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The effects of RANK blockade and osteoclast depletion in a model of pure osteoblastic prostate cancer metastasis in bone ☆

Peter G. Whang [a], Edward M. Schwarz [b], Seth C. Gamradt [a], William C. Dougall [c], Jay R. Lieberman [a,*]

[a] *Department of Orthopaedic Surgery, David Geffen School of Medicine at UCLA, Center for Health Sciences 76-134, 10833 LeConte Avenue, Los Angeles, CA 90095, USA*

[b] *The Center for Musculoskeletal Research, University of Rochester Medical Center, Rochester, NY, USA*

[c] *Amgen, Inc., Seattle, Washington, DC, USA*

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Abstract

Adenocarcinoma of the prostate exhibits a clear propensity for bone and is associated with the formation of osteoblastic metastases. It has previously been suggested that osteoclast activity may be necessary for the development of these osteoblastic metastases based on data from lytic and mixed lytic–blastic tumors. Here we investigate the effects of complete in vivo osteoclast depletion via the blockade of receptor activator of NF:κB (RANK) on the establishment and progression of purely osteoblastic (LAPC-9 cells) bone lesions induced by human prostate cancer cells using a SCID mouse intratibial injection model. The subcutaneous administration of the RANK antagonist (15 mg/kg) RANK:Fc did not prevent the formation of purely osteoblastic lesions, indicating that osteoclasts may not be essential to the initial development of osteoblastic metastases. However, RANK:Fc protein appeared to inhibit the progression of established osteoblastic lesions, suggesting that osteoclasts may be involved in the subsequent growth of these tumors once they are already present. In contrast, RANK:Fc treatment effectively blocked the establishment and progression of purely osteolytic lesions formed by PC-3 cells, which served as a positive control. These results indicate that in vivo RANK blockade may not be effective for the prevention of osteoblastic metastasis but may potentially represent a novel therapy that limits the growth of established metastatic CaP lesions in bone.

Keywords: Receptor activator of NF:κB (RANK); RANK ligand; RANK:Fc protein; Osteoclastogenesis; Bone metastasis

Introduction

Adenocarcinoma of the prostate (CaP) is the most common malignancy diagnosed in males and is currently the second leading cause of cancer death among men in the United States [20]. Since skeletal metastases are known to develop in as many as 80% of CaP patients, these bony lesions represent a considerable source of morbidity [5,11]. In contrast to most metastatic skeletal lesions, which are primarily osteolytic in nature, CaP metastases to bone are generally considered to be osteoblastic, and are characterized by the deposition of dense, sclerotic bone. Up to 95% of CaP bony metastases have been shown to be purely osteoblastic, with the remainder forming mixed blastic/lytic lesions [18,29].

The receptor activator of nuclear factor-κB ligand (RANKL) is the final effector molecule that regulates

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* Corresponding author. Tel.: +1 310 825 7687; fax: +1 310 206 0063.

E-mail address: jlieberman@mednet.ucla.edu (J.R. Lieberman).

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osteoclastogenesis and bone resorption [32]. Ligation of RANKL to its receptor RANK on the membrane of osteoclast precursors is both necessary and sufficient for the differentiation and activation of osteoclasts. This pathway is negatively regulated by osteoprotegerin (OPG), a soluble decoy receptor synthesized by osteoblasts and stromal cells that binds to RANKL and prevents its interaction with RANK, resulting in immediate abrogation of osteoclastogenesis and mature osteoclast apoptosis [15]. Similar events can be mediated by a recombinant soluble fusion protein consisting of the extracellular domain of RANK coupled with the Fc domain of the human IgG (RANK:Fc). By suppressing osteoclast maturation and reducing their resorptive activity, administration of RANK:Fc has been shown to minimize osteoclastic bone resorption in multiple murine models [6,27].

The role of osteoclastic bone resorption in the establishment and progression of CaP skeletal lesions and other osteoblastic metastases has not yet been fully elucidated. Because CaP cells are known to produce soluble RANKL, it has been hypothesized that by initiating the RANK/nuclear factor-κB cascade that underlies normal physiologic osteolysis, CaP cells may facilitate the entry of tumor cells into bone and liberate a number of growth factors stored in the mineralized bone matrix that may further support tumor propagation [1,33]. Patients with advanced metastatic CaP have been found to have increased levels of collagen breakdown products in their urine, suggesting that bone resorption may be essential for the generation or remodeling of bony lesions [10,16,31]. It is believed that the inhibition of osteoclast activity may preclude the establishment or at least diminish the progression of bony CaP lesions, and as a result the osteoclast has recently become a critical target for novel therapies designed to treat CaP-induced bone metastases. OPG has been shown to inhibit the initial formation and subsequent progression of both osteosclerotic and mixed lytic/blastic CaP lesions [35,36]. In contrast to these findings, we have previously demonstrated that the use of bisphosphonates to limit osteoclast function successfully prevented the development of osteolytic CaP lesions but did not restrict the growth of purely osteoblastic metastases in a murine intratibial injection model [22]. Thus, at this time it is unclear whether osteoclast-mediated bone resorption is obligatory for the development and/or progression of purely osteoblastic CaP skeletal lesions. The present study was designed to evaluate the efficacy of RANK:Fc in limiting the growth of purely osteoblastic and osteolytic bone lesions induced by human CaP cells using a SCID mouse bone metastasis model, and thereby determine whether osteoclast activity is essential for the formation of osteoblastic lesions.

Materials and methods

Cell lines and cell culture

Two human prostate cancer cell lines were used in this study, PC-3 (American Type Culture Collection) and LAPC-9, which when implanted into bone produce purely osteolytic (PC-3) and osteoblastic (LAPC-9) lesions [8,17]. Unless otherwise specified, all reagents and labware for cell culture were obtained from Gibco/Life Technologies (Rockville, MD) and Becton Dickson Labware (Franklin Lakes, NJ), respectively. Cells were cultured in Iscove's medium (Irvine Scientific, Irvine, CA) supplemented with 15% FBS and 1% glutamine. Cells were maintained at 37 °C in a humidified incubator with 5% CO_2.

Animal care

Eight-week-old male severe combined immunodeficient (SCID) mice were housed under pathogen-free conditions according to the protocol approved by the Chancellor's Animal Research Committee at the senior author's institution.

Suspension of prostate cancer cells

SCID mice with subcutaneous LAPC-9 or PC-3 tumors were anesthetized (100 mg ketamine and 10 mg xylazine/kg body weight) and euthanized. The skin overlying the tumors was shaved and prepped with 70% ethanol and Betadine. The tumor was explanted in a sterile fashion and minced in PBS (Invitrogen Life Technologies, Carlsbad, CA) using a sterile razor blade. This slurry was spun in a centrifuge at 1300 rpm for 5 min at room temperature. After the supernatant was aspirated, the pellet was resuspended in Iscove's medium and centrifuged again at 1300 rpm for 5 min at room temperature. The supernatant was aspirated once more, and the pellet was resuspended in a 0.1% Pronase E/Iscove's medium solution which had previously been filtered using a 0.22 μm Steriflip filter (Millipore Corp., Bedford, MA). This cell suspension was gently shaken on a rotating platform for 18 min at room temperature, placed on ice for 2 min, passed through a 70 μm cell strainer and centrifuged again at 1300 rpm for 5 min at room temperature. Following aspiration of the supernatant, the pellet was resuspended in Iscove's medium supplemented with 15% FBS and 1% glutamine, at which time the cells were plated in 10 ml culture dishes. 1 × fungizone (Invitrogen Life Technologies) was added to all of the plates, which were incubated at 37 °C in a humidified atmosphere with 5% CO_2 until the time of injection.

Tibial implantation of prostate cancer cells

Single-cell suspensions of LAPC-9 or PC-3 cells were combined with an equal amount of Matrigel (Collaborative Biomedical Products, Bedford, MA) so that 1×10^5 cells were present in 10 μl of the mixture [21]. Eight-week-old SCID mice were anesthetized (100 mg ketamine and 10 mg xylazine/kg body weight) and their left hindlimbs were shaved and prepped with 70% ethanol and Betadine. Using a no. 15 scapel blade, a 3 mm longitudinal incision was made through the skin and patellar ligament. With the knee flexed, a 0.5 in. 27 gauge needle was inserted through the tibial plateau and a 10 μl suspension containing either 1×10^5 LAPC-9 cells or a similar number of PC-3 cells was injected into the proximal tibia. The skin incision was closed with 5-0 Vicryl suture (Ethicon, Inc., Somerville, NJ). All animals were euthanized 8 weeks after tibial implantation at which time hindlimb tumor diameter was recorded using calipers.

Treatment protocols

In this study, SCID mice undergoing tibial implantation with either LAPC-9 cells (experimental groups) or PC-3 cells (positive control groups) were injected with a RANK:Fc fusion protein consisting of