Tumor-Derived Syndecan-1 Mediates Distal Cross-Talk with Bone that Enhances Osteoclastogenesis

Thomas Kelly,1 Larry J Suva,2 Kristy M Nicks,2 Veronica MacLeod,1 and Ralph D Sanderson3

1Department of Pathology, 2Center for Orthopedic Research, Departments of Orthopedic Surgery and Physiology and Biophysics, Winthrop P Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA
3Department of Pathology, Center for Metabolic Bone Disease, UAB Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL, USA

ABSTRACT

Tumor-stimulated bone resorption fuels tumor growth and marks a dramatic decline in the health and prognosis of breast cancer patients. Identifying mechanisms that mediate cross-talk between tumor and bone remains a key challenge. We previously demonstrated that breast cancer cells expressing high levels of heparanase exhibit enhanced shedding of the syndecan-1 proteoglycan. Moreover, when these heparanase-high cells are implanted in the mammary fat pad, they elevate bone resorption. In this study, conditioned medium from breast cancer cells expressing high levels of heparanase was shown to significantly stimulate human osteoclastogenesis in vitro ($p < .05$). The osteoclastogenic activity in the medium of heparanase-high cells was traced to the presence of syndecan-1, intact heparan sulfate chains, and heat-labile factor(s), including the chemokine interleukin 8 (IL-8). The enhanced osteoclastogenesis promoted by the heparanase-high cells results in a dramatic increase in bone resorption in vitro. In addition, the long bones of animals bearing heparanase-high tumors in the mammary fat pad had significantly higher numbers of osteoclasts compared with animals bearing tumors expressing low levels of heparanase ($p < .05$). Together these data suggest that syndecan-1 shed by tumor cells exerts biologic effects distal to the primary tumor and that it participates in driving osteoclastogenesis and the resulting bone destruction.

KEY WORDS: CD138; CYTOKINES; HEPARAN SULFATE PROTEOGLYCANs; BONE METASTASIS; TUMOR MICROENVIRONMENT; GROWTH FACTORS

Introduction

Bone metastasis is a frequent, often debilitating, and painful complication of breast cancer, yet the mechanisms by which breast cancer cells colonize bone to form metastases remain poorly understood. A number of molecules elaborated by breast cancer cells, including parathyroid hormone–related peptide (PTHrP), interleukin 8 (IL-8), IL-11, and many others, have been shown to stimulate osteoclastogenesis and the resulting bone destruction.

Once osteoclasts are activated to resorb bone, growth factors that are bound to the bone matrix and that can stimulate metastatic tumor cell growth are released. This positive-feedback loop produces a microenvironment within the bone marrow that has the potential to sustain tumor growth. The cross-talk between the breast cancer cells colonizing bone and the resident osteoclasts (and their precursors) is believed to be particularly important for the initial establishment of bone metastases, at least in animal models. For example, in various mouse models, treatment with bone-resorption-inhibiting bisphosphonates reduces the number of bone metastatic foci if administered prior to the arrival of the tumor cells in bone. However, bisphosphonates do not inhibit the growth of metastatic foci once they are established in bone. Thus, although bisphosphonates maintain bone density in breast cancer patients with bone metastases and have been shown to increase disease-free survival in premenopausal patients with estrogen-responsive early breast cancer, in animal models, bisphosphonates do not slow the growth of established lesions. This suggests that after becoming established, bone metastases may no longer require active bone resorption. The question of how osteoclastogenesis and bone resorption are stimulated initially by tumor cells prior to and after their arrival in the skeleton remains largely unanswered.

Heparanase is an enzyme that acts both at the cell surface and within the extracellular matrix to degrade polymeric heparan sulfate chains into shorter-chain-length, biologically active oligosaccharides of 18 to 20 sugar residues. In human breast
cancer, heparanase overexpression has been associated with increased metastasis and poor prognosis. Heparanase may contribute to disease progression through its well-established proangiogenic effects that drive vessel formation and fuel explosive tumor growth. In addition to stimulating tumor growth, heparanase has been implicated in local invasion and metastasis and is upregulated by estrogen.

One of the major heparan sulfates expressed on the surface of human breast cancer cells is syndecan-1. Syndecan-1 is a transmembrane (type I) heparan sulfate proteoglycan known to regulate multiple biologic functions, including cell–extracellular matrix adhesion, and it has a well-documented and important role as a growth factor coreceptor. In addition, syndecan-1 on the surfaces of cells can activate cell-surface integrins, thereby promoting breast tumor cell spread, migration, and angiogenesis. Syndecan-1 is overexpressed in the reactive stroma of bone marrow stroma of patients with multiple myeloma. In fact, the shedding of syndecan-1 from cells within primary human breast tumors has emerged as an important regulator of tumor microenvironment, where syndecan-1 is associated with enhanced tumor proliferation, angiogenesis, and the activity of matrix metalloproteinases (MMPs). When shed from the cell surface, syndecan-1 can facilitate the growth, angiogenesis, and metastasis of tumors. Importantly, the functional activities of syndecan-1 can be modulated by the enzymatic activity of heparanase, which specifically cleaves heparan sulfate chains.

Previously we demonstrated that bone resorption occurs before the appearance of overt bony metastases in animals bearing breast tumors overexpressing heparanase. Subsequently, we also demonstrated that breast cancer cells overexpressing heparanase release elevated levels of syndecan-1 into the growth medium and, moreover, that the overexpression of catalytically inactive heparanase did not increase the shedding of syndecan-1. These data suggest that factors may be released into the systemic circulation by breast cancer cells overexpressing heparanase that may stimulate distant osteoclastogenesis and the subsequent bone destruction. In the studies described here, increased osteoclast numbers and bone resorption were observed in the long bones of animals bearing human breast tumor mammary fat pad xenografts overexpressing active heparanase. In addition, in vitro osteoclastogenesis assays demonstrated that shed syndecan-1 was required for the enhanced osteoclastogenic activity of the tumor cells. These findings identify syndecan-1 that is shed by tumor cells expressing heparanase as a potent pro-osteoclastogenic agent.

Materials and Methods

Cell culture

The MDA MET-derived cell lines HPSE-Low, HPSE-High, M225, and M343 were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) at 37°C in sterile culture dishes as described previously. All cell lines were Mycoplasma-free.

Cells were subcultured by trypsinization in 0.5% trypsin (Sigma, St. Louis, MO) and 0.5 mM EDTA in Hank’s balanced salt solution (HBSS) without calcium or magnesium in a laminar flow hood during their logarithmic phase of growth. For human osteoclastogenesis assays, 48-hour conditioned media (containing serum) from HPSE-High, HPSE-Low, M225, and M343 cells were collected, diluted 50% in α modified essential medium (αMEM) and added to cultures of human peripheral blood mononuclear cells (as described below). We have shown previously that the 50% dilution of MDA MET-conditioned medium contains pro-osteoclastogenic activity, whereas 100% conditioned medium contains inhibitors that induce osteoclast progenitor death.

Heparanase activity assay

The heparanase activity assay used an immobilized [3H]heparan sulfate substrate and was performed as described previously. Purified recombinant heparanase (46 ng; generously provided by Dr Hua Quan Miao, ImClone Inc., New York, NY) was used as the positive control, and buffer was used as the negative control. Each sample was normalized to equal volume and tested in triplicate on at least two separate occasions.

IL-8 ELISA

The production and secretion of IL-8 was measured in conditioned medium using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. The commercially available IL-8 ELISA specifically detects human IL-8. IL-8 concentrations in conditioned media were calculated from a standard curve generated by adding recombinant IL-8 to the specific unconditioned medium and were considered undetectable if media concentrations were less than 0.3 pmol/L before correction for cell number. Murine NIH 3T3 cells tested with the IL-8 kit did not secrete IL-8 cross-reacting products. To block IL-8 activity, an IL-8 antibody (a kind gift of Dr Rakesh Singh, University of Nebraska, Omaha, NE, USA) was used as described previously. Briefly, 200 μg/ml of IL-8 antibody was added every other day to osteoclast cultures. All IL-8 biologic activity is inhibited by this treatment.

Western blots

Cells were extracted, and Western blot analysis was performed using 60 μg of protein per lane and a mouse monoclonal antibody directed against recombinant human heparanase, as described previously.

Tumor biology

Four- to 6-week-old female SCID mice were purchased from Harlan (Indianapolis, IN, USA) and allowed to acclimate for 7 days. In the two experiments, animals (five or four per group) received up to 2 × 10⁶ cells (HPSE-Low or HPSE-High) in each of four different injection sites within the mammary fat pads (two axillary and two abdominal), as described previously. All
Histology and osteoclast determination

Osteoclasts were identified in mouse long bones that were excised, fixed in 10% neutral-buffered formalin for 2 days, and decalcified in 5% formic acid with agitation until deemed clear by the ammonium oxalate endpoint test. The decalcified specimens then were dehydrated through graded ethanol and cleared in methyl salicylate before paraffin infiltration. Subsequently, the tissue was embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin (H&E) as described previously and tartrate-resistant acid phosphatase (TRACP) using the Acid Phosphatase Leukocyte Kit (Sigma) as described previously. Osteoclasts were identified as TRACP$^+$ multinucleated cells apposed to the bone surface, as described previously.

Isolation and culture of peripheral blood mononuclear cells and their differentiation to osteoclasts

Peripheral blood was collected from healthy donors (approved by the UAMS Institutional Review Board) using heparin as an anticoagulant and 200 ng/mL RANK-Fc to minimize any priming of osteoclast progenitors by endogenous RANKL, as described previously. Blood was diluted in sterile PBS (1:1) in a sterile hood. The blood-PBS solution was slowly layered over AccuPrep solution (Accurate Chemicals, Westbury, NY, USA) and then centrifuged at 400g in swinging buckets for 30 minutes at 21 °C. The peripheral blood mononuclear cell (PBMC) layer was collected and washed in five to six volumes of PBS, isolated by centrifugation at 140g, and resuspended in α-MEM containing 10% FBS. Cells were counted with a hemocytometer and plated in 48-well tissue culture plates at a concentration of 10$^6$ cells per well in 0.5 mL α-MEM. Using sterile forceps, one slice was placed in each well of a 48-well plate (containing 0.5 mL of α-MEM), and the plate was incubated at 37 °C for 30 minutes. Equilibration medium was then aspirated off, and PBMCs were added to the wells at a concentration of 1.0 × 10$^6$ cells per well in 0.5 mL volume. Precursors were allowed to adhere to the slices for 4 hours at 37 °C. Appropriate amounts of treatment medium were prepared, and 0.5 mL was added to wells in a replicate 48-well plate (lacking dentine slices) with 4 wells per treatment group. mCSF (25 ng/mL) was present in all treatment groups including control. The positive controls for stimulation of osteoclastic bone resorption was the addition of either RANKL (25 ng/mL) or IL-8 (10 ng/mL). Using sterile forceps, slices (with adherent cells) were transferred to the second 48-well plate, being careful not to invert the slices. Half the medium was exchanged three times per week. Cells were allowed to grow on dentine slices for 10 to 12 days, after which time the cultures were terminated. Dentine slices were fixed in 10% formalin and stained for TRACP. The ability to resorb bone were counted as osteoclasts. Bone-resorption area was measured using histomorphometry software (Osteomaster, Atlanta, GA, USA) after removal of the cells by sonication, as described previously.

Statistical analysis

Statistical significance between groups was determined by analysis of variance (ANOVA) prior to post hoc pair-wise multiple comparisons using the Student-Newman-Keuls method (SigmaStat, Aspire Software International, Ashburn, VA, USA); p < .05 was considered significant.

Results

Breast cancer frequently metastasizes to bone via a process that is apparently facilitated by increased bone turnover. In a previous study we demonstrated that primary breast tumor
xenografts formed in the mammary fat pads of SCID mice expressing high levels of heparanase stimulated bone resorption with no evidence of any detectable tumor cells within the bone.\textsuperscript{(12)} This study was performed to determine the mechanism for the distal osteolysis mediated by the heparanase-expressing tumor cells.

Osteotropic MDA-MET breast cancer cells\textsuperscript{(3)} were engineered to express high levels of wild-type heparanase (HPSE-High) or transfected with the empty vector (HPSE-Low).\textsuperscript{(12)} In addition, MDA-MET cells expressing catalytically inactive heparanases designated M225 [mutated proton donor site of the active site (Glu225 to Ala225)] and M343 [mutated nucleophilic residue of the active site (Glu343 to Ala343)] were prepared. Analysis by Western blot and heparanase activity assays confirmed that cells overexpressing wild-type heparanase had high levels of heparanase protein and high levels of heparan sulfate degrading activity, whereas cells expressing mutant heparanases had high levels of heparanase protein that was catalytically inactive and did not degrade heparan sulfate\textsuperscript{(29)} (Fig. 1).

Medium conditioned by HPSE-High cells enhanced osteoclastogenesis in vitro compared with medium conditioned by HPSE-Low cells, confirming our prior observations\textsuperscript{(12)} (Fig. 2). Moreover, pretreatment of the conditioned medium with heparinase III (HepIII), a bacterial enzyme that extensively degrades heparan sulfate, completely abolished the enhanced osteoclastogenic activity of medium from HPSE-High cells (Fig. 2). This demonstrates that the enhanced osteoclastogenic activity of HPSE-High cells requires intact heparan sulfate. However, boiling the conditioned medium greatly reduced the osteoclastogenic activity of all samples regardless of the presence or absence of HepIII, demonstrating the importance of other protein factors in the conditioned medium (Fig. 2). Together these results suggest that the enhanced osteoclastogenic effect in the conditioned medium from HPSE-High cells requires both intact heparan sulfate chains and heat-labile factors.
Next, osteoclasts were generated directly on human bone slices to measure the bone-resorbing activity of osteoclasts induced by medium from HPSE-High or HPSE-Low tumor cells. Consistent with the findings in Fig. 2, significantly more osteoclasts formed in response to medium from HPSE-High cells than medium from HPSE-Low cells (not shown), and the total area of bone resorbed by those osteoclasts was significantly greater than that in bone exposed to medium from HPSE-Low cells \( (p < .05; \text{Fig. 3}) \). However, osteoclasts generated in the presence of medium from HPSE-High cells created individual resorption pits that were not significantly different in area or depth from the pits formed by osteoclasts from HPSE-Low cells. In fact, the mean area of bone resorbed per osteoclast was 0.0036 ± 0.00067 mm\(^2\) for osteoclasts formed by medium from HPSE-high cells and 0.0032 ± 0.00053 mm\(^2\) for osteoclasts formed by medium from HPSE-Low cells, and these were not statistically different \( (p > .05) \). Similarly, no differences were observed in the number of nuclei per osteoclast in either HPSE-High or HPSE-Low conditioned-medium cultures (data not shown). Thus the enhanced bone resorption observed with the heparanase-expressing tumor cells is due to their impact on osteoclastogenesis (i.e., increased osteoclast numbers) and not an effect of any increase in the activity or size of the individual osteoclasts.

Based on the results in Figs. 2 and 3, we hypothesized that the distal osteolysis induced by tumors in the mammary fat pad of mice\(^{122}\) was due to increased osteoclast numbers in the long bones of mice bearing HPSE-High tumors compared with those from animals bearing HPSE-Low tumors. To test this, histomorphometric analysis of the long bones from tumor-bearing mice was performed. The results confirmed that significantly more osteoclasts per millimeter of bone surface were present in animals bearing tumors formed by HPSE-High cells compared with those bearing tumors formed by HPSE-Low cells \( (p < .05; \text{Fig. 4}) \). The animals with HPSE-High tumors also had increased serum levels of TRAP-5b and collagen telopeptides relative to levels of these markers in the sera of mice bearing tumors of HPSE-Low cells.\(^{122}\) These data demonstrate that the increased osteoclast number observed in HPSE-High tumor-bearing mice is reflected in the increased serum biochemical markers of bone resorption.

Because enzymatically inactive heparanase also may exert effects on cell behavior,\(^{9}\) the osteoclastogenic activity of medium conditioned by cells expressing enzymatically inactive heparanase was examined. The results demonstrated that medium conditioned by cells expressing enzymatically inactive heparanase does not enhance osteoclastogenesis beyond the level seen in medium from the HPSE-Low cells (Fig. 5).

In contrast to HepIII, which significantly degrades heparan sulfate into non–biologically active fragments, human heparanase cleaves heparan sulfate into biologically active fragments of 5 to 7 kDa.\(^{9}\) In addition, heparanase also enhances the expression and shedding of syndecan-1 from the cell surface.\(^{20}\) To determine whether heparan sulfate fragments or intact syndecan-1 were responsible for the increased osteoclastogenesis, syndecan-1 was immunoprecipitated from conditioned medium using the B-B4 antibody that recognizes the core protein of syndecan-1 (CD 138, Serotec, Raleigh, NC). This treatment removes all syndecan-1 from the medium to a level that is below the limit of detection of the syndecan-1 ELISA \( (\leq 17 \text{ ng/mL}) \). In contrast, HPSE-High conditioned medium

![Fig. 3. Medium from HPSE-High cells enhances bone resorption.](image)

![Fig. 4. Orthotopic HPSE-High breast tumors increase osteoclast numbers in long bones.](image)
contained 43 ng/mL syndecan-1 after immunoprecipitation by beads coated with non-immune IgG.

The absence of syndecan-1 significantly diminished the enhanced osteoclastogenic activity of medium conditioned by HPSE-High cells (Fig. 6A). Immunodepletion and subsequent HepIII treatment further reduced the extent of osteoclastogenesis induced by the conditioned medium to the levels observed for HPSE-Low cells (Fig. 6B). Interestingly, the reduction in osteoclastogenic activity owing to HepIII treatment and syndecan-1 depletion was not significantly different from the osteoclastogenic activity of immunodepleted syndecan-1 alone. Collectively, these data demonstrate that shed syndecan-1, and not the heparanase-generated fragments of heparan sulfate, is critical for the enhanced osteoclastogenic activity of the tumors formed by the HPSE-High cells.

Elevated levels of the alpha chemokine IL-8 are associated with the increased bone metastasis and osteolysis of human breast cancer cells. IL-8 is a heparan sulfate–binding osteoclastogenic factor and as such could cooperate with the heparan sulfate chains on the syndecan-1 shed by the HPSE-High cells. IL-8 is released into the medium by both HPSE-Low and HPSE-High cells. HPSE-High cells secrete 78 ± 2 ng/μg of IL-8 protein into conditioned medium compared with 32 ± 2 ng/μg secreted by HPSE-Low cells into the conditioned medium. Interestingly, IL-8 levels are also elevated in the sera of mice bearing tumors of HPSE-High cells (15 ± 2 ng/mL) compared with IL-8 levels in the sera of mice bearing HPSE-Low tumors (3 ± 1 ng/mL).

We and others have shown that IL-8 is a potent osteoclastogenic agent. Indeed, only 10 ng/mL of IL-8 is sufficient to stimulate osteoclastogenesis in in vitro preparations of PBMCs (Fig. 7). A function-blocking antibody to IL-8 can completely abrogate the osteoclastogenesis mediated by recombinant human IL-8 (Fig. 7A, B; compare IL-8− to IL-8 +). This IL-8 antibody also significantly suppresses the enhanced osteoclastogenic activity of the HPSE-High cells, demonstrating that IL-8 is an osteoclastogenic component of the conditioned medium (Fig. 7A). As expected, the same IL-8 antibody almost completely abrogated the osteoclastogenesis promoted by medium conditioned by HPSE-Low cells (Fig. 7A), demonstrating that IL-8 is the principal component of the osteoclastogenic activity in the medium from these cells. We investigated the combined effect of blockade of IL-8 and immunodepletion of syndecan-1 and found that the suppression of osteoclastogenesis was greatly enhanced over either treatment alone (Fig. 7B).

**Discussion**

This study addresses the mechanism(s) whereby expression of heparanase by tumor cells enhances systemic osteolysis. Using histomorphometric analysis of bones from an animal model of breast cancer and in vitro assays of osteoclastogenesis and bone resorption, we discovered that (1) mammary fat pad tumors expressing high levels of heparanase stimulate osteoclastogenesis in distant bones, (2) medium from cells expressing high levels of heparanase stimulates osteoclastogenesis in vitro and bone resorption at levels significantly above those of medium from cells expressing low levels of heparanase, and (3) the enhanced osteoclastogenesis requires the presence of syndecan-1.
can-1, a proteoglycan whose shedding from tumor cell surfaces is enhanced by the expression of heparanase and protein agents, such as IL-8. In addition, these effects depend on the expression of the enzymatically active form of heparanase, suggesting that they occur downstream of the heparanase degradation of heparan sulfate. These results reveal a novel mechanism whereby expression of heparanase by tumor cells upregulates the shedding of syndecan-1, which after entering the systemic circulation stimulates osteoclastogenesis and enhances bone destruction.

The finding that syndecan-1 shed by breast cancer cells participates in driving osteolysis adds to the growing list of important functions of this proteoglycan within the tumor microenvironment. For example, the expression of syndecan-1 by stromal cells and its shedding have been linked to proliferation and angiogenesis of breast cancer cells, which is at least in part due to enhanced fibroblast growth factor 2 (FGF-2) signaling. Increased shedding of syndecan-1 also has been associated with increased MCF-7 breast cancer cell invasion through matrigel, suggesting a role for shed syndecan-1 in the invasive phenotype of tumor cells. Interestingly, shed syndecan-1 also promotes the growth, metastasis, and angiogenesis observed in multiple myeloma, another cancer with an aggressive osteolytic phenotype.

Although our results demonstrate that the heparan sulfate chains of syndecan-1 are required for its effect in stimulating osteoclastogenesis, we cannot rule out the possibility that the core protein of syndecan-1 is also involved. This is particularly important in light of recent studies showing that the core protein of syndecan-1, even when added exogenously to cells, can activate αvβ3 and αvβ5 integrins. Importantly, the αvβ3 integrin is expressed on the surfaces of osteoclasts and inhibitors of this integrin can block metastasis to bone and tumor-related osteolysis. Thus it is compelled to speculate that the shed syndecan-1 activates the αvβ3 integrin on osteoclasts, thereby enhancing osteoclastogenesis.

The impact of shed syndecan-1 on osteoclastogenesis is important because it provides a novel mechanism for the extensive osteolysis seen in many breast cancer patients. Bone metastases frequently are associated with the later stages of disease progression and relapse and may not be evident when the cancer is first diagnosed, suggesting that the initial stages of bone metastasis may not be completed when patients are initially diagnosed.
Bone turnover appears to be particularly important during the earliest stages of the formation of bone metastases\(^{47,48}\) and is clearly involved in the initial colonization of bone by migrating breast cancer cells.\(^7\) Once resorption is activated, disseminated tumor cells relocate to the bone marrow microenvironment and eventually the bone itself. As the metastatic foci grow, heterogeneity again may develop, potentially including cells with different metastatic capabilities and potential.\(^5\)

Heparanase is upregulated in many tumor types and has been associated with increased metastasis and poor prognosis in breast cancer.\(^{10}\) It has been suggested that much of heparanase function is regulated by its remodeling of the extracellular matrix and by its effects on cell signaling.\(^{49}\) Our findings provide new insight into how heparanase regulates tumor behavior via upregulation of syndecan-1 shedding. Interestingly, enhanced syndecan-1 shedding depends on the heparan sulfate degrading activity of heparanase,\(^{29}\) consistent with our finding that heparanase enzymatic activity is required for the enhanced osteoclastogenic effect of the heparanase-high breast cancer cells.

Overall, these results suggest a model where heparanase upregulation by tumor cells degrades heparan sulfate chains and enhances syndecan-1 shedding. The shed and active syndecan-1 is free to diffuse within the tumor microenvironment or to enter the circulation and travel to distal tissues, where it affects the behavior of host cells, such as osteoclasts and their precursors. It is also possible that the distal effects on osteoclasts we have observed helps to prepare premetastatic niches that are receptive to incoming circulating tumor cells. Further investigation of the role of the heparanase–shed syndecan-1 axis in breast cancer progression should focus on disruption of heparanase function, which is likely to profoundly affect tumor growth, metastasis, and osteolysis.

Immunodepletion of syndecan-1 from the medium is sufficient to reduce osteoclastogenesis to control levels, suggesting that syndecan-1 is the specific heparan sulfate proteoglycan that stimulates the enhancement of osteoclastogenesis by HPSE-High cells. In addition, the background (and substantial) osteoclastogenic activity of conditioned medium from HPSE-Low cells (and heparanase mutant cells) is presumably due to the expression of the osteolytic cytokine IL-8 that is still expressed by these cells.\(^{33}\) Although syndecan-1 on the surfaces of myeloma cells can bind to osteoprotegerin (OPG), leading to its internalization and degradation,\(^{50}\) we do not believe that this inhibits IL-8-mediated osteoclastogenesis because we have shown previously that the addition of OPG (or RANK-Fc) did not inhibit IL-8-induced osteoclastogenesis in vitro.\(^{32,33}\) Moreover, in our in vivo model where we see enhanced osteoclastogenesis, the tumor cells are not present in the bone and thus are not likely altering local OPG levels in the bone. However, we cannot rule out the possibility that the clearance of OPG from the circulation by tumors distal to the
Collectively, these data demonstrate that shed syndecan-1 in the conditioned medium is not the sole agent stimulating osteoclastogenesis. Although syndecan-1 is the primary heparan sulfate proteoglycan stimulating osteoclastogenesis in the conditioned medium, boiling also abrogated osteoclastogenic activity. Heparan sulfate is impervious to boiling; however, proteins are not. This suggests that syndecan-1 acts in concert with other heparin-binding growth factors to stimulate the potent osteoclast formation observed. As mentioned earlier, an attractive candidate for this activity is IL-8. IL-8 is a CXC-chemokine that is activated by interaction with heparan sulfate(35) and that has been shown to directly stimulate osteoclast formation and activity. (32) Importantly, the heparanase-high cells were derived from the osteolytic IL-8 expressing MDA-MET human breast cancer cells. (3,12) As such, the heparanase-high (and heparanase-low) cells also produce significant and measurable levels of human IL-8 (HPSE-High: 78 ± 2 ng/µg of protein; HPSE-Low: 32 ± 5 ng/µg of protein). In addition, other heparin-binding growth factors may contribute to enhancing osteoclastogenesis or to creating an osteoclastogenic environment (e.g., FGFs, insulin-like growth factor–binding proteins, and platelet-derived growth factor). Binding of heparan sulfate of syndecan-1 to these factors can promote their interaction with high-affinity receptors on the cell surface and/or protect the factors from proteolytic degradation within the bone microenvironment. In addition, a region of the syndecan-1 core protein has been shown to activate αvβ3 integrin on cell surfaces. (25) Because activation of αvβ3 integrin on osteoclasts is critical for normal osteoclast function and bone resorption, (52) syndecan-1 also could be playing a role in stimulating osteoclastogenesis in vivo via the activity of its core protein interacting with osteoclast surface integrins.

Interestingly, after implantation in the mammary fat pad, serum IL-8 levels are measurable only in animals bearing HPSE-High tumors, suggesting a role for HPSE in the access of tumor-derived cytokines to the circulation (HPSE-High: 15 ± 2 ng/mL; HPSE-Low: 3 ± 1 ng/mL [at the detection limit of the assay]). Understanding of the combined effects of heparanase, syndecan-1, and IL-8 on breast cancer progression, development of bone metastases, and activation of bone resorption is currently the focus of intense investigation in our laboratory. Whatever the case, it is clear that the osteolytic phenotype of aggressive human breast cancer cells involves multiple factors, including, but not limited to, heparanase, syndecan-1, and IL-8.

The finding that syndecan-1 shed from the surface of tumor cells is another important mediator of osteoclastogenesis identifies the heparanase–syndecan-1–cytokine axis as a novel target for inhibiting osteoclastogenesis and ultimately for preventing the consequences of bone metastases. We and others have speculated that heparanase acts as a master regulator of the aggressive tumor phenotype (29) that is likely the result of heparanase effects on multiple cell behaviors, including the regulation of tumor osteolysis. Further investigation of the role of this important axis in breast cancer progression should focus on disruption of heparanase function, which is likely to profoundly affect tumor growth, metastasis, and osteolysis.

Disclosures

All the authors state that they have no conflicts of interest.

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