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Advances in Understanding Bone Cancer Pain

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Abstract Experimental animal models of bone cancer pain have emerged and findings have provided a unique glimpse into unraveling the mechanism that drives this debilitating condition. Key contributors to the generation and maintenance of bone cancer pain are tumor-induced osteolysis, tumor itself, and production of nociceptive mediators in the bone-tumor microenvironment. J. Cell. Biochem. 96: 682–688, 2005. © 2005 Wiley-Liss, Inc.

Key words: bone cancer; experimental model; neurochemical markers; osteolysis; pain

Patients with primary bone sarcomas and malignant tumors that have metastasized to bone are frequently confronted with poor quality of life (QOL). Detractors from life enjoyment can include skeletal fractures secondary to osteolysis, hypercalcaemia, neurologic compression, depression, insomnia, and bone cancer pain. Bone cancer pain is one of the most common symptoms presented by patients with cancer [Coyle et al., 1990; Mercadante, 1997; Portenoy and Lesage, 1999]. Metastatic breast and prostate carcinomas are principle contributors to the prevalence of cancer-induced bone pain. Skeletal complications, as sequelae of metastatic disease, manifest themselves in approximately 70% of patients with advanced breast or prostate carcinoma [Coleman, 1997]. Furthermore, skeletal metastases are discovered in greater than 90% of patients who die from breast or prostate carcinoma [Coleman, 1997]. With continued progression of malignancy, the degree of bone cancer pain deteriorates rapidly from a consistent, stable background pain to unpredictable pain characterized by episodic, breakthrough events [Portenoy and Hagen, 1990; Clohisy and Mantyh, 2003]. Breakthrough pain may be provoked by movement (incident pain) or may be spontaneous and unrelated to patient mobility [Portenoy and Lesage, 1999]. Mechanical allodynia is the painful perception of mechanical stimuli that are not normally perceived as noxious. Development of this type of pain punctuates the pathway of bone cancer pain. This acute form of movement-evoked pain can be generated by modest limb use, coughing, or turning in bed and has diminished responsiveness to conventional therapeutics.

ANIMAL MODELS OF BONE CANCER PAIN

Experimental models of bone cancer pain have been developed in mice and rats. These models study tumors at different anatomic sites and of varied histological origin. Contemporary experimental models are based on direct injection of cancer cells into the medulla of the femur, humerus, and calcaneus [Honore et al., 2000c; Cain et al., 2001; Mach et al., 2002; Medhurst et al., 2002; Wacnik et al., 2003]. Intramedullary injection was first introduced at the end of the last decade by Schwei et al. [1999] and has since evolved to incorporate a variety of tumor types used to observe the progression of bone cancer pain. For example, bone cancer pain has been studied using rat breast carcinoma (MRMT-1), murine fibrosarcoma (2472), murine breast carcinoma (4T1), hepatocellular carcinoma (HCa-1), and murine melanoma (B16).

There are two methods of introducing cancer cells directly into skeletal medulla. In rats, because of the large bone size, it is possible to inject tumor cells percutaneously into the tibia without performing a knee stifle [Medhurst

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et al., 2002]. With mice, because of diminutive bone size, tumor cells are injected into the humerus or femur after a surgical knee stifle [Wacnik et al., 2003; Goblirsch et al., 2004]. Direct, local intramedullary injection of tumor cells is advantageous over systemic intravenous or intracardiac administration because the skeletal site where the tumor develops is known, allowing for analysis of corresponding behavioral and neuroanatomic segments. Furthermore, intramedullary injection permits simultaneous and precise quantitative evaluation of site-specific pain behaviors, tumor growth, osteolytic bone destruction, bone-tumor microenvironment, and neurochemistry.

Assessment of cancer-induced bone pain in experimental animal models has been performed, based on behavioral analyses, neurochemical markers of peripheral and central nervous system pathology, radiographic imaging, and histology. From the behavioral standpoint, two types of bone cancer pain have been studied, ongoing pain and movement-evoked pain. Ongoing pain is measured in murine models by quantification of spontaneous guarding and flinching, or the duration and frequency, respectively that a mouse holds the tumor-affected limb aloft during a predetermined observation period. Movement-evoked bone pain is assessed by limb use in an open field and forced ambulation.

Radiographic and histologic analyses of osteolytic tumors in experimental models have consistently demonstrated that mature, multinucleated osteoclasts are stimulated by the release of tumor cytokines and growth factors. Upon activation, osteoclasts have been shown to cause cancer-induced osteolysis in bone [Clohisy and Ramnaraine, 1998]. Novel experimental models allow osteolytic bone destruction to be correlated to pain behaviors, neurochemical changes, and cellular reorganization of the spinal cord [Hukkanen et al., 1992; Honore et al., 2002; Sevcik et al., 2004].

NEUROCHEMISTRY AND BONE CANCER PAIN

Bone contains a highly concentrated mosaic of primary sensory afferent and sympathetic fiber innervation, particularly embedded in the non-mineralized, connective tissue sheath covering the external bone surface or periosteum and in the intramedullary marrow (Fig. 1) [Bjurholm et al., 1988; Mach et al., 2002]. Distribution of nerve fibers in mineralized, osseous bone is limited to blood vessels and Haversian canals [Hukkanen et al., 1992; Serre et al., 1999]. Cellular and neurochemical

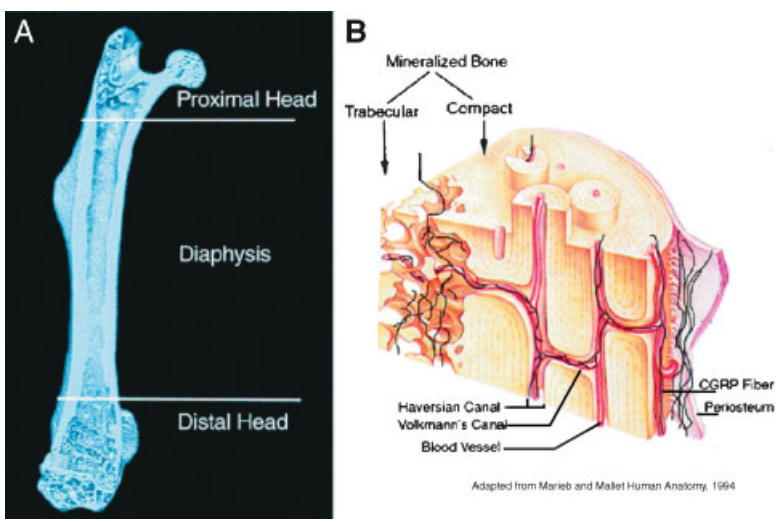

Fig. 1. Distribution of nerve fibers in bone. **A**: Microcomputerized tomography scan of the mouse femur subdivided according to proximal head, diaphysis, and distal head via presence of intramedullary trabecular bone. **B**: Schematic illustration of sensory fiber localization in periosteum, mineralized bone, and bone marrow. Sensory fiber concentration per unit area is greatest in the periosteum and concentrated less in the mineralized bone. The schematic is adapted from Marieb and Mallat, 1997. Reprinted with permission from Mach DB, Rogers SD, Sabino MC, Luger NM, Schwei MJ, Pomonis JD, Keyser CP, Clohisy DR, Adams DJ, O'Leary P, Mantyh PW. Origins of skeletal pain, sensory, and sympathetic innervation of the mouse femur. *Neuroscience*. 2002; 113(1):155–166. © 2002 IBRO.

characteristics of chronic pain can be detected in the dorsal root ganglia (DRGs) and at the site of primary sensory afferent innervation of the spinal cord. Central sensitization can be detected through evaluation of the neurochemistry and neural composition of the spinal cord.

Noxious stimulation of peripheral tissues initiates a cascade of nociceptive signals in the primary sensory afferent neurons. Pain signals are transduced from noiceptors in the primary afferent nerve fibers and transmitted to the DRGs, which house the nuclei and cellular bodies of sensory neurons. Lamina I spinal neurons in the dorsal horn of the spinal cord innervate the central nervous system and relay pain signals from DRGs to terminal processes in the brain after extensive excitatory or inhibitory processing [Urch, 2004].

Pain, as a sequela of injury or disease, can be characterized according to neuropeptide production in primary sensory afferent neurons and the spinal cord. Distinct neurochemical signatures have been suggested to characterize inflammatory, neuropathic, and tumorigenic pain. Experimental induction of inflammation via subdermal injection of complete Freund's adjuvant or intraplantar injection of capsaicin has been shown to upregulate substance P (SP), calcitonin gene-regulated peptide (CGRP), nerve growth factor (NGF), and pro-inflammatory cytokines in the primary afferent nerve fibers of DRGs and in laminae I and II of the dorsal spinal cord [Kuraishi et al., 1989; Donnerer et al., 1992; Smith et al., 1993; Galeazza et al., 1995; Honore et al., 2000b]. In contrast to pain produced by inflammation, neuropathic pain induced by peripheral sciatic transection and constriction or spinal nerve ligation exhibits downregulation of SP and CGRP in the primary afferents of the DRG and the superficial dorsal horn [Fitzgerald et al., 1985; Villar et al., 1991; Garrison et al., 1993]. Neuropathic pain is also associated with the upregulation of galanin (GAL) and NPY in the primary sensory afferents of the DRG and laminae I and II of the dorsal horn of the spinal cord [Garrison et al., 1991; Villar et al., 1991; Zhang et al., 1995a,b].

Tumorigenic pain can be delineated from inflammatory pain and neuropathy as a distinct condition based on alterations in the spinal cord. Cancer-induced bone pain leads to reorganization and sensitization of the central dorsal horn of the spinal cord. This condition is manifested as increased expression of the pro-hyperalgesic peptide dynorphin (DYN), enhanced neuronal activity monitored by elevated c-Fos expression, and profound astrocytosis [Mantyh et al., 1995; Honore et al., 2000a,b]. Recent data indicate that painless stimuli can stimulate release of SP from primary afferent sensory neurons of cancerous hind limbs, terminating in lamina I of the spinal cord [Schwei et al., 1999]. In contrast to inflammatory and neuropathic diseases, bone cancer pain does not produce significant expression of SP and CGRP markers in the dorsal horn of the spinal cord or GAL and NPY in the primary sensory afferent neurons. Importantly, however, expression of glial fibrillary acidic protein (GFAP), an astrocyte-specific cellular protein found in the supporting glial cells of the spinal cord, increases markedly in bone cancer pain (Fig. 2) [Honore et al., 2000b]. The evidence from experimental models, therefore, suggests that the neurochemical and cellular characteristics of bone cancer pain are unique when compared to inflammatory or neuropathic pain.

THERAPIES FOR CANCER-INDUCED BONE PAIN

Approximately 90% of cancer patients experience bone pain. Of these patients, 54% receive only temporary pain relief from conventional therapies [Meuser et al., 2001]. Permanent pain relief is often unobtainable and continues to be a challenging endeavor. The direction of available therapies are focused on eliminating tumor proliferation, reducing tumor-induced bone loss, intervening surgically to stabilize painful bones infiltrated with skeletal metastases, and administrating powerful pain medications. Treatment regimens can include monotherapy or concurrent combinations of nonsteroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase-2 (COX-2) inhibitors, chemotherapy, radiotherapy, nitrogen-containing bisphosphonates, and opioids. Medical management of bone cancer pain typically begins with NSAIDs or COX-2 inhibitors that are aimed at alleviating inflammatory states associated with bone pain. The potency of COX-2 inhibitors versus NSAIDs is similar; however, COX-2 inhibitors produce fewer gastrointestinal side effects.

Eradication of the tumor is usually approached with chemotherapy and radiotherapy management. External beam radiation is one

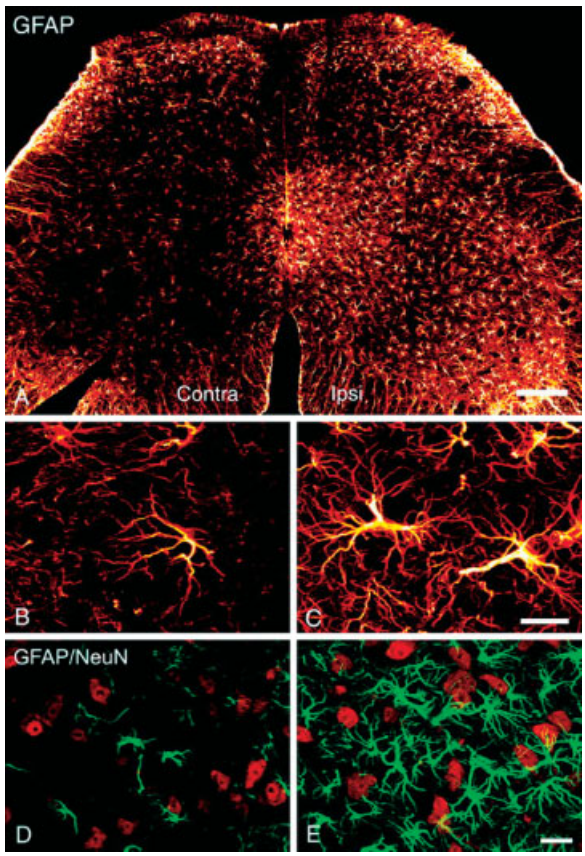

Fig. 2. Spinal cord astrogliosis as an indicator of bone cancer pain. Confocal image showing the profound unilateral discrepancy of GFAP staining (bright orange) in coronal sections of the fourth lumbar segment of the spinal cord after intramedullary injection of osteolytic sarcoma cells into the femur. GFAP upregulation, a marker of astrocyte hypertrophy, is nearly exclusive to the ipsilateral spinal cord of the tumor-bearing femur. Reprinted with permission from Schwei MJ, Honore P, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, Clohisy DR, Mantyh PW. Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. *J Neurosci.* 1999 Dec; 19(24):10886–10897. © 1999 Society for Neuroscience.

the most effective treatments found to alleviate tumor-induced bone pain, with 90% of patients receiving some pain relief and 50% of patients having complete relief. Unfortunately, more than 50% of patients who undergo radiation treatment and obtain pain relief will experience a relapse of pain equivalent to pretreatment levels [Tong et al., 1982]. The exact mechanism of radiation-induced pain relief is unknown. Hoskins et al. has suggested that decreased activity of osteoclasts in the bone-tumor microenvironment after radiation treatment is responsible for decreased bone destruction and serves as a predicting factor in the decreased pain response following radiation [Hoskin et al., 2000]. More recently, Goblirsch et al. has suggested that reduced tumor burden and reduced osteolysis are the principal contributors through which radiation improves cancer-induced bone pain (Fig. 3) [Goblirsch et al., 2004].

Administration of bisphosphonates and/or surgical stabilization address the painful, fragile condition of bones affected by tumor-induced bone loss and skeletal metastasis. Bisphosphonates, used initially to treat malignancy-

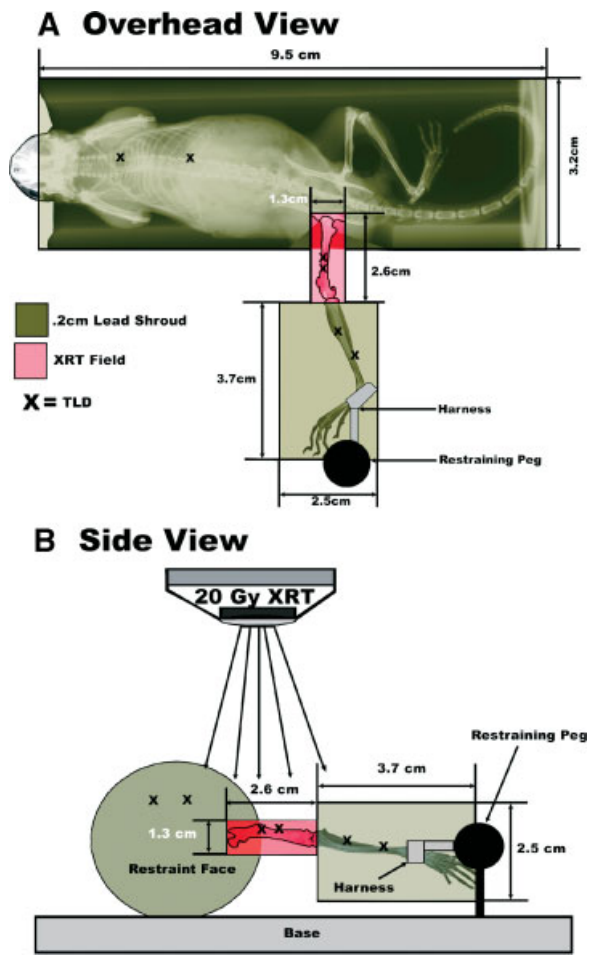

Fig. 3. Murine model of radiation treatment for bone cancer pain. A schematic representation of the lead-lined mouse restraining apparatus used to deliver a single, localized dose of orthovoltage radiation to tumor-bearing femora. The anatomic area exposed to radiation includes the entire femur (red shading). All other areas of the anesthetized mouse are protected from radiation. Lithium fluoride thermoluminescent dosimeters (TLDs) confirmed the actual radiation doses (**x**). Reprinted with permission from Goblirsch M, Mathews W, Lynch C, Alaei P, Gerbi BJ, Mantyh PW, Clohisy DR. Radiation treatment decreases bone cancer pain, osteolysis, and tumor size. *Radiat Res.* 2004 161:228–234. © 2004 Radiation Research Society.

associated humoral hypercalcaemia, have shown promise at decreasing cancer-induced skeletal complications. The suggested mechanism of bisphosphonates' action is a direct inhibition of osteoclast and osteoclast precursor cell activation [Hiraga et al., 2001; Vasikaran, 2001; Neville-Webbe et al., 2002]. Recent reports have shown that bisphosphonate treatment can significantly reduce the number of activated osteoclasts and osteolytic destruction of bone [Breuil et al., 1998; Boissier et al., 2000]. Furthermore, clinical trials have reported modest alleviation of bone pain in up to 50% of patients administered bisphosphonates [Coleman and Kaplan, 1993; Coleman, 2004]. The nitrogen-containing bisphosphonate, alendronate in particular has been shown to alleviate ongoing and movement-evoked pain in a murine model of femoral cancer [Sevcik et al., 2004]. The suggested mechanism for reductions in pain behavior is a product of bone resorption inhibition and mechanical stabilization [Hukkanen et al., 1992; Mach et al., 2002]. It has also been hypothesized that osteoclast inhibition causes an increase in bone-tumor microenvironment pH, resulting in a loss of sensory channel stimulation [Bassilana et al., 1997; Olson et al., 1998].

Chronic pain unresponsive to anti inflammatory agents, chemotherapy, radiotherapy, surgery, and/or bisphosphonates is typically combated with strong pain medications. Opioid management of advanced bone cancer pain is common and effective for pain relief. Unfortunately, opioid doses required to attenuate bone pain (120 mg/kg per day) can produce undesirable side effects such as confusion, somnolence, and constipation, that can severely diminish overall QOL [Baines, 1989; Lesage and Portenoy, 1999]. Opioid-treated patients with advanced bone pain are in a particularly vulnerable state. Within 4 weeks after seeing their physician, 73% of terminally ill patients receiving opioid treatment reported pain that was moderate to severe, and 40% of those patients with severe pain requested an increase in opioid treatment [Coyle et al., 1990]. Since opioids do not directly target the source of pain but act systemically via the central nervous system, the negative repercussions to organ systems can contribute significantly to poor QOL.

Significant progress has been made recently in examining potential new therapies. Recombinant osteoprotegerin (OPG-Fc) and other receptor activator of nuclear factor-kappaB ligand (RANKL) blockades inhibit bone resorption. The link between osteolysis and bone cancer pain was shown in studies where reduced ongoing and movement-evoked pain was noted after OPG-Fc was delivered to block bone cancer-induced bone pain and osteolysis [Simonet et al., 1997; Honore and Mantyh, 2000]. Clinical trials are currently underway using a human antibody against RANKL. Other laboratory research indicates that the transient receptor potential vanilloid type-1 (TRPV1) ion channel, endothelin A, and anti-nerve growth factor (anti-NGF) therapies relieve bone cancer pain (Fig. 4). It has been shown, using TRPV1 antagonists and TRPV1 knockout mice, that this acid-sensing ion channel contributes to bone cancer pain [Ghilardi et al., 2005]. Endothelin A, a receptor antagonist, has been shown to reduce pain [Peters et al., 2004] and treatment with anti-NGF antibody has been shown to reduce bone cancer pain [Sevcik et al., 2005].

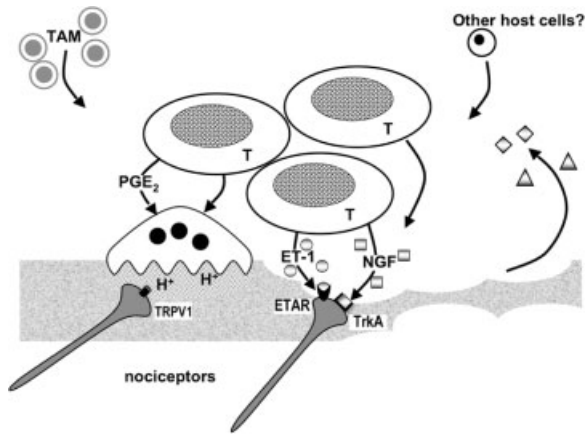

Fig. 4. Mechanisms of bone cancer pain. Tumor cells (T) act to cause pain in many ways. Production of prostaglandins (PGE_2) and other molecules by tumors, tumor-associated macrophages (TAM) and other host cells stimulate osteoclast-mediated bone resorption. Nociceptors in bone are stimulated via activation of transient receptor potential vanilloid type-1 (TRPV1), endothelin A receptor (ETAR), and TrkA receptor. Activation is directed by acid microenvironment (H^+), endothelin-1 (ET-1), and nerve growth factor (NGF), respectively.

CONCLUSION

Cancer-induced bone pain is a complex pain condition. Novel initiatives pursuing the etiology and treatment of bone cancer pain are

requisite to identify the mechanistic origins of this debilitating pain condition. Recent work, using experimental animal models that mimic patient-like states, have proven valuable by initiating study of bone cancer pain. From this research, induction of peripheral and central sensitization of the nervous system has been shown to originate from skeletal cancers. Continued investigations to elucidate molecular markers and mechanisms through which sensitization occurs will be important. Utilizing current and emerging animal models to test the efficacy of emerging therapies will direct future clinical management of this dreaded condition.

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