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Selective inactivity of TGF-β/decorin complexes

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Abstract Previous studies had shown that binding of TGF-β to the small proteoglycan decorin results in its inactivation. Indeed, in osteosarcoma cells the addition of decorin prevented the TGF-β1-mediated up-regulation of biglycan synthesis. However, the down-regulation of proteoglycan-100 remained unaltered. Even in the presence of a 100,000-fold molar excess of decorin, TGF-β1 was fully active in U937 monocytes with respect to the inhibition of cell proliferation. There was no inhibition of the TGF-β-mediated stimulation of the retraction of fibroblast-populated collagen lattices. Thus, the formation of TGF-β/decorin complexes leads to the neutralization of distinct effects only.

Key words: TGF-β; Decorin; Biglycan; Proteoglycan-100; Collagen-gel retraction

1. Introduction

The family of TGF-β polypeptides represents multifunctional cytokines which are involved in the modulation of the differentiated phenotype, in the regulation of cell proliferation and in the synthesis and deposition of extracellular matrix proteins (see [1–3] for reviews). Although different TGF-β isoforms are interchangeable in most in vitro assays, TGF-β1 was considered as playing a major role in stimulating the production of the extracellular matrix and regulating cell matrix interactions [4].

Strong evidence has been provided that TGF-β is an essential factor during tissue repair after injury [1–3]. For example, TGF-β has been shown to stimulate collagen-matrix contraction by fibroblasts [5] and to induce the expression of fibrillar collagens [6]. Somewhat contradictory results have been obtained with respect to the effects of TGF-β on small interstitial proteoglycan expression. An up-regulation of biglycan has consistently been noted. An increased production of the collagen-binding proteoglycan decorin has been observed in mesangial cells [7] but not in fibroblasts [8,9–11]. Another small proteoglycan, proteoglycan-100 (PG-100), was markedly down-regulated [9].

The beneficial effect of TGF-β on tissue repair can be counteracted by excessive matrix deposition [12]. In a rat model, the development of an acute proliferative glomerulonephritis could indeed be prevented by a neutralizing antiserum against TGF-β [13]. It has also been shown that small proteoglycans themselves form inactive complexes with the cytokine [14], and could therefore be of therapeutic value [15]. In this report data are presented which indicate that the interaction of decorin with TGF-β interferes with distinct functions of the growth factor.

2. Materials and methods

2.1. Materials

Human recombinant TGF-β1 was from Serva, and collagenase from Advance Biofactures (Lynbrook, NY). Calcitriol was kindly provided by Dr. M.R. Uskokovic, Hoffmann-La Roche. Decorin was purified under non-denaturing conditions from the secretions of cultured human skin fibroblasts as described [16] and characterized before and after the incubations by SDS-PAGE and Western blotting [17]. SDS-PAGE indicated that the preparations were at least 95% pure. Antisera against biglycan, decorin and PG-100 were those used previously [17].

2.2. Cell culture and metabolic labeling

Human osteosarcoma cells (MG-63), skin fibroblasts [9] and monocyte-like U937 cells [18] were cultured as described. Fibroblast-populated collagen lattices were prepared [19] with bovine serum albumin (BSA) in place of serum [9]. Before metabolic labeling, MG-63 cells were preincubated for 20 h with the indicated quantities of TGF-β and/or decorin in serum-free MEM medium containing 1% BSA. Subsequent labeling with [^{35}S]sulfate (4 h, 1.85 MBq/ml) was performed in the presence of identical concentrations of reagents. U937 cells (10^6 cells in 5 ml RPMI 1640 medium) received 10 μM calcitriol together with the indicated quantities of TGF-β and/or decorin for a period of 3 days.

2.3. Proteoglycan quantification

In most experiments cell-associated and secreted proteoglycans were sequentially immune precipitated with antisera against PG-100, decorin, and biglycan [17]. The precipitations of PG-100 and biglycan were at least 90% complete. Decorin was not investigated further because of incomplete precipitation in the presence of excess of the unlabeled proteoglycan.

2.4. Interaction of TGF-β with collagen-bound decorin

Purified acid-soluble type I collagen from calf skin, kindly provided by Dr. J. Rauterberg of this university, was dissolved in 10 mM acetic acid (2 mg/ml), sterilized and adjusted to 18 mM sodium phosphate, pH 7.4, 0.137 M NaCl, 3 mM KCl (PBS) and 1% BSA. One ml of the solution, containing 1.1 mg collagen, was incubated for 5 h at 37°C in the absence or presence of 0.1 μM decorin. Fibrils were collected by centrifugation, washed twice with PBS/1% BSA and suspended in 1 ml RPMI 1640 medium/10 μM calcitriol with or without 50 pM TGF-β. After 90 min at 37°C free TGF-β and TGF-β bound to the fibrils were separately quantitated by determining the growth-inhibitory effect on U937 cells. Prior to the assay, bound TGF-β was solubilized by digesting the washed collagen fibrils for 16 h at 37°C with 25 BTC-U of collagenase in 500 μl RPMI 1640 medium/10 μM calcitriol. In parallel experiments the quantity of collagen-bound decorin was obtained by including trace amounts of [^{35}S]sulfate-labeled decorin in the assay mixture.

3. Results

In MG-63 osteosarcoma cells TGF-β causes an up-regulation of biglycan, a marked down-regulation of PG-100 and a minor reduction of decorin synthesis [9]. Inactivation of TGF-β by decorin would therefore be expected to interfere with opposite effects on proteoglycan biosynthesis. In three independent experiments, however, we found that only the TGF-β-mediated

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Table 1
Influence of TGF-β and TGF-β/decorin complexes on biglycan and PG-100 biosynthesis in MG-63 cells

Addition		TGF-β free* (pM)	Biglycan	PG-100
TGF-β (pM)	Decorin (nM)		(10^{-3} × cpm/flask)	
None	none	–	49 (100)	144 (100)
None	100	–	54 (110)	188 (132)
200	none	200	88 (180)	44 (31)
20	none	20	80 (163)	114 (79)
2	none	2	64 (131)	153 (106)
200	100	3	50 (102)	46 (32)

*Calculated under the assumption of $K_d = 1.5 \times 10^{-9}$ M for the decorin–TGF-β interaction [14]. The concentration of decorin was calculated from the hexuronic acid content assuming 65 residues per molecule [17]. Values in brackets show percent of untreated control.

induction of biglycan biosynthesis (136–186% of control in the presence of 200 pM TGF-β) was sensitive to decorin addition (102–108% of control with 200 pM TGF-β/100 nM decorin). The inhibition of PG-100 biosynthesis (26–31% of control at the same TGF-β concentration) remained unaltered. One of the experiments is shown in Table 1. None of the treatments influenced the ratio between cell-associated and secreted proteoglycans after a labeling period of 4 h (data not shown).

It had been described previously [5] that TGF-β stimulates the contraction of fibroblast-populated collagen lattices. It was, therefore, of interest to study the influence of TGF-β/decorin complexes in this model where an insoluble matrix is present, comparable to the in vivo situation. Applying a 500-fold molar excess of decorin which is expected to decrease the quantity of free TGF-β by 98.5%, at the most a marginal neutralizing effect on the activity of the cytokine was found in two separate experiments (Table 2).

During the course of this study we found that picomolar doses of TGF-β exhibited a marked anti-proliferative effect on monocytic U937 cells, which was accompanied by an alkalisation of the culture medium. Using this extremely sensitive assay, in three independent experiments even a 100,000-fold excess of decorin was without influence on TGF-β activity (Table 3). Because of the possibility of proteolytic degradation of decorin during the 3 day incubation period, decorin was analyzed by SDS-PAGE and Western blotting. Neither in the presence of 10 pM TGF-β nor in its absence measurable degradation occurred.

In addition to the question of the biological activity of soluble TGF-β/decorin complexes, the problem has been considered whether collagen-bound decorin, i.e. the predominant in vivo form of the proteoglycan, retains its ability to interact with TGF-β. The U937 assay system was used to quantify TGF-β (50 fmol per assay) upon interaction with either free type I collagen (1.1 mg) or decorin-containing collagen (30 pmol/1.1 mg). In the case of decorin-free collagen 0.6 fmol of the cytokine were found in association with collagen, and 36.5 fmol in the supernatant. Decorin-containing collagen bound 2.0 fmol and 47.5 fmol were found in the supernatant. Thus, collagen-bound decorin is still able to interact with TGF-β albeit either with low affinity or with an unfavourable stoichiometry.

Table 2
Influence of TGF-β and TGF-β/decorin complexes on collagen gel retraction

Addition		Time (h)			
TGF-β (pM)	Decorin (nM)	13	21	30	72
		Gel area (% of zero time)			
None	none	25.0	20.7	11.1	4.2
200	none	12.0	8.3	3.3	0.5
20	none	19.0	14.4	9.2	3.9
2	none	22.0	18.0	11.6	4.2
200	100	13.0	8.8	3.9	0.7

4. Discussion

The main observation described in this paper concerns the neutralization of only a selected TGF-β1-mediated effect by decorin. In two experimental models a negative influence of decorin on TGF-β activity had been described. Under tissue culture conditions, i.e. under similar conditions as in this study, the expression of high levels of human decorin in CHO cells had a dramatic effect on cell growth ascribed to TGF-β binding and inactivation [14]. This observation, however, defies a simple interpretation since wild-type CHO cells themselves produce large quantities of decorin (unpublished observation). In a rat model, injection of massive doses of decorin (450 μg/dose) prevented excessive matrix accumulation in experimental glomerulonephritis [15]. In this system complex formation between TGF-β and decorin most likely occurs on solid phases thus withdrawing TGF-β from the signalling receptors. In the simple solid phase model used in this study, contraction of fibroblast-populated collagen lattices, no alteration of the TGF-β effect by decorin could be observed.

The failure to observe the neutralisation of all TGF-β-mediated effects by decorin cannot be explained by the endocytosis of the proteoglycan since its concentration is reduced by 3% only during the 4 h labeling period [17]. It is known that TGF-β binds to cell membrane receptors with high affinity [2]. Decorin-bound TGF-β should therefore be in an equilibrium with the receptor-bound cytokine. Although the respective quantitative data are not available, the consistent finding of an inactivation of TGF-β with respect to biglycan induction argues for a biologically relevant complex formation between decorin and TGF-β. The selective inactivity of TGF-β/decorin complexes,

Table 3
Influence of TGF-β and TGF-β/decorin complexes on the proliferation of U937 cells

Addition		TGF-β free* (pM)	Cell number (10^{-6} × ml^{-1})	pH
TGF-β (pM)	Decorin (nM)			
None	none	–	1.16 (100)	7.08
None	100	–	1.20 (103)	7.02
10	none	10	0.48 (41)	7.65
1	none	1	0.75 (65)	7.36
0.1	none	0.1	1.15 (99)	7.09
0.01	none	0.01	1.15 (99)	7.07
10	100	0.15	0.49 (42)	7.67
1	100	0.015	0.80 (69)	7.30

*Calculated under the assumption of $K_d = 1.5 \times 10^{-9}$ M. Values in brackets show percent of untreated control. Measurements were performed after 3 days of incubation.

however, cannot be explained unambiguously. There exist at least two signalling TGF-β receptors, both of which exhibit serine/threonine kinase activity and cooperate during binding and signal transfer [20,21]. Skin fibroblasts and MG-63 cells possess both receptors, whereas cross-linking experiments failed to detect the type II receptor in U937 cells [23]. According to the proposed necessity of receptor cooperation, the selective inactivity of TGF-β/decorin complexes should not be caused by selective binding to one of the receptors but by the modulation of other steps of the signalling cascade.

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