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Mechanical Allodynia and Thermal Hyperalgesia Induced by Experimental Squamous Cell Carcinoma of the Lower Gingiva in Rats

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Abstract: **We developed a rat model of oral cancer pain by inoculating cancer cells into the lower gingiva. A squamous cell carcinoma (SCC) derived from Fisher rats, SCC-158, was inoculated into the subperiosteal tissue on the lateral side of the lower gingiva in male Fisher rats. Inoculation of cancer cells induced marked mechanical allodynia and thermal hyperalgesia in the ipsilateral maxillary and mandibular nerve area. Infiltration of the tumor cells into the mandible and the completely encompassed inferior alveolar nerve was observed. Calcitonin gene–related peptide (CGRP)–, substance P (SP)–, ATP receptor ($P2X_3$)–, and capsaicin receptor (TRPV1)–immunoreactive cells strikingly increased in the small-cell group of trigeminal ganglia (TGs) after tumor cell inoculation. The TRPV1-immunoreactive cells also increased in the medium- and large-cell groups. Retrograde tracing combined with immunofluorescence techniques revealed the increased expression of peptides and the receptors in maxillary nerve afferent neurons. These results suggest that inoculation of SCC cells into the lower gingiva produces mechanical allodynia and thermal hyperalgesia, indicating the establishment of a novel rat model of oral cancer pain. Increased expression of CGRP, SP, $P2X_3$, and TRPV1 in the TG may be involved in the behavioral changes in this model.**

Perspective: ***To clarify the mechanisms of oral cancer pain, we examined the expression of calcitonin gene–related peptide, substance P, ATP receptor $P2X_3$, and capsaicin receptor TRPV1 in trigeminal ganglia. Characterizations of these molecular systems which mediate pain perception are important to develop novel clinical tools for promoting relief of oral cancer pain.***

Key words: *Oral cancer, CGRP, SP, P2X3, TRPV1.*

Pain due to malignant tumors occurs frequently in cancer patients, especially the intractable pain related to tumor progression and/or treatments. Recently, various types of animal models of cancer pain have been developed.[4,36,42,45,55] These models have demonstrated the distinct pharmacologic and neurochemical aspects of cancer pain, suggesting the existence of its inflammatory, neuropathic, and tumorigenic components.[36] However, different cancer models may have different underlying mechanisms based on the animal species, tumor types, and locations.[55]

Although its frequency is relatively low, pain is the main symptom leading oral cancer patients to physicians.[17,30] Local progress of oral cancer causes severe pain, which is increased by growth of the tumor or metastasis.[3,27] Although pain relief using nonnarcotic agents is normally effective at an early stage, many pa-

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tients subsequently require opiates and rapidly develop a tolerance.[9,27] Oral cancer pain impairs a patient's speech, swallowing, eating, drinking, and interpersonal relations and has a significant impact on his or her overall quality of life.[9] Development of adequate animal models of oral cancer pain is essential for discovering better therapeutic approaches to oral cancer pain.[30]

Calcitonin gene–related peptide (CGRP) is a mediator of pain transmission in the spinal cord and brain stem.[29,56] The contribution of the local production of CGRP to tumor-induced nociception has been reported.[54]

Substance P (SP) has been recognized as an important mediator in the pain pathway.[15] Up-regulations of this peptide have been shown in inflammatory pain models[10] and neuropathic cancer pain model.[45]

Extracellular adenosine triphosphate (ATP) has been implicated in nociceptive signaling in normal and pathologic pain states, including cancer-related pain.[11,46] The $P2X_3$ receptor, one of the ligand-gated ion channels activated by extracellular ATP, has been shown to be present in nociceptive afferent fibers in rats.[53]

The capsaicin (vanilloid) receptor subtype 1 (TRPV1) is an ion channel which can be activated by vanilloid compounds, protons, and heat.[8,48] Involvment of this receptor in inflammatory,[1] neuropathic,[23,41] and cancer pain[4,18] has been reported.

In the present study, we developed a rat model of oral cancer pain to investigate the mechanisms involved in the generation and maintenance of pain associated with oral cancer. We examined changes in the mechanical and thermal sensitivity of the facial skin to correlate with local tumor progression. In addition, to clarify the possible role of CGRP, SP, $P2X_3$, and TRPV1 in pain associated with oral cancer, we examined the expression of these peptides and receptors in the trigeminal ganglia (TGs) in our model. Characterization of these molecular systems which mediate pain perception is important in developing novel clinical tools for promoting the relief of oral cancer pain.

Materials and Methods

Experimental Animals

Eighty male Fisher rats (SLC, Hamamatsu, Japan) weighing around 190 g were used in this study. They were exposed to a light-dark cycle (L:D 12:12-h) and kept in a temperature-controlled room (23°C) with food and water ad libitum. This study was conducted under the auspices of the local animal ethics committee in accordance with the Guidelines for Animal Experiments of the Nagoya University Graduate School of Medicine (No. 16019), the Animal Protection and Management Law of the Japanese Government (No. 105), and the International Association for the Study of Pain.[57]

Cell Culture

The SCC-158 cells (squamous cell carcinoma derived from the external acoustic meatus of the Fisher rat) were provided by the Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan). They were cultured in Dulbecco's MEM (Nissui, Tokyo, Japan) with 10% fetal bone serum (IBL, Fujioka, Japan) and passaged weekly according to JCRB guidelines. Cells were detached by scraping and then centrifuged at 15,000 rpm. The pellet was suspended in 0.1 mol/L phosphate-buffered saline (PBS) (5×10^6 cells/100 μL) and then used for subcutaneous inoculation into the backs of Fisher rats under anesthesia with inhalation of diethyl ether. By day 14 after inoculation, the tumors had grown to approximately 10-15 mm in diameter. They were then extirpated and crushed with a metal mesh. A suspension was initially prepared at the concentration of 10^8 cells/mL in sterile PBS and finally 2.5×10^6 cells in 0.025 mL PBS and then used for subperiosteal inoculation into the lower gingiva.

Inoculation of Tumor Cells

Under anesthesia with an intraperitoneal injection of pentobarbital (50 mg/kg, Nembutal, Abbot Laboratories, Chicago, Ill), the tumor cells (2.5×10^6 cells in 0.025 mL PBS) were inoculated into the subperiosteal tissue of the rats on the lateral side of the lower gingiva with a 24-gauge needle. Control groups were injected with 0.025 mL PBS.

Behavioral Testing

The time courses of mechanical and thermal sensitivity were determined in a group of tumor cell–inoculated animals ($n = 5$) and a control group ($n = 5$). The analyses of these behavioral experiments were made by experimenters blind to the experimental conditions. Body weights were monitored daily during the experimental period.

Assessment of Mechanical Sensitivity

Mechanical sensitivity of the whisker-pad skin (maxillary nerve) area and submandibular skin (mandibular nerve) area was assessed by the use of von Frey hairs (North Coast Medical, Morgan Hill, Calif). Training sessions were carried out for 7 consecutive days to increase the sensitivity of the test.[47] Animals were restrained around the trunk with a towel and allowed to acclimate to their surroundings for 10-15 min before testing. The von Frey filaments were applied to the whisker-pad skin and submandibular skin areas. Areas both ipisilateral and contralateral to the tumor inoculation were examined. The frequency of nociceptive response (head withdrawal or vocalization) was counted from 10 trials. For each trial, the filament was applied at 5-s intervals. The nociceptive threshold was defined as the minimum pressure needed to evoke nociceptive responses in at least 60% of the trials.

The paw-withdrawal threshold value was recorded from the left hind paw of rats using a dynamic plantar aesthesiometer (Ugo Basile, Comerio, Italy). Animals were placed on a metal mesh floor covered with transparent plastic boxes, and allowed to acclimate to their surroundings for a minimum of 20 min before testing. A movable filament probe (0.5 mm diameter) was placed