Comparative Medicine
Copyright 2008
by the American Association for Laboratory Animal Science

Vol 58, No 3
June 2008
Pages 220–233

Animal Models of Cancer Pain

Cholawat Pacharinsak[1,*] and Alvin Beitz[2]

Modern cancer therapies have significantly increased patient survival rates in both human and veterinary medicine. Since cancer patients live longer they now face new challenges resulting from severe, chronic tumor-induced pain. Unrelieved cancer pain significantly decreases the quality of life of such patients; thus the goal of pain management is to not only to alleviate pain, but also to maintain the patient's physiological and psychological well-being. The major impediment for developing new treatments for cancer pain has been our limited knowledge of the basic mechanisms that drive cancer pain and the lack of adequate animal cancer pain models to study the molecular, biochemical and neurobiological pathways that generate and maintain cancer pain. However this situation has recently changed with the recent development of several novel animal models of cancer pain. This review will focus on describing these animal models, many of them in rodents, and reviewing some of the recent information gained from the use of these models to investigate the basic mechanims that underlie the development and maintenance of cancer pain. Animal models of cancer pain can be divided into the following five categories: bone cancer pain models, non-bone cancer pain models, cancer invasion pain models, cancer chemotherapeutic-induced peripheral neuropathy models, and spontaneous occurring cancer pain models. These models will be important not only for enhancing our knowledge of how cancer pain is generated, but more importantly for the development of novel therapeutic regimes to treat cancer pain in both domestic animals and humans.

Abbreviations: COX2, cyclooxygenase 2; DRG, dorsal root ganglion; NSAID, nonsteroidal antiinflammatory drug; TRPV1, transient receptor potential vanilloid 1

Pain is one of the most common and distressing symptoms experienced by both human and veterinary oncology patients with advanced cancer. In humans, unrelieved pain can disrupt and interfere with activities of daily living, quality of life, and mood,[26,173] and domestic animals typically are euthanized when pain is no longer adequately controlled.[138] New developments in cancer detection and therapy have occurred over the past decade. These developments are contributing to longer life expectancies and have raised important issues related to quality of life, as attention has focused increasingly on how to manage cancer pain effectively.[91,115,134] This increased attention is true in the fields of both human and veterinary medicine. Human cancer patients who are in advanced stages of the disease, particularly those with bone metastasis, report that they experience significant pain, and pain intensity appears to be related to the degree of bone destruction. Similarly, pain secondary to cancer in domestic animals is a key concern in veterinary practice and should be addressed promptly to alleviate suffering, stress, and anxiety and to improve quality of life. Not only do cancer patients have to deal with persistent pain, they also often experience 'breakthrough pain.' Breakthrough pain—intermittent episodes of extreme pain—occurs spontaneously or after movement or weight-bearing of the affected leg.[114,133] Canine osteosarcoma has many clinical and biological similarities to human osteosarcoma, and affected dogs show signs of both ongoing and breakthrough pain.[37]

In addition to cancer-induced pain, human patients also experience pain caused by the very therapies used to treat the cancer. Almost 30% of adult cancer patients and 60% of pediatric cancer patients who have undergone treatments that include radiation, chemotherapy, or surgery also have experienced pain resulting from these therapeutic procedures.[49,174] Whether radiation treatment and chemotherapy also induce pain in domestic animals is virtually impossible to address, because carefully controlled studies have not examined this issue.

Pain intensity varies among cancer patients and is dependent on a patient's pain sensitivity, the type of cancer, and the tumor location.[48,49] Cancer treatment guidelines provided by the World Health Organization have been used in oncology and pain treatment clinics.[20,25,92,113,117,119,163] Treatment of human cancer patients include the use of opioids, nonsteroidal antiinflammatory drugs (NSAIDs), corticosteroids, local anesthetics, antidepressants, and anticonvulsants either alone or in combination. Similarly in veterinary medicine, the goal of palliative therapy in cancer patients is to control pain from an incurable tumor and to support overall quality of life.[165] Therefore, opioids, NSAIDs, and bisphosphonates are used in addition to surgery and radiation therapy and are the drugs of choice in treating domestic animals with cancer pain, particularly those suffering from osteosarcoma and other forms of bone cancer.[108] Although these medicinal treatments are the best drugs available at the current time, they often fail to control pain effectively in many terminal cancer patients or they have significant side effects: for example, opioids may cause sedation, respiratory depression, and interfere with gastrointestinal motility; NSAIDs can interfere with coagulation pathways or cause gastric ulcers and renal toxicity.[45,55,84,90,147,172] Clearly more effective treatments with greater efficacy for cancer pain are needed.

Received: 1 Nov 2007. Revision requested: 29 Nov 2007. Accepted: 18 March 2008.
[1]Department of Comparative Medicine, Stanford University School of Medicine, Stanford, CA and [2]Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St Paul, MN
[*]Corresponding author. Email: beitz001@umn.edu

New Approaches Using Animal Models

Despite the need for new treatments, one of the great impediments for discovering novel analgesics is our inadequate understanding of the basic neurobiology of cancer pain generation and maintenance. Over the past 20 years, several new animal models have been developed and used to further investigate cancer pain, neuropathic pain, and inflammatory pain.[100] Study of experimental animal models has provided insight into the mechanisms that drive bone cancer pain and provides an opportunity for developing targeted therapies. In this review, we will first summarize recent findings related to the mechanisms that drive cancer pain, then briefly discuss the methods used to quantify pain in animals, and finally describe the different types of animal models of cancer pain that have been developed. We have divided our discussion to address models for pain due to bone cancer, nonbone cancer, cancer invasion, cancer chemotherapy-related peripheral neuropathy; and spontaneously occurring cancer.[19,76,100,185] The most commonly used animal models of cancer pain have been developed in rodents, and therefore much of this review focuses on rodent models. However, recently described naturally occurring tumor models in dogs and cats have been developed, and these models also will be presented.

Literature Identification

This systemic review was performed by searching the following databases: PubMed, AMED (Allied and Complementary Medicine Database), CancerLit, and ILAR Animal Models. Where possible, searches were performed from 1980 to 2008. The databases were searched by using the words 'cancer pain' or 'tumor nociception' as the main search terms in the title, abstract, or key words of an article. Searches were performed by using the terms 'cancer pain' or 'tumor nociception' alone or in combination with 1 or more of the following terms: animal models, veterinary medicine, domestic animals, rodent models, mice, rats, dogs, cats, survey, cross sectional, follow-up, prospective, longitudinal, case control, and control group. The following journals were searched manually: *Pain*, *Journal of Pain*, *European Journal of Pain*, and the *Journal of Cancer Pain and Symptom Palliation*. In addition, cancer sites on the Internet were investigated for more up-to-date information on the most recent cancer pain publications. When papers were found, they were hand searched for cross-references. To avoid problems with understanding a language other than English and different categorization of animal models of cancer pain, we included only papers in English. The data primarily were extracted by 1 of the authors (CP) and were checked by the other author (AJB).

Papers were excluded from this review if they: 1) did not describe original studies; 2) did not have a well-defined control group; 3) did not include a statement indicating that the study was approved by an IACUC committee;[53] or 4) were not focused on mechanisms of cancer pain. Inclusion in the review was based on the following criteria: 1) the study was well-controlled and included a well-defined control group; 2) the study involved a distinct animal model of cancer pain as compared with models of tumor growth or metastasis; 3) the study provided mechanistic data relevant to understanding the causes of cancer pain.

Mechanisms that Drive Cancer Pain

During the past 25 y, many references in the clinical literature have indicated that cancer pain is generated and maintained by 1 (or a combination) of the following anatomic mechanisms: compression of bone, soft tissue, or peripheral nerve; vascular occlusion; and tumor infiltration. In addition, cancer pain can arise as a result of diagnostic or therapeutic surgical procedures (such as biopsies and resection) or, particularly in people, as a side effect of toxicity relating to therapies used to treat cancer (for example, chemotherapy and radiation therapy). Although anatomic factors including compression, vascular occlusion, and tumor infiltration provide a mechanistic rationale that explains the basis of tumor-induced pain in gross pathologic terms, they fail to address the basic biochemical, molecular, and neurobiologic mechanisms that underlie the production of pain in patients with cancer. In this regard, tumor pain, particularly that from bone cancer, represents 1 of the most severe types of chronic pain in both humans and animals. Unfortunately because the mechanisms that generate cancer pain were poorly understood until recently, the management of cancer pain has been largely empirical and based on scientific studies of noncancerous conditions such as inflammatory pain, where knowledge of the nociceptive mechanisms is quite extensive.

During the past decade, this lack of knowledge of the molecular, biochemical, and neurobiologic mechanisms that generate cancer pain has begun to be addressed, with the recent development of cancer pain models,[72,110,148,154,171,175,179,181,183,184] which are described in more detail later. These studies have resulted in the beginning of a mechanism-based understanding of the factors that generate and maintain cancer-induced pain. In this regard, it is now recognized that tumor cells themselves release a number of mediators that directly affect primary afferent pain fibers.[6,11,61,179,180] However, in addition to cancer cells, tumors contain inflammatory cells and blood vessels, which often are found in close proximity to primary afferent nociceptors and release mediators that affect these nociceptors. Therefore cancer cells, inflammatory cells, and vascular cells release a variety of products, including prostaglandins, ATP, bradykinin, cytokines, chemokines, nerve growth factor, and several vascular factors including endothelin 1 and vascular endothelial growth factor (VEGF), that either excite or sensitize the nociceptor. Once the nociceptor is activated, it sends an excitatory signal to the spinal cord where the nociceptive information is processed and then relayed via the spinothalamic and spinocervicothalamic tracts to higher centers of the brain. Based on the recently acquired knowledge of nociceptive mediators released at the tumor site, newer studies using animal models of cancer pain have demonstrated that blocking tumor-associated mediators, including TNFα,[180] endothelin,[179] calcitonin gene-related peptide,[178] nerve growth factor,[151] or cyclooxygenase 2 (COX2),[143] significantly reduces tumor-induced nociception. Although this information holds promise for the development of new therapies for cancer pain, blocking these mediators individually is not sufficient to block cancer pain completely, indicating that tumor-induced pain is produced by multifaceted mechanisms. Several recent reviews[28,40,60,61,70,101-104,114,137,145,169,170,183] focus on the mechanisms of cancer pain and the mediators involved, and the reader is referred to these excellent sources for more complete summaries of this information.

Methods Used to Quantify Pain in Animals

Numerous behavioral tests are used to quantify pain in rats and mice, and some of these tests recently have been adapted

to test pain in dogs and cats. The 2 most common tests are the radiant heat paw-withdrawal test[64] and the von Frey test.[177] The radiant heat paw-withdrawal test is used to assess thermal sensitivity. In this test, a noxious stimulus, a high-intensity beam from a projector lamp bulb located below an unheated glass floor, is aimed at the plantar surface of the mid-hindpaw. The latency in seconds to withdrawal or pain behavior (vigorous shake) is measured and recorded as a measure of tumor-induced thermal hyperalgesia (an increased response to noxious heat stimuli) or allodynia (an increased response to nonnoxious heat stimuli). In the von Frey test, filaments of various thicknesses are applied against the central edge of the hindpaw. Paw withdrawal caused by the stimulation is registered as a response. Several other behavioral tests have been used to measure pain in animals, but because of lack of sufficient space to cover this topic adequately, we refer the reader to several papers and reviews[14,19,77,88,120,121] that address methods used to quantify pain in animals.

For measuring chronic pain conditions like tumor pain, various other measures have been proposed: self-administration of analgesic; conditioned place aversion; gait or weight-bearing disturbance; grip or bite force; grooming (scratching, licking, and biting) behavior; guarding (abnormal positioning); hindpaw lifting, flinching, or shaking (nocifensive behaviors); hypolocomotion; hypophagia and weight loss; inattention to novel stimuli, and ultrasonic vocalization. Unfortunately such spontaneous measures of chronic pain have only been used in a handful of studies involving animal models of cancer pain. As some authors have pointed out, "The greater practical demands associated with measuring spontaneous nociception in animals, combined with the lack of consensus over exactly which behavior(s) to measure, have conspired to favor the continuing and virtually exclusive measurement of hypersensitivity states."[120] If one agrees with the contention that spontaneous pain is a more serious clinical problem than hypersensitivity, then it seems logical that incorporating measures of spontaneous pain in the analysis of tumor pain in animal models would be more clinically relevant.[97]

Models of Cancer Pain

Bone cancer produces one of the most painful conditions that affects humans and animals. The pain from bone cancer also represents the most common pain in human patients with advanced cancer, because most common tumors, including breast, prostate, and lung cancers, have a remarkable propensity to metastasize to bone.[62,113] The first animal models developed to study cancer pain were models of primary and metastatic bone tumors. This advance was followed by the development of nonbone cancer pain models that mimic other types of malignant lesions, including pancreatic cancer and squamous cell carcinoma, as well as, benign, but painful, neuromas. In addition animal cancer pain models have been developed that replicate the pain caused by tumor invasion of peripheral nerves and the pain produced by cancer chemotherapy-related peripheral neuropathy. Finally naturally occurring tumors that arise spontaneously in animals are being used as more natural models of cancer pain. Each of these different models will be discussed in more detail in the following sections.

Models of bone cancer pain The most common presenting symptom of bone cancer is bone pain; the tumor grows, the pain becomes more severe.[32] As bone pain becomes more severe, bone remodeling occurs; as remodeling progresses, breakthrough pain, which is difficult to treat with standard therapies,[114] can occur during weight-bearing on or movement of the affected bone. Thus animal models that mimic both bone tumor pain and remodeling hold promise for understanding the mechanisms contributing to the development of tumor-induced bone pain. In this regard, a key advantage of recently developed animal models of bone cancer pain is that they share many characteristics that occur in the human bone cancer conditions, including the pain and skeletal remodeling that accompanies metastatic bone cancer.[33,115] These bone cancer pain models are based on intramedullary injection of cancer cells directly into bone. Consequently the location of the resulting bone tumor can be carefully controlled, unlike after systemic or intracardiac administration of tumor cells. In addition, these models allow easier assessment of tumor growth over time, as well as radiographic imaging, bone destruction observation, assessment of histopathologic changes, accurate site-specific behavioral analyses, and appraisal of both neurochemical and neuroanatomical changes that occur at the tumor site, in the dorsal root ganglion (DRG), and within the spinal cord and other levels of the CNS. Animal models of bone cancer pain have been developed in both mice and rats, and these models will be discussed separately. Because of their small bone size, mouse models usually are generated by surgically implanting tumor cells directly into the bone (that is, femur or humerus). In contrast, rat models generally are produced by percutaneous injection of cancer cells into bone, such as the tibia.[60] These models are summarized in Table 1.

Mouse models of pain from bone cancer. The first animal model of cancer pain was developed in 1998. In this mouse model, 10^5 (20 µl) fibrosarcoma cells (NCTC 2472) were implanted directly into the femur. A crucial component of this model is that the tumor cells are confined to the marrow space of the injected femur and do not invade adjacent soft tissues.[148] After injection, both ongoing and movement-evoked pain-related behaviors increase as the cancer cells proliferate and the tumor develops. These behaviors are correlated with the progressive tumor-induced bone destruction that ensues, and they seem to mimic those of patients with primary or metastatic bone cancer.[103] This model was used to obtain new information about the mechanisms that generate bone cancer pain.[148,182,183] This femur bone cancer model has been used to examine tumor-induced bone destruction, pain behaviors, and spinal cord neurochemical changes in the mouse (B6C3-Fe a/a and C3H/HeJ).[72,148] The animals with intramedullary femur tumors showed nocifensive behaviors (vocalization and guarding of the affected limb) and mechanical allodynia (a response to nonnoxious mechanical stimuli, such as light touch or palpation). This model also revealed important neurochemical changes in the spinal cord including 1) an increase in dynorphin (a prohyperalgesic neuropeptide) expression in deep laminae of the spinal cord dorsal horn; 2) an increase in c-fos expression (a marker of neuronal activation) in spinal cord lamina I; and 3) an internalization of substance P (an important neurotransmitter in nociception) receptors in the ipsilateral tumor-injected side of the spinal cord.[72,148] These spinal cord changes are normal after application of a noxious stimulus, but they also are present in cancer-affected animals after nonnoxious stimuli (in this case, palpation).

Another unique finding in tumor-bearing mice was massive astrocyte hypertrophy in the spinal cord dorsal horn, which is uncommon in inflammatory or neuropathic pain conditions and thus represents a unique signature of cancer pain. Although

Table 1. Models for pain from bone cancer

Model	Cancer type	Location	Reference(s)
Mouse (C3H/He; C3H/HeJ, B6C3-Fe-a/a, C3H/HeNCrl, Nude)	Sarcoma	Femur	45, 57, 58, 63, 69, 71, 82, 97, 98, 130, 148, 175
Mouse (C3H/He, B6C3fe/1)	Fibrosarcoma Melanoma	Calcaneous	81, 179, 180
Mouse (C3H/HeJ, B6C3fe/1)	Fibrosarcoma Melanoma Osteosarcoma	Humerus	21, 79, 176, 181
Mouse (C3H-SCID, C3H/HeJ)	Sarcoma Melanoma Colon adenosarcoma 4T1 breast cancer cells	Femur	46, 143, 144
Mouse (Swiss CD1)	XC Rous sarcoma virus-transformed rat fibroblasts (XC cells)	Intraplantar region	11
Mouse (C3H/HeJ)	Sarcoma	Tibia	111, 112
Mouse (C3H/HeJ)	Hepatosarcoma Sarcoma	Thigh, dorsum of the foot, intraplantar region	5, 89, 129, 149
Rat (Sprague Dawley, Wistar)	Mammary gland carcinoma cells (MRMT1, Walker 256)	Tibia Intraplantar region	13, 18, 50, 105, 110, 123
Rat (Sprague Dawley)	Mammary gland carcinoma cells (MRMT1)	Tibia	41, 42, 171, 172, 184
Rat (Copenhagen)	Prostate cancer cells (AT3.1, R3327)	Tibia	93, 192
Rat (Nude)	Breast cancer cells (MDA-MB 231) Human prostate cancer (CWR22)	Femoral artery	3, 12

this massive astrocyte hypertrophy was evident in the mouse femur bone cancer model, whether this increase is related to the generation or maintenance of bone cancer pain remains unclear. These profound neurochemical changes and reorganization of the spinal cord may be involved in central sensitization.[72,148] In human cancer patients, pain seems to relate closely to the degree of bone destruction.[101,104] Because osteoclast activity is crucial for bone resorption,[28,101,104] if osteoclast activity causing bone destruction could be inhibited, cancer-induced pain might be relieved.[30,60,69,70,164] On the basis of this concept, several investigators demonstrated that they could administer the novel analgesic osteoprotegerin ligand and successfully treat cancer pain in male C3H/HeJ mice.[69] Osteoprotegerin ligand is a member of the tumor necrosis factor family and blocks osteoclast activity that causes cancer-induced bone destruction. The study found that osteoprotegerin ligand inhibited both the pain-related behaviors and the neurochemical changes (increased dynorphin, c-fos expression, internalization of substance P receptors, and astrocyte hypertrophy) that occurred in cancer-inoculated mice.[69] Further, a recent study has shown that activation of the transient receptor potential vanilloid 1 (TRPV1) receptor plays a critical role in the generation of bone cancer pain. Bone cancer increases the expression of this protein within a distinct subpopulation of DRG neurons in mice with femur tumors.[125] This result, coupled with the finding that destruction of DRG neurons containing the receptor for TRPV1 in rats and dogs completely inhibits bone cancer pain in these animals, suggests that the development of drugs targeting these receptors will be effective in relieving bone cancer pain.[78]

Several studies have now examined the efficacy of morphine in treating bone cancer pain as compared with inflammatory pain. In human patients, systemic morphine dose-dependently attenuates pain-related behaviors. In mice with bone tumors, the effective morphine dose required to relieve tumor-induced pain was 10 times higher than that required to relieve inflammatory pain induced by injection of mice with complete Freund adjuvant, an

algesic agent.[98] This need for an increased dose is due to downregulation of μ opioid receptors in the DRG of tumor-affected animals compared with those with inflammatory pain, in which expression of μ opioid receptor actually is increased.[191]

Similarly, in a model that may be more relevant for canine osteosarcoma patients, implantation of fibrosarcoma cells into the humerus of mice produced forelimb hyperalgesia and required a 3-fold higher dose of morphine to effectively treat hyperalgesia as compared with the dose needed to reduce hyperalgesia induced by the injection of the algesic agent carrageenan into the humerus.[175,181] In addition to morphine, other μ agonists including fentanyl[45] and sufentanyl,[45] as well as antinerve growth factor,[63] effectively reduce pain-related behaviors in a dose-dependent manner. Cancer-induced hyperalgesia can also be attenuated by peripheral opioids[112] or cannabinoid receptor antagonists.[79] Although morphine seems to be relatively effective for the treatment of bone tumor-induced pain in these animal models, a recent study of chronic morphine administration using a mouse bone fibrosarcoma model found that chronic administration of morphine increased osteoclast activity, osteolysis, spontaneous fracture, quantities of the proinflammatory cytokine IL1β, and pain in mice with sarcoma.[82] Therefore long-term use of morphine to treat bone tumor pain may have the detrimental effect of increasing bone destruction and thereby causing additional pain. More investigation is necessary to determine whether this result also occurs in domestic animals.

The effects of radiotherapy on tumor-induced pain have been studied in mice implanted with sarcoma cells into the humerus.[176] Sarcoma cells (2×10^5 in 5 μl) were implanted into humeri of female C3H/HeJ mice. Seven days after 6-Gy radiation, the performance of tumor-implanted mice on both the rotarod and grip force tests showed significant improvement. A similar result was obtained when using the COX (cyclooxygenase) inhibitor, ketorolac. In addition, after radiation, the increased dynorphin levels and astrocyte hypertrophy seen with other mouse cancer pain models were both reduced.[176] When sarcoma cells (10^5 in 20 μl) were implanted into mouse femurs to examine the effects of 20-Gy and 30-Gy radiation as a means to control cancer pain, these radiation therapies effectively decreased cancer-induced osteolysis, reduced tumor size by 75%, and decreased bone cancer-related pain.[57-59] Radiation therapy also successfully controlled pain and skeletal fractures associated with femoral implantation of 4T1 breast carcinoma cells into mice.[59] These studies demonstrated that radiation therapy effectively decreased cancer-induced pain by direct effects on tumor cells.[57,58] Support for the effects of radiation therapy also comes from studies in which transplantable hepatocellular carcinoma cells, HCa-1, were injected into the periosteal membrane of the foot dorsum in C3H/HeJ mice.[129,149] In these studies, mice treated with radiotherapy showed decreased objective levels of pain (decrease mechanical hyperalgesia) beginning 3 d after irradiation compared with that in control nonradiated mice. Moreover, radiotherapy dramatically decreased expression of calcitonin gene related peptide in the spinal cord. Because this protein is known to play an important role in the development of hyperalgesia, the radiation-induced reduction of calcitonin gene related peptide correlates well with the decrease in tumor-induced pain and provides a mechanism by which radiation therapy is effective in reducing cancer pain. Finally, a recent study used a mouse bone tumor model to demonstrate that administration of the antiangiogenic inhibitor bevacizumab together with radiation therapy reduced the frequency of pain-associated behaviors, decreased levels of nociceptive protein expression in the spinal cord, maintained cortical integrity and decreased the density of microvessels as compared with the effects of single-modality treatments.[195]

In addition to murine models of bone cancer that use injection of cancer cells into the femur or the humerus, another model injects tumor cells directly into the calcaneous bone of the mouse hindpaw.[21,179] The advantages of this model are that it allows easy quantification of hyperalgesia, better microperfusion access to measure the release of pain mediators from the tumor, and easier electrophysiologic recordings of nerves innervating the tumor. It is extremely difficult to record from nerves innervating tumors located in the femur or humerus. This hindpaw bone tumor model has been used to record electrophysiologically from primary afferent fibers innervating the calcaneous tumor in male mice.[21,81] Mice with calcaneous tumors showed pain-related behaviors and mechanical hyperalgesia (an increased pain response to a mechanical stimulus that is normally painful), which could be measured easily. Electrophysiologic recordings from primary afferent fibers in control and hyperalgesic mice with tumor revealed the development of spontaneous activity in 34% of pain fibers (C fibers) adjacent to the tumor 9 to 17 d after implantation. The development of spontaneous activity and a decrease in thermal thresholds for activation in C fibers of mice with calcaneous bone tumors suggest activation and sensitization of a population of C fibers, which contribute to the observed behavioral hyperalgesia.[21] Central sensitization likely also occurs in these mice as well as in dogs and other domestic animals that have bone tumors, and this sensitization is probably maintained by the spontaneous activity of pain-transmitting C fibers.

When fibrosarcoma or osteosarcoma cells were implanted into the calcaneous bone of male B6C3fe/1 mice, these mice developed cancer-related bone destruction (similar to findings from the murine femur and humerus after implantation of fibrosarcoma cells into these bones) and thermal hyperalgesia.[179] Studies that have examined the mediators released at the tumor site have identified a large number of potential algogenic (pain-causing) substances released by the tumor itself or by the bone that is being broken down. For instance, the peptide endothelin 1, which is expressed by numerous tumor types, is release by fibrosarcoma cells and contributes to bone cancer pain.[6,34,86,179,193] High levels of endothelin 1 were found and activation of primary afferent fibers occurred in mice with calcaneous fibrosarcoma tumor but not in control mice implanted with nonpainful melanoma cells into the calcaneous bone.[179] The finding that hyperalgesia occurred only in fibrosarcoma-implanted mice and not in melanoma-implanted mice suggests that endothelin 1 contributes to cancer-related pain associated with fibrosarcoma tumors.[66,130,179] Fibrosarcoma-implanted mice also exhibited a significantly elevated level of TNFα, a proinflammatory cytokine released by fibrosarcoma cells as well as various other cell types.[180] Intraplantar injection of TNFα caused mechanical hyperalgesia in naive mice and increased hyperalgesia in mice implanted with fibrosarcoma cells into the calcaneous bone. Importantly intratumor injection of the soluble TNFα receptor antagonist blocked tumor-induced mechanical hyperalgesia in these mice indicating that TNFα is an important mediator involved in the development of bone tumor-induced mechanical hyperalgesia. This fact raises the possibility

that TNFα receptor antagonists may be useful in treating bone cancer pain.

Rat models of pain from bone cancer. A rat model of bone cancer pain was developed in 2002 by using mammary gland carcinoma cells.[110] In this model, 3×10^3 or 3×10^4 syngeneic MRMT1 mammary gland carcinoma cells were implanted into the tibias of female Sprague–Dawley rats. Animals inoculated with MRMT1 cells gradually showed signs of mechanical hyperalgesia in weight-bearing tests and developed mechanical allodynia as measured by using von Frey monofilaments on days 10 to 12 and 12 to 14 postimplantation, respectively. In MRMT1-injected rats, bone destruction was evident by day 15. The numbers of tartarate-resistant acid-phosphatase–positive polykaryocytes, which were activated by prostaglandins, cytokines, and growth factors from tumor cells, were also increased. Similar to findings from mouse cancer models, astrocyte hypertrophy was evident by day 17 based on the increased expression of glial fibrillary acidic protein, a marker of astrocyte activity, and this increased activation was specific to bone cancer. In this study,[110] rats implanted with 3×10^4 MRMT1 cells were euthanized on day 16 due to bone deterioration. No significant changes were found in either heat-killed MRMT1- or vehicle-treated groups, and weight loss and body temperature were unchanged in all groups. Because previous findings of bisphosphonate-induced reduction of bone pain suggested that osteoclasts play a critical role in bone pain, this model was used to address that question. The acidic environment created by osteoclasts, at least in part, contributes to the induction of hyperalgesia associated with bone tumors, due to upregulation of the acid-sensing channel ASICS in DRG neurons.[123]

With respect to the pain produced by the implantation of MRMT1 cells into the rat tibia, morphine dose-dependently attenuated mechanical allodynia and hyperalgesia, whereas the COX2 inhibitor celecoxib was ineffective, suggesting that prostaglandins may not contribute to cancer pain in the rat MRMT1 carcinoma model.[110,184] This ineffective COX2 treatment differs from other results, which showed that the COX2 inhibitor lumiracoxib administered twice daily for 10 d attenuated mechanical hyperalgesia and bone destruction in this tibia bone cancer model.[50] Although reconciling the findings of these 2 studies is difficult, the fact that the structure of lumiracoxib differs from those of standard COX2 inhibitors (for example, celecoxib) may account for the differences. In support of a positive effect of COX2 inhibitors on cancer pain, COX2 inhibitors have also been shown to attenuate pain in the femur bone cancer mouse model.[143] Because COX2 expression is upregulated in canine appendicular osteosarcomas,[122] COX2 inhibitors represent a reasonable first-line treatment for bone cancer pain in domestic animals.

In addition to behavioral studies in the rat tibia model, electrophysiologic recordings of MRMT1-injected animals showed that the receptive field size for superficial spinal cord neurons was enlarged and that nociceptive-specific neurons in the spinal cord, which normally only respond to noxious stimuli, were excited by nonnoxious stimuli.[171] The responses of superficial wide dynamic range neurons (WDR), generally excited by both nonnoxious and noxious stimuli, were dramatically enhanced, whereas deeper WDR neurons showed minimal changes, suggesting involvement of both ascending and descending facilitation pathways.[171,172] To further study central descending serotonergic pathway modulation in this model, a serotonergic receptor antagonist, ondansetron, was administered intrathecally in MRMT1-injected rats. Ondansetron significantly decreased responses to mechanical and thermal, but not electrical, stimuli in both tumor and naive animals, suggesting that descending serotonergic pathways can effectively modulate tumor-induced pain at the level of the spinal cord.[42] In MRMT1-injected rats, the antihyperalgesic drug gabapentin effectively attenuated pain-related behaviors, and electrophysiologic recordings of the spinal cord indicated reduced responses to electrical and mechanical but not thermal stimuli.[41] Although gabapentin is used widely to treat neuropathic pain, consistent but perhaps less compelling clinical evidence supports its use also for cancer pain. Therefore gabapentin should be considered as an alternative to standard opiates and NSAIDS.

Glial cells in the spinal cord reportedly play a role in enhancing pain and in the process of central sensitization.[150,186,187] Therefore, implantation of AT3.1 prostate cancer cells (3×10^5 in 10 µl) into the tibia of male Copenhagen rats was used to study spinal glial activation under conditions of bone cancer pain. The animals with implanted prostate cancer cells demonstrated several characteristics: pain-related behaviors including thermal hyperalgesia, mechanical hyperalgesia, and flinches; bone destruction 1 wk after tumor implantation; massive astrocyte hypertrophy in the ipsilateral side of the spinal cord; and upregulation of spinal cord IL1β, a proinflammatory cytokine.[192] Therefore studies in both mouse and rat models of bone cancer pain indicate that glial cell activation plays a critical role in the development of the chronic pain state associated with bone cancer. Because inhibition of spinal cord glial cell activation remarkably reduces neuropathic pain in several animal models,[95,116,127] the development of novel glial cell inhibitors likely will result in improved treatment of bone cancer pain within the next decade.

Skeletal metastasis is a serious complication of certain neoplastic diseases, including breast, prostate, and lung cancer.[16,31] To investigate metastatic bone cancer, MDA-MB231 human breast cancer cells (5×10^5 in 1 ml) were injected into the femoral arteries of nude rats.[12] Osteolytic lesions occurred exclusively in the femur, tibia, and fibula of these animals, and if the tumor cells were preincubated with an antibody against bone sialoprotein, osteolytic lesion size was reduced significantly. In contrast, R3327 prostate cancer cells were injected directly into the left cardiac ventricle, intravenously, or intraosseously into male Copenhagen rats for observation of metastatic lesions and their relationship to pain.[93,94] Bone lesions were observed in bone scans after intraosseous injection but not after intraventricular or intravenous injections of prostate cancer cells. The investigators concluded that the intraosseous administration of R3327 prostate cancer cells represents a useful and effective osteoblastic bone lesion model. Although these rat models mimic what occurs in metastatic cancer in both humans and domestic animals, they suffer from the fact that the tumors involve multiple bones at multiple sites and therefore the nociceptive responses and pain are difficult to evaluate.[93,94]

Compared with the mouse femur cancer model, the tumors in the rat models described above do not affect the joints, muscles, or ligaments, which offers some advantages. The rat model in which 3×10^3 MRMT1 cells are implanted into the tibia may be a more suitable cancer pain model than the mouse femur model because both mechanical allodynia and hyperalgesia developed in the rat model without significant undesirable side effects during the study's 20-d time course.[110] Moreover injection of tumor cells into rat bones is far easier than into mouse bones because of the rat's

larger bone size. However, the mouse models are advantageous because tumor experiments can be performed on knockout mice or transgenic mice that over- or underexpress various proteins to determine the role of these proteins in cancer-induced pain. Collectively, studies from both mice and rats suggest that different bone cancer models have different underlying mechanisms depending on species, tumor types, and tumor location and that the most appropriate model will depend on the experimental design and the questions addressed by a particular investigation. Nonetheless these recently introduced models of bone cancer pain are not only providing insight into the mechanisms that drive bone tumor pain but also are guiding the development of novel mechanism-based therapies to treat the pain and skeletal remodeling associated with both primary and metastatic bone cancer.

Models of pain from nonbone cancer Several models of cancer pain arising from tumors located outside of bone have been developed and are summarized in Table 2. These include models of pancreatic cancer, squamous cell carcinoma and neuroma. With respect to pancreatic cancer, the source of visceral cancer pain is often difficult to detect, and the clinical symptoms associated with visceral cancer usually are not noticed until the cancer has progressed to an advanced stage. Pancreatic cancer typically is detected during its late stages, and pain management becomes a factor in maintaining the quality of life for pancreatic cancer survivors. In humans, pancreatic cancer represents about 2% of new cancer cases.[74] Pancreatic cancer is slightly more common in cats than dogs, and there is no known underlying cause. A transgenic mouse model of cancer pain was developed recently in which pancreatic cancer arises due to expression of the simian virus 40 large T antigen under control of the elastase 1 promoter.[96] By use of this transgenic mouse model with spontaneous pancreatic cancer development, pain-related behaviors (hunching and vocalization) were quantified at early, intermediate, and late stages of cancer to investigate the involvement of the endogenous opioid system. Precancerous cellular changes were evident at 6 wk in these mice and included increases in microvascular density, macrophages that expressed nerve growth factor, and the density of sensory and sympathetic fibers that innervated the pancreas.[96,152] Changes in pain-related behaviors, such as morphine-reversible severe hunching and vocalization only became evident at 16 wk of age, by which time the pancreatic cancer was highly advanced; this pattern mimics what is observed in human patients, in whom pain typically is not evident until the cancer is quite advanced. Importantly, administration of the CNS-penetrating opioid antagonist naloxone, but not of an opioid antagonist incapable of crossing the blood brain barrier into the CNS, led to overt pain-related behaviors in mice with early-stage pancreatic cancer. The investigators concluded that a CNS opioid-dependent mechanism tonically modulates early- and late-stage pancreatic cancer pain.[51,96,152] Understanding the mechanisms that mask this pain in early-stage disease and drive this pain in late-stage disease may facilitate improved diagnosis, treatment, and care of patients with pancreatic cancer.

Pancreatic cancer cells that infiltrate the perineurium of local intrapancreatic nerves might cause pancreatic neuropathy[24] and therefore visceral pain. What actually causes pain in pancreatic cancer is unknown currently, but the generation and maintenance of pancreatic cancer-related pain may involve neurogenic inflammation,[38] and administration of vanilloid receptor antagonists might be an effective treatment of choice.[65]

Other models of nonbone cancer pain include squamous cell carcinoma[124] and benign neuromas.[43,167] In the rat orofacial cancer model, squamous carcinoma cells are injected into the subperiosteal tissue of the lower gingiva. Inoculation of cancer cells induces marked mechanical allodynia and thermal hyperalgesia in the ipsilateral maxillary and mandibular nerve, and these effects are associated with increased expression of calcitonin gene related peptide, substance P, $P2X_3$ receptors, and TRPV1 in the trigeminal ganglia.[124] Clearly identification of the upregulation of these proteins may lead to the development of novel therapeutics for the treatment of orofacial cancer.

In the tibial neuroma transposition model of neuroma pain, the tibial nerve is ligated and placed just superior to the lateral malleolus, and a neuroma is allowed to form.[43,167] Mechanical stimulation of the neuroma produces a profound withdrawal behavior in these rats. Although this model might be more representative of neuropathic pain than tumor pain, it does provide a useful tool to investigate the various mechanisms underlying the tenderness of the neuroma and mechanical hyperalgesia associated with neuropathic pain. Because tumors often invade nerves and establish neuropathic pain, the tibial neuroma transposition model is not an unreasonable model for investigating the mechanisms by which tumor-induced nerve injury causes pain.

Models of pain due to cancer invasion Cancer invasion of peripheral nerves often occurs in patients with vertebral metastasis or malignant lymphomas and during tumor progression as the tumor invades surrounding nerve bundles. Each of these conditions can lead to tumor-induced neuropathic pain syndromes.[106] Therefore, animal models that mimic cancer-induced neuropathic pain have been developed and can be broadly classified as cancer invasion pain models.[153] In an initial study from our group, we showed that implantation of fibrosarcoma cells near the sciatic nerve produced significant mechanical allodynia 11 to 23 d postimplantation, correlating with perineural invasion by tumor cells.[183] In a similar experimental design, MethA sarcoma cells were used to induce cancer-related nerve injury or neuropathy by implantation of these cells in close proximity to the sciatic nerve in male BALB/c mice. This model benefited from the slow progression of this rather destructive tumor,[153] but more importantly this experiment illustrated that cancer-related neuropathy causes spontaneous pain (paw lifting and guarding), thermal hyperalgesia, and allodynia. These responses are consistent with the increased pain that is associated with tumor invasion of nerves in human patients.[194] In addition, mechanical allodynia present on day 10 of the model changed to mechanical hyposensitivity by day 14. Damage to both myelinated and nonmyelinated fibers were more extensive in this cancer-induced neuropathy model than in the sciatic nerve ligation (chronic constriction injury) model, suggesting cancer-associated nerve compression differs mechanistically from nerve ligation. Similar to other previous mouse cancer models, animals injected with MethA cells showed upregulation of dynorphin, c-fos expression, and substance P in the spinal cord.[154]

Models of pain due to chemotherapy-related peripheral neuropathy As advances in cancer detection and treatment have increased the life expectancy of cancer patients, more attention to improving both human and animal patient quality of life is required. The major sources of cancer-induced pain in these patients are not only the cancer itself but also side effects of the various therapeutic treatments used, including radiation therapy, surgery,

Table 2. Models for pain from nonbone cancer

Model	Cancer type	Location	Reference(s)
Mouse (Transgenic)	Pancreatic cancer	Pancreas	96, 152
Rat (Fisher)	Squamous cell carcinoma	Gingiva	124
Rat (Sprague Dawley)	Neuroma	Tibia Sciatic nerve	43, 167

and chemotherapy.[68] Treatment-associated pain can impede the cancer patients' quality of life during the course of cancer treatments and, in the case of chemotherapy, may result in limiting the dose of the treatment. Patients may develop chemotherapy- or radiation-induced peripheral neuropathies that are as painful or more so than the original cancer that they were designed to treat. In addition, chemotherapy-induced neuropathy may persist well beyond the discontinuation of treatment (coasting).[136]

Chemotherapy-induced neuropathy varies depending on dose, treatment duration, and other concurrent or preexisting conditions of the patients. Because its underlying cause remains poorly understood, an additional type of cancer pain model was developed to investigate cancer chemotherapy-related pain; these models are summarized in Table 3. These models involve the induction of peripheral neuropathy by chemotherapeutic agents and were developed to elucidate the mechanistic-based pathophysiology of chemotherapeutic agent-induced neuropathy. The well-known cancer chemotherapeutic drugs vincristine,[1,159] paclitaxel,[22,23,118] and cisplatin[22,157] are used in both human and veterinary medicine and all yield neuropathic pain after extended use.[131] This portion of the review will focus on the models that involve these 3 chemotherapeutic agents.

Models of pain due to vincristine-related peripheral neuropathy. One of the most commonly used chemotherapeutic agents is vincristine, which belongs to the vinca alkaloid family,[67] and one of the main limiting complications of vincristine is that it causes painful peripheral neuropathy. Vincristine binds to intracellular tubulin and alters microtubular structures, causing a dose-dependent neuropathy. The signs of neuropathy start with paraesthesia, followed by hyperesthesia.[136] A dose of vincristine as low as 50 μg/kg produces mechanical hyperalgesia, allodynia, and thermal hypoalgesia in rats.[7] Intravenous vincristine induced mechanical hyperalgesia within 2 wk after initiation of the chemotherapy regimen in Sprague-Dawley rats.[1] However, 2 wk after discontinuation of vincristine, the signs of mechanical hyperalgesia were ablated.[1] Vincristine caused greater mechanical hyperalgesia in female than in male rats[75] and increased electrophysiologic responses to suprathreshold stimuli in C fiber nociceptors; both C and A fiber mean conduction velocities were slower; and no histopathologic changes were evident.[159,160] Therefore vincristine does not impair nociceptor function per se but rather interferes with mechanisms underlying responsiveness to suprathreshold stimuli.[160] Histopathologic examination of samples from animals treated with vincristine revealed a significant increase in the cross-sectional area of myelinated axons, a dramatic decrease in the number of axonal microtubules, and disorganization of microtubules of myelinated axons.[166] Continuous infusion of vincristine led to dose-dependent mechanical allodynia but not thermal hyperalgesia.[126] Mechanical allodynia started after 1 wk of vincristine infusion and returned to the baseline values by 4 wk, whereas cold allodynia occurred 1 wk after the infusion.[99] This mechanical hyperalgesia could be attenuated by morphine or lidocaine administration[126] but not by the μ opioid agonist DAMGO.[1] Because vincristine treatment produces different results in different experimental paradigms—hyperalgesia, hypoalgesia and allodynia, its mechanisms of action remain to be identified. However, recent work in human patients has shown that chronic vincristine-induced pain is associated with dysfunction in Aβ-, Aδ-, and C-caliber primary afferent fibers.[44] Deficits in Aβ fibers appear to precede and presage deficits in the other fiber types, whereas deficits in Aδ and C fiber function appear to be specifically associated with the generation of pain.

Models of pain due to paclitaxel-related peripheral neuropathy. Another chemotherapeutic drug that causes neuropathy is paclitaxel, which is used to treat various cancers including breast cancer, nonsmall cell lung cancer, head and neck cancer, melanoma, and ovarian cancer.[83,139,140,142,146,156,188] Numerous cancer patients who are treated with paclitaxel complain of tingling, numbness, and burning pain.[23,83,128,132,139,141,142,158,168,189] Paclitaxel, which is a vinca alkaloid, binds to tubulin blocking the polymerization of microtubules and interfering with mitosis, and reported side effects include myelosuppresion and sensory or sensory–autonomic neuropathy.[35,73,83,85,132,136]

In laboratory settings, paclitaxel-induced neuropathy lasted for several weeks and was mostly limited to peripheral nerves, with the adult rats showing no clinical systemic toxicity.[23,132] In addition, this agent induced mechanical and thermal hyperalgesia without motor deficits in Sprague–Dawley rats.[131,132] In contrast, in a different study paclitaxel produced motor neuropathy, gait disturbances, and abnormal rotarod performance during the light and dark cycles within 2 wk of treatment.[17] Electrophysiologic recording revealed decreases in H-wave amplitudes in the hindlimb and in action potentials in sensory nerves in the tail; paclitaxel affected all sensory modalities, especially those mediated by thick myelinated nerve fibers[27,136] and the effect could be blocked by intraperitoneal injection of gabapentin.[107] In addition, paclitaxel-treated animals demonstrated severe axonal degeneration and hypomyelination of the dorsal roots but not the ventral roots. This model revealed that paclitaxel produced minimal effects on the general health of the rats, similar to the pattern in human patients treated with the drug.[27] A recent study of the responses of 10 inbred mouse strains to paclitaxel injections showed that virtually all strains developed statistically significant mechanical allodynia, with 1 strain, DBA/2J, exhibiting especially robust changes. DBA/2J and C57BL/6J mice showed comparable cold allodynia, but neither strain showed evidence of thermal hyperalgesia.[155] In CD1 mice, paclitaxel reduced both peptide neurotransmitters, including substance P, in DRG and the action potential amplitude of the caudal nerve. These neurotoxic effects were prevented by administration of nerve growth factor.[4] Glu-

Table 3. Models for pain from peripheral neuropathy due to cancer chemotherapy

Model	Drug	References
Rat (Sprague Dawley)	IV vincristine	1, 7, 67, 75, 99, 126, 159, 160, 166
Mouse (CD1,ddY,C3H/He, C57BL/6, 129P3, A, AKR, C57BL/10, CBA, DBA/2, RIIIS, SM, BDF1)	IP paclitaxel	4, 107, 118, 155
Rat (Sprague Dawley, Wistar, Dark Agouti)	IP paclitaxel	9, 17, 23, 27, 39, 131, 132
Rat (Sprague Dawley, Wistar, CD1, Dark Agouti)	IP cisplatin	8, 10, 17, 36, 161, 162

tamate also may have a neuroprotective effect in preventing the neuropathy induced by paclitaxel administration.[17]

Models of pain due to cisplatin-related peripheral neuropathy. A chemotherapeutic drug commonly used for treating ovarian cancers and small cell lung cancer is cisplatin, which causes not only ototoxicity and nephrotoxcity but also neurotoxicity, such as peripheral polyneuropathy, mechanical hyperalgesia, and allodynia, in rats.[8] These neuropathy symptoms are described as numbness and tingling, and these symptoms can be severe with increasing cumulative doses.[56] Polyneuropathy caused by cisplatin can last for more than 10 y, and the severity of the resulting neuropathy depends on the dose and duration used.[157] In an animal model designed to mimic the condition in human patients, rats received 3 different cisplatin injections at a cumulative dose of 15 mg/kg. These animals showed mechanical allodynia and hyperalgesia that lasted as long as 15 d after injection.[8] Moreover this treatment regimen caused gait disturbance within 8 wk of administration.[17] The advantage of this particular rat model is that the animals were in good health throughout the study and that the cisplatin-induced sensory peripheral neuropathy symptoms progressed rapidly,[8] mimicking what is observed in human patients.

Other studies of cisplatin-induced neuropathy have shown impairment of rotarod analyses only during dark cycles, suggesting some proprioceptive loss, and glutamate was protective against these side effects.[17] Electrophysiologic recording revealed significant reduction of sensory nerve conduction velocity, but motor nerve conduction velocity was unaffected.[36] Histologically, cisplatin affects large axons with normal myelin levels, but has no effects on nonmyelinated axons. In addition, DRG apoptosis (cell death) may contribute in part to cisplatin neurotoxicity, which can be blocked by administration of a high dose of nerve growth factor. This result indicates that cisplatin induces apoptosis through mitochondrial stress pathways.[47,109] Cisplatin-induced neuropathy also was blocked by treatment with neurotrophic factor, the ACTH4-9 analog ORG 2766,[36,56] and recombinant human glial growth factor 2,[162] and the survival of large-fiber sensory neurons can be induced by administration of neurotrophin 3.[135] Although cisplatin-induced neuropathy progressed for 6 wk after discontinuation of the drug and slowly reversed over 3 mo, this side effect could be prevented by early decompressive surgery.[161]

Models of pain from spontaneous cancer A natural model of cancer pain involves using animals that have spontaneously occurring tumors. Such models have been used recently to evaluate improved therapeutic approaches to treating cancer pain. In this regard a spontaneous osteosarcoma canine model has been used to examine the effectiveness of targeting specific nociceptive neurons in the DRG as a novel method to treat bone cancer pain.[54,78] This study was conducted by targeting DRG axons expressing TRPV1. The hypothesis behind this investigation was that selectively ablating the DRG neurons expressing TRPV1 receptors would control chronic bone cancer pain, while leaving other sensory functions intact. The potent TRPV1 agonist resiniferatoxin (a capsaicin analogue) was administered intrathecally to target the TRPV1-expressing axons in the spinal cord dorsal horn, leaving other primary afferent neurons unaffected. After intrathecal administration, resiniferatoxin produced significant analgesia for as long as 14 wk postadministration in dogs with spontaneous bone cancer.[19] The concept of selective DRG neuron ablation to treat cancer pain is intriguing and has been used to target other neurons[2]. Such approaches have shown no serious side effects in either rats[80] or dogs[19] and represent novel future approaches for treatment of pain resulting from various types of cancer.

Conclusions

Experimental animal models are useful representations of the pain induced by human and veterinary tumors and thus allow the dissection of the molecular and cellular mechanisms contributing to cancer pain. The use of cancer pain models has provided insights into the mechanisms driving cancer pain and opportunities for developing targeted therapies. Because the development of cancer pain is a dynamic process, alleviating cancer pain based on disease progression may be more effective than simply administering analgesic drugs at the late stages of the process. At the beginning of tumor growth, as tumors start to proliferate, pronociceptive factors such as prostaglandin E_2 and endothelin ET, are released. Therefore, drugs such as COX inhibitors or ET antagonists may be effective treatments during this early period. Depending on the tumor type, cytokines and chemokines are released either from the tumor cells themselves or by the infiltrated immune cells.[190] Because many cytokines and chemokines can directly effect primary afferent pain fibers at the tumor site, knowing which cytokines are released by various tumor types enables the development of tumor-specific cancer pain therapies. Moreover, growing tumors often compress surrounding nerve bundles, and at this point, neuropathic pain medications such as gabapentin may provide improved analgesia. At later stages of bone tumor growth, osteoclasts typically proliferate, at which point medications blocking osteoclast activity, such as OPG and bisphosphonates, may yield effective pain attenuation.[15,29,52] When tumors fill the intramedullary canal and some tumor cells start to die, producing an acidic environment, TRPV1 or Acid-Sensitive Ionic Channel (ASIC) receptor antagonists may be advantageous in controlling cancer pain. As bone destruction becomes evident, ATP antagonists may block movement-related pain.[101,104,123]

Although further investigations clearly are needed to elucidate the biochemical and molecular mechanisms contributing to cancer pain, new therapies such as resiniferatoxin and substance P–saporin[2], which were developed in light of our current understanding of these mechanisms, appear promising cancer pain treatments. In addition, the recently constructed pain genes database, based on the 'knock out' of individual mouse genes, allows investigators to study pain-related phenotypes associated with specific genes. This new database therefore represents a useful and easy-to-use tool allowing pain researchers to generate novel hypotheses regarding the roles of these genes and their protein products in pain processing and modulation. Such information will be crucial to developing novel therapeutic drugs that specifically target particular genes for specific types of cancer pain.[87]

The development of these cancer pain animal models has come at a time when cancer patients are surviving longer, so cancer pain has become an important quality-of-life issue. Use of these models has revealed numerous features associated with pain-related behaviors and has provided insight into the neurochemical and neurophysiologic mechanisms that underlie cancer pain. Many of the features observed in these animal models are shared by human cancer patients that experience tumor pain. Some of these shared features include bone destruction, primary afferent neuron sensitization, and the reorganization and development of central sensitization in the spinal cord. Animal models of cancer pain have been developed to increase our basic knowledge of tumor pain in terms of anatomy, neurobiology, neurophysiology, genetics, psychology, pharmacology, and molecular mechanisms. These animal models will allow investigators and clinicians to gain new information, leading to improved understanding of the factors generating and maintaining cancer pain. This increased understanding of cancer pain mechanisms undoubtedly will lead to the development of novel therapeutic approaches and mechanism-based pharmacotherapeutic treatments.

Acknowledgments

Dr. Patrick Sharp (National University of Singapore) and Ms. Sheryl Holt (University of Minnesota) contributed to the final preparation of this manuscript. This work was partially supported by NIH grant CA084233.

References

1. **Aley KO, Reichling DB, Levine JD.** 1996. Vincristine hyperalgesia in the rat: a model of painful vincristine neuropathy in humans. Neuroscience **73:**259–265.
2. **Allen JW, Mantyh PW, Horais K, Tozier N, Rogers SD, Ghilardi JR, Cizkova D, Grafe MR, Richter P, Lappi DA, Yaksh TL.** 2006. Safety evaluation of intrathecal substance P–saporin, a targeted neurotoxin, in dogs. Toxicol Sci **91:**286–298.
3. **Andersen C, Bagi CM, Adams SW.** 2003. Intratibial injection of human prostate cancer cell line CWR22 elicits osteoblastic response in immunodeficient rats. J Musculoskelet Neuronal Interact **3:**148–155.
4. **Apfel SC, Lipton RB, Arezzo JC, Kessler JA.** 1991. Nerve growth factor prevents toxic neuropathy in mice. Ann Neurol **29:**87–90.
5. **Asai H, Ozaki N, Shinoda M, Nagamine K, Tohnai I, Ueda M, Sugiura Y.** 2005. Heat and mechanical hyperalgesia in mice model of cancer pain. Pain **117:**19–29.
6. **Asham E, Shankar A, Loizidou M, Fredericks S, Miller K, Boulos PB, Burnstock G, Taylor I.** 2001. Increased endothelin-1 in colorectal cancer and reduction of tumour growth by ET(A) receptor antagonism. Br J Cancer **85:**1759–1763.
7. **Authier N, Coudore F, Eschalier A, Fialip J.** 1999. Pain-related behaviour during vincristine-induced neuropathy in rats. Neuroreport **10:**965–968.
8. **Authier N, Fialip J, Eschalier A, Coudore F.** 2000. Assessment of allodynia and hyperalgesia after cisplatin administration to rats. Neurosci Lett **291:**73–76.
9. **Authier N, Gillet JP, Fialip J, Eschalier A, Coudore F.** 2000. Description of a short-term taxol-induced nociceptive neuropathy in rats. Brain Res **887:**239–249.
10. **Authier N, Gillet JP, Fialip J, Eschalier A, Coudore F.** 2003. An animal model of nociceptive peripheral neuropathy following repeated cisplatin injections. Exp Neurol **182:**12–20.
11. **Baamonde A, Lastra A, Fresno MF, Llames S, Meana A, Hidalgo A, Menendez L.** 2004. Implantation of tumoral XC cells induces chronic, endothelin-dependent, thermal hyperalgesia in mice. Cell Mol Neurobiol **24:**269–281.
12. **Bauerle T, Adwan H, Kiessling F, Hilbig H, Armbruster FP, Berger MR.** 2005. Characterization of a rat model with site-specific bone metastasis induced by MDA-MB231 breast cancer cells and its application to the effects of an antibody against bone sialoprotein. Int J Cancer **115:**177–186.
13. **Beyreuther BK, Callizot N, Brot MD, Feldman R, Bain SC, Stohr T.** 2007. Antinociceptive efficacy of lacosamide in rat models for tumor- and chemotherapy-induced cancer pain. Eur J Pharmacol **565:**98–104.
14. **Blackburn-Munro G.** 2004. Pain-like behaviours in animals–how human are they? Trends Pharmacol Sci **25:**299–305.
15. **Blair JM, Zhou H, Seibel MJ, Dunstan CR.** 2006. Mechanisms of disease: roles of OPG, RANKL, and RANK in the pathophysiology of skeletal metastasis. Nat Clin Pract Oncol **3:**41-49.
16. **Blouin S, Basle MF, Chappard D.** 2005. Rat models of bone metastases. Clin Exp Metastasis **22:**605–614.
17. **Boyle FM, Wheeler HR, Shenfield GM.** 1999. Amelioration of experimental cisplatin and paclitaxel neuropathy with glutamate. J Neurooncol **41:**107–116.
18. **Brigatte P, Sampaio SC, Gutierrez VP, Guerra JL, Sinhorini IL, Curi R, Cury Y.** 2007. Walker 256 tumor-bearing rats as a model to study cancer pain. J Pain **8:**412–421.
19. **Brown DC, Iadarola MJ, Perkowski SZ, Erin H, Shofer F, Laszlo KJ, Olah Z, Mannes AJ.** 2005. Physiologic and antinociceptive effects of intrathecal resiniferatoxin in a canine bone cancer model. Anesthesiology **103:**1052–1059.
20. **Bruera E, Lawlor P.** 1997. Cancer pain management. Acta Anaesthesiol Scand **41:**146–153.
21. **Cain DM, Wacnik PW, Eikmeier L, Beitz A, Wilcox GL, Simone DA.** 2001. Functional interactions between tumor and peripheral nerve in a model of cancer pain in the mouse. Pain Med **2:**15–23.
22. **Cavaletti G, Bogliun G, Crespi V, Marzorati L, Zincone A, Marzola M, Rota S, Galli A, Tredici P, Tredici G.** 1997. Neurotoxicity and ototoxicity of cisplatin plus paclitaxel in comparison to cisplatin plus cyclophosphamide in patients with epithelial ovarian cancer. J Clin Oncol **15:**199–206.
23. **Cavaletti G, Tredici G, Braga M, Tazzari S.** 1995. Experimental peripheral neuropathy induced in adult rats by repeated intraperitoneal administration of taxol. Exp Neurol **133:**64–72.
24. **Ceyhan GO, Michalski CW, Demir IE, Muller MW, Friess H.** 2008. Pancreatic pain. Best Pract Res Clin Gastroenterol **22:**31–44.
25. **Cleary JF.** 2000. Cancer pain management. Cancer Control **7:**120–131.
26. **Cleeland CS.** 1984. The impact of pain on the patient with cancer. Cancer **54:**2635–2641.
27. **Cliffer KD, Siuciak JA, Carson SR, Radley HE, Park JS, Lewis DR, Zlotchenko E, Nguyen T, Garcia K, Tonra JR, Stambler N, Cedarbaum JM, Bodine SC, Lindsay RM, DiStefano PS.** 1998. Physiological characterization of taxol-induced large-fiber sensory neuropathy in the rat. Ann Neurol **43:**46–55.
28. **Clohisy DR, Mantyh PW.** 2003. Bone cancer pain. Clin Orthop Relat Res **415(Suppl):**S279–S288.

29. **Clohisy DR, Mantyh PW.** 2004. Bone cancer pain and the role of RANKL/OPG. J Musculoskelet Neuronal Interact **4:**293–300.
30. **Clohisy DR, Ramnaraine ML.** 1998. Osteoclasts are required for bone tumors to grow and destroy bone. J Orthop Res **16:**660–666.
31. **Coleman RE.** 2000. Management of bone metastases. Oncologist **5:**463–470.
32. **Coleman RE.** 2001. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. Cancer Treat Rev **27:**165–176.
33. **Coleman RE, Rubens RD.** 1987. The clinical course of bone metastases from breast cancer. Br J Cancer **55:**61–66.
34. **Davar G.** 2001. Endothelin 1 and metastatic cancer pain. Pain Med **2:**24–27.
35. **De Brabander M, Geuens G, Nuydens R, Willebrords R, De Mey J.** 1981. Taxol induces the assembly of free microtubules in living cells and blocks the organizing capacity of the centrosomes and kinetochores. Proc Natl Acad Sci USA **78:**5608–5612.
36. **de Koning P, Neijt JP, Jennekens FG, Gispen WH.** 1987. ORG2766 protects from cisplatin-induced neurotoxicity in rats. Exp Neurol **97:**746–750.
37. **Dernell WS, Straw RC, Withrow SJ.** 2001. Tumors of the skeletal system. In: Withrow SJ, MacEwen EG, editors. Small animal clinical oncology. Philadelphia: WB Saunders. p 378–417.
38. **di Mola FF, di Sebastiano P.** 2008. Pain and pain generation in pancreatic cancer. Langenbecks Arch Surg 2008 [Epub].
39. **Dina OA, Chen X, Reichling D, Levine JD.** 2001. Role of protein kinase cepsilon and protein kinase A in a model of paclitaxel-induced painful peripheral neuropathy in the rat. Neuroscience **108:**507–515.
40. **Donovan-Rodriguez T, Dickenson AH, Urch CE.** 2004. Superficial dorsal horn neuronal responses and the emergence of behavioural hyperalgesia in a rat model of cancer-induced bone pain. Neurosci Lett **360:**29–32.
41. **Donovan-Rodriguez T, Dickenson AH, Urch CE.** 2005. Gabapentin normalizes spinal neuronal responses that correlate with behavior in a rat model of cancer-induced bone pain. Anesthesiology **102:**132–140.
42. **Donovan-Rodriguez T, Urch CE, Dickenson AH.** 2006. Evidence of a role for descending serotonergic facilitation in a rat model of cancer-induced bone pain. Neurosci Lett **393:**237–242.
43. **Dorsi MJ, Chen L, Murinson BB, Pogatzki-Zahn EM, Meyer RA, Belzberg AJ.** 2008. The tibial neuroma transposition (TNT) model of neuroma pain and hyperalgesia. Pain **134:**320–334.
44. **Dougherty PM, Cata JP, Burton AW, Vu K, Weng HR.** 2007. Dysfunction in multiple primary afferent fiber subtypes revealed by quantitative sensory testing in patients with chronic vincristine-induced pain. J Pain Symptom Manage **33:**166–179.
45. **El Mouedden M, Meert TF.** 2005. Evaluation of pain-related behavior, bone destruction, and effectiveness of fentanyl, sufentanil, and morphine in a murine model of cancer pain. Pharmacol Biochem Behav **82:**109–119.
46. **Erin N, Boyer PJ, Bonneau RH, Clawson GA, Welch DR.** 2004. Capsaicin-mediated denervation of sensory neurons promotes mammary tumor metastasis to lung and heart. Anticancer Res **24:**1003–1009.
47. **Fischer SJ, Podratz JL, Windebank AJ.** 2001. Nerve growth factor rescue of cisplatin neurotoxicity is mediated through the high affinity receptor: studies in PC12 cells and $p75^{null}$ mouse dorsal root ganglia. Neurosci Lett **308:**1–4.
48. **Foley KM.** 1996. Controlling the pain of cancer. Sci Am **275:**164–165.
49. **Foley KM.** 1999. Advances in cancer pain. Arch Neurol **56:**413–417.
50. **Fox A, Medhurst S, Courade JP, Glatt M, Dawson J, Urban L, Bevan S, Gonzalez I.** 2004. Antihyperalgesic activity of the COX2 inhibitor lumiracoxib in a model of bone cancer pain in the rat. Pain **107:**33–40.
51. **Freedman SD, Callery MP.** 2007. Central control of pancreatic cancer pain: protecting the enemy? Gastroenterology **132:**1191–1192.
52. **Fulfaro F, Casuccio A, Ticozzi C, Ripamonti C.** 1998. The role of bisphosphonates in the treatment of painful metastatic bone disease: a review of phase III trials. Pain **78:**157–169.
53. **Gebhart GF.** 1999. Animal models of pain. ILAR J **40**.
54. **Ghilardi JR, Rohrich H, Lindsay TH, Sevcik MA, Schwei MJ, Kubota K, Halvorson KG, Poblete J, Chaplan SR, Dubin AE, Carruthers NI, Swanson D, Kuskowski M, Flores CM, Julius D, Mantyh PW.** 2005. Selective blockade of the capsaicin receptor TRPV1 attenuates bone cancer pain. J Neurosci **25:**3126–3131.
55. **Giraudel JM, Diquelou A, Lees P, Toutain PL.** 2005. Development and validation of a new model of inflammation in the cat and selection of surrogate endpoints for testing antiinflammatory drugs. J Vet Pharmacol Ther **28:**275–285.
56. **Gispen WH, Hamers FP, Vecht CJ, Jennekens FG, Neyt JP.** 1992. ACTH/MSH like peptides in the treatment of cisplatin neuropathy. J Steroid Biochem Mol Biol **43:**179–183.
57. **Goblirsch M, Lynch C, Mathews W, Manivel JC, Mantyh PW, Clohisy DR.** 2005. Radiation treatment decreases bone cancer pain through direct effect on tumor cells. Radiat Res **164:**400–408.
58. **Goblirsch M, Mathews W, Lynch C, Alaei P, Gerbi BJ, Mantyh PW, Clohisy DR.** 2004. Radiation treatment decreases bone cancer pain, osteolysis and tumor size. Radiat Res **161:**228–234.
59. **Goblirsch M, Zwolak P, Ramnaraine ML, Pan W, Lynch C, Alaei P, Clohisy DR.** 2006. Novel cytosine deaminase fusion gene enhances the effect of radiation on breast cancer in bone by reducing tumor burden, osteolysis, and skeletal fracture. Clin Cancer Res **12:**3168–3176.
60. **Goblirsch MJ, Zwolak P, Clohisy DR.** 2005. Advances in understanding bone cancer pain. J Cell Biochem **96:**682–688.
61. **Goblirsch MJ, Zwolak PP, Clohisy DR.** 2006. Biology of bone cancer pain. Clin Cancer Res **12:**6231s–6235s.
62. **Guise TA, Kozlow WM, Heras-Herzig A, Padalecki SS, Yin JJ, Chirgwin JM.** 2005. Molecular mechanisms of breast cancer metastases to bone. Clin Breast Cancer **5 Suppl:**S46–S53.
63. **Halvorson KG, Kubota K, Sevcik MA, Lindsay TH, Sotillo JE, Ghilardi JR, Rosol TJ, Boustany L, Shelton DL, Mantyh PW.** 2005. A blocking antibody to nerve growth factor attenuates skeletal pain induced by prostate tumor cells growing in bone. Cancer Res **65:**9426–9435.
64. **Hargreaves K, Dubner R, Brown F, Flores C, Joris J.** 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain **32:**77–88.
65. **Hartel M, di Mola FF, Selvaggi F, Mascetta G, Wente MN, Felix K, Giese NA, Hinz U, Di Sebastiano P, Buchler MW, Friess H.** 2006. Vanilloids in pancreatic cancer: potential for chemotherapy and pain management. Gut **55:**519–528.
66. **Hasue F, Kuwaki T, Yamada H, Fukuda Y, Shimoyama M.** 2004. Inhibitory actions of endothelin 1 on pain processing. J Cardiovasc Pharmacol **44:**S318–S320.
67. **Higuera ES, Luo ZD.** 2004. A rat pain model of vincristine-induced neuropathy. Methods Mol Med **99:**91–98.
68. **Honore P.** 2006. Behavioral assessment of neuropathic pain in preclinical models. Drug Dev Res **67:**302–307.
69. **Honore P, Luger NM, Sabino MA, Schwei MJ, Rogers SD, Mach DB, O'keefe PF, Ramnaraine ML, Clohisy DR, Mantyh PW.** 2000. Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord. Nat Med **6:**521–528.
70. **Honore P, Mantyh PW.** 2000. Bone cancer pain: from mechanism to model to therapy. Pain Med **1:**303–309.
71. **Honore P, Rogers SD, Schwei MJ, Salak-Johnson JL, Luger NM, Sabino MC, Clohisy DR, Mantyh PW.** 2000. Murine models of inflammatory, neuropathic and cancer pain each generates a unique set of neurochemical changes in the spinal cord and sensory neurons. Neuroscience **98:**585–598.

72. **Honore P, Schwei J, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, Clohisy DR, Mantyh PW.** 2000. Cellular and neurochemical remodeling of the spinal cord in bone cancer pain. Prog Brain Res **129:**389–397.
73. **Horwitz SB.** 1992. Mechanism of action of taxol. Trends Pharmacol Sci **13:**134–136.
74. **Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ.** 2007. Cancer statistics, 2007. CA Cancer J Clin **57:**43–66.
75. **Joseph EK, Levine JD.** 2003. Sexual dimorphism for protein kinase Cε signaling in a rat model of vincristine-induced painful peripheral neuropathy. Neuroscience **119:**831–838.
76. **Joshi SK, Honore P.** 2006. Animal models of pain for drug discovery. Expert Opin Drug Discov **1:**323–334.
77. **Jourdan D, Ardid D, Eschalier A.** 2001. Automated behavioural analysis in animal pain studies. Pharmacol Res **43:**103–110.
78. **Karai L, Brown DC, Mannes AJ, Connelly ST, Brown J, Gandal M, Wellisch OM, Neubert JK, Olah Z, Iadarola MJ.** 2004. Deletion of vanilloid receptor 1-expressing primary afferent neurons for pain control. J Clin Invest **113:**1344–1352.
79. **Kehl LJ, Hamamoto DT, Wacnik PW, Croft DL, Norsted BD, Wilcox GL, Simone DA.** 2003. A cannabinoid agonist differentially attenuates deep tissue hyperalgesia in animal models of cancer and inflammatory muscle pain. Pain **103:**175–186.
80. **Khasabov SG, Ghilardi JR, Mantyh PW, Simone DA.** 2005. Spinal neurons that express NK1 receptors modulate descending controls that project through the dorsolateral funiculus. J Neurophysiol **93:**998–1006.
81. **Khasabov SG, Hamamoto DT, Harding-Rose C, Simone DA.** 2007. Tumor-evoked hyperalgesia and sensitization of nociceptive dorsal horn neurons in a murine model of cancer pain. Brain Res **1180:**7–19.
82. **King T, Vardanyan A, Majuta L, Melemedjian O, Nagle R, Cress AE, Vanderah TW, Lai J, Porreca F.** 2007. Morphine treatment accelerates sarcoma-induced bone pain, bone loss, and spontaneous fracture in a murine model of bone cancer. Pain **132:**154–168.
83. **Kohler DR, Goldspiel BR.** 1994. Paclitaxel (taxol). Pharmacotherapy **14:**3–34.
84. **KuKanich B, Lascelles BD, Papich MG.** 2005. Pharmacokinetics of morphine and plasma concentrations of morphine-6-glucuronide following morphine administration to dogs. J Vet Pharmacol Ther **28:**371–376.
85. **Kumar N.** 1981. Taxol-induced polymerization of purified tubulin mechanism of action. J Biol Chem **256:**10435–10441.
86. **Kurbel S, Kurbel B, Kovacic D, Sulava D, Krajina Z, Dmitrovic B, Sokcevic M.** 1999. Endothelin-secreting tumors and the idea of the pseudoectopic hormone secretion in tumors. Med Hypotheses **52:**329–333.
87. **Lacroix-Fralish ML, Ledoux JB, Mogil JS.** 2007. The pain genes database: an interactive web browser of pain-related transgenic knockout studies. Pain **131:**3.e1–3.e4.
88. **Lariviere WR, Wilson SG, Laughlin TM, Kokayeff A, West EE, Adhikari SM, Wan Y, Mogil JS.** 2002. Heritability of nociception. III. Genetic relationships among commonly used assays of nociception and hypersensitivity. Pain **97:**75–86.
89. **Lee BH, Seong J, Kim UJ, Won R, Kim J.** 2005. Behavioral characteristics of a mouse model of cancer pain. Yonsei Med J **46:**252–259.
90. **Lees P, Landoni MF, Giraudel J, Toutain PL.** 2004. Pharmacodynamics and pharmacokinetics of nonsteroidal antiinflammatory drugs in species of veterinary interest. J Vet Pharmacol Ther **27:**479–490.
91. **Lesage P, Portenoy RK.** 1999. Trends in cancer pain management. Cancer Control **6:**136–145.
92. **Levy MH.** 1996. Pharmacologic treatment of cancer pain. N Engl J Med **335:**1124–1132.
93. **Liepe K, Geidel H, Haase M, Hakenberg OW, Runge R, Kotzerke J.** 2005. New model for the induction of osteoblastic bone metastases in rat. Anticancer Res **25:**1067–1073.
94. **Liepe K, Runge R, Kotzerke J.** 2005. The benefit of bone-seeking radiopharmaceuticals in the treatment of metastatic bone pain. J Cancer Res Clin Oncol **131:**60–66.
95. **Lin CS, Tsaur ML, Chen CC, Wang TY, Lin CF, Lai YL, Hsu TC, Pan YY, Yang CH, Cheng JK.** 2007. Chronic intrathecal infusion of minocycline prevents the development of spinal-nerve ligation-induced pain in rats. Reg Anesth Pain Med **32:**209–216.
96. **Lindsay TH, Jonas BM, Sevcik MA, Kubota K, Halvorson KG, Ghilardi JR, Kuskowski MA, Stelow EB, Mukherjee P, Gendler SJ, Wong GY, Mantyh PW.** 2005. Pancreatic cancer pain and its correlation with changes in tumor vasculature, macrophage infiltration, neuronal innervation, body weight, and disease progression. Pain **119:**233–246.
97. **Luger NM, Honore P, Sabino MA, Schwei MJ, Rogers SD, Mach DB, Clohisy DR, Mantyh PW.** 2001. Osteoprotegerin diminishes advanced bone cancer pain. Cancer Res **61:**4038–4047.
98. **Luger NM, Sabino MA, Schwei MJ, Mach DB, Pomonis JD, Keyser CP, Rathbun M, Clohisy DR, Honore P, Yaksh TL, Mantyh PW.** 2002. Efficacy of systemic morphine suggests a fundamental difference in the mechanisms that generate bone cancer versus inflammatory pain. Pain **99:**397–406.
99. **Lynch JJ 3rd, Wade CL, Zhong CM, Mikusa JP, Honore P.** 2004. Attenuation of mechanical allodynia by clinically utilized drugs in a rat chemotherapy-induced neuropathic pain model. Pain **110:**56–63.
100. **Ma C.** 2007. Animal models of pain. Int Anesthesiol Clin **45:**121–131.
101. **Mantyh PW.** 2004. A mechanism-based understanding of bone cancer pain. Novartis Found Symp **261:**194–214; discussion 214–219, 256–261.
102. **Mantyh PW.** 2006. Cancer pain and its impact on diagnosis, survival, and quality of life. Nat Rev Neurosci **7:**797–809.
103. **Mantyh PW, Clohisy DR, Koltzenburg M, Hunt SP.** 2002. Molecular mechanisms of cancer pain. Nat Rev Cancer **2:**201–209.
104. **Mantyh PW, Hunt SP.** 2004. Mechanisms that generate and maintain bone cancer pain. Novartis Found Symp **260:**221–238; discussion 238–240, 277–279.
105. **Mao-Ying QL, Zhao J, Dong ZQ, Wang J, Yu J, Yan MF, Zhang YQ, Wu GC, Wang YQ.** 2006. A rat model of bone cancer pain induced by intratibia inoculation of Walker 256 mammary gland carcinoma cells. Biochem Biophys Res Commun **345:**1292–1298.
106. **Martin LA, Hagen NA.** 1997. Neuropathic pain in cancer patients: mechanisms, syndromes, and clinical controversies. J Pain Symptom Manage **14:**99–117.
107. **Matsumoto M, Inoue M, Hald A, Xie W, Ueda H.** 2006. Inhibition of paclitaxel-induced A-fiber hypersensitization by gabapentin. J Pharmacol Exp Ther **318:**735–740.
108. **Mayer MN, Grier CK.** 2006. Palliative radiation therapy for canine osteosarcoma. Can Vet J **47:**707–709.
109. **McDonald ES, Windebank AJ.** 2002. Cisplatin-induced apoptosis of DRG neurons involves BAX redistribution and cytochrome C release but not FAS receptor signaling. Neurobiol Dis **9:**220–233.
110. **Medhurst SJ, Walker K, Bowes M, Kidd BL, Glatt M, Muller M, Hattenberger M, Vaxelaire J, O'Reilly T, Wotherspoon G, Winter J, Green J, Urban L.** 2002. A rat model of bone cancer pain. Pain **96:**129–140.
111. **Menendez L, Lastra A, Fresno MF, Llames S, Meana A, Hidalgo A, Baamonde A.** 2003. Initial thermal heat hypoalgesia and delayed hyperalgesia in a murine model of bone cancer pain. Brain Res **969:**102–109.
112. **Menendez L, Lastra A, Hidalgo A, Meana A, Garcia E, Baamonde A.** 2003. Peripheral opioids act as analgesics in bone cancer pain in mice. Neuroreport **14:**867–869.
113. **Mercadante S.** 1997. Malignant bone pain: pathophysiology and treatment. Pain **69:**1–18.
114. **Mercadante S, Arcuri E.** 1998. Breakthrough pain in cancer patients: pathophysiology and treatment. Cancer Treat Rev **24:**425–432.
115. **Mercadante S, Fulfaro F.** 2007. Management of painful bone metastases. Curr Opin Oncol **19:**308–314.

116. **Meunier A, Latremoliere A, Dominguez E, Mauborgne A, Philippe S, Hamon M, Mallet J, Benoliel JJ, Pohl M.** 2007. Lentiviral-mediated targeted NF-κB blockade in dorsal spinal cord glia attenuates sciatic nerve injury-induced neuropathic pain in the rat. Mol Ther **15:**687–697.
117. **Miguel R.** 2000. Interventional treatment of cancer pain: the fourth step in the World Health Organization analgesic ladder? Cancer Control **7:**149–156.
118. **Mimura Y, Kato H, Eguchi K, Ogawa T.** 2000. Schedule dependency of paclitaxel-induced neuropathy in mice: a morphological study. Neurotoxicology **21:**513–520.
119. **Mishra S, Bhatnagar S, Singhal AK.** 2004. Recent trends in cancer pain management. Indian J Med Paediatric Oncol **25:**22–28.
120. **Mogil JS, Crager SE.** 2004. What should we be measuring in behavioral studies of chronic pain in animals? Pain **112:**12–15.
121. **Mogil JS, Wilson SG, Bon K, Lee SE, Chung K, Raber P, Pieper JO, Hain HS, Belknap JK, Hubert L, Elmer GI, Chung JM, Devor M.** 1999. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. Pain **80:**67–82.
122. **Mullins MN, Lana SE, Dernell WS, Ogilvie GK, Withrow SJ, Ehrhart EJ.** 2004. Cyclooxygenase 2 expression in canine appendicular osteosarcomas. J Vet Intern Med **18:**859–865.
123. **Nagae M, Hiraga T, Yoneda T.** 2007. Acidic microenvironment created by osteoclasts causes bone pain associated with tumor colonization. J Bone Miner Metab **25:**99–104.
124. **Nagamine K, Ozaki N, Shinoda M, Asai H, Nishiguchi H, Mitsudo K, Tohnai I, Ueda M, Sugiura Y.** 2006. Mechanical allodynia and thermal hyperalgesia induced by experimental squamous cell carcinoma of the lower gingiva in rats. J Pain **7:**659–670.
125. **Niiyama Y, Kawamata T, Yamamoto J, Omote K, Namiki A.** 2007. Bone cancer increases transient receptor potential vanilloid subfamily 1 expression within distinct subpopulations of dorsal root ganglion neurons. Neuroscience **148:**560–572.
126. **Nozaki-Taguchi N, Chaplan SR, Higuera ES, Ajakwe RC, Yaksh TL.** 2001. Vincristine-induced allodynia in the rat. Pain **93:**69–76.
127. **Nutile-McMenemy N, Elfenbein A, Deleo JA.** 2007. Minocycline decreases in vitro microglial motility, β1 integrin, and Kv1.3 channel expression. J Neurochem **103:**2035–2046.
128. **Ohtsu T, Sasaki Y, Tamura T, Miyata Y, Nakanomyo H, Nishiwaki Y, Saijo N.** 1995. Clinical pharmacokinetics and pharmacodynamics of paclitaxel: a 3-hour infusion versus a 24-hour infusion. Clin Cancer Res **1:**599–606.
129. **Park HC, Seong J, An JH, Kim J, Kim UJ, Lee BW.** 2005. Alteration of cancer pain-related signals by radiation: proteomic analysis in an animal model with cancer bone invasion. Int J Radiat Oncol Biol Phys **61:**1523–1534.
130. **Peters CM, Lindsay TH, Pomonis JD, Luger NM, Ghilardi JR, Sevcik MA, Mantyh PW.** 2004. Endothelin and the tumorigenic component of bone cancer pain. Neuroscience **126:**1043–1052.
131. **Polomano RC, Bennett GJ.** 2001. Chemotherapy-evoked painful peripheral neuropathy. Pain Med **2:**8–14.
132. **Polomano RC, Mannes AJ, Clark US, Bennett GJ.** 2001. A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug paclitaxel. Pain **94:**293–304.
133. **Portenoy RK, Hagen NA.** 1990. Breakthrough pain: definition, prevalence, and characteristics. Pain **41:**273–281.
134. **Portenoy RK, Lesage P.** 1999. Management of cancer pain. Lancet **353:**1695–1700.
135. **Pradat PF, Kennel P, Naimi-Sadaoui S, Finiels F, Scherman D, Orsini C, Delaere P, Mallet J, Revah F.** 2002. Viral and nonviral gene therapy partially prevents experimental cisplatin-induced neuropathy. Gene Ther **9:**1333–1337.
136. **Quasthoff S, Hartung HP.** 2002. Chemotherapy-induced peripheral neuropathy. J Neurol **249:**9–17.
137. **Regan JM, Peng P.** 2000. Neurophysiology of cancer pain. Cancer Control **7:**111–119.
138. **Rollin BE.** 2003. Oncology and ethics. Reprod Domest Anim **38:**50–53.
139. **Rowinsky EK, Chaudhry V, Cornblath DR, Donehower RC.** 1993. Neurotoxicity of taxol. J Natl Cancer Inst Monogr **15:**107–115.
140. **Rowinsky EK, Donehower RC.** 1993. The clinical pharmacology of paclitaxel (taxol). Semin Oncol **20:**16–25.
141. **Rowinsky EK, Donehower RC.** 1995. Paclitaxel (taxol). N Engl J Med **332:**1004–1014.
142. **Rowinsky EK, Eisenhauer EA, Chaudhry V, Arbuck SG, Donehower RC.** 1993. Clinical toxicities encountered with paclitaxel (taxol). Semin Oncol **20:**1–15.
143. **Sabino MA, Ghilardi JR, Jongen JL, Keyser CP, Luger NM, Mach DB, Peters CM, Rogers SD, Schwei MJ, de Felipe C, Mantyh PW.** 2002. Simultaneous reduction in cancer pain, bone destruction, and tumor growth by selective inhibition of cyclooxygenase 2. Cancer Res **62:**7343–7349.
144. **Sabino MA, Luger NM, Mach DB, Rogers SD, Schwei MJ, Mantyh PW.** 2003. Different tumors in bone each give rise to a distinct pattern of skeletal destruction, bone cancer-related pain behaviors and neurochemical changes in the central nervous system. Int J Cancer **104:**550–558.
145. **Sabino MA, Mantyh PW.** 2005. Pathophysiology of bone cancer pain. J Support Oncol **3:**15–24.
146. **Sahenk Z, Barohn R, New P, Mendell JR.** 1994. Taxol neuropathy. Electrodiagnostic and sural nerve biopsy findings. Arch Neurol **51:**726–729.
147. **Saito O, Aoe T, Yamamoto T.** 2005. Analgesic effects of nonsteroidal antiinflammatory drugs, acetaminophen, and morphine in a mouse model of bone cancer pain. J Anesth **19:**218–224.
148. **Schwei MJ, Honore P, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, Clohisy DR, Mantyh PW.** 1999. Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. J Neurosci **19:**10886–10897.
149. **Seong J, Park HC, Kim J, Kim UJ, Lee BW.** 2004. Radiation-induced alteration of pain-related signals in an animal model with bone invasion from cancer. Ann N Y Acad Sci **1030:**179–186.
150. **Sessle BJ.** 2007. Glia: non-neural players in orofacial pain. J Orofac Pain **21:**169–170.
151. **Sevcik MA, Ghilardi JR, Peters CM, Lindsay TH, Halvorson KG, Jonas BM, Kubota K, Kuskowski MA, Boustany L, Shelton DL, Mantyh PW.** 2005. AntiNGF therapy profoundly reduces bone cancer pain and the accompanying increase in markers of peripheral and central sensitization. Pain **115:**128–141.
152. **Sevcik MA, Jonas BM, Lindsay TH, Halvorson KG, Ghilardi JR, Kuskowski MA, Mukherjee P, Maggio JE, Mantyh PW.** 2006. Endogenous opioids inhibit early-stage pancreatic pain in a mouse model of pancreatic cancer. Gastroenterology **131:**900–910.
153. **Shimoyama M, Tanaka K, Hasue F, Shimoyama N.** 2002. A mouse model of neuropathic cancer pain. Pain **99:**167–174.
154. **Shimoyama M, Tatsuoka H, Ohtori S, Tanaka K, Shimoyama N.** 2005. Change of dorsal horn neurochemistry in a mouse model of neuropathic cancer pain. Pain **114:**221–230.
155. **Smith SB, Crager SE, Mogil JS.** 2004. Paclitaxel-induced neuropathic hypersensitivity in mice: responses in 10 inbred mouse strains. Life Sci **74:**2593–2604.
156. **Socinski MA, Langer CJ.** 1999. Single-agent paclitaxel and paclitaxel/non-platinum combination therapy in advanced non-small cell lung cancer. Semin Oncol **26:**51–61, discussion 71-72.
157. **Strumberg D, Brugge S, Korn MW, Koeppen S, Ranft J, Scheiber G, Reiners C, Mockel C, Seeber S, Scheulen ME.** 2002. Evaluation of long-term toxicity in patients after cisplatin-based chemotherapy for nonseminomatous testicular cancer. Ann Oncol **13:**229–236.
158. **Tamura T, Sasaki Y, Nishiwaki Y, Saijo N.** 1995. Phase I study of paclitaxel by 3-hour infusion: hypotension just after infusion is one of the major dose-limiting toxicities. Jpn J Cancer Res **86:**1203–1209.
159. **Tanner KD, Levine JD, Topp KS.** 1998. Microtubule disorientation and axonal swelling in unmyelinated sensory axons during vincristine-induced painful neuropathy in rat. J Comp Neurol **395:**481–492.

160. **Tanner KD, Reichling DB, Levine JD.** 1998. Nociceptor hyperresponsiveness during vincristine-induced painful peripheral neuropathy in the rat. J Neurosci **18:**6480–6491.
161. **Tassler P, Dellon AL, Lesser GJ, Grossman S.** 2000. Utility of decompressive surgery in the prophylaxis and treatment of cisplatin neuropathy in adult rats. J Reconstr Microsurg **16:**457–463.
162. **ter Laak MP, Hamers FP, Kirk CJ, Gispen WH.** 2000. rhGGF2 protects against cisplatin-induced neuropathy in the rat. J Neurosci Res **60:**237–244.
163. **Thomas EM, Weiss SM.** 2000. Nonpharmacological interventions with chronic cancer pain in adults. Cancer Control **7:**157–164.
164. **Thompson SW, Tonge D.** 2000. Bone cancer gain without the pain. Nat Med **6:**504–505.
165. **Thrall DE, LaRue SM.** 1995. Palliative radiation therapy. Semin Vet Med Surg (Small Anim) **10:**205–208.
166. **Topp KS, Tanner KD, Levine JD.** 2000. Damage to the cytoskeleton of large diameter sensory neurons and myelinated axons in vincristine-induced painful peripheral neuropathy in the rat. J Comp Neurol **424:**563–576.
167. **Tyner TR, Parks N, Faria S, Simons M, Stapp B, Curtis B, Sian K, Yamaguchi KT.** 2007. Effects of collagen nerve guide on neuroma formation and neuropathic pain in a rat model. Am J Surg **193:**e1–e6.
168. **Ueda H.** 2006. Molecular mechanisms of neuropathic pain—phenotypic switch and initiation mechanisms. Pharmacol Ther **109:**57–77.
169. **Urch C.** 2004. The pathophysiology of cancer-induced bone pain: current understanding. Palliat Med **18:**267–274.
170. **Urch CE.** 2004. Current and potential treatment for cancer-induced bone pain. Am Acad Hospice Palliative Med Bull **1-3:**7.
171. **Urch CE, Donovan-Rodriguez T, Dickenson AH.** 2003. Alterations in dorsal horn neurones in a rat model of cancer-induced bone pain. Pain **106:**347–356.
172. **Urch CE, Donovan-Rodriguez T, Gordon-Williams R, Bee LA, Dickenson AH.** 2005. Efficacy of chronic morphine in a rat model of cancer-induced bone pain: behavior and in dorsal horn pathophysiology. J Pain **6:**837–845.
173. **Valeberg BT, Rustoen T, Bjordal K, Hanestad BR, Paul S, Miaskowski C.** 2008. Self-reported prevalence, etiology, and characteristics of pain in oncology outpatients. Eur J Pain **12:**582–590.
174. **Vecht CJ.** 2000. Cancer pain: a neurological perspective. Curr Opin Neurol **13:**649–653.
175. **Vermeirsch H, Nuydens RM, Salmon PL, Meert TF.** 2004. Bone cancer pain model in mice: evaluation of pain behavior, bone destruction, and morphine sensitivity. Pharmacol Biochem Behav **79:**243–251.
176. **Vit JP, Ohara PT, Tien DA, Fike JR, Eikmeier L, Beitz A, Wilcox GL, Jasmin L.** 2006. The analgesic effect of low-dose focal irradiation in a mouse model of bone cancer is associated with spinal changes in neuromediators of nociception. Pain **120:**188–201.
177. **von Frey M.** 1922. Verspätete schmerzempfindungen. Z Gesamte Neurol Psychiatr **79:**324–333.
178. **Wacnik PW, Baker CM, Herron MJ, Kren BT, Blazar BR, Wilcox GL, Hordinsky MK, Beitz AJ, Ericson ME.** 2005. Tumor-induced mechanical hyperalgesia involves CGRP receptors and altered innervation and vascularization of DsRed2 fluorescent hindpaw tumors. Pain **115:**95–106.
179. **Wacnik PW, Eikmeier LJ, Ruggles TR, Ramnaraine ML, Walcheck BK, Beitz AJ, Wilcox GL.** 2001. Functional interactions between tumor and peripheral nerve: morphology, algogen identification, and behavioral characterization of a new murine model of cancer pain. J Neurosci **21:**9355–9366.
180. **Wacnik PW, Eikmeier LJ, Simone DA, Wilcox GL, Beitz AJ.** 2005. Nociceptive characteristics of tumor necrosis factor α in naive and tumor-bearing mice. Neuroscience **132:**479–491.
181. **Wacnik PW, Kehl LJ, Trempe TM, Ramnaraine ML, Beitz AJ, Wilcox GL.** 2003. Tumor implantation in mouse humerus evokes movement-related hyperalgesia exceeding that evoked by intramuscular carrageenan. Pain **101:**175–186.
182. **Wacnik PW, Stool L, Lauretti GR, Kitto KF, Ramnaraine ML, Mantyh PW, Beitz AJ, Wilcox GL.** 1998. A practical model of cancer pain: comparing different hindlimb sites of melanoma cell implantation. Soc Neurosci Abstr (Los Angeles, CA) **24:**628.
183. **Wacnik PW, Wilcox GL, Clohisy DR, Ramnaraine ML, Eikmeier LJ, Beitz AJ.** 2000. Cancer pain mechanisms and animal models of cancer pain. Proc 9th World Congress Pain Progress Pain Res Manage **16:**615–637.
184. **Walker K, Medhurst SJ, Kidd BL, Glatt M, Bowes M, Patel S, McNair K, Kesingland A, Green J, Chan O, Fox AJ, Urban LA.** 2002. Disease modifying and anti-nociceptive effects of the bisphosphonate zoledronic acid in a model of bone cancer pain. Pain **100:**219–229.
185. **Wang LX, Wang ZJ.** 2003. Animal and cellular models of chronic pain. Adv Drug Deliv Rev **55:**949–965.
186. **Watkins LR, Hutchinson MR, Ledeboer A, Wieseler-Frank J, Milligan ED, Maier SF.** 2007. Norman Cousins lecture. Glia as the 'bad guys:' implications for improving clinical pain control and the clinical utility of opioids. Brain Behav Immun **21:**131–146.
187. **Watkins LR, Milligan ED, Maier SF.** 2001. Glial activation: a driving force for pathological pain. Trends Neurosci **24:**450–455.
188. **Wiernik PH, Schwartz EL, Einzig A, Strauman JJ, Lipton RB, Dutcher JP.** 1987. Phase I trial of taxol given as a 24-hour infusion every 21 days: responses observed in metastatic melanoma. J Clin Oncol **5:**1232–1239.
189. **Wiernik PH, Schwartz EL, Strauman JJ, Dutcher JP, Lipton RB, Paietta E.** 1987. Phase I clinical and pharmacokinetic study of taxol. Cancer Res **47:**2486–2493.
190. **Wood LJ, Nail LM, Gilster A, Winters KA, Elsea CR.** 2006. Cancer chemotherapy-related symptoms: evidence to suggest a role for proinflammatory cytokines. Oncol Nurs Forum **33:**535–542.
191. **Yamamoto J, Kawamata T, Niiyama Y, Omote K, Namiki A.** 2008. Downregulation of mu opioid receptor expression within distinct subpopulations of dorsal root ganglion neurons in a murine model of bone cancer pain. Neuroscience **151:**843–853.
192. **Zhang RX, Liu B, Wang L, Ren K, Qiao JT, Berman BM, Lao L.** 2005. Spinal glial activation in a new rat model of bone cancer pain produced by prostate cancer cell inoculation of the tibia. Pain **118:**125–136.
193. **Zhou QL, Strichartz G, Davar G.** 2001. Endothelin 1 activates ET(A) receptors to increase intracellular calcium in model sensory neurons. Neuroreport **12:**3853–3857.
194. **Zhu Z, Friess H, diMola FF, Zimmermann A, Graber HU, Korc M, Buchler MW.** 1999. Nerve growth factor expression correlates with perineural invasion and pain in human pancreatic cancer. J Clin Oncol **17:**2419–2428.
195. **Zwolak P, Dudek AZ, Bodempudi VD, Nguyen J, Hebbel RP, Gallus NJ, Ericson ME, Goblirsch MJ, Clohisy DR.** 2008. Local irradiation in combination with bevacizumab enhances radiation control of bone destruction and cancer-induced pain in a model of bone metastases. Int J Cancer **122:**681–688.