreted stTβR-I might serve as a chaperone, for instance, by recognizing and interacting with ligand-bound TβR-II to stabilize the ligand-bound complex and prevent its dissociation and degradation, and may cooperate to facilitate the recruitment of the membrane-anchored TβR-I to form the signaling heteromeric complex. The soluble form of receptor may then dissociate and allow the membrane-anchored TβR-I to form a more stable complex with ligand-bound TβR-II to allow transphosphorylation and initiation of the signaling cascade. The concept of agonist activity of a natural stTβR-I to potentiate TGF-β1 actions has important clinical implications for its potential role in a wide range of biological processes, such as growth and development, wound repair, and pathogenesis of fibrotic diseases. The present studies demonstrate upregulation of stTβR-I expression in the developing kidney, potentially to enhance TGF-β signaling and thereby amplify TGF-β actions during renal development.

In summary, three variant forms of TβR-I were isolated from a rat neonatal kidney library, and data presented in this report demonstrate for the first time...
the existence of a naturally occurring variant TβR-I transcript whose nucleotide sequence and deduced amino acid sequence predict a soluble form of ALK-5. Physiological functions of these variant receptor forms are not yet known, but their variable in vivo expression may represent a mechanism conferring multiple cellular responses to TGF-β1. Moreover, the biological importance of a soluble TβR-I is yet to be determined, but the findings here suggest that it may serve as a natural potentiator of TGF-β signaling. The molecular cloning and identification of a naturally occurring stTβR-I adds a new level of complexity to our present knowledge of the TGF-β receptor system and will facilitate further investigations to expand our understanding of the mechanism of TGF-β1 actions.

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