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Reduction of bone cancer pain by CB1 activation and TRPV1 inhibition

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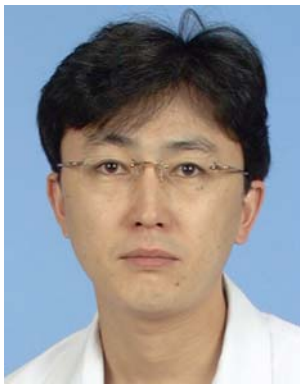

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As advances in cancer detection and treatment have increased the life expectancy of cancer patients, more attention to improving patient quality of life is needed. Among the various types of cancer pain, bone cancer pain is often debilitating, difficult to treat, and insufficiently relieved. Recently, a preclinical model of bone cancer pain has been developed [1]. This model has begun to provide insights into the mechanisms by which tumors cause pain and how cancer-pain-related sensory information is processed. Here we present new strategies for bone-cancer pain based on our data obtained from analyses of a mouse model.

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Murine model of bone-cancer pain

The murine model of bone-cancer pain was created by injecting osteosarcoma cells into the intramedullary space of the femur [1]. Although bone marrow cells fill the intramedullary space in the normal mouse femur, they are largely replaced by tumor cells in the femur at day 14 after osteosarcoma injection (Fig. 1). This model features a reproducible pattern and rate of tumor growth within the intramedullary cavity following implantation of sarcoma cells [2]. Figure 2 shows pain-related behavior before, and at days 7, 14, and 21 after sarcoma or sham injection. The mice with sham injection exhibit no detectable pain-related behavior throughout the observation period. On the other hand, mice with osteosarcoma injection exhibit spontaneous flinches and impaired weight-bearing at day 7. These pain-related behaviors remained unchanged at days 14 and 21. Mice with sarcoma injection also exhibited impaired limb use during spontaneous ambulation at day 7. At days 14 and 21, more pronounced impairment of limb use was observed.

Transient receptor potential vanilloid subfamily 1 (TRPV1)

TRPV1 is expressed predominantly in unmyelinated neurons and is activated not only by capsaicin but also by noxious heat, protons, and membrane-derived lipids [3]. Because these stimuli cause pain in vivo, this sensitivity of TRPV1 to multiple types of noxious stimuli might explain the properties of so-called polymodal nociceptors. TRPV1 is essential for selective modalities of pain as well as for tissue-injury-induced thermal hyperalgesia [4]. Pharmacological studies have also suggested a contribution of

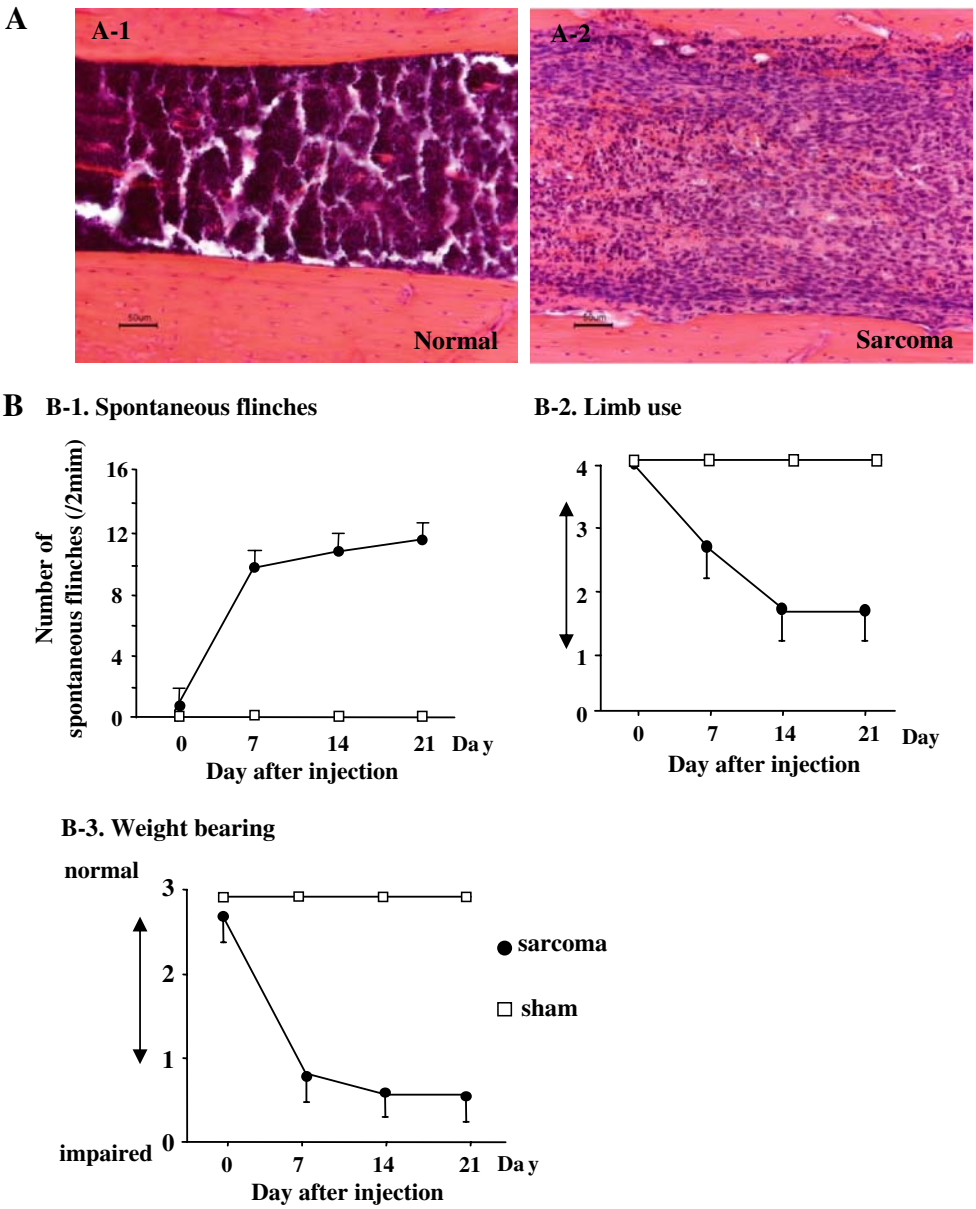

Fig. 1 **a** Hematoxylin and eosin (H&E) staining of normal (*A-1*) and sarcoma-bearing femur (14 days after sarcoma injection) (*A-2*). Note replacement of the darkly stained marrow cells with the more lightly stained sarcoma cells in the sarcoma-bearing femur. **b** Time course of bone-cancer pain-related behaviors. All behaviors were analyzed before (day 0), and on days 7, 14, and 21 after sarcoma or sham injection. *B-1* the number of spontaneous flinches during a 2-min observation period. *B-2* limb use score during spontaneous ambulation. *B-3*, weight-bearing score during spontaneous standing. *Sarcoma* mice with sarcoma injection; *normal* untreated mice; *sham* mice with sham injection

TRPV1 to hyperalgesia observed in neuropathic pain and diabetes [5, 6]. In addition, change in the pattern of expression and up-regulation of TRPV1 in dorsal root ganglion (DRG) neurons can contribute to abnormal increase in pain transmission. However, the potential role of TRPV1 in bone-cancer pain is unknown. and we therefore examined its involvement.

Analgesic effects of TRPV1 inhibition on bone-cancer pain [7]

Systemic administration of a potent TRPV1 antagonist, 5-iodoresiniferatoxin, reduced bone-cancer-related pain behaviors without producing any observable side effects in a dose-dependent fashion at day 14 after sarcoma injection.

Altered expression of TRPV1 in DRG neurons in bone-cancer pain [7]

TRPV1 level was significantly increased in DRG neurons and peripheral axons ipsilateral to the site of osteosarcoma injection in a transcription-dependent fashion at day 14 after injection. Sarcoma injection increased the number of TRPV1-positive neurons, and a TRPV1-positive profile was observed not only in small neurons but also in medium to large neurons in ipsilateral DRGs. The percentage of the ratio of TRPV1-positive neurons was notably increased in

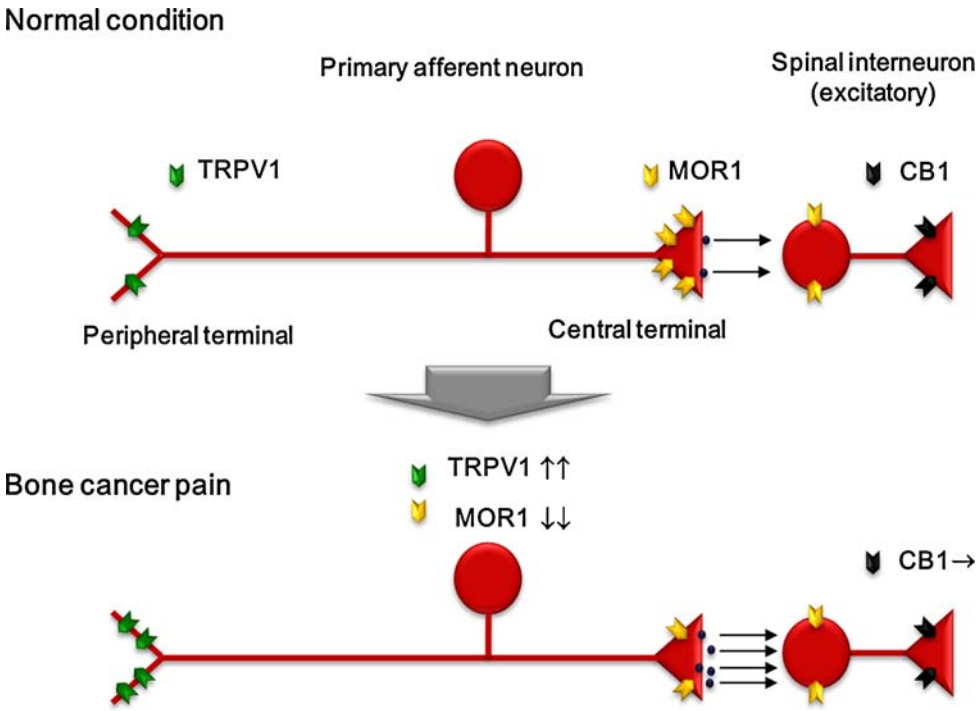

Fig. 2 Possible mechanism of bone-cancer pain. Under a bone-cancer condition, transient receptor potential vanilloid subfamily 1 (TRPV1) is up-regulated in the peripheral neurons and sensitized. On the other hand, mu-opioid receptor (MOR) is down-regulated in the peripheral neurons. Canabinoid receptor type 1 (CB_1) is located in the axon terminals of excitatory spinal interneuron, and its expression is preserved under a bone-cancer condition

neurons positive for neurofilament protein 200 (NF200) (a marker of neurons with myelinated fibers) and in neurons positive for calcitonin-gene-related peptide (CGRP) (a marker of peptidergic neurons) but not in neurons positive for isolectin B4 (IB4) (a marker of non-peptidergic neurons).

Increased excitability of TRPV1 [8]

Many chemical mediators released from tumor and inflammatory cells can be involved in bone-cancer pain. Among the various chemical mediators, endothelin-1 (ET-1) is released by osteosarcoma cells, and activation of endothelin type A receptor (ETA) by ET-1 generates bone-cancer pain. We performed an in vitro experiment on interaction of TRPV1 with ET-1 and found that ET-1 sensitized TRPV1 in an ETA–protein kinase C (PKC)-dependent manner and that the thermal threshold of TRPV1 was decreased below normal body temperature.

Rescue of decreased analgesic effect of morphine by a TRPV1 antagonist

The analgesic effect of intrathecal morphine in bone-cancer pain is decreased compared with that in nonmalignant inflammatory pain. Decreased analgesic effect of morphine in bone-cancer pain is associated with down-regulation of the μ opioid receptor in TRPV1-positive DRG neurons [9]. The combination of morphine and a TRPV1 antagonist, SB366791, was shown to have a potent analgesic effect on bone-cancer pain, and a subanalgesic dose of SB366791 therefore potentiated the reduced analgesic effect of morphine [10]. Thus, the combination of morphine and a TRPV1 antagonist can rescue the decreased analgesic effect of morphine.

Our results show that TRPV1 is an important molecule in the generation of bone-cancer pain and a target for its treatment.

Cannabinoid receptor type I (CB1)

Recently, there has been increasing interest in the use of cannabinergic-system-based drugs for pain management. Cannabinoids exert their effects via activation of CB1 and CB2, which are coupled to $G_{i/o}$ protein. The role of spinal CB1 in nociceptive transmission has been extensively studied. Activation of spinal CB1 has been shown to inhibit glutamatergic excitatory postsynaptic currents in spinal cord slices of naïve rats [11] and c-fiber-evoked neuronal response of the dorsal horn [12–14]. It has also been shown that spinal CB1 activation reduced several types of pain [15, 16]. However, the role of spinal CB1 in bone-cancer pain has not been elucidated. Therefore, we focused on spinal CB1 to develop a novel strategy for treating bone-cancer pain.

Analgesic effect of spinal CB1 activation on bone-cancer pain

Spinal CB1 activation by the CB1 agonist arachidonyl-2-chloroethylamide reduced bone-cancer-related pain behaviors in a dose-dependent manner without inducing catalepsy or motor impairment, which were associated with systemic administration of CB1 agonists. However, spinal inhibition of the metabolism of two endogenous cannabinoids, anandamide and 2-arachidonoyl glycerol, and inhibition of spinal CB1 did not affect bone-cancer pain [17]. These results suggest that nociceptive input by bone cancer does not activate the spinal endocannabinoid system involving CB1.

Expression of CB1 in the spinal cord in a murine model of bone-cancer pain

In the dorsal horn of the spinal cord, CB1 was mainly expressed in specific laminae, including the superficial dorsal horn, deep layer of the dorsal horn, dorsolateral funiculus, and around the central canal. In the superficial dorsal horn, which plays an important role in the processing of nociceptive transmission from the periphery to the central nervous system, CB1 was expressed in lamina I and lamina II outer and the ventral part of lamina II inner but not in the dorsal part of lamina II inner. Dorsal root rhizotomy did not alter expression of CB1 in the dorsal horn, and CB1 was not colocalized with any neuronal makers of primary afferents, including CGRP, IB4, and cholera toxin β subunit. In addition, CB1 was expressed within the axon terminals of the spinal interneurons rather than in the dendritic processes. In contrast to MOR expression, CB1 expression in the superficial dorsal horn ipsilateral to the site of implantation of sarcoma cells was preserved, compared with that contralateral to the site of implantation.

The results of our study demonstrate that spinal CB1 activation by an exogenously administered CB1 agonist reduced bone-cancer-related pain behaviors, including behaviors related to spontaneous pain and movement-evoked pain. Presynaptic inhibition of spinal neurons may contribute to spinal CB1 activation-induced analgesia.

Mechanism and possible therapeutic strategies of bone-cancer pain

The possible mechanism of bone-cancer pain based on our data is shown in Fig. 2. TRPV1 is an important molecule in the generation of bone-cancer pain and a target for its treatment. CB1, but not MOR, expression is preserved in the dorsal horn of the spinal cord under a bone-cancer pain-related condition. Therefore, spinal CB1 activation may be effective for treating bone-cancer pain compared with MOR activation. The reduced analgesic effect of morphine can be rescued by its combination with a TRPV1 antagonist. In addition to our findings, other researchers have shown that bradykinin, prostaglandin E2, ET-1, nerve growth factor, and osteoclasts are important molecules in the generation of bone-cancer pain. It is expected that these findings will lead to mechanism-based treatment resulting in improved quality of life of bone-cancer patients.

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