TRPV4 MEDIATES CANCER-INDUCED BONE PAIN

1+ Wakabayashi H, 2Wang Lee Yang, 3Matsubara T, 5Mizuno A, 3Suzuki M, 1Uchida A, 2Yoneda T
1Orthopedic Surgery Mie University Graduate School of Medicine, 2Department of Biochemistry Osaka University Graduate School of Dentistry, 3Department of Pharmacology, Jichi Medical School
email address: whiroki@clin.medie-u.ac.jp

Introduction
Bone pain is frequently accompanied with cancer metastasis to bone and recognized as a major clinical problem as survival of cancer patients prolongs. Bone pain can be caused by noxious chemical and/or mechanical stimuli produced by cancer cells and associated inflammatory cells. It is among such a noxious chemical stimulus. Our earlier studies showed that the acidic microenvironment caused by H+ release by bone-resorbing osteoclasts induced bone pain through an activation of the acid sensing receptors including the transient receptor potential vanilloid 1 (TRPV1). TRPV4 is a transducer of hypo-osmotic stimuli in primary nociceptive afferents. Recent studies using TRPV4-deficient mice suggest a critical role of TRPV4 in mechanosensing. Since TRPV4 has 45% homology with TRPV1, it is plausible to hypothesize that TRPV4 is involved in the pathogenesis of cancer-induced bone pain. In the present study, we investigated the role of TRPV4 in the induction of bone pain associated with cancer colonization in bone.

Materials and Methods
Animal model of cancer-induced bone pain
Lewis lung cancer cells (1X10^5 cells/10 µl PBS) were inoculated into the bone marrow cavity of right tibiae in mice under general anesthesia. Left tibiae received PBS. Tumor growth was monitored weekly by radiological examinations.

Pain-related behavior assays
Three behavioral assays including the plantar test, grip force test and hind-limb lifting (flinching) test were conducted in tumor-bearing tibiae compared with non-tumor-bearing tibiae. In the plantar test, the paw withdrawal latency in response to thermal stimuli was monitored as an indicator for hyperalgesia. The grip force test is an assay of movement-related hyperalgesia that is known clinically as a specific type of 'breakthrough pain'. To examine spontaneous sign of cancer-induced bone pain, the cumulative duration of repetitive and spontaneous lifting of the tumor-inoculated hind-limbs (flinching) was measured. These assays were performed at day 0, 7 and 14 of tumor cell inoculation by three investigators who have no knowledge of experiments.

Immunohistochemical examination
To examine markers for neural activation by noxious stimuli in the primary afferent neurons, we performed immunohistochemical examination in the primary afferent neurons (dorsal root ganglia).

Results
Animal model of cancer-induced bone pain
Radiological examinations demonstrated that inoculated Lewis lung cancer cells aggressively colonized and destroyed bone. The tumor-inoculated hind-limbs displayed much shorter paw withdrawal latency than the hind-limbs without tumor cells (Fig 1). Grip force of hind-limbs inoculated with tumor cells was decreased compared with non-tumor-bearing hind-limbs as the tumor enlarged. Tumor cell-inoculated hind-limbs exhibited increased duration of lifting as the tumor grew. Flinching was increased in tumor-inoculated hind-limbs 14 days after cell inoculation.

Hyperalgesia in TRPV4-deficient mice
Paw withdrawal latency of the hind-limbs inoculated with tumor cells did not differ from the hind-limbs without tumor cells in TRPV4-/- mice (Fig 2). These results were in marked contrast to those seen in WT mice (see Fig 1). Similarly, there was no difference in grip force between hind-limbs with and without tumor cells. Flinching was significantly decreased in tumor-inoculated hind-limbs compared with WT mice.

There were no differences in tumor growth in bone and area of osteolytic lesions between WT and TRPV4-/- mice.

Phosphorylated-ERK Expression in DRG
Phosphorylated-ERK (p-ERK) is a widely used molecular marker for neural activation by noxious stimuli. The expression of p-ERK immunoreactive neurons was increased in the DRG of tumor-bearing side compared with the DRG of non-tumor-bearing side in WT mice. p-ERK immunoreactive neurons were significantly decreased in tumor-bearing side in TRPV4-/- mice compared with WT mice (Fig 3).

Activating transcription factor 3 (ATF-3) is widely-used as a neuronal injury marker. The expression of ATF-3 immunoreactive neurons was increased in the tumor-bearing side DRG compared with the non-tumor-bearing side DRG in WT mice. However, TRPV4-/- mice showed no differences in ATF-3 expression in the tumor and non-tumor-bearing side DRG.

Discussion and Conclusions
Our results suggest that TRPV4 as well as TRPV1 plays an important role in inducing bone pain associated with cancer cell colonization in bone. TRPV4 might be a new molecular target for the development of novel analgesics in cancer-induced bone pain.

References
3.TRPV4 mediates pain-related behavior induced by mild hypertonic stimuli. Pain 118, 70-79, 2005