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Heparanase inhibits osteoblastogenesis and shifts bone marrow progenitor cell fate in myeloma bone disease ☆

Jian Ruan [a,b], Timothy N. Trotter [a], Li Nan [a], Rongcheng Luo [b], Amjad Javed [c,d], Ralph D. Sanderson [a,c], Larry J. Suva [e], Yang Yang [a,c,*]

[a] Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA
[b] Department of Oncology, Nanfang Hospital, Guangzhou, China
[c] Comprehensive Cancer Center and the Center for Metabolic Bone Disease, University of Alabama at Birmingham, Birmingham, AL, USA
[d] Department of Oral and Maxillofacial Surgery, School of Dentistry, University of Alabama at Birmingham, Birmingham, AL, USA
[e] Department of Orthopaedic Surgery, Center for Orthopaedic Research, University of Arkansas for Medical Sciences, Little Rock, AR, USA

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ABSTRACT

A major cause of morbidity in patients with multiple myeloma is the development and progression of bone disease. Myeloma bone disease is characterized by rampant osteolysis in the presence of absent or diminished bone formation. Heparanase, an enzyme that acts both at the cell-surface and within the extracellular matrix to degrade polymeric heparan sulfate chains, is upregulated in a variety of human cancers including multiple myeloma. We and others have shown that heparanase enhances osteoclastogenesis and bone loss. However, increased osteolysis is only one element of the spectrum of myeloma bone disease. In the present study, we hypothesized that heparanase would also affect mesenchymal cells in the bone microenvironment and investigated the effect of heparanase on the differentiation of osteoblast/stromal lineage cells. Using a combination of molecular, biochemical, cellular and in vivo approaches, we demonstrated that heparanase significantly inhibited osteoblast differentiation and mineralization, and reduced bone formation in vivo. In addition, heparanase shifts the differentiation potential of osteoblast progenitors from osteoblastogenesis to adipogenesis. Mechanistically, this shift in cell fate is due, at least in part, to heparanase-enhanced production and secretion of the Wnt signaling pathway inhibitor DKK1 by both osteoblast progenitors and myeloma cells. Collectively, these data provide important new insights into the role of heparanase in all aspects of myeloma bone disease and strongly support the use of heparanase inhibitors in the treatment of multiple myeloma.

Introduction

Multiple myeloma (MM) is a hematologic malignancy characterized by the development of progressive and destructive osteolytic bone disease that is associated with diminished numbers of marrow stromal cells and osteoblasts [17,27]. Despite recent advances in treatment strategies myeloma remains largely incurable, with renal failure and immunosuppression as well as bone destruction as the major causes of morbidity [11,14,27]. Numerous studies have shown that the rampant osteolysis in myeloma results from the uncoupling of osteoclastic bone resorption and osteoblastic bone formation [14,17,27]. However, the molecular mechanisms regulating these events are not fully understood.

Heparanase is an enzyme that cleaves the heparan sulfate chains of proteoglycans into shorter chain length oligosaccharides [2,32] and is upregulated in a variety of human tumors, including myeloma [5,9,10,15,19,21,29]. We have demonstrated that increased levels of heparanase dramatically enhance myeloma tumor growth, angiogenesis, and the spontaneous metastasis of tumor cells to bone [18,26,33,35]. Recently, we reported that the expression of heparanase by myeloma cells markedly increased local and systemic osteolysis [36].

However, whether heparanase also contributes to the decreased osteoblast compartment common in myeloma bone disease remains unknown. In the present study, we determined the mechanism(s) by which heparanase modifies the development and/or activity of mesenchymal lineage cells that differentiate into osteoblasts and adipocytes in the bone marrow microenvironment.

Methods

Cells and reagents

The CAG myeloma cell line was established at the University of Arkansas for Medical Sciences (Little Rock, AR) as described previously

* Corresponding author at: Department of Pathology, University of Alabama at Birmingham, 1824 6th Avenue South, WTI 320A, Birmingham, AL 35294, USA. Fax: +1 205 996 6119.
E-mail address: yangyang@uab.edu (Y. Yang).

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[3]. CAG cells have a low level of endogenous heparanase expression and were previously transfected with empty vector (HPSE-low) or with vector containing the cDNA for the active form of human heparanase (HPSE-high) [16,25,33,35]. The murine C3H10T1/2 and ST2 pre-osteoblast cell lines were obtained from ATCC (Manassas, VA) and cultured as described below.

Recombinant human heparanase (rHPSE) and heparanase antibodies were kindly provided by Dr. Israel Vlodavsky (Technion, Haifa, Israel). Dickkopf1 (DKK1) inhibitor was purchased from Millipore (Billerica, MA); active and total β-catenin antibodies were purchased from Cell Signaling (Danvers, MA); human osteocalcin and mouse peroxisome proliferator-activated receptor gamma (PPARγ) antibodies were obtained from Abcam (Cambridge, MA); and mouse Runt-related transcription factor 2 (Runx2) antibody was purchased from MBL (Woods Hole, MA). Human and mouse DKK1 ELISA kits were obtained from R&D Systems (Minneapolis, MN). ALP and Oil Red O staining kits and β-actin antibody were purchased from Sigma (St. Louis, MO); and the Von Kossa staining kit was from Polysciences (Warrington, PA).

Animals

All animals were used in this study according to the NIH Guide for the Care and Use of Laboratory Animals and were approved under local institutional guidelines for the humane use of animals in research. SCID (CB.17 scid/scid) and C57BL/6 mice were purchased from Harlan Laboratories, Inc. (Indianapolis, IN) and housed in individual cages (5 per cage) in temperature (22 °C) and humidity (50%) controlled rooms having a 12 h light/12 h dark cycle with food and water ad libitum. All animal experiments were performed under a UAB IACUC approved protocol.

SCID-hu model, myeloma bone marrow specimens and immunohistochemistry

The SCID-hu is a well described animal model in which human fetal long bones (Advanced Bioscience Resources, Inc., Alameda, CA) are implanted subcutaneously on each side of the dorsum of SCID mice [33,34,37]. 10^5 CAG HPSE-low or HPSE-high cells were injected directly into the cut end of one human bone graft (primary bone) in each mouse, whereas the contralaterally implanted human bones were not injected with tumor cells (7 mice in each group). Eight weeks after the injection of tumor cells, the mice were euthanized. Tumor-injected human bones and non-injected contralateral human bones were collected and fixed in 10% neutral-buffered formalin and embedded in paraffin as described [36]. The paraffin-embedded bone sections were then stained with human osteocalcin antibody according to the manufacturer's recommendations and the numbers of osteocalcin positive osteoblasts on the surface of trabecular bones were counted [25,33].

Twenty eight paraffin-embedded bone marrow core biopsy specimens of myeloma patients, obtained from the Department of Pathology at UAB, were stained for both heparanase and osteocalcin. The

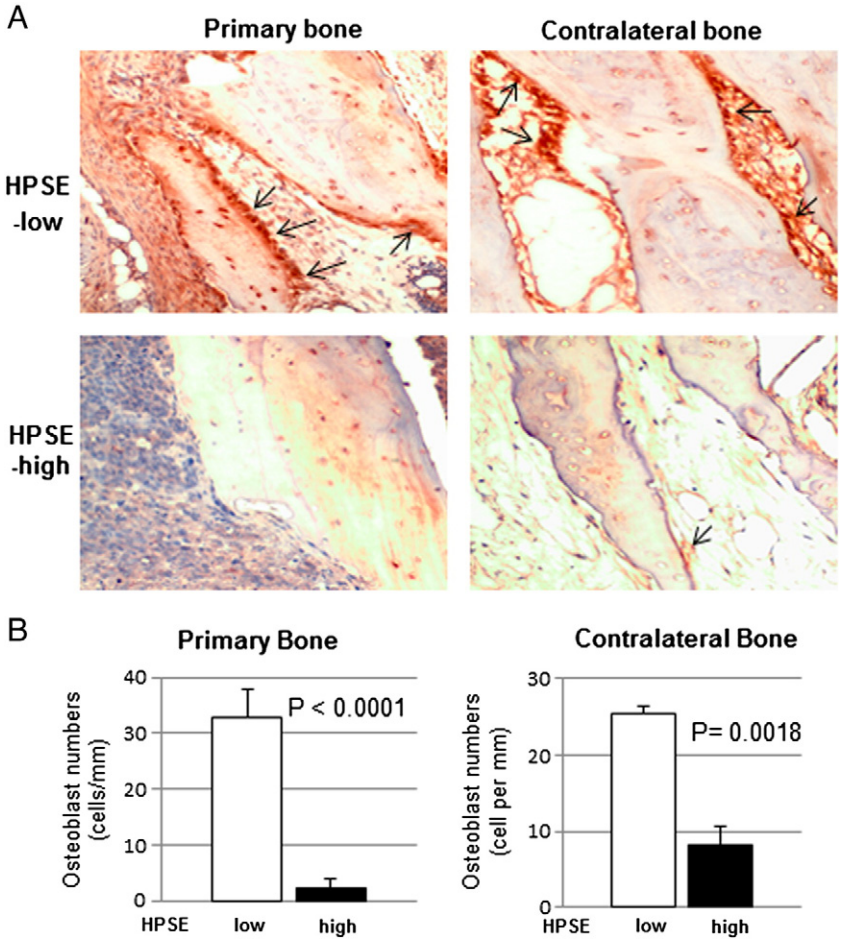

Fig. 1. Heparanase suppresses bone formation in animal model of multiple myeloma. Human fetal bones were implanted into SCID mice. (A) Primary bone (injected with HPSE-low [top-left] or HPSE-high [bottom-left] myeloma cells) and contralateral bone (uninjected) were harvested and stained for human osteocalcin. The brown-colored cells along the bone surface are osteoblasts (arrows). A-top: Primary and contralateral bones harvested from the mice bearing HPSE-low tumors. Many osteoblasts are visible on the bone surface. A-bottom: Primary and contralateral bones harvested from mice bearing HPSE-high tumors. Original magnification, ×400. (B) The number of osteoblasts on bone surface was measured in both primary and contralateral bones of the mice bearing HPSE-low or HPSE-high tumors (Mean ± SEM per mm of bone surface; n = 7). Significance is indicated for each comparison in each panel.