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Correlation of the size of type II transforming growth factor β (TGF-β) receptor with TGF-β responses of isolated bovine articular chondrocytes

H L Glansbeek, P M van der Kraan, E L Vitters, W B van den Berg

Abstract

***Objectives*—Transforming growth factor β (TGF-β) is a multipotent regulator of cell proliferation and extracellular matrix production. The effect of TGF-β on chondrocyte matrix production was studied in relation to the expression of TGF-β binding proteins. The effect of TGF-β on proteoglycan synthesis of isolated articular chondrocytes depended on the culture period. Proteoglycan synthesis of chondrocytes which were cultured for one day was inhibited by TGF-β whereas proteoglycan synthesis of chondrocytes cultured in monolayer for seven days or longer was stimulated by TGF-β. To investigate if this differential response is related to a distinct expression of TGF-β receptors, this parameter was studied by affinity labelling.**

***Methods*—Chondrocytes were incubated with 100 pM TGF-β labelled with iodine-125. Crosslinking was performed using 0·25 mM disuccinimidyl suberate. Membrane proteins were extracted and analysed by denaturating sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography.**

***Results*—Freshly isolated and cultured chondrocytes expressed types I, II, and III TGF-β receptors. The type II TGF-β receptor of cultured chondrocytes appeared to be about 15 kilodaltons smaller than the type II TGF-β receptor expressed on freshly isolated chondrocytes, however.**

***Conclusions*—As the type II TGF-β receptor appears to be involved in signal transduction, this change in size of the type II TGF-β receptor might be related to the differential effect of TGF-β on proteoglycan synthesis of freshly isolated and cultured bovine articular chondrocytes.**

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Transforming growth factor β (TGF-β) is a multipotent regulator of cell proliferation and extracellular matrix synthesis.[1 2] It is produced as a latent, high molecular weight complex[2 3] which can be activated by proteolytic cleavage or extremes of pH.[2] Active TGF-β is a 25 kilodalton polypeptide consisting of two identical subunits of 112 amino acids linked by disulphide bonds.[1 2] Transforming growth factor β might be an important regulator of articular chondrocyte metabolism during joint diseases, as high concentrations of latent TGF-β (300 ng/g) are present in articular cartilage[4] and active TGF-β is found in the synovial fluid of patients with rheumatoid arthritis or osteoarthritis.[5]

The reported effects of TGF-β on chondrocyte metabolism are contradictory. Stimulating and inhibitory effects of TGF-β on proteoglycan and DNA synthesis of articular chondrocytes have been described.[6–9] We have shown that the effects of TGF-β on proteoglycan and DNA synthesis of isolated bovine articular chondrocytes are related to differences in culture time.[10] Transforming growth factor β inhibits proteoglycan and DNA synthesis of isolated bovine articular chondrocytes which have been cultured for one day ('freshly isolated chondrocytes'), whereas TGF-β stimulates proteoglycan and DNA synthesis of bovine articular chondrocytes cultured for seven days or longer ('cultured chondrocytes'). This effect appeared to be the result of phenotypical changes between freshly isolated and cultured chondrocytes.

Effects of TGF-β are mediated by cell surface receptors. Articular chondrocytes express at least four distinct types of TGF-β binding proteins: type I (53 kilodalton), type II (70–100 kilodalton), type III (250–350 kilodalton), and type V (400 kilodalton).[1 11] Other types of TGF-β binding proteins, like the type VI TGF-β receptor, are described on several cell types,[12–14] but it is not known if these are also present on chondrocytes. Little is known about the function of these different binding proteins, though on a large variety of cell types it was shown that the type I and II receptors are important in signal transduction.[15–21] Involvement of the type II TGF-β receptor in signal transduction is also suggested by the presence of a serine/threonine kinase domain.[22] The type V receptor might also have a function in signal transduction as it contains a serine/threonine kinase domain,[23] and TGF-β resistant tumour cell lines have been described which have no expression of type V receptors, but a normal expression of the other TGF-β receptors.[11] The type III receptor, also called betaglycan, probably does not have a role in signal transduction as it has a small intracellular domain[24 25] and cells are described which lack betaglycan but have a normal response on TGF-β.[26 27]

The aim of this study was to investigate if the differential effect of TGF-β on proteoglycan synthesis of freshly isolated and cultured articular chondrocytes is related to differences in TGF-β receptor expression.

Methods

ISOLATION OF BOVINE ARTICULAR CHONDROCYTES

Articular cartilage chondrocytes were isolated from bovine metacarpophalangeal joints. Cartilage slices were incubated for 48 hours in RPMI DM (Flow Laboratories) supplemented with 1 mg/ml clostridium collagenase (371 U/mg, Worthington Biochemical) at 37°C in a humidified 5% CO_2 atmosphere. After incubation with collagenase, chondrocytes were washed three times with collagenase free medium. Cells were seeded at a density of 5×10^5 cells/ml in 24 well cluster dishes (1 ml/cluster, Costar) and grown for one day ('freshly isolated chondrocytes') or 14 days ('cultured chondrocytes') in medium supplemented with 20% fetal calf serum (FCS) (Flow Laboratories). The culture medium was changed every other day. The cell number doubled during culture in about one week, after which confluence was reached.

EFFECT OF TGF-β ON PROTEOGLYCAN SYNTHESIS

Recombinant TGF-β1 (Serva) was solubilised in 4 mM HCl with 0·1% bovine serum albumin (Sigma). Freshly isolated and cultured chondrocytes were incubated for 24 hours in the presence of TGF-β (5 ng/ml, 200 pM). Four hours before the end of the incubation period, 370 kBq [^{35}S]sulphate (Du Pont de Nemours) was added. After the incubation period, the culture medium was treated with papain (1 mg/ml papain (Sigma), 0·2 M NaCl, 0·1 M sodium acetate, 10 mM L-cysteine hydrochloride (Sigma), 50 mM Na_2EDTA, 50 μg/ml chondroitin sulphate carrier (Sigma), pH 6·0) for two hours at 60°C. The glycosaminoglycans were precipitated by incubation (two hours, 37°C) with 0·1% cetylpyridinium chloride (Sigma). After centrifugation (15 minutes, room temperature, 10 000 *g*) the pellet was washed three times with 0·05% cetylpyridinium chloride. The pellet was solubilised for two hours at 60°C with Lumasolve (Perstorp Analytical) and after the addition of scintillation fluid was counted in a liquid scintillation counter.

The Wilcoxon's rank sum test was used to test statistical significance. Differences were significant when the p value was less than 0·05.

COLLAGENASE TREATMENT OF CULTURED CHONDROCYTES

Chondrocytes, cultured for 14 days in medium supplemented with 20% FCS, were incubated for 48 hours in RPMI DM supplemented with 1 mg/ml clostridium collagenase (371 U/mg) at 37°C in a humidified 5% CO_2 atmosphere. After incubation with collagenase, chondrocytes were washed three times with collagenase free medium. Cells were seeded at a density of 5×10^5 cells/ml in 24 well cluster dishes (1 ml/cluster) and grown for one day, after which the effect of TGF-β on the proteoglycan synthesis was measured.

^{125}I LABELLING OF TGF-β

Transforming growth factor β1 was labelled with iodine-125 using the oxidising agent 1,3,4,6-tetrachloro-3α,6α-diphenyl glycoluril (Iodogen; Pierce) according to the method described by Salacinski *et al.*[28] Transforming growth factor β (5 μg in 100 μl sodium phosphate buffer (0·05 M, pH 7·4)) was incubated in the presence of 18·5 MBq carrier free iodine-125 labelled sodium iodide (Amersham) in a reaction vial coated with 2 μg Iodogen. After an incubation of 15 minutes at room temperature, the reaction was terminated by the addition of 1 M KI. Radiolabelled TGF-β was separated from free iodine using a Sephadex G25 column which was equilibrated with 4 mM HCl containing bovine serum albumin (BSA) (0·1% w/v).

AFFINITY LABELLING

Affinity labelling was performed according to the method of Massagué and Like.[29] Chondrocytes were washed with binding buffer (128 mM NaCl, 5 mM KCl, 1·2 mM $CaCl_2$, 1·2 mM $MgSO_4$, 50 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulphonate, pH 7·5), after which they were incubated with 100 pM [^{125}I]TGF-β in binding buffer, supplemented with 5 mg/ml BSA, for three hours at 4°C. Competition studies were performed in the presence of 4 nM non-radiolabelled TGF-β. After the incubation period the buffer was discarded and the crosslink reaction was performed in binding medium supplemented with 0·25 mM disuccinimidyl suberate (Pierce) for 30 minutes at 4°C. After washing cells with binding buffer the cells were incubated (16 hours, 4°C) with extraction buffer (1% v/v Triton X-100, 1 mM EDTA, 10 mM TRIS, and 1 mM *p*-methyl sulphonyl fluoride). Detergent soluble material was analysed by denaturing sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) using prefabricated 5–20% gradient gels (Bio-Rad) and by autoradiography. Autoradiograms were scanned using an automatic gel scanner (LKB Ultroscan XL). Sizes of binding proteins were determined using prestained molecular weight markers (Bio-Rad).

Results

As shown previously,[10] TGF-β inhibited proteoglycan synthesis of freshly isolated chondrocytes whereas proteoglycan synthesis of cultured chondrocytes was stimulated by TGF-β (fig 1). It was suggested that this phenomenon is the result of phenotypical changes occurring during culture. The possibility that the lack of stimulation on

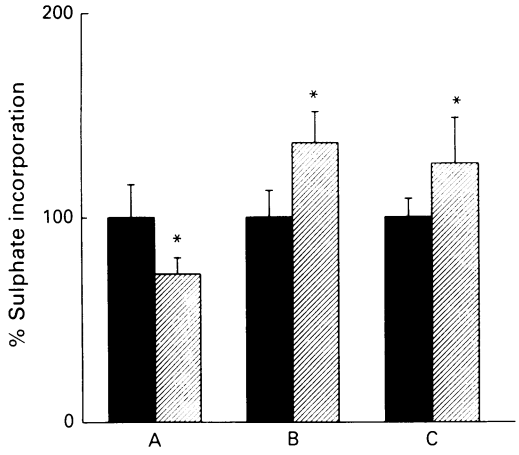

Figure 1 Effect of transforming growth factor β (TGF-β) on the proteoglycan synthesis of freshly isolated articular chondrocytes (A), cultured chondrocytes (B), and cultured chondrocytes after collagenase treatment (C). Chondrocytes were incubated with (shaded) or without (solid black) 200 pM TGF-β for 24 hours. During the last four hours the culture medium was supplemented with 370 kBq [^{35}S]sulphate. After incubation proteoglycans were precipitated and ^{35}S content was measured. Experiments were performed in quintuple. Without a TGF-β incubation the ^{35}S incorporation of cultured chondrocytes and cultured chondrocytes after collagenase treatment was comparable with the ^{35}S incorporation of freshly isolated chondrocytes. Results are mean (SD) of a representative experiment of four. Significant differences are indicated with an asterisk.

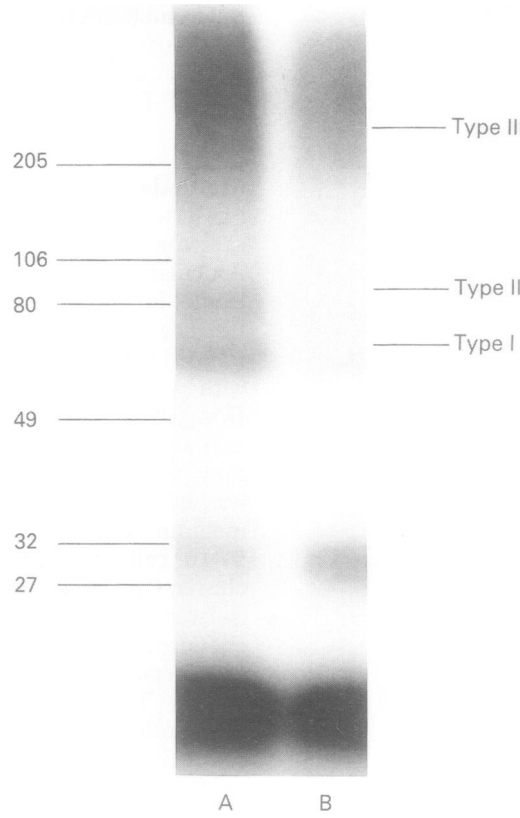

Figure 2 Affinity labelling of cultured chondrocytes without (A) and in the presence (B) of 4 nM non-radiolabelled transforming growth factor β (TGF-β). Chondrocytes were incubated with 100 pM [^{125}I]TGF-β. Crosslinking was performed using 0·25 mM disuccinimidyl suberate. Membrane proteins were extracted and analysed by denaturating sodium dodecylsulphate polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers and bands corresponding with the affinity labelled types I, II, and III TGF-β receptors are indicated.

freshly isolated chondrocytes was a result of the collagenase treatment, which was used to isolate chondrocytes from cartilage, still remained, however. To exclude this possibility, cultured chondrocytes were treated with collagenase, seeded in the original density of 5×10^5 cell/ml and cultured for 24 hours, after which the effect of TGF-β on the proteoglycan synthesis was measured. As shown in fig 1, collagenase treatment of cultured chondrocytes did not alter the effect of TGF-β, showing that the differential effect of TGF-β was indeed a result of phenotypical changes.

To investigate if this effect was related to alterations in TGF-β receptor expression, affinity labelling was performed. After SDS-PAGE and autoradiography, several bands were demonstrated. The sizes of these bands correspond with sizes of the affinity labelled type I (65 kilodalton), type II (85 kilodalton), and type III (diffuse high molecular band 250–350 kilodalton) TGF-β receptors (fig 2). It might be possible that the diffuse high molecular band also includes the affinity labelled types V (400 kilodalton) and VI (180 kilodalton) binding proteins, but that the separation capacity in this region of the gel was not enough to distinguish them from the type III receptor. Studies in which affinity labelling was performed in the presence of 4 nmol/l non-radiolabelled TGF-β showed that these affinity labelled proteins are specific TGF-β binding proteins as competition between [^{125}I]TGF-β and non-radiolabelled TGF-β for binding to these receptors was demonstrated (fig 2). In addition to the types I, II, and III TGF-β receptors, we showed a band of about 30 kilodalton, corresponding to a binding protein of 18 kilodalton. This binding protein appears not to be a specific TGF-β binding protein, as we could not show competition between radiolabelled and non-radiolabelled TGF-β for binding to this protein (fig 2).

Although isolated and cultured chondrocytes expressed the 18 kilodalton binding protein and types I, II, and III TGF-β receptors, a difference in TGF-β receptor expression was shown between these chondrocytes. A small but significant difference was observed in the size of the type II TGF-β receptors, whereas no other reproducible differences were observed in TGF-β receptor expression. The type II TGF-β receptor of freshly isolated chondrocytes appears to be about 15 kilodalton larger than the type II receptor of cultured chondrocytes (fig 3). The variation in size of the type II receptor was also shown after scanning autoradiograms with an automatic gel scanner (fig 4). The ratio between the intensities of bands showed variation between experiments. We could not show a correlation between the ratio of the intensities of the type I and type II receptors and the effects of TGF-β on chondrocytes, however. The TGF-β receptor expression of cultured chondrocytes which were treated with collagenase was also analysed. We were not able to show differences between TGF-β receptor expression of cultured chondrocytes and cultured chondrocytes which were treated with collagenase (data not shown).

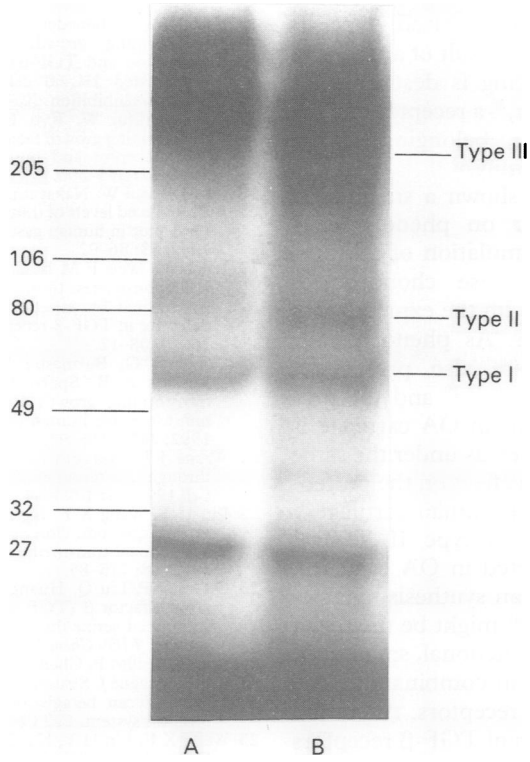

Figure 3 Affinity labelling of freshly isolated (A) and cultured (B) chondrocytes. Chondrocytes were incubated with 100 pM [^{125}I]TGF-β. Crosslinking was performed using 0·25 mM disuccinimidyl suberate. Membrane proteins were extracted and analysed by denaturating sodium dodecylsulphate polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers and bands corresponding with the affinity labelled types I, II, and III TGF-β receptors are indicated. A representative autoradiogram of three runs is shown.

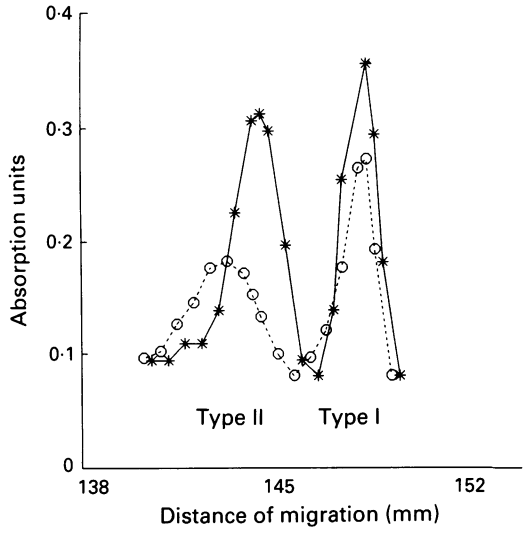

Figure 4 Scan of autoradiogram. Autoradiograms were scanned using an automatic gel scanner. Absorption units were normalised for background. The relative positions of the type I and type II transforming growth factor β receptors of freshly isolated (○) and cultured (*) chondrocytes are shown.

Discussion

Proteoglycan synthesis of isolated articular chondrocytes has been reported to be stimulated and inhibited by TGF-β.[6-9] We have shown that these differential findings may be the result of differences in culture period.[10] Proteoglycan synthesis of bovine articular chondrocytes which were cultured for one day ('freshly isolated chondrocytes') was inhibited, whereas the proteoglycan synthesis of chondrocytes cultured for seven or more days ('cultured chondrocytes') was stimulated by TGF-β.

In this study we excluded the possibility that this was an effect of the collagenase treatment used to isolate chondrocytes from cartilage, suggesting that the differential effect of TGF-β on freshly isolated and cultured chondrocytes is a result of phenotypical changes occurring during culture. We showed that freshly isolated chondrocytes are normal differentiated chondrocytes as they have the typical polygonal morphology of differentiated chondrocytes and produced predominantly large proteoglycans, both characteristics of differentiated chondrocytes.[10] During culture, phenotypical changes occur as monolayer cultured chondrocytes had an altered morphology and synthesised, in addition to large proteoglycans, a considerable amount of smaller proteoglycans.

The observation that proteoglycan synthesis of normal differentiated chondrocytes is inhibited by TGF-β is in agreement with earlier observations.[6 30] Proteoglycan synthesis of articular chondrocytes cultured in agarose, a system in which chondrocytes maintain their differentiated phenotype, was inhibited by TGF-β.[6] Moreover, proteoglycan synthesis of intact murine articular cartilage, cultured for one day, was also inhibited by TGF-β.[30] As the effects of TGF-β are mediated by its cell surface receptors, we investigated whether the dissimilar effect of TGF-β on proteoglycan synthesis of freshly isolated and cultured chondrocytes could be the result of differences in TGF-β receptor expression.

Using affinity labelling we were able to show that the type II TGF-β receptor of cultured chondrocytes is about 15 kilodaltons smaller than the type II TGF-β receptor of freshly isolated chondrocytes. No other reproducible variations in TGF-β receptor expression were found. This observation suggests a relation between the size of the TGF-β type II receptor and the effect of TGF-β on the proteoglycan synthesis of articular chondrocytes. As the type II TGF-β receptor has been shown to play a part in mediating inhibition of proteoglycan synthesis on chondrocytes,[31 32] we postulate that the small sized type II TGF-β receptor on cultured chondrocytes is non-functional, leading to stimulating effects of TGF-β by way of the type I or other TGF-β receptors. The existence of comparable non-functional, small sized type II TGF-β receptors on other cell types has been reported earlier. Resistance to growth inhibition by TGF-β was correlated with the expression of non-functional small sized type II TGF-β receptors on mutant bovine endothelial cells,[33] mutant mink lung epithelial cells,[19] and human colon carcinoma cells.[34] As the TGF-β type II receptor is a glycoprotein, with about 15 kilodalton of N-linked carbohydrate,[1 35] it is possible that the difference in molecular weight between normal and small sized type II receptors is a result of alterations in glycosylation. Normal glycosylation was shown on the altered type II TGF-β receptors on mink lung epithelial cells,[35] however, suggesting that other mechanisms may be involved. The difference

between normal and small sized type II receptors might also be the result of alternative splicing. Alternative splicing is described for the type II activin receptor,[36] a receptor like the type II TGF-β receptor, belonging to the serine/threonine kinase family.[13]

In this study we have shown a small sized type II TGF-β receptor on phenotypically altered chondrocytes. Stimulation of the proteoglycan synthesis of these chondrocytes appears to be correlated with the expression of this small sized receptor. As phenotypically changed chondrocytes are also present in osteoarthritic (OA) cartilage[37–39] and the proteoglycan synthesis of human OA cartilage is stimulated by TGF-β, whereas under the same conditions TGF-β had no effect on the proteoglycan synthesis of normal human cartilage,[40] non-functional, small sized type II TGF-β receptors might be expected in OA cartilage. The increased proteoglycan synthesis, characteristic of OA cartilage,[41 42] might be the result of the expression of non-functional, small sized type II TGF-β receptors in combination with functional type I TGF-β receptors. Alteration of expression and function of TGF-β receptors could be a mechanism of chondrocytes in pathological cartilage to initiate the repair process. Interference with cellular physiology by pharmacological modulation of TGF-β receptor expression might provide new directions for the stimulation of tissue repair.

1 Massaqué J. The transforming growth factor-β family. *Annu Rev Cell Biol* 1990; **6:** 597–641.
2 Barnard J A, Lyons R M, Moses H L. The cell biology of transforming growth factor β. *Biochim Biophys Acta* 1990; **1032:** 79–87.
3 Derynck R, Jarrett J A, Chen E Y, Goeddel D V. The murine transforming growth factor-β precursor. *J Biol Chem* 1986; **261:** 4377–9.
4 Morales T I, Joyce M E, Sobel M E, Roberts A B. Autocrine production of TGFβ by calf articular cartilage [abstract]. *Trans Orthop Res Soc* 1990; **15:** 109.
5 Fava R, Olsen N, Keski-Oja J, Moses H, Pincus T. Active and latent forms of transforming growth factor β activity in synovial effusions. *J Exp Med* 1989; **169:** 291–6.
6 Redini F, Galera P, Mauviel A, Layau G, Pujol J P. Transforming growth factor β stimulates collagen and glycosaminoglycan biosynthesis in cultured rabbit articular chondrocytes. *FEBS Lett* 1988; **234:** 172–6.
7 Skantze K, Brinckerhoff C E, Collier J P. Use of agarose culture to measure the effect of transforming growth factor β and epidermal growth factor on rabbit articular chondrocytes. *Cancer Res* 1985; **45:** 4416–21.
8 O'Keefe J R, Crabb I D, Puzas J E, Rosier R N. Differential expression of transforming growth factor-beta receptors and biological effects in chondrocytes [abstract]. *Trans Orthop Res Soc* 1990; **15:** 114.
9 Vivien D, Galéra P, Lebrun E, Loyau G, Pujol J. Differential effects of transforming growth factor-β and epidermal growth factor on the cell cycle of cultured rabbit articular chondrocytes. *J Cell Physiol* 1990; **143:** 534–45.
10 van der Kraan P, Vitters E, van den Berg W. Differential effect of transforming growth factor β on freshly isolated and cultured articular chondrocytes. *J Rheumatol* 1992; **19:** 140–5.
11 O'Grady P, Huang S S, Huang J S. Expression of a new type high molecular weight receptor (type V receptor) of transforming growth factor β in normal and transformed cells. *Biochem Biophys Commun* 1991; **179:** 378–85.
12 MacKay K, Danielpour D. Novel 150- and 180-kDa Glycoproteins that bind transforming growth factor (TGF)-β1 but not TGF-β2 are present in several cell lines. *J Biol Chem* 1991; **266:** 9907–11.
13 Massagué J. Receptors for the TGF-β family. *Cell* 1992; **69:** 1067–70.
14 Segarini P R, Ziman J M, Kane C J M, Dasch J R. Two novel patterns of transforming growth factor β (TGF-β) binding of cell surface proteins are dependent upon the binding of TGF-β1 and indicate a mechanism of positive cooperativity. *J Biol Chem* 1992; **267:** 1048–53.
15 Myoken Y, Kan M, Sato G H, McKeehan Wl, Sato J D. Bifunctional effects of transforming growth factor-β (TGF-β) on endothelial cell growth correlate with phenotypes of TGF-β binding sites. *Exp Cell Res* 1990; **191:** 299–304.
16 Falk L A, de Benedetti F, Lohrey N, *et al.* Induction of transforming growth factor-β1 (TGF-β1) receptor expression and TGF-β1 protein production in retinoic acid-treated HL-60 cells: Possible TGF-β1-mediated autocrine inhibition. *Blood* 1991; **77:** 1248–55.
17 Ito M, Yasui W, Kyo E, *et al.* Growth inhibition of transforming growth factor β on human gastric carcinoma cells: receptor and postreceptor signalling. *Cancer Res* 1992; **52:** 295–300.
18 Ito M, Yasui W, Nakayama H, Yokozaki H, Ito H, Tahara E. Reduced levels of transforming growth factor-beta type I receptor in human gastric carcinomas. *Jpn J Cancer Res* 1992; **83:** 86–92.
19 Laiho M, Weis F M B, Boyd F T, Ignotz R A, Massagué J. Responsiveness to transforming growth factor-β (TGF-β) restored by genetic complementation between cells defective in TGF-β receptors I and II. *J Biol Chem* 1991; **266:** 9108–12.
20 Geiser A G, Burmester J K, Webbink R. Webbink R, Roberts A B, Sporn M B. Inhibition of growth by transforming growth factor-β following fusion of two nonresponsive human carcinoma cell lines. *J Biol Chem* 1992; **267:** 2588–93.
21 Wrana J L, Attisano L, Cárcamo J, *et al.* TGFβ signals through a heteromeric protein kinase receptor complex. *Cell* 1992; **71:** 1003–14.
22 Lin H Y, Wang X F, Ng-Eaton E, Weinberg R A, Lodish H F. Expression cloning of the TGF-β type II receptor, a functional transmembrane serine/threonine kinase. *Cell* 1992; **68:** 775–85.
23 O'Grady P, Liu Q, Huang S S, Huang J S. Transforming growth factor β (TGF-β) type V receptor has a TGF-β stimulated serine/threonine-specific autophosphorylation activity. *J Biol Chem* 1992; **267:** 21033–7.
24 López-Casillas F, Cheifetz S, Doody J, Andres J L, Lane W S, Massagué J. Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-β receptor system. *Cell* 1991; **67:** 785–95.
25 Wang X F, Lin H Y, Ng-Eaton E, Downward J, Lodish H F, Weinberg R A. Expression cloning and characterization of the TGF-β type III receptor. *Cell* 1991; **67:** 797–805.
26 Massagué J, Cheifetz S, Boyd F T, Andres J L. TGF-β receptors and TGF-β binding proteoglycans: recent progress in identifying their functional properties. *Ann NY Acad Sci* 1990: **593:** 59–72.
27 Segarini P R, Rosen D M, Seyedin S M. Binding of transforming growth factor β to cell surface proteins varies with cell type. *Mol Endocrinol* 1989; **3:** 261–71.
28 Salacinski P R P, McLean C, Sykes J E C, Clement-Jones V V, Lowry P J. Iodination of proteins, glycoproteins, and peptides using a solid-phase oxidizing agent, 1,3, 4,6-tetrachloro-3α,6α-diphenyl glycoluril (Iodogen). *Anal Biochem* 1981; **117:** 136–46.
29 Massagué J, Like B. Cellular receptors for type β transforming growth factor. *J Biol Chem* 1985; **260:** 2636–45.
30 van der Kraan P M, Vitters E L, van den Berg W B. Inhibition of proteoglycan synthesis by transforming growth factor β in anatomically intact articular cartilage of murine patellae. *Ann Rheum Dis* 1992; **51:** 643–7.
31 O'Keefe R J, Rosier R N, Puzas J E. Differential expression of biological effects in maturationally distinct subpopulations of growth plate chondrocytes. *Connect Tissue Res* 1990; **24:** 53–66.
32 Rosier R N, O'Keefe R J, Crabb I D, Puzas J E. Transforming growth factor: an autocrine regulator of chondrocytes. *Connect Tissue Res* 1989; **20:** 295–301.
33 Fafeur V, O'Hara B, Böhlen P. The 85-kD TGF-β receptor subtype as a mediator of TGF-β1 in endothelial cells. *J Cell Biochem* 1991; **15B:** 172.
34 Murthy U, Anzano M A, Greig R G. Expression of TGF-α/EGF and TGF-β receptors in human colon carcinoma cell lines. *Int J Cancer* 1989; **44:** 110–5.
35 Laiho M, Weis F M B, Massague J. Concominant loss of transforming growth factor (TGF)-β receptor types I and II in TGF-β resistant cell mutants implicates both receptor types in signal transduction. *J Biol Chem* 1990; **265:** 18518–24.
36 Attisano L, Wrana J L, Cheifetz S, Massagué J. Novel activin receptors: distinct genes and alternative mRNA splicing generate a repertoire of serine/threonine kinase receptors. *Cell* 1992; **68:** 97–108.
37 Gay S, Müller D K, Lemmen C, Remberger K, Matzen K, Kühn K. Immunohistological study on collagen in cartilage-bone metamorphosis and degenerative osteoarthritis. *Klin Wochenschr* 1976; **54:** 969–76.
38 Adam M, Deyl Z. Altered expression of collagen phenotype in osteoarthritis. *Clin Chim Acta* 1983; **133:** 25–32.
39 Von der Mark K, Kirsch T, Aigner T, *et al.* The fate of chondrocytes in osteoarthritic cartilage. Regeneration, dedifferentiation, or hypertrophy. In: Kuettner E, ed. *Articular cartilage and osteoarthritis*. New York: Raven, 1992: 221–34.
40 Lafeber F P J G, van der Kraan P M, Huber-Bruning O, van den Berg W B, Bijlsma J W J. Osteoarthritic human cartilage is more sensitive to transforming growth factor β than normal cartilage. *Br J Rheumatol* 1993; **32:** 281–6.
41 Mankin H J, Johnson M E, Lippiello L. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. III. Distribution and metabolism of amino sugar containing macromolecules. *J Bone Joint Surg [Am]* 1981; **63:** 131–9.
42 Sandy J D, Adams M E, Billingham M E J, Plaas A, Muir H. In-vivo and in-vitro stimulation of chondrocyte biosynthetic activity in early experimental osteoarthritis. *Arthritis Rheum* 1984; **27:** 388–97.