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Decorin Inhibits Cell Migration Through a Process Requiring Its Glycosaminoglycan Side Chain

Blandine Merle,[1]* **Laurence Durussel,**[1] **Pierre D. Delmas,**[1] **and Philippe Clézardin**[2]

[1]INSERM Research Unit 403, Pavillon F, Hôpital Edouard Herriot, 69437 Lyon Cédex 03, France
[2]INSERM Research Unit 403, Faculté de Médecine Laënnec, 69372 Lyon Cédex 08, France

Abstract Several studies overwhelmingly support the notion that decorin (DCN) is involved in matrix assembly, and in the control of cell adhesion and proliferation. However, nothing is known about the role of DCN during cell migration. Cell migration is a tightly regulated process which requires both adhesion (at the leading edge of the cell) and de-adhesion (at the trailing edge of the cell) from the substratum. We have determined in this study the effect of DCN on MG-63 osteosarcoma cell migration and have analyzed whether its effect is mediated by the protein core and/or the glycosaminoglycan side chain. DCN impeded the migration-promoting effect of matrix molecules (fibronectin, collagen type I) known to interact with the proteoglycan. Conversely, DCN did not counteract the migration-promoting effect of fibrinogen lacking proteoglycan affinity. DCN bearing dermatan-sulfate chains (i.e., skin and cartilage DCN) was about 20-fold more effective in inhibiting cell migration than DCN bearing chondroitin-sulfate chains (i.e., bone DCN). In addition, chondroitinase AC-treatment of cartilage DCN (which specifically removes chondroitin-sulfate chains) did not attenuate the inhibitory effect of this proteoglycan, while cartilage DCN deprived of both chondroitin- and dermatan-sulfate chains failed to alter cell migration promoted by either fibronectin or its heparin- and cell-binding domains. These data assert that the dermatan-sulfate chains of DCN are responsible for a negative influence on cell migration. However, isolated glycosaminoglycans failed to alter cell migration promoted by fibronectin, indicating that strongly negatively charged glycosaminoglycans alone cannot account for the impaired cell motility seen with DCN. Overall, these results show that the inhibitory action of DCN is dependent of substratum binding, is differentially mediated by its glycosaminoglycan side chains (chondroitin-sulfate vs. dermatan-sulfate chains), and is independent of a steric hindrance effect exerted by its glycosaminoglycan side chains. J. Cell. Biochem. 75:538–546, 1999.

Key words: decorin; cell migration; glycosaminoglycans

Decorin (DCN) is a small leucine-rich proteoglycan expressed in a wide range of connective tissues, including skin, cartilage and bone tissues [for review, see Hocking et al., 1998]. It is composed of a 40-kDa core protein substituted with a single glycosaminoglycan (GAG) chain which is tissue-type specific. In skin, DCN bears dermatan-sulfate chains [Choi et al., 1989], while in bone it exists as a a chondroitin-sulfate proteoglycan [Franzen et al., 1984]. In cartilage, DCN carries dermatan-sulfate/chondroitine-sulfate chains [Choi et al., 1989]. DCN is an important regulator of matrix assembly because it binds through its core protein to collagen types I and II and inhibits collagen fibrillogenesis both in vitro [Vogel et al., 1984] and in vivo [Danielson et al., 1997]. DCN also interacts with other collagens, such as collagen types V, VI, and XIV [Hocking et al., 1998]. The core protein of DCN mediates the attachment of collagen type VI to striated collagen fibrils [Bidanset et al., 1992a], while its GAG side chain interacts with collagen type XIV [Font et al., 1993]. However, it has become apparent that DCN interacts with a wide variety of proteins that are involved not only in matrix assembly, but also in the control of cell adhesion and proliferation. For instance, the protein core of DCN interacts with the heparin-binding and the cell-binding domains of fibronectin (FN) and inhibits cell adhesive properties of this extracellular matrix protein [Winnemöller et al., 1991; Bidanset at al., 1992b]. Similarly, DCN with dermatan- or chondroitin-sulfate chains inhibits cell adhesion to thrombospon-

Abbreviations used: DCN, decorin; FN, fibronectin; GAG, glycosaminoglycan.

*Correspondence to: B. Merle, INSERM Research Unit 403, Pavillon F, Hôpital Edouard Herriot, Place d'Arsonval, 69437 Lyon Cédex 03, France. E-mail: merle@lyon151.inserm.fr

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din-1 by binding to a KKTR-dependent cell adhesive site present in the N-terminal domain of thrombospondin-1 [Winnemöller et al., 1992; Merle et al., 1997]. Several lines of evidence indicate that DCN also regulate cell proliferation. DCN overexpression in Chinese hamster ovary cells inhibits cell proliferation by virtue of its ability to bind and neutralize transforming growth factor-β activity (TGF-β) [Yamaguchi et al., 1990]. Interestingly, DCN isolated from bovine bone extract rather increases the bioactivity of TGF-β in MC3T3-E1 osteoblastic cells [Takeuchi et al., 1994]. Also, DCN gene expression is markedly up-regulated during quiescence [Mauviel et al., 1995] and ectopic expression of DCN protein core induces growth suppression in a variety of tumor cells, including human Saos-2 osteosarcoma, HT-1080 fibrosarcoma, and WiDr colon carcinoma cells [Santra et al., 1995, 1997]. Besides the neutralization of TGF-β activity [Yamaguchi et al., 1990], the activation of the epidermal growth factor receptor and induction of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ are possible mechanisms by which DCN causes growth suppression [Santra et al., 1997; Moscatello et al., 1998].

Although the studies discussed above overwhelming support the notion that DCN modulates cell adhesion and proliferation, nothing is known about the role of DCN during cell migration. Cell migration is a tightly regulated process which requires both adhesion (at the leading edge of the cell) and de-adhesion (at the trailing edge of the cell) from the substratum [Huttenlocher et al., 1995]. Because cell adhesion is a prerequisite for migration [Huttenlocher et al., 1995] and DCN modulates cell adhesion [Winnemöller et al., 1991, 1992; Bidanset at al., 1992b; Merle et al., 1997], we have determined in this study the effect of DCN on cell migration and have analyzed whether its effect is mediated by the protein core and/or the GAG side chain. We report that intact DCN inhibits cell migration through a process requiring its GAG side chain.

MATERIALS AND METHODS

Proteins and Peptides

DCN was extracted from bovine long bones as previously described [Merle et al., 1997]. Bovine fetal skin and cartilage DCN were generously provided by Drs. H.U. Choi and L.C. Rosenberg (Montefiore Medical Center, Bronx, NY) [Choi et al., 1989]. Collagen type I was a gift from Dr. B. Kehrel (Münster, Germany). Human fibronectin and bovine vitronectin were obtained from Sigma (L'Isle d'Abeau, France). Human fibrinogen was purchased from Diagnostica Stago (Gennevilliers, France). Purified α-chymotryptic fragments of 40 kDa (CS1 heparin-binding domain) and 120 kDa (cell-binding domain) from human fibronectin were purchased from Chemicon (Temecula, CA).

Cells

Human MG-63 osteosarcoma and MDA-MB-231 breast carcinoma cells were obtained from the American Type Culture Collection. Cells were cultured in RPMI medium containing 10% (vol./vol.) fetal calf serum. For cell migration assays, cells were harvested with trypsin/EDTA then resuspended in RPMI medium containing 0.1% (mass/vol.) BSA.

Cell Migration Assay

The haptotactic motility experiments were performed using Bio-Coat cell migration chambers (Becton Dickinson) which consists of a 24-well companion plate (lower chamber) with cell culture inserts (upper chamber) containing a 8-μm diameter pore size membrane. The experimental procedure used was essentially as previously described [Magnetto et al., 1998]. Briefly, the under surface of the membrane (0.33 cm^2) was coated with varying concentrations of vitronectin, collagen type I, fibrinogen, fibronectin, or its proteolytic fragments in the presence or absence of increasing concentrations of DCN (20 μl/insert), and incubated overnight at room temperature. Cells resuspended in RPMI/BSA medium were added to the insert (upper chamber; 50,000 cells/ 0.5 ml); the same medium was also placed in the lower chamber (0.75 ml/well). After a 4-h incubation at 37°C in a 5% $C0_2$ incubator, the upper surface of the porous membrane was wiped with a cotton-tipped applicator to remove nonmigratory cells and the migrant cells on the under surface were fixed and stained with Diff-Quick stain (Baxter). The membranes were mounted on glass slides and the cells from six random microscopic fields (400× magnification) were counted. All experiments were run in duplicate and results were expressed as the number of migrating cells per mm^2.

Chondroitinase Treatment

DCN was digested with chondroitin ABC lyase (0.02 to 2 mU enzyme/µg DCN; EC 4.2.2.4., from *Proteus vulgaris*, ICN) in Tris buffer (10 mM Tris, pH 7.4) for 60 min at 37°C to remove chondroitin- and dermatan-sulfate chains. Alternatively, DCN was treated for 60 min at 37°C with chondroitin AC lyase (0.02 to 2 mU enzyme/µg DCN; EC 4.2.2.5., from *Arthrobacter aurescens*, ICN) to specifically remove chondroitin-sulfate chains. Untreated and chondroitinase-treated DCN were subjected to electrophoresis on a 10% SDS-polyacrylamide gel (Laëmmli) and gels were subsequently stained with Coomassie Blue or Alcian Blue.

RESULTS

DCN Impedes the Migration of Human MG-63 Osteosarcoma Cells Promoted by FN

FN alone promoted the migration of human MG-63 osteosarcoma cells in a dose-dependent manner, reaching a plateau at 1.2 µg/insert (i.e., 3.6 µg/cm^2; data not shown). MG-63 cells were unable to migrate on DCN purified from cartilage. By contrast, preincubation of FN with increasing concentrations of cartilage DCN dose-dependently inhibited MG-63 cell migration promoted by FN, reaching 80% inhibition with 1 µg/insert (i.e., 3 µg/cm^2) of proteoglycan (Fig. 1A,B). Similar to cartilage DCN, both skin and bone DCN inhibited MG-63 cell migration induced by FN (1.2 µg/insert; Fig. 1A). Half-maximal inhibition (IC_{50}) achieved with skin, cartilage and bone DCN was 0.1, 0.25, and 4.5µg/insert (i.e., 0.3, 0.76, and 13.6 µg/cm^2), respectively.

GAG Side Chains Mediate the Inhibitory Effect of DCN on MG-63 Cell Migration Promoted by FN

The observation that cartilage DCN was 18-fold more potent than bone DCN in inhibiting FN-induced cell migration (Fig. 1) suggested that the inhibitory activity of DCN was, at least in part, dependent on its GAG moiety. To address this question, cartilage DCN was first digested with chondroitinase then incubated with FN to perform cell migration assays. Chondroitinase AC-treatment of cartilage DCN (which specifically digests chondroitin-sulfate chains) only partially removed GAG chains as judged by Alcian blue staining of electrophoresed untreated (Fig.2a, lane A) and chondroitinase AC-treated DCN (Fig. 2a, lane B). Removal of chondroitin-sulfate chains by chondroitinase-AC treatment was, however, confirmed by the appearance of a 48-kDa Coomassie blue stained band corresponding to the protein core of cartilage DCN (Fig. 2a, lane D). Chondroitinase ABC-treatment of cartilage DCN (which digests both chondroitin- and dermatan-sulfate chains) totally removed GAG chains as judged by Alcian blue (Fig. 2a, lane C) and Coomassie blue staining of electrophoresed chondroitinase-treated DCN (Fig. 2a, lane E). Under these experimental conditions, chondroitinase ABC-treatment of cartilage DCN abolished the inhibitory activity of native DCN on FN-induced migration of MG-63 cells, while chondroitinase AC-treatment did not (Fig. 2b).

DCN Binds to the Cell- and Heparin-Binding Domains of FN, and Inhibits the MG-63 Cell Migration-Promoting Effect of These FN Domains

DCN core protein interacts with the heparin-binding and RGD-dependent cell-binding domains of FN [Schmidt et al., 1991; Winnemöller et al., 1991]. These FN domains are required for both cell adhesion and migration [Fukai et al., 1991]. Cell migration experiments were therefore conducted with a 40-kDa and a 120-kDa proteolytic fragments containing the heparin-binding domain and the RGD-dependent cell-binding domain of FN, respectively. Both fragments induced MG-63 cell migration in a dose-dependent manner, the 120-kDa fragment being as effective as native FN to support cell migration (optimal concentration 0.288 µg/insert, 0.9 µg/cm^2, data not shown). Higher concentrations of the 40-kDa fragment were required to fully support MG-63 cell migration (optimal concentration 2 µg/insert, 6.1 µg/cm^2). Preincubation of the heparin- and cell-binding domains of FN with increasing concentrations of cartilage DCN resulted in a dose-dependent inhibition of MG-63 cell migration (Fig. 3). Half-maximal inhibition (IC_{50}) achieved with cartilage DCN on MG-63 cell migration induced by the cell- and heparin-binding domains of FN, and native FN was 0.06, 0.15, and 0.25 µg/insert (i.e., 0.18, 0.45, and 0.76 µg/cm^2), respectively. In addition, chondroitinase ABC-treatment of cartilage DCN abolished the inhibitory activity of native DCN on the migration-promoting effect of cell- and heparin-binding domains of FN, while chondroitinase AC-treatment of cartilage DCN did not (Fig. 4).

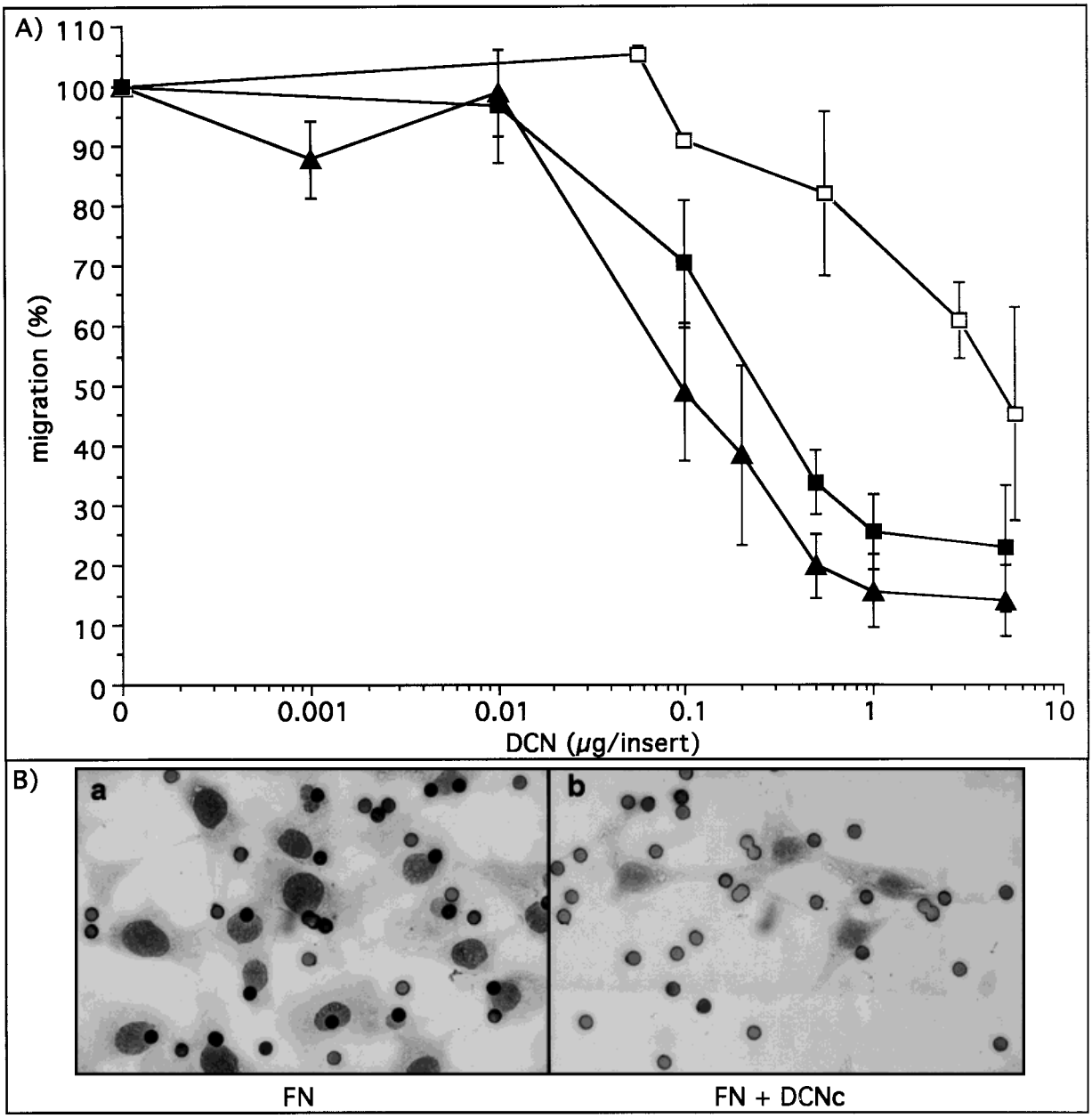

Fig. 1. Skin, cartilage, and bone DCN inhibit the migration of human MG-63 cells promoted by FN. **A**: Cell migration assays were performed with FN (1.2 μg/insert) in the absence or presence of increasing concentrations (0.01 to 5 μg/insert) of cartilage DCN (■), skin DCN (▲), or bone DCN (□). After a 4-h incubation, migrating cells were fixed, stained, and counted. Cell migration induced by FN alone was considered as a positive control and the number of cells that migrated under these experimental conditions was set to 100%. Results obtained in the presence of DCN were expressed as a percentage of the positive control. Results are the mean ± SE of two to eight separate experiments. **B**: MG-63 cell migration on FN (1.2 μg/insert) in the absence (a) or presence (b) of cartilage DCN (1 μg/insert). 400× magnification.

DCN Impedes Cell Migration Promoted by Vitronectin and Collagen Type I, but Not Fibrinogen

Because DCN interacts with several extracellular matrix components, the effect of cartilage DCN on MG-63 cell migration induced by vitronectin (0.5 μg/insert; 1.5 μg/cm^2), collagen type I (0.75 μg/insert; 2.3 μg/cm^2), and fibrinogen (1 μg/insert; 3 μg/cm^2) was tested. As shown in Figure 5, cartilage DCN dose-dependently inhibited cell migration induced by vitronectin and collagen type I, whereas fibrinogen-induced mi-

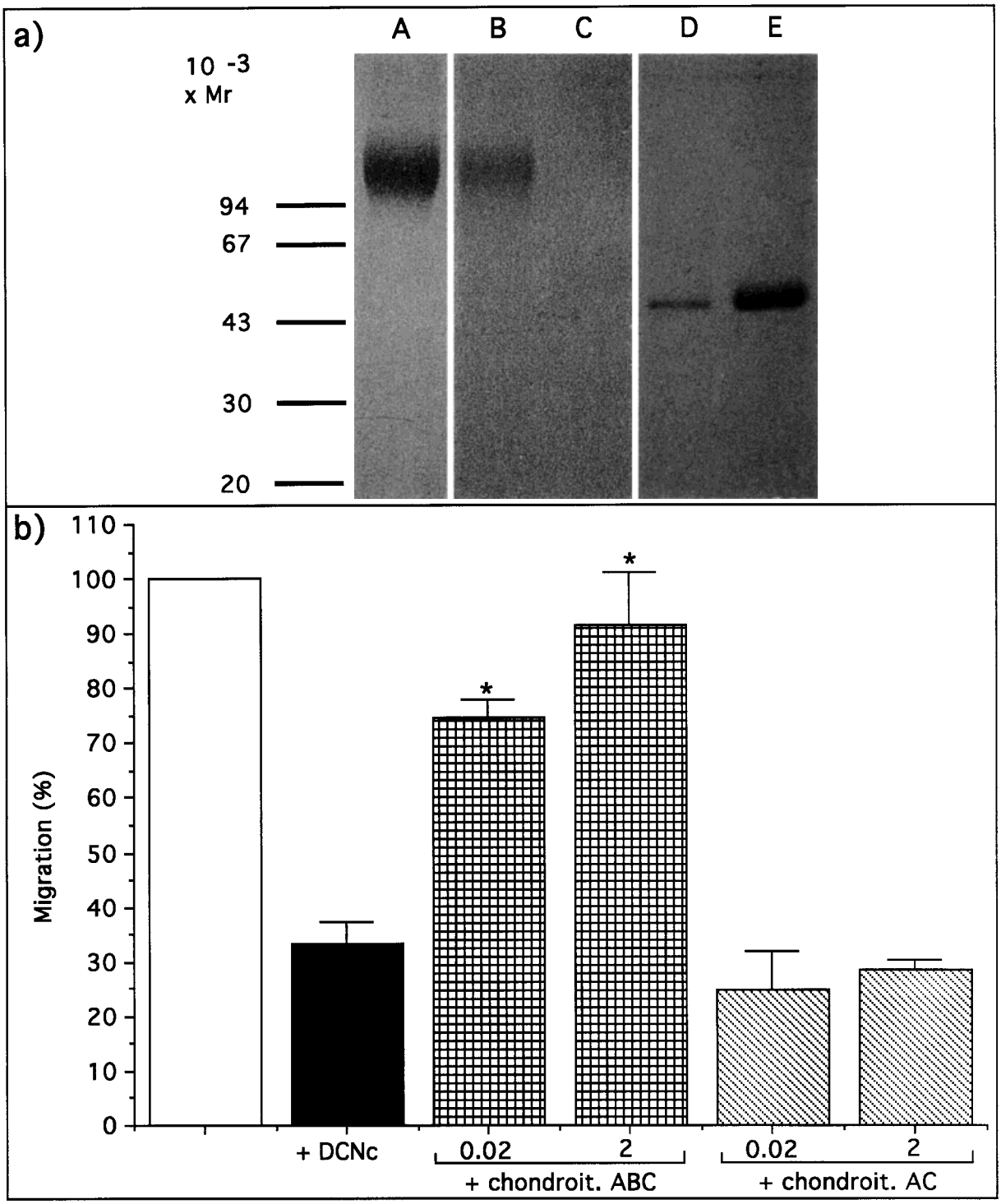

Fig. 2. GAG side chains mediate the inhibitory activity of DCN on the migration-promoting effect of FN. **a**: Untreated and chondroitinase-treated cartilage DCN were subjected to electophoresis on a 10% SDS-polyacrylamide gel then stained with Alcian blue (lanes A–C) or Coomassie blue (lanes D–E). **Lane A**: Untreated DCN. **Lanes B,D**: Chondroitinase AC-treated DCN. **Lanes C,E**: Chondroitinase ABC-treated DCN. **b**: Cell migration assays were performed with FN (1.2 µg/insert) in the absence (□) or presence of native cartilage DCN (1 µg/insert; ■). Alternatively, FN was incubated with cartilage DCN pretreated with 0.02 and 2 mU/µg of chondroitinase ABC (▦) or chondroitinase AC (▧). After a 4-h incubation, migrating cells were fixed, stained, and counted. FN-induced cell migration in the absence of DCN was considered as a positive control and the number of cells that migrated under these experimental conditions was set to 100%. Results obtained in the presence of untreated and chondroitinase-treated DCN were expressed as a percentage of the positive control. Results are the mean ± SE of three separate experiments. $*P < 0.05$ using a Mann-Whitney test.

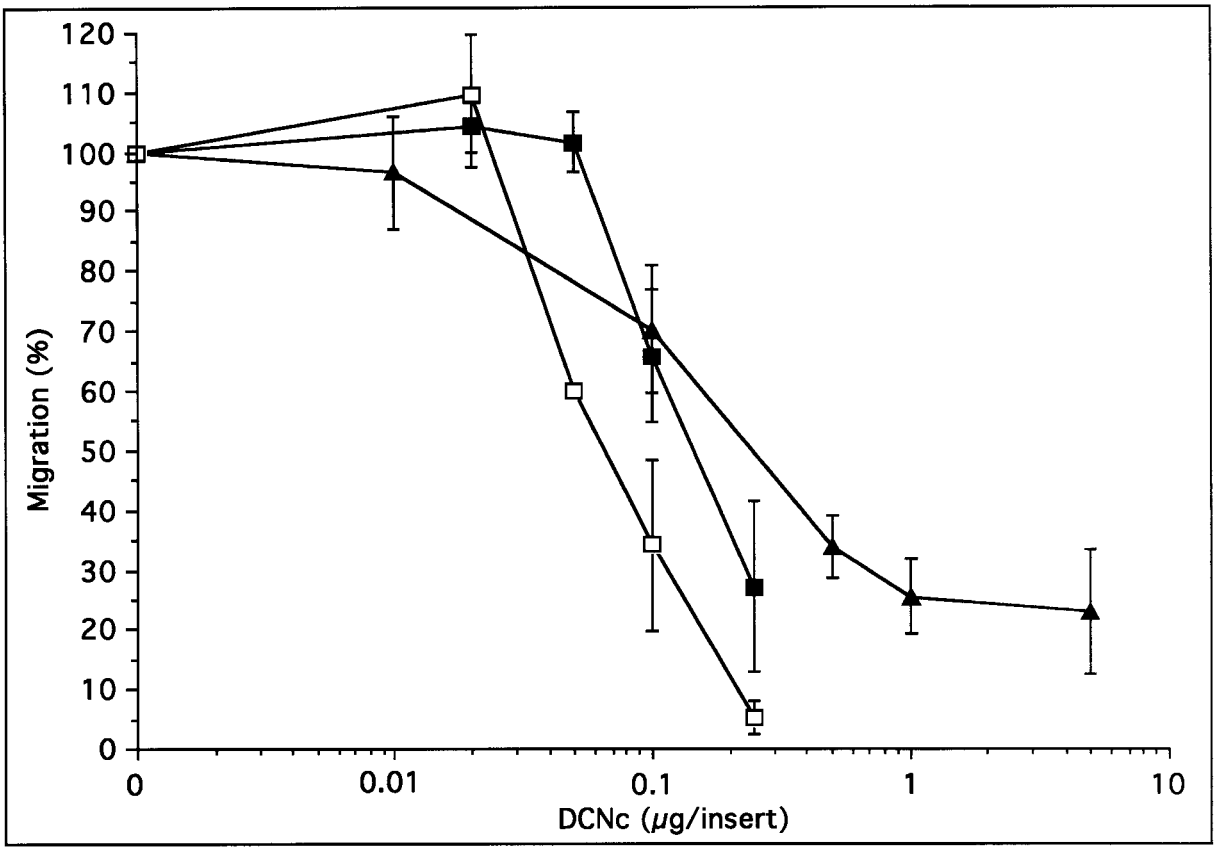

Fig. 3. DCN inhibits MG-63 cell migration promoted by the cell- and heparin-binding domains of FN. Cell migration assays were performed with native FN (1.2 μg/insert) (▲), the cell-binding domain of FN (0.288 μg/insert; □) or its heparin-binding domain (2 μg/insert; ■) in the absence or presence of increasing concentrations of cartilage DCN (DCNc). After a 4-h incubation, migrating cells were fixed, stained, and counted. Cell migration induced by FN and its cell-binding and heparin-binding domains were considered as positive controls and the number of cells that migrated under these experimental conditions was set to 100%. Results obtained in the presence of DCNc were expressed as a percentage of each positive control. Results are the mean ± SE of three to four separate experiments.

gration of MG-63 cells was unaffected. Half-maximal inhibition (IC_{50}) achieved with cartilage DCN on MG-63 cell migration induced by vitronectin was as effective as for FN (0.25 μg/insert; 0.76 μg/cm^2; Fig. 5). Higher concentrations of cartilage DCN were required to inhibit collagen-induced MG-63 cell migration (IC_{50} = 0.5 μg/insert or 1.5 μg/cm^2). The effect of cartilage DCN on FN-induced cell migration was also investigated using human MDA-MB-231 breast carcinoma cells. Cartilage DCN dose-dependently inhibited MDA-MB-231 cell migration promoted by FN (1.2 μg/insert; 3.6 μg/cm^2) with an IC_{50} of 0.15 μg/insert (0.45 μg/cm^2; results not shown).

DISCUSSION

It has become apparent that DCN is involved in matrix assembly, and in the control of cell adhesion and proliferation [for review, see Hocking et al., 1998]. Surprisingly, nothing is known about the role of DCN during cell migration. Here, we report for the first time that intact DCN impedes cell migration. The inhibitory effect of DCN on cell motility likely reflects a general property shared by most proteoglycans. For instance, modular proteoglycans such as aggrecan and versican inhibit neural crest cell migration [Perris et al., 1996]. A large chondroitin sulfate proteoglycan purified from embryonic quail also inhibits spreading and migration of neural crest cells [Kerr et al., 1997]. In addition, biglycan, a small leucine-rich proteoglycan sharing 57% amino acid homology with decorin [Hocking et al., 1998], is selectively expressed at the edges of lamellopodia of migrating endothelial cells [Kinsella et al., 1997], and we have observed that the MG-63 cell migration-

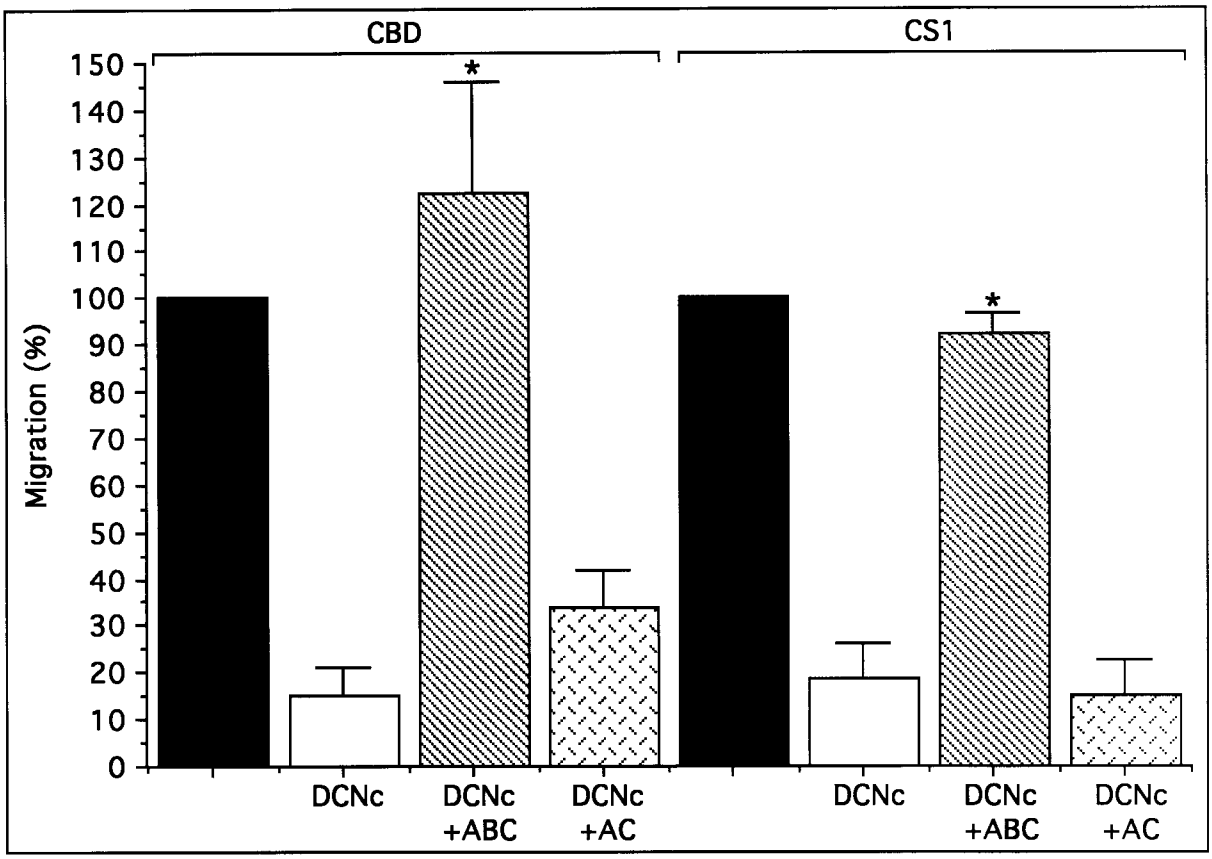

Fig. 4. GAG side chains mediate the inhibitory activity of DCN on the migration-promoting effect of the cell- and heparin-binding domains of FN. Cell migration assays were performed with the cell-binding domain (CBD) or the heparin-binding domain (CS1) of FN in the absence (■) or presence of cartilage DCN (DCNc) either untreated (□) or treated with chondroitinase ABC (2 mU/μg; ▨) or chondroitinase AC (2 mU/μg; ▩). After a 4-h incubation, migrating cells were fixed, stained, and counted. Cell migration induced by CBD or CS1 in the absence of DCNc was considered as a positive control and the number of cells that migrated under these experimental conditions was set to 100%. Results obtained in the presence of untreated and chondroitinase-treated DCNc were expressed as a percentage of each positive control. Results are the mean ± SE of three to seven separate experiments. $*P < 0.05$ using a Mann-Whitney test.

promoting effect of FN was also strongly impaired in the presence of biglycan (unpublished results). Beside the fact that modular and small proteoglycans inhibit cell migration [Perris et al., 1996; Kerr et al., 1997; Kinsella et al., 1997, and this study], it is also most conceivable that this inhibitory effect occurs on both normal and malignant cells. How does DCN inhibit cell migration? In the present study, DCN inhibited the migration-promoting effect of matrix molecules (fibronectin, type I collagen, vitronectin) known to interact with the proteoglycan [Vogel et al., 1984; Winnemöller et al., 1991; Bidanset at al., 1992b; Huttenlocher et al., 1996]. Conversely, DCN did not counteract the migration-promoting effect of fibrinogen lacking proteoglycan affinity. These results suggest therefore that the inhibitory effect of DCN is dependent of substrate binding. DCN bearing dermatan-sulfate chains (i.e., skin and cartilage DCN) was about 20-fold more effective in inhibiting cell migration than DCN bearing chondroitin-sulfate chains (i.e., bone DCN; Fig. 1). In addition, chondroitinase AC-treatment of cartilage DCN (which specifically removes chondroitin-sulfate chains) did not attenuate the inhibitory effect of this proteoglycan, while cartilage DCN deprived of both chondroitin- and dermatan-sulfate chains failed to alter cell migration promoted by either fibronectin or its heparin- and cell-binding domains (Figs. 2 and 4). These data assert that the dermatan-sulfate chains of cartilage DCN are responsible for a negative influence on cell migration. However, they do not rule out the role of chondroitin sulfate chains of bone DCN in inhibiting cell migration. The core

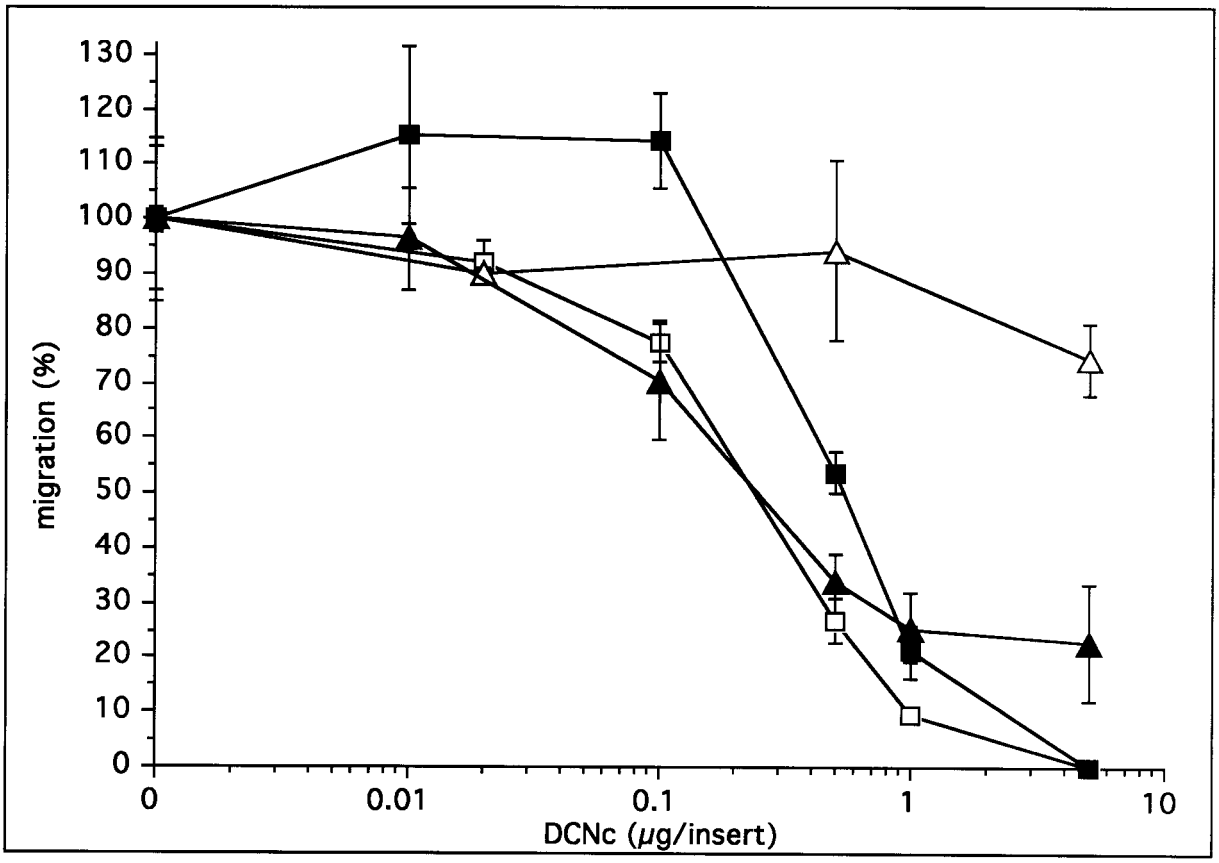

Fig. 5. Comparative effects of DCN on MG-63 cell migration promoted by fibronectin, vitronectin, fibrinogen, and collagen type I. Cell migration assays were performed with vitronectin (0.5 μg/insert; □), collagen type I (0.75 μg/insert; ■), fibrinogen (1 μg/insert;△), and fibronectin (1.2 μg/insert;▲) in the absence or presence of increasing concentrations (0.01 to 5 μg/insert) of cartilage DCN (DCNc). After a 4-h incubation, migrating MG-63 cells were fixed, stained, and counted. Cell migration induced by vitronectin, type I collagen, fibrinogen, or fibronectin in the absence of DCNc was considered as a positive control and the number of cells that migrated under these experimental conditions was set to 100%. Results obtained in the presence of DCNc were expressed as a percentage of each positive control. Results are the mean ± SE of one representative experiment out of three separate experiments.

protein of DCN interacts with fibronectin and collagen type I [Vogel et al., 1984; Winnemöller et al., 1991]. It is therefore possible that, upon association of the core protein with the substrate (fibronectin or collagen type I), the dermatan-sulfate chain of DCN could exert its effect through steric hindrance or by modulating the activity of cell-surface components involved in the process of migration. Isolated strongly negatively charged glycosaminoglycans alone failed to alter MG-63 cell migration promoted by fibronectin (our unpublished results). Thus, a steric hindrance effect exerted by the GAG side chain cannot account for the inhibitory action of DCN on cell migration. It has been recently demonstrated that the small leucine-rich proteoglycan osteoadherin directly binds to integrin $\alpha_v\beta_3$ [Wendel et al., 1998]. A chondroitin sulfate GAG binding site has also been identified in integrin $\alpha_4\beta_1$ [Iida et al., 1998]. It is therefore possible that the GAG side chain of DCN binds directly to integrins and interferes with integrin function during cell motility. Moreover, our data do not preclude a possible contribution of the core protein in this inhibitory process. DCN core protein suppresses tumor cell growth by directly activating the EGF receptor [Moscatello et al., 1998]. MG-63 osteosarcoma cells express the EGF receptor [Mioh et al., 1989]. In addition, the convergence of growth factor-mediated and adhesion-mediated signaling pathways may be required for cell migration. For instance, EGF stimulates the motility of human pancreatic carcinoma cells on vitronectin through activation of integrin $\alpha_v\beta_5$ [Klemke et al., 1994]. It is therefore tempting to speculate that the core

protein of DCN activates the EGF receptor while its GAG side chain interfers with integrin function. Clarifying the exact roles of the DCN core protein and its GAG moiety and their respective receptors in cell migration will undoubtedly be an important task for the future.

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REFERENCES

Bidanset DJ, Guidry C, Rosenberg LC, Choi HU, Timpl R, Hook M. 1992a. Binding of the proteoglycan decorin to collagen type VI. J Biol Chem 267:5250–5256.

Bidanset DJ, Lebaron R, Rosenberg L, Murphy-ullrich JE, Hook M. 1992b. Regulation of cell substrate adhesion: Effects of small galactosaminoglycan-containing proteoglycans. J Cell Biol 118:1523–1531.

Choi HU, Johnson TL, Pal S, Tang L, Rosenberg L, Neame PJ. 1989. Characterization of the dermatan sulfate proteoglycans, DS-PGI and DS-PGII, from articular cartilage and skin isolated by octyl-sepharose chromatography. J Biol Chem 264:2876–2884.

Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV. 1997. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. J Cell Biol 136:729–743.

Font B, Aubert-Foucher E, Goldschmidt D, Eichenberger D, van der Rest M. 1993. Binding of collagen XIV with the dermatan sulfate side chain of decorin. J Biol Chem 268:25015–25018.

Franzén A, Heinegard D. 1984. Characterization of proteoglycans from the calcified matrix of bovine bone. Biochem J 224:59–66.

Fukai F, Suzuki H, Suzuki K, Tsugita A, Katayama T. 1991. Rat plasma fibronectin contains two distinct chemotactic domains for fibroblastic cells. J Biol Chem 266:8807–8813.

Hocking AM, Shinomura T, McQuillan DJ. 1998. Leucine-rich repeat glycoproteins of the extracellular matrix. Matrix Biol 17:1–9.

Huttenlocher A, Sandborg RR, Horwitz AF. 1995. Adhesion in cell migration. Curr Opin Cell Biol 7:697–706.

Huttenlocher A, Werb Z, Tremble P, Huhtala P, Rosenberg L, Damsky CH. 1996. Decorin regulates collagenase gene expression in fibroblasts adhering to vitronectin. Matrix Biol 15:239–250.

Iida J, Meijne AM, Oegema TR, Yednock TA, Kovach NL, Furcht LT, McCarthy JB. 1998. A role of chondroitin sulfate glycosaminoglycan binding site in alpha4-beta1 integrin-mediated melanoma cell adhesion. J Biol Chem 273:5955–5962.

Kerr RSE, Newgreen DF. 1997. Isolation and characterization of chondroitin sulfate proteoglycans from embryonic quail that influence neural crest cell behavior. Dev Biol 192:108–124.

Kinsella MG, Tsoi CK, Järvelainen HT, Wight TN. 1997. Selective expression and processing of biglycan during migration of bovine aortic endothelial cells. J Biol Chem 272:318–325.

Klemke RL, Yebra M, Bayna EM, Cheresh DA. 1994. Receptor tyrosine kinase signalling required for integrin $\alpha_v\beta_5$ directed cell motility but not adhesion to vitronectin. J Cell Biol 127:859–866.

Magnetto S, Bruno-Bossio G, Voland C, Lecerf J, Lawler J, Delmas P, Silverstein R, Clézardin P. 1998. CD36 mediates binding of soluble thrombospondin-1 but not cell adhesion and haptotaxis on immobilized thrombospondin-1. Cell Biochem Funct 16:211–221.

Mauviel A, Santra M, Chen YQ, Uitto J, Iozzo R. 1995. Transcriptional regulation of decorin gene expression. Induction by quiescence and repression by tumor necrosis factor α. J Biol Chem 270:11692–11700.

Merle B, Malaval L, Lawler J, Delmas PDD, Clézardin P. 1997. Decorin inhibits cell attachment to thrombospondin-1 by binding to a KKTR-dependent cell adhesive site present within the N-terminal domain of thrombospondin-1. J Cell Biochem 67:75–83.

Mioh H, Chen JK. 1989. Differential inhibitory effects of TGF-beta on EGF-, PDGF-, and HBGF-1-stimulated MG63 human osteosarcoma cell growth: possible involvement of growth factor interactions at the receptor and postreceptor levels. J Cell Physiol 139:509–516.

Moscatello DK, Santra M, Mann DM, McQuillan DJ, Wong AJ, Iozzo RV. 1998. Decorin suppresses tumor cell growth by activating the epidermal growth factor receptor. J Clin Invest 101:406–412.

Perris R, Perissinoto D, Pettway Z, Bronner-Fraser M, Mörgelin M, Kimata K. 1996. Inhibitory effect of PG-H/aggregan and PG-M/versican on avian neural crest cell migration. FASEB J 10:293–301.

Santra M, Skorski T, Calabretta B, Lattime EC, Iozzo R. 1995. De novo decorin gene expression suppresses the malignant phenotype in human colon cancer cells. Proc Natl Acad Sci USA 92:7016–7020.

Santra M, Mann DM, Mercer EW, Skorski T, Calabretta B, Iozzo RV. 1997. Ectopic expression of decorin protein core causes a generalized growth suppression in neoplastic cells of various histogenetic origin and requires endogenous p21, an inhibitor of cyclin-dependent kinases. J Clin Invest 100:149–157.

Schmidt G, Hausser H, Kresse H. 1991. Interaction of the small proteoglycan decorin with fibronectin. Biochem J 280:411–414.

Takeuchi Y, Kodama Y, Matsumoto T. 1994. Bone matrix decorin binds transforming growth factor-β and enhances its bioactivity. J Biol Chem 269:32634–32638.

Vogel KG, Paulsson M, Heinegard D. 1984. Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. Biochem J 223:587–597.

Wendel M, Sommarin Y, Heinegard D. 1998. Bone matrix proteins: Isolation and characterization of a novel cell-binding keratan sulfate proteoglycan (osteoadherin) from bovine bone. J Cell Biol 141:839–847.

Winnemöller M, Schmidt G, Kresse H. 1991. Influence of decorin on fibroblast adhesion to fibronectin. Eur J Cell Biol 54:10–17.

Winnemöller M, Schön P, Visher P, Kresse H. 1992. Interactions between thrombospondin and the small proteoglycan decorin: Interference with cell attachment. Eur J Cell Biol 59:47–55.

Yamaguchi Y, Mann DM, Ruoslahti E. 1990. Negative regulation of transforming growth factor-β by the proteoglycan decorin. Nature 346:281–284.