



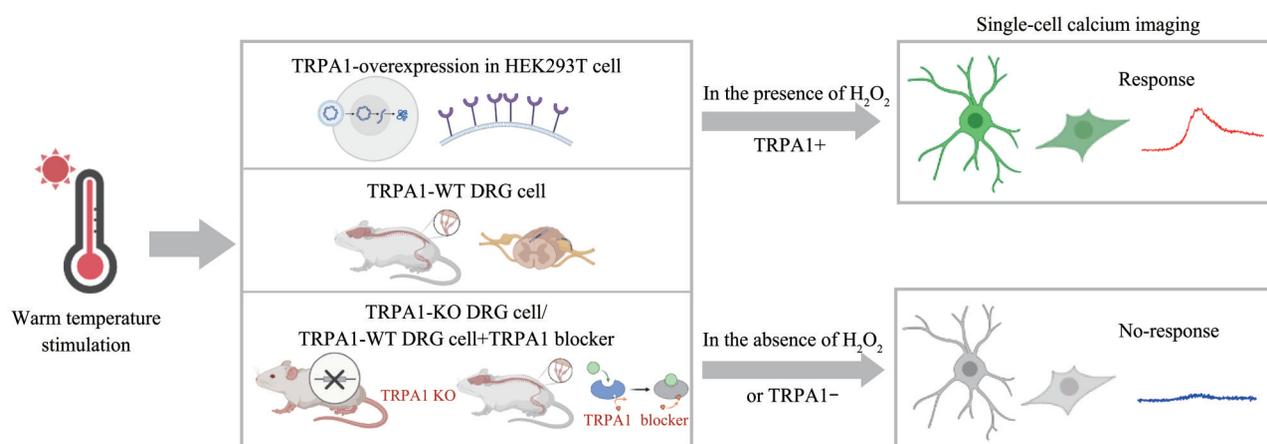
Mechanism Study of Warm Transduction From Keratinocytes to Downstream TRPA1 in DRG Neurons*

ZHENG Tian-Yuan^{1)**}, WANG Lei^{1)**}, JIANG Yan²⁾, LIU Xiao-Ling^{1)***}

¹⁾School of Life Sciences, Beijing University of Chinese Medicine, Beijing 102488, China;

²⁾School of Pharmaceutical Sciences, Tsinghua University, Beijing 100084, China)

Graphical abstract



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Abstract Objective The warm sensors located in keratinocytes have the ability to directly detect warm temperature, with TRPA1 in dorsal root ganglion (DRG) neurons being a potential mediator of the downstream transduction process. The present study aimed to elucidate the mechanism by which warm information is transmitted from keratinocytes to TRPA1. **Methods** TRPA1-transfected HEK293T cells as well as DRG neuron cells from wild-type and TRPA1 knockout mice were cultured separately, and stimulated with warm temperatures using a perfusion apparatus. Single cell calcium imaging was used to monitor calcium influx during stimulation, and the role of H_2O_2 in this process was also examined. Additionally, RNA-seq analysis was performed on primary keratinocytes cultured at different temperatures to identify potential candidates responsible for keratinocytes-TRPA1 warm transduction. **Results** Our finding indicated that TRPA1-transfected HEK293T cells or DRG neurons could be activated by warm temperature in the presence of H_2O_2 . However, when TRPA1 was knocked out or blocked by HC-030031, the H_2O_2 -potentiated warm response was significantly reduced. Moreover, Chemokine C-C motif ligand 2 (CCL2) and decorin (DCN), two H_2O_2 related

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** These authors contributed equally to this work.

*** Corresponding author.

Tel: 86-18801126360, E-mail: liuxiaoling66@bucm.edu.cn

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factors, exhibited different expression patterns in keratinocytes cultured at 33°C and 37°C, respectively. This result is consistent with previous research showing that mice stimulated at 37°C induced more DRG activation than those stimulated at 33°C. **Conclusion** H₂O₂ can potentiate the TRPA1-dependent warm response, and H₂O₂-related factors in keratinocytes can be affected by warm temperature, suggesting the possible role of H₂O₂ related factors in warm transduction from keratinocytes to downstream TRPA1 in DRG neurons.

Key words H₂O₂, chemokine C—C motif ligand 2, decorin, dorsal root ganglion, TRPA1, keratinocyte, warm temperature sensation
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Mammals are believed to perceive environmental temperature through specialized thermosensitive sensory neurons located in the trigeminal ganglia (TG) and dorsal root ganglia (DRG), which extend their peripheral nerves into the skin and express various thermosensors, including TRPV1 (>43°C)^[1], TRPV2 (>52°C)^[2], TRPM3 (>40°C)^[3], TRPM2 (>42°C)^[4], TRPM8 (<28°C)^[5-6] and TRPA1 (<17°C)^[7]. Despite this, a growing body of research suggested that these thermo-TRP channels may not directly sense temperature, as they exhibited different sensitivity patterns between *in vitro* and *in vivo* conditions. For instance, TRPA1, which is known to be activated by cold *in vitro*, has been reported to be involved in noxious heat sensation *in vivo* when combined with TRPM3 and TRPV1^[8]. Although TRPV1 can only be activated by heat *in vitro*, TRPV1 knockout mice were found to be defective in warm sensation^[9]. DRG-expressing TRPM2 was shown to influence the warm sensation of mice *in vivo*, but only in the presence of H₂O₂^[4]. While Paricio-Montesinos *et al.*^[10] pointed out that warm-responsive TRP Channels including TRPV1 and TRPM2 are not absolutely required for warmth perception, but loss or local pharmacological silencing of the cool-driven TRPM8 channel abolished the ability to detect warm. Additionally, these thermo-TRP channels could also be activated by chemical stimuli other than temperature^[11-12]. Taken together, these observations suggest that these thermo-TRP channels may be responsible for the downstream transduction of temperature sensation rather than directly sensing temperature themselves.

Thermo-sensitive DRG nerve endings are located within the skin, which may have a temperature similar to that of the human body (37°C). Compared to heat or cold temperature, warm temperature sensation seems to be more paradoxical that the subtle temperature deviations (*e.g.* 32°C–34°C) at the skin surface can effectively alter the local temperature

directly. This can lead to activation of the thermo-TRPs residing in the nerve endings when the temperature is above 37°C. It has been postulated that keratinocytes themselves may sense subtle temperature changes occurring at the skin surface, then indirectly activate downstream sensory neurons for generating warm sensation. TRPV3^[13-14], TRPV4^[15-16] as well as STIM1^[17] are expressed in keratinocytes rather than DRG neurons, and can be activated by warm temperature, indicating the role of keratinocytes in warm temperature sensitivity. Considering no synaptic connection has been found between keratinocytes and DRG neurons^[18], it has been proposed that keratinocytes may release certain signal factors and indirectly activate downstream DRG neurons through transduction channels expressed in them^[19-20]. Previous research has suggested an indirect role of DRG-expressed TRPA1 in warm sensation *in vivo*^[17]. However, the mechanism for warm transduction from keratinocytes to downstream TRPA1 is unclear. Therefore, our study aimed to investigate the probable signal pathway for warm transduction from keratinocytes to downstream TRPA1 channels.

1 Materials and methods

1.1 DRG culture

Wild type or TRPA1 knockout mice were anesthetized and decapitated. DRG neurons were collected under a microscope with the aid of an ice bag positioned under the spinal cord. The neurons were then gathered into a 1.5 ml tube containing DMEM medium without serum. The tube was briefly spun to allow the DRG neurons to settle at the bottom. The medium was then removed, and 1 ml of fresh medium was used to wash the neurons. The medium was again removed, and 500 µl of collagenase was added to the tube. The DRG neurons were then incubated at 37°C for 45 min to digest with collagenase. At the same time, in a separate 1.5 ml

tube, 20 μ l of papain (LS003126, Worthington) and 20 μ l of activating buffer were combined and activated at 37°C for 30 min. After the 45-min digestion period, the DRG neuron-containing tube was briefly spun, and the medium was removed. The activated papain medium was then added to 1 ml of DMEM F-12 medium (Gibco, C11330500BT) and combined with the DRG neurons before being incubated at 37°C for an additional 30 min. The supernatant medium was removed, 1 ml of fresh DMEM F-12 medium was added and mixed followed by 1 ml bovine serum albumin (BSA) added from the bottom. The tube was centrifuged at 1 000 r/min for 10 min at room temperature, and the supernatant was discarded. The DRG neurons were then suspended in DMEM F-12 medium with horse serum, penicillin/streptomycin (P/S), and nerve growth factor (NGF). Finally, 30 μ l of the neuron-containing medium was placed onto a coverslip pre-coated with poly-D-lysine (PDL) and laminin for 1 h, respectively.

1.2 Keratinocytes culture

Keratinocytes used in this study were obtained from neonatal mice and cultured according to a previously established protocol^[17]. Briefly, Newborn mice were decapitated firstly. After being soaked into 10% povidone, 70% ethanol and Dulbecco's phosphate-buffered saline (DPBS) for 5 min, respectively, the back skin was removed, stripped of fat and floated (epidermis facing upward) in 0.25% trypsin for 1 h at 37°C. Epidermal layer of the skin was peeled off, cut into smaller pieces in Cnt-02 medium (CELLnTEC, supplemented with 10% fetal bovine serum (FBS) and 1 \times P/S), and then stirred for 30 min at room temperature in a 100 ml bottle to obtain single cells. After being filtered through a 70 μ m cell strainer and spun down at 1 000 r/min for 3 min, the cells were resuspended in CnT-02 medium without additional serum or P/S, seeded into 6-well plates coated with fibronectin and collagen IV at a concentration of 10 mg/L, and incubated at 33°C and 37°C respectively with 5% CO₂ for 2 d. The keratinocytes were used for RNA-seq analysis, while the supernatant medium from 33°C and 37°C were collected for calcium imaging of TRPA1-transfected HEK293T cells.

1.3 Fura-2 single-cell Ca²⁺ imaging

The warm-induced response in DRG neurons and TRPA1-transfected HEK293T cells were evaluated

using single-cell Ca²⁺ imaging as described previously^[17]. In brief, cultured cells were rinsed twice with KRH buffer (Krebs-Ringer-Hepes), loaded with fura-2 calcium indicator for 30 min, rinsed again with KRH buffer, and then imaged using a Nikon microscope with a 20 \times objective. The perfusion system was used and the temperature of the bath solution (KRH buffer) was adjusted using a CL-100 temperature controller (Warner Instruments) and a SC-20 Solution In-Line Heater/Cooler (Harvard Apparatus), and monitored using a thermistor at the perfusion outlet. The intracellular Ca²⁺ concentration was determined as the 340 nm/380 nm fluorescence intensity ratio (F_{340}/F_{380}) of fura-2. For H₂O₂-potentiated warm response, H₂O₂ was incorporated into the KRH buffer at a final concentration of 400 μ mol/L as suggested by previous studies^[8]. The cells were incubated in H₂O₂-contained KRH buffer, and then tested the warm response by temperature controller.

1.4 RNA extraction and RNA-seq analysis

RNA was isolated from keratinocytes using the RNA miniprep kit (Axygen, 04113KD1), and assessed for quality using Nanodrop. The samples were then sent to Beijing Genomics Institute for RNA-Seq analysis. The sequencing data was analyzed using the company's web-based tools and following their provided protocols.

1.5 Data analysis

Data in all figures are shown as mean \pm SEM. The number of separate experiments was labeled in the figure legend. One-way ANOVA with Dunn's comparison between samples was applied to all data, and the statistical significance was set at $P < 0.05$.

2 Results

2.1 H₂O₂ was needed for warm-induced TRPA1 response in HEK293T cells

Given the involvement of TRPA1 in warm transduction *in vivo*^[17], we explored the role of TRPA1 in warm response at cellular level. Regrettably, we did not observe any reaction when exposed to temperatures below 42°C (Figure 1a, c). Interestingly, a considerable thermal response was elicited in the presence of H₂O₂, with a response threshold value of approximately 38°C (Figure 1b, c). This finding suggests that H₂O₂ plays a role in inducing TRPA1 response to warm stimuli.

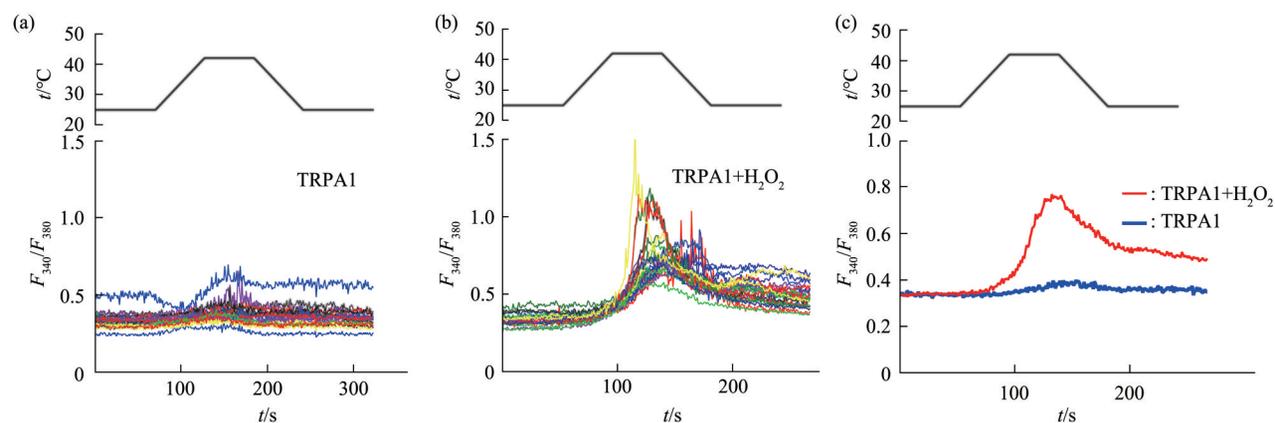


Fig. 1 H₂O₂ was needed for warm-induced TRPA1 response in HEK293T cells

(a) The HEK293T cells transfected with TRPA1 showed no response to warm stimulation. (b) In the presence of H₂O₂ (400 μmol/L), TRPA1 could be activated by warm temperature below 42°C. (c) Representative average traces of TRPA1 with or without H₂O₂ in response to a warm stimulation to ~42°C in the presence of 1.3 mmol/L Ca²⁺.

2.2 H₂O₂ was needed for warm-induced DRG responses

We next test the role of H₂O₂ in warm-induced TRPA1 response in primary DRG neuron cells which were responsible for warm transduction *in vivo*. Initially, DRG neurons were cultured, then stimulated with warm temperatures below 42°C using a perfusion apparatus. Simultaneously, calcium influx of DRG neurons was monitored by calcium imaging. As we expected, no significant responses were observed in the range of warm temperatures (Figure 2a). This implied that DRG neurons could not be activated by warm temperature directly, and warm transduction through DRG neurons may be mediated by other factors. Interestingly, significant calcium influx responses were induced by warm temperature in the presence of H₂O₂ (Figure 2b). Specifically, both the number and peak height of calcium influx for warm-responding DRG neurons were significantly increased in the presence of H₂O₂ (Figure 2c, d), suggesting the pivotal role of H₂O₂ in warm-induced DRG responses. This result aligns with our findings in TRPA1-transfected HEK293T cells, which has prompted us to further investigate the role of TRPA1 in DRG neurons regarding this warm response.

2.3 The role of TRPA1 in H₂O₂ potentiated warm responses of DRG neurons

We then tried to ascertain whether TRPA1 was

accountable for the H₂O₂-mediated augmentation of warm responses in DRG neurons. The DRG neurons were stimulated by warm temperature firstly followed by benzyl isothiocyanate (BITC), a pungent chemical that could causes selective activation of TRPA1 and could not activated other reported warm sensors such as TRPV3, TRPM2 and TRPM8 (Figure 2e). The result showed significant H₂O₂-potentiated warm responses of DRG neurons, which were also activated by BITC (Figure 2b). Consequently, TRPA1-knockout DRG neurons were cultured and subjected to calcium imaging to assess their H₂O₂-potentiated warm responses. As anticipated, the TRPA1-knockout DRG neurons demonstrated significantly fewer H₂O₂-potentiated warm responses in comparison to wild-type DRG neurons (Figure 3). Furthermore, the proportion of H₂O₂-potentiated warm responses in DRG neurons that were insensitive to BITC was comparable to that observed in TRPA1-knockout DRG neurons (Figure 3c). Similarly, the inclusion of TRPA1 blocker HC-030031 in the calcium imaging buffer resulted in a considerable reduction in H₂O₂-potentiated warm responses (Figure 4), which was consistent with the findings from TRPA1-knockout DRG neurons, highlighting the role of TRPA1 in H₂O₂-potentiated warm responses of DRG neurons.

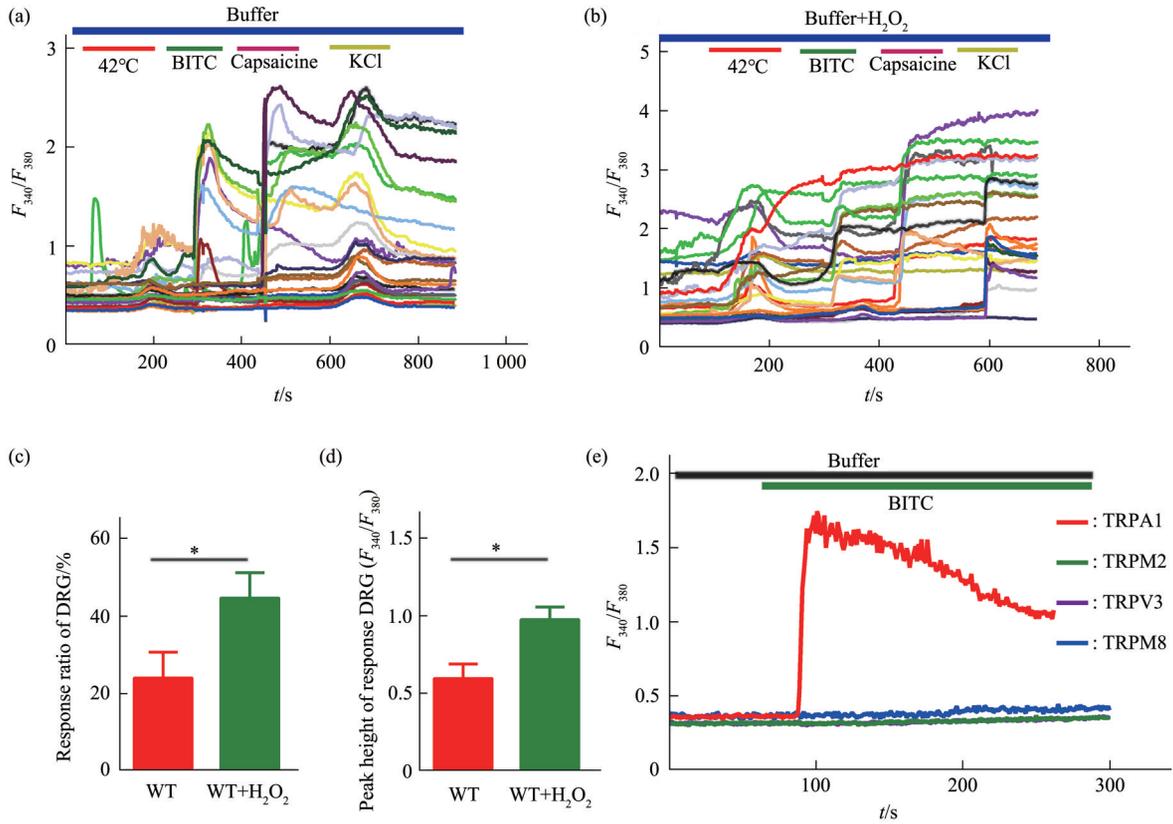


Fig. 2 Warm induced DRG responses could be potentiated by H₂O₂

(a) The DRG neurons showed no significant response to warm temperatures. (b) In the presence of H₂O₂ (400 μmol/L), significant DRG responses were observed upon exposure to warm temperatures. BITC (TRPA1 agonist, 500 μmol/L), Capsaicin (TRPV1 agonist, 10 μmol/L) and KCl (75 mmol/L) were applied to each sample after warm stimulation. (c) The ratios of warm-induced DRG responses were compared between DRG neurons treated with (*n*=6 coverslips) or without (*n*=3 coverslips) H₂O₂. (d) The peak height values were calculated for neurons that responded to warm stimulation and compared between DRG neurons treated with or without H₂O₂. Data for (c) and (d) were expressed as $\bar{x} \pm s$. **P*<0.05. (e) BITC could not activate TRPM2, TRPV3 and TRPM8 ion channels which were also reported to have warm sensation.

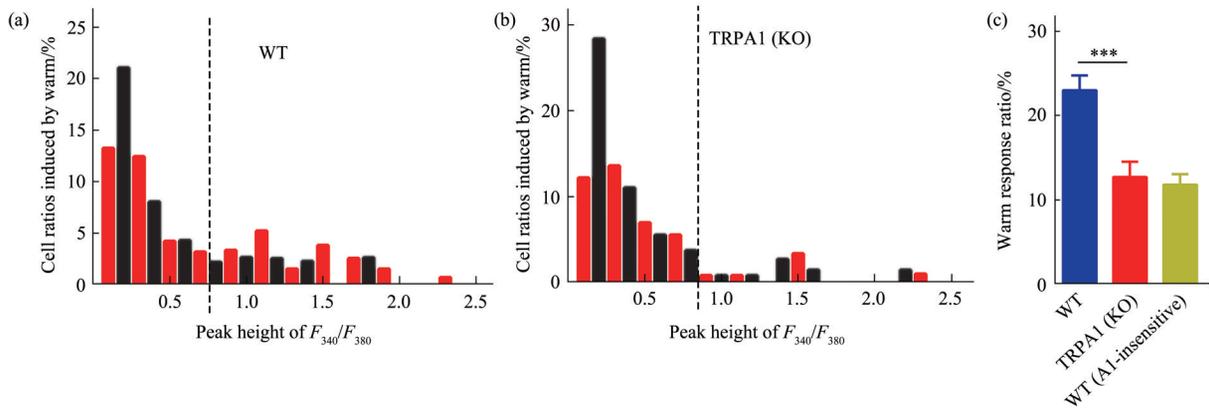


Fig. 3 TRPA1 was responsible for a portion of H₂O₂ potentiated warm induced DRG responses

(a,b) Wild type (a) and TRPA1 knockout (b) DRG neurons were cultured and stimulated with warm temperature in the presence of 400 μmol/L H₂O₂. The response peak height of F_{340}/F_{380} was calculated for each cell by subtracting the baseline value from the maximum value corresponding to the warm response, and the peak height value ranged between 0 and 2.5. The proportion of cells within each small range of peak height was determined with a resolution of 0.1. A positive warm response was defined as a response that exceeded the mean response of TRPA1 (KO) DRG response by at least five times the standard deviation. Analysis of positive warm response DRG neurons was performed on the right side of the dashed line. (c) The warm response ratio potentiation by H₂O₂ was compared between wild type (*n*=4 coverslip) and TRPA1 knockout (*n*=3 coverslip) DRG neurons. WT (A1-insensitive) referred to DRG neurons that could be activated by warm temperature in the presence of H₂O₂, but not by TRPA1 agonist BITC. Data are expressed as $\bar{x} \pm s$. ****P*<0.001.

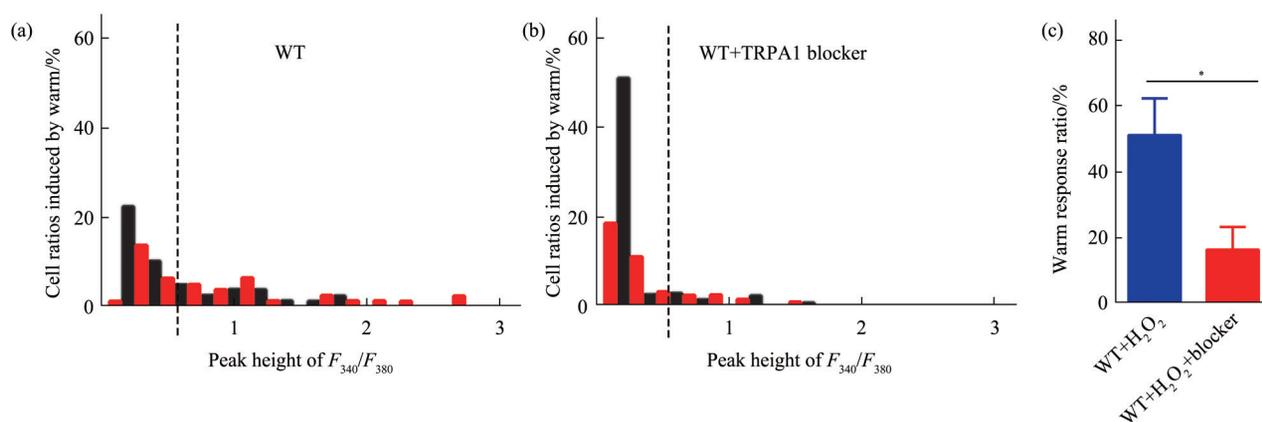


Fig. 4 There were less H₂O₂ potentiated warm responses of DRG neurons in the presence of TRPA1 blocker

(a,b) H₂O₂ potentiated warm responses of wild type DRG neurons was monitored by single cell calcium imaging in the presence of TRPA1 blocker (a, HC-030031, 100 μmol/L) or absence (b). (c) The analysis of positive warm response DRG neurons was conducted on the right side of the dashed line. The warm response ratio of wild type DRG neurons with HC-030031 (*n*=3 coverslip) was compared to that without HC-030031 (*n*=5 coverslip) to assess the potentiating effect of H₂O₂. Data are expressed as $\bar{x} \pm s$. **P*<0.05.

2.4 Potential candidates for keratinocyte-TRPA1 mediated downstream warm transduction

Given that H₂O₂ was needed for warm-induced activation of TRPA1-positive DRG neurons, we investigated whether keratinocyte-derived factors, especially those related to H₂O₂, may contribute to the keratinocyte-DRG warm transduction pathway. To achieve this, we cultured keratinocytes at 33°C and 37°C respectively and screened for potential candidates. Our findings revealed several oxidative stress and TRPA1 related factors that exhibited differential expression in keratinocytes cultured at varying warm temperatures. Specifically, we observed that keratinocytes cultured at 37°C released significantly more chemokine C-C motif ligand 2 (CCL2) and decorin (DCN) compared to those cultured at 33°C (Figure 5). This aligns with our previous results, which indicated that warm stimulation at 37°C on the skin of mice induced greater downstream DRG activation compared to stimulation at 33°C, and that TRPA1 knockout mice exhibited reduced differences in DRG activation^[16]. These findings suggested a possible involvement of CCL2 and DCN in TRPA1-mediated downstream warm transduction.

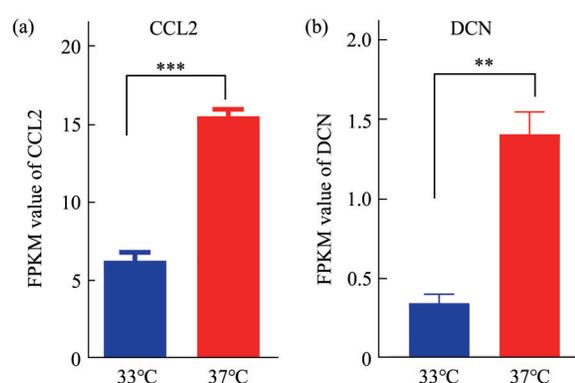


Fig. 5 CCL2 and DCN expressions were significantly different in keratinocytes between various cultured temperatures

(a, b) Statistical significance was performed between 33°C (*n*=3 coverslips) and 37°C (*n*=3 coverslips) for CCL2 (a) and DCN (b). Data are expressed as $\bar{x} \pm s$. ***P*<0.01, ****P*<0.001.

3 Discussion

In this study, we revealed that DRG neurons could be activated by warm temperature in the presence of H₂O₂, and TRPA1 was involved in this response. CCL2 and DCN in keratinocytes may serve as the upstream factor responsible for TRPA1-dependent warm transduction. H₂O₂, a peroxide and

oxidizing agent, has been reported to act as an agonist of TRPA1^[21-23]. Actually, TRPA1 itself cannot be activated by heat or warm temperature, but was involved in noxious heat escape behaviors of planarians, which were mediated by H₂O₂ and reactive oxygen species^[24]. This suggested that H₂O₂ played a role in TRPA1-mediated heat sensation *in vivo*, and that TRPA1 may not function as a direct temperature sensor, but rather as a downstream transducer of temperature sensation. Vandewauw *et al.*^[8] have also reported that TRPA1 could be activated by heat in the presence of H₂O₂, and that these responses occur exclusively in TRPA1 agonist AITC-sensitive neurons, which could be fully suppressed by the TRPA1 blocker HC-030031. Our study provides additional evidence that TRPA1 can also be activated by warm temperature in the presence of H₂O₂. The concentration of H₂O₂ we used in this study was according to previous researches^[8, 21]. Actually, H₂O₂ concentration has been measured up to 100 μmol/L in situations of physiological stress^[21], suggesting relatively higher concentration of H₂O₂ was needed for its related studies. Interestingly, unlike heat-activated neurons, a subset of DRG neurons were insensitive to BITC but still exhibited warm responses in the presence of H₂O₂, indicating the possible existence of additional candidates for H₂O₂-potentiated DRG warm responses.

Keratinocytes have been suggested to be responsible for warm temperature sensitivity due to their expression of warm sensors such as TRPV3, TRPV4, and STIM1^[14, 16-17]. As there is no synaptic connection between keratinocytes and downstream DRG neurons^[18], it has been proposed that keratinocytes may release certain factors upon stimulation by warm temperatures, subsequently transducing the temperature signal to downstream DRG neurons. However, TRPV3 and TRPV4 knockout mice displayed only minor defects in warm sensation *in vivo*^[16, 25-26]. Although STIM1 was shown to play a pivotal role in optimal preference temperature for warm sensation in mice, the mechanism by which keratinocytes transmit temperature information to DRG neurons remains unclear. TRPA1 knockout mice exhibited a reduction in DRG activation induced by warm stimulation of keratinocytes, as well as a defect in warm preference *in vivo*^[17]. Our results demonstrated that significant

differences existed in H₂O₂ related factors such as CCL2 and DCN expression in keratinocytes when cultured at 33°C and 37°C. This is consistent with our previous findings of DRG *in vivo* calcium imaging. Specifically, stimulation at 37°C on the upper epidermis of mice hind paws induced a greater number of DRG activations than stimulation at 33°C^[17]. Indeed, previous studies by Trevisan *et al.*^[24] have shown that oxidative stress by-products mediate infraorbital nerve-evoked pain-like behaviors through TRPA1. Local CCL2 release was identified as a major contributing mechanism, with antibody-mediated inhibition of CCL2 significantly reducing superoxide dismutase activity and hydrogen peroxide levels. CCL2 was most likely located upstream of the cascade of cellular and molecular events that drive TRPA1-dependent pain-like behaviors^[23]. DCN could increase the intracellular Ca²⁺ levels and induced the production of reactive oxygen species (ROS) in a concentration-dependent manner^[27]. We thus suppose that H₂O₂ related factors in keratinocytes may be affected by warm temperature, and then influence the amount of oxidative stress by-products around TRPA1 in DRG neurons, which lead to downstream warm transduction from keratinocytes to TRPA1 in DRG neurons. Considering this is a preliminary study for the role of CCL2 and DCN in warm transduction, further research is still required to investigate how the different expression pattern of factors like CCL2 and DCN are induced by warm temperatures, and how signals are transduced to downstream H₂O₂-potentiated TRPA1 activation.

4 Conclusion

Our research suggests that TRPA1 in DRG neurons could be activated by warm temperatures in the presence of H₂O₂. The oxidative stress-related factors CCL2 and DCN in keratinocytes seemed to be potential mediators of the warm transduction pathway between keratinocytes and TRPA1 in DRG neurons.

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References

- [1] Caterina M J, Schumacher M A, Tominaga M, *et al.* The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*, 1997, **389**(6653): 816-824
- [2] Caterina M J, Rosen T A, Tominaga M, *et al.* A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature*, 1999, **398**(6726): 436-441
- [3] Vriens J, Owsianik G, Hofmann T, *et al.* TRPM3 is a nociceptor channel involved in the detection of noxious heat. *Neuron*, 2011, **70**(3): 482-494
- [4] Tan C H, McNaughton P. The TRPM2 ion channel is required for sensitivity to warmth. *Nature*, 2016, **536**(7617): 460-463
- [5] McKemy D D, Neuhauss W M, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature*, 2002, **416**(6876): 52-58
- [6] Peier A M, Moqrich A, Hergarden A C, *et al.* A TRP channel that senses cold stimuli and menthol. *Cell*, 2002, **108**(5): 705-715
- [7] Story G M, Peier A M, Reeve A J, *et al.* Anktm1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell*, 2003, **112**(6): 819-829
- [8] Vandewauw I, De Clercq K, MuLier M, *et al.* A TRP channel trio mediates acute noxious heat sensing. *Nature*, 2018, **555**(7698): 662-666
- [9] Yarmolinsky D A, Peng Y, Pogorzala L A, *et al.* Coding and plasticity in the mammalian thermosensory system. *Neuron*, 2016, **92**(5): 1079-1092
- [10] Paricio-Montesinos R, Schwaller F, Udhayachandran A, *et al.* The sensory coding of warm perception. *Neuron*, 2020, **106**(5): 830-841.e3
- [11] Jordt S E, Bautista D M, Chuang H, *et al.* Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature*, 2004, **427**(6971): 260-265
- [12] Caterina M J, Schumacher M A, Tominaga M, *et al.* The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*, 1997, **389**(6653): 816-824
- [13] Peier A M, Reeve A J, Andersson D A, *et al.* A heat-sensitive TRP channel expressed in keratinocytes. *Science*, 2002, **296**(5575): 2046-2049
- [14] Moqrich A, Hwang S W, Earley T J, *et al.* Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. *Science*, 2005, **307**(5714): 1468-1472
- [15] Mihara H, Boudaka A, Sugiyama T, *et al.* Transient receptor potential vanilloid 4 (TRPV4)-dependent calcium influx and ATP release in mouse oesophageal keratinocytes. *J Physiol*, 2011, **589**(14): 3471-3482
- [16] Lee H, Iida T, Mizuno A, *et al.* Altered thermal selection behavior in mice lacking transient receptor potential vanilloid 4. *J Neurosci*, 2005, **25**(5): 1304-1310
- [17] Liu X, Wang H, Jiang Y, *et al.* STIM1 thermosensitivity defines the optimal preference temperature for warm sensation in mice. *Cell Res*, 2019, **29**(2): 95-109
- [18] Chateau Y, Misery L. Connections between nerve endings and epidermal cells: are they synapses?. *Exp Dermatol*, 2004, **13**(1): 2-4
- [19] Mandadi S, Sokabe T, Shibasaki K, *et al.* TRPV3 in keratinocytes transmits temperature information to sensory neurons *via* ATP. *Pflugers Arch*, 2009, **458**(6): 1093-1102
- [20] Miyamoto T, Petrus M J, Dubin A E, *et al.* TRPV3 regulates nitric oxide synthase-independent nitric oxide synthesis in the skin. *Nat Commun*, 2011, **2**: 369
- [21] Andersson D A, Gentry C, Moss S, *et al.* Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *J Neurosci*, 2008, **28**(10): 2485-2494
- [22] Arenas O M, Zaharieva E E, Para A, *et al.* Activation of planarian TRPA1 by reactive oxygen species reveals a conserved mechanism for animal nociception. *Nat Neurosci*, 2017, **20**(12): 1686-1693
- [23] White F A, Wilson N M. Chemokines as pain mediators and modulators. *Curr Opin Anaesthesiol*, 2008, **21**(5): 580-585
- [24] Trevisan G, Benemei S, Materazzi S, *et al.* TRPA1 mediates trigeminal neuropathic pain in mice downstream of monocytes/macrophages and oxidative stress. *Brain*, 2016, **139**(5): 1361-1377
- [25] Marics I, Malapert P, Reynders A, *et al.* Acute heat-evoked temperature sensation is impaired but not abolished in mice lacking TRPV1 and TRPV3 channels. *PLoS One*, 2014, **9**(6): e99828
- [26] Miyamoto T, Petrus M J, Dubin A E, *et al.* TRPV3 regulates nitric oxide synthase-independent nitric oxide synthesis in the skin. *Nat Commun*, 2011, **2**: 369
- [27] Adam M, Saller S, Strobl S, *et al.* Decorin is a part of the ovarian extracellular matrix in primates and may act as a signaling molecule. *Hum Reprod*, 2012, **27**(11): 3249-3258

TRPA1传导皮肤角质细胞温热信息的机制研究*

郑天元^{1)**} 王磊^{1)**} 江艳²⁾ 刘晓玲^{1)***}

(¹⁾ 北京中医药大学生命科学院, 北京 102488;

(²⁾ 清华大学药学院, 北京 100084)

摘要 **目的** 探究角质细胞中的温热信息是如何被传导至下游背根神经节 (DRG) 中的 TRPA1 离子通道。**方法** 采用单细胞钙离子成像技术, 利用 TRPA1 过表达系统以及野生型和 TRPA1 基因敲除小鼠, 联合 TRPA1 激动剂 (BITC) 和抑制剂 (HC-030031) 检测 H₂O₂ 在 DRG 神经元温热感知中的作用及其与 TRPA1 的相关性。在不同温热条件下培养皮肤角质细胞, 并提取角质细胞 RNA; 采用 RNA-seq 分析比较不同温度培养的角质细胞基因表达异同, 筛选皮肤角质细胞传导温热信息的候选因子。**结果** 过表达 TRPA1 以及 DRG 神经元中的 TRPA1 均可在 H₂O₂ 存在情况下被温热激活, 而温热范围内不同温度可影响皮肤角质细胞中与 H₂O₂ 产生有关的趋化因子配体 2 (CCL2) 和核心蛋白聚糖 (DCN) 基因的表达。**结论** H₂O₂ 相关因子可能参与 TRPA1 介导的温热传导过程。

关键词 过氧化氢, 趋化因子配体2, 核心蛋白聚糖, 背根神经节, 瞬时受体电位A1通道, 皮肤角质细胞, 温热感知
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** 并列第一作者。

*** 通讯联系人。

Tel: 18801126360, E-mail: liuxiaoling66@bucm.edu.cn

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