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De novo decorin gene expression suppresses the malignant phenotype in human colon cancer cells

(proteoglycan/transfection/cell growth/transformation)

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ABSTRACT **The rapid progress in the cloning of proteoglycan genes has enabled investigators to examine in depth the functional roles these polyhedric molecules play in the control of cell proliferation. Decorin, a leucine-rich proteoglycan expressed by most connective tissues, is a prototype molecule that regulates cellular growth via two mechanisms: modulation of growth factor activity and matrix assembly. We now provide direct evidence that human colon cancer cells stably transfected with decorin cDNA exhibit a marked suppression of the transformed phenotype: the cells have a reduced growth rate *in vitro*, form small colonies in soft agar, and do not generate tumors in *scid/scid* mice. Several independent clones are arrested in the G_1 phase of the cell cycle, and their growth suppression can be restored by treatment with decorin antisense oligodeoxynucleotides. These effects are independent of growth factors and are not due to either clonal selection or integration site of the decorin gene. These findings correlate well with the observation that decorin gene expression is markedly up-regulated during quiescence. Decorin thus appears to be one component of a negative loop that controls cell growth.**

Decorin, a leucine-rich proteoglycan (1, 2) with a ubiquitous tissue distribution (3), has been implicated in the control of cell proliferation (4) by two key observations: overexpression of decorin in Chinese hamster ovary cells inhibits cell proliferation (5) and this biologic effect is mediated by a decorin-induced block of transforming growth factor β (TGF-β) activity (6). These reports correlate quite well with the findings of enhanced decorin gene expression during quiescence in mesenchymal cells (7, 8) and suppression of this expression upon viral transformation (7). We showed that decorin is abnormally expressed in the stroma of human colon cancer (9), that these effects are induced by tumor cell-secreted cytokines acting in a paracrine fashion (10, 11), and that a concurrent hypomethylation of the decorin gene occurs in the connective tissue cells surrounding the tumor (12). More recently, we completed the structural and functional characterization of both the human (13) and murine (14) decorin genes, discovered key regulatory elements that govern their transcriptional machineries (15, 16), and unraveled the patterns of developmental expression that support a role for decorin in epithelial/mesenchymal interactions and organ shaping (14). Despite the above mentioned reports of an association between altered decorin gene expression and growth regulation, there has been no explicit evidence involving this proteoglycan in the regulation of the transformed phenotype. Here we provide direct evidence for a growth-regulating activity of the human decorin proteoglycan. When decorin is ectopically expressed in colon carcinoma cells, there is a marked suppression of the transformed phenotype: the cells manifest a reduced rate of growth *in vitro*, form small colonies in soft agar, and do not generate tumors in *scid/scid* mice. These effects are independent of growth factor activity, clonal selection, or specific integration site. Interestingly, several independent clones are arrested in the G_1 phase of the cell cycle, and their growth can be restored by exposure to antisense decorin oligodeoxynucleotides (aDcns). We propose that decorin belongs to a class of molecules directly involved in growth suppression and that cancer growth may be modulated by an abnormal expression of decorin in the reactive connective tissue stroma.

MATERIALS AND METHODS

Materials. All reagents were of molecular biology grade; [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq) was obtained from Amersham. Carrier-free [^{35}S]sulfate (≈50 Ci/mg of S) was purchased from ICN.

Construction of Decorin Expression Vector and Generation of Stably Transfected Clones. The 5′ untranslated region of the published decorin cDNA (1) is quite complex and harbors the 3′ end of an alternatively spliced exon enriched in GpC dinucleotides (13). In initial transfection experiments, this cDNA was not efficiently expressed, a phenomenon likely due to the negative transcriptional effects of methylation on a G + C-rich sequence in the proximal 5′ untranslated region (15). To circumvent this problem, this region was eliminated by *Msp* I digestion. The resulting construct was treated with Klenow polymerase and ligated to the *Nhe* I-digested decorin coding sequence. The modified full-length decorin cDNA of ≈1.5 kb was digested with *Eco*RI, then digested with *Bam*HI, and finally ligated to the 3′ end of the human cytomegalovirus early gene promoter/enhancer in a eukaryotic expression vector (Invitrogen) (Fig. 1*A*). Proper orientation of the insert was verified by DNA sequencing. About ≈10^7 cells were transfected with 20 μg of DNA (15), and after 2 days in nonselective medium to allow expression of the transfected gene, the cells were passaged and cultured with G418 (600 μg/ml). Independent colonies were isolated by ring cloning, transferred to microtiter wells, and expanded in the same G418 concentration.

Metabolic Labeling, Immunoblotting, and RNA Blotting Analyses. Cultures were metabolically labeled with [^{35}S]sulfate (50 μCi/ml) for 24 hr, and medium and cell layers were analyzed separately by SDS/PAGE and fluorography (17).

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Abbreviations: TGF-β, transforming growth factor β; aDcn, antisense decorin oligodeoxynucleotide; sDcn, sense decorin oligodeoxynucleotide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MTS/PMS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium, inner salt/phenazine methosulfate.

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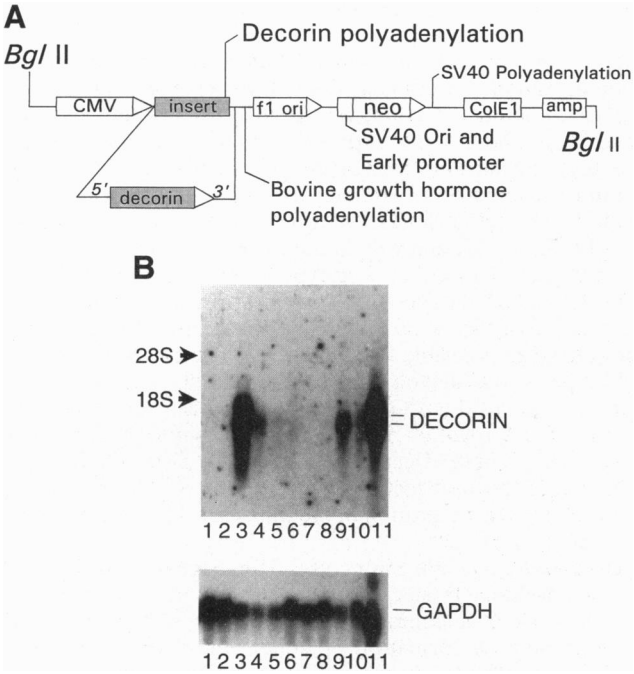

FIG. 1. Decorin transfection construct (*A*) and Northern blotting of transfected clones (*B*). (*A*) Schematic representation of the construct harboring the full-length decorin cDNA and including the signal peptide and two polyadenylylation signals, one from native decorin and one from bovine growth hormone. These two polyadenylylation signals should give two transcripts of ≈1.6 and 1.8 kb, as shown in *B*. CMV, human cytomegalovirus major intermediate early promoter/enhancer region; f1 ori, f1 origin for rescue of single strand; neo, neomycin resistance marker; ColE1, ColE1 origin of replication from pUC19; amp, ampicillin-resistant gene; SV40, simian virus 40; ori, origin of replication. (*B*) Autoradiogram of a typical RNA blotting (10 μg per lane) using either decorin (*Upper*) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (*Lower*) as labeled probe. Lanes: 1 and 2, wild-type colon cancer WiDr cells; 3–10, individual stably transfected clones; and 11, human diploid fibroblast cultures as positive control. Blots were purposely overexposed to detect clones expressing low decorin levels. For better visualization of the two decorin transcripts, see Fig. 5*B*.

Immunoblotting (18) was done with an antibody against the amino-terminal peptide of human decorin (1) before and after chondroitinase ABC digestion. Total RNA (19) was electrophoresed in formaldehyde/1% agarose gels, transferred to nitrocellulose filters, and hybridized to multiprime-labeled (Stratagene) decorin cDNA or GAPDH under high stringency (14).

Cell Proliferation Assays. To establish the growth rates and kinetics of stably transfected clones, we used two independent approaches: cell counting and a nonradioactive cell proliferation assay (20), a colorimetric method based on the use of a modified tetrazolium/formazan [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium, inner salt/phenazine methosulfate] (MTS/PMS) assay. In this method the amount of formazan product is time-dependent and proportional to the number of viable cells (20).

Growth of Transfected Clones in Soft Agar and in Immunocompromised Animals. *In vitro* tumorigenicity was tested by growth in a soft agar colony assay. The flasks were covered with 6 ml of Dulbecco's modified Eagle medium (DMEM)/0.5% agar/10% fetal bovine serum. The middle layer contained ≈10^5 cells in 2 ml of DMEM/0.33% agar/10% fetal bovine serum, while the top layer consisted of 2.5 ml of DMEM/10% fetal bovine serum. Cells were incubated in 37°C and humidified 5% CO_2/air for 14 days, stained with a vital tetrazolium dye (21), and photographed. *In vivo* tumorigenicity was investigated by injecting 10^6 cells s.c. into CB.17 *scid/scid* mice with severe combined immunodeficiency (22). The growth of tumor was recorded at weekly intervals for up to 16 weeks, and tumors were excised and processed for routine histopathology.

aDcn Treatment. aDcns were tested to determine whether the effects of decorin transfection were specific. aDcn 5′-GTCATCAGGAACTTCTGG-3′ antisense to the sequence encoding amino acids 11–16 and the sense decorin oligodeoxynucleotide (sDcn) 5′-GATGAGGCTTCTGGGATA-3′, encoding amino acids 4–9, were selected for testing. Cells were treated for 24 hr with increased concentrations (20–160 μg/ml) of aDcn or sDcn in the presence of Polybrene at 30 μg/ml to enhance uptake (23). Cell growth was measured as above, and total RNA was isolated and hybridized to ^{32}P-labeled decorin cDNA.

RESULTS

Expression of Decorin in Human Colon Cancer Cells. The rationale for investigating the effects of ectopic expression of decorin proteoglycan in human colon cancer WiDr cells is

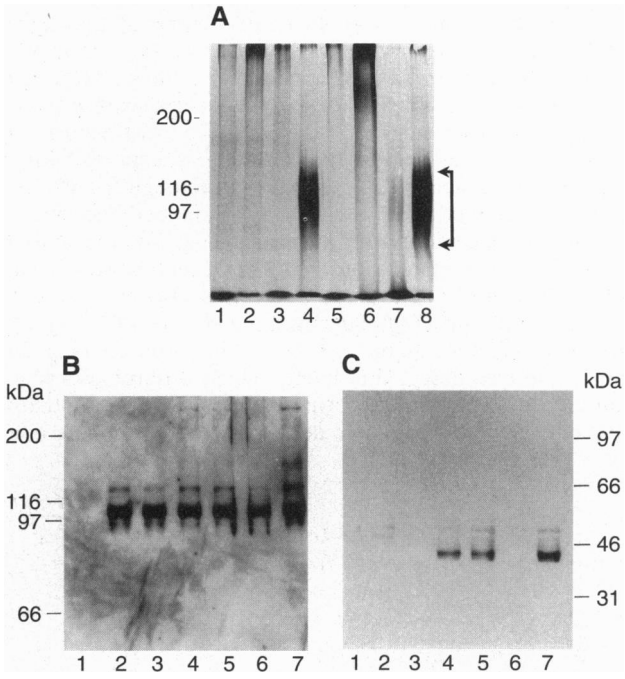

FIG. 2. Autoradiogram of SDS/PAGE electrophoresis of [^{35}S]sulfate-labeled proteoglycans (*A*) and immunoblotting analyses (*B*) before or after (*C*) chondroitinase ABC treatment. (*A*) Proteins extracted from wild-type cells (lanes 1 and 2), stably transfected wD1 clone (lanes 3 and 4), pcDNA3 vector-transfected WiDr as a negative control (lanes 5 and 6), or diploid fibroblasts as a positive control (lanes 7 and 8) were resolved in SDS/5% PAGE. Lanes 1, 3, 5, and 7 are from cell extracts, whereas lanes 2, 4, 6, and 8 are from medium. Molecular mass markers (kDa) are indicated at left, and migration of decorin is indicated at right by bracketed arrowheads. (*B*) Proteins from medium of wild-type WiDr culture (lane 1), stably decorin-transfected wD1 clone (lane 2), wD5 clone (lanes 3 and 4), wD10 clone (lanes 5 and 6), and human diploid fibroblast (lane 7) were separated in a SDS/5% PAGE and immunoblotted as described. The bands were detected by chemiluminescence (18). (*C*) Immunoblotting analysis of total medium proteins after chondroitinase ABC digestion. Equal amounts of proteins were separated in a SDS/10% PAGE, immunoblotted, and probed with the antidecorin antiserum. Lanes: 1, wild-type cells; 2 and 3, pcDNA3-transfected cells; 4 and 5, decorin-transfected clones wD1 and wD10, respectively; 6, negative clone wD14; and 7, human diploid fibroblasts as a positive control. Notice the ≈42-kDa protein typical of deglycosylated decorin (1, 2) in only the two clones wD1 and wD10 (lanes 4 and 5) and fibroblasts (lane 7).

based on the fact that these cells have the unique property of synthesizing perlecan proteoglycan exclusively (17, 24), a large heparan sulfate proteoglycan of basement membranes and extracellular matrices (25). The endogenous decorin gene is silenced by methylation of its transcriptional control regions (12). In addition, the growth properties of these cells are not affected by TGF-β (26), thus allowing the possibility of studying decorin effects independently from TGF-β-induced effects. When cells were transfected with a vector containing the full-length human decorin cDNA driven by a potent viral promoter (Fig. 1*A*), several stably transfected clones resistant to G418, an analogue of the antibiotic neomycin, expressed decorin transcripts at various levels (Fig. 1*B*). Nine independent clones (out of >40) were selected for further studies. Interestingly, several clones showed transcripts of both 1.6 and 1.8 kb, as predicted from use of the native and bovine growth hormone polyadenylylation signal, respectively. The proteoglycan nature of the transfected product was investigated by SDS/PAGE (Fig. 2*A*) and immunoblotting before (Fig. 2*B*) or after (Fig. 2*C*) chondroitinase ABC digestion. The results showed that both wild-type and the mock-transfected cells synthesized only perlecan as a [^{35}S]sulfate-labeled proteoglycan at the top of the separating gel (Fig. 2*A*, lanes 1 and 2, and lanes 5 and 6, respectively). In contrast, one of the highly expressing clones, designated wD1, synthesized a proteoglycan that migrated as a broad band centered at ≈100 kDa (Fig. 2*A*, lane 4) with features identical to native decorin synthesized by human skin fibroblasts (Fig. 2*A*, lane 8). The vast majority of decorin was released into the medium as expected for a properly folded and fully glycosylated proteoglycan (5). To determine whether this proteoglycan was indeed decorin, we used a monospecific antiserum directed against the amino-terminal end of human decorin (1). The results showed that all clones that expressed decorin transcripts also expressed an immunoreactive proteoglycan migrating at ≈100 kDa (Fig. 2*B*, lanes 2–6), a feature shared by human skin fibroblasts (Fig. 2*B*, lane 7) and also detected in human gingival fibroblasts when treated with the same antiserum (27). After chondroitinase ABC digestion, the immunoreactive proteoglycans were converted to a major protein of ≈42 kDa (Fig. 2*C*) typical of decorin protein core.

***De Novo* Synthesis of Decorin Suppresses Cell Growth in Human Colon Carcinoma Cells Independently of TGF-β Activity, Clonal Selection, or Specific Integration Site.** To determine whether the expression of a chondroitin sulfate proteoglycan had any effect on the growth properties of human colon carcinoma cells, we determined the growth kinetics of several transfected clones. All three clones that we studied in detail (wD1, wD5, and wD10) demonstrated a lack of growth as compared with either wild-type or mock-transfected cells (Fig. 3*A* and *B*). (*i*) These effects were not mediated by TGF-β because a 24-hr incubation with TGF-β at either 1 or 10 ng/ml showed no appreciable changes in growth kinetics in either wild-type or transfected clones (data not shown). (*ii*) These effects were not due to clonal selection, inasmuch as pooling all the clones together inhibited growth similarly (data not shown). (*iii*) These effects were not due to a specific integration site of the transfected DNA because Southern blotting showed random integration of the transfected DNA constructs (Fig. 3*C*).

Decorin Expression Suppresses Tumorigenicity of Human Colon Carcinoma Cells Both *in Vitro* and *in Vivo*. It is well established that anchorage-independent growth in semisolid medium and the formation of tumor xenograft in immunocompromised hosts are two of the most useful parameters for evaluating the malignant potential of a given cell population. With this in mind, we tested wild-type and transfected clones for their ability to grow in soft agar or generate tumors in *scid/scid* mice. The stably expressing clones (Fig. 4 *C–E*) generated only small colonies when compared with either the wild-type (Fig. 4*A*) or mock-transfected (Fig. 4*B*) cells. The maximal size of the decorin-transfected clones was on the order of ≈20 cells per colony or ≈10-fold less than the wild type. These results were corroborated by the lack of tumor formation (zero/eight mice) when 10^6 wD1 and wD10 clone cells were injected s.c. into *scid/scid* mice. No tumors were detected after 16 weeks, whereas wild-type cells showed tumor xenograft (four/four mice) at ≈4 weeks after injection (data not shown). These results corroborate the growth kinetics data

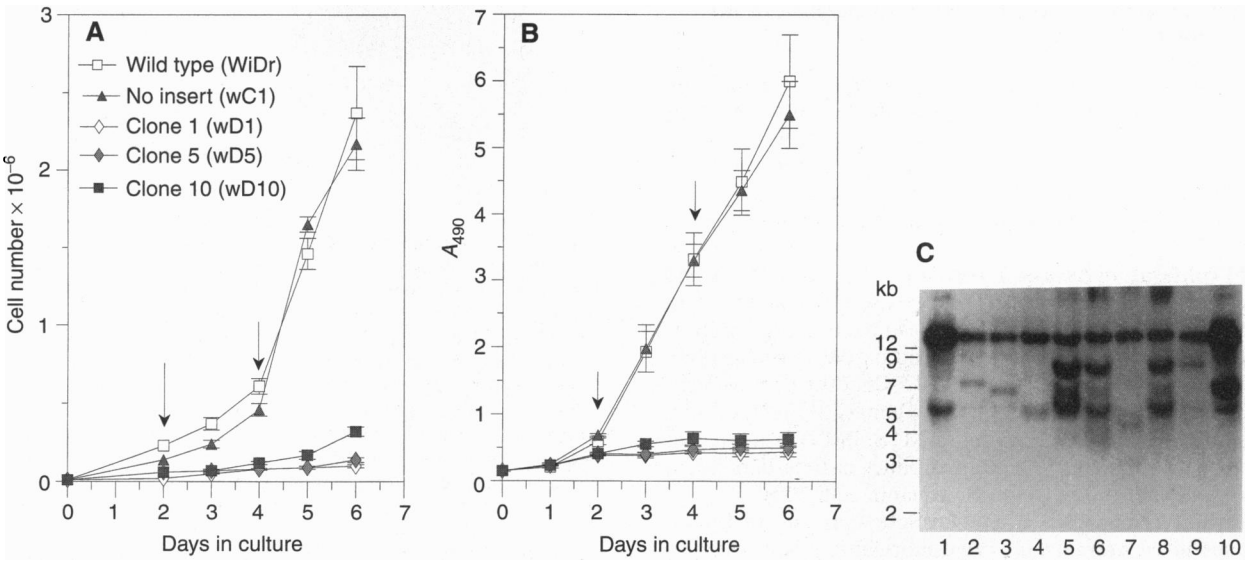

FIG. 3. Growth curve (*A*), proliferation assay (*B*), and Southern blotting analysis (*C*) of transfected and wild-type cells. (*A*) Number of viable cells as determined by hemocytometer. (*B*) Number of proliferating cells as detected by a modified tetrazolium/formazan assay (20). Approximately 5×10^5 cells per well were incubated in 96-well culture plates; then a freshly prepared sample (20 μl) of combined MTS/PMS solution was added to each well and incubated for 1 hr at 37°C in humidified 5% CO_2/atmosphere. Absorbance at 490 nm was then measured. Vertical arrows, addition of fresh medium. Values in *A* and *B* derive from means ± SD of quadruplicate determinations. (*C*) Autoradiogram of Southern blotting of total genomic DNA digested with *Pst* I and hybridized with ^{32}P-labeled decorin cDNA. The endogenous gene is contained in two major bands of ≈5 and 14 kb, as shown in wild-type cells (lane 1). Notice that all stably transfected clones contain additional bands of various sizes, indicating random integration of the exogenous decorin construct. Lanes: 2, clone wD1; 3, wD5; 4, wD9; 5, wD10; 6, wD12; 7, wD13; 8, wD17; 9, wD21; and 10, wD22.

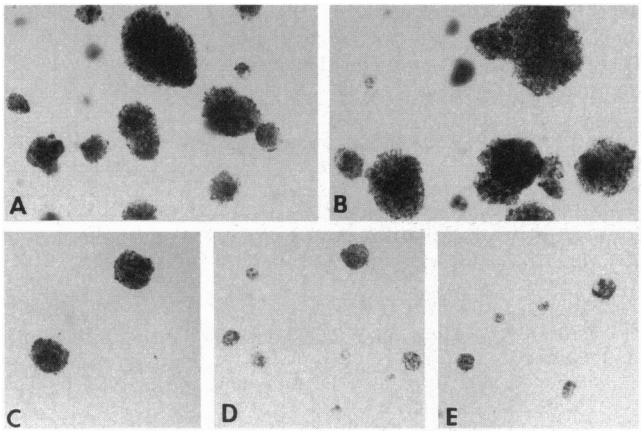

FIG. 4. Soft-agar colony formation of wild-type WiDr colon carcinoma cells (*A*), mock-transfected cells (*B*), and stably transfected clones wD10- (*C*), wD5- (*D*), and wD1- (*E*) expressing various levels of decorin proteoglycan. Cells were seeded in a sandwich of soft agar (0.5% bottom layer and 0.3% top layer) and cultured for 2 weeks. Plates were stained with a vital tetrazolium dye (21) and photographed.

presented above and indicate that the *de novo* decorin expression suppresses the growth and tumorigenicity of colon cancer cells.

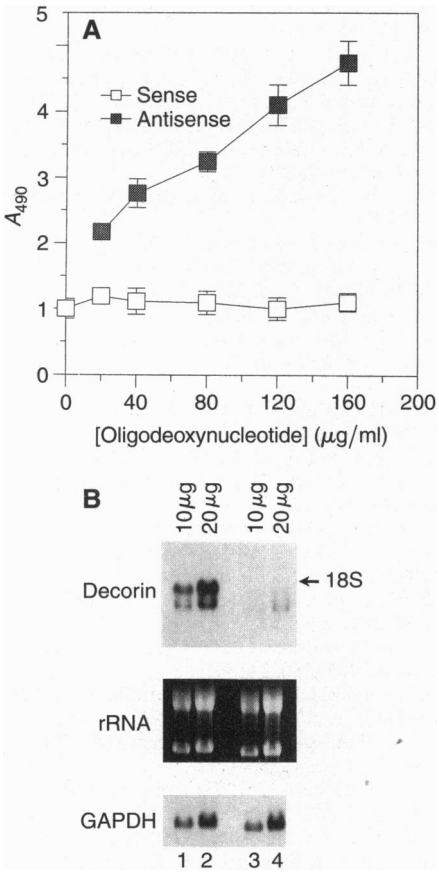

FIG. 5. Effect of sense decorin oligonucleotide (sDcn) and aDcn on cell proliferation (*A*) and decorin expression (*B*). wD1 cells were treated for 24 hr with the designated concentrations of aDcn or sDcn and tested for proliferation assay and steady-state levels of decorin and GAPDH mRNA. Notice a dose–response effect on cell proliferation induced by aDcn treatment and the concurrent suppression (>95%) of exogenous decorin gene expression by aDcn (lanes 3 and 4) vs. sDcn (lanes 1 and 2). In contrast, both ribosomal RNA and GAPDH levels were unaffected by aDcn treatment.

Restoration of Cell Growth in Stably Transfected Clones Upon Treatment with aDcns. To ascertain the specificity of the effects reported above, we treated clone wD1 with aDcn complementary to a sequence in exon 2, immediately after the glycosaminoglycan binding site. The data (Fig. 5*A*) showed a dose-dependent derepression of tumor cell growth induced by aDcn when compared with sDcn. In support of these results was the observation that aDcn markedly suppressed the steady-state level of decorin-specific transcripts (Fig. 5*B*, lanes 3 and 4) as compared with sDcn-treated cells (Fig. 5*B*, lanes 1 and 2). No effects were seen with the wild-type cells from either sDcn or aDcn treatment (data not shown). Further experiments with four different and unrelated oligodeoxynucleotides, based on the primary structure of human perlecan cDNA, showed no appreciable changes in growth kinetics (data not shown). Taken together, these data strongly indicate that decorin is specifically and directly involved in the growth control of human colon carcinoma cells.

Decorin-Expressing Cells Are Blocked in Cell Cycle Progression. Next we wanted to determine whether the inhibitory effects of decorin were due to a block in the onset of DNA synthesis and whether this block could be reversed by abrogating decorin gene expression. The growth state of the cells was therefore tested by fluorescent-activated cell sorting (FACS). Most wild-type cells were actively synthesizing DNA with ≈52% in S and G_2 phases (Fig. 6*A*), while the transfected clones wD1 (Fig. 6*D*) and wD10 (data not shown) were blocked in the G_1 phase of the cell cycle. This block could be specifically abrogated by treatment with aDcn (Fig. 6*F*), in contrast to the sDcn treatment, which showed no changes (Fig. 6*E*). As expected, the wild-type cells showed no changes with either treatment (Fig. 6 *B* and *C*). These results demonstrate a

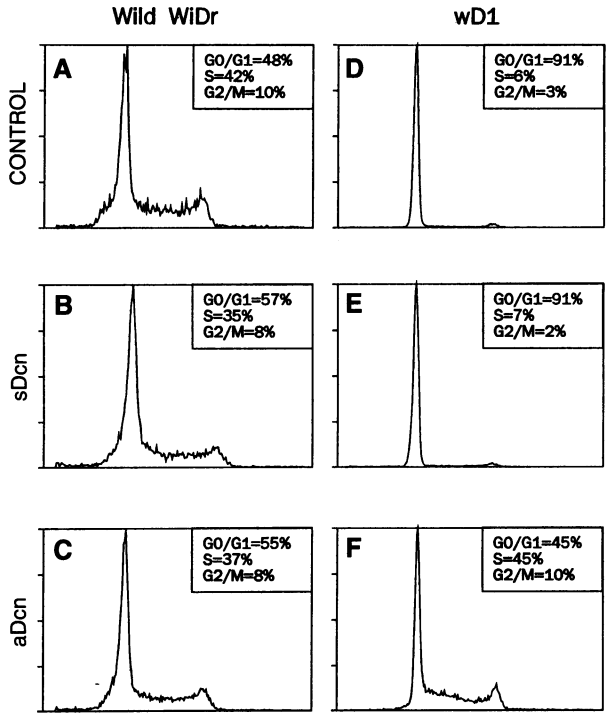

FIG. 6. Fluorescence-activated cell sorter (FACS) analysis of the DNA content of wild-type (*A–C*) and stably transfected clone wD1 (*D–F*), with or without either sDcn or aDcn, as indicated at left. About 10^6 cells were analyzed by FACS after a 24-hr incubation with either medium alone (*A* and *D*) or medium supplemented (160 μg/ml) with sDcn (*B* and *E*) or aDcn (*C* and *F*). Notice the restoration of cell growth and cell cycle features upon aDcn treatment (*F*) when compared with sDcn treatment (*E*). The ordinate indicates amount of DNA as a function of fluorescence. Relative fraction of cells in each phase of the cell cycle is indicated as percentage.

specific and reversible effect of decorin in suppressing the growth of colon carcinoma cells.

DISCUSSION

The results of this study directly implicate decorin in processes regulating the growth and maintenance of the malignant phenotype in human colon carcinoma cells. *De novo* expression of decorin, a gene that is totally suppressed in the wild-type cells via methylation of its control regions (9), leads to an arrest in the G_1 phase of the cell cycle. During this process, the neoplastic cells cease to proliferate and lose their ability to form large colonies in semisolid medium and to induce tumor xenograft in immunocompromised hosts. Our results also show that the decorin-mediated effects are independent of growth factor modulation, inasmuch as TGF-β affects the growth properties of neither parental nor transfected cells. This agrees with our previous study, which has shown that TGF-β does not affect the growth of WiDr colon carcinoma cells (26). The data point toward a specific and direct effect of decorin as another tumor-suppressor gene. This growth suppression can be reversibly abrogated by blocking decorin transcription via aDcns, a process that leads to a complete restoration of the overall growth abilities of the transfected clones. Fundamental questions are how this secreted product modifies growth rates and tumorigenic potential of transformed cells, and whether this growth suppression is also operational in normal diploid cells. Interestingly, quiescent WI38 lung fibroblasts harbor at least 10 separate genes, named quiescins, that are expressed at levels much higher than rapidly proliferating cells (7). Decorin is one of these gene products together with other matrix proteins including collagen type I, III, and VI. Because most of these proteins can bind decorin (28, 29), they may belong to a family of genes that is coordinately regulated during cell cycle progression. We have found that when normal diploid skin fibroblasts cease to proliferate, decorin levels are markedly enhanced, and these changes persist for up to 16 days after confluence vs. cells harvested in their logarithmic phase of growth (16). Nuclear run-off analyses indicated enhanced transcriptional activity of the endogenous gene, a trait that could be reversed by tumor necrosis factor α through a direct down-regulation of decorin promoter activity (16). Similarly, in rat vascular smooth muscle cells, decorin mRNA levels are significantly increased in density-arrested cultures (8). Collectively, these findings suggest that decorin is involved in a negative loop—perhaps by interacting with a specific signal-transducing pathway, inasmuch as decorin is a secreted molecule. Interestingly, the free ectodomain of syndecan, a proteoglycan that inhibits tumor cell growth (30) and collagen gel invasion (31), can suppress the growth of mouse mammary tumor cells (32). Therefore, the reported overexpression of decorin in the tumor stroma of colon cancer patients (9–11) could be reinterpreted as representing a protective, antiproliferative mechanism developed by the organism against tumor growth and invasion.

In conclusion, we establish a growth-regulating activity for decorin that, therefore, should be included as a component of the negative circuit that suppresses growth in proliferating eukaryotic cells. We propose that decorin is an antiproliferative molecule, a possible tumor-suppressor gene, synthesized by the host stromal cells to counteract and block tumor cell growth *in vivo*. Our studies also suggest that (*i*) animals with targeted deletion of the decorin gene may have an increased propensity to develop tumors, (*ii*) boosting endogenous decorin gene expression may be therapeutically beneficial and (*iii*) alternatively, pharmacological delivery of recombinant decorin may have beneficial therapeutic effects in the treatment of human cancer.

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1. Krusius, T. & Ruoslahti, E. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7683–7687.
2. Fisher, L. W., Termine, J. D. & Young, M. F. (1989) *J. Biol. Chem.* **264,** 4571–4576.
3. Bianco, P., Fisher, L. W., Young, M. F., Termine, J. D. & Robey, P. G. (1990) *J. Histochem. Cytochem.* **38,** 1549–1563.
4. Ruoslahti, E. & Yamaguchi, Y. (1991) *Cell* **64,** 867–869.
5. Yamaguchi, Y. & Ruoslahti, E. (1988) *Nature (London)* **336,** 244–246.
6. Yamaguchi, Y., Mann, D. M. & Ruoslahti, E. (1990) *Nature (London)* **346,** 281–284.
7. Coppock, D. L., Kopman, C., Scandalis, S. & Gilleran, S. (1993) *Cell Growth Differ.* **4,** 483–493.
8. Asundi, V. K. & Dreher, K. L. (1992) *Eur. J. Cell Biol.* **59,** 314–321.
9. Adany, R., Heimer, R., Caterson, B., Sorrell, J. M. & Iozzo, R. V. (1990) *J. Biol. Chem.* **265,** 11389–11396.
10. Iozzo, R. V. (1985) *J. Biol. Chem.* **260,** 7464–7473.
11. Iozzo, R. V., Sampson, P. M. & Schmitt, G. (1989) *J. Cell. Biochem.* **39,** 355–378.
12. Adany, R. & Iozzo, R. V. (1991) *Biochem. J.* **276,** 301–306.
13. Danielson, K. G., Fazzio, A., Cohen, I., Cannizzaro, L. A., Eichstetter, I. & Iozzo, R. V. (1993) *Genomics* **15,** 146–160.
14. Scholzen, T., Solursh, M., Suzuki, S., Reiter, R., Morgan, J. L., Buchberg, A. M., Siracusa, L. D. & Iozzo, R. V. (1994) *J. Biol. Chem.* **269,** 28270–28279.
15. Santra, M., Danielson, K. G. & Iozzo, R. V. (1994) *J. Biol. Chem.* **269,** 579–587.
16. Mauviel, A., Santra, M., Chen, Y. Q., Uitto, J. & Iozzo, R. V. (1995) *J. Biol. Chem.* **270,** 11692–11700.
17. Iozzo, R. V. (1984) *J. Cell Biol.* **99,** 403–417.
18. Murdoch, A. D., Liu, B., Schwarting, R., Tuan, R. S. & Iozzo, R. V. (1994) *J. Histochem. Cytochem.* **42,** 239–249.
19. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **102,** 156–159.
20. Buttke, T. M., McCubrey, J. A. & Owen, T. C. (1993) *J. Immunol. Methods* **157,** 233–240.
21. Schaeffer, W. J. & Friend, K. (1976) *Cancer Lett.* **1,** 259–262.
22. Bosma, G. C., Custer, R. P. & Bosma, M. J. (1983) *Nature (London)* **301,** 527–530.
23. Wagner, R. W. (1994) *Nature (London)* **372,** 333–335.
24. Murdoch, A. D., Dodge, G. R., Cohen, I., Tuan, R. S. & Iozzo, R. V. (1992) *J. Biol. Chem.* **267,** 8544–8557.
25. Iozzo, R. V., Cohen, I. R., Grässel, S. & Murdoch, A. D. (1994) *Biochem. J.* **302,** 625–639.
26. Dodge, G. R., Kovalszky, I., Hassell, J. R. & Iozzo, R. V. (1990) *J. Biol. Chem.* **265,** 18023–18029.
27. Larjava, H., Häkkinen, L. & Rahemetulla, F. (1992) *Biochem. J.* **284,** 267–274.
28. Vogel, K. G., Paulsson, M. & Heinegård, D. (1984) *Biochem. J.* **223,** 587–597.
29. Iozzo, R. V. & Cohen, I. (1993) *Experientia* **49,** 447–455.
30. Leppa, S., Mali, M., Miettinen, H. M. & Jalkanen, M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 932–936.
31. Liebersbach, B. F. & Sanderson, R. D. (1994) *J. Biol. Chem.* **269,** 20013–20019.
32. Mali, M., Andtfolk, H., Miettinen, H. M. & Jalkanen, M. (1994) *J. Biol. Chem.* **269,** 27795–27798.