J. Biochem. 119, 743-748 (1996)

Effect of Exogenous Decorin on Cell Morphology and Attachment of Decorin-Deficient Fibroblasts[1]

Jianguo Gu and Yoshinao Wada[2]

Department of Molecular Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 590-02

Received for publication, October 25, 1995

We have reported deficient expression of decorin on skin fibroblasts from a patient with carbohydrate-deficient glycoprotein syndrome type I [Gu, J. and Wada, Y. (1995) *J. Biochem.* 117, 1276-1279]. The characteristics of fibroblasts from this patient included increased cell spreading and reduced proliferation. We analyzed the expression of other extracellular matrix proteins by Western and Northern blot analyses, and found that adhesion molecules, fibronectin, and type I collagen, were increased, whereas an anti-adhesion molecule, tenascin, was decreased, like decorin. Subsequently, decorin was purified from bovine tendons, and cultured with these fibroblasts in fibronectin-depleted culture medium. Exogenous decorin inhibited cell attachment to a plastic culture dish in a dose-dependent manner, while dermatan sulfate did not. The cell morphology was markedly normalized by decorin, but proliferation was not restored. These findings suggest that decorin exhibits an anti-adhesion property in a fibroblast culture system and that the deficiency is responsible for the morphological change observed in this patient's fibroblasts.

Key words: adhesion molecule, cell attachment, cell spreading, decorin, fibroblast.

The interaction of a cell with its surrounding extracellular matrix (ECM) regulates cell behavior and tissue architecture. There are two groups of ECM proteins, classified as to their adhesive or anti-adhesive properties in the cell-matrix interaction (*1*), and the balance between these properties changes according to cell behavior (*2-4*). For example, an adhesive molecule, fibronectin, that facilitates cell attachment to the matrix is greatly reduced or even absent after transformation of various cultured cells (*3*). On the other hand, anti-adhesive molecules decrease the adhesive force of the ECM, thereby allowing cell migration and division, as has been demonstrated for tenascin (*4, 5*).

A line of evidence has indicated that proteoglycans also play a major role in the cell-matrix interaction (*6-9*). An example is decorin, a small leucine-rich proteoglycan that consists of a protein core of about 36 kDa with a single glycosaminoglycan (GAG) chain substituted near the amino terminus and with three asparagine-linked oligosaccharide chains (*10*). Decorin binds to transforming growth factor β (TGF-β) through its core structure and modulates this ubiquitous growth regulatory factor (*11, 12*). Interestingly, in turn, TGF-β down-regulates decorin mRNA in fibroblasts (*13*). In addition to the binding to TGF-β, decorin binds to ECM proteins and affects their biological functions; binding to fibronectin inhibits cell adherence to fibronectin (*14-16*), and binding to the collagen fibril surface inhibits fibrillogenesis *in vitro* (*17, 18*). These data suggest specific roles of decorin in ECM formation and tissue remodeling *in vivo*. In fact, decorin expression is prominent in organ-lining elements such as mesothelia, parenchymal capsules and meninges in the developing mouse, supporting the notion that decorin is involved in multiple organ development and shaping (*19*).

Under pathological conditions, deficient expression of the decorin gene has been detected in cultured skin fibroblasts from patients with Marfan syndrome (*20*) and infantile progeroid syndrome (*21*), although their pathogenetic roles are unknown. Very recently, we reported a marked deficiency of decorin expression along with an increase in another proteoglycan biglycan in a patient with carbohydrate-deficient glycoprotein syndrome (CDGS) type-I (*22*). It appears that decorin deficiency is associated with multisystemic manifestations such as dysmorphy in this disease, of which the etiology remains to be clarified (*23*). Herein we analyze the expression of various ECM proteins relevant to cell adhesion in the patient's fibroblasts, and the effect of exogenous decorin on these decorin-deficient cells.

MATERIALS AND METHODS

Materials—Polyclonal rabbit antisera against human fibronectin and tenascin were purchased from Chemicon International Inc. (Temecula, CA, USA); a monoclonal

[1] This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas, No. 07259224, from the Ministry of Education, Science and Culture of Japan.

[2] To whom correspondence should be addressed. Tel: +81-725-56-1220, Fax: +81-725-57-3021

Abbreviations: ECM, extracellular matrix; TGF-β, transforming growth factor β; CDGS, carbohydrate-deficient glycoprotein syndrome; DS, dermatan sulfate; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

antibody against human type I collagen from Fujiyakuhin Kogyo (Toyama); a horseradish peroxidase-conjugated antibody to rabbit or mouse IgG from Dako (Glostrup, Denmark); chondroitin ABC lyase and gelatin cellulofine from Seikagaku Kogyo (Tokyo); Dig RNA Labeling and BrdU ELISA kits from Boehringer (Mannheim, Germany); the pT7-Blue T vector from Novagen (Madison, WI, USA); dermatan sulfate (DS, bovine mucosa) from Sigma; and Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) from Gibco-BRL (Gaithersburg, MD, USA).

Cell Culture—Fibroblasts were isolated from outgrowths of a forearm skin specimen from a five-year-old female with type-I CDGS or from child controls of 2-10 years old. All cells in this study were cultured in DMEM supplemented with 10% FBS, and used between passages 5 and 15.

Nucleotide Probes and Northern Blot Analysis—The template cDNA sequences of tenascin and the α1(I) chain of procollagen (COL1A1) for preparation of the cRNA probes were generated by RT-PCR of mRNA from human skin fibroblasts using synthetic oligonucleotides specific for bases 241-261 (sense) and 580-600 (antisense) of tenascin (EMBL/GenBank/DDBJ X56160 by Zardi, L.), and bases 194-204 (sense) and 879-899 (antisense) of COL1A1 (EMBL/GenBank/DDBJ X07884 by Prockop, D.J.), and the amplified products were cloned into the pT7-Blue T vector. Template cDNAs for decorin, biglycan and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were described previously (*22*). For fibronectin, the pBluescript vector containing the 4122-4686 sequence was prepared from the full length cDNA. Digoxigenin-labeled cRNA probes were generated by using the DIG RNA Labeling kit with T7 RNA polymerase. Extraction of total RNA, electrophoresis and hybridization were carried out as described previously (*22*). For reprobing, the membranes were washed with distilled water and then equilibrated with 2× SSC for 5 min each, and then incubated in 0.1×SSC containing 0.1% SDS at 100°C for 5 min to remove the preceding probe.

Preparation of ECM Proteins and Western Blotting Analysis—For the analysis of secreted proteins in conditioned medium, confluent cells were cultured in serum-free medium in a 100 mm dish for 24 h, and then the medium was recovered and 70% saturated with ammonium sulfate (*22*).

For the analysis of matrix proteins, cells were cultured in fibronectin-free medium. Fibronectin-free FBS was prepared in advance by the following procedure; 20 ml of FBS was applied to a Sepharose-4B column (1×2.6 cm), to remove insoluble components, and then applied to a gelatin cellulofine column (1×5.2 cm). The eluent was depleted of fibronectin. The medium of the subconfluent cells was replaced with medium containing 10% fibronectin-free FBS, followed by culturing for another three days. The ECM proteins were extracted with sodium deoxycholate and hypotonic buffer as described in an earlier report (*24*). Matrix proteins obtained from 100 mm culture dishes were dissolved in 10 mM Tris-HCl, pH 6.8, 50 mM NaCl, 0.5 mM EDTA, and 0.1% SDS.

Proteins, at 3 μg per lane, were electrophoresed on a 7.5% sodium dodecyl sulfate polyacrylamide gel, and immunoreactive bands were visualized with the horseradish peroxidase-conjugated antibody against primary antibodies. Fibronectin and tenascin were analyzed under reducing conditions, whereas type I collagen was analyzed under non-reducing conditions because of the immunoreactivity of the antibody.

Isolation of Decorin from Bovine Tendons—Decorin was isolated from bovine tendons as described previously (*25*), with some modifications. Briefly, minced tissue was extracted with 4 M guanidine-HCl, and then the extract was dialyzed against 7 M urea, 0.05 M sodium acetate, and 0.2 M NaCl, pH 6.0. Dialyzed samples were applied to a DE52 cellulose column and eluted with a linear gradient of NaCl from 0.2 to 1.0 M. The fractions containing decorin were dialyzed and applied to a second DE52 column to remove other proteins as completely as possible. The second DE52 cellulose chromatography was performed with a linear gradient of NaCl from 0.25 to 1.0 M. The eluent was examined for purity by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). For subsequent studies, purified decorin was dissolved in phosphate-buffered saline (PBS) at the concentration of 7 mg/ml.

Exposure of Cells to Exogenous Decorin—Fibroblasts were suspended in a medium containing 10% fibronectin-free FBS and various concentrations, 0, 0.6, 3, 20, and 40 μg/ml, of decorin, and then seeded onto plastic culture dishes. The number of attached cells was determined 6 h after plating. The culture medium was replaced with fresh medium containing the same concentration of decorin 24 h after plating. The cells were cultured for another 24 h and then photographed under a phase-contrast microscope.

RESULTS

Expression of ECM Protein Genes in Decorin-Deficient Fibroblasts—We have reported that decorin gene expression was markedly suppressed, while that of biglycan was increased in cultured skin fibroblasts from a patient with CDGS (*22*). In the present study, the expression of other ECM proteins relevant to cell adhesion was analyzed in the same cells. Compared with fibroblasts from a child control, the fibronectin and α1(I) procollagen mRNA levels markedly increased, while the mRNA of an anti-adhesive

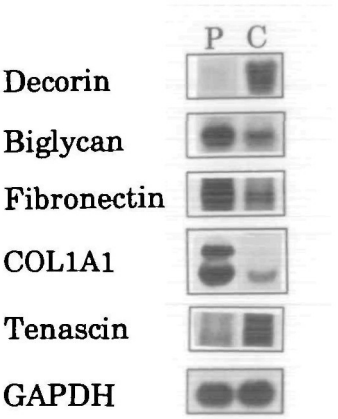

Fig. 1. Expression of ECM protein genes in cultured skin fibroblasts. Total cellular RNA (3 μg) was analyzed by Northern hybridization, successively, using digoxigenin-labeled cRNAs for decorin, biglycan, fibronectin, α1(I) procollagen (COL1A1), and tenascin. GAPDH served as the reference gene C, control (representative of several control children analyzed); P, CDGS patient.

protein, tenascin, decreased sharply (Fig. 1).

ECM Proteins in the Matrix and Conditioned Medium of a Fibroblast Culture—The altered expression of these proteins was substantiated by Western blot analysis of the ECM proteins secreted into the matrix and conditioned medium. In either preparation, increased expression of fibronectin and type I collagen, and decreased expression of tenascin were detected at the protein level (Fig. 2).

Decorin Purified from Bovine Tendons—Decorin was purified from bovine tendons. The prepared sample gave a diffuse band migrating between 97-200 kDa on SDS-PAGE, with Coomassie Blue staining (Fig. 3). Removal of the glycosaminoglycan moiety gave discrete double bands corresponding to 42 and 44 kDa, a similar pattern to that of human fibroblast decorin (*26*). The purity was high. These results allowed the use of the bovine protein for the succeeding study, because the structure is highly conserved between the human and bovine species in both the amino acid sequence and post-translational modification (*19*).

Features of the Decorin-Deficient Fibroblasts—The patient's fibroblasts exhibited abnormal features in shape and proliferation. The cells had a broader surface area and looked laminar (Fig. 4a). They proliferated slowly and showed arrested growth at a low saturation density due to the expanded surface area of individual cells. The doubling time for the patient's fibroblasts was more than twice the normal 48 h for control cells. At confluence, the cell

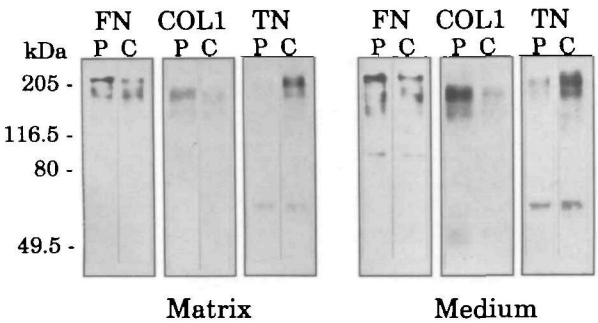

Fig. 2. **Western blot analysis of ECM proteins secreted by fibroblasts.** ECM proteins were extracted from the matrix (3 columns on left) or conditioned medium (3 columns on right) as described under "MATERIALS AND METHODS." Three micrograms dissolved in reducing (fibronectin and tenascin) or non-reducing (type I collagen) sample buffer was electrophoresed on a 7.5% SDS-polyacrylamide gel and then transferred to a nylon membrane. Fibronectin (FN), type I collagen (COL I), and tenascin (TN) were detected at about 220, 180, and 240 kDa, respectively. C, control; P, CDGS patient.

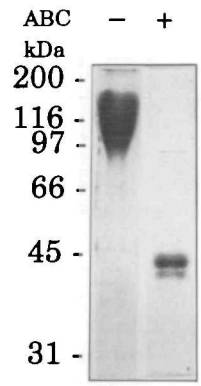

Fig. 3. **SDS-PAGE of the purified decorin.** Bovine decorin was purified from tendons and then loaded on a 10% reducing SDS-polyacrylamide gel. The band was stained with Coomassie Blue. A diffuse band migrating between 90-200 kDa (left lane, 5 μg loaded), and discrete bands migrating at 42 and 44 kDa (right lanes, 1 μg loaded) were observed before and after digestion with chondroitin ABC lyase (ABC), respectively.

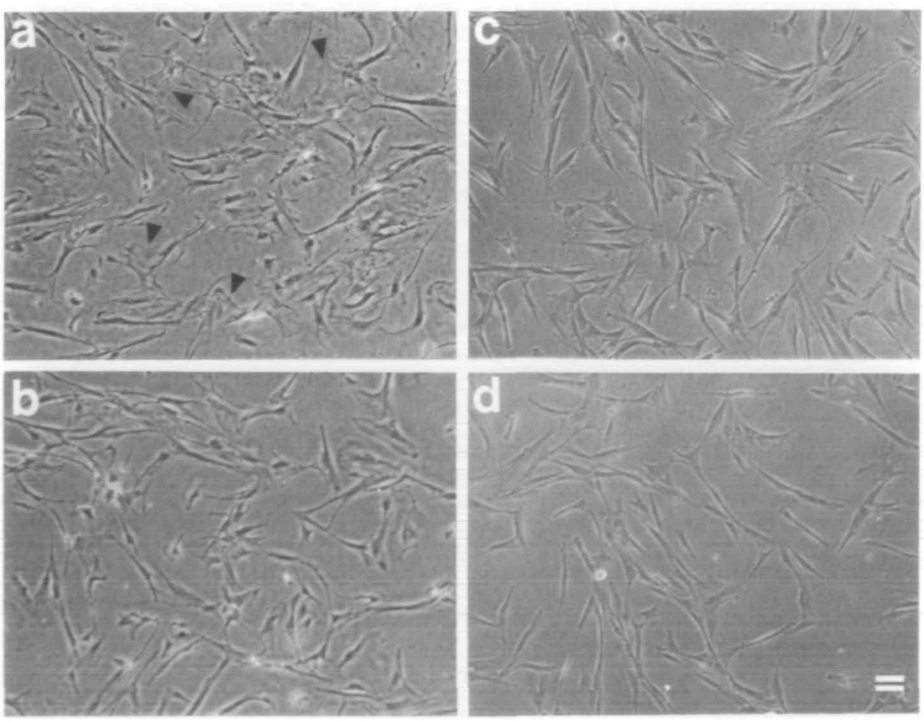

Fig. 4. **The effects of exogenous decorin on cell morphology.** Cultured skin fibroblasts were exposed to bovine decorin (20 μg/ml) for 48 h as described under "MATERIALS AND METHODS." A significant proportion of the patient's cells has a broadened surface area and appear laminar, as indicated by arrowheads, when cultured in the absence of decorin (a). These laminar-shaped cells are not recognizable in the culture with decorin (b). A small number of control cells also exhibits the laminar morphology (c), but such cells are not observed following exposure to exogenous decorin (d). Bar, 200 μm. Patient's cells (a, b), control cells (c, d).

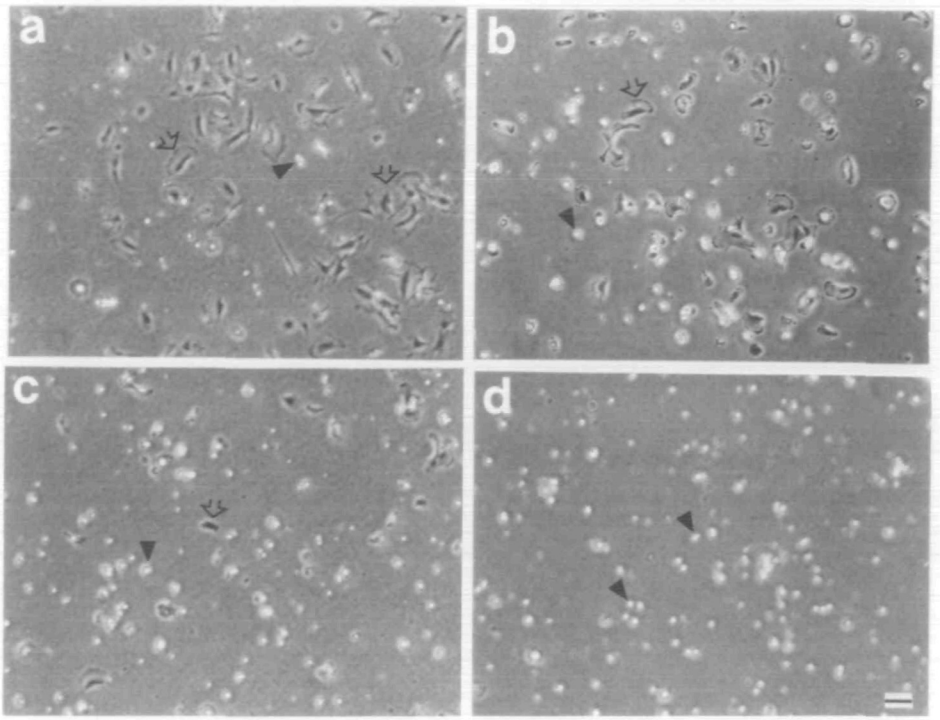

Fig. 5. **The effects of exogenous decorin on cell attachment to plastic culture dishes.** The patient's fibroblasts were suspended in 10% fibronectin-free FBS containing various concentrations of bovine decorin. The final concentrations of decorin in the medium were 0, 0.6, 3, and 20 μg/ml for panels a, b, c, and d, respectively. Cells were photographed 6 h after plating. The white dots are unattached cells floating in the medium. Some of the attached cells are indicated by open arrows. Bar, 200 μm.

TABLE I. **Inhibition of the attachment of the patient's fibroblasts to plastic culture dishes by exogenous decorin.** Fibroblasts were suspended in 10% fibronectin-free FBS medium containing various concentrations of decorin, 20 μg/ml of DS (dermatan sulfate), or 20 μg/ml of BSA, and then seeded onto plastic dishes. 100 cells in each dish were counted at 6 h after plating.

Concentration of decorin (μg/ml, final concentration)	Number of attached cells (in 100 cells)
0	95
0.6	42
3	14
20	5
40	0
0 +20 μg/ml DS	92
0 +20 μg/ml BSA	93

numbers in 100 mm dish were $1\text{-}1.5\times10^6$ and $3\text{-}5\times10^6$ for the patient's and control fibroblasts, respectively.

Effects of Exogenous Decorin on the Adhesion of Decorin-Deficient Fibroblasts to Plastic Culture Plates and on Cell Shape—The effects of various concentrations of decorin on the patient's fibroblasts were examined in fibronectin-free culture medium. At 6 h after plating, only 5% of cells had attached to the plate in the presence of 20 μg/ml of decorin, whereas about 95% adhered in the absence of exogenous decorin (Fig. 5). A similar degree of inhibition of cell attachment was observed in the control cells (data not shown). Neither bovine serum albumin (BSA) nor DS exerted such an effect at the same concentration, and the inhibition of cell attachment by decorin was dose-dependent (Table I). By 12 h after plating, most cells had adhered to the plates even in the presence of 20 μg/ml decorin.

After 24 h incubation, the medium was replaced with fresh medium containing the same concentration of decorin, and the culture was continued for another 24 h. At the end of the last culture in the presence of 20 μg/ml decorin, the population of cells with a laminar shape had markedly decreased, and the surface area of most cells was quite small compared with that of the cells observed under the conditions without decorin (Fig. 4b). This morphological change was also observed, even minimally, in control cells (Fig. 4d). Exogenous decorin did not affect proliferation, as measured by BrdU incorporation, in either control or patient's fibroblasts (data not shown).

DISCUSSION

Increased and decreased expressions of biglycan and decorin, respectively, were reported in the patient's fibroblasts previously (*22*). In the same cells, the synthesis of adhesive proteins, fibronectin and type I collagen, was increased, while that of an anti-adhesive protein, tenascin, was decreased in this study. These findings apparently indicated that the balance between the adhesive and anti-adhesive properties of ECM of cultured fibroblasts shifted toward the former, and therefore the increased cell spreading was presumably due to the aberrant expression of ECM constituents. TGF-β up-regulates fibronectin, tenascin, type I collagen and biglycan, and down-regulates decorin in fibroblasts (*13, 22, 27-29*). These effects have also been confirmed in human skin fibroblasts in culture (our unpublished observation). Most of the changes of the ECM protein expression observed in the patient's fibroblasts can be primarily explained by such direct stimulation by TGF-β. However, the suppression of tenascin, which was expected to be up-regulated by TGF-β, could not be explained by the same mechanism. Secondary effects as well as primary ones of TGF-β would underlie the ECM changes in the patient's fibroblasts.

CDGS is an etiologically unknown, multisystemic disease with severe nervous system involvement and autosomal recessive inheritance (*23*). It shows different presentations during different age periods. During infancy and early childhood liver dysfunction, pericardial effusion and focal fat deposition are observed. In later childhood and adolescence, stationary mental retardation, ataxia, and peripheral neuropathy are only slowly progressive (*30*). The seemingly best clue for elucidating the primary cause is the biochemical hallmark of the disease, *i.e.* *N*-glycosylation deficiency of serum glycoproteins (*31, 32*), but enzymatic studies on the glycosylation pathway have failed to reveal the primary defect, except for the *N*-acetylglucosaminyltransferase II deficiency in very rare variant type II (*33*). The patient analyzed in this study is a typical case of the most common isoform type I of CDGS. Recently, linkage analysis has revealed the genetic locus for type I CDGS in the short arm of chromosome 16 (*34*). None of genes of the ECM molecules analyzed in this study are located in this region.

The patient's fibroblasts have another cytological characteristic, *i.e.* impaired proliferation. Among the ECM proteins analyzed herein, increased expression of fibronectin and type I collagen has also been reported in normal senescent fibroblasts and in fibroblasts from patients with Werner syndrome or a progeria (*35, 36*). These senescent cells also proliferate slowly. Therefore, a mechanism leading to both impaired proliferation and overexpression of adhesive proteins was suggested in CDGS as well as in these pathological and physiological conditions, although CDGS is not a progeria and the cells studied here were still within 15 passages.

The decorin deficiency in the cultured skin fibroblasts in the present CDGS case was severe compared with that in the reported cases of other diseases. We therefore utilized these cells for investigation of the effect of exogenous decorin. Administration of decorin inhibited the cell attachment 6 h after plating, indicating the anti-adhesive property of decorin in the fibroblast culture. Since fibronectin had been removed from the culture medium in advance, decorin might have exhibited its inhibitory activity toward early cell attachment, before the production of fibronectin, without interfering with a fibronectin-mediated adhesion system.

Exogenous decorin dramatically reversed the abnormal morphology caused by enhanced spreading of the patient's fibroblasts. This finding also supported the anti-adhesive function of this protein in fibroblasts. Yamaguchi and Ruoslahti reported that decorin overexpression enhanced cell spreading and impaired the proliferation in Chinese hamster ovary (CHO) cells (*37*), and later demonstrated that the effects were mediated by the inhibition of TGF-β activity (*11*). The observed effect of exogenous decorin in the present study appeared to contradict their results. However, such seemingly inconsistent findings were also made in other studies. Decorin enhances TGF-β activity by promoting its binding to TGF-β receptors in osteoblastic cells (*12*), while TGF-β is negatively regulated by decorin in CHO cells (*11*). Moreover, even with the same CHO cell line, exogenous decorin did not enhance cell spreading, but, instead, reduced focal adhesion formation on a fibronectin-coated substrate (*16*). These contradictory results indicate that the cellular effects of decorin vary according to the cell type and the experimental conditions employed. For correction of the cell shape observed in the present study, a direct or indirect mechanism affecting the ECM architecture should be considered as follows. (i) Decorin reduces the rate of fibril formation by binding to ECM components such as fibronectin and collagens. (ii) Decorin controls the gene expression relevant to the synthesis or degradation of ECM molecules by modulating the activity of cytokines including TGF-β. The latter mechanism involving metalloproteinase induction has been demonstrated for an anti-adhesion molecule, SPARC (*38*).

The authors would like to thank Dr. K. Sekiguchi for the helpful comments and for providing the full length cDNA of human fibronectin, and Y. Nakayama and M. Sakamoto for their excellent technical assistance. The fibroblasts from the patient with CDGS were provided by Prof. S. Okada of Osaka University Medical School.

REFERENCES

1. Sage, E.H. and Bornstein, P. (1991) Extracellular proteins that modulate cell-matrix interactions. *J. Biol. Chem.* **266**, 14831-14834
2. Sage, H., Vernon, R.B., Funk, S.E., Everitt, E.A., and Angello, J. (1989) SPARC, a secreted protein associated with cellular proliferation, inhibits cell spreading *in vitro* and exhibits Ca^{2+}-dependent binding to the extracellular matrix. *J. Cell Biol.* **109**, 341-356
3. Hynes, R.O., Martin, G.S., Shearer, M., Critchley, D.R., and Epstein, C.J. (1976) Viral transformation of rat myoblasts: Effects on fusion and surface properties. *Dev. Biol.* **48**, 35-46
4. Erickson, H.P. and Bourdon, M.A. (1989) Tenascin: An extracellular matrix protein prominent in specialized embryonic tissues and tumors. *Annu. Rev. Cell Biol.* **5**, 71-92
5. Mackie, E.J., Halfter, W., and Liverani, D. (1988) Induction of tenascin in healing wounds. *J. Cell Biol.* **107**, 2757-2767
6. Knox, P. and Wells, P. (1979) Cell adhesion and proteoglycans. I. The effect of exogenous proteoglycans on the attachment of chick embryo fibroblasts to tissue culture plastic and collagen. *J. Cell Sci.* **40**, 77-88
7. Brennan, M.J., Oldberg, Å, Hayman, E.G., and Ruoslahti, E. (1983) Effect of a proteoglycan produced by rat tumor cells on their adhesion to fibronectin-collagen substrata. *Cancer Res.* **43**, 4302-4307
8. Ruoslahti, E. (1991) Proteoglycans in cell regulation. *J. Biol. Chem.* **264**, 13369-13372
9. Ruoslahti, E. and Yamaguchi, Y. (1991) Proteoglycans as modulators of growth factor activities. *Cell* **64**, 867-869
10. Krucius, T. and Ruoslahti, E. (1986) Primary structure of an extracellular matrix proteoglycan core protein deduced from cloned cDNA. *Proc. Natl. Acad. Sci. USA* **83**, 7683-7687
11. Yamaguchi, Y., Mann, D.M., and Ruoslahti, E. (1990) Negative regulation of transforming growth factor-β by the proteoglycan decorin. *Nature* **346**, 281-284
12. Takeuchi, Y., Kodama, Y., and Matsumoto, T. (1994) Bone matrix decorin binds transforming growth factor-β and enhances its bioactivity. *J. Biol. Chem.* **269**, 32634-32638
13. Kähäri, V.-M., Larjava, H., and Uitto, J. (1991) Differential regulation of extracellular matrix proteoglycan (PG) gene expression. *J. Biol. Chem.* **266**, 10608-10615
14. Schmidt, G., Hausser, H., and Kresse, H. (1991) Interaction of the small proteoglycan decorin with fibronectin: Involvement of the sequence NKISK of the core protein. *Biochem. J.* **280**, 411-414
15. Winnemöller, M., Schmidt, G., and Kresse, H. (1991) Influence of decorin on fibroblast adhesion to fibronectin. *Eur. J. Cell Biol.* **54**, 10-17
16. Bidanset, D.J., LeBaron, R., Rosenberg, L., Murphy-Ullrich, J.E., and Hook, M. (1992) Regulation of cell substrate adhesion: Effects of small galactosaminoglycan-containing proteoglycans. *J.*

Cell Biol. **118**, 1523-1531
17. Vogel, K.G., Paulsson, M., and Heinegård, D. (1984) Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. *Biochem. J.* **223**, 587-597
18. Bidanset, D.J., Guidry, C., Rosenberg, L.C., Choi, H.U., Timpl, R., and Hook, M. (1992) Binding of the proteoglycan decorin to collagen type VI. *J. Biol. Chem.* **267**, 5250-5256
19. Scholzen, T., Solursh, M., Suzuki, S., Reiter, R., Morgan, J.L., Buchberg, A.M., Siracusa, L.D., and Iozzo, R.V. (1994) The murine decorin: Complete cDNA cloning, genomic organization, chromosomal assignment, and expression during organogenesis and tissue differentiation. *J. Biol. Chem.* **269**, 28270-28281
20. Pulkkinen, L., Kainulainen, K., Krusius, T., Mäkinen, P., Schollin, J., Gustavsson, K.-H., and Peltonen, L. (1990) Deficient expression of the gene coding for decorin in a lethal form of Marfan syndrome. *J. Biol. Chem.* **265**, 17780-17785
21. Beavan, L.A., Quentin-Hoffmann, E., Schönherr, E., Snigula, F., Leroy, J.G., and Kresse, H. (1993) Deficient expression of decorin in infantile progeroid patients. *J. Biol. Chem.* **268**, 9856-9862
22. Gu, J. and Wada, Y. (1995) Aberrant expression of decorin and biglycan genes in the carbohydrate-deficient glycoprotein syndrome. *J. Biochem.* **117**, 1276-1279
23. Jaeken, J., Hagberg, B., and Strømme, P. (1991) Clinical presentation and natural course of the carbohydrate-deficient glycoprotein syndrome. *Acta Paediatr. Scand. Suppl.* **375**, 6-13
24. Hedman, K., Kurkinen, M., Alitalo, K., Vaheri, A., Johansson, S., and Hook, M. (1979) Isolation of the pericellular matrix of human fibroblast cultures. *J. Cell Biol.* **81**, 83-91
25. Vogel, K.G. and Evanko, S.P. (1987) Proteoglycans of fetal bovine tendon. *J. Biol. Chem.* **262**, 13607-13613
26. Glössl, J., Beck, M., and Kresse, H. (1984) Biosynthesis of proteodermatan sulfate in cultured human fibroblasts. *J. Biol. Chem.* **259**, 14144-14150
27. Allen-Hoffmann, B.L., Crankshaw, C.L., and Mosher, D.F. (1988) Transforming growth factor β increases cell surface binding and assembly of exogenous (plasma) fibronectin by normal human fibroblasts. *Mol. Cell. Biol.* **8**, 4234-4242
28. Pearson, C.A., Pearson, D., Shibahara, S., Hofsteenge, J., and Chiquet-Erusmann, R. (1988) Tenascin: cDNA cloning and induction by TGF-β. *EMBO J.* **7**, 2977-2982
29. Jimenez, S.A., Varga, J., Olsen, A., Li, L., Diaz, A., Herhal, J., and Koch, J. (1994) Functional analysis of the human α1(I) procollagen gene promoter. Differential activity in collagen-producing and non-producing cells and response to transforming growth factor-β1. *J. Biol. Chem.* **269**, 12684-12691
30. Hagberg, B.A., Blennow, G., Kristiansson, B., and Stibler, H. (1993) Carbohydrate-deficient glycoprotein syndromes: Peculiar group of new disorders. *Pediatr. Neurol.* **9**, 255-262
31. Wada, Y., Nishikawa, A., Okamoto, N., Inui, K., Tsukamoto, H., Okada, S., and Taniguchi, N. (1992) Structure of serum transferrin in carbohydrate-deficient glycoprotein syndrome. *Biochem. Biophys. Res. Commun.* **189**, 832-836
32. Yamashita, K., Ideo, H., Ohkura, T., Fukushima, K., Yuasa, I., Ohno, K., and Takeshita, K. (1993) Sugar chains of serum transferrin from patients with carbohydrate-deficient glycoprotein syndrome. *J. Biol. Chem.* **268**, 5783-5789
33. Jaeken, J., Schachter, H., Carchon, H., de Cock, P., Coddeville, B., and Spik, G. (1994) Carbohydrate-deficient glycoprotein syndrome type II: A deficiency in Golgi localised *N*-acetyl-glucosaminyltransferase II. *Arch. Dis. Child.* **71**, 123-127
34. Martinsson, T., Bjursell, C., Stibler, H., Kristiansson, B., Skovby, F., Jaeken, J., Blennow, G., Strömme, P., Hanefeld, F., and Wahlström, J. (1994) Linkage of a locus for carbohydrate-deficient glycoprotein syndrome type I (CDG1) to chromosome 16p, and linkage disequilibrium to microsatellite marker D16S406. *Hum. Mol. Genet.* **3**, 2037-2042
35. Murano, S., Thweatt, R., Shmookler Reis, R.J., Jones, R.A., Moerman, E.J., and Goldstein, S. (1991) Diverse gene sequences are overexpressed in Werner syndrome fibroblasts undergoing premature replicative senescence. *Mol. Cell Biol.* **11**, 3905-3914
36. Rasoamanantena, P., Thweatt, R., Labat-Robert, J., and Goldstein, S. (1994) Altered regulation of fibronectin gene expression in Werner syndrome fibroblasts. *Exp. Cell Res.* **213**, 121-127
37. Yamaguchi, Y. and Ruoslahti, E. (1988) Expression of human proteoglycan in Chinese hamster ovary cells inhibits cell proliferation. *Nature* **336**, 244-246
38. Tremble, P.M., Lane, T.F., Sage, E.H., and Werb, Z. (1993) SPARC, a secreted protein associated with morphogenesis and tissue remodeling, induces expression of metalloproteinases in fibroblasts through a novel extracellular matrix-dependent pathway. *J. Cell Biol.* **121**, 1433-1444