Heparanase Enhances Syndecan-1 Shedding

A NOVEL MECHANISM FOR STIMULATION OF TUMOR GROWTH AND METASTASIS

Received for publication, December 7, 2006, and in revised form, March 7, 2007 Published, JBC Papers in Press, March 8, 2007, DOI 10.1074/jbc.M611259200

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When shed from the cell surface, the heparan sulfate proteoglycan syndecan-1 can facilitate the growth, angiogenesis, and metastasis of tumors. Here we report that tumor cell expression of heparanase, an enzyme known to be a potent promoter of tumor progression and metastasis, regulates both the level and location of syndecan-1 within the tumor microenvironment by enhancing its synthesis and subsequent shedding from the tumor cell surface. Heparanase regulation of syndecan-1 is detected in both human myeloma and breast cancer cell lines. This regulation requires the presence of active enzyme, because mutated forms of heparanase lacking heparan sulfate-degrading activity failed to influence syndecan-1 expression or shedding. Removal of heparan sulfate from the cell surface using bacterial heparitinase dramatically accelerated syndecan-1 shedding, suggesting that the effects of heparanase on syndecan-1 expression by tumor cells may be due, at least in part, to enzymatic removal or reduction in the size of heparan sulfate chains. Animals bearing tumors formed from cells expressing high levels of heparanase or animals transgenic for heparanase expression exhibited elevated levels of serum syndecan-1 as compared with controls, indicating that heparanase regulation of syndecan-1 expression and shedding can occur in vivo and impact cancer progression and perhaps other pathological states. These results reveal a new mechanism by which heparanase promotes an aggressive tumor phenotype and suggests that heparanase and syndecan-1 act synergistically to fine tune the tumor microenvironment and ensure robust tumor growth.

Heparanase is an endo-β-D-glucuronidase that releases 5–7 kDa fragments of heparan sulfate from intact heparan sulfate chains of proteoglycans and is known to have multiple important roles in promoting tumor growth, angiogenesis, and metastasis (1). In general, it is thought that the enzymatic activity of heparanase is necessary for remodeling of the extracellular matrix and particularly the subendothelial basement membrane of endothelial cells prior to their migration during angiogenesis (1). Heparanase can also liberate a number of heparan sulfate-bound pro-angiogenic growth factors (e.g. fibroblast growth factor-2, vascular endothelial growth factor (VEGF)) from the extracellular matrix to indirectly enhance endothelial cell migration and proliferation (2, 3). Fragments of heparan sulfate generated by heparanase retain biological activity and can act as potent promoters of growth factor activity (2, 4). In addition, heparanase has functions not related to its enzymatic activity. For example, VEGF expression is up-regulated 3–6-fold in several heparanase-transfected tumor cell lines. This effect does not require the active form of the enzyme and occurs via the Src pathway (5). The non-enzymatic form of heparanase can also promote cell adhesion (6) and Akt signaling as well as phosphoinositol 3-kinase- and p38-dependent endothelial cell migration and invasion (7). Thus, heparanase clearly can impact tumor progression via both enzymatic and non-enzymatic mechanisms.

Heparan sulfate binds to and regulates the activity of many effector molecules capable of initiating signaling pathways critical to cancer inception, growth, and progression (e.g. growth factors, chemokines, angiogenic factors) (8). Heparan sulfate is prevalent in many cancers and particularly abundant in multiple myeloma, where most of the tumor cells express the proteoglycan on the cell surface (9). In addition to expression on the cell surface, syndecan-1 is shed from the surface of the tumor cells as an intact ectodomain bearing the extracellular portion of the core protein with attached heparan sulfate chains. Shed syndecan-1 can become lodged and incorporated into the bone marrow extracellular matrix that supports the tumor or remain as a soluble component within the bone marrow plasma. In addition, shed syndecan-1 accumulates in the sera of myeloma patients, where high serum levels of syndecan-1 reflect a high tumor burden and predict poor prognosis (10, 11).

Recently, we demonstrated that both soluble syndecan-1 and heparanase can promote myeloma tumor growth and metastasis-1. This work was supported by National Institutes of Health Grants CA 103054 and CA 055819 (to R. D. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The on-line version of this article (available at http://www.jbc.org) contains supplemental data.

2 The abbreviations used are: VEGF, vascular endothelial growth factor; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline.
sis in vivo (12, 13). These results suggest that syndecan-1 and heparanase acting together serve as master regulators of the myeloma tumor microenvironment within the bone marrow niche by binding to and regulating the activity of multiple growth-promoting factors whose actions are key to the progression of this cancer (14, 15). We now report the unexpected finding that heparanase acts on tumor cells to regulate both the amount and location of syndecan-1 within tumors by enhancing synthesis and shedding of the proteoglycan. Thus, in addition to its role in remodeling tumor extracellular matrix and releasing fragments of heparan sulfate, heparanase also enhances levels of the soluble, tumor-promoting form of syndecan-1. This novel function of heparanase further underscores the importance of the interplay between the syndecan-1 heparan sulfate proteoglycan and heparanase in driving aggressive tumor growth and metastasis.

**EXPERIMENTAL PROCEDURES**

*Cells and Transfections*—CAG cells were established from a bone marrow aspirate of a myeloma patient at Arkansas Cancer Research Center as previously described (16). ARH-77 cells were obtained from the American Type Culture Collection (Manassas, VA). MDA-MET cells were derived from MDA-MB-231 human breast adenocarcinoma cells based on their ability to home to and grow in bone (17). CAG and ARH-77 cells were grown in RPMI medium supplemented with 10% fetal bovine serum. MDA-MET cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. For karyotyping of ARH-77 cells (wild-type, control-transfected, or heparanase-transfected), the cells growing in culture were exposed to colcemid for 1 h, washed with alkaline Pucks saline solution, and treated with trypsin to remove the cells from the flasks. The cells were treated in hypotonic solution (0.05 M KCl) and fixed in Carnoy’s fixative (3:1 methanol:acetic acid). Cell suspensions were dropped onto slides and trypsin G-banded. Ten G-banded metaphase cells were counted and three cells karyotyped for each cell line.

For transfections, human heparanase cDNA was subcloned in the sense direction into pcDNA3 or pIRE2-enhanced green fluorescent protein vectors (Clontech, Palo Alto, CA) and stably transfected into the above-described cell lines using Lipofectamine (Invitrogen) and Opti-MEM I (Invitrogen) according to the manufacturer’s instructions. Following transfection, cells were selected by growth in G418 followed by sorting for green fluorescence (Amersham Biosciences). For flow cytometry, cell suspensions were washed in PBS and stained with antibodies to human syndecan-1 (CD138-fluorescein isothiocyanate or CD138 phycoerythrin; Serotec). Samples were analyzed using a FACSscan flow cytometer (BD Biosciences).

For phenotypic analysis of ARH-77 cells, the levels of a series of markers (CD19, CD20, CD38, CD45, and CD52) were examined using marker-specific antibodies directly conjugated to fluorescein isothiocyanate or phycoerythrin. Cells were harvested from culture, washed in PBS, and stained at 4 °C with antibodies or isotype-matched control antibodies. After staining, cells were fixed in 1% paraformaldehyde and analyzed by flow cytometry.

*Quantification of Syndecan-1*—Equal numbers of cells were plated in flasks, and after 2 days in culture, the levels of shed syndecan-1 accumulated in the conditioned medium were assessed by enzyme-linked immunosorbent assay (ELISA) using an Eli-pair kit from Diaclone (Cell Sciences, Inc., Norwood, MA). The standard curve was linear between 8 and 256 ng/ml, and all samples were diluted to concentrations within that range. For some experiments, cells (106 cells/ml) were plated on a 12-well plate in complete RPMI medium with the subsequent addition of PBS (control) or 10 μg/ml recombinant human heparanase. The cells were incubated for 2 h at 37 °C in 5% CO2, with another addition of recombinant heparanase or PBS administered after 24 h of cell culture. At time points 4, 24, 48, and 72 h after the addition of heparanase, 200 μl of cell suspensions were collected from each well, centrifuged to remove cells, and the supernatant stored at 4 °C prior to analysis of syndecan-1 levels by ELISA. At termination of the experiment, the remaining cells were fixed with 3.7% formaldehyde, and the level of cell-surface syndecan-1 was determined by flow cytometry using antibody B-B4 against human syndecan-1 (CD138, Serotec). To determine the effects of removal of heparan sulfate chains on syndecan-1 shedding, control or heparanase-transfected cells (106/ml) in serum-free RPMI medium were plated on a 12-well plate, and 5 milliunits/ml of bacterial heparitinase (Seikagaku Corporation, Tokyo, Japan) or PBS was added and cells incubated at 37 °C, 5% CO2. Additional heparitinase (5 milliunits/ml) or PBS was added each hour during

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buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 0.2 mM phenylmethylsulfonic acid, 10 μg/ml leupeptin), and incubated on ice for 30 min. Lysates were centrifuged at 13,000 × g at 4 °C for 20 min and supernatants frozen. Proteins isolated from cell lines were quantified by a BCA protein assay reagent kit (Pierce). For heparanase detection, equal amounts of protein (50 μg/lane) were loaded onto 4–12% gradient SDS-polyacrylamide gels, transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH), probed with affinity-purified rabbit antibody against heparanase (19), and followed by a horseradish peroxidase-conjugated donkey anti-rabbit IgG. For syndecan-1 detection, samples were run on 4–15% SDS-PAGE, transferred to a positively charged nylon membrane (NitranSPC; Schleicher & Schuell) and probed with mouse anti-human syndecan-1 (CD138 antibody clone B-B4; Serotec, Raleigh, NC) and a horseradish peroxidase-conjugated secondary anti-mouse antibody (GE Healthcare). Immunoreactive bands were detected using enhanced chemiluminescence (Amersham Biosciences). For flow cytometry, cell suspensions were washed in PBS and stained with antibodies to human syndecan-1 (CD138-fluorescein isothiocyanate or CD138 phycoerythrin; Serotec). Samples were analyzed using a FACSscan flow cytometer (BD Biosciences).

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the first 6 h of incubation and 200 μl of conditioned medium collected at appropriate intervals. After 24 h, the remaining conditioned medium was collected, the cells fixed in 3.7% formaldehyde, and the level of cell-surface syndecan-1 determined by flow cytometry. Levels of syndecan-1 in conditioned medium were determined by ELISA as described above. Experiments were also performed on ARH-77 cells using exogenous bovine heparan sulfate (5 μg/ml) with or without pretreatment with recombinant heparanase or heparitinase. Cells were analyzed as described above for levels of syndecan-1 at the cell surface or in the medium 24, 48, and 72 h after addition of the heparan sulfate. Syndecan-1 levels were also measured with or without the addition for 24 h of the Src inhibitor PP2 (4 μg/ml).

Syndecan-1 levels in sera from severe combined immunodeficient mice bearing tumors formed by either CAG control or CAG cells transfected with heparanase (12) were also determined by ELISA and resulting values normalized by the level of serum κ light chain, a marker of whole animal tumor burden. ELISA was performed on duplicate samples following the manufacturer’s instructions, and the absorbance at 450 nm was determined with an Auto-Reader II ELISA (Ortho Diagnostic Systems, Raritan, NJ). To determine levels of murine syndecan-1 present in heparanase transgenic and control mice, sera from these animals were diluted to a final concentration of 2 m urea, 100 mM sodium-acetate, pH 4.5, and dot-blotted onto a Nytran SPC membrane (Schleicher & Schuell). Blots were probed with rat anti-mouse antibody (281.2) against murine syndecan-1 biotinylated anti-rat secondary antibody (Vector Laboratories, Burlingame, CA) and visualized using a biotin-avidin ABC kit (Vector Laboratories).

For gene array analysis, total RNA was isolated from neo- or heparanase-transfected CAG or ARH-77 cells using the RNeasy mini kits (Qiagen, Valencia, CA). Gene expression profiling was performed with the Affymetrix U133 Plus, version 2.0 microarray platform (Santa Clara, CA) using methods previously described (20). Determination of heparan sulfate proteoglycan mRNA levels was performed with Affymetrix microarray suite GCOS1.1 software.

RESULTS

Heparanase Enhances the Synthesis and Shedding of Syndecan-1—In previous studies, we demonstrated that transfection of the gene for human heparanase into CAG myeloma cells enhances tumor growth and metastasis in vivo compared with controls (12). Gene array analysis of the heparanase-transfected and control vector-only-transfected CAG cells revealed that, when heparanase was expressed, levels of syndecan-1 transcription increased 38% over that of controls (data not shown). To determine whether this altered transcription led to higher levels of cell surface syndecan-1, cells were immunostained and examined by flow cytometry. Surprisingly, despite the increase in transcription in cells expressing heparanase, cell surface levels of syndecan-1 were essentially identical to that of controls (Fig. 1A). However, analysis of conditioned medium 48 h after plating cells reveals that the level of shed syndecan-1 is 48% higher in heparanase-transfected versus control-transfected cells (Fig. 1B). Analysis of heparanase-transfected MDA-MET breast cancer cells demonstrates a similar (33%) increase in syndecan-1 shedding as compared with controls (Fig. 1B), suggesting that the effect seen in myeloma cells is not cell type-specific. To determine whether the enhanced syndecan-1 shedding requires the activity of the heparanase enzyme, CAG cells expressing heparanase mutated at the active site of the enzyme were generated (mutated at amino acid 225 (Met-225) or 343 (Met-343)). Western blotting reveals that the mutated forms of heparanase are expressed, but as expected, activity assays indicate that they lack enhanced heparan sulfate-degrading activity as compared with controls (Fig. 1C). When cells expressing mutated heparanase were analyzed, both the cell surface syndecan-1 (not shown) and shed syndecan-1 levels were similar to those of control cells (Fig. 1B), indicating that heparanase activity is required to promote enhanced synthesis and shedding of syndecan-1 in these cells.

As an independent confirmation of heparanase effects on syndecan-1 shedding, we introduced recombinant heparanase to wild-type CAG cells growing in culture. Over a 48-h time period, accelerated shedding was noted, and by 72 h, the amount of shed syndecan-1 that accumulated in the medium was twice that of cells not exposed to exogenous heparanase (Fig. 2). Flow cytometry analysis at the 72 h time point revealed nearly equivalent levels of cell surface syndecan-1 on PBS-treated versus recombinant heparanase-treated cells (mean fluorescence intensity of 224.1 versus 200.4, respectively). This parallels what is seen when heparanase levels are increased by cell transfection (Fig. 1) and confirms a role for heparanase in promoting syndecan-1 shedding.

Enzymatic Degradation of Heparan Sulfate Chains Enhances Shedding of Syndecan-1 from the Cell Surface—Western blots of heparanase-transfected CAG cells growing in vitro (Fig. 1A, inset) or growing as tumors in vivo (see Ref. 12) demonstrate that the molecular size of syndecan-1 is substantially reduced as compared with controls. Presumably, this is due to the effect of heparanase enzymatic activity that shortens heparan sulfate chains. Thus, the mechanism responsible for enhanced syndecan-1 shedding on the heparanase transfectants could be related to a reduction in the amount of heparan sulfate present on the proteoglycan. To assess this possibility, the control CAG cells were exposed to bacterial heparitinase to strip heparan sulfate chains from the cell surface. This caused a rapid up-regulation of syndecan-1 shedding that was evident as early as 2 h after the addition of the enzyme (Fig. 3A). After 24 h, shedding in control-transfected cells was elevated >4-fold as compared with the same cells not exposed to heparitinase. An even more dramatic effect was seen on heparanase-transfected cells. Following the addition of heparitinase, these cells exhibited a 6-fold increase in syndecan-1 shedding over untreated cells (note the difference in the scale of the y-axes between panels A and B of Fig. 3). This enhanced shedding occurred in the absence of any change in the level of cell surface syndecan-1 (not shown).

Heparanase Initiates Expression of Syndecan-1 in a Syndecan-1-negative Cell Line—To further explore the relationship between heparanase and syndecan-1 expression, ARH-77 cells (an Epstein-Barr virus-transformed human B cell line lacking
expression) were transfected with the cDNA for human heparanase. This resulted in a dramatic induction of syndecan-1 cell surface expression, thereby transforming the cell from syndecan-1-negative to syndecan-1-positive (Fig. 4). This shift from syndecan-1-negative to syndecan-1-positive was also seen in a repeat transfection experiment of wild-type ARH-77 cells using a different vector carrying the heparanase cDNA (not shown). Because of this unexpected initiation of expression of syndecan-1 by a previously syndecan-1-negative cell line, we examined the cell karyotype to exclude the possibility of cell line cross-contamination. Results revealed that ARH-77 control-transfected and heparanase-transfected cells showed the presence of a similar pattern of numerical and structural chromosome aberrations similar to the ARH-77 wild-type cell line (not shown). Phenotypic analysis by flow cytometry with cell surface markers CD19, CD20, CD38, CD45, and CD52 indicated that both control and heparanase-transfected cells expressed the same levels of these markers as are detected on the ARH-77 wild-type cells (not shown). Thus, the induction of syndecan-1 expression that occurs when ARH-77 cells express heparanase is not accompanied by a global change in cell phenotype.

The data presented above on CAG cells suggests that the mechanism for up-regulation of syndecan-1 expression may be related to the degradation of heparan sulfate chains by heparanase. However, because the control ARH-77 cells do not...
express syndecan-1 prior to their expression of heparanase, it raised the possibility that other heparan sulfate proteoglycans might be present on these cells that, when degraded by enzyme, would induce syndecan-1 expression. Microarray analysis of the ARH-77 control cells reveals them to be absent of expression of the extracellular proteoglycan perlecan as well as all four members of the syndecan family (syndecan-1, -2, -3, and -4) (see supplemental Table 1). For the other major family of cell surface heparan sulfate proteoglycans, the glypicans (glypican-1, -2, -3, -4, -5, and -6), there was only a very weak detection signal on the gene chip for glypican-2 and glypican-4 (see supplemental Table 1). To determine whether even this very low level of proteoglycan when degraded by enzyme could promote syndecan-1 expression, the ARH-77 control cells were treated separately with either recombinant heparanase or heparitinase. When assessed for syndecan-1 expression, it was found that the cells remained negative even after 72 h of exposure to enzyme (data not shown). As a further test, bovine heparan sulfate was separately with either recombinant heparanase or heparitinase. When assessed for syndecan-1 expression, it was found that the ARH-77 control cells were treated with heparanase or heparitinase and added to control ARH-77 cells. When assessed at 24, 48, and 72 h after the addition of the heparan sulfate fragments, all of the cells remained negative for syndecan-1 expression (data not shown). Together, these data indicate that the induction of syndecan-1 expression in the ARH-77 cells is not due to the heparan sulfate-degrading activity of heparanase.

Expression of Heparanase in Vivo Enhances Levels of Shed Syndecan-1—In a previous study, we demonstrated that tumors formed from CAG cells expressing high levels of heparanase grow and metastasize to bone much more readily than CAG tumors expressing low levels of heparanase (12). To examine the levels of syndecan-1 shed by these tumors, we analyzed serum samples from mice that bore tumors formed from heparanase-transfected or control-transfected cells. Soluble syndecan-1 levels were much higher in animals with tumors formed from heparanase-transfected cells, a finding consistent with the fact that these animals had a significantly higher tumor burden than animals bearing tumors from control-transfected cells. To adjust for this difference in tumor burden, levels of serum syndecan-1 were normalized to the tumor burden by dividing the syndecan-1 concentration by the level of serum k light chain, a well-established marker of tumor burden in this model (13). Results show that, even after normalizing for tumor burden, there remains a significant enhancement of syndecan-1 levels in the sera of animals bearing tumors expressing heparanase as compared with animals bearing control tumors (Fig. 5).

To determine whether the elevated shedding of syndecan-1 in response to heparanase expression was limited to tumor cells or might be more widespread, we analyzed sera from mice carrying the transgene for heparanase. As compared with sera from control animals, heparanase transgenic mice have much higher levels of detectable syndecan-1 (Fig. 6).

**DISCUSSION**

The present study reveals that human heparanase, an important regulator of tumor behavior, elevates the expression and shedding of syndecan-1, a proteoglycan known to promote the growth and metastasis of tumors. This is a novel and important discovery, because it reveals a new mechanism by which heparanase accomplishes its well documented tumor-enhancing properties. Our results demonstrate that (i) expression of heparanase enhances the synthesis and shedding of syndecan-1, (ii) expression of heparanase can initiate syndecan-1 expression even in a cell line normally negative for syndecan-1, (iii) the effect of heparanase on syndecan-1 shedding requires the active form of the enzyme, and (iv) the regulation of syndecan-1 by heparanase extends to tumors growing in vivo and to heparanase-transgenic animals. Because our previous studies demonstrate that soluble (shed) syndecan-1 can actively promote myeloma tumor growth, angiogenesis, and metastasis, the finding that heparanase promotes syndecan-1 synthesis and shedding suggests that these two molecules act synergistically to fuel and accelerate tumor progression.

Although the mechanism of heparanase-mediated regulation of syndecan-1 is not yet clear, data from the syndecan-1-negative ARH-77 cells is intriguing, because it indicates heparanase has a direct effect on initiating syndecan-1 expression. Heparanase can directly enhance VEGF expression via activation of Src family members (5). However, in both heparanase-transfected ARH-77 and CAG cells, the Src inhibitor PP2 failed.
to block up-regulation of syndecan-1 expression over a 72-h period (data not shown). This suggests that the mechanism leading to the initiation of syndecan-1 expression is not the same as the mechanism that enhances VEGF expression. A direct effect of heparanase on syndecan-1 expression is also supported by the finding that ARH-77 cells lack appreciable heparan sulfate proteoglycan expression and do not up-regulate syndecan expression in response to exogenous heparanase or heparitinase. Even the addition to cells of heparan sulfate that had been degraded by heparanase or heparitinase did not stimulate syndecan-1 expression. Thus, the target of heparanase in up-regulating syndecan-1 expression in ARH-77 cells is not heparan sulfate. This may also be the case with the CAG cell line, which in contrast to the ARH-77 cells, expresses syndecan-1 prior to transfection with the gene for heparanase. However, because exogenous heparanase or heparitinase increases the shedding of syndecan-1 from CAG cells (Figs. 2 and 3), we cannot rule out the possibility that, in cells expressing syndecan-1, there is a feedback loop that stimulates syndecan-1 expression once shedding is elevated. This might occur in cells programmed to maintain a certain level of the proteoglycan on the cell surface. It is possible that both mechanisms, a direct up-regulation of syndecan-1 expression and an indirect up-regulation in response to enhanced shedding, contribute to the effects seen in CAG cells.

Regardless of the mechanism that controls syndecan-1 expression, the critical discovery of the present work is that, as heparanase levels rise (as they often do in tumors), syndecan-1 shedding increases. This unveils an important new mechanism by which heparanase promotes tumor aggressiveness. Studies on samples from myeloma patients reveals that a high level of shed syndecan-1 in the serum is an indicator of high tumor burden and poor prognosis (10, 11) and that high heparanase enzyme activity in the bone marrow of myeloma patients correlates with high tumor microvessel density, which is also an indicator of poor prognosis (21). Using in vivo models of myeloma, we have demonstrated that elevation of expression of either soluble syndecan-1 or heparanase promotes tumor growth, angiogenesis, and metastasis (12, 13). Our finding that heparanase is responsible, at least in part, for the up-regulation of syndecan-1 shedding in tumors indicates that this may be an important mechanism by which heparanase promotes tumor growth. This notion is further supported by the demonstration

**FIGURE 4.** Heparanase induces expression of syndecan-1 on ARH-77 cells. A, ARH-77 cells that lack syndecan-1 expression were transfected with vector only (shaded) or vector containing the cDNA for human heparanase. Cells were stained with B-B4 and analyzed by flow cytometry. B, immunofluorescence staining of unfixed heparanase-transfected ARH-77 cells with antibody B-B4 reveals syndecan-1 localization on the cell surface. FITC, fluorescein isothiocyanate.

**FIGURE 5.** Tumors formed by heparanase-transfected cells shed higher levels of syndecan-1 than tumors formed by control cells. A, sera from animals bearing tumors of heparanase-transfected or control-transfected CAG myeloma cells were analyzed for levels of syndecan-1 (n = 8 in each group, p = 0.001). B, because animals with tumors formed from heparanase-transfected cells have a greater tumor burden than that of animals bearing tumors formed from control cells, the levels of serum syndecan-1 were normalized using the levels of serum κ light chain. This is shown as the ratio of serum syndecan-1 to κ light chain (p < 0.02).
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Although heparanase and heparan sulfate proteoglycans have multiple effects within the tumor microenvironment, the most substantial impact of the heparanase/heparan sulfate axis in cancer and other diseases may be related to its role in angiogenesis. Heparan sulfate acts as a positive modulator of VEGF/VEGF receptor signaling by potentiating VEGF binding to its receptors and possibly by directly binding to VEGF receptors as well (28–31). Additional important functions of heparan sulfate include its role in binding to and establishing gradients of VEGF required to promote proper endothelial branching (32) and the ability of heparanase to salvage VEGF by reactivating it following oxidative damage (33). Because VEGF and heparan sulfate are important regulators of tumor angiogenesis, our present finding that heparanase increases the levels of extracellular syndecan-1 heparan sulfate coupled with the finding of Vlodavsky’s group that heparanase increases the levels of VEGF points to these events as major components that condition the tumor microenvironment to drive angiogenesis. The resulting synergism between heparanase, heparan sulfate, and VEGF likely occurs on several levels including (i) the heparanase-enhanced shedding of syndecan-1 results in abnormally high levels of the proteoglycan being trapped within the tumor microenvironment where it binds VEGF and other growth factors (e.g. fibroblast growth factor-2) thereby creating a pro-angiogenic reservoir, (ii) this concentration of growth factors leads to the establishment of gradients that promote angiogenesis and/or vasculogenesis and, (iii) subsequent cleavage of heparan sulfate chains by extracellular heparanase releases fragments of heparan sulfate with attached growth factors that stimulate vascular growth thereby promoting tumor growth and survival. An additional possibility is that, once modified by heparanase, the heparan sulfate chains that remain on syndecan-1 have an enhanced ability to bind to and activate growth factors such as fibroblast growth factor-2 (4).

The synergistic role between heparanase and heparan sulfate in driving angiogenesis and other microenvironmental interactions indicates their key role in regulating events that accompany cancer, inflammation, and other diseases. The current finding that heparanase promotes syndecan-1 expression and shedding gives new insight into how heparanase promotes an aggressive tumor phenotype and provides additional rationale for targeting heparanase as a cancer therapy.

Acknowledgment—We thank Larry Suva (University of Arkansas for Medical Sciences) for use of MDA-MET cells.

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FIGURE 6. Syndecan-1 is elevated in the serum of heparanase-transgenic mice. Serum was collected and pooled from four heparanase transgenic mice and four control mice. The designated amount of serum was dot-blotted and probed with antibody 281–2 against mouse syndecan-1. The blot reflects an ~4 times higher level of serum syndecan-1 in heparanase transgenic mice as compared with controls.

that shed syndecan-1 in the circulation is elevated in animals bearing tumors formed from cells expressing high levels of heparanase (Fig. 5).

Additionally, the observation that heparanase-transgenic mice have elevated syndecan-1 in their serum (Fig. 6) implies that heparanase may also regulate syndecan-1 in pathological events that are non-cancer in nature. Heparanase transgenic mice display a number of irregular growth features, including excess branching and widening of mammary gland ducts, accelerated rate of hair growth, enhanced trabecular bone formation and cortical bone thickening, and resistance to induction of amyloidosis (22–25). Interestingly, heparanase has been implicated in the pathogenesis of inflammatory disease, as has shed syndecan-1, which can promote inflammation as well as bacterial pathogenesis (1, 26, 27). It will be important to determine whether heparanase-enhanced shedding of syndecan-1 plays a role in these conditions.

Because syndecan-1 is the predominant and, in many cases, the sole heparan sulfate proteoglycan expressed on myeloma cells, this study does not address in detail whether the effect of heparanase expression seen here is specific for syndecan-1 or whether the enzyme up-regulates expression and shedding of other proteoglycans. Following transfection of the ARH-77 cells with the gene for heparanase, through gene array analysis, we detected high levels of mRNA for syndecan-1 along with very low levels of mRNA for both syndecan-2 (called present in only one of three probes) and syndecan-4 (see supplemental Table 1). Although 10-fold lower than syndecan-1 levels, this does suggest that heparanase expression may have a global effect on heparan sulfate proteoglycan expression that will be more evident in other cell types where syndecan-1 is not the predominant proteoglycan.
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