Bone Cancer Pain: From Mechanism to Model to Therapy

Prisca Honore, PharmD, PhD, and Patrick W. Mantyh, PhD
Neurosystems Center and Departments of Preventive Sciences, Psychiatry, Neuroscience, and Cancer Center, University of Minnesota, Minneapolis; and VA Medical Center, Minneapolis, Minnesota

ABSTRACT

Although bone cancer pain can be severe and is relatively common, very little is known about the basic mechanisms that generate and maintain this debilitating pain. To begin to define the mechanisms that give rise to bone cancer pain, a mouse model was developed using the intramedullary injection and containment of osteolytic sarcoma cells in the mouse femur. These tumor cells induced bone destruction as well as ongoing and movement-evoked pain behaviors similar to that found in patients with bone cancer pain. In addition, there was a significant reorganization of the spinal cord that received sensory input from the cancerous bone, and this reorganization was significantly different from that observed in mouse models of chronic neuropathic or inflammatory pain. To determine whether this mouse model of bone cancer could be used to define the basic mechanisms giving rise to bone cancer pain, we targeted excessive osteoclast activity using osteoprotegerin, a secreted decoy receptor that inhibits osteoclast activity. Osteoprotegerin blocked excessive tumor-induced, osteoclast-mediated bone destruction, and significantly reduced ongoing and movement-evoked pain, and the neurochemical reorganization of the spinal cord. These data suggest that this model can provide insight into the mechanisms that generate bone cancer pain and provide a platform for developing and testing novel analgesics to block bone cancer pain.

Key Words: Astrocyte Hypertrophy; Bone; C-fos Protein; Dynorphin; Osteolysis; Osteoprotegerin; Persistent Pain

As advances in cancer detection and therapy have extended the life expectancy of cancer patients, there is increasing focus on improving the patient’s quality of life. More than 1.2 million cases of cancer will be diagnosed in 2000 in the United States alone. Approximately 30% to 50% of all cancer patients will experience pain, and between 75% and 90% of patients with advanced cancer will experience significant, life-altering, cancer-induced pain. One common type of cancer pain that is difficult to treat is bone cancer pain [1–3]. This pain occurs in patients with primary bone cancer (sarcoma or hematologic malignancies) and more commonly in patients with cancer that has metastasized to bone from distant sites such as the breast, prostate, ovary, and lung [4].

The most common symptom of bone cancer is pain [5–7]. This pain is dull, constant, increases with time, and is exacerbated by use of involved portions of the skeleton. As bone cancer progresses, intermittent episodes of extreme pain occur spontaneously, or after weight bearing or movement of the affected limb [8–10]. Bone cancer pain is commonly associated with radiographic evidence of cancer-induced bone destruction. It has been suspected that all painful bone cancers, whether primary or metastatic, induce pain at least in part by stimulating bone destruction, as the extent of bone destruction is usually correlated with ongoing pain and with the severity and frequency of intermittent episodes of incapacitating pain [6,7].

Existing pharmacological treatments for bone cancer pain can be ineffective, burdensome to administer, and fraught with side effects [11,12]. The neurobiologic bases for these treatments are empirical and based on scientific advances in understanding the pathophysiology of other painful conditions. The greatest obstacle to developing new
treatments for persistent cancer pain and/or optimally coordinating existing treatments is a paucity of knowledge of the basic neurobiology of cancer pain. There is no well-accepted animal model of cancer pain, and the majority of what we know about the neurochemistry of cancer pain has been obtained from clinical studies on how to best manage pain in patients with cancer. Developing and characterizing a model of bone cancer pain and defining the cellular and neurochemical changes responsible for bone cancer pain would significantly improve the probability of identifying novel treatments for this condition.

Model of Bone Cancer Pain

Over the past decade, a major thrust in pain research has been to shift the process of discovering novel analgesics from one based on empirical observations, to one based on a mechanistic understanding of the biology involved in the generation and maintenance of different pain states. In an attempt to define the mechanisms that are important in the generation and maintenance of bone cancer pain, we hypothesized that cancer-induced bone destruction contributes to the pain. The murine model was induced by injecting murine osteolytic sarcoma cells into the intramedullary space of the murine femur (Figure 1); this model appears to share many similarities with bone cancer pain seen in humans [13].

A critical step was to ensure that the osteolytic cancer cells are confined within the intramedullary space of the femur and do not invade adjacent soft tissues. To accomplish this, following injection of sarcoma cells into the intramedullary canal of the femur, the injection hole in the bone was sealed by placement of an amalgam plug. This confinement of the tumor to the intramedullary space of the bone more closely mimics the condition in patients with primary or metastatic bone cancers.

As the tumor cells grew into the intramedullary space of the femur and replaced the normal bone marrow, bone destruction was monitored using radiographic analysis. By day 6, a small number of pits of bone destruction were detectable and from that point on, bone destruction progressed with increased pitted appearance, loss of medullary bone, and erosion of cortical bone up to displaced skeletal fracture at day 21 after sarcoma cell injection (Figure 1). At the same time, we observed an increase in the number of osteoclasts in the sarcoma-injected bone. Parallel to the increased bone destruction, tumor-bearing mice showed signs of ongoing and stimulus-evoked pain behaviors (Figure 2). In addition, in the ipsilateral spinal cord segments that receive primary afferent input from the cancerous femur, we detected several notable neurochemical changes that peaked at lumbar level L4, the major termination site of sensory fibers that innervate the femur. First, there was an increase in the pro-hyperalgesic peptide dynorphin in the deep dorsal horn [14–22]. Second, there was an increase in the basal number of immediate early gene protein product C-fos positive neurons located in the deep dorsal horn [23–31]. Third, there was a massive astrocytic hypertrophy (Figure 3). Lastly, signs of primary afferent sensitization were also observed as a normally non-

Figure 1 Sarcoma-induced bone destruction in the mouse femur. Low power radiograph of the murine pelvis and hind limbs after a unilateral injection of sarcoma cells into the distal part of the femur, and obturation of the injection site with an amalgam plug (A, see arrow). Note the loss of mineralized bone (white) in the proximal and distal head of the cancerous femur with the amalgam plug, in contrast to the normal contralateral femur. High-resolution radiographs of sarcoma-injected femora (0–3) showing the progressive loss of bone due to tumor growth. Bone destruction was quantified on a 0-to-3 scale based on the loss of bone. Images 0 to 3 are examples of each state of destruction: (0) normal bone with no signs of destruction; (1) bone destruction just detectable; (2) loss of medullary bone and erosion of cortical bone; (3) full thickness cortical bone loss and displaced skeletal fracture. Line bar = 2mm.
noxious palpation of the femur induced release of substance P, substance P receptor internalization [32–36], and C-fos expression [29, 31, 37–40] in lamina I spinal neuron. All these changes were correlated to the extent of bone destruction [13].

In assessing any experimental animal model, it is important to determine how well the model approximates the human disease. The most common symptom of bone metastases in humans is bone pain. Bone destruction can lead to pathological fractures and/or hypercalcemia [6, 7, 41, 42]. Over weeks or months, as the tumor grows and stimulates bone destruction, pain progressively becomes more severe [8, 9]. With increased bone destruction and time, the pain intensifies and can incapacitate the affected individual. As bone destruction progresses, acute pain is frequently observed when the involved bone is moved or palpated. In humans, the extent of bone destruction, and particularly ongoing osteolytic activity, is correlated with the severity and frequency of breakthrough pain [43].

The murine model appears to mirror many of the features found in humans with bone cancer-induced pain. The osteolytic sarcoma cell line aggressively destroys bone and provides localized pathologic findings similar to that found in human osteolytic bone cancer [44–46]. Mice with bone cancer exhibit painful behaviors and these behaviors correlate with the extent of bone destruction [13]. Severe acute pain is also observed in mice once significant bone destruction has occurred, as normally non-noxious palpation of the affected bone results in behaviors indicative of severe pain; this severe pain is again correlated with the extent of bone destruction.

**Neurochemical Signature of Inflammatory, Neuropathic, and Bone Cancer Pain**

The mouse model of cancer pain induced behavioral, cellular, and neurochemical changes that were correlated with the degree of tumor growth and bone destruction [13]; but it remained unclear whether cancer pain is a unique type of pain or merely a subtype of inflammatory or neuropathic pain. Previous studies, performed primarily in the rat, have suggested that there are distinct differences in the neurochemical changes that occur in the spinal cord and primary afferent neurons in inflammatory and neuropathic pain states [47]. These findings have generated significant interest, as these neurochemical changes may be involved in the different clinical symptoms. Interestingly, these neurochemical differences mirror the fact that many analgesics are most efficacious in specific types of persistent pain [48].

In comparing the neurochemical changes that take place in mouse models of bone cancer pain with those that occur in inflammatory or neuropathic pain [49], the most remarkable finding is how distinct the changes are with each type of persistent pain state. For example, in the inflammatory model, there was a significant up regulation of SP and CGRP in the dorsal horn, while there was a down regulation of these same primary afferent neurotransmitters in the neuropathic models. In
contrast, in the cancer pain model, there was no significant change in either of these neurotransmitters. Likewise galanin and neuropeptide Y, two neuropeptides, were dramatically upregulated in DRG neurons in models of neuropathy, whereas no change was observed in these neurotransmitters in cancer pain.

Even more dramatic were the different neurochemical changes that each pain state induced in the spinal cord. The greatest change we observed in the spinal cord in response to cancer pain was a massive astrogliosis that was not observed in models of inflammatory or neuropathic pain [49]. These data argue that cancer pain is not merely a form of inflammation or neuropathy pain, but rather, is a distinct pain entity. Determining which neurochemical changes are involved in generating and/or maintaining each type of persistent pain should provide a mechanistic platform for designing therapies specifically targeting these different pain states.

**Mechanism-based Therapy for Bone Cancer Pain**

To begin to define the mechanisms that give rise to bone cancer pain, we targeted excessive osteoclast activity using osteoprotegerin (OPG), a secreted decoy receptor that inhibits osteoclast activity [50]. Osteoclasts are the body’s principal bone-resorbing cell; and it has recently been shown that the formation, survival, and bone-resorbing activity of these cells can be potently inhibited by the soluble tumor necrosis factor receptor (TNFR) family molecule osteoprotegerin (OPG) [51–53]. Soluble TNFRs are thought to bind to their cognate ligands, thus sequestering their ligands and preventing them from activating their cellular targets. A major action of OPG appears to be that it binds to and/or sequesters OPG ligand (OPGL). In the adult, one of the richest sources of OPGL is the osteoblast, a cell critically involved in skeletal homeostasis. Osteoblasts participate directly in bone formation and indirectly by regulating, via OPGL production, osteoclast-mediated bone resorption, as OPGL potently stimulates osteoclast development and activates mature, pre-existing osteoclasts [51,53]. Thus, OPG rapidly inhibits osteoclast activity so that 2 days following a single injection of OPG, there is a significant inhibition of bone resorption [52,54]. In sarcoma-bearing mice that received OPG treatment, there was near total elimination of both tumor-induced bone destruction and osteoclasts at sites of tumor. In addition, OPG significantly reduced, by approximately one-half, both ongoing and movement-evoked pain behaviors and the neurochemical reorganization of the spinal cord. These results show that osteoclast-induced bone destruc-

![Figure 3](http://painmedicine.oxfordjournals.org/)

**Figure 3** Confocal image showing astrocyte hypertrophy with immunostaining for glial fibrillary acidic protein (GFAP) in coronal sections of the L4 spinal cord, 21 days following injection of osteolytic sarcoma cells into the intramedullary space of the femur. Note that the upregulation of GFAP is almost exclusively ipsilateral to the femur with tumor-induced bone destruction. This confocal image was taken from a 60-μm thick tissue and is projected from 6 optical sections acquired at 4-μm intervals with a 20× lens, scale bar = 200 μm.
tation plays a role in bone cancer pain. There are several mechanisms by which osteoclasts may be involved in generating and maintaining bone cancer pain. For osteoclasts to resorb bone, they must maintain an extracellular microenvironment at acidic pH (4.0–5.0) at the osteoclast-mineralized bone interface [55]. A population of sensory neurons that innervate bone expresses acid-sensing ion channels and vanilloid receptors [56]. These neurons could be excited and/or sensitized if exposed to the osteoclast-induced acidic extracellular microenvironment. Sensory neurons have been shown to be excited and/or sensitized by growth factors [57,58], and a variety of growth factors reside in bone and are released during cancer-induced bone resorption. Additionally, as the tumor continues to induce excessive osteoclast bone resorption, over time the bone is weakened, becomes mechanically compromised, and ultimately will fracture.

There is a portion of bone cancer pain that continues despite inhibition of cancer-induced bone resorption, as OPG administration reduces but does not completely abolish the pain behaviors (Figure 2). Previous studies have suggested that there is relatively little direct sensory or sympathetic innervation of tumors [59], although both mineralized bone and bone marrow receive sensory and sympathetic innervation [60–62]. Malignant cells are known to secrete prostaglandins, endothelins, cytokines, epidermal growth factor, transforming growth factor, and platelet-derived growth factor; and many of these factors have been shown to excite primary afferent nociceptors [63–69]. Our data suggest that there is a component of bone cancer pain caused by tumor-secreted factors that directly sensitizes and/or activates primary afferent fibers that innervate bone and induce central sensitization in the spinal cord.

Summary

The mouse model of bone cancer pain shares many features with bone cancer pain encountered in humans. Thus, in addition to bone destruction, there is also peripheral sensitization of primary afferent fibers and central sensitization with an accompanying neurochemical reorganization of the spinal cord. While osteoclasts play a significant role in bone cancer pain, other factors are clearly involved in generating and maintaining bone cancer pain. The mouse model of bone cancer pain should begin to allow definition of the basic mechanisms that generate and maintain bone cancer pain, which should permit the development of novel, mechanism-based therapies.

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