International Journal of Oral Biology, Vol. 31, No. 4, December 31 2006, p. 135-140

International Journal of Oral Biology

Expression of TRP Channels in Mouse Dental Papilla Cell-23 (MDPC-23) Cell Line

Myoung Sang Shin, Kyu Young Yeon, Seog Bae Oh, and Joong Soo Kim*

Department of Physiology, School of Dentistry and Dental Research Institute, Seoul National University, Seoul 110-749, Korea

(Received September 27, 2006 ; Accepted October 26, 2006)

Temperature signaling can be initiated by members of transient receptor potential (thermo-TRP) channels. Hot and cold substances applied to teeth usually elicit pain sensation. Since odontoblasts constitute a well-defined layer between the pulp and the mineralized dentin, being first to encounter thermal stimulation from oral cavity, they may be involved in sensory transduction process, in addition to their primary function as formation of dentin. We investigated whether thermo-TRP channels are expressed in a odontoblast cell line, MDPC-23. The expressions of thermo-TRP channels were examined using reverse transcription polymerase chain reaction (RT-PCR), immunohistochemistry, fluorometric calcium imaging. Analysis of RT-PCR revealed mRNA expression of TRPV1, TRPV2, TRPV4 and TRPM8, but no TRPV3, TRPA1. Immunohistochemical approach failed to detect TRPV1 expression. Whereas the application of 4-phorbol-12,13-didecanoate(10 μM, a TRPV4 agonist), menthol(1 mM, a TRPM8 agonist) and icilin (10 μM, a TRPM8 agonist) produced the enhancement of intracellular calcium concentration, capsaicin(1 μM, a TRPV1 agonist) did not. Our results suggest that subfamily of thermo-TRP channels expressed in odontoblasts may serve as thermal or mechanical transducer in teeth.

Keywords: MDPC-23, odontoblast, TRP channels

Introduction

Odontoblasts originating from the neural crest are the cells responsible for the dentin formation during tooth development (Lumsden, 1988). Main functions of odontoblasts are synthesis and extracellular deposition of a type I collagen-rich matrix referred to as predentin, and then formation of dentin by the mineralization of this matrix (Qin *et al*, 2004). In their proximal ends, long processes penetrate through the nonmineralized predentin and into dentinal tubules. In addition to its critical role in dentinogenesis, it has long been postulated that the odontoblast may also be involved in sensory transduction in teeth (Matthews *et al*, 1996).

The mouse dental papilla cell-23 (MDPC-23) cell line was developed as a spontaneously immortalized cell line derived from 18- to 19-days CD-I fetal mouse molar dental papillae (Hanks *et al*, 1998a). MDPC cells are unique in that the cells are smaller than fibroblasts, are epithelioid in shape, and have multiple small cell membrane processes from the time they are plated until they form multilayered nodules. The cell line synthesizes the proteins, dentin sialoprotein (DSP) and a moderately phosphorylated rat phosphophoryn (DPP-MP), which are thought to be dentin-specific and synthesized mainly by odontoblasts. They also synthesize other proteins common to mineralized tissues such as alkaline phosphatase (ALP), type I collagen, osteopontin (OPN), and osteocalcin (OCN), but is negative for dentin matrix protein-1 (DMP-1) (Hanks *et al*, 1998b). These results strongly support the notion that MDPC-23 cells may serve as an odontoblast-like cells for *in vitro* studies. Thus, MDPC-23 cell provide a valuable cell model for studies of functional roles of odontoblasts such as identification of various specific molecules involved in dentinogenesis. However, to date, little is known whether MDPC-23 cells have characteristics as a sensory transducer.

*Corresponding author: Joong Soo Kim, D.D.S & Ph.D., Department of Physiology, College of Dentistry, Seoul National University, 28-2 Yeongeon-Dong, Chongno-Ku Seoul 110-749, Korea., Tel.: +82-2-740-8657; Fax.: +82-2-762-5107; E-mail: joongkim@snu.ac.kr

Several phenotypes of the transient receptor potential (TRP) family of ion channels are directly related to the cellular mechanisms for nociceptive and thermosensitive functions in the sensory system (Pedersen *et al*., 2005). The superfamily of TRP channels has been divided into six subfamilies in mammals, TRPC, TRPV, TRPM, TRPA, TRPP and TRPML according to structural similarities and modes of activation. Some of them mediate sensory transduction of cold, heat, mechanical, osmotic or noxious stimuli (Clapham, 2003). Since tooth pain is commonly induced by the presence of hot or cold foods in the oral cavity, temperature-sensitive TRP channels in odontoblasts might be involved in the pain transduction in the teeth. This study was aimed to determine the expression of temperature-sensitive TRP channel in an odontoblast cell line, MDPC-23 cells.

Materials and Methods

Cell culture

MDPC-23 cells were cultured with minimum essential medium α (α-MEM) containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine (Gibco BRL, Grand Island, NY, USA). The MDPC-23 cells were maintained in a 5% CO_2 incubator at 37°C. Media were changed every two days and conditions of the cells were monitored under phase contrast microscopy.

Reverse Transcription Polymerase Chain reaction (RT-PCR)

Total RNA was isolated from rat TG neurons and MDPC-23 cells using a TRI reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction, treated with RNase-free DNase I for 30 min, and then heat-inactivated for 10 min at 75°C. Single-stranded cDNA was synthesized from 3-5 μg of the total RNA with the Superscript First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Primers sets and annealing temperatures are listed in Table 1. The PCR amplifications (initial Taq activation step at 95°C for 5 min, then 95°C for 30 s, annealing temperature for 30 s, 72°C for 50 s, and 72°C for 10 min) were performed for 35-40 cycles using Taq™ (TaKaRa Shuzo, Shiga, Japan). The house-keeping gene GAPDH was used as a positive control. PCR products were separated on 1.5% agarose gels in the presence of ethidium bromide.

Immunohistochemistry

All rinsing and incubations were performed with phosphate buffered saline (PBS). Cell samples were fixed in 4% paraformaldehyde for 10 min. After rinsing during 15 min, cells were treated with blocking solution (2% BSA, 5% FBS, 0.1% PBST) at room temperature for 1 hour. Samples were then incubated overnight at 4°C with primary antibody (guinea pig anti-TRPV1 (1:500; Chemicon, Temecula, CA, USA). The cells were washed three times with PBS and then incubated with FITC-conjugated donkey anti-guinea pig (Jackson ImmunoResearch, West Grove, PA, USA) at 1:200 respectively for 1 hour at room temperature. After washing with PBS, the samples were covered with a Vectashield mounting media (Vector Laboratories, Inc., Burlingame, CA, USA) and visualized under a confocal microscope using the appropriate filter sets (LSM 5 PASCAL; Carl Zeiss, Germany). The primary antibodies were omitted as negative control (data not shown).

Intracellular calcium imaging

MDPC-23 cells were loaded with fura-2 AM (4 uM: Molecular Probes, Eugene, OR, USA) and 0.01% Pluronic F-127 (Molecular Probes, Eugene, OR, USA) for 45 min at 37°C. Loading and experiments were performed in a balanced salt solution [BSS (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose]. Followed by rinsing and incubation for 30 min to allow hydrolysis of the fura-2 AM. The coverslips were mounted onto the chamber, which then was placed onto the inverted microscope (Olympus IX70, Japan), and perfused continuously by BSS at 2 ml/min. All measurements were made at 36°C (temperature controller PTC-20: ALA Scientific Instrument Inc.,

Table 1. The sequence of the primers used for RT-PCR

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	Annealing Temperature (°C)	size (bp)
rTRPV1	TGA CAA CAC CAA GTT CGT GAC A	CGA TCA CCT CCA GAA CCG AGT T	61	301
rTRPV2	GAA TTC TCA GGA CCG TAC CAG C	GCA ACG ACG GTG AAG ATG AAC	59	281
rTRPV3	CCA AGC TGT GCT TGT GAT ACT G	GCC TAT GGT GAG CTT GAA GAG C	59	330
rTRPV4	GCT GCA AGC ATT ACG TGG A	GGC GAA AGA CCG TCA TTG TTA	59	373
rTRPM8	CTG TGG CCT CGT ATC GTT TAG G	GCC GGA ATA CAA TAC CCG CTA T	61	331
rTRPA1	ACA AGA AGC ACC AAA CAT TGA CAC A	TTA ACT GCG TTT AAG ACA AAA TTC C	59	242
mTRPV1	AGC GAG TTC AAA GAC CCA GA	TTC TCC ACC AAG AGG GTC AC	64	232
mTRPV2	CAG AAG GCT CCA CTG GAA AG	CAC CAC AGG CTC CTC TTC TC	60	201
mTRPV3	ACG GTG GAG AAC GTC TCC	TGT CCG TCT TAT GGG TCC	59	239
mTRPV4	ACA ACA CCC GAG AGA ACA CC	TGA ACT TGC GAG ACA GAT GC	64	221
mTRPM8	CTG TGG CCT CGT ATC ATT TAG G	GCC GGA ATA CAA TAC CCG CTA T	59	331
mTRPA1	ACA AGA AGT ACC AAA CAT TGA CAC A	TTA ACT GCG TTT AAG ACA AAA TTC C	59	242

USA). Cells were illuminated with a 175W xenon arc lamp, and excitation wavelengths (340/380 nm) were selected by a Lambda DG-4 monochromator wavelength changer (Shutter Instrument, Novato, CA, USA). Intracellular free calcium concentration ($[Ca^{2+}]_i$) was measured by digital video microfluorometry with an intensified CCD camera (CasCade, Roper Scientific, Trenton, NJ, USA) coupled to a microscope and software (Metafluor, Universal Imaging Corp., PA, USA) on a Pentium 4 computer.

Drugs

Capsaicin, (-)menthol (1 mM) and 4-phorbol-12,13-didecanoate (4α-PDD) were purchased from Sigma (St. Louis, MO, USA). Icilin (10 μM) was acquired from Tocris (Ellisville, MO, USA). Ionomycin (4 μM) was purchased from Molecular Probes (Eugene, OR, USA). Capsaicin and menthol stock were made in ethanol and stored at -20°C. 4α-PDD, icilin and ionomycin were dissolved in dimethyl-sulfoxide (DMSO) to make stock solution and kept at -20°C. The drugs were diluted to their final concentration with the extracellular solution, and then applied by gravity through a bath perfusion system. The bath solution was continuous during the experiment at a rate of 2 ml/min.

Results

We first determined the mRNA expressions of temperature-sensitive TRP channels in MDPC-23 cell lines. RT-PCR investigation of the TRP channels profile of MDPC-23 showed that these cells constitutively expressed genes coding for TRPV1, TRPV2, TRPV4 and TRPM8 (Fig. 1A). However, TRPV3 and TRPA1 mRNAs were not found in MDPC-23, although they were readily detected in rat trigeminal ganglion (TG) neurons which were well-known to express temperature-sensitive TRP channel (Fig. 1B) (Park *et al*, 2006; Liedtke *et al*., 2000). TRPM8 mRNA was expressed at low levels in MDPC-23 cells.

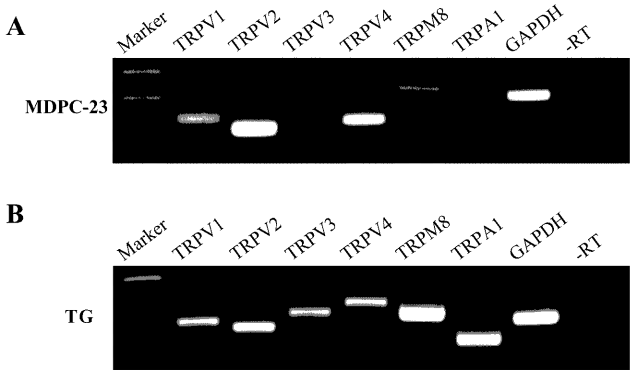

Fig. 1. Thermo-TRP gene is expressed in MDPC-23 cells.
Using gene-specific primers, TRPV1, TRPV2, TRPV4, TRPM8 transcripts are detected in MDPC-23 cells (A) as well as in rat TG (B). TRPV2 mRNA, highly expressed, and TRPM8 mRNA, expressed at lower levels in MDPC-23 (A). As a negative control, reverse transcriptase was omitted from the reaction (-RT). GAPDH was used as a positive control. Predicted product sizes are 232 bp (TRPV1), 201 bp (TRPV2), 221 bp (TRPV4), 331 bp (TRPM8), 242 bp (TRPA1), 316 bp (GAPDH), respectively.

We then examined the expression of TRPV1 at the protein level using immunocytochemistry. We first determined the specificity of TRPV1 antibody using TG neurons. TRPV1 immunoreactivity was observed in different subpopulations of TG neurons, being predominantly expressed in small sensory neurons rather than large neurons (Bae *et al*., 2004) (Fig. 2B). However, using the same antibody, TRPV1 was not detected in MDPC-23 cells. TRPV2, TRPV4, TRPM8 proteins could not be assessed because of the absence of specific antibodies at this moment.

Functional expression of TRPV1, TRPV4 and TRPM8 in MDPC-23 cells were investigated by calcium imaging. Application of capsaicin (1 μM), a ligand for TRPV1 channel, for 2 min did not enhance the concentration of intracellular calcium ($[Ca^{2+}]_i$) in all cells tested (n=29/29). MDPC-23 cells were challenged with a 30% hypotonic solution for 1min 30 s, rinsed for 2 min, and then challenged with an isotonic solution containing 4α-PDD (10 μM) for 2 min. As for TRPV4, we used hypotonic solution and 4α-PDD which was shown to activate TRPV4 in the previous studies (Alessandri-Haber *et al*, 2003). 22 out of 34 MDPC-23 cells tested (64%) showed a small increase in $[Ca^{2+}]_i$ with the 30% hypotonic stimulation, with 40% of those MDPC-23 cells (9/22) sensitive to both the 30% hypotonic and 4α-PDD stimuli. MDPC-23 cells responsive to both stimuli were likely to express TRPV4. When we applied agonists for TRPM8, (-) menthol (1 mM) and icilin (10 μM), $[Ca^{2+}]_i$ was increased in MDPC-23 cells (n=30/30) (Fig. 2). At the end of each experiment, ionomycin (4 μM), a calcium ionophore, was added to confirm the viability of the cells tested. We only included ionomycin-responsive cells in the analysis.

Discussion

In the present study, we determined expression of temperature-sensitive TRP channels in MDPC-23 cells, an odontoblast cell line, by the combination of molecular and functional analyses. It was found that TRPV4 and TRPM8 are functionally expressed in MDPC-23 cell lines, whereas TRPV1 is not functional in terms of calcium response. mRNA of TRPV3 and TRPA1 was not constitutively expressed.

mRNA Levels were not homogenous throughout the temperature-sensitive TRP channels studied in this work. While TRPV1, TRPV2, TRPV4 and TRPM8 were constitutively expressed in MDPC cell line, TRPV3 and TRPA1 mRNAs were not detected. These results suggest that although they all belong to subfamily of TRP channels, their expression are under distinctive transcriptional regulation, and might have different functional roles as sensory trans-

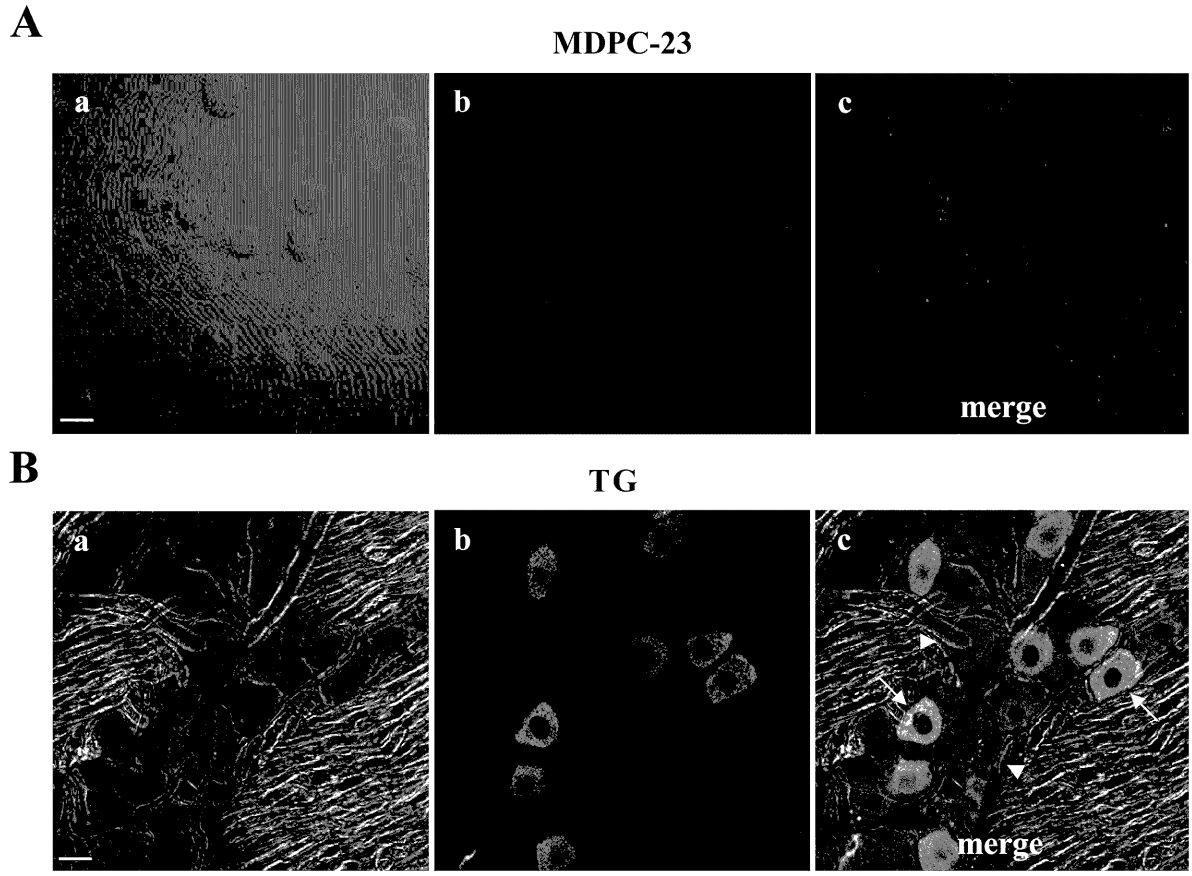

Fig. 2. Immunohistochemistry of TRPV1 protein in MDPC-23 cells. FITC-conjugated anti-TPPV1 antibody (green) were used for immunostaining of the MDPC-23 cells. Specific staining for TRPV1 is absent in MDPC-23 cells (A). Rat TG was used as a positive contol (B). The Ab stained cell of smaller diameter (arrows) and non-stained cells of large diameter (arrowheads). Scale bar=20 μm. a; DIC, b; FITC, c; Overlay

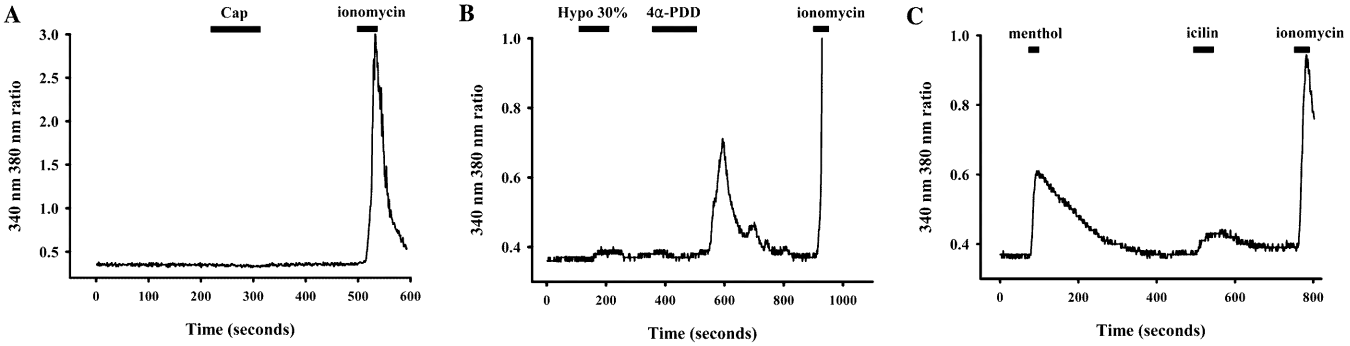

Fig. 3. Calcium transients evoked by thermo-TRP ligands. **(A)** Applications of capsaicin (1 μM) was not increase in $[Ca^{2+}]_i$ in MDPC-23 , **(B)** example of 30% hypotonic solution and 4α-PDD evoked Ca^{2+} transients in MDPC-23 cells **(C)** Ca influx induced to TRPM8 agonists, menthol (1 mM), and icilin (10 μM).

ducers for temperature detection in odontoblasts. Moreover, TRPV1 protein is not present in the cells, in spite of mRNA expression of TRPV1. Further work is required to elucidate the mechanism by which expression of TRPV1 is regulated in these cells.

One of the most prominent cellular events followed by the activation of thermo-TRP channels, which belong to non-selective cation channels, is the increase of $[Ca^{2+}]_i$. We therefore determined functional expression of temperature-sensitive TRP channels using ratiometric calcium imaging experiments. When the effects of capsaicin (a TRPV1 agonist) were examined as a screen for the presence of

functional TRPV1, we failed to detect increase of $[Ca^{2+}]_i$. It was thought that TRPV1 was not functional since TRPV1 was not detected at protein level. This is inconsistent with previous report (Okumura *et al*., 2005). It is not clear at this moment why the results of both experiments are different. There might be difference in results depending on species and cell types used for the study.

Mechanical stimulation of odontoblasts and fluid flow in dentinal tubules are known to elicit nociceptive responses ((Dong *et al*., 1985; Jyvasjarvi and Kniffki, 1989). TRPV4 is known to be activated by hypotonicity, heat, and possibly by an unidientified intracellural ligand (Nilius *et al*., 2003; Suzuki *et al*., 2003). Thus, we hypothesized that TRPV4 expreesed in odontoblasts may serve as a transducer for mechanical stimuli. Hypotonic solution and 4α-PDD induced increase in $[Ca^{2+}]_i$ by entry of extracellular Ca^{2+} in MDPC-23 cells. 4α-PDD that does not activate protein kinase C is reported to be an agonist of TRPV4 (Watanabe *et al*., 2002). Our results were in agreement with the previous reports which showed that 4α-PDD was more efficacious than a 30% hypotonic stimulus in TRPV4-expressing CHO cells (Watanabe *et al*., 2002). Our results demonstrating calcium response by TRPV4 ligands suggest that TRPV4 might act as a transducer of osmotic stimulation and, thereby be involved in the transmission of tooth pain.

TRPM8 is also likely to be functionally expressed in MDPC-23 cells, given that cooling compounds, menthol and icilin, produced calcium responses. Icilin is potential ligand for TRPA1 as well as TRPM8. Because TRPA1 mRNA was not detected from RT-PCR analysis, it was thought that icilin only activated TRPM8 in MDPC-23 cells in our experiments. Interestingly, menthol evoked very small cationic currents when we performed whole-cell patch clamp recordings (data not shown).

It was interesting to note that while some temperature-sensitive TRP channels such as TRPM8 and TRPV4 are functional, TRPV1 is not in MDPC-23 cells. Several factors may be involved for TRP channels to be functional. First, the degree of differentiation could affect on this phenomenon. Up-regulation of expression level by other factors such as bacterial and/or other components may be required. In line with this possibility, it has been demonstrated that lipoteichoic acid (LTA), a component released from bacteria wall may affect functional characteristics of human odontoblasts (Durand *et al*., 2006).

In this study, we used MDPC-23, cell rather than odontoblasts that were acutely isolated from mammalian animal teeth such rats and human. Although MDPC-23 cells are immortalized odontoblast-like cell line derived molar dental papillae, it is possible that they may be different biological characteristics from odontoblast itself because they lost cellular polarity and displays biological variability including growing faster and changing in phenotypes over time while maintained in culture conditions. Thus, we are now further studying on the functional expression of thermo-TRP channels in native odontoblasts.

In summary, we show that subfamily of thermo-TRP channels are functionally expressed in MDPC-23 cell lines. Likewise, thermo-TRP channels expressed in odontoblasts may serve as thermal transducer in teeth.

Abbreviations

Balanced salt solution, BSS; phosphate buffered saline, PBS; Reverse transcription-polymerase chain reaction, RT-PCR; trigeminal ganglion, TG; mouse dental papilla cell-23, MDPC-23; transient receptor potential vanilloid 1, TRPV1; transient receptor potential vanilloid 2, TRPV2; transient receptor potential vanilloid 3, TRPV3; transient receptor potential vanilloid 4, TRPV4, transient receptor potential melastatin 8, TRPM8; transient receptor potential Ankyrin 1, TRPA1.

Acknowledgement

This research was supported by the Korea Research Foundation Grant (MOEHRD)(KRF-2006-E00143), Republic of Korea.

Reference

Alessandri-Haber, N., J. J. Yeh, A. E. Boyd, C. A. Parada, X. Chen, D. B. Reichling, and J. D. Levine.: Hypotonicity induces TRPV4-mediated nociception in rat. Neuron. 39:497-511, 2003.

Bae, Y. C, Oh, J. M, Hwang, S. J, Shigenaga, Y, Valtschanoff, J. G.: Expression of vanilloid receptor TRPV1 in the rat trigeminal sensory nuclei. J. Comp. Neurol. 478(1): 62-71, 2004.

Clapham, D. E.: TRP channels as cellular sensors. Nature. 426:517-524, 2003.

Dong WK, Chudler EH, Martin RF.: Physiological properties of intradental mechanoreceptors. Brain, Res. 334(2):389-395, 1985.

Durand SH, Flacher V, Romeas A, Carrouel F, Colomb E, Vincent C, Magloire H, Couble ML, Bleicher F, Staquet MJ, Lebecque S, Farges JC.: Lipoteichoic acid increases TLR and functional chemokine expression while reducing dentin formation in in vitro differentiated human odontoblasts. J. Immunol. 176(5):2880-2887, 2006

Hanks, C. T., Z. L. Sun, D. N. Fang, C. A. Edwards, J. C. Wataha, H. H. Ritchie, and W. T. Butler.: Cloned 3T6 cell line from CD-1 mouse fetal molar dental papillae. Connect. Tissue. Res. 37:233-249, 1998a.

Hanks, C. T., D. Fang, Z. Sun, C. A. Edwards, and W. T. Butler.: Dentin-specific proteins in MDPC-23 cell line. Eur. J. Oral. Sci. 106 Suppl. 1:260-266, 1998b.

Jyvasjarvi E, Kniffki KD.: Afferent C fibre innervation of cat

tooth pulp: confirmation by electrophysiological methods. J. Physiol. **411**: 663-675, 1989

Liedtke, W., Y. Choe, M. A. Marti-Renom, A. M. Bell, C. S. Denis, A. Sali, A. J. Hudspeth, J. M. Friedman, and S. Heller.: Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell. **103**:525-535, 2000.

Lumsden, A. G.: Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. Development. 103 Suppl:155-169, 1988.

Matthews B, Andrew D, Amess TR, Ikeda H, Vongsavan N.: The functional properties of intradental nerves. In: Shinmono M, Maeda T, Suda H, Takahashi K (eds) Dentin/pulp complex. Quintessence Publishing Co, Tokyo, 146-153, 1996

Nilius, B., H. Watanabe, and J. Vriens.: The TRPV4 channel: structure-function relationship and promiscuous gating behaviour. Pflugers. Arch. **446**:298-303, 2003.

Okumura, R., K. Shima, T. Muramatsu, K. Nakagawa, M. Shimono, T. Suzuki, H. Magloire, and Y. Shibukawa.: The odontoblast as a sensory receptor cell? The expression of TRPV1 (VR-1) channels. Arch. Histol. Cytol. **68**:251-257, 2005.

Park, C. K., M. S. Kim, Z. Fang, H. Y. Li, S. J. Jung, S. Y. Choi, S. J. Lee, K. Park, J. S. Kim, and S. B. Oh.: Functional expression of thermo-transient receptor potential channels in dental primary afferent neurons: implication for tooth pain. J. Biol. Chem. **281**:17304-17311, 2006.

Pedersen, S. F., G. Owsianik, and B. Nilius.: TRP channels: an overview. Cell. Calcium. **38**:233-252, 2005.

Qin, C., O. Baba, and W. T. Butler.: Post-translational modifications of sibling proteins and their roles in osteogenesis and dentinogenesis. Crit. Rev. Oral. Biol. Med. **15**:126-136, 2004.

Suzuki, M., A. Mizuno, K. Kodaira, and M. Imai.: Impaired pressure sensation in mice lacking TRPV4. J. Biol. Chem. **278**:22664-22668, 2003.

Watanabe, H., J. B. Davis, D. Smart, J. C. Jerman, G. D. Smith, P. Hayes, J. Vriens, W. Cairns, U. Wissenbach, J. Prenen, V. Flockerzi, G. Droogmans, C. D. Benham, and B. Nilius.: Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives. J. Biol. Chem. **277**:13569-13577, 2002.