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Unfortunately, the development of or progression of skeletal cancer is almost certainly a harbinger of future problems. These problems are defined by the complications that develop as skeletal cancers progress, and include bone cancer pain, skeletal fracture, hypercalcemia, and the need for palliative radiation therapy and/or surgical stabilization of bones incapable of maintaining their mechanical integrity. The most common problem among these is bone cancer pain.

Bone cancer pain can be the first symptom of metastatic cancer or, more commonly, a symptom of advanced cancer. Bone cancer pain symptoms dominate quality of life, as patients with advanced cancers cope with their final years and months. Unfortunately, the vast majority of patients with bone cancer cannot be cured of their disease, and as a result, treatment modalities and decisions focus on palliation of cancer symptoms.

Bone cancer pain is the most common form of cancer-associated pain. This type of pain is often under-treated or not responsive to available therapies. The onset and progression of bone cancer pain is often insidious. Treatments initially focus on prescription of anti-inflammatory agents and the use of osteoclast-inhibiting agents. As patients survive, bone cancer pain is frequently no longer responsive to these simple measures. Narcotics and external beam radiation are then recommended. This more intense level of treatment is usually effective initially, but if patients survive additional months or years, the pain often recurs and intensifies. Modalities used to treat this higher level of pain are limited, and their morbidities are significant. Remaining options include significant escalation of pharmaceuticals, radiation, and/or surgical treatment of painful or mechanically incompetent bones. Morbidities for these treatments are high. Treatment with escalation of significant doses of narcotics can be complicated by the life-disrupting problems of constipation, confusion, lethargy, and risk of falling. Radiation can cause local skin irritation, myelosuppression, and nerve injury. Surgery in medically impaired cancer patients has significant risks, including problems with blood loss, wound healing, failure of surgical reconstruction, and even perioperative death.

Abstract

Cancer-induced bone diseases are common and can have a devastating impact at the end of life. One of the most difficult sequelae of cancer is metastases to the skeleton, an event that results in bone destruction and bone cancer pain. Bone cancer pain is usually progressive as the disease advances, and is particularly difficult to treat. Recently, experimental models of bone cancer pain have been developed and have provided seminal insight in understanding the pathophysiology of bone cancer pain. Animal models of bone cancer provided the finding that bone destruction (osteolysis) is associated with pain, and it has been determined that cancer-induced osteolysis is mediated by osteoclasts. Having established that RANK ligand contributed to cancer-induced osteoclastogenesis, it was determined that disruption of the RANKL-RANK axis with OPG inhibited tumor-induced osteoclastogenesis and decreased bone cancer pain.

Keywords: Bone Cancer, Pain, Osteoprotegerin, Osteolysis

Bone cancer pain and the role of RANKL/OPG

D.R. Clohisy and P.W. Mantyh

Department of Orthopaedic Surgery, University of Minnesota, Minneapolis, USA

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The tragic plight of cancer patients with the potential to survive months or years while battling bone cancer pain can be attributed to two clear failures in health professionals' attempts to provide palliative care. The first failure is a paucity of treatments capable of halting the progression of bone cancers. The second is the absence of pain therapies that have a known mechanistic basis of action. Obviously, enormous resources have targeted improving this first point of failure. For decades, investigations regarding mechanism of cancer spread, invasion and growth have been commonplace. In contrast, until recently there has been very little basic laboratory investigation focused on understanding the mechanisms of development and maintenance of bone cancer pain.

**Experimental model of bone cancer pain**

Recent work has provided experimental models of bone cancer pain in mice and rats. In one murine model, cancer cells (termed 2472 sarcoma cells) are injected directly into mouse femora. In this experimental model, bone cancers grow at the site of injection, destroy bone, and increase osteoclast number. As tumors progress, animals manifest behavioral and neurochemical characteristics of bone cancer pain.

Initial investigation with this experimental model focused on defining the cellular means through which cancers caused osteolysis. Findings indicated that tumors increase the number and size of osteoclasts at sites of tumor, showed that osteoclasts were required for cancer-induced bone destruction, determined that osteoclast number increased because of accelerated osteoclastogenesis, and determined that the precursor cells that formed osteoclasts at sites of tumor were post-mitotic osteoclast precursor cells. The experimental model of bone cancer pain rapidly accelerates between 7 and 10 days after intraosseous cancer cell injection. Accelerated tumor growth continues until 21 days after injection. Study of animals' behavior revealed that tumor-bearing mice exhibited behavioral changes exemplified by ongoing pain, activity-induced pain, and pain following non-painful limb stimulation (allodynia). The presence of these pain behaviors was supported by characterization of neural composition and neurochemistry of the peripheral and central nervous system.

When animals had bone cancer pain, no obvious alteration in innervation of bone or periosteum was appreciated, and there was no impact on the percentage of small diameter DRG neurons that expressed substance P (31%) or IB4-
expressing neurons (73%). In contrast, composition of the spinal cord was altered in mice with bone cancer pain. Specifically, spinal cords from these animals had three dramatic changes: astrocyte hypertrophy without neuronal loss, expression of the prohyperalgesic peptide dynorphin, and increased expression of c-fos in the dorsal horn and deep within the spinal cord (Figure 2). To evaluate for pain after non-painful limb stimulation, dorsal root ganglia (DRGs) were studied immediately following gentle manipulation of cancerous limbs. This prompted robust internalization of substance P and dramatic increase in lamina I neuron expression of c-fos, a finding that was specific to bone-residing and not soft tissue cancers.

When compared to neurochemical findings characteristic of inflammatory or neuropathic pain, the neurochemical features of bone cancer pain are unique. For example, in neuropathic pain, astrocyte hypertrophy occurs in combination with neuronal loss and decreased substance P levels. In contrast, bone cancer pain model exhibited astrocyte hypertrophy without neuronal loss and no change in substance P levels. Inflammatory pain states are characterized by increased substance P, calcitonin gene-related peptide and substance P in the spinal cord. Bone cancer pain did not exhibit any of these changes.

**OPG treatment reduces bone cancer pain**

Based on behavioral and neurochemical data, it was appreciated that progressive abnormalities in spinal cord neurochemistry correlated with the extent of bone destruction. This observation generated the hypothesis that cancer-induced osteolysis caused bone cancer pain. As molecular characterization of the bone cancer microenvironment revealed that sites of painful, osteolytic bone cancers had increased levels of RANK and RANKL (Figure 1), the possibility was raised that disruption of the RANK-RANKL axis via OPG treatment may reduce cancer-induced, osteoclast-mediated osteolysis and pain.

To determine if disruption of the RANK-RANKL interaction influenced cancer-induced osteolysis and bone cancer...
pain, mice were treated with OPG (OPG-Fc) while still in the early stages of bone cancer development. Treatment with OPG early in the development of bone cancer decreased tumor osteolysis. Bone destruction was significantly less in OPG-treated mice compared to vehicle-treated mice (Figure 3), with the majority of femora from OPG-treated mice showing no evidence of cancer-induced osteolysis. Histologic evaluation of vehicle- and OPG-treated mice demonstrated no significant reduction in tumor burden, but a dramatic reduction in osteoclast number (Figure 4). These findings indicated that the RANK-RANKL axis was pathogenic in the development of 2472 cancer-induced osteolysis.

Treatment with OPG early in the development of bone cancer also decreased bone cancer pain. Based on evaluation of pain behaviors, treatment with OPG provided significant reduction in pain, as manifested by a 73% reduction in activity-related guarding, a 44% decrease in the number of flinches, and a 30% decrease in palpation-induced pain behaviors. Improvements in pain behaviors were accompanied by normalization of several of the neurochemical characteristics of bone cancer pain (Figure 5). Specifically, there was a reduction in astrocyte hypertrophy, decreased spinal cord expression of c-fos and an absence of spinal cord dynorphin. In addition, following non-painful palpation of cancerous limbs, there was significant reduction in substance P receptor internalization and c-fos expression in laminae I of the spinal cord.

When mice with advanced bone cancer pain were treated with OPG, an impressive reduction in pain was also realized (Figure 7). Twelve days after intraosseous injection of 2472...
cells, mice had significant osteolysis and significant pain. Treatment of these animals with OPG halted cancer-induced bone destruction, stabilized behavioral measures of pain, and prompted corrective neurochemical reorganization of the spinal cord\textsuperscript{14}. Bone destruction ceased after treatment with OPG, as the extent of destruction nine days after treatment (day 21) with OPG was equivalent to the extent of bone destruction noted when OPG treatment began (day 12). Vehicle-treated animals experienced progressive bone destruction through the duration of the experiment.

Figure 5. OPG blocks both spontaneous and evoked pain behaviors seen in sarcoma-injected mice. Data include limb use score (0-4) during ambulation in an open field (a), the number of spontaneous flinching behaviors in a 2-minute observation period (b), nocifensive behavior score obtained during a normally non-noxious palpation (c), and the number of flinching behaviors in a 2-minute observation period after the completion of a normally non-noxious palpation (d) 17 days after sham or sarcoma injection into the femora of mice that subsequently received vehicle or OPG. Daily treatment with OPG significantly reduces spontaneous and evoked noxious behaviors or behaviors indicative of pain. Data represent mean ± SEM. Dashed lines, baseline values. *, P<0.05; **, P<0.01; and ***, P<0.001; one-way ANOVA and Fisher’s PLSD (brackets and downward arrows indicate groups being compared).
Measures of ongoing pain behaviors and activity-related pain behaviors demonstrated the presence of significant pain 12 days after tumor cell injection. These abnormalities stabilized following OPG treatment but progressed following vehicle treatment.

Several neurochemical measures of pain that were abnormal 12 days after tumor cell inoculation improved following OPG treatment, but not vehicle treatment. These measures included reduced spinal cord expression of c-fos, reduced spinal cord expression of dynorphin, and reduced spinal cord astrocyte hypertrophy. In addition, OPG treatment reduced cancer-induced sensitization of primary afferent neurons observed after non-painful palpation of cancerous limbs. Following non-painful palpation, OPG-treated mice had reduced pain behaviors, attenuated c-fos expression in laminae I-II, and no substance P internalization in the spinal cord following non-painful palpation, when compared to vehicle-treated animals (Figure 6).

Taken in total, these findings revealed that OPG treatment had profound corrective influences on bone cancer pain behaviors, on neurochemical alterations of the spinal cord, and on cancer-induced osteolysis.

**Potential mechanisms of OPG action on pain.**

Since this initial demonstration that osteolysis is linked to bone cancer pain, additional reports have confirmed that a reduction in cancer-induced osteolysis decreases bone cancer pain. It therefore seems that one mechanism through which OPG treatment reduces pain is via its known effect on osteoclast formation, survival, and activity. It is unclear, however, how osteoclastic bone resorption causes pain. Several possibilities should be considered. First, osteoclasts themselves may be responsible for nociceptive stimulation, which could reflect osteoclast-mediated direct injury
to nociceptive neurons within bone or, alternatively, could reflect exposure of acid-sensing neuronal channels to the acidic pH of the osteoclast resorption bay. A second possibility is that osteoclasts themselves do not cause pain, but rather osteoclast-mediated bone loss decreases the mechanical integrity of the cancer-ridden bone, resulting in mechanical nociceptor stimulation.

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