Binding Affinity of Transforming Growth Factor-β for Its Type II Receptor Is Determined by the C-terminal Region of the Molecule*

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Transforming growth factor-β (TGF-β) isoforms have differential binding affinities for the TGF-β type II receptor (TβRII). In most cells, TGF-β1 and TGF-β2 bind to TβRII with much higher affinity than TGF-β3. Here, we report an analysis of the effect of TGF-β structure on its binding to TβRII by using TGF-β mutants with domain deletions, amino acid replacements, and isoform chimeras. Examination of the binding of TGF-β mutants to the recombinant extracellular domain of TβRII by a solid-phase TGF-β/TβRII assay demonstrated that only those TGF-β mutants containing the C terminus of TGF-β1 (TGF-β1(-Δ69–73), TGF-β1-(Trp71), and TGF-β2/β1-(83–112)) bind with high affinity to TβRII, similar to native TGF-β1. Moreover, replacement of only 6 amino acids in the C terminus of TGF-β1 with the corresponding sequence of TGF-β2 (TGF-β1/β2-(91–96)) completely eliminated the high affinity binding of TGF-β1. Proliferation of fetal bovine heart endothelial (FBHE) cells was inhibited to a similar degree by all of the TGF-β mutants. However, recombinant soluble TβRII blocked the inhibition of FBHE cell proliferation induced by TGF-β mutants retaining the C terminus of TGF-β1, consistent with the high binding affinity between these TGF-β molecules and TβRII. It was further confirmed that the TGF-β2 mutant with its C terminus replaced by that of TGF-β1 (TGF-β2/β1-(83–112)) competed as effectively as TGF-β1 with 125I-TGF-β1 for binding to membrane TβRI and TβRII on FBHE cells. These observations clearly indicate that the domain in TGF-β1 responsible for its high affinity binding to TβRII, both the soluble and membrane-bound forms, is located at C terminus of the molecule.

Transforming growth factor-βs (TGF-βs) are multifunctional peptides that regulate many cellular processes, including growth, differentiation, inflammation, immunosuppression, and expression of extracellular matrix proteins (1–4). The biologically active mammalian TGF-βs are dimeric molecules (usually homodimers) consisting of disulfide-linked monomers that are derived by cleavage of the 112 C-terminal amino acids from a precursor polypeptide (1, 3). Three distinct isoforms of TGF-β (β1, β2, and β3) have been identified in mammalian cells (1). They show remarkable structural homology between each other, including 9 conserved cysteine residues that form four intrachain disulfide bonds (Cys3-Cys39, Cys15-Cys76, Cys44-Cys109, and Cys48-Cys111) and one interchain disulfide bond at Cys77, and high amino acid sequence identity: >98% among the same isoforms across species and 71–76% among different isoforms. The existence of these highly conserved TGF-β isoforms across species implies very important specific roles for each isoform in vivo. Indeed, although TGF-β isoforms have a similar receptor-binding pattern and are indistinguishable in most in vitro biological assays, recent studies indicate that the TGF-β isoforms can also have selective actions in appropriate systems. For example, TGF-β1 and TGF-β3, but not TGF-β2, strongly inhibit the growth of some endothelial cells (5, 6) and hematopoietic cells (7, 8). Similarly, TGF-β1 inhibits growth of the human colorectal cancer cell line LS513, while TGF-β2 has almost no effect on the growth of these cells (9). In contrast, TGF-β2 and TGF-β3, but not TGF-β1, inhibit the survival of cultured chick ciliary ganglionic neurons (10).

The differences in potency of TGF-β isoforms may correlate with specificity in the interactions between these isoforms and their cell-surface receptors.

The signaling receptors for TGF-β, type I receptors (TβRI) (53 kDa) and type II receptors (TβRII) (70–85 kDa), both contain extracellular ligand-binding, transmembrane, and intracellular Ser/Thr kinase domains (11, 12). It has been shown that TβRI and TβRII associate as interdependent components of a heteromeric complex: TβRI requires TβRII to bind ligand, and both receptors are required for inhibition of cell growth and gene expression by TGF-β (13–17). TGF-β binding to TβRII is required to initiate the TGF-β signaling pathway. Transfection of TβRII into the human breast cancer cell line MCF-7 lacking TGF-β receptors results in the appearance of not only TβRII, but also TβRI, suggesting that TβRII requires TβRI for transport to the cell surface or TGF-β binding, or both (18).

Two other cell-surface TGF-β-binding proteins of known structure are betaglycan (also called type III receptor (TβRIII)) (250–350 kDa) and endoglin (190 kDa) (19–23). Betaglycan is a membrane-bound proteoglycan having a large extracellular domain and a very short cytoplasmic tail (41 amino acids) with no apparent signaling motif. Betaglycan is involved in presentation of TGF-βs to TβRI and TβRII for signaling (24). Endoglin is a disulfide-linked homodimeric glycoprotein composed of two subunits of 95 kDa, showing ~70% structural homology to betaglycan at the transmembrane and short cytoplasmic regions of each subunit (22). It is expressed at high levels in

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vascular endothelial cells (21). The function of endoglin is currently unclear, although its expression is suggested to be critical to placental development (25), and mutation of the endoglin gene has been linked to hereditary hemorrhagic telangiectasia type 1 (26).

Accumulated evidence has shown that TGF-β receptors and membrane-binding proteins have different binding affinities for the TGF-β isoforms. In general, TβRII and TβRII bind more efficiently to TGF-β1 and TGF-β3 than to TGF-β2 (27, 28). Betaglycan has approximately equal binding affinity for all three TGF-β isoforms (24), whereas endoglin binds only to TGF-β1 and TGF-β3, but not to TGF-β2 (22). The difference in receptor binding affinity between TGF-β isoforms may result from conformational differences in their structure or from the specificity of amino acid side chains. TGF-β isoforms share 71–78% identity in their amino acid sequence. A comparison of the solution structure of TGF-β1 with the crystal structure of TGF-β2 has indicated that the backbone structures of TGF-β1 and TGF-β2 are almost identical.² There are, however, several regions of variation in conformation and/or mobility between these isoforms that may be related to their functions (29, 30). In addition, distribution of charged residues on the protein surface varies considerably (30). In our previous studies, TGF-β mutants including isoform chimeras, amino acid substitutions, or deletions were used to identify the specific amino acids (positions 45 and 47) in TGF-β2 responsible for its high affinity binding to α₂(macro)globulin (α₂M) (31, 32) and another region (amino acids 69–73) in TGF-β1 responsible for its binding to receptors on LS513 colon carcinoma cells (33). In this study, we have applied the same techniques and utilized recombinant soluble human TβRII to delineate the specific region(s) in the TGF-β molecule responsible for its high affinity binding to TβRII. Our results show that a C-terminal domain containing amino acids 92, 94, and 98 of TGF-β1 is necessary for specifying its high affinity binding to TβRII.

MATERIALS AND METHODS

Cell Culture

Chinese hamster ovary cells, NIH 3T3 cells, MvILu mink lung epithelial cells, and fetal bovine heart endothelial (FBHE) cells were purchased from American Type Culture Collection (Rockville, MD) and cultured as described (31, 34). MvILu and FBHE cell growth inhibition assays (34, 35) were used to determine the bioactivity of TGF-β mutants and the ability of soluble TβRII to block TGF-β activity.

Synthesis and Purification of Recombinant TGF-β Mutants

All of the recombinant TGF-β mutants were expressed as part of latent TGF-β including the entire coding sequence of the TGF-β gene. Construction of expression plasmids for TGF-β2/β1 (40–82), TGF-β2/β1 (40–47), TGF-β1/β1 (Δ69–73), and TGF-β1/β1 (Δ56, 57) has been previously described (31–33). Expression constructs of TGF-β1/β2 (40–112), TGF-β1/β2 (83–112), TGF-β1/β2 (91–96), TGF-β2/β1 (83–112), and TGF-β2/β1 (40–82) were generated using a two-step overlapping polymerase chain reaction method (31). Recombinant TGF-βs were expressed in NIH 3T3 or Chinese hamster ovary cells, purified by sequential high pressure liquid chromatography (31), and quantitated by silver staining on 10% Tricine gels (Novel Experimental Technology, San Diego, CA). The MvILu cell growth inhibition assay was used to determine the bioactivity of TGF-β mutants in each stage of expression and purification (35).

Synthesis and Purification of Soluble Extracellular Domain of TβRII

The cDNA for the extracellular domain (amino acids 1–159) of the human TGF-β type II receptor (36) was expressed in NIH3T3 mouse myeloma cells or a baculovirus expression system and purified by affinity chromatography using either a TGF-β1 column or a monoclonal anti-human soluble TβRII antibody column (37). The purity of the protein was verified by SDS-polyacrylamide gel electrophoresis and silver staining.

Solid-phase TGF-β/TβRII Binding Assays

Two solid-phase TGF-β/TβRII binding assays were used to measure the binding affinity of TGF-βs for TβRII.

TGF-β Antibody Detection Method—This method was established based on the sandwich enzyme-linked immunosorbent assay for TGF-β. All of the reaction buffers have been described previously (38). 96-well plates (Nunc Maxisorb, Nunc Inc.) were coated with 100 µl of bovine serum albumin in blocking buffer. TGF-β2s at concentrations ranging from 0 to 20,000 pg/ml were blocked to the coated plates and incubated at room temperature for 1 h. After extensive washing, 100 µl of antibodies (100 ng/ml) to TGF-β1 (or TGF-β2 conjugated with horseradish peroxidase (R&D Systems, Minneapolis, MN) was added to the plates and incubated at room temperature for another hour. The plates were washed with washing buffer and further with distilled water. Peroxidase substrate 3,3',5,5'-tetramethylbenzidine solution (1-Step® Turbo TMB, Pierce) was added to the plates (100 µl/well) to react with horseradish peroxidase at room temperature for 30 min and further developed by adding 100 µl of 1 N H2SO4 to produce a bright yellow solution. TGF-β binding was quantified by measurement of the absorbance of the solution at 450 nm.

Binding Competition Assay Using Biotinylated TGF-β—Reaction buffers have been described (38). 96-well Nunc Maxisorb plates were coated with 100 µl of 50 ng/ml soluble TGF-β2 at 4 °C overnight and then blocked with bovine serum albumin as described above. TGF-βs at serially diluted concentrations ranging from 0 to 500 ng/ml mixed with a fixed amount (5 ng/ml) of biotinylated TGF-β1 (R&D Systems) were added to the TβRII-coated plates and incubated at room temperature for 1 h. After extensive washing, 100 µl/well avidin-conjugated alkaline phosphatase (Pierce) in 1:1000 dilution in Tris-buffered saline (0.3% crystalline bovine serum albumin, 10 mM Tris, 150 mM NaCl, pH 7.4) was added to the plates and incubated at room temperature for 1 h. The plates were washed with washing buffer followed by distilled water. 100 µl/well alkaline phosphatase substrate, p-nitrophenyl phosphate (1 mg/ml in 10 mM diethanolamine solution) (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD), was added to the plates and incubated at room temperature until a bright yellow color developed. The signal from biotinylated TGF-β1 bound to TβRII was determined by measurement of the absorbance of the reaction mixture at 405 nm.

FBHE Cell Growth Inhibition Assay

The FBHE cell growth inhibition assay (34) was performed in 0.2% fetal bovine serum medium, in which TGF-β1 and TGF-β2 showed similar activity on inhibition of FBHE cell growth (32). In this assay, 10 µM TGF-βs, which inhibits 90% incorporation of [3H]thymidine (DuPont NEN) at 0.25% × 106 FBHE cells in 48-well dishes together with increasing concentrations of soluble TβRII ranging from 24 to 6000 ng/ml.

Receptor Cross-linking Competition Analysis

FBHE cells were cross-linked with 25 µM [35S]labeled TGF-β1 (39) and nonradioactive TGF-βs at concentrations of 25, 50, 100, 250, and 500 pg/ml as described (40, 41). Proteins in the cell lysate were reduced and separated on 5% SDS-polyacrylamide gel. [35S]TGF-β1-cross-linked receptors were visualized by autoradiography with a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA).

RESULTS

Differential Binding Affinity of TGF-β Isoforms for Soluble Type II Receptor—TGF-β1 and TGF-β3 bind with much higher affinity than TGF-β2 to the type II receptor (28). To identify specific high affinity epitopes of TGF-β1, we utilized the recombinant extracellular domain of the human type II receptor for solid-phase TGF-β/TβRII binding assays. We first measured the binding of native TGF-β1 and TGF-β2 in this assay. In each experiment, the plates were coated with an increasing amount (5, 10, 25, 50, or 100 ng/well) of TβRII, and the binding of TGF-β to the receptor was quantitated using a specific antibody against TGF-β1 or TGF-β2. Each antibody detects the corresponding TGF-β isoform with the same efficiency, as demonstrated in enzyme-linked immunosorbent assay experiments.² As shown in Fig. 1, TGF-β1 binds soluble TβRII at all

² M. L.-S. Tsang, unpublished data.
Fig. 1. Differential affinity binding of TGF-β isoforms to soluble TβRII.
96-well plates were coated with 0, 5, 10, 25, 50, or 100 ng/well recombinant soluble TβRII. Increasing concentrations of TGF-β1 (A) or TGF-β2 (B) were added to the plates for binding. TGF-β binding to TβRII on the plates was detected using TGF-β1- or TGF-β2-specific antibodies conjugated with horseradish peroxidase. Dose-response curves were measured by the absorbance of the horseradish peroxidase substrate 3,3',5,5'-tetramethylbenzidine chromophore measured at 450 nm. All samples were tested in triplicate, and the standard error of TGF-β binding was <5% of the mean value for each sample.

of the different coating concentrations, whereas under the same conditions, the binding of TGF-β2 is detectable only at the highest coating concentration of TβRII (100 ng/well). As a control, TGF-β1 or TGF-β2 was added to the plates without the coating of soluble TβRII. No significant binding was detected, suggesting that TGF-β binding in this assay is TβRII-dependent. These data are consistent both with the higher binding affinity of TGF-β1 for TβRII expressed on the cell membrane (28) and with the affinity of soluble TβRII for TGF-β isoforms reported recently by Lin et al. (42), where the apparent dissociation constant (Kd) for binding to TGF-β1 was ~200 pm, while that for TGF-β2 was undetectable.

Fig. 1 also shows that the binding of TGF-β1 increases with the coating concentration of TβRII in the range of 5–25 ng/well, but does not change significantly when the coating concentration increases further from 25 to 100 ng/well. Our interpretation of these results is that the amount of recombinant TβRII coated on the plates at higher concentrations (25, 50, and 100 ng/well) is in excess for TGF-β1 binding and that, at these concentrations, the TGF-β1-binding signal is determined by the TGF-β1 concentration alone. In contrast, at lower coating concentrations (5 and 10 ng/well) of TβRII, the TGF-β1-binding signal is a function not only of the TGF-β1 concentration, but also of the amount of recombinant TβRII coated on the plates.

The binding of TGF-β3 to recombinant TβRII was not measured by this method because of lack of appropriate antibodies. However, we show below that TGF-β3 binds to recombinant soluble TβRII in a ligand binding competition assay with an affinity similar to that of TGF-β1 (see Fig. 3D).

Analysis of Binding Affinities of TGF-β Mutants for Recombinant TβRII—To determine the specific domain(s) in the TGF-β1 molecule responsible for the high affinity binding of TGF-β1 to TβRII, we generated a series of TGF-β mutants by recombinant techniques (Fig. 2). Each TGF-β mutant was expressed as part of its latent complex including the entire proregion of TGF-β to ensure the correct folding of the mature active TGF-β molecule. Since specific antibodies against TGF-β1 or TGF-β2 do not discriminate between most of the TGF-β chimeras, we developed a novel competition assay to determine the binding affinity of these mutants for soluble TβRII by using a fixed amount (5 ng/ml) of biotinylated TGF-β1 and increasing the amount of unlabeled TGF-β mutants. To increase the sensitivity of competition by TGF-β mutants, we coated 96-well plates with 5 ng/well TβRII, at which concentration TβRII is not in excess for TGF-β1 binding (Fig. 1A). As expected, TGF-β1, but not TGF-β2, competes with biotinylated TGF-β1 for binding to soluble TβRII. Four groups of TGF-β mutants were then tested for their ability to compete with biotinylated TGF-β1 for binding to soluble TβRII. Fig. 3A shows that the two TGF-β1 mutants TGF-β1-(Δ69–73) and TGF-β1-(Trp72)2, which were designed to investigate the possibility that the exposed loop of TGF-β centered at amino acid 71 participates in TGF-β receptor binding (33), competed with biotinylated TGF-β1 for binding to TβRII, suggesting that this loop is not an important epitope. Fig. 3D shows that two chimeras, TGF-β2/β1-(40–82) and TGF-β2/β1-(40–47), in which the high affinity αM-binding domain in TGF-β2 was replaced by the corresponding domain in TGF-β1 (32), failed to compete with biotinylated TGF-β1 for binding to TβRII. This result suggests that the region responsible for the high affinity binding of TGF-β1 to TβRII does not overlap with the domain determining the binding affinity for αM.

We further tested other chimeras (TGF-β1/β2-(40–112), TGF-β1/β2-(83–112), TGF-β1/β2-(91–96), and TGF-β2/β1-(83–112)) in which the C-terminal and N-terminal domains of TGF-β1 and TGF-β2 have been substituted. Fig. 3C shows that TGF-β2/β1-(83–112) effectively competes with biotinylated TGF-β1 for binding to soluble TβRII, suggesting that the C terminus of TGF-β1 is the region responsible for the high affinity binding of TGF-β1 to soluble TβRII. The mirror-image chimera TGF-β1/β2-(83–112) and another chimera, TGF-β1/β2-(91–96), which contains only 6 amino acids of TGF-β2 substituted into the C terminus of TGF-β1, also did not compete, providing further evidence for the importance of the C terminus of TGF-β1. Fig. 3D shows that TGF-β3 also competes with biotinylated TGF-β1, whereas TGF-β2/β3-(40–82) does not, in agreement with the result shown in Fig. 3B.

Blocking Effect of Soluble TβRII on Inhibition of FBHE Cell Proliferation by TGF-β Mutants—To evaluate further the importance of the C terminus of TGF-β1 in binding to soluble TβRII, we tested the ability of soluble TβRII to reverse the inhibition of growth of FBHE cells by TGF-β chimeras modified at the C-terminal region. FBHE cells were selected for this assay because they express very little betaglycan (34), allowing soluble TβRII to efficiently block access of TGF-β1 and TGF-β mutants to cell membrane TβRII and thus reverse the inhibition of FBHE cell proliferation induced by TGF-β. In Mv1Lu cells, which express a high level of betaglycan, very high amounts of soluble TβRII are required to block the activity of TGF-β (data not shown). In this study, 10 psTGF-β3s, which results in ~90% inhibition of [3H]thymidine incorporation in FBHE cells, was added to these cells together with increasing amounts of soluble TβRII. Fig. 4 shows that recombinant soluble TβRII efficiently blocks the inhibition of FBHE cell growth induced by TGF-β1 and TGF-β2/β1-(83–112), but not by TGF-β2 and TGF-β1/β2-(83–112). In the absence of added TGF-β, the growth of FBHE cells is not affected by recombinant soluble TβRII (data not shown). These data clearly indicate...
that soluble TβRII has high affinity only for those TGF-β molecules containing the C terminus (amino acids 83–112) of TGF-β1.

Binding Competition of TGF-β Mutants with 125I-TGF-β1 for Membrane TGF-β Receptors on FBHE Cells—To confirm that the C terminus (amino acids 83–112) of TGF-β1 is important for TGF-β1 high affinity binding to cell membrane TβRII, receptor cross-linking competition experiments were performed. FBHE cells were cross-linked with 25 pM 125I-labeled TGF-β1 in the presence of 500, 250, 100, 50, or 25 pm nonradioactive TGF-β1, TGF-β2, or TGF-β2/β1-(83–112). Fig. 5 shows that TGF-β1 and TGF-β2/β1-(83–112), but not TGF-β2, compete with 125I-labeled TGF-β1 for binding to TβRI and TβRII on FBHE cells. In contrast, TGF-β2/β2-(83–112) is ineffective (data not shown). Since it is known that recruitment of TβRI to the TGF-β receptor complex occurs only after binding of TGF-β1 to TβRII (13–17), competition of 125I-labeled TGF-β1 binding to TβRI by nonradioactive TGF-β3 is dependent on the affinity of these TGF-βs to bind TβRII. These data thus confirm that the C terminus of TGF-β1 is important for its high affinity binding not only to soluble TβRII, but also to its membrane-bound form.

**DISCUSSION**

We have shown that the C-terminal region (amino acids 83–112) of TGF-β1 is essential for the high affinity binding of TGF-β1 to recombinant soluble TβRII and have confirmed this finding in living cells. Since most cells express multiple TGF-β receptors, we have used recombinant soluble TβRII, containing only the extracellular domain of this receptor, to investigate the binding pattern of the TGF-β mutants. This method is simple and straightforward and avoids interference from other TGF-β-binding proteins such as betaglycan on the cell membrane.

Comparison of the crystal structure of TGF-β2 (43, 44) with the NMR solution structure of TGF-β1 (29, 30) has indicated that the structures of these two isoforms differ in several regions including amino acids 8–12, 70–76, and 90–98. The C-terminal region (amino acids 83–112) of TGF-β1 reported here, which specifies the high affinity binding of TGF-β1 to TβRII, includes one of these domains (amino acids 90–98). Moreover, Flanders et al. (45) observed that among polyclonal antibodies raised against a series of synthetic peptides corresponding to different regions of TGF-β1, only the antibody to peptide 78–109 effectively blocked binding of TGF-β1 to receptors. Recently, Postlethwaite and Seyer (46) reported that a
7-residue peptide containing amino acids 89–95 of TGF-β1 or larger peptides containing these 7 residues, but not peptides representing other regions of TGF-β1, stimulated chemotactic migration of neutrophils, monocytes, and foreskin fibroblasts. Collectively, these data strongly suggest that the C terminus of TGF-β1 is an important domain for receptor binding.

The data here show that exchange of the C-terminal regions of TGF-β1 and TGF-β2 (i.e. TGF-β1/β2-(83–112) and TGF-β2/β1-(83–112)) can switch binding patterns of TGF-β isoforms for TβRII, and TGF-β1 and TGF-β3 bind similarly to TβRII. This suggests that amino acids identical in the C-terminal regions of TGF-β1 and TGF-β3, but different in TGF-β2, might be responsible for the differential binding affinities of TGF-β isoforms for TβRII. Comparison of the amino acid sequences of the C-terminal regions (amino acids 83–112) of the three TGF-β isoforms shows that there are only 3 amino acids (positions 92, 94, and 98) that fit into this category. In TGF-β1 and TGF-β3, residues 92, 94, and 98 are valine, arginine, and valine, respectively; in TGF-β2, they are isoleucine, lysine, and isoleucine, respectively (Fig. 6B). Confirming this hypothesis, we were able to demonstrate that the ability of TGF-β1 to bind to soluble TβRII was markedly reduced by substitution of only 6 amino acids (positions 91–96) of TGF-β2 into TGF-β1 (TGF-β1/β2-(91–96)). Whether these residues interact with TβRII directly or whether they can indirectly affect the conformation of the TβRII-binding site on TGF-β is still unknown. Even though the three amino acid substitutions in TGF-β isoforms at positions 92, 94, and 98 are very conservative, change(s) in side chains of these amino acids could affect the conformation of the whole protein. However, this is unlikely since we know that the backbone structures of TGF-β1 and TGF-β2 are almost identical. Also, it is noteworthy that residues 91–98 compose the most extended and flexible region in TGF-β and undergo rapid local internal motion, which is not found in any other regions (30). Thus, it is more likely that this domain is directly rather than indirectly involved in TGF-β binding to TβRII. Whether all 3 amino acids (residues 92, 94, and 98) are critical for high affinity binding of TGF-β1 and TGF-β3 to TβRII, is under further investigation.

Recently, it has been demonstrated that whereas TGF-β1 binds to TβRII expressed alone on cells, TGF-β2 binds only to cells on which both TβRI and TβRII are coexpressed, and then with an affinity comparable to that of TGF-β1 binding (47). These data are consistent with the equal ability of TGF-β1 and TGF-β2 to inhibit proliferation of FBHE cells and the selective ability of soluble TβRII to compete for TGF-βs containing the C terminus of TGF-β1. These data also suggest that the confor-
Epitope Mapping of TGF-β1 High Affinity for TβRII

30661

The biological effect of a particular TGF-β isoform on a given target cell is the product of 1) the selective “presentation” of the isoform to the different binding receptors, governed by the accessory proteins including membrane proteins (e.g. betaglycan) and extracellular proteins (e.g. α2M); and 2) the signaling pathway triggered by the receptor combination that is activated by the TGF-β isoform presented in this manner. Although many models have been proposed for the interactions between TGF-β receptors and between TGF-β ligands and receptors (52, 53), they are all based on data from experiments in which receptors have been overexpressed on the surface of cells transfected with respective cDNAs. The stoichiometry of complexes of TGF-β receptors with or without ligand in vivo is still not well understood. The goals of our research are to identify the specific regions of TGF-β involved in binding to its signal-receptor as well as to accessory proteins that control the accessibility of TGF-β to its signaling receptors. The strategy we have reported here involves study of TβRII as a single component for analysis of ligand binding in vitro and it also extends the observations in more complex cellular systems. Applying this strategy to other TGF-β receptors and accessory proteins will elucidate the complex interactions among ligands, receptors, and other binding proteins, which underlie the different biological potencies of TGF-β isoforms. Moreover, the demonstration of similar three-dimensional structures for TGF-β2 and osteogenic protein 1, a distantly related member of the TGF-β superfamily with ~30% amino acid identity to TGF-βs, suggests that these structure-function studies of TGF-βs will also identify important receptor-binding epitopes in other members of the TGF-β superfamily (54).

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