Differential Regulation of Extracellular Matrix Proteoglycan (PG) Gene Expression

TRANSFORMING GROWTH FACTOR-β1 UP-REGULATES BIGLYCAN (PGI), AND VERSICAN (LARGE FIBROBLAST PG) BUT DOWN-REGULATES DECORIN (PGII) mRNA LEVELS IN HUMAN FIBROBLASTS IN CULTURE*

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Proteoglycans (PGs) comprise a group of extracellular matrix macromolecules which play an important role in matrix biology. In this study, normal human skin and gingival fibroblasts were incubated with transforming growth factor-β1 (TGF-β1), and the expression of three PGs, viz. biglycan (PGI), decorin (PGII), and versican (a large fibroblast proteoglycan) was examined. The results indicate that TGF-β1 (5 ng/ml) markedly increased the expression of biglycan (up to 24-fold) and versican (up to 6-fold) mRNAs and the enhancement of biglycan expression was coordinate with elevated type I procollagen gene expression in the same cultures. In contrast, the expression of decorin mRNA was markedly (up to ~70%) inhibited by TGF-β1. The response to TGF-β1 was similar in both skin and gingival fibroblasts, although the gingival cells were clearly more responsive to stimulation by TGF-β1 with respect to biglycan gene expression. Analysis of 35S-labeled proteoglycans in the culture media of skin and gingival fibroblasts also revealed stimulation of biglycan and versican production, and reduction in decorin production. Quantitation of both 35S-sulfate and [3H]leucine-labeled decorin in cell culture media by immunoprecipitation revealed a 50% reduction in decorin production in cell cultures treated with TGF-β1. This TGF-β1-elicted reduction was accompanied by an apparent increase in the size of the decorin molecules, although the size of the core protein was not altered, as judged by Western immunoblotting following chondroitinase ABC digestion. Analysis of proteoglycans in the matrix and membrane fractions also revealed increased amounts of versican in cultures treated with TGF-β1. These results indicate differential regulation of PG gene expression in fibroblasts by TGF-β1, and these observations emphasize the role of PGs in the extracellular matrix biology and pathology.

The extracellular matrix of connective tissue is composed of a variety of macromolecules that interact with each other and with connective tissue cells. As a result of these interactions, the matrix macromolecules provide structural integrity to the tissues, and also mediate regulation of cell growth, migration, and differentiation. Among the major classes of extracellular matrix components are the collagens, elastic fibers, non-collagenous glycoproteins (such as fibronectin), hyaluronic acid, and various proteoglycans (1, 2). Among the proteoglycans (PGs),1 several distinct molecules have been recently partially characterized through cDNA cloning of the core protein sequences (3, 4). For example, two small proteoglycans, biglycan (PGI) and decorin (PGII), contain two and one chondroitin/dermatan sulfate glycosaminoglycan side chains, respectively, attached to a core protein of approximately 45 kDa (3, 6). The core protein sequences have been postulated to play a role in molecular interactions and specifically to facilitate the binding of these macromolecules to other matrix components, such as the collagens and fibronectin.

The entire core protein structure of the large chondroitin sulfate proteoglycan expressed by human fibroblasts, versican, has also been characterized by cDNA cloning. The deduced amino acid sequence consists of several functional domains, including hyaluronic acid binding region, epidermal growth factor-like repeats, a lectin-like sequence, and a complement regulatory protein-like domain (7). Versican has been postulated to function in cell recognition, possibly by connecting extracellular matrix components and cell surface glycoproteins (7). Collectively, these observations suggest that proteoglycan macromolecules play an important role in extracellular matrix assembly in situations involving tissue development and repair. A variety of polypeptide growth factors have been demonstrated to modulate the remodeling of connective tissues (6, 9). One of them, transforming growth factor-β (TGF-β), has been implicated in the regulation of extracellular matrix formation. Specifically, TGF-β has been shown to enhance gene expression of several extracellular matrix components, including type I, III, IV, and V collagens, fibronectin, thrombospondin, osteonectin, and osteopontin (10–17). On the other hand, TGF-β inhibits extracellular

1 The abbreviations used are: PG, proteoglycan; TGF-β, transforming growth factor-β; 1 × SSC (standard saline citrate), 0.15 M NaCl, and 15 mM sodium citrate, pH 6.8; HGF, human gingival fibroblasts; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; kb, kilobase; HSFG, heparan sulfate PG.

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matrix degradation by decreasing the synthesis and secretion of proteases, such as plasminogen activator, stromelysin, and collagenase (18–20), and by stimulating expression of protease inhibitors, such as plasminogen activator inhibitor (21) and tissue inhibitor of metalloproteinase (20).

Several studies have suggested that TGF-β also modulates the synthesis of PGs. Specifically, studies utilizing both human skin fibroblasts and arterial smooth muscle cells in culture have revealed stimulation of PG synthesis by TGF-β, as detected by incorporation of radioactive glucosamine and sulfate (22–24). In addition, two recent studies have shown that TGF-β increases the synthesis of small chondroitin/dermatan sulfate proteoglycans in several different types of cells, including kidney and lung fibroblasts, lung epithelial cells, preadipocytes, skeletal muscle myoblasts, and kidney mesangial cells (25, 26). These observations indicate that PG expression is susceptible to modulation by TGF-β.

In this study, we have specifically examined the expression of three PG macromolecules, decorin, biglycan, and versican, in cultured normal human skin and gingival fibroblasts. The results indicate that TGF-β1 exhibits differential modulation of the core protein mRNAs and the synthesis of these three macromolecules. Specifically, TGF-β1 up-regulated biglycan and versican gene expression, and the up-regulation of biglycan expression was coordinated with the enhancement of type I procollagen gene expression. In contrast, decorin mRNA levels, as well as its production at the protein level, were markedly reduced by TGF-β1.

**MATERIALS AND METHODS**

**Cells**—Human skin fibroblast cultures were established from specimens obtained from cosmetic surgery. These studies primarily dealt with two human skin fibroblast strains (HSF1 and HSF2) established from two 50-year-old females, respectively. Human gingival fibroblast (HGF) cultures were established from healthy gingival tissue of a 27-year-old female. Cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1 mM sodium pyruvate, 2 mM glutamine, antibiotics (50 μg/ml streptomycin, 50 units/ml penicillin), and 10% heat-inactivated fetal calf serum. All tissue culture supplies were purchased from GIBCO. The skin fibroblasts were used in passages 5–15, and the gingival cell cultures were used in passages 8–15.

**Northern Analyses**—Cell cultures in early confluence were rinsed twice with phosphate-buffered saline (PBS), and then placed in DMEM plus 1% fetal calf serum supplemented with various concentrations of TGF-β1 purified from bovine bone (kindly provided by Dr. David Olsen, Collagen Corporation, Redwood City, CA). Total RNA was isolated from cell cultures after incubation with TGF-β1 for the time periods varying from 1 to 48 h (27). Total RNA, 12 μg/lane, was then fractionated on 2.2% agarose gels containing 2.2 M formaldehyde, transferred to nylon filters (Zeta Probe, Bio-Rad), and immobilized by UV-linking (Stratalinker, Stratagene, La Jolla, CA), followed by heating at 80 °C for 30 min under vacuum. The filters were then prehybridized and hybridized in 50% formamide, 0.25 M NaH₂PO₄, pH 7.2, 0.25 M NaCl, 7% (v/v) sodium dodecyl sulfate (SDS), and 1 mM EDTA, with cDNA probes labeled by nick translation with [α-32P]dCTP and [α-35S]dGTP (28). Following hybridizations at 42 °C for 18 h, the filters were washed in solutions with decreasing ionic strength and increasing temperature, and the final stringency of the washes was 0.1× standard saline citrate (SSC), 0.1% SDS, at 65 °C. The [32P]cDNA–mRNA hybrids were visualized by autoradiography, and the corresponding levels of mRNAs were quantitated by scanning densitometry at 633 nm, using a HeNe laser scanner. The corresponding levels of mRNAs were quantitated by scanning densitometry at 633 nm, using a HeNe laser scanner.

**Immunoprecipitation of Decorin**—Media from cell cultures labeled with [35S]sulfate or [3H]leucine for 48 h in the absence or presence of TGF-β1 (5 ng/ml) were used for immunoprecipitation of decorin. Polyclonal antiserum, which was raised against a synthetic amino-terminal peptide corresponding to amino acid residues 1–15 of human decorin (5), was added to the cell culture medium in the final protein concentration of 900 μg/ml. The samples were then mixed overnight at 4 °C, after which protein A-Sepharose (final concentration 4%) was added, and the incubation was continued overnight at 4 °C. Decorin immunoprecipitates were then washed three times with 0.2 M NaCl and 0.1% Tween 20 in 50 mM Tris-HCl buffer, pH 8.0, followed by a rinse with 0.5 M NaCl and 0.1% Tween 20 in the same buffer. The radioactivity was then determined by liquid scintillation counting and the samples were processed for gel electrophoresis, as described previously (33), except that no SDS was added to the running buffer. Agarose gels were air-dried on Gel-Bond (Bioproducts, Rockland, ME) and directly exposed against X-Omat film (Eastman Kodak, Rochester, NY). The agarose gel electrophoresis was performed as described previously (33), except that SDS-PAGE gels were fixed, enhanced (Fluoro-Hance, RPI Corp., Mt. Prospect, IL), and exposed against X-Omat film (Eastman Kodak, Rochester, NY). The agarose gel electrophoresis was performed as described previously (33), except that no SDS was added to the running buffer. Agarose gels were air-dried on Gel-Bond (Bioproducts, Rockland, ME) and directly exposed against X-Omat film.

**Immunoprecipitation of Versican**—Media from cell cultures labeled with [35S]sulfate or [3H]leucine for 48 h in the absence or presence of TGF-β1 (5 ng/ml) were used for immunoprecipitation of versican. Polyclonal antiserum, which was raised against the synthetic amino-terminal peptide corresponding to amino acid residues 1–15 of human decorin (5), was added to the cell culture media in the final protein concentration of 900 μg/ml. The samples were then mixed overnight at 4 °C, after which protein A-Sepharose (final concentration 4%) was added, and the incubation was continued overnight at 4 °C. Decorin immunoprecipitates were then washed three times with 0.2 M NaCl and 0.1% Tween 20 in 50 mM Tris-HCl buffer, pH 8.0, followed by a rinse with 0.5 M NaCl and 0.1% Tween 20 in the same buffer. The radioactivity was then determined by liquid scintillation counting and the samples were processed for gel electrophoresis, as described (34).
Incubation of human skin fibroblasts with TGF-α1, in the concentration range 0.1–5 ng/ml, resulted in a dose-dependent increase in type I procollagen mRNA levels (Fig. 1), demonstrating that under the experimental conditions used the cells were clearly responsive to TGF-α1. Rehybridization of the same Northern filter with a biglycan cDNA revealed a parallel increase in the corresponding mRNA levels (Fig. 1). Rehybridization of the same filter with the GAPDH cDNA did not reveal significant differences in the corresponding mRNA levels in response to TGF-α1 (Fig. 1). Quantitation of the mRNA levels by scanning densitometry of the autoradiograms revealed that the highest increase in proc1(1) collagen and biglycan mRNA levels was noted in the presence of 5 ng/ml of TGF-β1. However, after correction of the values for the GAPDH mRNA levels, the proc1(1) collagen and biglycan mRNA levels were increased approximately 4-fold in cultures treated either with 1 or 5 ng/ml of TGF-β1, as compared with the corresponding values noted in the untreated control cultures.

To examine the possibility that the levels of mRNAs encoding other PG core protein sequences were similarly altered by TGF-β1, Northern hybridizations were also performed with the decorin cDNA. In contrast to observations on biglycan mRNAs, the decorin gene expression was markedly decreased after exposure of the cells to 1 or 5 ng/ml of TGF-β1, and the decorin mRNA levels in the presence of 5 ng/ml of TGF-β1 were reduced to about 50% of those noted in untreated control cells (Fig. 1).

In further studies, the effects of TGF-β1 were examined in cultures of two different human skin fibroblast strains (HSFI and HSF2). These cell cultures were incubated for 24 h with or without TGF-β1 (5 ng/ml). The alterations detected in biglycan, decorin and proc1(1) collagen mRNA levels were similar in both cell strains (Fig. 2 and Table 1). Again, the increase in biglycan mRNA levels (up to 5.6-fold) closely paralleled that noted in proc1(1) collagen mRNA levels (up to 5.6-fold). In contrast, a marked decrease, ~70%, was detected in decorin mRNA levels.

The effect of TGF-β1 on the expression of the large fibroblast proteoglycan, versican, core protein gene was also examined in the same RNA preparations by Northern hybridizations. The levels of the two versican core protein mRNAs (~9 and 10 kb, respectively), were relatively low, yet detectable, in both skin fibroblast strains (Fig. 2). A significant increase, up to 5.8-fold, was noted in HSFI cultures in the presence of 5 ng/ml of TGF-β1 when the mRNA levels were corrected for the GAPDH mRNA levels in the same samples. However, in HSFI cultures, no clear response could be detected in 9- and 10-kb versican mRNAs, but an enhanced hybridization signal could be detected in the 5-kb region,
with that of type I collagen, a time course experiment was performed with gingival fibroblasts studied above. As shown in Fig. 4, the mRNA levels for proc(1)I collagen and biglycan appeared to be coordinately up-regulated up to 24 h of incubation with TGF-β1 (5 ng/ml). At the same time, versican mRNA levels increased to a lesser degree up to 12 h of incubation but remained unaltered thereafter. In contrast, decorin mRNA levels steadily decreased between 1 and 48 h of incubation with TGF-β1 (Fig. 4B).

**TGF-β1 Modulation of Soluble PG Synthesis**—To further examine whether the alterations noted in PG core protein mRNA levels by TGF-β1 might be accompanied by similar changes in the synthesis of the corresponding macromolecules, cultured skin fibroblasts (HSF2) were biosynthetically labeled for 48 h with [35S]sulfate. Six major radioactive bands were detected in the medium compartment of dermal fibroblast cultures, when analyzed by SDS-PAGE or agarose gel (Fig. 5, A and B). The PGs were tentatively identified as decorin, biglycan, versican (2 bands), and two large heparan sulfate PGs (HSPG1 and HSPG2), according to their sizes and sensitivity to enzymatic cleavage by chondroitinases and heparitinase, as well as by immunoblotting (see below). SDS-PAGE separated decorin and biglycan from large Fgs (Fig. 5A), which were more easily distinguishable in agarose gels (Fig. 5B).

Enzymatic digestions were used for further identification of the radiolabeled macromolecules. Specifically, treatment of decorin, biglycan, or versican with chondroitinase ABC removed the GAG side chains resulting in free glucuronic/iduronic acid-galactosamine disaccharides that were not detectable in the SDS-PAGE (Fig. 5A). Digestion of versican with chondroitinase AC completely removed the GAG side chains consisting of glucuronic acid-galactosamine repeats, while GAG chains in biglycan and decorin were only partially digested due to the presence of varying amounts of iduronic acid-rich repeats (Fig. 5B). Heparitinase digestion completely

**Fig. 4. Effects of TGF-β1 on the expression of proc(1)I collagen, biglycan, decorin or versican core protein, and GAPDH mRNAs as function of incubation time.** Human gingival fibroblasts were incubated with TGF-β1 (5 ng/ml), and mRNA levels were determined by Northern hybridization at time points indicated (A). The corresponding mRNA abundance, expressed as relative densitometric units (U)/12 µg of total RNA analyzed/lane, and corrected for the abundance of GAPDH mRNA in the same time points, is indicated (B).
TGF-β and Proteoglycan Gene Expression

FIG. 5. Effects of TGF-β1 on PGs in the culture media of human skin fibroblasts. Fibroblasts (HSF2) were metabolically labeled with [35S]sulfate for 48 h in the presence (+) or absence (−) of 5 ng/ml TGF-β1. The medium samples were dialyzed against deionized water, lyophilized, and treated with chondroitinase ABC (ABC), AC (AC), and heparitinase (Hep), or left untreated (C). Proteoglycans were then fractionated in either 7% SDS-PAGE (A) or 0.75% agarose gels (B), as described under "Materials and Methods." Lanes with samples treated with chondroitinase AC and ABC were exposed about twice the time shown for other lanes. Molecular weight standards (Std) are shown in panel A. The positions of undigested versican, biglycan, decorin, and heparan sulfate proteoglycans (HSPGs) are indicated by arrows.

removed the sulfated glucuronic/iduronic acid-glucosamine rich repeats in the heparan sulfate macromolecules (Fig. 5B). Treatment of dermal fibroblasts (HSF2) with TGF-β1 (5 ng/ml) resulted in reduction in the amount of decorin secreted into the culture media (Fig. 5A). However, the apparent molecular mass of decorin appeared to be increased by about 20 kDa in TGF-β1-treated cell cultures. The expression of biglycan was very low in untreated control cells, but could be markedly increased by incubation with TGF-β1 (Fig. 5A). The synthesis of versican was also stimulated by TGF-β1 while no effect was seen on the two large HSPGs secreted into the medium (Fig. 5B).

Synthesis of radiolabeled decorin was also quantitated by immunoprecipitation using an antibody specific for decorin core protein sequences. Determination of 35S radioactivity in the material precipitated with a decorin-specific antibody revealed that TGF-β1 reduced the total radioactivity, on per cell basis, to approximately 50% of that noted in untreated control cells both in skin fibroblast and gingival fibroblast cultures (Table II). To study if TGF-β1 specifically reduced the synthesis of decorin core protein, the cells were also labeled with [3H]leucine, followed by antibody precipitation. Immunoprecipitation of the [3H]leucine-labeled material in the culture media of TGF-β1-treated cells with a decorin-specific antibody also revealed an approximately 50% decrease in the radioactivity incorporated into the antibody precipitable material as compared to untreated control cultures, indicating that TGF-β1 reduced the synthesis of decorin core protein molecules. The relative cell number in cultures treated with TGF-β1 were significantly increased in comparison to untreated control cultures (Table II).

Fractionation of the 35S-labeled material in the immunoprecipitate by SDS-PAGE confirmed that TGF-β1 reduced the incorporation of 35S-sulfate into bands migrating in the region consistent with decorin molecules (Fig. 6). As noted above, the apparent molecular weight of the decorin molecules synthesized in the presence of TGF-β1 appeared to be slightly higher than that of molecules synthesized in control skin fibroblast cultures. Also, in gingival fibroblast cultures, immunoprecipitations revealed an increase in the molecular weight of decorin as a result of TGF-β1 treatment (Fig. 6). This increase was somewhat less than that noted in dermal fibroblasts which in the absence of TGF-β1 produced a smaller molecular weight decorin than synthesized by gingival cells. The analysis of the decorin core protein by immunoblotting after chondroitinase ABC digestion revealed the presence of two closely migrating bands as noted previously (34), and there was no difference in size of the core protein between cells incubated with or without TGF-β1 (Fig. 7).

Effects of TGF-β1 on PGs in Matrix and Cell Membrane Fractions—Cell membranes of skin fibroblasts (HSF2) extracted by 2% Triton X-100 were found to contain mainly three PGs, namely two heparan sulfate PGs (HSPG3 and 4) and versican, HSPGs being the major membrane associated PGs (Figs. 8 and 9B). Although the amount of radioactivity incorporated into HSPGs was about the same in control and TGF-β1-treated cultures, clearly more radioactively labeled versican was detected in the membrane fraction in TGF-β1 treated cultures (Fig. 8). The membrane fraction HSPGs (HSPG3 and 4) showed electrophoretic mobilities slightly different from those seen in the medium and matrix fractions (HSPG1 and 2) (Fig. 9B). The cell matrix fraction was found to contain more PGs in the cultures incubated with TGF-β1 (Fig. 9, A and B), and four large PGs were definitely associated with the fibroblast matrix. Two large chondroitin sulfate proteoglycans and two large HSPGs were detected. The core proteins in the two CSPGs were identified as versican by immunoblotting (Fig. 7). Since the size of the HSPGs in the matrix fraction was about the same as the size of HSPGs in the medium, they most likely represent HSPG1 and HSPG2, distinct from the membrane-bound HSPG3 and HSPG4. Decorin and biglycan were, however, undetectable in fibroblast matrix fraction. Comparison of the PG patterns in control and TGF-β1-treated cultures revealed that the relative amounts of versican and HSPG1 were increased in the matrix fraction of the TGF-β1-treated cultures (Fig. 9B). Treatment of fibroblast matrix with chondroitinase ABC resulted in release of two versican core proteins (Fig. 9A), identified by Western immunoblotting. Whether these two versican core proteins are related to two different sized versican core protein mRNAs is not known.

DISCUSSION

Activation of connective tissue cells is a critical feature in tissue development and repair, and several growth factors have been implicated in these processes (8, 9). Numerous studies have indicated that TGF-β stimulates connective tissue formation in vivo and in vitro, primarily by enhancing the expression of several genes coding for extracellular matrix components (10–17). Recent studies have also demonstrated that TGF-β stimulates the synthesis of PGs by several different cell types in culture, including human skin fibroblasts and aortic smooth muscle cells (22–26). However, the precise identity of the PGs being synthesized in response to TGF-β1 has not been elucidated in detail. In this study, we have examined the effects of TGF-β1 on the expression of three
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Effects of TGF-β1 on the synthesis of decorin in human fibroblast cultures

Cultures of human skin fibroblasts (HSF2) or human gingival fibroblasts (HGF) were incubated either with $^{35}$SO$_4$ or $[^{3}H]$leucine, without (−) or with (+) TGF-β1 (5 ng/ml). The radioactivity in secreted macromolecules precipitable by decorin-specific antibodies was determined by liquid scintillation counting and expressed as cpm. The values were corrected for relative cell number in the same cultures, as determined by a dye binding assay and expressed as absorbance at 595 nm (see “Materials and Methods”). Specifically, the relative cell numbers were as follows: HSF2 cultures labeled with $^{35}$SO$_4$, 0.700 ± 0.057 and 0.973 ± 0.124 ($p < 0.01$), the same cell strain labeled with $[^{3}H]$leucine, 0.292 ± 0.022 and 0.444 ± 0.003 ($p < 0.001$); HGF cells, 0.594 ± 0.024 and 0.927 ± 0.024 ($p < 0.001$), in the absence and presence of TGF-β1, respectively.

<table>
<thead>
<tr>
<th>TGF-β1</th>
<th>$^{35}$SO$_4$</th>
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<tr>
<td>HSF2</td>
<td>5.796 ± 1.367</td>
<td>2.986 ± 176*</td>
<td>11.680 ± 779</td>
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<td>(1.00 ± 0.24)</td>
<td>(0.58 ± 0.03)</td>
<td>(1.00 ± 0.07)</td>
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<tr>
<td>HGF</td>
<td>4.390 ± 373</td>
<td>2.292 ± 57**</td>
<td>ND*</td>
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<td>(1.00 ± 0.69)</td>
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*The values are mean ± S.D. from four parallel cultures. The numbers in parentheses indicate the relative values in TGF-β1 treated cultures, as compared to untreated control cultures (1.00). The asterisks denote statistically significant differences from the corresponding controls; * $p < 0.01$, ** $p < 0.001$, by Student’s paired t test.

*The ratios are calculated from mean cpm values obtained with $^{35}$SO$_4$ and $[^{3}H]$leucine.

ND, not determined.

In contrast to biglycan and versican, the mRNA levels and the synthesis of decorin were markedly reduced in fibroblast cultures by TGF-β1. Interestingly, accompanied by reduced synthesis of decorin, a change in the size of the molecules was noted. Specifically, the decorin molecules synthesized by skin fibroblasts in the presence of TGF-β1 were ~20 kDa larger than those synthesized by control cells, and this change was likely to reflect the presence of larger individual GAG side chains since the ratio of $^{35}$SO$_4$/[$^{3}H$]leucine incorporation was increased by about 12% in the presence of TGF-β1 (Table II). At the same time, decorin molecules synthesized by gingival fibroblasts in the absence of TGF-β1 was somewhat larger than that synthesized by skin fibroblasts. This finding is in accordance with a previous report.

Fig. 6. Effect of TGF-β1 on the synthesis of decorin in fibroblasts. Human skin (HSF1) and gingival (HGF) fibroblasts were labeled for 48 h with $^{35}$SO$_4$ in the presence or absence of TGF-β1 (5 ng/ml). Radiolabeled decorin in the culture media was immunoprecipitated with a decorin core protein-specific antibody and analyzed by 7% SDS-PAGE and visualized by fluorography.

Fig. 7. Western immunoblotting analysis of decorin (left panel) and versican (right panel) core proteins in the medium and matrix portions, respectively, of human gingival fibroblasts. The cells were cultured in the absence (+) or presence (+) of TGF-β1 (5 ng/ml) for 24 h. Aliquots were digested with chondroitinase ABC and then fractionated in 7.5% SDS-PAGE gels, and transferred to Zeta-probe membrane. Immunodetection of decorin and versican core proteins was performed, as described under “Materials and Methods.” The migration positions of the core proteins and those of molecular weight markers are indicated.

Table II

Effects of TGF-β1 on the synthesis of decorin in human fibroblast cultures

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<thead>
<tr>
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The core protein sequences of decorin and biglycan display a series of tandem repeats of 24 amino acids, and these repeats are characterized by leucine in conserved positions (5, 6). Similar features have been previously detected in a diverse collection of proteins, and it has been postulated that the repeat domains play a role in molecular interactions, specifically facilitating the binding of these proteins to other macromolecules. The core proteins of biglycan and decorin have been shown to bind to other extracellular matrix molecules, including collagens, fibronectin, and other PGs (3). In a recent study, the relative binding affinities of biglycan and decorin to type I collagen were tested (40). It was found that only decorin was readily bound to type I collagen under the conditions tested indicating strong, specific interactions between type I collagen fibrils and the core protein of decorin, as noted earlier in vivo (41). Decorin has also been shown to inhibit fibrillogenesis of both type I and II collagens isolated from bovine tendon and cartilage, respectively (42). Thus, the reduction of decorin production by TGF-β1 may result in enhanced fibrillogenesis of newly synthesized type I and II collagen molecules during development and repair of relevant tissues.

One of the physiologic roles of PGs revolves around their participation in cell regulation. Specifically, the PGs have been implicated in cell adhesion and in the control of cell proliferation. In their role as adhesion molecules, it has been suggested that PGs provide an auxiliary binding mechanism that complements the more specific integrin-mediated adhesion. It has also been suggested that soluble proteoglycans may inhibit cell adhesion by directly binding to the glycosaminoglycan-binding sites in fibronectin and the collagens, thus making them inaccessible to the cell surface PGs (3, 4). Although cell culture findings should be interpreted with caution, it is likely that simultaneous down-regulation of decorin expression and enhancement of type I collagen gene expression demonstrated in this study have a functional role in matrix remodeling. It should be noted, however, that under the experimental conditions used in our study, we could not detect any decorin or biglycan in the matrix fraction of the skin fibroblasts cultured either in the presence or absence of TGF-β1. However, decorin has been previously demonstrated to colocalize with type I collagen fibers in the skin (41). Thus, further studies utilizing fibroblast culture systems together with collagen matrices are necessary to elucidate the mechanisms leading to decorin and biglycan deposition in the extracellular space. Such systems have been recently utilized to examine the influence of collagen lattice on the expression of decorin by cultured fibroblasts (43).

Several observations in the literature suggest that PGs play a role in the control of cell proliferation. Some of these regulatory effects are probably mediated by growth factors, such as basic fibroblast growth factor and TGF-β, which bind to PGs in the extracellular matrices (44, 45). A recent study also demonstrated that the expression of decorin in Chinese hamster ovary cells resulted in changes in growth characteristics of these cells (46). Specifically, the expression of recombinant decorin converted them from a transformed cell phenotype into morphologically normal cells, and this change was accompanied by reduced saturation density in culture. This effect has been shown to result from the ability of decorin core protein to bind TGF-β, a mitogen for these cells (47). The results of our study, utilizing normal skin and gingival fibroblasts in culture, demonstrate a marked reduction in the decorin expression as a result of exposure to TGF-β1. Consistent with the role of decorin in the regulation of cell proliferation is our observation that down-regulation of de-
corin gene expression in fibroblastic cells by TGF-β1 was associated with increased cell number.

In contrast to decorin expression, TGF-β1 significantly increased the expression of biglycan and versican. The enhancement of the synthesis of these two macromolecules was associated with the elevation of type I procollagen gene expression in the same cells. These findings are in accordance with a recent study which showed simultaneous up-regulation of versican and type I collagen gene expression in de-differentiating chondrocytes (48). The precise interactions of biglycan and versican with type I collagen remains to be elucidated. However, the observations of parallel up-regulation of the expression of these genes suggest important biological functions, such as directed molecular interactions, for these macromolecules in situations involving activation of connective tissue formation by TGF-β1, as in wound healing and fibrotic skin diseases (49-51). In this context, it is of interest to note that deficient expression of decorin has been observed in Marfan syndrome (52). In analogy with other extracellular matrix molecules, genetic disorders affecting decorin or other PGs are likely to yield important information on the physiological role of these macromolecules.

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