A Cell Surface Chondroitin Sulfate Proteoglycan, Immunologically Related to CD44, Is Involved in Type I Collagen-mediated Melanoma Cell Motility and Invasion

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Abstract. The metastatic spread of tumor cells occurs through a complex series of events, one of which involves the adhesion of tumor cells to extracellular matrix (ECM) components. Multiple interactions between cell surface receptors of an adherent tumor cell and the surrounding ECM contribute to cell motility and invasion. The current studies evaluate the role of a cell surface chondroitin sulfate proteoglycan (CSPG) in the adhesion, motility, and invasive behavior of a highly metastatic mouse melanoma cell line (K1735 M4) on type I collagen matrices. By blocking mouse melanoma cell production of CSPG with *p*-nitrophenyl β-D-xylopyranoside (β-D-xyloside), a compound that uncouples chondroitin sulfate from CSPG core protein synthesis, we observed a corresponding decrease in melanoma cell motility on type I collagen and invasive behavior into type I collagen gels. Melanoma cell motility on type I collagen could also be inhibited by removing cell surface chondroitin sulfate with chondroitinase. In contrast, type I collagen-mediated melanoma cell adhesion and spreading were not affected by either β-D-xyloside or chondroitinase treatments. These results suggest that mouse melanoma CSPG is not a primary cell adhesion receptor, but may play a role in melanoma cell motility and invasion at the level of cellular translocation. Furthermore, purified mouse melanoma cell surface CSPG was shown, by affinity chromatography and in solid phase binding assays, to bind to type I collagen and this interaction was shown to be mediated, at least in part, by chondroitin sulfate. Additionally we have determined that mouse melanoma CSPG is composed of a 110-kD core protein that is recognized by anti-CD44 antibodies on Western blots. Collectively, our data suggests that interactions between a cell surface CD44-related CSPG and type I collagen in the ECM may play an important role in mouse melanoma cell motility and invasion, and that the chondroitin sulfate portion of the proteoglycan seems to be a critical component in mediating this effect.

TUMOR cell metastasis involves a complex series of interdependent events, one of which involves the adhesion of tumor cells to extracellular matrix (ECM)[1] components (Liotta et al., 1983). Although traditionally considered as structural elements of the ECM, it is now appreciated that various collagen types promote the adhesion and migration of normal and transformed cells (Aumailley and Timpl, 1986; Rubin et al., 1981; Dedhar et al., 1987; Herbst et al., 1988; Chelberg et al., 1989). Additionally, certain collagen types have been shown to promote directional motility of normal and transformed cells, thus, potentially contributing to the invasive process (Herbst et al., 1988, Chelberg et al., 1989). The motility of normal and transformed cells may be directed by an adhesive gradient of substratum-bound attractant, and this process is termed haptotaxis (McCarthy and Furcht, 1984).

Tumor cells adhere and move on ECM components via multiple cell surface receptors that interact with distinct domains on ECM proteins. In addition to the well known integrin receptor model for cell-ECM interactions (Buck and Horwitz, 1987, Hynes, 1987, Ruoslahti, 1988), substantial evidence suggests an important role for cell surface proteoglycans (PGs) in mediating cell adhesion to ECM components (Hook et al., 1984; Couchman and Hook, 1988; Ruoslahti, 1988; Wight, 1989). Both cell surface heparan sulfate proteoglycans (HSPG) and chondroitin sulfate proteoglycans (CSPG) have been implicated in modulating cell adhesion (Lark et al., 1985), but each has distinct effects on cell adhesion (Ruoslahti, 1988, Gallagher, 1989). While cell surface HSPG has been associated with the formation of tight cell adhesion contacts on ECM components (Lark et al., 1985;

1. *Abbreviations used in this paper*: CSPG, chondroitin sulfate proteoglycan; ECM, extracellular matrix; HSPG, heparan sulfate proteoglycan; PG, proteoglycan.

Woods et al., 1986), cell surface CSPG has been implicated in weakening cell adhesion (Lark et al., 1985, Culp et al., 1978). Although the molecular mechanism by which CSPG disrupts cell adhesion is not understood, CSPG may facilitate cell detachment from the ECM, and thereby promote tumor cell motility and subsequent invasion.

To further evaluate the potential role of cell surface CSPG in tumor cell motility and invasion, we studied the effect of *p*-nitrophenyl β-D-xylopyranoside (β-D-xyloside), an agent than uncouples chondroitin sulfate addition to the protein core (Schwartz, 1977), on the invasion of melanoma cells into type I collagen gels. Others have shown that the inhibition of CSPG synthesis by β-D-xyloside causes a dose-dependent inhibition of invasive morphogenetic events such as the branching of salivary glands (Thompson and Spooner, 1982) and ureteric buds (Klein et al., 1989). Our studies show that β-D-xyloside inhibits the invasion of mouse melanoma cells into type I collagen gels, suggesting a role for cell surface CSPG in contributing to the invasive behavior of these cells. Similarly, haptotactic motility of melanoma cells on type I collagen was inhibited by either β-D-xyloside or chondroitinase pretreatment. However, type I collagen-mediated melanoma cell adhesion or spreading was not affected by these treatments. These data suggest that while melanoma CSPG may be important in cell motility at the level of translocation, it is apparently not a primary cell adhesion receptor. We have isolated and partially characterized a cell surface CSPG from mouse melanoma cells that interacts with type I collagen of the ECM and has properties consistent with localization as an integral membrane component. Furthermore, our data indicates that the 110-kD core protein of mouse melanoma CSPG is immunologically related to the CD44 antigen, implicated in several aspects of cell–cell and cell–ECM interactions (Gallatin et al., 1989; Haynes et al., 1989; Jalkanen et al., 1986; Miyake et al., 1990) and most recently implicated in mediating the metastatic behavior of certain carcinoma cell lines (Günthert et al., 1991). Thus, mouse melanoma cells express a cell surface CD44-related CSPG molecule that may play an important role in collagen-mediated melanoma cell motility and invasion.

Materials and Methods

Cell Culture

A highly metastatic clone (M4) of the K1735 mouse melanoma, was generously provided by Dr. I. J. Fidler (M.D. Anderson Hospital Cancer Center, Houston, TX). This melanoma cell line was maintained by in vitro culture in DMEM (Sigma Chemical Co., St. Louis, MO) supplemented with 10% heat-inactivated calf serum (Sigma Chemical Co.). The number of in vitro passages was limited to eight in order to minimize phenotypic drift. There was no detectable change in the metastatic phenotype of these cells over this time, as measured by an experimental metastasis assay (McCarthy et al., 1988*b*).

Invasion Gels

Gels composed of type I collagen were prepared under sterile conditions using a modified protocol as described by Shor (1980). Type I collagen (Collagen Corp., Palo Alto, CA; 3.3 mg/ml Vitrogen in 0.013 M HCl) was mixed to a final concentration of 2.2 mg/ml in DMEM (Dulbecco's modified Eagle's medium; Sigma Chemical Co.), 10% PBS (vol/vol), and 0.225% $NaHCO_3$ (wt/vol). Reagents were stored at 4°C to prevent premature fibril assembly. Aliquots of 1.5 ml of this solution were added to each well of a 6-well plate (total area per well of 9.6 cm^2; Costar, Cambridge, MA), carefully swirled to allow uniform distribution and allowed to solidify for 1 h at 37°C. The gels were overlaid with 2 ml DMEM (pH 7.4) overnight, then reequilibrated with fresh DMEM for 4 additional hours the next morning. Confluent cells were harvested with trypsin (0.25%)/EDTA (1 mM), pelleted by centrifugation (1,500 rpm, 5 min) and resuspended in DMEM containing 2.5% heat-inactivated calf serum. The DMEM used to equilibrate the gels was removed and a 2-ml cell suspension containing 5×10^4 cells was added to the top of the gels. The plates were swirled gently to obtain an even distribution of cells on the gel surface and incubated at 37°C in a humidified, 5% CO_2 incubator.

Cell invasion was measured after 48–72 h using an inverted phase-contrast microscope (Nikon Diaphot) equipped with a calibrated fine focus knob, at 200× magnification. The fine focus knob was calibrated using a collagen gel sandwiched between two coverslip-separated glass slides to account for the refractive properties of the gel. The number of invading cells was determined at successive 20-μm (1 cell diameter) intervals in five randomly selected fields. Quantitation was made at increasing depths within the gels until no additional cells were detected. The total number of cells detected in five fields at a particular level was multiplied by a constant (65.36) to obtain the number of cells at each interval per cm^2 of gel area. Data are presented as the total number of cells penetrating the gel per cm^2 of surface area, calculated by totaling the number of cells per cm^2 at successive levels. Results are reported as means, plus or minus standard errors of the means, of duplicate gels.

Cell Migration, Adhesion, and Spreading Assays

Cell motility was assayed in 48-well microchambers (Neuroprobe, Bethesda, MD), utilizing 8-μm pore size polyvinyl pyrrolidone-free polycarbonate filters (Nucleopore, Pleasanton, CA). The undersides of the filters were precoated with either type I collagen or fibronectin at 1 μg/ml (a concentration chosen on the basis of half maximal migration promoting activity of the proteins), as described previously (McCarthy et al., 1986; Chelberg et al., 1989). The lower wells of the Boyden chambers were filled with DMEM supplemented with 20 mM Hepes, 5 mg/ml BSA. Cells were released with 5 mM EDTA in HBSS (Ca,Mg free), washed and resuspended to a final concentration of 4×10^5 cells/ml in DMEM, 20 mM Hepes, 5 mg/ml BSA. Cells were added to the upper wells at 2×10^4 cells/well and migration to the underside of the precoated filter was measured after 4 h at 37°C.

Cell adhesion to protein-coated substrata was performed as described previously (McCarthy et al., 1986; Chelberg et al., 1989) using 1 μg/ml type I collagen or fibronectin. Briefly, for the adhesion assays, cell cultures were radiolabeled for the final 18 h with 2 μCi/ml ^{3}H-thymidine, released with 5 mM EDTA in HBSS, washed, and resuspended to a final concentration of 5×10^4 cells/ml in adhesion medium (DMEM supplemented with 20 mM Hepes, 5 mg/ml BSA [fatty acid free; Sigma Chemical Co.]). Approximately 5×10^3 cells/well were added in 100 μl of adhesion medium and allowed to adhere to protein coated substrata for 45 min. After gentle washing (4×) adherent cells were solubilized in 150 μl of 0.5N NaOH containing 1% SDS, and radioactivity was quantitated with a Beckman LS 3801 liquid scintillation counter.

Measurement of cell spreading was performed as described (Chelberg et al., 1989) by measuring the area of cells plated onto protein-coated substrata. Briefly, cells prepared as described above were incubated in 24-well tissue culture plates (Costar) coated with fibronectin or type I collagen for 90 min, and then fixed by the addition of PBS containing 1–2% glutaraldehyde at 37°C. The cells were stained by the addition of a 0.5% solution of Coomassie R-250 stain in 95% ethanol. Cells were viewed on a Nikon Diaphot inverted phase microscope connected to an Optomax System IV image analysis system integrated with an Apple IIe computer. Cell area was determined for at least 30 randomly selected cells per culture, and each experimental condition was performed in duplicate.

β-D-Xyloside and Chondroitinase ABC Pretreatment

For the invasion assays, cells were seeded onto the gels as described above in the presence of 0, 0.05, 0.1, 0.5 or 1.0 mM *p*-nitrophenyl-β-D-xylopyranoside [β-D-xyloside] (Sigma Chemical Co.) or *p*-nitrophenyl-α-D-xylopyranoside [α-D-xyloside] (Koch-Light Ltd., Suffolk, England). For motility, adhesion and spreading assays, or before proteoglycan extraction, cells were exposed to β-D-xyloside for 48 h to inhibit CSPG synthesis by replacing the medium of cell cultures, at 50% confluency, with DMEM containing 2.5% heat-inactivated calf serum, with or without 1 mM β-D-xyloside. α-D-xyloside does not inhibit CSPG synthesis and was used as a control in invasion and motility assays. 1 mM α or β-D-xyloside was also included in the medium during the assays. β-D-xyloside was also added to

the ^{35}S-sulfate labeling medium before proteoglycan extraction such that the cells were exposed to the drug for a total of 48 h (see below). Levels of protein synthesis were examined for cells pretreated (or not) with β-D-xyloside. Briefly, cells grown in 96-well plates (Costar, Cambridge MA) were exposed (or not) to 1.0 mM β-D-xyloside for 48 h and 5 μCi/ml ^{3}H-leucine was added to each of triplicate wells for the final 6 h. Levels of ^{3}H-leucine incorporation into cell-associated proteins were determined by harvesting cells with a Brandell cell harvester (Gaithersburg, MD), and the values were compared for cells exposed (or not) to β-D-xyloside on a per cell basis. The latter value was obtained by counting cells/well (in triplicate), or determining relative DNA synthesis (0.2 μCi/μl ^{3}H-thymidine label added for final 18 h). Radioactivity was quantitated by liquid scintillation spectroscopy (Beckman LS 3801).

For chondroitinase ABC treatment, cells were pretreated for 15 min with 0.1 U/ml chondroitinase ABC (Sigma Chemical Co.) before motility and adhesion assays, as described by Saunders and Bernfield (1988). Chondroitinase ABC enzyme (0.1 U/ml) was also included in the cell medium during migration and adhesion assays. The absence of proteases in the chondroitinase ABC enzyme was verified by a lack of digestion of azocasein after a 60-min incubation with the enzyme at 37°C (Tomarelli et al., 1949). Chondroitinase ABC enzyme activity was verified for the assay conditions used, under conditions of Saito et al. (1968).

Proteoglycan Extraction

Prior to extraction, melanoma cell proteoglycans (PGs) were preferentially labeled with ^{35}S-sulfate for 18 h by replacing the medium in 80% confluent cultures with RPMI 1640 containing 0.1 mM Na_2SO_4, 0.36 mM glutamine, and 50 μCi/ml $Na_2{}^{35}SO_4$ (Carrier free, Specific Activity 43 Ci/mg; ICN Biomedicals, Irvine, CA). An extraction protocol utilizing detergent-mediated cell lysis was followed to specifically enrich for intact plasma membrane-associated PG (Yanagashita et al., 1987). To remove peripheral, extrinsically associated PG, the medium of ^{35}S-labeled cell cultures was replaced with 30 ml DMEM containing 50 μg/ml heparin for a 30-min incubation at 18°C (Yanagashita and Hascall, 1984). The heparin extract was removed and 30 ml cellular extraction buffer (0.15 M NaCl, 10 mM Tris, 5 mM $MgCl_2$, 2 mM EDTA, 0.25 mM DTT, 1 mM PMSF, 1% Triton X-100, pH 7.2) was added to each roller bottle and incubated in a rolling apparatus at 37°C for 15 min (Carey and Todd, 1986). The cellular extracts, containing plasma membrane-associated and intracellular PG, were centrifuged at 1,500 rpm for 5 min to remove insoluble material. The remaining extracellular matrix-cytoskeletal-associated PG were solubilized in 4 M guanidine containing 50 mM NaAcetate, pH 5.8, 2% Triton X-100, 10 mM EDTA, 1 mg/ml benzamidine, 0.1 M 6-aminohexanoic acid, for a 30-min incubation at 25°C (Oegema et al., 1979). All extracts were dialyzed in small pore dialysis tubing (mol wt cut off 3,500) against successive changes of 0.5 M NaAcetate, pH 6.8, 0.1 M NaSulfate containing 10 mM EDTA, 0.1 mM PMSF, 10 mM 6-aminohexanoic acid (Oegema et al., 1979) until no radioactivity was observed in the dialysis buffer.

Purification of Detergent-extracted CSPG

Detergent-extracted ^{35}S-PGs were dialyzed into DEAE buffer (0.15 M Tris, 6.0 M Urea, 0.1 M NaCl, 0.01 M EDTA, 0.01 M 6-aminohexanoic acid, 0.2% Triton X-100, 0.1 mM PMSF, pH 7.0), and purified by HPLC (Beckman Model 110 A) with a 7.5 × 75 mm TSK DEAE 5PW anion exchange column (BioRad Laboratories, Richmond, CA) using a linear salt gradient from 0.1 to 0.8 M NaCl over a 45-min period as described (Klein et al., 1989). The salt gradient was monitored by conductivity measurements using a Radiometer Conductivity Meter (model CDM 83). Comparisons were made to standards of known NaCl concentration in DEAE buffer to calculate the actual salt concentrations of the fractions. To insure adequate separation of ^{35}S-PG, HPLC-DEAE chromatographic peaks were each rechromatographed on the same column before further characterization. Recovery of radioactivity from the HPLC-DEAE columns was 90–95%.

Before iodination, DEAE-HPLC-separated CSPG was further purified on Sepharose CL-4B (Sigma Chemical Co.) columns equilibrated and eluted with 0.05 M sodium acetate, pH 7.0, containing 4 M guanidine, 1% Triton X-100, and 0.04% sodium azide at a flow rate of 3 ml/h (Klein et al., 1986). Fractions of 1.0–2.0 ml were collected with an 85–95% recovery of radioactivity. Rat chondrosarcoma chondroitin sulfate PGs (Oegema et al., 1975) associated with 4% hyaluronic acid and glucuronolactone were used to mark the column void (V_o) and total (V_t) volumes, respectively. CSPG core protein was labeled with 2.5 mCi ^{125}I on Iodobeads (Pierce Chemical Co., Rockford, IL) in 0.05 M Tris, pH 7, 0.5% CHAPS. ^{125}I-CSPG was bound to DEAE-Spetra/Gel M (Spectrum, Los Angeles, CA), washed with DEAE buffer (see above) to remove unbound ^{125}I, eluted with DEAE buffer containing 1.0 M NaCl, and dialyzed extensively in deionized water, 1 mM PMSF. Radioactivity per sample was quantitated by gamma ray scintillation (TM Analytic, Gamma Trac 1193). To visualize CSPG core protein by autoradiography, ^{125}I-CSPG was digested with chondroitinase ABC (Seikagaku America Inc., Rockville, MD) and analyzed by 6–15% SDS gel electrophoresis under nonreducing conditions (McCarthy et al., 1988*a*). Gels were dried under vacuum and used for autoradiography using Kodak XAR5.

Characterization of Detergent-extracted CSPG

The hydrodynamic properties of detergent-extracted ^{35}S-PG and ^{35}S-glycosaminoglycans (GAGs) were evaluated by gel filtration on 0.9 × 110 cm Sepharose CL-6B (Sigma Chemical Co.) columns. The columns were equilibrated and eluted with 0.5 M sodium acetate, pH 7.0, containing 0.2% CHAPS, at a flow rate of 3 ml/h (Klein et al., 1986). ^{35}S-GAGs were released from PG protein cores by alkaline borohydride reduction, neutralized, and desalted on Sephadex G-50 (Sigma Chemical Co.) columns, as previously described (Oegema et al., 1979). ^{35}S-GAGs were recovered from the column V_o with <5% of alkali-released material included in the column. The heparan sulfate and chondroitin sulfate content of ^{35}S-GAG samples were determined by sequential nitrous acid deaminative cleavage and chondroitinase ABC treatment, respectively, as previously described (Brown et al., 1981). The hydrodynamic properties of ^{35}S-chondroitin/dermatan sulfate recovered after nitrous acid treatment were determined on Sepharose CL-6B columns. The C4:6 ratio of oligosaccharides generated by chondroitinase ABC digestion was determined by descending paper chromatography using C4, C6, and unsulfated oligosaccharide standards (Saito et al., 1968). The absence of iduronic acid residues within these chains was confirmed by equivalent sensitivity of chondroitin/dermatan sulfate to chondroitinase ABC (Sigma Chemical Co.), which digests chondroitin sulfate and dermatan sulfate and chondroitinase AC II (Sigma Chemical Co.), which digests only chondroitin sulfate. Detergent-extracted ^{35}S-macromolecules resistant to the effects of nitrous acid and chondroitinase ABC digestion were considered "^{35}S-glycoproteins" (Brown et al., 1981), and this population never exceeded 10% of any extract examined.

Type I Collagen Affinity Chromatography and Binding Assays

For affinity chromatography, type I collagen (Vitrogen) was covalently coupled to Reacti-Gel according to manufacturers instructions (Pierce Chemical Co.). Final concentration bound was 0.175 mg type I collagen/ml gel. Columns (30 ml bed volume) were equilibrated with 50 mM Tris, pH 6.8, 0.5% CHAPS, 0.05 M NaCl, 0.01 M 6-amino hexanoic acid, 0.1 mM PMSF, 1.0 mM NEM, and 0.02% azide. Detergent-extracted ^{35}S-CSPG was applied to the columns, washed with three column volumes of buffer and eluted with a salt gradient from 0.05 to 1.0 M NaCl. To assure that the binding of CSPG was specific to type I collagen, ^{35}S-CSPG was applied to duplicate columns prepared without type I collagen. Recovery of radioactivity from these columns was 90–95%.

Solid phase binding assays were performed with Immulon 1 plates as a support (Skubitz et al., 1988). The substrata were prepared, as in the adhesion assays, by coating the surface of the wells with intact type I collagen (0.2 μg/well) or BSA (0.2 μg/well; fatty acid free; Sigma Chemical Co.). The plates were blocked for 1 h with 0.1 M carbonate buffer (Engvall and Perlmann, 1972) containing 5 mg/ml BSA, then washed 5× with deionized water, and ^{35}S-CSPG was added to the wells in binding buffer (50 mM Tris, pH 6.8, 50 mM NaCl, 5 mg/ml BSA). The plates were incubated for 2 h at 37°C, washed (×5) with binding buffer, and the bound ^{35}S-CSPG was solubilized with 0.5 N NaOH containing 1% SDS.

Octyl Sepharose Chromatography

Detergent-extracted ^{35}S-CSPG, purified by ion exchange chromatography, was resuspended in Octyl Sepharose buffer (4.0 M Guanidine HCl, 20 mM Tris, pH 6.8). Samples were applied to a 5-ml Octyl Sepharose CL-4B (Sigma Chemical Co.) column at a flow rate of 0.5 ml/min. Hydrophobic CSPG were then eluted with a linear gradient of 0 to 0.5% Triton X-100 in Octyl Sepharose buffer (Yanagashita et al., 1987). 2 ml-fractions were analyzed for ^{35}S-radioactivity and percent Triton X-100 by absorbance at 280 nm.

Immunoblots

For identification of CSPG core protein by immunoblot, detergent-extracted

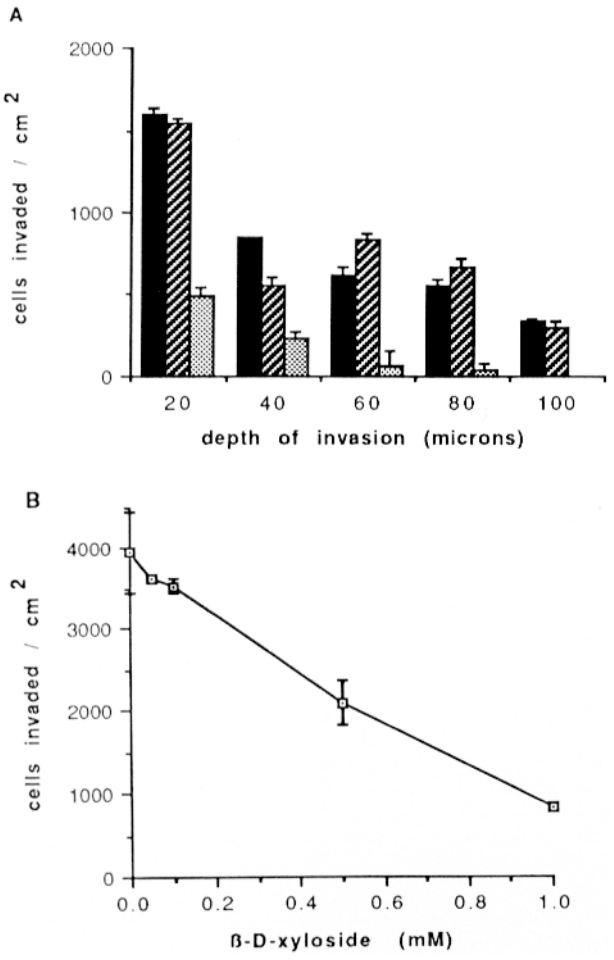

Figure 1. β-D-xyloside inhibits melanoma cell invasion into type I collagen gels. Native type I collagen gels were prepared (2.2 mg/ml final concentration) as described in Materials and Methods. (*A*) Shown are the invasion profiles for mouse melanoma cells plated on the surface of gels in the absence (*solid bars*) or presence of 1.0 mM α-D-xyloside (*cross hatched bars*) or 1.0 mM β-D-xyloside (*dotted bars*). (*B*) The total number of cells invading these gels in the presence (or absence) of indicated concentrations of β-D-xyloside was determined. The data are presented as the mean number of total cells invading per square cm at day 3 of the assay (performed in duplicate gels), plus or minus the standard errors of the means.

DEAE-HPLC-purified CSPG was digested with chondroitinase ABC (Seikagaku America Inc., Rockville, MD) and electrophoresed on 7.5 or 10% SDS-PAGE, under reducing conditions as described previously (McCarthy et al., 1988*a*). After electrophoresis, proteins were transferred onto nitrocellulose (Micron Separations Inc., Westboro, MA) in a transfer chamber over 2–3 h at 70 V in 0.025 M Tris and 0.192 M glycine (Towbin et al., 1979). The transfer of proteins was monitored by including prestained protein molecular weight standards (Amersham Corp., Arlington Heights, IL) on each gel. After transferring, the blot was placed in a blocking solution for 2 h at 25°C consisting of TBS (20 mM Tris, 0.5 M NaCl), 5% blotto, and 1% normal goat serum. The transfer was incubated for 2 h at 25°C, then washed for 20 min in TTBS (TBS, 0.2% TWEEN 20). The transfers were probed for 2 h in TTBS, 1% blotto at 25°C with a rabbit polyclonal antibody that recognizes the unsaturated uronic acid residues that remain attached to the PG core protein after chondroitinase ABC digestion (Couchman et al., 1985). Duplicate blots were probed with rat anti-CD44 mAbs, IM7 (Picker et al., 1989) (kindly provided by Dr. Eugene Butcher), KM81, KM114, KM201, and KM703 (Miyake et al., 1990) (kindly provided by Dr. Paul Kincade). Antibodies against the human melanoma proteoglycan (MPG) were also tested, including mAb 9.2.27 (Bumol et al., 1984) and a rabbit polyclonal antibody, RαMPG (Spiro et al., 1988) (kindly provided by Dr. Robert Spiro). As controls, normal rat sera and an isotype matched rat anti-α4 integrin antibody, P s/2 (provided by Dr. Paul Kincade) were used. After two 10-min washes in TTBS, goat antirabbit-, antirat- or antimouse peroxidase conjugate (Organon Tecknika Corp., West Chester, PA) was bound in TTBS, 1% blotto for 1 h at 25°C. Blots were washed twice in TTBS and then once in TBS for 10 min each wash. Membranes were then developed in 100 ml enzyme/substrate buffer (TBS, 25 mg 3,3′ DAB tetrahydrochloride, 20 μl H_2O_2) until signal/noise ratio was at desired level. The reaction was stopped by rinsing blots with water.

Results

Cell Surface Chondroitin Sulfate Proteoglycans Play a Role in Melanoma Cell Motility and Invasion

Three-dimensional gels composed of type I collagen were used to monitor melanoma cell invasion. Approximately 77% of highly metastatic mouse melanoma cells applied to each invasion gel invaded these structures to a maximum level of 100 μm by the third day of incubation. Of the total cells that invaded these gels, ~30% penetrated to the first level (20 μm), while the remaining levels contained progressively decreasing numbers of melanoma cells (Fig. 1 *a*, *solid bars*). To evaluate the potential role of CSPGs in mediating invasive behavior, melanoma cell invasion was observed in the presence of increasing concentrations of β-D-xyloside. Melanoma cell invasion was inhibited by β-D-xyloside in a concentration-dependent fashion (Fig. 1*b*), with ~80% inhibition observed at the highest concentration of β-D-xyloside tested (1.0 mM). The number of cells detected at each level of the invasion gel was drastically reduced in the presence of 1.0 mM β-D-xyloside (Fig. 1*a*, *dotted bars*). No inhibition was observed in the presence of α-D-xyloside (Fig. 1*a*, *cross-hatched bars*), an inactive analogue of β-D-xyloside that does not interfere with CSPG synthesis (Robinson et al., 1975). The inhibitory effects of β-D-xyloside were predominantly at the level of entry of these cells into the gels, since the depth of the furthest moving cells in the presence of β-D-xyloside was relatively unaltered compared to untreated cells.

To determine whether or not β-D-xyloside was cytotoxic, melanoma cells in culture were preincubated with 1.0 mM β-D-xyloside for 48 h, released and seeded onto the gels in the absence of additional β-D-xyloside. The number of cells invading per cm^2 was not significantly different for cells pretreated with β-D-xyloside (3,431 ± 138) as for cells that had not been exposed to this drug (4,052 ± 500). Furthermore, regardless of β-D-xyloside treatment, cells were determined to be 95% viable by trypan blue exclusion and protein synthesis was not affected (data not shown). Collectively, these results indicate that β-D-xyloside–mediated inhibition of melanoma cell invasion is reversible and not due to a cytotoxic effect of the drug.

To further evaluate the role of cell surface CSPG in melanoma cell invasion, the effects of β-D-xyloside on melanoma cell motility were examined in Boyden chamber type migration assays. Melanoma cell motility in response to type I collagen or fibronectin was inhibited by 50% in the presence of 1.0 mM β-D-xyloside (Fig. 2 *a*) and not affected by the inactive analogue, α-D-xyloside (data not shown). In contrast, β-D-xyloside did not affect melanoma cell adhesion to type I collagen- or fibronectin-coated substrata (Fig. 2 *b*). Also, by directly measuring the cytoplasmic area of adherent cells, we

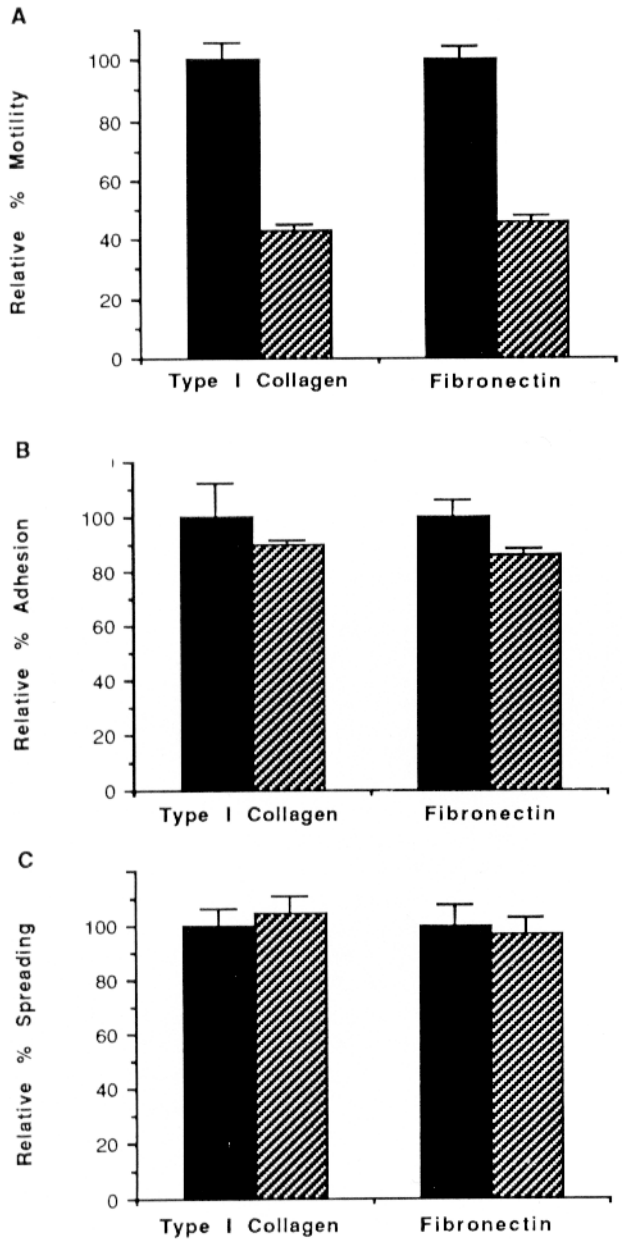

Figure 2. β-D-Xyloside inhibits melanoma cell migration, but not adhesion or spreading on adhesion promoting proteins of the extracellular matrix. Mouse melanoma cells were pretreated for 48 h without (*solid bars*) or with 1 mM β-D-xyloside (*cross hatched bars*). (*A*) Cells were examined for the ability to migrate in the Boyden Chamber Assay in response to 1 μg/ml of type I collagen or fibronectin. (*B*) Cells radiolabeled with ^{3}H-thymidine for the final 18 h before the assay were released from the flask with EDTA, and examined for the ability to adhere on substrata coated with 10 μg/ml of type I collagen or fibronectin. (*C*) Cell spreading on 10 μg/ml type I collagen or fibronectin was quantitated by directly measuring the cytoplasmic area of adherent cells. Data represent the mean percentage of triplicate determinations plus or minus the standard errors of the means.

determined that β-D-xyloside–pretreated cells spread to the same extent on type I collagen- or fibronectin-coated substrata as untreated counterparts (Fig. 2 *c*). Thus, in the presence of β-D-xyloside, the cells were still able to adhere and undergo cytoskeletal reorganization, indicating that melanoma cell surface CSPG is not required for these processes. These results suggest that the inhibition of melanoma cell invasion by β-D-xyloside reflects a specific effect on cell motility in response to extracellular matrix (ECM) components such as type I collagen and fibronectin.

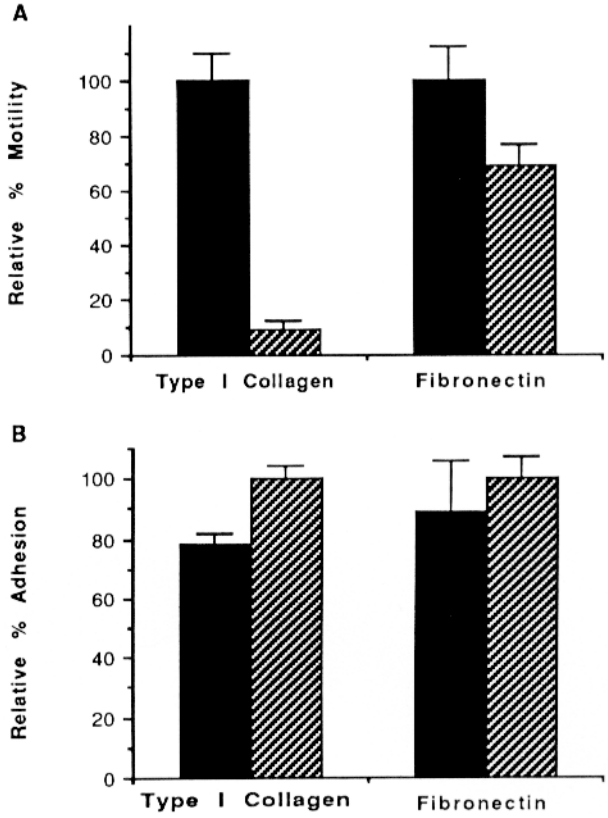

Figure 3. Removal of cell surface CSPG by chondroitinase ABC inhibits melanoma cell motility, but not adhesion on type I collagen or fibronectin. Mouse melanoma cells were pretreated for 15 min with 0 (*solid bars*) or 0.1 U/ml chondroitinase ABC (*cross hatched bars*). (*A*) Cells were examined for the ability to migrate in a Boyden chamber assay in response to 10 μg/ml type I collagen or fibronectin. (*B*) Cells radiolabeled with ^{3}H-thymidine, were examined for the ability to adhere to substrata coated with 10 μg/ml type I collagen or fibronectin.

To further investigate the role of melanoma cell surface CSPG in cell motility, we measured melanoma cell motility in Boyden chambers, after treatment with chondroitinase ABC (Fig. 3 *a*). Cell migration in the presence of chondroitinase ABC (0.1 U/ml) was inhibited by 90% on type I collagen and by 30% on fibronectin, suggesting an important role for CSPG in mediating motile behavior on ECM components. As was observed for β-D-xyloside pretreatment, melanoma cell adhesion to type I collagen and fibronectin was not inhibited in the presence of chondroitinase ABC (Fig. 3 *b*). The inhibition of motility observed in the presence of chondroitinase ABC was attributed to the action of the chondroitinase ABC enzyme, since proteases were not detectable in the enzyme mixture (data not shown, see Materials and Methods). Thus, both β-D-xyloside and chondroitinase ABC treatments inhibited melanoma cell motility but not cell adhesion or spreading on type I collagen, suggesting that CSPG may play a role in melanoma cell motility and invasion

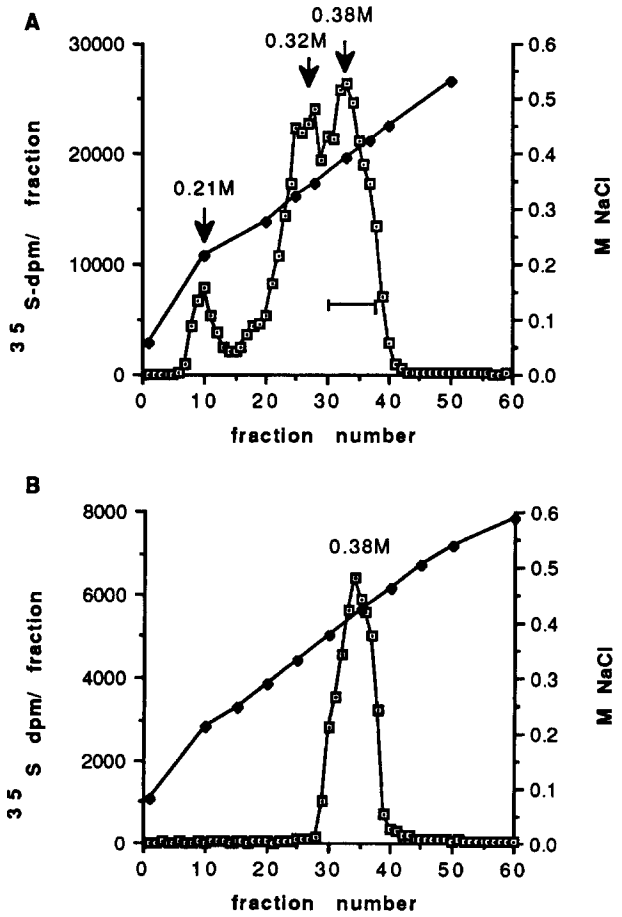

Figure 4. Purification of detergent-extracted ^{35}S-proteoglycans by HPLC-DEAE chromatography. (*A*) ^{35}S-proteoglycans were extracted with 1% Triton X-100 and applied to an HPLC-DEAE column in DEAE buffer (50 mM Tris, pH 7.0, 6 M Urea, 0.2% CHAPS, 0.1 M NaCl) and eluted with a NaCl gradient generated by HPLC. Radioactivity was monitored for each 1-ml fraction (*open squares*) and the salt gradient was monitored by conductivity measurements (*solid diamonds*). (*B*) The fractions eluting at 0.38 M NaCl (Fig. 4 *A*) were pooled (as indicated by the *bar*) and rechromatographed on the same column to insure maximum purity prior to further characterization.

at the level of cellular translocation, rather than as a primary cell adhesion receptor.

β-D-Xyloside Alters CSPG Production by Highly Metastatic Melanoma Cells

Considering our results indicating a role for cell surface CSPG in mediating melanoma cell motility and invasion, PGs synthesized by mouse melanoma cells were isolated and partially characterized. PGs may be associated with an adherent cell as integral or peripheral membrane components, or they may be deposited into the ECM (Hook et al., 1984; Yanagashita and Hascall, 1984). We followed an extraction protocol to preferentially isolate cell surface integral membrane components (Yanagashita et al., 1987). Approximately 20% of the ^{35}S-PG could be extracted by treatment of the cell cultures with low levels of heparin (50 μg/ml), indicating that these molecules were noncovalently associated with the plasma membrane or the ECM (Hook et al., 1984). The majority (65%) of the ^{35}S-PGs were extracted with

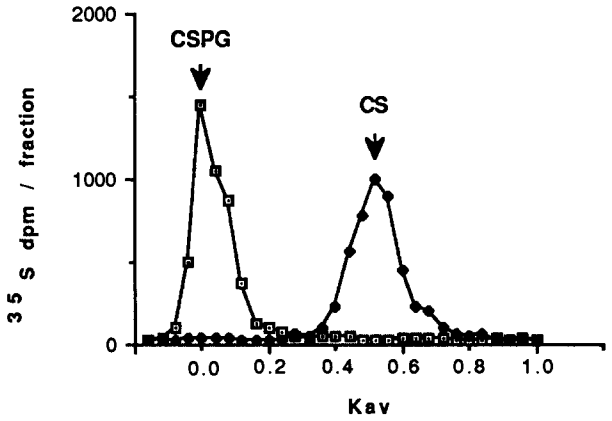

Figure 5. Elution of DEAE-purified CSPG on Sepharose CL-6B columns. Detergent-extracted CSPG, purified by ion exchange chromatography, was chromatographed on a Sepharose CL-6B column in the presence of 0.2% CHAPS both before (*open squares*) and after (*solid diamonds*) release of ^{35}S-GAGs by alkaline β-elimination. Radioactivity was monitored by liquid scintillation for each 1.0–2.0 ml fraction. K_{av}'s were determined by elution relative to rat chondrosarcoma chondroitin sulfate proteoglycan and glucuronolactone used to mark the column void and total volumes, respectively (see Materials and Methods). The eluting material (*solid diamonds*) was pooled and presence of chondroitin sulfate was verified by nitrous acid resistance and chondroitinase ABC sensitivity.

nonionic detergent, comprising those macromolecules intrinsically associated with the cell surface or with intracellular compartments. The remaining 15% of ^{35}S-PGs were detergent insoluble and required guanidine-HCl for solubilization, suggesting that they were associated with the detergent-resistant cytoskeleton or ECM.

To further characterize melanoma cell surface CSPG, detergent extracts of $^{35}SO_4$-labeled melanoma cell cultures were purified twice by HPLC-DEAE column chromatography. The initial chromatograph of this extract contained three partially resolved peaks as shown in Fig. 4 *a*. These peaks were pooled and rechromatographed on HPLC-DEAE, as shown for the material eluting at 0.38 M NaCl (Fig. 4 *b*). ^{35}S-CSPG eluted exclusively at 0.38 M and consisted of 85–90% chondroitin sulfate, as determined by sensitivity to chondroitinase ABC and resistance to nitrous acid. The remaining two peaks contained ^{35}S-glycoproteins (0.21 M) or ^{35}S-HSPGs (0.32 M) and were not further characterized in these studies.

HPLC-DEAE–purified CSPG accounted for 48% of the total detergent-extracted PG and contained exclusively chondroitin-4-sulfate, determined by descending paper chromatography, with no iduronate modifications, as indicated by >95% sensitivity of nitrous acid–resistant molecules to either chondroitinase ABC or AC. ^{35}S-CSPG eluted from a Sepharose CL-6B column between K_{av} 0–0.2 (Fig. 5). ^{35}S-Chondroitin sulfate was released from the PG by alkaline borohydride treatment. This treatment shifted the elution volume from a Sepharose CL-6B column to a K_{av} of 0.55 (Fig. 5), confirming that the ^{35}S-radiolabel was incorporated into O-linked carbohydrates.

The proportion of ^{35}S-PG in detergent extracts of cells treated with β-D-xyloside was similar (i.e., 65%) to that obtained from untreated cell cultures, as determined by the

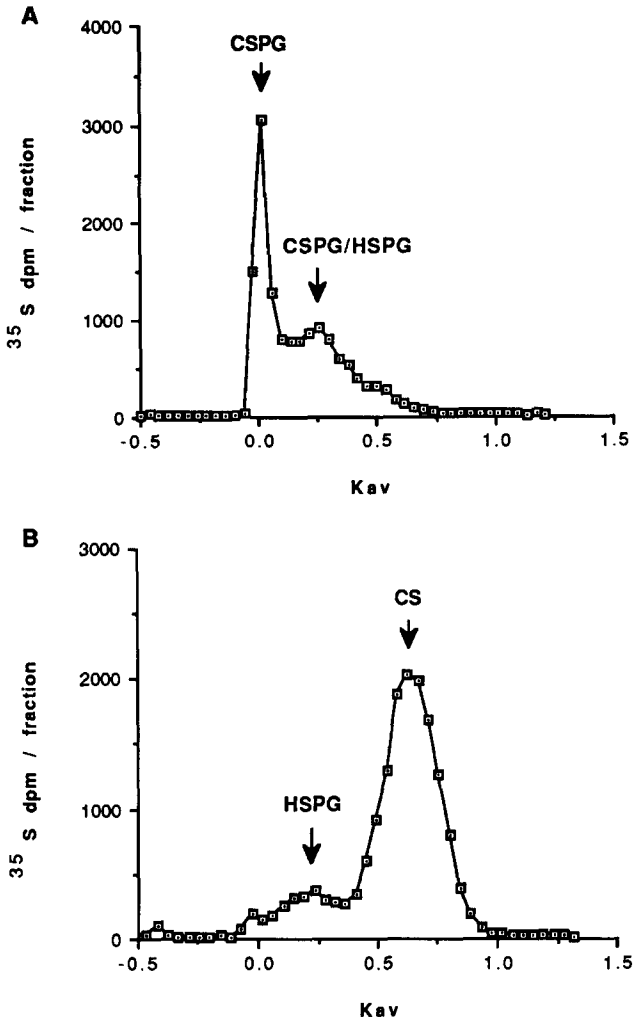

Figure 6. β-D-xyloside alters size distribution of detergent-extracted ^{35}S-labeled macromolecules. Detergent-extracted ^{35}S-proteoglycans were suspended in CL-6B buffer (0.5 M sodium acetate containing 0.2% CHAPS, pH 7.0) and applied to a 0.9 × 110 cm Sepharose CL-6B column. The CL-6B elution profiles are shown of ^{35}S-labeled macromolecules from detergent-extracts of melanoma cells cultured in the absence (*A*) or presence (*B*) of 1.0 mM β-D-xyloside. The eluting peaks were pooled and analyzed for GAG type by nitrous acid or chondroitinase ABC digestions as described in Materials and Methods.

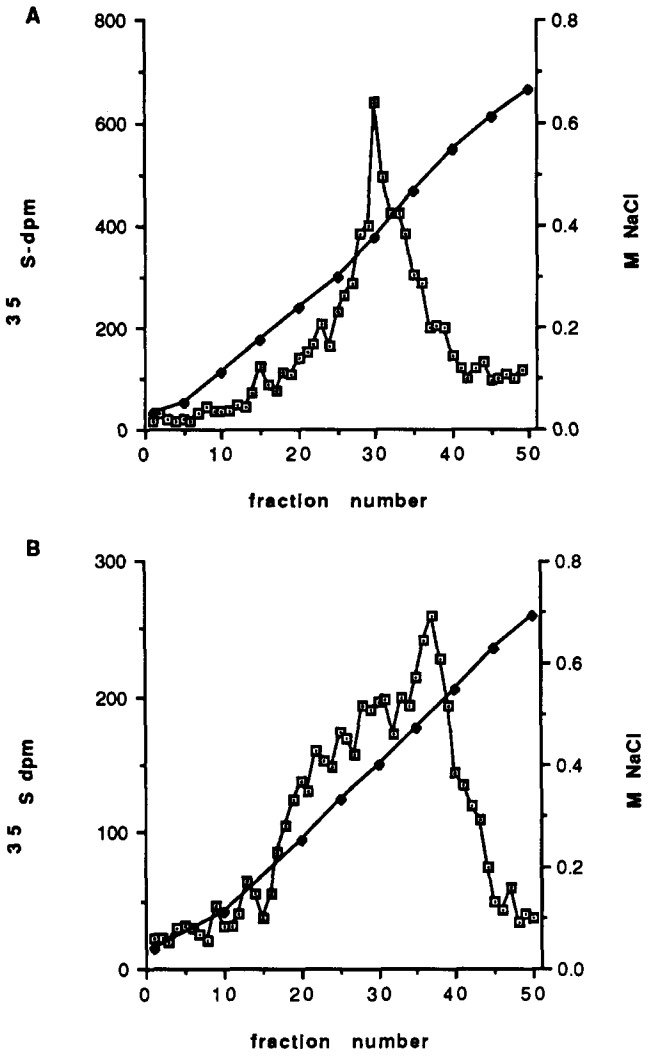

Figure 7. ^{35}S-CSPG binds to a type I collagen affinity column. (*A*) Detergent-extracted HPLC-DEAE purified ^{35}S-CSPG or (*B*) alkaline borohydride-released, nitrous acid-resistant chondroitin sulfate was applied to a type I collagen affinity column (0.175 mg/ml) in 50 mM Tris, pH 6.8, 50 mM NaCl, 0.5% CHAPS and eluted with a NaCl gradient. Radioactivity was monitored for each 2–3-ml fraction (*open squares*) and the salt gradient was monitored by conductivity measurements (*solid diamonds*).

amount of radioactivity recovered in detergent extracts on a per cell basis (data not shown). However, the hydrodynamic properties of DEAE-purified PG from detergent extracts of melanoma cells treated with or without 1 mM β-D-xyloside were markedly different. Detergent-extracted ^{35}S-PG from untreated or treated cell cultures were batch eluted on DEAE, pooled, and compared by Sepharose CL-6B chromatography. ^{35}S-PG from untreated cells eluted from a Sepharose CL-6B column as two partially overlapping peaks at K_{av} 0 and 0.2, containing a mixture of CSPG and HSPG (Fig. 6 *a*). In contrast, for PG isolated from β-D-xyloside–treated cell cultures, the ^{35}S-macromolecules eluting at K_{av} 0–0.2 contained HSPG, determined by 95% nitrous acid sensitivity, whereas chondroitin sulfate eluted at K_{av} 0.6 (Fig. 6 *b*). Alkaline borohydride treatment of β-D-xyloside–initiated chondroitin sulfate did not alter the K_{av} of the eluting material on a Sepharose CL-6B column (not shown), confirming that the chondroitin sulfate synthesis in the β-D-xyloside–treated cultures was uncoupled from core protein synthesis. Furthermore, the amount of nitrous acid–sensitive ^{35}S-proteoglycans recovered from unfractionated cell extracts of either culture condition differed by <10% (data not shown), suggesting that HSPG synthesis was not altered in the presence of β-D-xyloside. These results demonstrate that 1.0 mM β-D-xyloside effectively uncoupled chondroitin sulfate synthesis from the core protein, while the attachment of heparan sulfate to the core protein was essentially uninterrupted.

Detergent-extracted CSPG Produced by Melanoma Cells Binds to Type I Collagen and Has Properties Consistent with Plasma Membrane Intercalation

The above results strongly support a role for melanoma cell

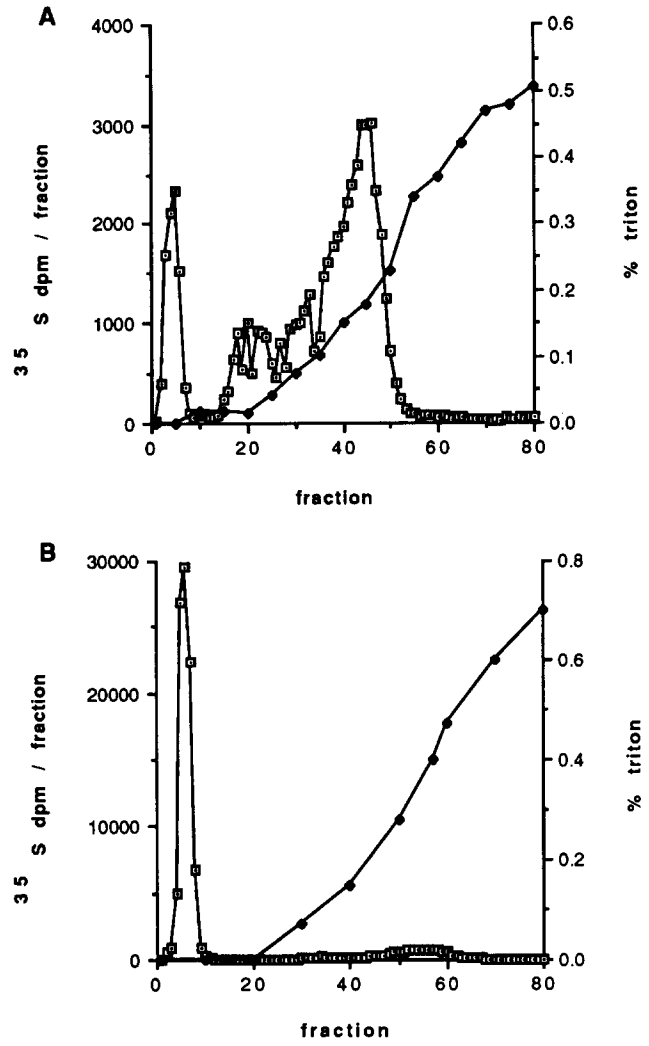

Figure 8. Detergent-extracted murine melanoma CSPG binds Octyl Sepharose. (*A*) HPLC-DEAE-purified ^{35}S-CSPG was resuspended in Octyl Sepharose buffer (4.0 M Guanidine HCL, 20 mM Tris, pH 6.8) and applied to a 5-ml Octyl Sepharose column at a flow rate of 0.5 ml/min. Hydrophobic CSPG were then eluted with a linear gradient of Triton X-100 from 0 to 0.5% in Octyl Sepharose buffer. Fractions of 2 ml each were analyzed for ^{35}S radioactivity (*open squares*) by liquid scintillation and percent Triton X-100 by absorbance at 280 nm (*solid diamonds*). (*B*) HPLC-DEAE-purified ^{35}S-CSPG was briefly digested with trypsin before Octyl Sepharose chromatography.

surface CSPG in type I collagen-mediated melanoma cell motility and invasion. By utilizing a solid phase binding assay, containing type I collagen coated onto microtiter wells, we determined that DEAE-HPLC-purified CSPG bound to type I collagen in a concentration dependent and saturable fashion (data not shown). HPLC-DEAE-purified CSPG also bound to a type I collagen affinity column in the presence of 0.5% CHAPS and was eluted from the column by 0.4 M NaCl (Fig. 7 *a*). The binding of ^{35}S CSPG was specific to type I collagen since it failed to bind to an affinity column prepared without type I collagen. To further evaluate the mechanism by which CSPG bound to type I collagen, chondroitin sulfate released from the protein core by alkaline β-elimination and treated with nitrous acid to remove any contaminating heparan sulfate, was applied to the type I collagen affinity column. The nitrous acid-resistant chondroitin sulfate bound to the type I collagen affinity column and eluted as a broad peak with 0.1–0.4 M NaCl (Fig. 7 *b*), which was much more heterogenous that that observed for intact CSPG. These studies demonstrate that mouse melanoma cell surface CSPG binds directly to type I collagen and that this interaction is mediated, at least in part, by chondroitin sulfate.

Hydrophobic chromatography has been used by other investigators to identify potential intercalated cell surface proteoglycans (Yanagashita et al., 1987). Approximately 80% of detergent-extracted HPLC-DEAE-purified ^{35}S-CSPG bound to an Octyl Sepharose column and was eluted with concentrations of Triton-X-100 that were close to the critical micellar concentration of this detergent (Fig. 8 *a*). In contrast, detergent-extracted CSPG that had been treated briefly with low levels of trypsin failed to bind this column (Fig. 8 *b*). These results are consistent with the hypothesis that a hydrophobic domain within the detergent-extracted CSPG protein core mediates binding to the hydrophobic column. Importantly, ^{35}S-chondroitin sulfate macromolecules isolated from β-D-xyloside cultures also failed to bind this column (not shown), further demonstrating that β-D-xyloside treatment prevents the expression of intact cell surface CSPG in mouse melanoma cells.

To determine the apparent molecular weight of the mouse melanoma CSPG core protein, detergent-extracted, HPLC-purified ^{125}I-labeled CSPG was digested with chondroitinase ABC and analyzed by 6–15% SDS gel electrophoresis under nonreducing conditions (Fig. 9, lane *A*). A single CSPG core protein was observed at ~110 kD by autoradiography, with a slight but noticeable increase in molecular mass observed upon reduction of this protein (not shown). No radioactivity was observed in lanes that contained heparatinase-digested ^{125}I-CSPG (Fig. 9, lane *B*), undigested ^{125}I-CSPG (Fig. 9, lane *C*), or chondroitinase ABC (Fig. 9, lane *D*). To confirm that the 110-kD band was a core protein of CSPG, chondroitinase digests of melanoma CSPG from similar gels were electroblotted onto nitrocellulose and probed with a polyclonal antibody that recognizes the unsaturated bonds of chondroitin sulfate-associated uronic acid residues that remain after chondroitinase ABC digestion (Couchman et al., 1985). Such blots indicate a single band at ~110 kD specifically detected by the antibody (Fig. 10 *A*, lane *2*). The specificity of the reaction is indicated by the failure of this antibody to recognize undigested CSPG (Fig. 10 *A*, lane *1*) or chondroitinase ABC (Fig. 10 *A*, lane *3*).

The relationship of mouse melanoma CSPG to other previously described cell surface PGs was evaluated by probing duplicate Western blots with antibodies against the human melanoma proteoglycan that has a 250-kD core protein (Bumol et al., 1984) and CD44, an 80–95-kD glycoprotein that may contain chondroitin sulfate modifications (Jalkanen et al., 1988; Brown et al., 1991). IM7, an anti-CD44 mAb (Picker et al., 1989) recognizes a single band at ~110 kD in the lane containing chondroitinase-digested CSPG (Fig. 10 *B*, lane *4*), suggesting that murine melanoma CSPG is immunologically related to CD44. No immunological reactivity was observed in lanes containing undigested CSPG (Fig. 10 *B*, lane *5*) or chondroitinase ABC (Fig. 10 *B*, lane *6*). Similar results were obtained with four additional mAbs, KM81, KM114, KM201, and KM703, that detect different epitopes on CD44

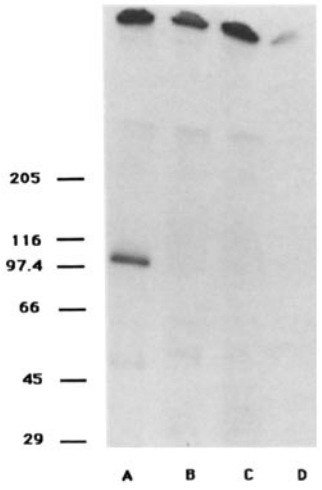

Figure 9. Identification of 110-kD core protein of mouse melanoma CSPG by autoradiography. ^{125}I-labeled DEAE-HPLC purified CSPG was digested with chondroitinase ABC and analyzed by 6-15% SDS-PAGE under nonreducing conditions. (Lane *A*) chondroitinase ABC digested CSPG; (*B*) heparatinase digested CSPG; (*C*) undigested CSPG; (*D*) chondroitinase ABC.

(Miyake et al., 1990 [data not shown]). Chondroitinase-digested CSPG was not recognized by antibodies against MPG, monoclonal 9.2.27 (Bumol et al., 1984) or polyclonal RαMPG (Spiro et al., 1988) or by preimmune rat sera or rat anti-α4 integrin antibody, P s/2 (data not shown). These results indicate that the 110-kD core protein of mouse melanoma CSPG is immunologically related to CD44, but not to the 250-kD CSPG core protein identified on human melanoma cells.

Discussion

Our studies demonstrate an important role for a cell surface CD44-related CSPG in mouse melanoma cell motility and invasion into type I collagen matrices. By pharmacologically blocking mouse melanoma cell production of CSPG with β-D-xyloside, we observed a corresponding decrease in melanoma cell migration and invasive behavior on type I collagen. Melanoma cell motility on type I collagen could also be inhibited by enzymatically removing cell surface chondroitin sulfate with chondroitinase ABC. In contrast, type I collagen–mediated cell adhesion and spreading were not affected by either β-D-xyloside or chondroitinase treatments. Purified mouse melanoma cell surface CSPG was shown to bind to type I collagen and to have properties consistent with localization as an integral plasma membrane component. Furthermore, we have identified a mouse melanoma CSPG with a 110-kD core protein that is recognized by antibodies against CD44 on Western blots, but not by antibodies against the 250-kD CSPG core protein identified on human melanoma cells. Collectively, our data suggests that mouse melanoma cell surface CSPG may interact with type I collagen of the ECM and subsequently mediate melanoma cell motility and invasion within three dimensional collagen gels in vitro.

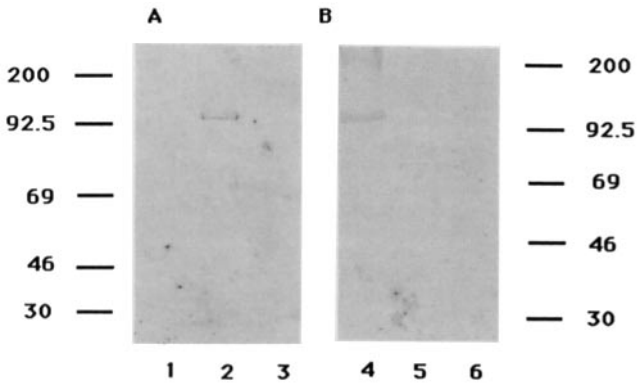

Figure 10. Mouse melanoma cells express a 110-kD CD44-related CSPG core protein. Detergent-extracted, DEAE-HPLC–purified CSPG was digested with chondroitinase ABC and electrophoresed under (*A*) reducing conditions on 7.5% SDS-PAGE or (*B*) nonreducing conditions on 10% SDS-PAGE. Proteins on these gels were transferred to nitrocellulose and probed with (*A*) a polyclonal antibody that recognizes the unsaturated bonds of chondroitin sulfate-associated uronic acid residues that remain after chondroitinase ABC digestion (Couchman et al., 1985) or (*B*) IM7, a rat mAb against CD44 (Picker et al., 1989). (Lanes *1* and *5*) undigested CSPG; (*2* and *4*) chondroitinase-digested CSPG; (*3* and *6*) chondroitinase ABC.

Several lines of evidence from other investigators support the hypothesis that cell surface CSPG contributes to the motile behavior of normal and transformed cells. Wounded endothelial cell cultures have been observed to rapidly change proteoglycan production from heparan sulfate to chondroitin sulfate coincident with the onset of motility (Kinsella and Wight, 1986). CSPGs have also been implicated in regulating the migration of cardiac mesenchymal cells (Funderburg and Markwald, 1986) and neural crest cells (Perris and Johnson, 1987). Furthermore, human melanoma CSPG has been localized to microspikes, a cell surface microdomain (Garrigues et al., 1986), and has been implicated to play a role in human melanoma cell adhesion and metastatic behavior (Bumol et al., 1984). Despite the widely recognized relationship between CSPG and cell motility, the precise mechanism through which CSPG mediates cell motility is not yet understood.

Cell surface CSPG does not appear to be a primary melanoma cell adhesion receptor for type I collagen, since cell adhesion and spreading on type I collagen were unaffected by β-D-xyloside or chondroitinase ABC treatments. Other cell surface receptors that may mediate the adhesion of moving cells include integrins, the major family of receptors known to mediate cell adhesion (Ruoslahti and Pierschbacher, 1987; Albelda and Buck, 1990; Humphries, 1990). Cell surface HSPGs have also been shown to mediate cell attachment to ECM proteins (Lark et al., 1985; LeBaron et al., 1988; McCarthy et al., 1986; Woods et al., 1986; Rogers et al., 1987; Saunders and Bernfield, 1988). HSPGs have been associated with newly formed adhesions of adherent cells (Lark et al., 1985), while older focal adhesions, found at the trailing edge of a moving cell, are primarily composed of CSPG (Culp et al., 1978). While certain cell surface HSPGs and integrins may be involved in cell motility by promoting cell adhesion and spreading at the leading edge of a moving cell, our data is consistent with the hypothesis that cell surface CSPG may disrupt these adhesive contacts and promote the release of the trailing edge of a moving cell, as has been previously proposed by Culp et al. (1978).

The molecular mechanism by which mouse melanoma CSPG modulates cell adhesion during cell motility has not been clearly defined. Several studies have indicated that CSPG, isolated from a wide variety of sources, exerts antagonistic effects on cell adhesion to ECM proteins such as fibronectin, collagen, and laminin (Knox and Wells, 1979;

Rich et al., 1981; Brennan et al., 1983; Rosenberg et al., 1985; Yamagata et al., 1989). CSPG may interfere with cell attachment either by competing with other cell surface receptors for binding to ECM molecules at the GAG binding sites and/or by masking the integrin binding sites (Ruoslahti, 1988). By weakening cell adhesion to other ECM components, CSPG may participate in cell motility by facilitating cell detachment (Culp et al., 1986; Ruoslahti, 1988). In this regard, the ability of CS on the surface of melanoma cells to bind type I collagen may be important for bringing cell surface CSPG into a close association with other adhesion receptors such as integrins. The close proximity of cell surface CSPG to other cell adhesion receptors may serve to disrupt the association of receptors at that site of the cell membrane and subsequently alter the adhesive contacts. Alternatively, mouse melanoma CSPG could serve to transmit additional signals to the interior of the cell, thereby indirectly modulating cell adhesion and migration (Yamagata et al., 1989). Further study of the molecular and cellular basis of cell surface CSPG interactions with ECM components will be required to understand the role of cell surface CSPG in melanoma cell motility and invasion.

In addition to demonstrating an immunological relationship, the structural and functional properties of mouse melanoma CSPG are similar to CD44, a cell surface molecule that has been implicated in mediating cell–cell and cell–ECM interactions (Gallatin et al., 1989; Haynes et al., 1989; Jalkanen et al., 1988; Miyake et al., 1990). Although the 110-kD core protein of mouse melanoma CSPG is slightly larger than the well characterized 80–95-kD CD44 glycoprotein, larger forms of CD44 have recently been identified that have various carbohydrate modifications, such as CS addition (Jalkanen et al., 1988; Brown et al., 1991), or result from alternative splicing of mRNA coding for CD44 (Brown et al., 1991; Günthert et al., 1991). The class III ECM receptor (ECMRIII [Wayner and Carter, 1987]), recently identified as CD44 (Gallatin et al., 1989), has been shown to bind to collagen, although it does not directly mediate cell adhesion (Wayner, 1987), similar to the functional properties currently identified for mouse melanoma CSPG. Furthermore, CD44 has been shown to be associated with the cytoskeleton (Jacobson et al., 1984; Kalomiris and Bourguignon, 1988) and to play a role in cell movement (Jacobson et al., 1984). Importantly, a variant form of CD44, containing an additional extracellular domain, has recently been implicated in mediating the metastatic behavior of certain rat carcinoma cell lines (Günthert et al., 1991). Additional studies that further define the structural similarities of the mouse melanoma CSPG core protein and CD44, as well as studies of this particular CSPG in low metastatic counterparts of the K1735 melanoma and normal melanocytes, will help to elucidate the mechanism by which mouse melanoma CSPG mediates tumor cell motility and invasion.

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