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Heparanase Degrades Syndecan-1 and Perlecan Heparan Sulfate

FUNCTIONAL IMPLICATIONS FOR TUMOR CELL INVASION*

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Heparanase (HPSE-1) is involved in the degradation of both cell-surface and extracellular matrix (ECM) heparan sulfate (HS) in normal and neoplastic tissues. Degradation of heparan sulfate proteoglycans (HSPG) in mammalian cells is dependent upon the enzymatic activity of HPSE-1, an endo-β-D-glucuronidase, which cleaves HS using a specific endoglycosidic hydrolysis rather than an eliminase type of action. Elevated HPSE-1 levels are associated with metastatic cancers, directly implicating HPSE-1 in tumor progression. The mechanism of HPSE-1 action to promote tumor progression may involve multiple substrates because HS is present on both cell-surface and ECM proteoglycans. However, the specific targets of HPSE-1 action are not known. Of particular interest is the relationship between HPSE-1 and HSPG, known for their involvement in tumor progression. Syndecan-1, an HSPG, is ubiquitously expressed at the cell surface, and its role in cancer progression may depend upon its degradation. Conversely, another HSPG, perlecan, is an important component of basement membranes and ECM, which can promote invasive behavior. Down-regulation of perlecan expression suppresses the invasive behavior of neoplastic cells *in vitro* and inhibits tumor growth and angiogenesis *in vivo*. In this work we demonstrate the following. 1) HPSE-1 cleaves HS present on the cell surface of metastatic melanoma cells. 2) HPSE-1 specifically degrades HS chains of purified syndecan-1 or perlecan HS. 3) Syndecan-1 does not directly inhibit HPSE-1 enzymatic activity. 4) The presence of exogenous syndecan-1 inhibits HPSE-1-mediated invasive behavior of melanoma cells by *in vitro* chemoinvasion assays. 5) Inhibition of HPSE-1-induced invasion requires syndecan-1 HS chains. These results demonstrate that cell-surface syndecan-1 and ECM perlecan are degradative targets of HPSE-1, and syndecan-1 regulates HPSE-1 biological activity. This suggest that expression of syndecan-1 on the melanoma cell surface and its degradation by HPSE-1 are important determinants in the control of tumor cell invasion and metastasis.

Most of the molecular events associated with tumor growth, neovascularization, and metastasis are influenced by interactions between cancer cells and their extracellular matrix (ECM)[1] components. Heparan sulfate proteoglycans (HSPG), along with other proteoglycans and structural proteins, are key components of the cell surface and ECM (1–5). Heparan sulfate (HS) polysaccharide chains in the ECM both bind and regulate the activities of numerous signaling molecules (4, 6–12). Realization that cell-surface HSPG mediate extracellular information has raised questions about their selective degradation at the HS chains level.

Heparanase (HPSE-1), is an endo-β-D-glucuronidase that cleaves HS at specific intrachain sites, resulting in the formation of fragments of appreciable size (10–20 sugar units; see Refs. 13–15). Of note, HPSE-1 is distinguishable from other HS-degradative enzymes, such as heparitinases and heparinases from *Flavobacterium heparinum*, or endoglucosaminidases, which are eliminases that cleave HS into di- or tetrasaccharide units (16, 17). Increased levels of HPSE-1 activity are associated with several tumor types (14, 18–20), including brain metastatic melanoma (15, 21–23), and have been implicated in tumor angiogenesis and metastasis (14). Tumor cells transfected with HPSE-1 cDNA become more invasive and acquire a highly metastatic phenotype (24). Our laboratory has demonstrated that astrocytes produce HPSE-1 and could contribute to the brain metastatic specificity of melanoma cells (25). Furthermore, release of HS-bound paracrine growth factors affects the metastatic phenotype (26). Additionally, HPSE-1 releases ECM-resident angiogenic factors *in vitro*, and its overexpression induces an angiogenic response *in vivo* (14).

Syndecan-1, a transmembrane HSPG that binds both growth factors and ECM components, acts as a co-receptor for multiple growth factor receptors (2, 4–6, 27). Syndecan-1 expressed at the cell surface is typically down-regulated in metastatic phenotypes. However, soluble syndecan-1 expressed in tumors has been directly associated with increased invasion and cancer progression (28–30).

Perlecan, the most abundant ECM HSPG of epithelial and endothelial basement membranes, has been shown to promote

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[1] The abbreviations used are: ECM, extracellular matrix; HPSE-1, heparanase; bFGF, basic fibroblast growth factor; CHAPS, 3-[(3-chloroamidopropyl)dimethylammonio]-1-propane sulfonate; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; FITC-HS, FITC-labeled heparan sulfate; HPLC, high pressure liquid chromatography; HRP, horseradish peroxidase; HSPG, heparan sulfate proteoglycans; HS, heparan sulfate glycosaminoglycan chains; b-HS, biotinylated HS; mAb, monoclonal antibody; PBS, phosphate-buffered saline; VEGF, vascular endothelial growth factor; ELISA, enzyme-linked immunosorbent assay.

invasive behavior (31). Down-regulation of perlecan expression inhibits tumor growth and angiogenesis *in vivo* (32) and suppresses the invasive behavior of melanoma cells *in vitro* (33). A recent study (27) has demonstrated that HS degradation uncovers cryptic HS fragments that participate in controlling tumor cell growth and metastasis. Enzymatic degradation of HSPG HS chains by HPSE-1 is therefore likely to be involved in fundamental biological processes ranging from normal development to cancer metastasis. Although the precise mechanisms by which they promote invasion are unknown, perlecan and other cell-surface/ECM HSPG may enhance metastasis by binding chemokines and growth factors which can then be released by enzymatic degradation of HS.

Although several studies have reported HPSE-1 cloning (24, 34–36), molecular properties (13–14, 24, 34–36), expression (25, 34–39), localization (34, 38), and involvement in cancer progression (13–14, 23), heparanase targets have not been thoroughly investigated. In the present study we have: 1) investigated HPSE-1 degradation of cell-surface HS in metastatic melanoma cells; 2) determined whether purified syndecan-1 and perlecan HS are degradative targets of HPSE-1; 3) evaluated the relationship between syndecan-1 and the enzymatic activity of HPSE-1; and 4) assessed the modulation of HPSE-1-induced invasion by syndecan-1 including the requirement for syndecan-1 HS chains.

EXPERIMENTAL PROCEDURES

Materials—Heparin, HS, and trypsin-EDTA were acquired from Sigma. [^{35}S]Sulfate (43 Ci/mg) was purchased from ICN (Irvine, CA). DMEM, Ham's F-12 nutrient medium were purchased from Invitrogen, and FBS was from HyClone Laboratories (Logan, UT). WiDr cells were obtained from the American Tissue Culture Collection (Manassas, VA); guanidine hydrochloride was from Invitrogen, and CHAPS was from Roche Applied Science. Heparitinase (*F. heparinum*, EC 4.2.2.8), chondroitinases AC (*Arthrobacter aurescens*; EC 4.2.2.5), and ABC (*Proteus vulgaris*; EC 4.2.2.4) were from Seikagaku America, Inc. (Ijamsville, MD). Transwell cell culture chambers were purchased from Corning Glass, and Matrigel™ was obtained from Discovery Labware. All other chemicals used were reagent-grade or better.

Tissue Culture—Early passage melanoma cells with high metastatic abilities compared with parental cell lines of murine (B16B15b line; see Refs. 21–23) and human origin (70W line; see Refs. 21–23), were maintained as monolayer cultures in a 1:1 (v/v) mixture of DMEM/F-12 supplemented with 5% FBS (B16B15b) or 10% (v/v) FBS (70W). Human colorectal adenocarcinoma cells (WiDr) were maintained in RPMI supplemented with 10% (v/v) FBS. Cells were maintained at 37 °C in a humidified 5% CO_2, 95% air (v/v) atmosphere and passaged using EDTA (2 mM in PBS) or trypsin-EDTA (70W, WiDr) before reaching confluence. WiDr cells were chosen as a source for perlecan because it is the predominant cell-associated, high M_r proteoglycan they produce (40–41).

Immunofluorescent Staining—Cells were plated at 10% density on glass coverslips in DMEM/F-12, 5% (v/v) FBS, allowed to attach, and grown for 24 h. Cells were then switched to DMEM/F-12, 5% FBS (v/v) containing Hepes (10 mM, pH 6.3). Subsequently, recombinant HPSE-1 (200 ng/ml) was added to the appropriate wells, and cells were incubated for 24 h at 37 °C. Cell-surface HS was detected with an anti-heparan sulfate mAb 10E4 (1:50 dilution; Seikagaku America, Falmouth, MA) followed by incubation with biotinylated anti-mouse IgM (BioGenex Laboratories, San Ramon, CA) and rhodamine red-x-streptavidin (1:400 dilution; Accurate Inc., Westbury, NY). Cells stained for cell-surface syndecan-1 were plated as above and immunostained with anti-syndecan-1 mAb 281.2 (1:100 dilution; see Ref. 30) followed by a biotinylated goat anti-rat IgG (1:400 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) and rhodamine red-x-streptavidin (Molecular Probes, Eugene, OR). Digital images were produced on an axioplan fluorescent microscope (Carl Zeiss Microimaging Inc., Thornwood, NY) with advanced spot imaging program (Bio-Rad) using identical conditions for all photographs and antibodies used.

Preparation of ^{35}S-Labeled HS and Column Chromatography—WiDr cells were used as a source of HS because the predominant HSPG produced by WiDr cells is perlecan (40–41). WiDr cells were incubated overnight (18 h) at 37 °C in a humidified 5% CO_2, 95% air (v/v) atmosphere in RPMI media lacking sulfate and supplemented with 33 mM $MgCl_2$, 15 mM Hepes, 1.2 gm/ml sodium bicarbonate, pH 7.3, containing penicillin G (5 units), and penicillin-streptomycin sulfate (5 μg). This resulted in a final concentration of 10 μM sulfate in the labeling media. The labeling media contained 400 μCi/ml $H_2{}^{35}SO_4$ for a final specific activity of 40 μCi/nmol. At the end of the labeling period, the media were collected and precipitated with trichloroacetic acid-phosphotungstic acid as described previously (42). The material in the pellet was subjected to incubation for 48 h at 37 °C in 0.1 M NaOH, 0.25 M sodium borohydride (β-elimination). At the end of the incubation, the solution was neutralized with 1 N acetic acid and desalted over a Sephadex G-50 column (0.5 × 10 cm) eluted in PBS. The material eluting in the void volume was pooled and defined as glycosaminoglycan. In both the secreted and extracellular matrix fractions, >95% was HS as determined by sensitivity to nitrous acid degradation (42). This preparation also was sensitive to bacterial heparitinases (17) but was resistant to chondroitinases AC and ABC digestion (21). Specific activity was estimated to be ~2.6 × 10^8 dpm/μg HS.

[^{35}S]HS from WiDr cell-derived perlecan (1 pg) was incubated in the presence or absence of HPSE-1 (200 ng) in 250 mM NaCl, 50 mM sodium acetate, pH 5.0, for 24 h at 37 °C. Digestion of perlecan WiDr HS was analyzed by liquid chromatography using a Sephacryl 300S HR column (1.0 × 30 cm, Amersham Biosciences) (21). The resin was eluted with 4 M guanidine hydrochloride, 0.5% (w/v) CHAPS, 50 mM sodium acetate, pH 6.0, at a flow rate of 0.4 ml/min; and fractions (0.4 ml) were collected. Aliquots were then taken for determination of radioactivity by liquid scintillation counting. Column void volume (V_o) and total volume (V_T) were assessed by the elution positions of blue dextran and vitamin B_{12}.

Syndecan-1 Isolation and Removal of Core Protein or HS Chains—Syndecan-1 was isolated from the culture media conditioned by CAG myeloma cells transfected with a cDNA construct coding for the extracellular domain of the human syndecan-1 core protein. Similar studies using a cDNA coding for the extracellular domain of murine syndecan-1 have shown that it is secreted into the culture media as an intact molecule bearing HS chains (30). For syndecan-1 isolation, culture media were brought to a final concentration of 2 M urea and 50 mM sodium acetate, pH 4.5, and incubated with DEAE-Sepharose fast flow beads overnight (18 h) at 4 °C with gentle rocking. Beads were harvested by centrifugation and washed in PBS, and syndecan-1 was eluted in PBS containing 1 M NaCl. Where indicated, syndecan-1 was treated to digest either the core protein or HS before chemoinvasion assays. To digest HS, syndecan-1 (250 ng) was incubated for 16 h at 37 °C with either HPSE-1 in 50 mM sodium acetate, pH 5.5, or with heparitinase (1 mg/ml) in heparitinase buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 4 mM $CaCl_2$, 70 μl final volume), and then the enzymes were inactivated for 10 min at 50 °C (43). For experiments requiring free HS chains, syndecan-1 (250 ng) was treated as above for β-elimination (48 h at 37 °C in 0.1 M NaOH, 0.25 M sodium borohydride) to digest the core protein. Alternatively, syndecan-1 core protein was digested with Pronase (5 mg/ml; Sigma) for 16 h at 37 °C (44), and Pronase was then inactivated for 15 min at 100 °C. Results shown are from multiple experiments.

HPSE-1 Digestion of Syndecan-1 and Western Blotting—Purified syndecan-1 (250 ng) was digested with active recombinant HPSE-1 (0–200 ng) in sodium acetate (80 mM), NaCl (250 mM) buffer, pH 4.5, for 24 h at 37 °C. Samples were then incubated for 10 min at 100 °C with Laemmli sample buffer (45) and separated on a 4–20% Criterion gel (Tris-HCl, Bio-Rad). Syndecan-1 was transferred to a zeta probe membrane (Bio-Rad). The membrane was incubated in a blocking reagent (3% (w/v) non-fat dry milk, 0.5% (w/v) bovine serum albumin in PBS, pH 7.5) for 1 h, and then 0.3% (w/v) Tween 20 buffer was added for an additional 30 min. Syndecan-1 core protein was labeled using anti-syndecan-1 mAb BB-4 (1:200 dilution; Accurate Chemical & Scientific Corp., Westbury, NY) in blocking reagent, washed for 1 h with 6–8 changes of 0.5% Igepal (CA-630, Sigma) in Tris buffer (20 mM Tris, 150 mM NaCl, pH 7.4), and incubated with horseradish peroxidase anti-mouse IgG (1:10,000 dilution; Accurate Chemical & Scientific Corp). It then was washed and developed using the SuperSignal® West Femto maximum sensitivity substrate (Pierce). Labeling was detected and quantified using a Versadoc imaging system (Bio-Rad).

Chemoinvasion Assay—The invasive properties of melanoma cells were assayed by use of invasive chambers followed by fluorescence plate scanner analysis as described (21–23, 39). Briefly, cell invasion was assayed by using Transwell cell culture chambers (8 μm pore size, 6.5 mm diameter or 12 μm pore size, 12 mm diameter) coated with Matrigel™ (diluted 1:30 with cold DMEM/F-12, 100–300 μl final coating volume) that was applied to the upper filter surface and allowed to dry before use. In assays where Matrigel™ was pretreated with HPSE-1, Matrigel™-coated filters were washed 3 times with DMEM/F-12 and

then incubated with HPSE-1 (300 ng/ml, 100 μl final volume in 10 mM Hepes/DMEM/F-12, pH 6.8) for 16 h at 37 °C and then washed 10 times with DMEM/F-12, pH 7.4, before adding cells for the indicated treatments. For invasion assays, cells were added to the upper chambers (4.0×10^4 cells/6.5 mm diameter chambers or 1.2×10^4 cells/12 mm diameter chambers) with indicated amounts of purified HPSE-1 and/or isolated syndecan-1. In some experiments, syndecan-1 (50 ng/ml), digested to remove core protein/HS chains, or mock controls including incubated enzyme/reagents and buffer were added with cells (300 μl) to the upper compartment of the chemoinvasion chambers (12 μm pore) in the presence of HPSE-1 (300 ng/ml). The lower chambers contained *N*-formyl-L-methyl-L-leucine-L-phenylalanine (5 nM) in DMEM/F-12 with 10% (v/v) FBS as chemoattractant. Cells were incubated in invasive chambers for 24–48 h at 37 °C in a humidified 5% CO_2, 95% air (v/v) atmosphere. Cells that invaded through the membrane were released from the lower side of the filter and the lower well by trypsin-EDTA (10 min treatment; Sigma) after washing with 2 mM EDTA in PBS, pH 7.4. They were then centrifuged and resuspended in calcein-AM (2 μg/ml; Molecular Probes, Eugene, OR) in Hanks' balanced salt solution. Cells with dye were incubated 40 min at 37 °C and read in a 96-well plate using a Cytofluor 4000 multiwell fluorescent plate reader (Applied Biosystems, Foster City, CA). Alternatively, to quantify cell invasion, non-invasive cells were removed from the upper chambers using cotton swabs, and invasive cells present on filters in lower chambers were visualized using Diff-Quick Stain Kit (IMEB Inc., San Marcos, CA) according to the manufacturer's instructions, photographed with a Sony Digital Camera at fixed focus, using a Zeiss Axiovert 25 microscope (Zeiss, Microimaging Inc., Thornwood, NY), and counted.

HPSE-1 Assay—HPSE-1 activity was determined by degradation of FITC-HS using high speed gel permeation column chromatography (see Refs. 21–23; HPLC) as described previously (24, 39). Enzymatic activity assays were performed at HPSE-1 optimal pH of 4.5 and at pH 6.8. 70W melanoma cells rapidly acidify their medium, often to below pH 6.8 within 1 day in culture. Briefly, FITC-HS (6 μg in 100 μl total volume) was incubated with purified and active recombinant HPSE-1 and with or without purified syndecan-1 at indicated concentrations for 18–24 h at 37 °C in 100 μl of sodium acetate (50 mM, pH 4.5) or in reaction buffer (heparin degrading kit adjusted to pH 6.8 with 1 M Tris-HCl; Takara.Mirus.Bio). Reaction was terminated by adding heparin (100 μg) and by heating samples for 5 min at 100 °C. Internal standards (4-aminobenzoic acid (3 μg/ml) and ovalbumin (3 μg/ml) in 1 M Tris-HCl, pH 8.0) were added to adjust pH and to provide quality controls for HPLC. HS products yielded by heparanase reaction were analyzed by size exclusion chromatography performed on an HP1090 liquid chromatograph with a photodiode array detector (Agilent Technologies Inc., Wilmington, DE) and an HP1046 programmable fluorescence detector (Agilent Technologies Inc.). Samples were injected on a TSK-GEL 3000SW_{XL} column (5 μm, 7.8 mm $\times$ 30 cm) with a TSK-GEL SW_{XL} guard column (5 μm, 6.0 mm x 4.0 cm) from Tosoh Bioscience (Montgomeryville, PA) at ambient temperature (25 °C). Samples were isocratically eluted using Tris-HCl (25 mM), NaCl (150 mM), pH 7.5, at a flow rate of 0.5 ml/min with a sample splitter placed between the column and detectors to maintain a column pressure of less than 70 bar. Fluorescence was monitored with excitation at 490 nm and emission at 520 nm, and then data were processed and peaks integrated using HP ChemStation software (Agilent Technologies). HPSE-1 activity was determined by measuring the decrease in fluorescence intensity in the first one-half area of the intact FITC-HS peak chromatogram in comparison to no HPSE-1 (negative control).

Alternatively, a commercial HS degrading enzyme assay kit (Takara.Mirus.Bio, Madison, WI) was used to determine purified HPSE-1 activity, in the presence or absence of isolated syndecan-1. Concentrations of syndecan-1 and HPSE-1 were incubated with biotinylated-HS (b-HS) as indicated and detected by an ELISA-type assay according to manufacturer's instructions. Where indicated, buffer used for HPSE-1 cleavage of bHS was adjusted to pH 6.0 (or pH 6.8) with 1 M Tris-HCl. Activity was determined by comparison to known amounts of HPSE-1 present in this assay.

RESULTS

HPSE-1 Degrades Cell-surface HS in Melanoma Cells—To determine the effect of HPSE-1 on cell-surface HS, HS expression before and after treatment of melanoma cells with purified and active recombinant HPSE-1 was measured. HS expression of murine brain metastatic melanoma cells (B16B15b) was readily visualized using a monoclonal antibody to HS (Fig. 1, *A* and *C*). Fig. 1 shows treatment of melanoma cells with HPSE-1 at both acidic (6.3) and physiologic pH (7.4). We chose to incubate the cells at an acidic pH because tumors often generate an acidic extracellular pH, and human HPSE-1 is more effective at this pH (15, 24). B16B15b cells expressed high levels of cell-surface HS at both pH conditions. Treatment with HPSE-1 (24 h at 37 °C, pH 6.3) resulted in a significant reduction of the cell-surface HS (Fig. 1*B*). HPSE-1 activity at pH 7.4 was sufficiently low that we were unable to detect a significant reduction in HS expression by immunofluorescence analysis (Fig. 1*D*). This confirms previous reports of lower HPSE-1 activity at physiologic (pH 7.4) *versus* acidic pH (26, 47). A more extensive digestion of HS may be needed to detect changes in HS expression through immunofluorescence than would be required for HPSE-1-induced activity in biological determinations such as invasion assays.

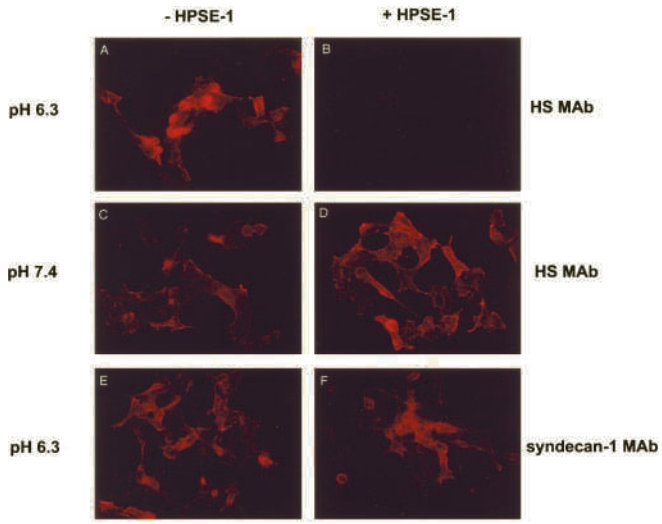

FIG. 1. **Cell-surface HS is degraded by HPSE-1, but syndecan-1 core protein is not removed by HPSE-1 treatment.** Melanoma (brain metastatic B16B15b) cells were plated on glass coverslips in DMEM/F-12 with 10% FBS and incubated for 24 h at 37 °C, 5% CO_2. The medium was then removed and replaced with DMEM/F-12 at pH 6.3 (*A* and *B*) or pH 7.4 (*C* and *D*) in the absence (*A, C,* and *E*) or presence (*B, D,* and *F*) of purified recombinant HPSE-1 (200 ng/ml). Subsequently, cells were surface-labeled with anti-HS mAb (*A–D*) followed by biotinylated anti-mouse IgM and rhodamine red-x-streptavidin. Additionally, cells were surface-labeled with anti-syndecan-1 mAb (281.2) (*E* and *F*) followed by biotinylated anti-rat immunoglobin and rhodamine red-x-streptavidin. Digital images were produced on an Axioplan fluorescent microscope (Carl Zeiss Inc., Thornwood, NY) with an advanced spot imaging program using identical conditions for all photographs and antibodies used.

Of relevance, syndecan-1 is a cell-surface HSPG expressed on B16B15b cells (Fig. 1*E*; data not shown). Syndecan-1 is lost from the cell surface under a number of physiological conditions (5). Furthermore, degradation of HS can result in changes in syndecan-1 distribution (5, 47). To determine whether removal of HS resulted in a loss of cell-surface syndecan-1 core protein, B16B15b cells were treated with HPSE-1, and syndecan-1 expression was detected using a specific monoclonal antibody to its core protein. Syndecan-1 core protein was still expressed on the cell surface of HPSE-1-treated cells at levels undistinguishable from non HPSE-1-exposed cells (Fig. 1, *E* and *F*). However, under the same conditions there was a significant reduction in cell-surface HS (Fig. 1, *A* and *B*).

HPSE-1 Degrades Syndecan-1 and WiDr Perlecan HS—HPSE-1 is known to not only promote cell invasion but also to induce angiogenesis by modulating growth factor activity and bioavailability (14, 39). However, little is known of its direct action on specific HSPG targets and their modulation/interac-

tions during HPSE-1-driven invasive events. To determine precisely which HSPG are digested by HPSE-1, we incubated purified preparations of syndecan-1 and perlecan HS with purified active recombinant HPSE-1. Syndecan-1 was digested with increasing concentrations of HPSE-1, and its core protein was detected by Western blot analysis. Syndecan-1 was detected as a broad band at ~220 kDa (Fig. 2). The broad band and large molecular weight are due to the presence of HS chains and aggregation of the syndecan-1 core protein as reported previously (48, 49). HPSE-1 exposure (50 ng for 24 h at 37 °C) of syndecan-1 resulted in a discrete degradation of its HS giving two different sized bands (Fig. 2). These bands were still aberrantly large indicating that HS chains and protein core aggregation still influenced protein mobility. Furthermore, HPSE-1 characteristically digests HS chains at discrete interior sites rather than acting by a progressive removal of sugar subunits from the chains (13–15, 50). Because the number of cleavage sites/HS chain is also finite, studying HPSE-1 enzymatic activity requires extended incubations to detect degradation of HS chains by chromatographic methods (24, 35, 46, 51). Digestion with increasing amounts of HPSE-1 (200 ng) using the same conditions resulted in further digestion of syndecan-1 to an ~120-kDa band (Fig. 2*A*). Studies have shown that small quantities of chondroitin sulfate chains and incompletely digested HS fragments attach to syndecan-1 core protein and prevent the formation of a tight band (52).

We digested WiDr HS preparations with purified HPSE-1. WiDr cells secrete primarily one type of HSPG as large intact proteoglycan (41) previously identified as perlecan (40). Perlecan is also the major HSPG present in the basal lamina and in Matrigel™, a commercial basement membrane used as coating ECM barrier in *in vitro* chemoinvasion assays. ^{35}Sulfate-labeled HS from WiDr cells-derived perlecan was exposed to HPSE-1, and radiolabeled HS digestion products were separated by Sephacryl 300S HR column chromatography. Undigested perlecan HS eluted significantly before HPSE-1-digested perlecan HS products (Fig. 3), demonstrating that HPSE-1 degraded perlecan-derived HS into smaller fragments. Similar results were obtained using HS from WiDr cell-secreted ECM (data not shown).

Syndecan-1 Inhibits HPSE-1 Stimulation of Melanoma Cell Invasion—The effects of syndecan-1 and HPSE-1 on the invasive capacity of melanoma cells were determined by seeding cells (highly brain metastatic 70W) onto Matrigel™-coated filters in Transwell units. In this assay, cells in the top well unit adhere to and penetrate the coated filters and then enter the lower chamber (22). Melanoma cells incubated with syndecan-1 and/or HPSE-1 were added to the upper chambers and allowed to invade for 48 h before analysis. HPSE-1 added with cells stimulated invasion through the Matrigel™ barrier 8-fold over untreated cells (Fig. 4). Importantly, co-incubation of exogenously added syndecan-1 with HPSE-1 inhibited heparanase-induced invasion. Presence of syndecan-1 alone in the upper chambers did not affect the basal invasion of cells. These data indicate that addition of soluble syndecan-1 ectodomain can act to inhibit HPSE-1-specific stimulation of melanoma cell invasion *in vitro* (Fig. 4).

The inhibition of HPSE-1-stimulated invasion is dependent upon the concentration of syndecan-1 present. First, an HPSE-1 concentration (100 ng/ml) was used to stimulate maximal penetration of 70W cells through the invasion chambers. Second, by using syndecan-1 at a similar concentration (100 ng/ml), inhibition of invasion was observed ($p < 0.001$ compared with HPSE-1 exposure (100 ng/ml); Table I). Syndecan-1 also inhibited HPSE-1-induced invasion when a 10-fold lower syndecan-1 concentration (10 ng/ml; $p < 0.001$ compared with

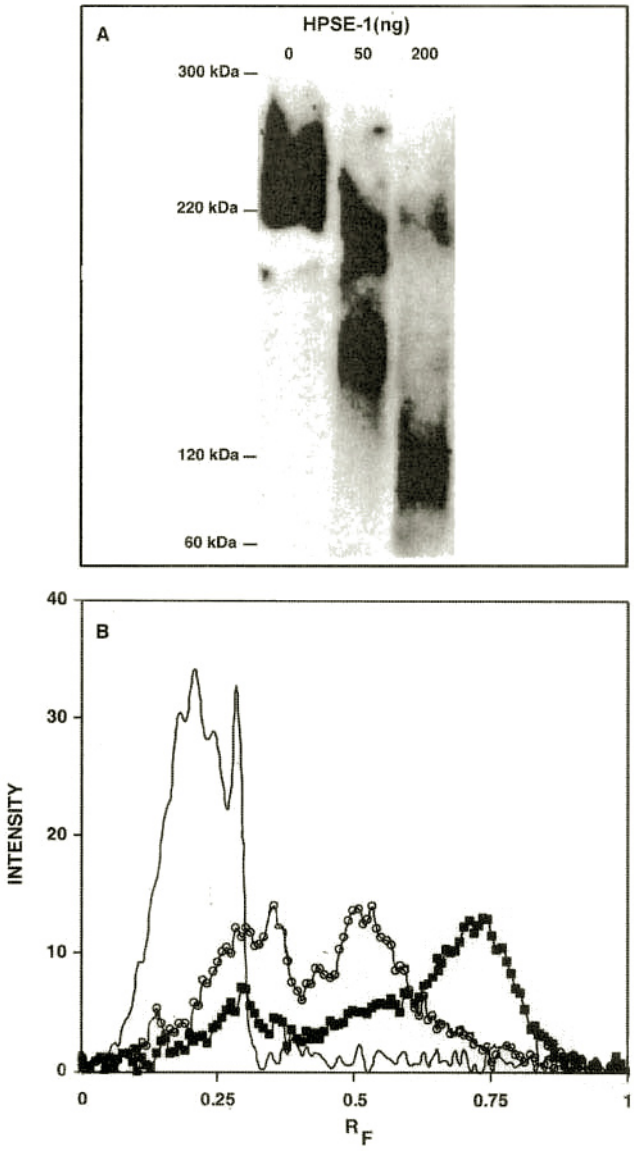

FIG. 2. **Purified syndecan-1 is degraded by HPSE-1.** *A*, syndecan-1 (250 ng/assay) was degraded with increasing concentrations of HPSE-1 (0, 50, or 200 ng/assay) for 24 h at 37 °C in 20 mM sodium acetate, pH 4.5, and 250 mM NaCl. Syndecan-1 was analyzed by Western blotting analysis with an anti-syndecan-1 core protein antibody (BB-4) and HRP-anti-mouse IgG mAb followed by using the SuperSignal West Femto maximum sensitivity substrate. Bands were visualized on a Versadoc imaging system. Results shown were consistently obtained in four independent experiments. *B*, quantification of size change was determined by analyzing the Western blotting for optical density of syndecan-1 staining *versus* R_F values using Quantity One quantitation software for treatment with 0 (—), 50 (○), and 200 ng (■) of HPSE-1.

HPSE-1 (100 ng/ml)) was present in these assays. This is a significantly lower molar amount of syndecan-1 (10 ng/ml or 0.23 nM) than HPSE-1 (100 ng/ml or 1.7 nM). Lower concentrations of syndecan-1 (1 ng/ml or 23 pM) were unable to inhibit HPSE-1-stimulated invasion.

HPSE-1 could be degrading both the matrix and syndecan-1 during the invasion assay. We therefore pretreated the Matrigel™ coating the invasion chamber with HPSE-1 (300 ng/ml) to digest HS in the matrix, and then extensively washed the Matrigel™ prior to adding cells and syndecan-1. HPSE-1 digestion of the matrix prior to the initiation of the invasion assay increased the number of invading cells (Table II). Syn-

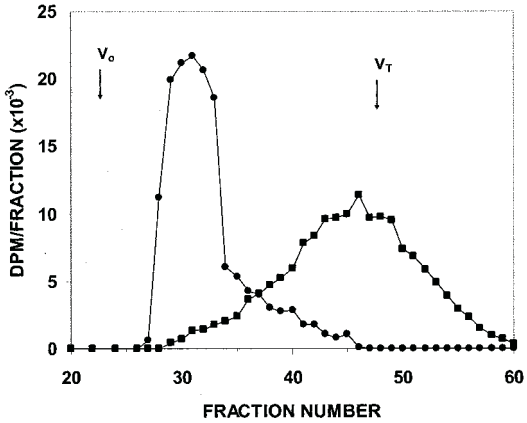

FIG. 3. **WiDr HS is degraded by HPSE-1 and WiDr HS degradation is not inhibited by syndecan-1.** [$^{35}SO_4$]HS from WiDr cells was treated in the presence (■) or absence (●) of HPSE-1 (200 ng/assay for 24 h at 37 °C, pH 4.5). The products were then separated by Sephacryl 300S HR chromatography in 4 M guanidine-HCl, 0.1% (w/v) CHAPS, in 50 mM sodium acetate (pH 6.0; 0.4 ml fractions). Elution of the [$^{35}SO_4$]HS was detected by liquid scintillation counting. Column V_o and V_T values are indicated by *arrows*.

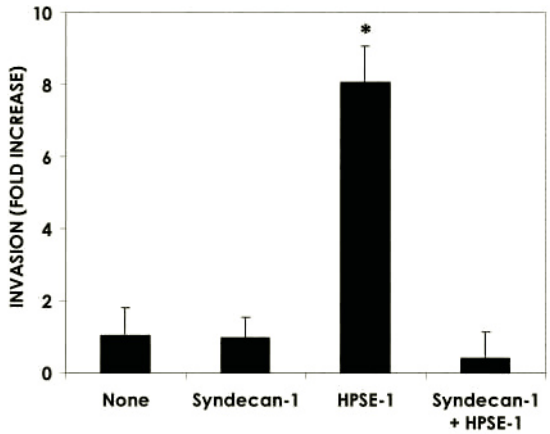

FIG. 4. **Syndecan-1 inhibits HPSE-1-stimulated invasion of melanoma cells.** Brain metastatic melanoma cells (70W) were placed in invasion chambers (8 μm pores) in the presence or absence of HPSE-1 (100 ng/ml or 1.7 nM), syndecan-1 (200 ng/ml or 2.3 nM), or both, and incubated in DMEM/F-12 for 24 h at 37 °C. At that time, the medium in the upper chambers was exchanged for fresh DMEM/F-12, and invasion assays were continued for additional 24 h. A trypsinization step was then applied releasing invasive cells from lower wells and Transwell filters (see "Experimental Procedures"). Cells were subsequently labeled with calcein-AM for 30 min at 37 °C and detected using a Cytofluor fluorescence plate reader. Cell numbers were calculated from a standard curve of known number of cells and increased invasions (fold) are reported. *Bars* represents the mean ± S.D. of triplicate determinations (*, $p < 0.001$). Student's t test was used as statistical method.

decan-1 added with the cells at the beginning of the invasion assay inhibited the increased invasion through HPSE-1-pretreated matrix.

Syndecan-1 is composed of a core protein and HS chains which may act independently or in concert to inhibit HPSE-1-induced melanoma cell invasion. To examine these possibilities, we first degraded either the core protein or the HS chains prior to their addition to the invasion assays. Second, enzymes were inactivated prior to their addition to the invasion assay and did not interfere with it (Table III). Heparitinase leaves the core protein intact but cleaves the HS chains into di- and tetrasaccharides that are too small to facilitate biological activity, such as growth factor binding. Syndecan-1 core protein from heparitinase-treated syndecan-1 was unable to inhibit the

TABLE I
Syndecan-1 inhibition of HPSE-1-stimulated invasion

HPSE-1	Syndecan-1	Invasion (cells/mm^2) mean ± S.D.
ng/ml		
0	0	13 ± 2
100	0	54 ± 1[a]
100	0.001	62 ± 16[a]
100	0.01	47 ± 7[a]
100	0.1	59 ± 9[a]
100	1	42 ± 6[a]
100	10	13 ± 3[b]
100	100	17 ± 6[b]

[a] $p < 0.005$ when compared with negative control (no HPSE-1 and no syndecan-1).
[b] $p < 0.001$ when compared with positive control (HPSE-1 (100 ng/ml) with no syndecan-1). Student's t test was used as statistical method.

TABLE II
Syndecan-1 inhibition of melanoma invasion through HPSE-1-pretreated Matrigel™ invasion chambers

HPSE-1 pretreatment	Syndecan-1	Invasion (cells/mm^2) mean ± S.D.
ng/ml		
0	0	38 ± 4.0
300	0	78 ± 2.3[a]
0	100	42 ± 1.1
300	100	18 ± 2.1

[a] $p < 0.005$ when compared with negative control (no HPSE-1 pretreatment and no syndecan-1 treatment). Student's t test was used as statistical method.

HPSE-1-induced melanoma cell invasion (Table III). However, syndecan-1 pretreated with HPSE-1 was still capable of inhibiting the HPSE-1-induced invasion. HPSE-1 cleaves HS chains into relatively large fragments that could retain biological activity. HPSE-1 may also leave sufficient HS attached to the core protein to allow binding of growth factors or ECM components to partially denuded core protein. As an alternative approach, we digested syndecan-1 core protein by using the β-elimination procedure or by Pronase incubation, both of which leave the HS chains intact. Free HS chains from syndecan-1 inhibited the HPSE-1-induced invasion. Together, these data indicate that HS chains are required for syndecan-1 to inhibit HPSE-1-induced invasion and that syndecan-1 HS chains digested with HPSE-1 still retain their active sites.

To evaluate if other HSPG would also inhibit HPSE-1-stimulated invasion, WiDr perlecan HS was added with melanoma cells in invasion assays. Unlike syndecan-1 HS, WiDr perlecan HS did not inhibit HPSE-1-stimulated invasion nor change the basal invasive values of these cells (Fig. 5).

Syndecan-1 Does Not Directly Inhibit HPSE-1 Activity—Mechanisms by which syndecan-1 inhibits invasion in melanoma cells are presently not known (5). We have shown that syndecan-1 can inhibit invasion through HPSE-1-pretreated Matrigel™ where soluble HPSE-1 has been removed prior to the start of the invasion assay (Table II), indicating that syndecan-1 does not directly inhibit HPSE-1 activity. However, syndecan-1 could be acting on HPSE-1 bound to the matrix which cannot easily be removed by washing. Therefore, to directly assess if syndecan-1 acted as an inhibitor of HPSE-1 enzymatic activity, we measured heparanase activity in the presence of syndecan-1 using several sources and derivatives of HS, FITC-labeled HS and biotinylated HS. The first assay consisted of preparing FITC-HS and analyzing HPSE-1 degradation by HPLC analysis (Refs. 24 and 39; see also "Experimental Procedures"). FITC-HS was incubated alone (negative control), with HPSE-1 (positive control), syndecan-1, or with

TABLE III
HPSE-1, heparitinase, β-elimination, or Pronase Syndecan-1 pretreatments, effects on HPSE-1-induced melanoma cell invasion

Syndecan-1	HPSE-1	Invasion (fold increase), enzymatic pretreatment[a]			
		β-Elimination	Pronase	Heparitinase	HPSE-1
ng/ml					
0	0	1.0 ± 0.13	1.0 ± 0.27	1.0 ± 0.14	ND[b]
0	300	2.0 ± 0.13[c]	2.1 ± 0.07[c]	2.4 ± 0.50[c]	ND[b]
50	300	1.0 ± 0.19	1.1 ± 0.18	2.6 ± 0.32[c]	0.7 ± 0.10

[a] Syndecan-1 or mock buffer was pretreated with indicated enzymes for 16 h; enzymes were then inactivated, and digested syndecan-1 was added to invasion assays (see "Experimental Procedures").
[b] ND, not determined.
[c] $p < 0.05$ when compared with negative control (no HPSE-1 treatment). Student's t test was used as statistical method.

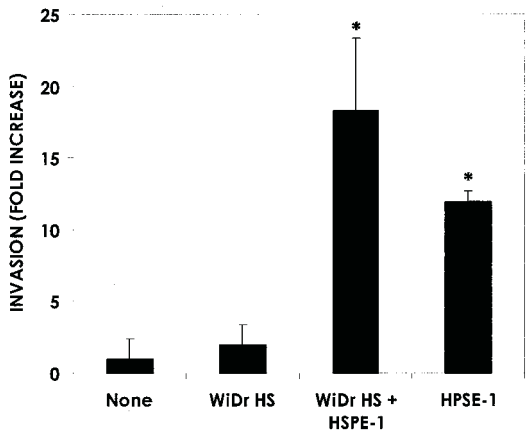

FIG. 5. **WiDr perlecan HS does not inhibit HPSE-1-mediated invasion of melanoma cells.** Brain metastatic melanoma cells (70W) were placed in invasion chambers (0.8 μm diameter pores) in the presence or absence of HPSE-1 (100 ng/ml), WiDr perlecan HS (8 ng/ml), or both, and incubated in DMEM/F-12 for 24 h at 37 °C. At that time, the medium in upper invasive chambers was exchanged for fresh DMEM/F-12, and invasion assays were continued for additional 24 h at 37 °C. Cells that invaded through the invasion chambers were stained with Diff-Quick staining kit, photographed, and counted (see "Experimental Procedures"). *Bars* represent the mean ± S.D. of triplicate determinations (*, $p < 0.05$). Student's t test was used as a statistical method.

both syndecan-1 and HPSE-1. Syndecan-1 and HPSE-1 concentrations in this assay were in the same range as the ones used in invasion assays. HPSE-1 concentration was chosen to readily detect any inhibition of activity (Fig. 6*C*) with heparanase activity subsequently analyzed by HPLC. By using this approach, FITC-HS degraded by HPSE-1 had a longer retention time than the control, undegraded FITC-HS (Fig. 6) (24, 39). When syndecan-1 was co-incubated in HPSE-1 activity assays, FITC-HS was specifically degraded, indicating that HPSE-1 was still active in the presence of syndecan-1. Similar results were obtained when syndecan-1 was predigested by HPSE-1 and then added to the reaction mixture (data not shown).

To further investigate any changes in HPSE-1 activity in response to syndecan-1, we used an ELISA at concentrations where we could detect a 2-fold decrease in HPSE-1 enzymatic activity at very low HPSE-1 concentrations. In this assay, HPSE-1 degrades a b-HS fragment. Undegraded b-HS is bound to a bFGF ELISA plate, and its degradation is detected by HRP-streptavidin. The b-HS fragment is specifically designed so that when it is degraded it is not bound and results in a lower HRP signal. By this methodology, we were able to distinctly measure HPSE-1 activity and detect a 50% decrease of its enzymatic activity at pH 6.0 (Fig. 7*B*). Accordingly, HPSE-1 concentration in this assay was lower than the one used in the invasion assay. Importantly, syndecan-1 did not inhibit HPSE-1 activity even at concentrations at much higher ratios than HPSE-1 concentrations used in invasion assays (Fig. 7). We also performed this assay at pH 7.4 resulting in negligible HPSE-1 activity. Again, this confirms previous reports (26, 47) of lower HPSE-1 activity at physiologic (pH 7.4) *versus* acidic pH. Furthermore, because 70W melanoma cells used in this assay rapidly acidify the culture medium, we have performed experiments using intermediate pH conditions (pH 6.8). As expected, HPSE-1 was less active at pH 6.8 (Fig. 8*B*) than at pH 7.4 (Fig. 8*C*). Although the overall level of FITC-HS degradation was lower at pH 6.8, HPSE-1 had the same level of enzymatic activity in the presence (0.2 ± 0.1 fraction HS digestion) or absence of syndecan-1 (0.2 ± 0.1 fraction HS digestion; Fig. 8*A*). Combined, these data demonstrate that HPSE-1 enzymatic activity is not being directly inhibited by syndecan-1 in invasion assays.

DISCUSSION

In this report we demonstrate for the first time that HS from purified syndecan-1 and perlecan are degradative targets of heparanase (HPSE-1). Furthermore, this work provides the first evidence: 1) that HPSE-1 cleaves HS present on the cell surface of metastatic melanoma cells; 2) that HPSE-1 cleaves HS of purified syndecan-1 and HS chains isolated from the medium and matrix of WiDr cells, which primarily secrete perlecan; 3) that syndecan-1 does not inhibit HPSE-1 digestion of HS; and 4) that syndecan-1 inhibits the HPSE-1-mediated invasive effects of melanoma cells by *in vitro* chemoinvasion assays. This inhibition is dependent upon syndecan-1 HS chains. Our results demonstrate that both syndecan-1 and perlecan are degraded by HPSE-1 as purified HS entities and confirm the role of syndecan-1 as an important modulator of tumor cell invasion.

Invasion and metastasis are characteristic features of malignant tumors and are among the greatest impediments to curing cancer. Substantial evidence accumulated over the last 3 decades indicates that HSPG acts to inhibit invasion by promoting tight cell-cell and cell-ECM interactions (1, 3, 5). Degradation of HS chains weakens cell-cell and cell-matrix adhesion in melanoma and other cells (28, 53–54). Furthermore, studies documenting a diminished quantity and quality of HS isolated from transformed cells have demonstrated a reduction in cellular adhesive capacities (1, 3, 5). The results of our HS immunostaining experiments confirm a role for HPSE-1 in the enzymatic degradation of HS. It is therefore conceivable that HPSE-1 could play a role in restructuring HS and thus promote metastatic spread.

HSPG can act both as reservoirs of growth factors (14, 31) and as co-receptors for ligand binding and subsequent intracellular signaling (1, 3). These heparin-binding factors are involved in growth, invasion, angiogenesis, and tumor progression (1–3, 5). Free HS chains bind ligands and replace the biological activities of cell-surface HSPG depending on their size and sequence (1–5). HS degradative enzymes, such as heparitinases, heparinases from *F. heparinum*, or endoglucosaminidases, cleave HS to di- and tetrasaccharides (16–17, 27), which are too short for growth factor and ECM ligand

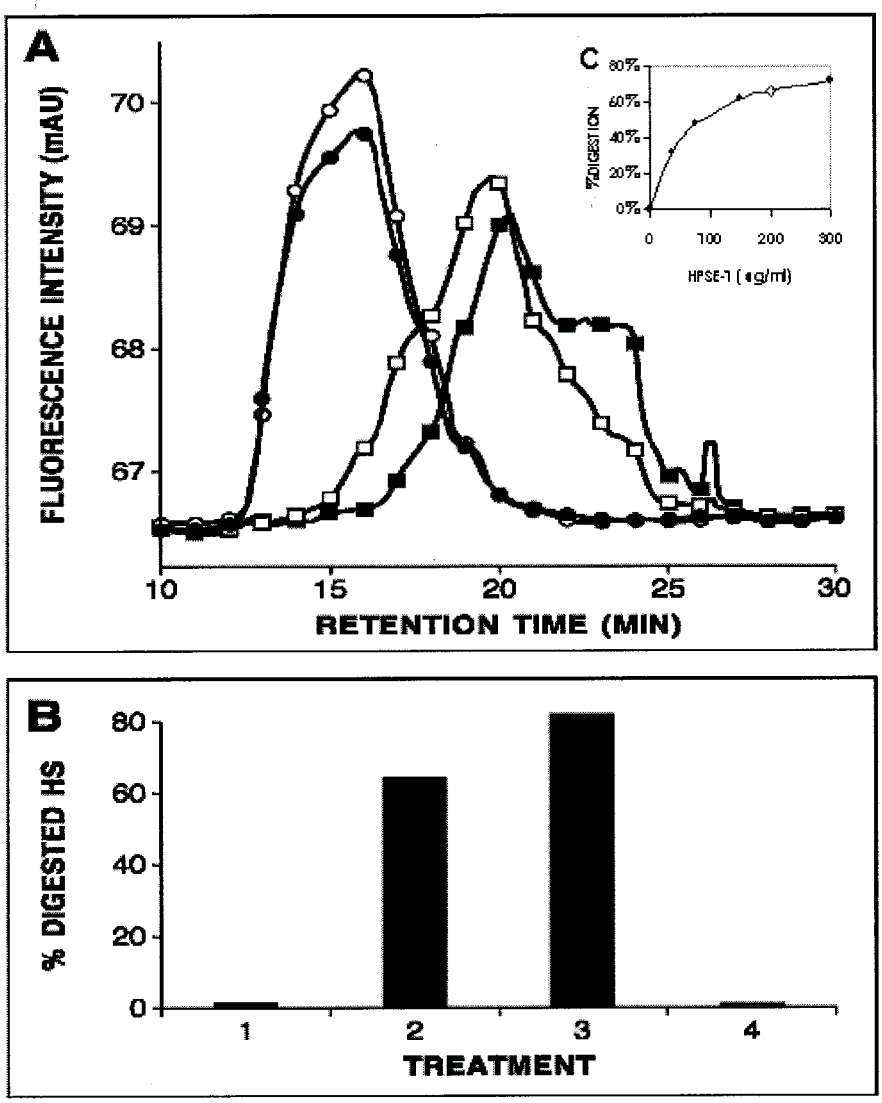

FIG. 6. **Syndecan-1 does not inhibit HPSE-1 enzymatic degradation of FITC-HS at pH 4.5.** *A*, FITC-HS was incubated alone (○), or in the presence of HPSE-1 (□), HPSE-1 and syndecan-1 (■), or syndecan-1 (●) in sodium acetate (50 mM, pH 4.5) for 24 h at 37 °C. Reaction products were then separated by HPLC (see "Experimental Procedures"). *B*, percentage of digested FITC-HS derived from HPLCs alone (*column 1*) or when HPSE-1 (*column 2*), HPSE-1 and syndecan-1 (*column 3*), or syndecan-1 (*column 4*), respectively, were added. *C*, HPSE-1 at indicated concentrations was incubated with FITC-HS, and the percentage of degraded FITC-HS was calculated as the area of the curve that shifted to the right of non-treated control curve. The *open symbol* is the concentration of HPSE-1 used in *A* and *B*. Standard deviations (±2–5% of values) were calculated but not shown for clarity purposes.

binding. On the other hand, HPSE-1 is an endo-β-D-glucuronidase that cleaves HS at specific intrachain sites resulting in fragments of appreciable size (10–20 sugar units; see Refs. 13–15). Cleavage depends on the correct HS sequence (51, 55) and possibly steric hindrance of the long HS chains that are attached to the core protein. HPSE-1 also requires long incubation periods, even at its pH optimum, to achieve substantial degradation of HS (24, 35, 37, 46). HPSE-1 is therefore a highly regulated enzyme that makes selective cuts at specific sequences. At least some HS fragments released by HPSE-1 cleavage of proteoglycans are able to bind ligands, although HPSE-1 degradation may alter activation and binding of growth factors to either the free HS chains or the HS fragments remaining on the core protein. For example, bFGF requires only a 6–8-mer of HS, whereas FGF-8b needs longer chains (14-mer) to bind and maintain its biological function (56). Our results show that HPSE-1 is capable of removing HS chains from both matrix and cell-surface proteoglycans. Our data also suggest that the longer HS fragments produced by HPSE-1 maintain biological activity, unlike the short fragments produced by heparitinase. This confirms the notion that HPSE-1, which degrades HS into significant size fragments, may aid modulation of growth factor activities in metastasis.

Syndecan-1 is a cell-surface HSPG present at the cell-cell contact sites, which functions in cellular adhesive, invasive, and metastatic processes (1, 5). It is a significant component of HSPG on brain metastatic melanoma cells and may act in multiple ways to promote tumor progression in these cells. Cell-surface syndecan-1 binds ECM components (including collagen and fibronectin) and various growth factors (such as bFGF, VEGF (57), keratinocyte growth factor, and others) via its HS chains. The HS chains of syndecan also function as a storage site for cytokines (1, 5) and act to promote binding of growth factors to receptors (58).

HPSE-1 degradation of syndecan-1 HS modifies cellular responses by releasing associated growth factors (59) and disrupting interactions with ECM and other cells (15). Additionally, HPSE-1 degradation of syndecan-1 HS can interfere in co-receptor function either by blocking formation of the tertiary complexes (HSPG-kinase receptor-ligand) or by releasing free HS chains that can then promote ligand binding and signaling. In our system, syndecan-1 inhibits HPSE-1-induced invasion, suggesting one mechanism by which syndecan-1 down-regulation induces tumor progression. Furthermore, our experiments suggest that HS chains are crucial for this biological activity because either free HS chains obtained by β-elimination of syndecan-1 or HS chains resulting from HPSE-1 degradation of syndecan-1 inhibited HPSE-1-induced invasion. According to our activity assays, syndecan-1 is not acting to inhibit HPSE-1 enzymatic degradation either at the optimal pH of the enzymes

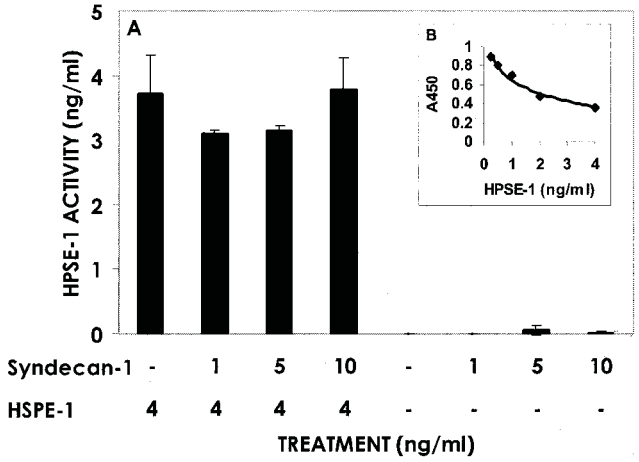

FIG. 7. **Syndecan-1 does not inhibit HPSE-1 activity using biotinylated HS as a substrate.** *A*, HPSE-1 and syndecan-1 were incubated with biotinylated HS (*b-HS*) at indicated concentrations, and then undigested b-HS was bound to bFGF ELISA plates (Takara.Mirus.Bio, Madison, WI). Biotinylated HS, digested by HPSE-1, remained unbound and discarded during PBS washes. Bound b-HS was detected with HRP-avidin followed by a colorimetric assay as per the manufacturer's instructions (see "Experimental Procedures"). HPSE-1 activity was determined by comparison with known HPSE-1 concentrations. *B*, HPSE-1 concentration curve (0.125–4 ng/ml) used to calculate HPSE-1 activity. Absorbance at 450 nm was plotted against HPSE-1 concentration and fit (4 parameter equation) with Kaleidagraph (Synergy Software, Reading, PA) according to manufacturer's instructions.

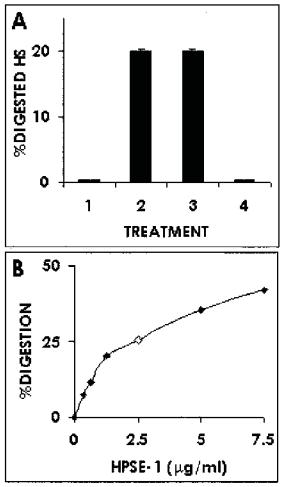

FIG. 8. **Syndecan-1 does not inhibit HPSE-1 enzymatic degradation of FITC-HS at pH 6.8.** *A*, FITC-HS was incubated alone (*column 1*), or in presence of HPSE-1 (*column 2*), HPSE-1 and syndecan-1 (*column 3*), or syndecan-1 (*column 4*), in reaction buffer (pH 6.8, Takara.Mirus.Bio) for 18 h at 37 °C. Reaction products were then separated by HPLC (see "Experimental Procedures"). Results are reported as percentage of digested FITC-HS. *B*, HPSE-1 was incubated at indicated concentrations with FITC-HS. Percentage of degraded FITC-HS was calculated as area of the curve that shifted to the right compared with non-HPSE-1-treated samples (control curve). The *open symbol* represents HPSE-1 concentration used in *A*. Standard deviations (±2–5% of values) were calculated but not shown for clarity purposes.

nor at pH 6.8 which is seen in cultured 70W melanoma cells and in cancer. This suggests that syndecan-1 is acting through other mechanisms that could include the following: 1) the production of HS fragments that activate bFGF mitogenicity thus bypassing the need for cell-surface syndecan-1 (60); 2) diminishing tight adhesion to the underlying ECM; and/or 3) increasing melanoma cell motility.

There is mounting evidence for the involvement of perlecan in tumor progression and vascularization (2, 3). The emerging view from several investigations is that an aggressive behavior in tumor cells coincides with an augmented synthesis and secretion of this potent and ubiquitous molecule (3). Moreover, the source of perlecan is dual because both cancer and stromal cells synthesize it. Thus, growth of neoplastic cells may be promoted by a relatively high and constant supply of this large macromolecule that consists of HS and a core protein having cell-adhesive motifs. In addition, perlecan HS binds growth factors, *i.e.* FGF-7 for epithelial cells and bFGF for mesenchymal cells (2, 3, 32). As with syndecan-1, digestion of perlecan HS by HPSE-1 can release active growth factors to bind to cells, stimulating angiogenesis, growth, migration, and invasion. In our hands, perlecan HS fragments did not inhibit invasion in the same manner as syndecan-1 HS fragments when tested both directly and indirectly. Purified perlecan HS fragments from WiDr cells did not inhibit melanoma cell invasion. Perlecan is the primary HSPG in Matrigel™, thus present and degraded when HPSE-1 was added in the invasion assays. Accordingly, we were able to detect enhanced invasive effects with HPSE-1 and the subsequent decrease in invasion due to syndecan-1 in the presence of HPSE-1-generated HS degradative products from Matrigel™. During tumor cell invasion, perlecan may bind a wider range of growth factors than the limited set in Matrigel™ and thus may be able to promote invasion *in vivo*. This indicates that distinct substrates are able to differentially modify the invasive behavior of tumor cells.

Although the full extent of HPSE-1 action in regulating brain metastatic melanoma (or tumor metastasis in general) is not completely understood, this enzyme likely participates in several key events. First, prior to gaining entrance into the vasculature, brain metastatic melanoma cells must degrade interstitial stroma and ECM that is rich in perlecan. Second, HPSE-1 produced by neoplastic and surrounding inflammatory cells is thought to be an important participant in angiogenesis because it can release heparin-binding growth factors (such as FGFs and/or VEGF). Third, HPSE-1 produced by endothelial cells can also aid in remodeling the ECM surrounding the developing vessel (61). Considering our results, it is also plausible to propose a pH-dependent action by HPSE-1 in invasive mechanisms. At neutral pH, HPSE-1 may be primarily inactive. However, under conditions where pH becomes acidic (as in invasion and inflammatory processes), HPSE-1 can become activated and degrade HS of HSPG, *i.e.* syndecan-1 and perlecan.

Besides perlecan and syndecan-1, other proteoglycans should be considered as potential substrates of HPSE-1 degradation because different HSPG may contain the same HS sequences (62). HPSE-1 can act on multiple HSPG both at the cell surface and in the ECM in a cell type-specific fashion, which may then influence tumor progression. For example, syndecan-4 is essential for cell adhesion and signaling through interaction with integrins (63). Therefore, degradation of syndecan-4 HS can affect cell behavior important for tumor progression. Furthermore, once released by HPSE-1, HS can have similar biological activities despite their origin from different HSPG (64).

In conclusion, perlecan and syndecan-1 HS degradation not only confers an invasive advantage, but it may be an essential component of the multifactorial and highly complex processes that lead to tumor growth and metastasis. The metastatic phenotype is considered decisive for tumor progression, and several key molecules involved in these complex biological events are potential candidates for therapeutic intervention. HPSE-1 should be considered one of these. Therapies focusing

on abrogating HPSE-1 expression and action through the use of specific inhibitors may prove to be beneficial and potentially improve cancer treatment.

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