

# The Neurobiology of Cancer Pain

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## Abstract

The global burden of cancer pain is enormous and opioids, despite their side effects, remain the primary therapeutic approach. The cause of cancer pain is unknown. Mechanisms driving cancer pain differ from those mechanisms responsible for inflammatory and neuropathic pain. The prevailing hypothesis put forward to explain cancer pain posits that cancers generate and secrete mediators which sensitize and activate primary afferent nociceptors in the cancer microenvironment. Moreover, cancers induce neurochemical reorganization of the spinal cord, which contributes to spontaneous activity and enhanced responsiveness. The purpose of this review, which covers clinical and preclinical studies, is to highlight those peripheral and central mechanisms responsible for cancer pain. The challenges facing neuroscientists and clinicians studying and ultimately treating cancer pain are discussed.

## Keywords

cancer pain, cancer, tumor, pain, sensory system

The global burden of cancer pain is enormous. Patients are living longer with cancer and many endure cancer pain for extended durations. The etiology of cancer pain remains unknown. Accordingly, a targeted approach to cancer pain remains elusive. In this article, I review the pain-producing mechanisms secondary to cancer. I do not review pain that results from cancer treatment, that is, pain resulting from radiation, chemotherapy, or surgery. The pain-producing mechanisms I review pertain to cancer patients prior to treatment or after failed treatment. This latter group of cancer patients includes those patients with viable carcinoma; carcinoma in some patients can be controlled for years.

The prevailing hypothesis put forward to explain cancer pain posits that cancers generate and secrete mediators. These putative mediators subsequently sensitize and activate primary afferent nociceptors in the cancer microenvironment. Cancer pain has been proposed to result from tissue destruction and nerve compression; however, this hypothesis is not consistent with clinical findings or with preclinical data. Cancer pain has also been described as inflammatory pain. However, non-steroidal anti-inflammatory drugs are clinically ineffectual. Moreover, preclinical studies provide ample evidence that cancer pain is distinguishable from inflammatory pain and is generally a unique form of pathologic pain.

Cancers exhibit phenotypic and genomic heterogeneity. These unpredictable differences throw up challenges to clinicians and scientists alike. Pain in patients varies by the histologic type of cancer, the site involved (i.e., primary versus metastatic sites), and whether the involved

site is required for musculoskeletal function. Histologically different cancers involving disparate anatomic sites produce a different pain phenotype (Figure 1). This finding is not only a clinical observation but aligns with findings from preclinical models that have been generated using different histologic types of cancer (e.g., sarcoma, melanoma, and adenocarcinoma). Different cancers inoculated into the same anatomic site produce different pain related behavior. These different types of cancer also produce distinct neurochemical reorganization of the spinal cord (Sabino and others, 2003). While the prevalence of cancer pain depends on the histologic type and anatomic site, more than 50% of cancer patients experience pain (van den Beuken-van Everdingen and others, 2007) (Figure 2).

I first review the relevant clinical studies and the scientific findings in these investigations that extend our understanding of the etiology of cancer pain. I then review the preclinical studies and summarize the scientific findings about the etiology of cancer pain garnered from animal models.

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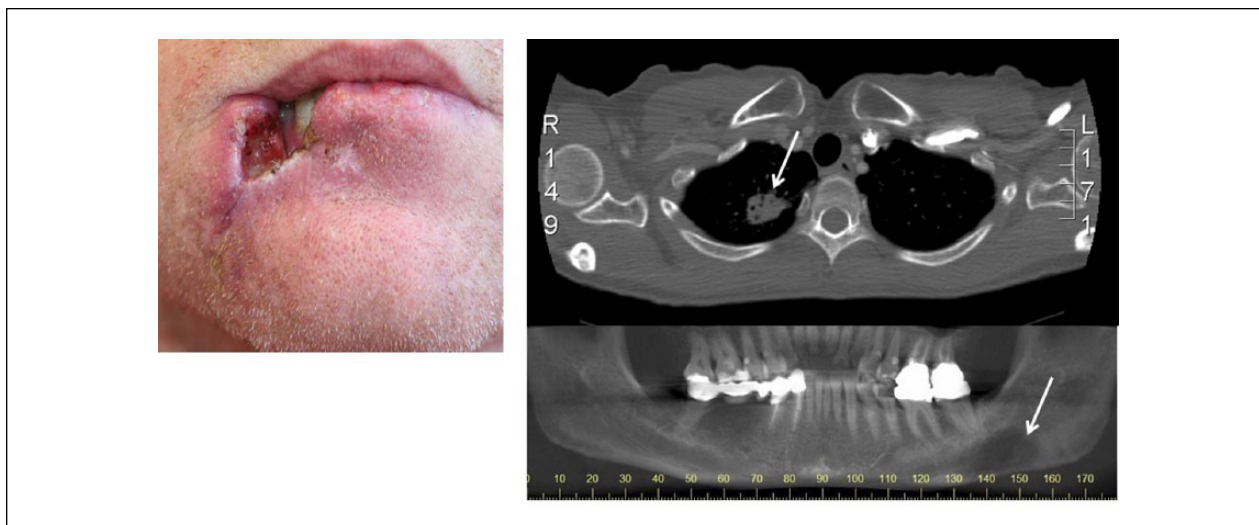
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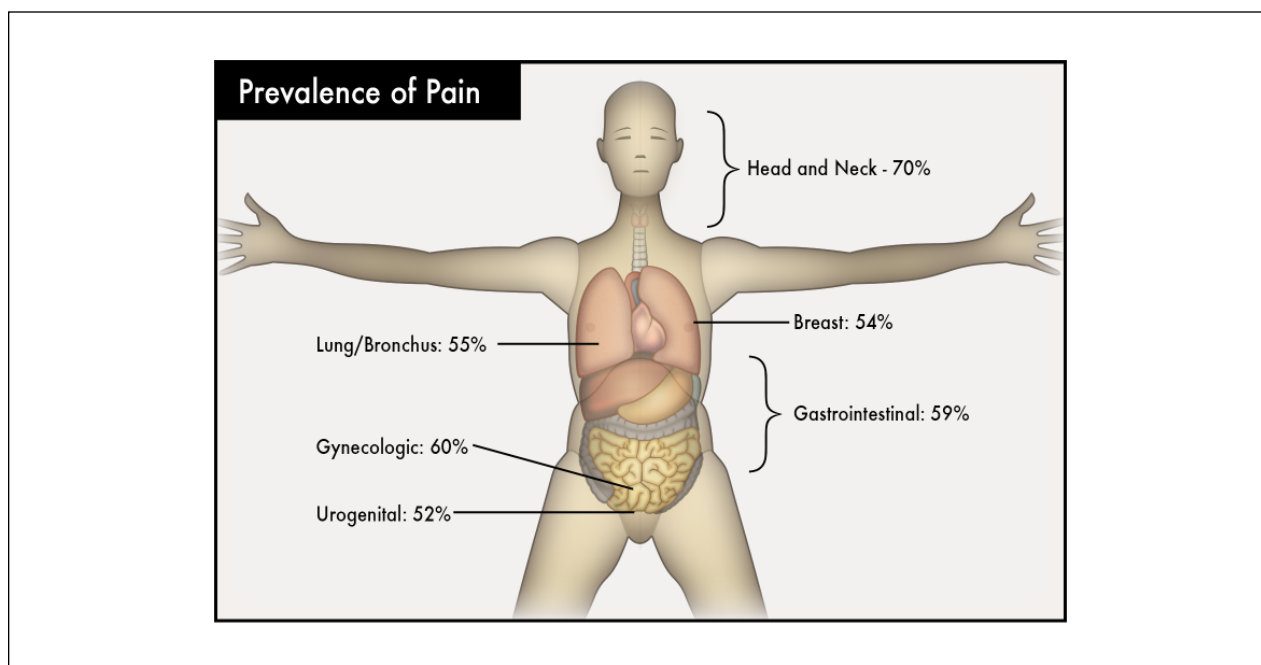
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**Figure 1.** Cancer pain can depend on the histologic type and the anatomic site involved. The patient pictured on the left has a lower lip squamous cell carcinoma. Despite significant pain he delayed his treatment. Ultimately, he sought treatment given his incapacity to eat, drink, or talk secondary to function-induced pain. On the right is an axial CT scan of a 52-year-old woman diagnosed with squamous cell carcinoma of the lung. Her initial complaint was pain in the jaw that turned out to be a metastasis of the lung cancer to her jaw. She had no pain in the lung.



**Figure 2.** The prevalence of pain based on the histologic type of cancer. The prevalence of pain is greater than 50% for all types of cancer (van den Beuken-van Everdingen and others, 2007).

## Clinical Studies

While clinical studies and trials can provide insight into the basic mechanisms of disease, most clinical trials on cancer pain lend limited insight into the neurobiology of

cancer pain. The investigators who design and conduct these trials should be commended; cancer pain trials are of the most difficult clinical studies. The medical condition of patients with late-stage cancer makes recruitment challenging. Dosing of experimental drugs in this population

can be difficult. A few of the clinical trials that do provide information regarding possible mechanisms will be reviewed.

### **Ketamine**

Ketamine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, and opioids have been combined and tested in cancer pain patients. The approach of NMDA receptor blockade is supported by preclinical cancer models that suggest that NMDA receptor activity, as measured by receptor phosphorylation, facilitates bone cancer pain (Gu and others 2010; Zhang and others 2008). Two randomized controlled trials that met appropriate Cochrane criteria concluded that ketamine improves the effectiveness of morphine in the treatment of cancer pain (Bell and others 2012). These results suggest that human cancers might be secreting mediators that activate the NMDA receptor leading to sensory neuron sensitization or activation. Also, possibly by antagonizing the NMDA receptor in the setting of cancer opioid receptors retain their sensitivity to opioid agonists.

### **Opioids**

Opioids, which can be delivered to patients by multiple routes, are the most commonly used and most effective analgesics for the management of cancer pain. Clinical trials that address the effectiveness of opioids for cancer pain are the most comprehensive. Four studies, which met the design criteria for Cochrane review measured the analgesic effect of opioids in cancer patients (Zeppetella and Davies 2013). All four studies looked at the use of oral transmucosal fentanyl citrate for the management of breakthrough cancer pain. Titration of oral transmucosal fentanyl citrate was the focus of one study while another study compared oral transmucosal fentanyl citrate with morphine. In a third study, oral transmucosal fentanyl citrate was compared with placebo. Oral transmucosal fentanyl citrate was shown to be effective for management of breakthrough cancer pain; it lowered pain intensity and increased pain relief at each of the time points. Global assessment also showed oral transmucosal fentanyl citrate to be effective. While the conclusion is that oral transmucosal fentanyl citrate can be effective for the management of breakthrough cancer pain, clinicians should remember that genetic variation in the catechol-*O*-methyltransferase gene affects cancer patients' response to morphine, which might explain the non-responding patient (Rakvag and others, 2008).

There are a number of drawbacks with opioids, including tolerance following administration of escalating doses. Practitioners, patients, and family caregivers are

often faced with concern for addiction in patients requiring treatment for cancer pain; however, addiction is rarely a problem in cancer patients. Studies in preclinical cancer mice have demonstrated that there are decreased motivational properties of morphine in cancer mouse models. Using the place preference paradigm investigators have demonstrated that mice suffering from cancer pain do not develop a preference for the environment associated with morphine (Betourne and others 2008). The authors proposed that secretion of anti-opioid neuropeptides within the nucleus accumbens contributed to decreased reward associated with morphine.

### **Spinal Cord Stimulation**

Cancer pain leads to pathologic changes in the spinal cord. Spinal cord stimulation (SCS) using electrotherapy has been proposed as an approach to reverse pathology-induced neurochemical changes in the dorsal horn. SCS has been used for difficult neuropathic pain conditions such as complex regional pain syndrome and has been investigated for cancer pain. While no randomized clinical trials are available, four clinical studies with a total of 92 participants have been published (Lihua and others 2013). In these studies the investigators reported pain scores before-and-after SCS. In two studies, pain relief was achieved in more than three quarters of patients by the end of the follow-up period. In all four of the studies, analgesic use was reduced. There are rare side effects associated with SCS; infection at the site of probe placement is most common. While efficacy is suggested based on these limited studies no conclusive recommendation can be made with regard to the use of SCS in cancer pain patients.

### **Acupuncture**

Acupuncture has been investigated for the management of cancer pain. While human studies have demonstrated that acupuncture is effective in reducing cancer pain, effective controls for acupuncture studies are nearly impossible (Paley and others 2011). Therefore, the results of acupuncture for cancer pain are difficult to assess. Electroacupuncture (EA) produces antinociception in female and male cancer mice; however, the effect in males does not last as long as in females (Smeester and others 2012). An experimental strength of this study is that the authors studied two separate cancer mouse models, a fibrosarcoma and an osteosarcoma. The authors use cells syngeneic with the mouse lines; therefore, the model does not suffer from the drawbacks of using athymic mice. When athymic mice are used for the orthotopic mouse model the role of cell-mediated immunity in cancer pain is lost. EA is antinociceptive in both cancer

models. EA significantly reduces neutrophil count and PGE<sub>2</sub> within the cancer microenvironment. While multiple mechanisms for acupuncture are proposed this work demonstrates that an anti-inflammatory effect contributes to the reduction in cancer pain. However, to demonstrate cause and effect of EA on inflammation in this cancer model, a pharmacological approach is required.

## Preclinical Studies

### *Interpretation and the Translational Potential of Preclinical Cancer Pain Studies*

Cancer pain is a complex pathologic process. Cancer cells produce mediators that recruit and affect other cells within the cancer microenvironment, including nerves and immune cells. Cancer pain involves interactions and crosstalk between the cancer, the primary afferent nociceptor, and the immune system. Cancer induces plasticity in the peripheral and central nervous system. Cancer-evoked responses in rodent models mirror some of the changes seen clinically.

When evaluating the results from preclinical cancer pain models, the reader should consider the following: (1) the proliferative, invasive, and metastatic potential of the cell line; (2) the anatomic site that is inoculated and how closely the site relates to where patients develop cancer; (3) the behavioral test that is used and its relevance to human reports of pain; (4) whether the behavioral test is reflexive or operant; (5) the genetic profile of the animal; and (6) the time between cancer inoculation and the behavioral measurement. More complex factors such as social interaction and environmental enhancement, which are difficult to control across studies, will also impact on cancer growth and pain behavior (Cao and others 2010, Vachon and others 2013). Most cancer pain models, whether bone or soft tissue exhibit heat hyperalgesia and mechanical allodynia.

Similar to most pain studies, the preclinical tests used in cancer models often do not align with the complaints of patients. For example, how often does a patient with either a primary or metastatic cancer in the femur complain of heat hyperalgesia in the foot? Yet, heat hyperalgesia is a common reported finding in bone cancer models. Because of the high prevalence of bone cancer pain the most common rodent cancer pain model is generated by inoculating cancer cells into a long bone. This model requires surgical manipulation including an arthrotomy; therefore, post-surgical pain might have an impact on the tested behavior. In a cancer pain model the control should include inoculation of the benign counterpart of the cancer. For example, if squamous cell carcinoma is inoculated, an approach used to create a soft tissue cancer model, then benign keratinocytes should be

inoculated. Inoculation of benign keratinocytes in the region of a nerve leads to hypersensitivity (Radtko and others 2010). A rarely used, but clinically relevant and worthwhile model is an abdominal carcinomatosis model in which carcinoma cells are inoculated into the abdomen. The measured behavior is abdominal mechanical allodynia or hunching behavior. A limitation which needs to be considered when interpreting the results of preclinical cancer pain studies is that for most of the published studies a single cancer cell line is used to produce the model. Because of the phenotypic and genotypic heterogeneity of cancers within a single histologic type the applicability of the molecular and behavioral results are limited.

### *Cancer Pain Is Distinct from Inflammatory Pain and Neuropathic Pain*

Cancer pain is often cited incorrectly as inflammatory pain; evidence from clinical studies and preclinical models strongly suggest that cancer pain can exist in a sterile tissue environment. Compelling evidence that cancer pain is not inflammatory pain is the meager evidence supporting the role of non-steroidal anti-inflammatory drugs; their role in clinical management of cancer pain is often considered controversial (Mercadante and Giarratano 2013). Cancer pain, as compared to inflammatory and neuropathic pain, induces a distinct set of neurochemical changes in the spinal cord and sensory neurons (Honore and others, 2000). Spinal cord plasticity in inflammatory pain is characterized by increased expression of the following: substance P, substance P receptor, calcitonin gene-related peptide (CGRP), and protein kinase C $\gamma$ . In the neuropathic pain model spinal cord plasticity is characterized by decreased substance P, decreased CGRP, increased galanin and increased neuropeptide Y. In a bone sarcoma model, spinal cord plasticity is characterized by spinal astrocyte hypertrophy, increased c-Fos expression, and an increase in dynorphin (DYN)-immunoreactive neurons. In a cancer model that is produced by implanting fibrosarcoma cells into and around the mouse calcaneus bone wide dynamic range dorsal horn neurons are sensitized to mechanical, heat and cold stimuli, electrophysiologic findings that differ from those found in inflammatory and neuropathic models. High threshold nociceptive neurons are not sensitized in the cancer model (Khasabov and others 2007).

Subtle differences in activation of spinal cord microglia and astrocytes differentiate cancer pain from neuropathic pain. Transient microglial activation and prolonged astrocyte proliferation is a hallmark of neuropathic pain and represents a critical step toward spinal cord plasticity. In two separate cancer pain models cancer astrocyte activation occurs independently of microglial

activation (Hald and others 2009). Spinal GFAP expression is reduced with a synthetic cannabinoid agonist (i.e., WIN 55, 212-2) in a cancer pain model but not a neuropathic pain model (Hald and others 2008)

Cancer induces decreased expression of the  $\mu$ -opioid receptor on dorsal root ganglia (DRG), which is a distinguishing feature between cancer pain and inflammatory pain (Yamamoto and others 2008). In a preclinical cancer model the intensity of cancer pain is greater than inflammatory pain; the morphine ED<sub>50</sub> for the cancer pain model is three times that for the carrageenan-injected mice (Wacnik and others 2003). Finally, cancer pain and inflammatory pain have been distinguished using a pharmacologic approach (Harano and others 2010). Additional preclinical models of cancer pain demonstrate differences between cancer pain and inflammatory pain (Shimoyama and others 2002).

### ***Cannabinoids and the Endogenous Cannabinoid System***

Cannabinoids have been studied extensively in different cancer pain models, including the following: a bone cancer model produced by inoculating a murine fibrosarcoma cell line into the humerus, femur, tibia, or calcaneus bone of mice; a bone cancer model produced by inoculating osteosarcoma or melanoma cells in the mouse tibia; a bone cancer model produced by inoculating breast carcinoma cells in the rat tibia; and a soft tissue cancer model produced by inoculating human oral squamous cell carcinoma cells into the mouse paw. Both cannabinoid receptor subtypes (i.e., CBR1 and CBR2) have analgesic roles in the cancer models. In preclinical models CBR agonists reverse cancer-induced pain with similar efficacy to opiates. Both CBR1 and CBR2 play roles at the periphery and spinal levels.

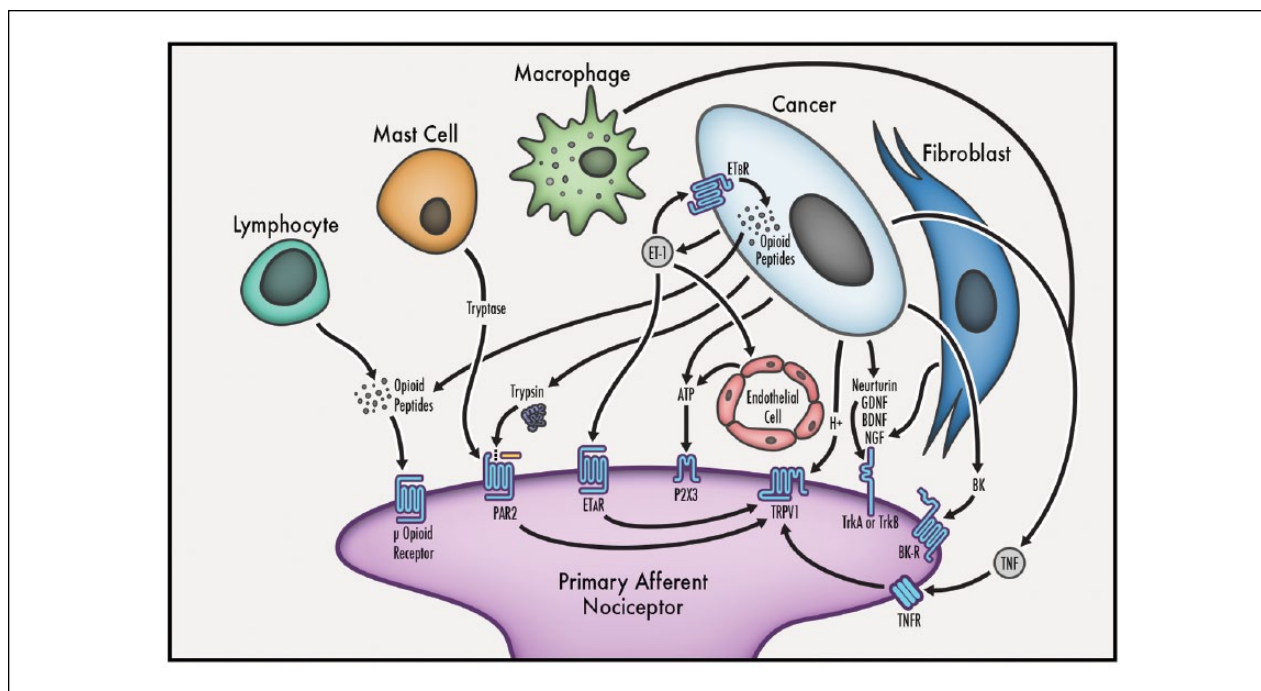
Cannabinoids potentially exert their antinociceptive action through reduced tumor burden, limitation of inflammatory mediators, endogenous opioid secretion, receptor and ion channel function in either primary afferent nociceptors or central neurons. There has been some question as to the role of the CBR1 and CBR2 receptors in the cancer microenvironment. Activation of peripheral CBR2 receptors reduce cancer burden and reduce cancer pain (Guerrero and others 2008; Lozano-Ondoua and others 2010; Saghafi and others 2011). In a fibrosarcoma mouse model, both CBR1 and CBR2 receptors have an antinociceptive role (Khasabova and others 2011b). Both CBR1 and CBR2 agonists reduce cancer-induced mechanical allodynia; co-injection of the two agonists have a synergistic effect which is independent of an opioid mechanism. Previously, Khodorova demonstrated that activation of CBR2 receptors on keratinocytes leads to opioid secretion (Ibrahim and others 2005). The

neurobiologic actions of CBR agonists in cancer pain likely include (1) CBR1-mediated spinal presynaptic inhibition (Furuse and others 2009); (2) CBR1-mediated peripheral afferent nociceptor inhibition (Kehl and others 2003; Hamamoto and others 2007, Guerrero and others 2008; Potenzieri and others 2008); (3) CBR2-mediated spinal NMDA receptor regulation (Gu and others 2011); (4) CBR2-mediated spinal opioid secretion (Curto-Reyes and others, 2010); (5) peripheral CBR1-mediated opioid secretion; and (6) peripheral CBR2-mediated opioid secretion (Curto-Reyes and others 2010; Guerrero and others 2008; Saghafi and others 2011).

An attractive strategy for the treatment of cancer pain is to exploit the endogenous analgesic system in the cancer microenvironment with opioids and cannabinoids. Preclinical studies suggest that the peripheral endocannabinoid system is a promising target for managing bone cancer pain (Khasabova and others 2011a). A local peripheral increase of 2-arachidonoyl glycerol (2AG) decreases mechanical hyperalgesia secondary to fibrosarcoma inoculated into the calcaneus bone. Activation of peripheral CBR2 receptors but not CBR1 receptors produces analgesic efficacy similar to morphine. There is an increase in CBR2 receptors in the plantar skin over the paw tumor. In a similar bone cancer pain model cutaneous hyperalgesia depends on the level of anandamide (AEA) in the paw skin. Fatty acid amide hydrolase (FAAH) activity and mRNA in the DRG ipsilateral to the affected paw contributes to increased FAAH activity in cancer microenvironment. The anti-hyperalgesia action of AEA and FAAH inhibition is blocked by a CBR1 antagonist. AEA and FAAH inhibition affects calcium ion transduction, which the investigators measured in DRG neurons co-cultured with fibrosarcoma cells (Khasabova and others 2008). The co-culture system provides a strategic experimental approach to understand the electrophysiologic response of neurons to mediators that are secreted by cancer. Reduced binding of AEA to FAAH is analgesic in a cancer pain model (Khasabova and others 2013). These preclinical studies reinforce that cannabinoids remain a good target for control of cancer pain and have shown promise in clinical studies (Portenoy and others 2012).

### ***Secretion of Mediators by the Cancer That Sensitize or Activate Primary Afferent Neurons***

The prevailing hypothesis for cancer pain is that cancers produce and secrete algogenic mediators that sensitize and/or activate primary afferent nociceptors within the cancer microenvironment (Figure 3). A formidable challenge in studying cancer pain research is the dynamic and complex interaction of cells within the cancer microenvironment. Carcinogenesis involves the recruitment of



**Figure 3.** A model of the cancer microenvironment and possible mechanisms that generate cancer pain. The primary hypothesis underlying cancer pain is that the cancer produces and secretes mediators that sensitize and/or activates primary afferent neurons within the microenvironment. Other constituent cells, which can be recruited by chemoattractants or mediators released by the cancer, including lymphocytes, mast cells, macrophages and fibroblasts also secrete mediators which modulate cancer pain. For example, in prostate cancer fibroblasts are responsible for secreting NGF. Mechanical stimulation of endothelial cells induces the release of ATP; this effect is sensitized by ET-1 (Joseph and others, 2013). Opioids can be secreted into the cancer microenvironment by the cancer or other cells including lymphocytes. BDNF = brain derived neurotrophic factor; BK = bradykinin; BK-R = bradykinin receptor; ET-1 = endothelin-1; ET<sub>A</sub>R = endothelin A receptor; ET<sub>B</sub>R = endothelin B receptor; GDNF = glial derived neurotrophic factor; NGF = nerve growth factor; PAR2 = protease activated receptor 2; TrkA = tyrosine kinase receptor A; TrkB = tyrosine kinase receptor B; TRPV1 = transient receptor potential vanilloid 1.

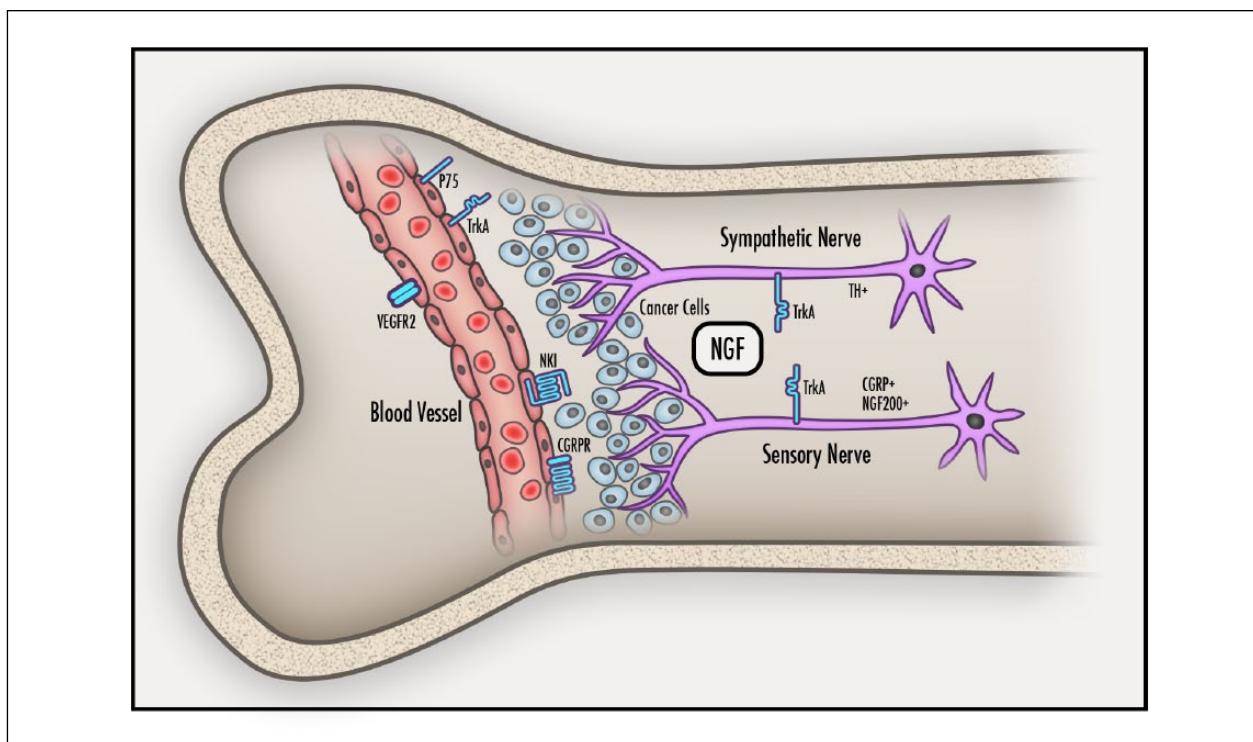
neurons, lymphocytes, endothelial and fibroblasts to the cancer microenvironment, which then secrete pain-modulating mediators.

Much of our evidence that mediators contribute to pain is based on a change in pain behavior following administration of an antagonist. While a drug might have an antinociceptive effect in a cancer pain model, the drug might also involve a reduction in cancer proliferation. Reduced cancer proliferation would reduce cancer burden and decrease the amount of algogenic mediators secreted by the cancer. Therefore, investigators are encouraged to measure the anti-proliferative effect of a drug, either *in vitro* or *in vivo* but preferably both, along with the antinociceptive effect. Some of the key mediators that contribute to cancer pain will be reviewed below.

### Neurotrophic Factors

Neurotrophic factors, which can be secreted by the cancer or constituent cells in the cancer microenvironment, contribute to cancer pain, perineural invasion, and locoregional recurrence. Different cancers express and secrete different neurotrophic factors and also express their

cognate receptors. Breast cancer expresses brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (Vanhecke and others 2011). The best described neurotrophic factor that has a role in cancer pain is nerve growth factor (NGF). Oral squamous cell carcinoma produces NGF and neurturin (Ye and others 2012). The source of NGF in the cancer microenvironment could be the carcinoma itself (Ye and others 2011). Constituent cells can also secrete NGF, which is the case with prostate cancer (Halvorson and others 2005). NGF sequestration with an antibody is highly effective in reversing cancer pain in pre-clinical cancer models (Mantyh and others 2010; Ye and others 2011). In a mouse bone sarcoma pain model anti-NGF reverses cancer-induced changes in the spinal cord (Sevcik and others 2005). In this model, anti-NGF does not have an effect on cancer progression nor does anti-NGF affect sensory or sympathetic innervation of the cancer in the bone or overlying skin. On the other hand, sensory nerve sprouting occurs in an orthotopic breast cancer model; NGF is expressed and secreted by the breast cancer cells and associated stromal cells. Sensory fibers that are CGRP<sup>+</sup>/Trk A<sup>+</sup>/GAP43<sup>+</sup> sprout (Bloom and others 2011). Anti-NGF reverses spinal cord markers consistent with



**Figure 4.** Angiogenesis and neurogenesis in the cancer microenvironment are linked processes that contribute to cancer pain. Newly forming blood vessels and nerves interact on a molecular and anatomic basis. VEGF and NGF share common signal transduction pathways. NGF, which induces both angiogenesis and neurogenesis, is secreted by cancers or cancer-associated cells (Nico and others, 2008). Endothelial cells express the NGF receptors, p75 and TrkA receptor, as well as the receptor for VEGF, VEGFR2. SP and CGRP act on their respective receptors, NK1 and CGRPR, on blood vessels to induce endothelial cell proliferation and blood vessel formation. NGF acts through TrkA to generate sensory and sympathetic nerve fibers that innervate the cancer microenvironment; both fiber types contribute to cancer pain. CGRP = calcitonin gene-related protein; CGRPR = calcitonin gene related protein receptor; NGF, nerve growth factor; NK1 = neurokinin-I receptor; SP = substance P; TH = tyrosine hydroxylase; TrkA = tyrosine kinase receptor A; VEGF = vascular endothelial growth factor; VEGFR = vascular endothelial growth factor receptor.

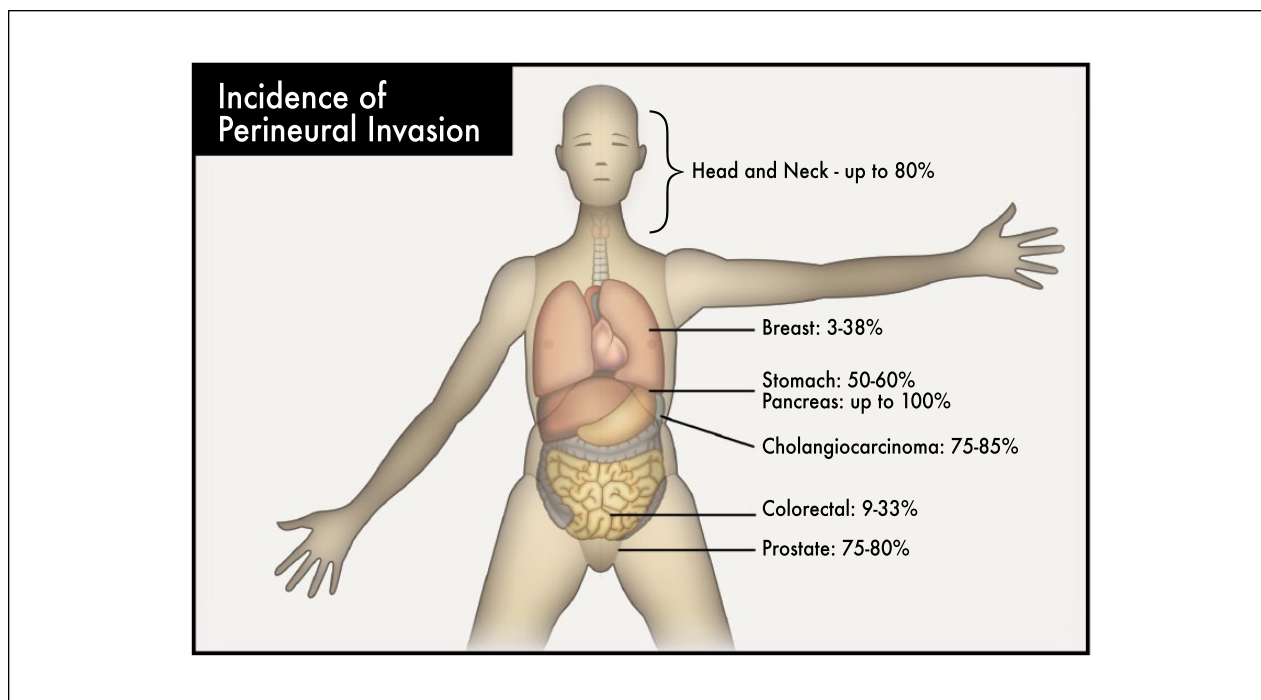
spinal cord plasticity, including DYN and c-Fos expression (Jimenez-Andrade and others 2011). In a soft tissue oral squamous cell carcinoma model, anti-NGF reduces cancer pain, cancer progression and cachexia (Ye and others 2011). Cancers are notoriously vascular. The processes of angiogenesis and neurogenesis have overlapping signaling pathways (Nico and others 2008). In certain models NGF secretion by the cancer induces both angiogenesis and neurogenesis (Mapp and Walsh 2012) (Figure 4). Angiogenesis and perivascular nerve growth are linked in a prostate cancer bone metastasis model (Jimenez-Andrade and others 2011). However, this finding is not upheld in all cancer models. In a mouse cancer model that is produced by inoculating fibrosarcoma cells into the hindpaw there is an increase in CGRP<sup>+</sup> nerve fibers and a decrease in CD-31<sup>+</sup> blood vessels (Wacnik and others, 2005).

### ATP

One of the early studies demonstrating the role of ATP and the P2X<sub>3</sub> receptor in cancer pain uses a fibrosarcoma

bone (calcaneous) cancer mouse model (Gilchrist and others 2005). The authors analyze the neuronal population in the skin overlying the tumor: P2X<sub>3</sub> expression increases in CGRP<sup>+</sup> neuronal fibers. Cancer induces P2X<sub>3</sub> receptor up-regulation and electrophysiologic sensitization (i.e., ATP-induced transient current) which increases by more than 50% (Wu and others 2012). This work uses a rat bone cancer pain model produced by inoculating Walker 256 breast sarcomatous cells. A317491, an antagonist of the P2X<sub>3</sub> receptor, attenuates bone cancer pain when injected locally or intrathecally. The P2X<sub>3</sub> and P2X<sub>2/3</sub> receptor antagonist, AF353, reduces cancer-induced electrical, mechanical, and thermal stimuli evoked dorsal horn hyperexcitability (Kaan and others 2010).

Nodose ganglia cocultured with fibrosarcoma cells show that P2X-mediated responses were highly variable and demonstrated biphasic desensitization kinetics with both fast and slow currents (Chizhnikov and others 2009). Inhibition of ATP-activated currents by opioids had a strong dependence on desensitization kinetics. In some neurons sensitivity to opioid agonists was



**Figure 5.** The incidence of perineural invasion based on the anatomic location of cancer (Bapat and others, 2011).

completely lost. These authors used an appropriate, but rarely employed, control: coculturing neurons with rapidly proliferating, but benign, fibroblasts. The neurons that were cocultured with the fibroblasts did not exhibit the desensitization kinetics observed with the neurons cocultured with the cancer. Potentially ATP that is produced at high levels by the cancer and then secreted into the cancer microenvironment could affect opioid responsiveness in cancer patients.

### Endothelin

The role of endothelin-1 (ET-1) was first described in a cancer pain model by Mantyh and his colleagues (Wacnik and others 2001). The role of ET-1 in cancer pain is now well established in multiple cancer pain models. The role of ET-1 in cancer pain is reviewed elsewhere (Hans and others 2009). Recently, an interesting mechanism of ET-1 sensitization of ATP release by endothelium is reported (Joseph and others 2013). Released ATP activates  $P2X_{2/3}$  receptors on nociceptors to induce pain. Given that cancers are characterized by angiogenesis and are highly vascular this is a compelling mechanism potentially contributing to cancer pain (Figures 3 and 4).

### Protons

Cancers generate an acidic microenvironment, which could activate transient receptor potential vanilloid 1 (TRPV1) or acid-sensing ion channels (ASICs). In a bone

cancer pain model the acidic microenvironment contributes to ASICs up-regulation and cancer pain behavior (Nagae and others 2007). The role of TRPV1 in cancer pain is demonstrated using pharmacology across a number of cancer pain models (Ghilardi and others 2005; Honore and others 2009; Karai and others 2004; Niiyama and others 2009; Shinoda and others 2008). In a rat bone cancer pain model, TRPV1 on DRGs are overexpressed via formaldehyde (Han and others 2012). Lipophilic substances that activate TRPV1 are secreted by sarcoma cells (Lautner and others 2011). Plasticity within the associated ganglia includes up-regulation of TRPV1 (Asai and others 2005). In a trigeminal cancer pain model that is produced by inoculating squamous cell carcinoma into the gingiva of a rat mechanical allodynia and thermal hyperalgesia occur along with increased expression of CGRP, substance P,  $P2X_3$ , and TRPV1 in the associated trigeminal ganglia (Nagamine and others 2006).

### Formaldehyde

Endogenous formaldehyde, which is produced by lysine-specific demethylase 1 (LSD1) in certain cancers, contribute to pain. LSD1 and endogenous formaldehyde are up-regulated in breast and prostate cancer. In a bone cancer pain model produced by inoculating MRMT-1 breast cancer cells in the bone marrow of rats, endogenous formaldehyde is increased in the bone marrow, sera, and tumor tissues of the rat bone cancer pain model. Systemic injection of the LSD1 inhibitor, pargyline, in this cancer pain model



reduces cancer pain behavior; pargyline did not have a proliferative effect on the cancer cells (Liu and others 2013).

### **Proteases**

Cancers produce and secrete proteases, which are responsible for destruction of tissue and expansion of the cancer. Proteases have been measured in the cancer microenvironment of human patients, in cancer cell culture and in the tissues of cancer mouse models (Hardt and others 2011). Moreover, using pharmacologic and genetic approaches the role of protease activated receptor-2 (PAR2) has been demonstrated in cancer pain (Lam and Schmidt 2010; Lam and others 2012).

### **Miscellaneous Mediators**

There are other algogenic mediators produced by cancers that have not been mentioned above. In an orthotopic mouse lung cancer model the tumor tissue has increased levels of tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), interleukin-1 $\beta$  (IL1 $\beta$ ), and IL6. TNF $\alpha$  contributes to cancer-induced heat hyperalgesia and nociceptor sensitization (Constantin and others 2008). Bradykinin (BK) also has a role in cancer pain. In a mouse melanoma model BK is secreted by the melanoma and activates both B<sub>1</sub> and B<sub>2</sub> receptors to produce cancer pain (Fujita and others 2010). The B<sub>1</sub> receptor is implicated in bone cancer pain (Sevcik and others 2005). Granulocyte colony stimulating factor (GCSF) and granulocyte macrophage colony stimulating factor (GMCSF) are secreted into the cancer microenvironment and receptors for these mediators are expressed on peripheral nerves innervating the cancer microenvironment (Bali and others 2013). GMCSF sensitizes nerves in the cancer microenvironment and leads to the release of CGRP. If signaling between cancer and the nerves via GCSF and GMCSF is interrupted there is a decrease in cancer induced pain. Interestingly disruption of the signaling leads to a decrease in cancer growth. This article is one of several recent papers that have shown that activity and crosstalk between cancer and the nerve is involved with cancer proliferation (Brener and others 2009; Feng and others 2011; Mayordomo and others 2012). These results suggest that receptors on primary afferents in the cancer microenvironment could not only be targets for cancer pain but also targets for cancer proliferation.

### **Interactions between Cancer and Sensory Neurons**

The interaction between cancer and surrounding sensory nerves likely contributes to pain. A classic paper in the cancer pain field is one which is produced by inoculating

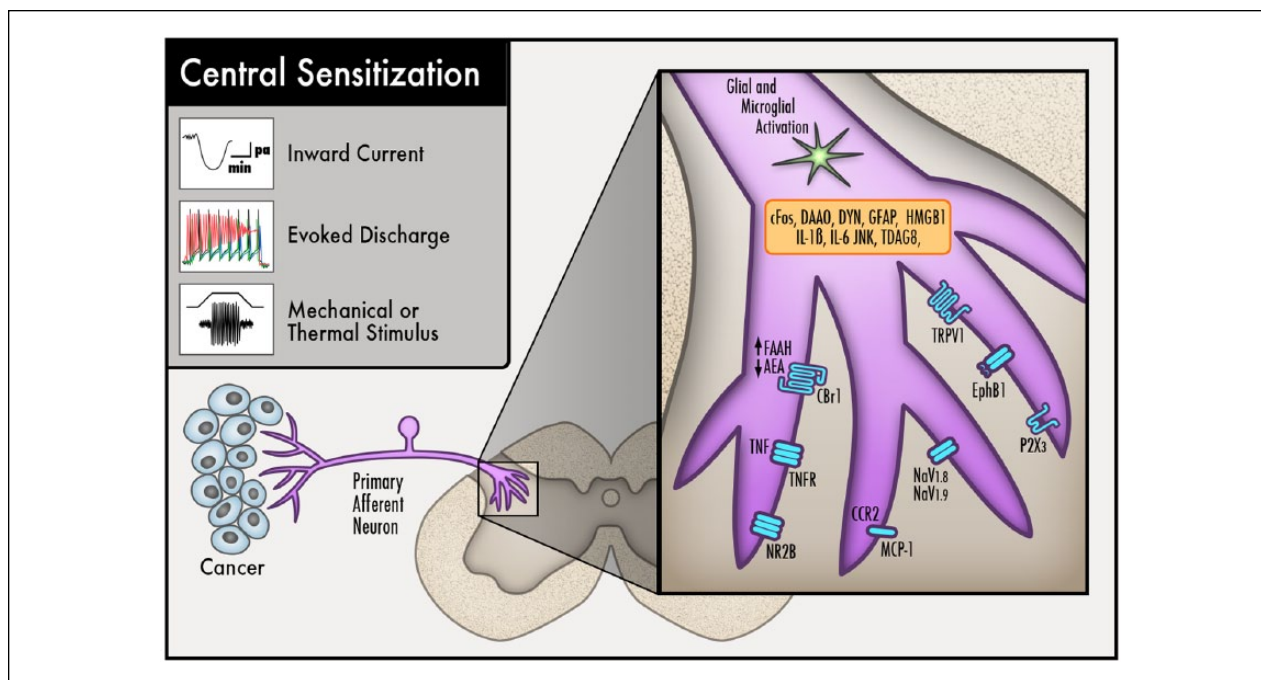
fibrosarcoma cells in and around the calcaneus bone of a mouse. C fibers adjacent to the tumor show spontaneous activity and increased response to heat. The animals displayed mechanical hypersensitivity. There is a significant increase in epidermal nerve fibers early during cancer growth. However, with time (16-24 days after implantation) there is a decrease in the epidermal nerve fibers (Cain and others 2001). In a rat bone cancer model the dorsal root ganglia overexpress tetrodotoxin resistant sodium channels NaV1.8 and in NaV1.9 (Qiu and others 2012).

A coculture system of cancer cells and neurons provides an experimental strategy for studying plasticity of neurons following exposure to mediators that are released from cancer. Using this approach, investigators demonstrate changes in sensory neurons, including the release of CC chemokine ligand 2 (CCL2), which results in increases in voltage-gated Ca<sup>2+</sup> channels (Khasabova and others 2007). Using a similar non-contact co-culture system, proteases released from squamous cell carcinoma up-regulate PAR2 in neurons (Lam and Schmidt 2010).

Perineural invasion (PNI), first described in head and neck cancer, generates cancer pain (Neumann 1862). The molecular mechanism of PNI, which involves neurotrophic factors, associated receptors and chemokines, are reviewed elsewhere (Bapat and others 2011). Cancers have differing proclivities for perineural invasion (Figure 5). Certain cancers which are notoriously painful, such as head and neck or pancreatic, have high rates of perineural invasion. Neurons are not simply bystanders within the cancer microenvironment and there is clear evidence that cross talk between the cancer and neuron contributes to carcinogenesis (Magnon and others 2013). PNI likely involves a molecular and physical interaction between cancer cells and neurons. Integrins, such as the  $\alpha$ 6 integrin adhesion receptor, are involved with PNI and mediate interaction between the cancer and peripheral nerves. In a bone cancer model inoculated with human cancer cells that express mutated  $\alpha$ 6 integrin, the mice show a decrease in bone fractures, a decrease in tumor cell migration within the bone and a decrease in cancer pain behavior. These findings are compelling and suggest that by inhibiting urokinase-type plasminogen activator, which cleaves and activate  $\alpha$ 6 integrin receptor, bone cancer pain could be reduced (King and others 2008).

### **Spinal Cord Changes**

Cancer induces spinal cord plasticity characterized by the following overexpression of nociceptive mediators and receptors, electrophysiologic changes and glial activation (Figure 6). Mantyh proposes a spinal “neurochemical signature” of bone cancer pain, which includes massive astrocyte hypertrophy, internalization of the substance P receptor, c-Fos expression in lamina one dorsal



**Figure 6.** Cancer induces spinal cord changes. Cancer leads to dorsal horn plasticity which includes up-regulation of pain-related mediators, up-regulation of pain-related receptors, glial and microglial activation, and electrophysiologic changes (i.e., greater amplitude of induced inward currents, evoked discharges, increased response of nociceptive neurons to heat and mechanical stimuli). The up-regulated nociceptive-related mediators are depicted in the orange oval. Cancer decreases AEA signaling through up-regulation of FAAH. The depicted changes are based on work from multiple cancer pain models. Please see the text for details and references. AEA = anandamide; CBr1 = cannabinoid receptor 1; CCR2 = chemokine C-C motif receptor 2; DAAO = D-Amino acid oxidase; DYN = dynorphin; EphB1 = ephrin B ligand receptor; FAAH = fatty acid amide hydrolase; GFAP = glial fibrillary acidic protein; HMGB1 = high-mobility group protein 1; JNK = c-jun N-terminal kinase; MCP-1 = monocyte chemoattractant protein-1; NR2B = NMDA (N-methyl-D-aspartate) receptor subunit; TDAG8 = T-cell death-associated gene 8; TNF = tumor necrosis factor; TNFR = tumor necrosis factor receptor; TRPV1 = transient receptor potential vanilloid 1.

horn neurons and increased dynorphin, a prohyperalgesic peptide (Schwei and others 1999). Similar dorsal horn changes (i.e., increase in c-Fos positive cells; up-regulation of substance P, CGRP, and DYN) occur in a mouse sarcoma model in which the sarcoma cells are inoculated into the region of the sciatic nerve, rather than into bone (Shimoyama and others, 2005). In a peritoneal carcinomatosis model generated by inoculating the abdominal cavity of mice with gastric carcinoma cells there is up-regulation of substance P and an increase in c-Fos positivity in the spinal cord; the model also shows up-regulation of the  $\mu$  opiate receptor in the DRG (Suzuki and others 2012). In an orofacial cancer pain model produced by injecting Walker carcinoma sarcoma 256B cells into the vibrissal pads of rats, c-Fos expression in the medullary dorsal horn increases. In this orofacial cancer pain model the rats display mechanical allodynia and have difficulty with ingesting food and have prolonged facial grooming periods. Interestingly in this rat orofacial cancer pain model there is a hyposensitivity to both mechanical and thermal stimulation in the center of the

tumor; however, hypersensitivity occurs along the tumor front (Ono and others 2009).

The spinal cord neurochemical signature along with the associated behavior in cancer pain models can be pharmacologically reversed. Ibandronate (a bisphosphonate that inhibits osteoclasts), osteoprotegerin and a cyclooxygenase-2 (COX-2) inhibitor decrease the neurochemical signs of central sensitization in a mouse bone sarcoma model (Halvorson and others 2008; Honore and others 2000; Sabino and others 2002). Similar to the effect of ibandronate in a rat bone cancer pain model, risedronate (also a bisphosphonate) decreases bone cancer-related bone destruction, pain related behavior and spinal expression of GFAP in a murine bone cancer pain model (Hald and others 2009). Spinal cord plasticity that occurs in the bone cancer model can also be reversed with radiation to the bone cancer site. This finding is consistent with clinical experience which shows that radiation of the cancer site, whether the cancer is in soft tissue or bone, leads to a reduction in pain. Bone cancer-induced spinal cord plasticity, including glial activity, DYN,

COX-2, and chemotactic cytokine receptor (CCR2) expression, are reduced with radiation of the primary site of bone cancer (Vit and others, 2006). In a rat model of tibial bone cancer electroacupuncture reverses cancer-induced neurochemical changes including up-regulation of IL-1 $\beta$ . Intrathecal injection of an IL-1 $\beta$  receptor antagonist inhibits cancer-induced hyperalgesia (Zhang and others 2007). Not all forms of analgesic treatment for cancer pain reverse the associated spinal cord pathophysiologic changes. Sustained morphine administration reverses cancer pain behavior in a rat cancer model produced by inoculating MRMT-1 cells. Dorsal horn plasticity, however, remains unchanged (Urch and others 2005).

C-Jun N-terminal kinase (JNK; a subgroup of the MAPK pathway) activation in both neurons and astrocytes within the spinal cord is associated with bone cancer pain in a rat model created by injecting rat mammary gland carcinoma cells. A JNK inhibitor reduces the cancer-induced mechanical allodynia (Wang and others 2012a). T cell death associated gene eight (TDAG8), which is involved with complete Freund's adjuvant-induced chronic inflammatory pain, contributes to bone cancer pain through the protein kinase A (PKA) signaling pathway. Spinal TDAG8 expression increases in a rat bone (tibia) cancer (Walker 256 cells) model. Administration of H89, a PKA inhibitor, attenuates bone cancer pain in this model (Hang and others 2012). The same rat bone cancer model shows increased expression of spinal CCR2 (Hu and others 2013). Intrathecal anti-CX3CR1 neutralizing antibody in a rat tibial bone cancer (Walker 256 mammary gland carcinoma cells) pain model reduces cancer pain behavior (Yin and others 2010). Intrathecal administration of a  $\kappa_2$ -opioid agonist and IL-10 synergistically reduce bone cancer pain (Kim and others 2011). The role of C-fibers in cancer pain is shown in a rat cancer model using epidurally administered resiniferatoxin (RTX), which leads to long-lasting segmental analgesia (Szabo and others, 1999).

EphrinB-EphB receptor signaling is involved in bone cancer pain and morphine tolerance in the cancer pain model. The Eph receptors are receptor tyrosine kinases, which are involved in progression of human malignancies. EphB receptor signaling is involved in neuropathic pain and pain associated with opiate withdrawal. EphB receptors and the respective ligands ephrinBs mediate spinal cord transmission in the setting of cancer. Spinal inhibition of the EphB1 receptor relieves bone cancer pain and restores morphine induced analgesia in this model; spinal cord neurochemical changes are also reversed (Liu and others 2011). Descending modulation of serotonin-dependent spinal processing contributes to cancer-induced bone pain (Donovan-Rodriguez and others 2006). Intrathecal injection of mGluR-3 agonists and

mGluR-5 antagonists inhibit spontaneous behavior in a bone cancer pain model that was produced by inoculating the mouse femur with sarcoma cells (Ren and others 2012). WNT signaling in the spinal cord mediates bone cancer pain. In a bone cancer pain model there is an increase in the expression of WNT in the spinal cord dorsal horn neurons as well as primary sensory neurons and astrocytes. WNT signaling activation stimulates production of IL-18, TNF $\alpha$ , NR2B glutamate receptor, and Ca<sup>2+</sup>-dependent signals through the  $\beta$ -catenin signaling pathway in the spinal cord. Blockade of WNT signaling in the spinal cord reduces neurochemical alterations consistent with mouse bone cancer pain (Zhang and others 2013). Cancers induce production of other spinal nociceptive mediators which are depicted in Figure 6 (Tong and others 2010).

### *Cancer-Induced Electrophysiologic Changes in the Spinal Cord*

The cancer-induced molecular changes within the spinal cord result in electrophysiologic changes which are distinct relative to the changes induced by neuropathic or inflammatory pain (Figure 6). In the setting of cancer the receptive field of superficial dorsal horn neurons enlarges. In a rat cancer pain model produced with MRMT1 mammary cancer cells dorsal horn neurons undergo changes which contribute to pain: they become hyperexcitable, the ratio of wide dynamic range to nociceptive specific neurons increases, and the wide dynamic range neurons have an increased response to mechanical, thermal and electrical (A $\beta$ -, C fiber-, and post-discharge evoked response) stimuli. One drawback of this study is that the sham-operated animals are produced by injecting growth media. A proper control consists of inoculation of benign cells rather than cell culture media alone (Urch and others 2003). Gabapentin normalizes hyperexcitable superficial dorsal horn neurons and reduces electrical evoked and mechanical evoked responses in the spinal cord (Donovan-Rodriguez and others 2005). Systemic gabapentin reduces mechanical allodynia in a cancer pain mouse model that is produced by inoculating melanoma into the hind paw; importantly, repeated administration of gabapentin does not induce tolerance (Kuraishi and others 2003).

Electrophysiology demonstrates cancer-induced changes in substantia gelatinosa (lamina II) neurons. In this study adult mice were inoculated with sarcoma into the femur. The mice demonstrated hyperalgesia to mechanical stimuli of the skin of the ipsilateral paw. Lamina II neurons in spinal cord slices were studied using whole cell voltage clamp recording techniques. The substantia gelatinosa neurons exhibited spontaneous

excitatory postsynaptic currents (EPSCs). The amplitudes of spontaneous EPSCs increase in cancer bearing mice; however, there are no changes in passive membrane potential of substantia gelatinosa neurons. These findings demonstrate that in the bone cancer pain model spinal synaptic transmission is enhanced and involves A $\delta$  and C fibers in the substantia gelatinosa across lumbar levels (Yanagisawa and others 2010).

### *Spinal Astrocyte Activation*

Cancer activates spinal astrocytes leading to astrocyte hypertrophy and proliferation. Reactive astrogliosis occurs in different cancer pain models (Hald and others 2009; Honore and others 2000; Zhang and others 2005). Spinal glia activation in a murine bone cancer pain model depends on TNF $\alpha$  receptors 1 and 2 (Geis and others 2010). Cancer also leads to molecular changes within astrocytes that contribute to cancer pain. One example is D-amino acid oxidase (DAAO), which is found within spinal cord astrocytes. DAAO catalyzes oxidation of amino acids to hydrogen peroxide. DAAO expression and activity is induced in a rat bone cancer model. Intrathecal injection of siRNA/DAAO reduces mechanical allodynia. Subcutaneous and intrathecal injection of a DAAO inhibitor, CBIO, blocks cancer-induced mechanical allodynia in a dose- and time-dependent manner. Subcutaneous injection of CBIO reduces the production of hydrogen peroxide in the spinal cord and reduces GFAP expression in the spinal cord; moreover, CBIO prevents morphine tolerance when the two drugs are used together (Huang and others 2012). The role of TLR4 on microglia is demonstrated in a rat cancer pain model produced by injection of Walker 256 cells into the tibia. In this model intrathecal administration of TLR 4 siRNA reduces the following: expression of TLR4, expression of spinal microglial markers and pro-inflammatory cytokines and behavioral hypersensitivity (Lan and others 2010; Li and others 2013).

Cancer-induced spinal glial changes are reversed with the glial modulating agent propentofylline (PPF). In a rat tibial cancer model produced with Walker 256 cells microglia and astrocytes are activated in the spinal cord ipsilateral to the cancer. Intrathecal PPF relieves cancer-induced pain and inhibits activation of spinal glial cells and the expression of glia-associated pro-inflammatory cytokines, including IL-1 $\beta$ , IL-6, and TNF $\alpha$  (Yao and others 2011). PPF works across spinal segments. The drug suppresses glia activation in a rat orofacial cancer pain model produced with Walker 256 cells (Sago and others 2012). Extracellular signal-regulated kinase (ERK) activation in spinal microglia and astrocytes also mediates cancer-induced bone pain. Activation of ERK1/2 occurs in microglia and astrocytes in this cancer model and intrathecal injection of a selective MEK (ERK

kinase) reverses cancer-induced mechanical allodynia (Wang and others 2012b).

## **Future Directions and Challenges in the Field of Cancer Pain Research**

The ardor of many scientists over the past decade has improved our understanding of cancer pain. These advances have primarily resulted from improved understanding of cancer biology and the interaction between cancers and neurons. We now recognize that cancers and neurons act reciprocally on each other. Adrenergic and cholinergic stimulation are necessary for carcinogenesis in some organs (prostate) (Magnon and others 2013). Additionally, there is overlap between pain-related and cancer apoptotic genes. JNK has been implicated in both tumor growth and cancer pain. Systemic injections of inhibitors of JNK reduce cancer-related mechanical allodynia, heat hyperalgesia, and tumor growth both in vitro and in vivo (Gao and others 2009). TPRV1 activation on astrocytoma induces endoplasmic reticulum-mediated cell death (Stock and others 2012).

Cancer pain will continue to plague patients and become increasingly prevalent as cancer therapy extends the duration that patients are able to live with cancer. Some types of cancers are now often curable (e.g., testicular and certain lymphomas). With the implementation of genomic analysis subsets of previously incurable cancers now exhibit improved cure rates (e.g., non-small lung cancers with ALK gene rearrangement that respond to Crizotinib) (Kwak and others 2010). Survival rates for a handful of cancers including oral cancer have not improved. Unfortunately, oral cancer happens to be notoriously painful; it is arguably the most painful and debilitating type of cancer. Genomic heterogeneity of certain cancers remains one of the most significant challenges for treatment and palliation. Histologically and clinically, certain cancers might appear to be identical; however, at a genomic level they display significant heterogeneity. In addition, the genetic makeup of the host has a great influence on patient response to morphine in the setting of cancer. According to the current prevailing explanation, mediators secreted by painful cancers are the primary cause of pain. If this explanation is correct then histologically similar cancers in the same anatomic location might produce a variety of mediators depending on genomic differences in the tumor. Accordingly a single analgesic therapy is unlikely to exhibit equal efficacy for different cancer types and for cancers in different patients.

Most of the studies reviewed are based on preclinical models. We now recognize that cancer pain in patients is intimately tied to other symptoms, including anxiety, depression, and sleep deprivation in the form of a

symptom cluster. Somatic and limbic systems converge and affect pain processing. Characterization of higher order cognitive and emotional processes in preclinical cancer models is difficult. In addition, emotions associated with a painful or rapid demise could alter pain perception and the study of cancer pain. Translating results from preclinical models to patient will subsequently be confounded with additional challenges. Improved relief of cancer pain might require pharmacologic and non-pharmacologic antagonism of mechanisms in the neurosensory system and mechanisms integral to cancer proliferation. Improved understanding and improved treatment for cancer pain will likely emerge from teams of investigators. Clinicians, neuroscientists, cancer and cell biologists and psychologist studying the problem at the molecular, preclinical or patient level will undoubtedly be required to tackle the problem.

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