Betaglycan has multiple binding sites for transforming growth factor- β 1

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The transforming growth factor- β (TGF- β)-binding site in betaglycan, the type III TGF- β receptor, has been variously assigned to the C-terminal half and N-terminal one-third of the extracellular domain. In this study, we show that there are at least two TGF- β -binding sites in betaglycan. Bacterially expressed fragments bg1,2 and bg3, which represent the N-terminal two-thirds and C-terminal one-third of betaglycan extracellular domain, both competed for the binding of 125 I-TGF- β to mink lung epithelial cells. 125 I-bg1,2 bound to immobilized TGF- β with an

affinity about 4-fold higher than bg3 had. Both bg3 and bg1,2 enhanced the bioactivity of TGF- β . The whole ectodomain of betaglycan was more active than either bg3 or bg1,2 in the assays. The binding of 125 I-bg3 to TGF- β was inhibited by bg1,2, and vice versa. The binding of 125 I-bg3 and 125 I-bg1,2 to TGF- β was also inhibited by the small decorin family of proteoglycans. These results indicate that there are at least two binding sites for TGF- β in betaglycan and that these sites recognize the same or overlapping sites in TGF- β .

INTRODUCTION

Transforming growth factor- β (TGF- β) is a disulphide-linked homodimer of a 25 kDa polypeptide that regulates cell proliferation and differentiation, and extracellular matrix formation [1,2].

TGF- β acts through a cellular receptor system that comprises at least three receptors: a 53 kDa type I receptor, a 75 kDa type II receptor and a type III receptor, betaglycan [3]. The cytoplasmic regions of the type I and type II receptors contain a serine/threonine kinase domain [4–7]. TGF- β acts by binding initially to the type II receptor. The type I receptor is then recruited to the TGF- β -type II receptor complex and becomes phosphorylated by the type II receptor, in which the serine/threonine kinase is constitutively active, resulting in stimulation of the downstream signal-transduction cascade [8].

Betaglycan is a transmembrane proteoglycan [9,10] originally identified because its core protein binds TGF- β with high affinity [11]. The deduced primary structure of rat betaglycan reveals a 853 amino acid precursor protein with a large extracellular domain [12,13]. It contains a region similar to endoglin in the N-terminal one-third, and a region similar to uromodulin in the C-terminal one-third [14,15]. Betaglycan is the major TGF- β -binding molecule on most cell types. However, many cell types that respond to TGF- β , such as haematopoietic cells, do not appear to have detectable amounts of betaglycan [16]. Such cells are particularly resistant to TGF- β 2, which is known to depend on betaglycan for high-affinity binding to the type I and II receptors [17,18]. Thus the function of betaglycan appears to be to deliver TGF- β to the signalling receptors [19–21].

Betaglycan binds TGF- β through the core protein [9,10]. The localization of the TGF- β -binding site in betaglycan is unclear. In a previous study, our laboratory localized the TGF- β -binding site of betaglycan into a recombinant fusion protein from the C-terminal one-third (bg3, amino acids 543–769) of the extracellular domain [22]. Pepin and co-workers, using COS-1 cells transfected with various betaglycan deletion mutants, came to a similar

conclusion [23]. However, López-Casillas et al. placed the binding site in the N-terminal half of the molecule [24].

We show here that betaglycan has at least two distinct binding sites for TGF- β . The fragments carrying these binding sites compete with one another, with decorin-type proteoglycans and with an inhibitory anti-TGF- β monoclonal antibody for the TGF- β binding, suggesting that all of these agents bind to the same or adjacent sites in TGF- β .

MATERIALS AND METHODS

Materials

Recombinant human TGF- β 1 was generously provided by Dr. Daniel Twardzik (Oncogen, Seattle, WA, U.S.A.). Neutralizing chicken anti-(human TGF- β 1) polyclonal antibody was purchased from R&D Systems (Minneapolis, MN, U.S.A.) and mouse anti-(bovine TGF- β) monoclonal antibody was from Genzyme (Cambridge, MA, U.S.A.). Bolton–Hunter-labelled 125 I-human TGF- β 1, carrier-free Na 125 I, [3 H]thymidine and [35 S]sulphate were from DuPont–NEN (Burlington, MA, U.S.A.). Bacterial expression vector pQE8, 9, 11 and 42, *Escherichia coli* M15 host cells and Ni-nitrilotriacetate–agarose were from Qiagen (Chatsworth, CA, U.S.A.). Disuccinimidyl suberate (DSS) was from Pierce Chemical Co. (Rockford, IL, U.S.A.), and isopropyl β -D-thiogalactopyranoside (IPTG) was from Diagnostic Chemicals (Charlottetown, Canada). All other chemicals were purchased from Sigma (St. Louis, MO, U.S.A.).

Mink lung epithelial cells (Mv1Lu) were from the American Type Culture Collection (CCL 64). Rat smooth muscle cells were derived from rat aorta and the cells between 5 and 20 passages were used.

PD-10 columns were from Pharmacia (Uppsala, Sweden), Immulon-2 96-well microtitre plates were from Dynatech Laboratories (Chantilly, VA, U.S.A.), and SDS/polyacrylamide precast gels were from Novex (San Diego, CA, U.S.A.). The core proteins for human decorin, biglycan and fibromodulin were

Abbreviations used: TGF- β , transforming growth factor- β ; DSS, disuccinimidyl suberate; IPTG, isopropyl β -D-thiogalactopyranoside; BSA, bovine serum albumin.

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expressed bacterially as fusion proteins with maltose-binding protein and purified as described previously [25].

Betaglycan fusion proteins

Betaglycan fragments were cloned into the pQE vector and expressed as fusion proteins with a cassette of six histidines.

The bg1, bg2 and bg3 constructs were generated as described previously [22]. Briefly, various portions of the DNA sequences from rat betaglycan were amplified by PCR (Coy, Model TempCycler) with an appropriate tag (*Bam*HI site) using cDNA derived from rat smooth muscle cells as a template. The bg1 fragment (amino acids 24–265) was obtained from the PCR products by *Bam*HI digestion, bg2 (amino acids 285–513) by *Bcl*II/*Sal*I digestion and bg3 (amino acids 543–769) by *Bam*HI digestion. The bg1 and bg3 fragments were subcloned into the *Bam*HI site of pQE8 vector and bg2 into the *Bam*HI/*Sal*I site of pQE11.

The bg1,2 (amino acids 24–513), bg2,3 (amino acids 285–769) and the whole ectodomain of betaglycan bg1,2,3 (amino acids 24–769) fragments were constructed from bg1, bg2 and bg3, and subcloned into the *Bam*HI/*Sal*I site of pQE8, the *Bam*HI site of pQE11, and the *Bam*HI/*Sal*I site of pQE42 respectively. The cDNAs for the bg1,2 Δ C (amino acids 24–369), bg1,2 Δ NC (amino acids 136–369) and bg1,2 Δ N (amino acids 136–513) fragments were amplified by PCR with *Bgl*II and *Sal*I restriction site tags using pQE8 carrying bg1,2 cDNA as a template, and subcloned into the *Bam*HI/*Sal*I site of the pQE42 vector.

The end construct was sequenced by using Sequenase (USB, Cleveland, OH, U.S.A.). The plasmids were transformed into M15 host cells, and positive clones were selected and tested for the expression of the protein product.

The fusion proteins were expressed and purified according to Qiagen's manual. The resulting fusion proteins were reduced with 0.1 M dithiothreitol (DTT) at 37 °C for 2 h, diluted to 50 μ g/ml of protein, and renatured by incubating at room temperature for 16 h with 3 mM reduced glutathione and 0.3 mM oxidized glutathione in 0.1 M Tris (pH 8.0)/10 mM EDTA/1–2 M urea [26]. After renaturation, the proteins were concentrated using Centricon 10 or 30. The yield of the purified fusion protein was usually 5–10 mg from 1 litre of bacterial culture. A fusion protein of the 11th type III domain of fibronectin (III₁₁) was prepared identically and used as a control.

Assays for binding and affinity cross-linking of ¹²⁵I-TGF- β

Binding assays and affinity labelling of ¹²⁵I-labelled TGF- β were performed as described previously [27]. Briefly, confluent monolayers of mink lung epithelial cells were washed once with binding buffer (25 mM Hepes, pH 7.4, 125 mM NaCl, 5 mM MgSO₄, 5 mM KCl and 1 mM CaCl₂) containing 2 mg/ml bovine serum albumin (BSA), ¹²⁵I-labelled TGF- β was added to a final concentration of 100 pM, and the cells were incubated at 4 °C for 4 h in the presence or the absence of inhibitors.

For the binding assay, the cells were washed four times with binding buffer and solubilized with 1 % Triton X-100/10 % glycerol/25 mM Hepes (pH 7.5)/1 mg/ml BSA. The radioactivities of the aliquots of soluble fractions were measured by γ -counter.

For cross-linking, the washed cells were cross-linked with 0.15 mM DSS at 4 °C for 15 min, and soluble fractions of the collected cells were analysed by SDS/PAGE (4–12 % gels) under reducing conditions. After electrophoresis, the gels were fixed in 10 % isopropanol/10 % acetic acid for 20 min and dried by gel-dryer, followed by fluorography and autoradiography using Biomax film (Kodak, Rochester, NY, U.S.A.).

Iodination of betaglycan fragments and solid-phase binding of ¹²⁵I-betaglycan fragments to TGF- β

Iodination of betaglycan fragments was carried out using Iodogen according to previous protocols [22] and purified by PD-10 column. Specific radioactivity of the labelled betaglycan fragments was 30000 to 100000 c.p.m./ng of protein. Initial precipitability in trichloroacetic acid was more than 90 %.

For the binding assay of ¹²⁵I-betaglycan fragment to TGF- β , a 96-well microtitre plate was coated with 3 μ g/ml TGF- β in 0.1 mM NaHCO₃ buffer, pH 9.5, overnight at 4 °C, and blocked with the solid-phase binding buffer (Tris-buffered saline containing 1 % BSA) at 37 °C for 2 h. ¹²⁵I-betaglycan fragment was added and the well was incubated at 37 °C for 6 h in the absence or the presence of competitors. After washing the well five times with the same binding buffer, the radioactivity of each well was measured by γ -counter.

Mink lung epithelial cell growth-inhibition assay

The bioassay for TGF- β was performed on mink lung epithelial cells as described previously [22].

[³⁵S]Sulphate labelling

For another bioassay for TGF- β , rat smooth muscle cells were metabolically labelled with [³⁵S]sulphate to analyse the effect of TGF- β on the synthesis of proteoglycans [28,29]. Confluent monolayers of the cells were serum-starved for 24 h in Dulbecco's modified Eagle's medium containing 0.5 % foetal calf serum, and washed once with the incubation medium (antibiotics-free Dulbecco's modified Eagle's medium containing 0.1 % human serum albumin). [³⁵S]Sulphate was added to a final concentration of 200 μ Ci/ml, and the cells were incubated with betaglycan fragments in the absence or the presence of 30 pM TGF- β . After incubation for 16–24 h, the culture media were collected and analysed by SDS/PAGE (4–20 % gels) under reducing conditions. Autoradiography was performed according to the standard method described above.

RESULTS

Inhibition of TGF- β binding to mink lung epithelial cells by betaglycan fragments

Previous results have shown that the binding of TGF- β is inhibited by the bg3 fragments of the betaglycan [22]. As shown in Figure 1(a), ¹²⁵I-TGF- β binding to these cells was inhibited not only by bg3 but also by bg1,2, bg2,3 and bg1,2,3 (see Figure 2 for the positions of the fragments in betaglycan sequences). In accordance with earlier results [22], bg1 and bg2 were inactive. Since the bg1,2 fusion was active, we also tested a mixture of bg1 and bg2 and found it to be inactive. The results in Figure 1 also show that a mixture of the active bg3 fragment with the inactive bg2 inhibited TGF- β binding to the same extent as bg3 alone. This result makes it unlikely that the inactivity of the bg1/bg2 mixture would be due to a neutralizing effect of the fragments on one another that might have arisen, for example, from an interaction between the His tails of the fragments.

The III₁₁ control fragment from fibronectin had no effect. Fragment bg1,2 Δ N(136–513) was as effective as bg1,2. Deletion of 147 amino acids from the C-terminus of bg1,2 (bg1,2 Δ C) significantly reduced the activity. Titration showed that the inhibitory effect of bg3, bg1,2 and bg1,2,3 was dose dependent and that the inhibitory effect of bg1,2 was about 5-fold more potent than that of bg3, and bg1,2,3 was several-fold more active than bg1,2 (Figure 1b).

Figure 1 Inhibition of the binding of ^{125}I -labelled TGF- β 1 to mink lung epithelial cells by various betaglycan fragments

(a) Mink lung epithelial cells in 48-well dishes were incubated for 4 h at 4 °C with ^{125}I -labelled TGF- β 1 (100 pM) and various betaglycan fragments (800 nM), and the radioactivity bound to the cells was measured. The results are expressed as percentages of TGF- β 1 binding in the absence of betaglycan fragments. Results from a representative experiment out of several are shown. (b) Inhibition of the binding of ^{125}I -TGF- β 1 to mink lung epithelial cells: dose dependency of the effect of betaglycan fragments. The binding of ^{125}I -labelled TGF- β 1 (100 pM) to mink lung cells was measured in the presence of various concentrations of bg3, bg1,2 and bg1,2,3 as indicated in the Figure. Results for bg3 and bg1,2 were from at least two separate experiments; the results for bg1,2,3 were from a single experiment, due to the small amounts of purified proteins. NS, non-specific binding.

Solid-phase binding of ^{125}I -labelled betaglycan fragments to immobilized TGF- β

Microtitre binding assays showed that ^{125}I -labelled bg3, bg1,2 and bg2,3 bind specifically to TGF- β , whereas no binding of ^{125}I -labelled bg1 or bg2 was seen (Figure 3).

Effect of betaglycan fragments on the binding of TGF- β to its receptors

Affinity cross-linking showed that the bg3 and bg1,2 fragments slightly enhanced the binding of TGF- β to the three receptors at low concentrations (1–5 pM) of the fragments, whereas at higher concentrations (25 pM or higher) the fragments inhibited the binding to all three receptors (Figure 4). The binding to betaglycan, in particular, was strongly inhibited.

Effect of betaglycan fragments on TGF- β -induced growth inhibition in mink lung epithelial cells

The betaglycan fragments had no effect on the growth of mink lung epithelial cells in the absence of TGF- β (Figure 5), but bg3,

Figure 2 Recombinant betaglycan fragments

The constructs of various betaglycan fragments are shown schematically. The bar represents the coding region of rat betaglycan. The signal sequence (dotted box), endoglin-related region (hatched box), uromodulin-related region (shaded box) and transmembrane region (filled box) are shown. The amino acid positions of each fragment are indicated in parentheses.

Figure 3 Binding of ^{125}I -labelled betaglycan fragments to immobilized TGF- β 1

Various betaglycan fragments were iodinated as described in the Materials and methods section, and the ^{125}I -labelled fragments (500 pM) were incubated for 6 h at 37 °C in microtitre wells that had been coated with 3 $\mu\text{g}/\text{ml}$ TGF- β 1 in the presence or the absence of 400-fold molar excess (200 nM) of unlabelled betaglycan fragment. Total and non-specific binding was measured. The results are expressed as percentages of the input radioactivity and are representative of several experiments performed in duplicate. NS, non-specific binding.

bg1,2, bg1,2 Δ N and bg1,2,3 enhanced the growth-inhibitory effect of TGF- β in these cells, and bg1,2 Δ C and bg1,2 Δ NC also had some activity (Figure 6a). bg1, bg2 and the control protein were inactive. This enhancing effect of bg3, bg1,2 and bg1,2,3 was dose dependent, and bg1,2,3 was 5–10 fold more active than was bg1,2, which was several-fold more active than bg3 (Figure 6b). The effect of the fragments was specific for TGF- β , because it was abolished by neutralizing anti-TGF- β antibody (Figure 5; [22]).

Effect of betaglycan fragments on the ability of TGF- β to stimulate proteoglycan synthesis

TGF- β is known to stimulate the synthesis of proteoglycans [1,2]. bg1,2 enhanced the stimulatory effect of TGF- β (30 pM) on the synthesis of proteoglycan in rat smooth muscle cells, whereas III₁₁ control protein had no effect (Figure 7). bg3 was also active,

Figure 4 Effect of betaglycan fragments on the affinity cross-linking of ^{125}I -labelled TGF- β 1 to cellular receptors

Mink lung epithelial cells in 48-well dishes were affinity labelled with ^{125}I -labelled TGF- β 1 (100 pM) for 4 h at 4 °C with or without various concentrations of bg3 and bg1,2, followed by cross-linking with DSS. The cells were solubilized and analysed by SDS/PAGE as described in the Materials and methods section. The protein molecular-mass markers are shown to the left. I R, TGF- β type I receptor; II R, TGF- β type II receptor.

Figure 5 Specificity of the effect of betaglycan fragments on TGF- β -induced growth suppression

The mink lung cell assay was performed in the absence or the presence of bg1,2 (625 nM), bg1,2,3 (625 nM), TGF- β 1 (30 pM) or neutralizing anti-TGF- β 1 polyclonal antibody (25 $\mu\text{g}/\text{ml}$) as indicated in the figure. Bars show means $\pm$ S.D. from experiments performed in triplicate.

but the enhancing effect was less potent than that of bg1,2 (results not shown). The effect of bg1,2 was specific for TGF- β , because it was abolished by neutralizing anti-TGF- β antibody. These results are consistent with the results from the mink lung cell growth-inhibition assay shown above.

Comparison of the TGF- β -binding specificities of the betaglycan fragments

We next examined whether the binding of bg3 to immobilized TGF- β could be inhibited by bg1,2, and vice versa. Figure 8(a) shows that the binding of ^{125}I -bg3 to TGF- β is inhibited not only by bg3 but also by bg1,2 and bg2,3. The inhibition was concentration dependent, and bg1, bg2 and the III₁₁ control protein had little or no effect. Conversely, the binding of ^{125}I -bg1,2 to immobilized TGF- β was inhibited not only by bg1,2 but

Figure 6 Effect of betaglycan fragments on TGF- β bioactivity in mink lung cell growth-inhibition assay

(a) Mink lung epithelial cells in 96-well plates were incubated overnight at 37 °C with 625 nM betaglycan fragments in the absence or the presence of 30 pM TGF- β 1. [^3H]Thymidine was added for 3–5 h, and the radioactivity incorporated into the cells was measured as described in the Materials and methods section. Bars show means $\pm$ S.D. from experiments performed in triplicate. (b) Dose dependency of the effect of betaglycan fragments on TGF- β 1-induced growth suppression. Mink lung cell growth-inhibition assay was performed in the presence of 30 pM TGF- β 1 and various concentrations of bg3, bg1,2 and bg1,2,3 fragments as indicated in the Figure. The results are expressed as percentages of the radioactivity in the presence of 30 pM TGF- β 1 without betaglycan fragments. Bars show means $\pm$ S.D. from experiments performed in triplicate.

also by bg3 and bg2,3, whereas bg1 and bg2 were only marginally active and the III₁₁ control protein was inactive (Figure 8b). The concentrations of the betaglycan fragment required for 50% inhibition of the binding were ~ 40 nM for bg3 and ~ 10 nM for bg1,2. The affinity constants calculated from these results were 6.7×10^7 (l/mol) for bg3 and 2.7×10^8 (l/mol) for bg1,2 respectively [30].

Competition for the binding of ^{125}I -labelled bg3 or bg1,2 to immobilized TGF- β by small proteoglycans or monoclonal anti-TGF- β antibody

It was previously shown that the binding of bg3 to immobilized TGF- β can be inhibited by fusion proteins representing the core proteins of the small proteoglycans decorin, biglycan or fibromodulin [22]. The results in Table 1 show that the proteoglycans

Figure 7 Effect of betaglycan fragments on TGF- β -induced proteoglycan synthesis in cultured rat smooth muscle cells

Rat smooth muscle cells in 48-well dishes were metabolically labelled for 16–24 h by [35 S]sulphate in the absence or the presence of the bg1,2 fragment (625 nM, lanes 2, 5 and 8), control III₁₁ fibronectin fragment (625 nM, lane 4), TGF- β 1 (30 pM, lanes 3, 4, 5, 7 and 8; 400 pM, lane 6) or neutralizing anti-TGF- β 1 polyclonal antibody (25 μ g/ml, lanes 7 and 8) as indicated in the Figure. Equal amounts of conditioned media were analysed by SDS/PAGE followed by autoradiography. The position of the 210 kDa protein molecular-mass marker is indicated in the Figure. The sulphated band migrating at $\sim$ 210 kDa is apparently biglycan [29].

also inhibit the binding of 125 I-bg1,2 to TGF- β , whereas the maltose-binding protein used as control had no effect.

Moreover, monoclonal anti-TGF- β antibody (Genzyme), which is known to inhibit the TGF- β binding to its receptors and to neutralize the bioactivities of TGF- β , also inhibited the binding of 125 I-bg3 or 125 I-bg1,2 to TGF- β , whereas control mouse IgG was inactive (results not shown).

DISCUSSION

We show here that there are at least two independently active TGF- β -binding sites in betaglycan, one in the N-terminal two-thirds of the extracellular domain of betaglycan and another in the C-terminal one-third. These binding sites exhibit similar binding specificities to one another and to small proteoglycans of

Table 1 Inhibition of the binding of 125 I-labelled bg3 or bg1,2 to immobilized TGF- β 1 by decorin-type proteoglycans

125 I-labelled bg1,2 was incubated in microtitre wells coated with TGF- β 1 (3 μ g/ml) in the presence of 200 nM of bg1,2, maltose-binding protein (MBP) or fusion proteins of decorin, biglycan and fibromodulin with MBP. The binding is expressed as percentages of control binding in the absence of the MBP fusion proteins. Each value shows a mean from duplicate wells in a representative experiment.

Sample	125 I-bg1,2 (% bound)
Control	100.0
bg1,2	34.2
MBP	107.2
Decorin	65.2
Biglycan	33.1
Fibromodulin	25.1

the decorin family, but the betaglycan fragments potentiate the biological activities of TGF- β .

The main result of this study is to delineate the presence of two independent binding sites in betaglycan for TGF- β . This conclusion is based on the activities of fragments representing the N-terminal and C-terminal binding sites. Each of the fragments inhibited the binding of TGF- β to intact cells, bound directly to TGF- β in solid-phase binding assays, interfered with affinity labelling of the TGF- β receptors by radioactive TGF- β , and enhanced the bioactivity of TGF- β in two different bioassays. Thus betaglycan clearly contains two binding sites for TGF- β .

The N-terminal and C-terminal binding sites, while showing similar characteristics in terms of the binding and biological effects on TGF- β action, differ in affinity. First, the N-terminal bg1,2 fragment was several-fold more effective in inhibiting the binding of TGF- β to mink lung epithelial cells than bg3 was. Secondly, bg1,2 bound to immobilized TGF- β with an affinity higher than that of bg3 in a solid-phase binding assay. Finally, bg1,2 was more active than bg3 in enhancing the bioactivity of TGF- β in mink lung epithelial cells. Assuming that the relative activities of the fragments are representative of those of the binding sites in the intact molecule, these results suggest that, of the two binding sites, the N-terminal site may be the main binding site for TGF- β . Moreover, our results show that the

Figure 8 Inhibition of the binding of 125 I-labelled bg3 or bg1,2 to immobilized TGF- β 1 by betaglycan fragments

(a) 125 I-labelled bg3 was incubated for 6 h at 37 °C in microtitre wells coated with TGF- β 1 (3 μ g/ml) in the presence of various concentrations of betaglycan fragments. The binding is expressed as percentages of control binding in the absence of unlabelled betaglycan fragments. (b) The solid-phase binding of 125 I-labelled bg1,2 fragment to TGF- β 1-coated wells was measured, and the results were shown as described above.

whole ectodomain of betaglycan binds more strongly than does either bg3 or bg1,2 alone, indicating that both the N-terminal and C-terminal binding sites are necessary for full activity. It may be that the intact ectodomain folds in a manner that is more favourable for high-affinity binding than do the smaller fragments. Alternatively, it could be that each site of the ectodomain can bind to its own subunit in the dimeric TGF- β molecule.

The N-terminal binding site requires a large portion of the betaglycan molecule to be active. We were able to truncate the 490 amino acid bg1,2 fragment by 116 amino acids at the N-terminus with complete retention of activity. However, deletion of 147 amino acids from the C-terminus of bg1,2 greatly reduced the activity. Moreover, cleaving the bg1,2 fragment in half gave two inactive fragments. The binding site that López-Casillas and co-workers localized essentially overlaps with our bg1,2 and bg1,2 Δ N fragment [24]. It is possible that ternary structure in the N-terminal two-thirds of betaglycan is important for the binding (see below). This requirement of a large portion of the betaglycan sequence for the activity of the N-terminal binding site explains why this site was not detected by Fukushima et al. and Pepin et al. in the N-terminal two-thirds of betaglycan [22,23].

Our results show that the fragments representing the N-terminal and C-terminal binding sites enhance the bioactivity of TGF- β . This enhancing effect was observed not only in growth-inhibitory activity of TGF- β but also in the stimulating effect of TGF- β on the matrix synthesis. This result is in apparent disagreement with our affinity-labelling results, which showed that the bg1,2 and bg3 fragments inhibit the binding of TGF- β to the receptors at high concentrations of the fragments, although they appear to enhance slightly the binding at lower concentrations. The possible explanation for the seemingly discrepant results is that betaglycan fragments preferentially inhibit TGF- β binding to betaglycan as compared with its binding to type I and type II receptors. Thus, the binding to the signalling receptor is relatively unimpaired even in the presence of high concentrations of betaglycan fragments. This, together with some unknown effects of TGF- β -betaglycan fragment complex on the signalling receptors, could somehow stimulate signal transduction. Alternatively, differences in the experimental conditions under which the assays are conducted might explain the discrepant results.

Our results are in contrast to the report by López-Casillas and co-workers, who showed that soluble betaglycan reduces the growth-inhibitory effect of TGF- β in mink lung epithelial cells [24]. The reason for this discrepancy is unknown, but they used baculoviral betaglycan, which includes the whole extracellular domain, and it may be that TGF- β -binding proteins of this variety give variable results in the bioassays. Thus decorin has been found to inhibit TGF- β activity [31], stimulate it [32] or have no consistent effect on it [25].

The binding specificities of the N-terminal and C-terminal binding sites appear to be similar, because fragments representing these sites inhibited one another's binding to TGF- β . The binding of the betaglycan fragments was also inhibited by bacterial fusion proteins containing the core proteins of small proteoglycans belonging to the decorin family. These findings suggest that betaglycan and the decorin-type proteoglycans all bind to the same or adjacent sites in TGF- β . While the N-terminus of the betaglycan ectodomain displays no apparent sequence similarity to the decorin-type core proteins, the C-terminal end does contain a motif, like the decorin-type proteoglycan, that is leucine rich and has been proposed to be a potential TGF- β -binding sequence [33]. However, the involvement of this motif, if any, in TGF- β binding is unknown, and it may be that ternary structure, rather

than any particular sequence of the core proteins, is required for the binding activity.

In summary, both the N-terminal and C-terminal portions of the extracellular domain of betaglycan can independently bind TGF- β ; these findings appear to reconcile the current disagreement regarding the localization of the TGF- β -binding sites in betaglycan.

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