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TRPV3 is highly activated in keratinocytes from patients with atopic dermatitis and contributes to warmth-evoked pruritogens release and itch behaviors

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INTRODUCTION

- **Atopic dermatitis (AD)**
  - Chronic inflammatory skin disease characterized by severe itching.
  - Chronic itch : Significant impact on measures of quality of life, such as sleep.
  - Itch in AD is characteristically triggered or aggravated under the innocuous warm or at nighttime.
- **Transient receptor potential vanilloid 3 (TRPV3)**
  - Function as non-selective cation-permeable Ca^{2+} channels.
  - Activated by innocuous warming temperatures with a threshold of activation of ~33˚C.
  - Most abundantly expressed in keratinocytes of the skin and hair follicles.
  - Barely detected in trigeminal and dorsal root ganglia in rodents.
- **Gain of function mutations of TRPV3**
  - Spontaneously hairless and AD-like dermatitis phenotypes with pruritus in the DS-Nh mice and WBN/kob-HT rats.
  - Development of pruritic dermatitis phenotype in $TRPV3^{Gly573Ser}$ transgenic DS mice.
  - $TRPV3^{Gly573Ser}$ mice on the Th-1 biased C57BL/6J mice developed scratching behavior despite the absence of dermatitis.
  - A cause of Olmsted syndrome, a rare genodermatosis characterized by palmoplantar keratoderma, periorificial keratosis, and severe pruritus in human.
- However, the mechanism by which TRPV3 activation in keratinocytes induces itch sensation are unclear.

→ **Hypothesis.** TRPV3 in keratinocytes may be involved in the warmth-provoked itch in AD.

OBJECTIVE

- **To investigate the role of TRPV3 on keratinocyte in heat-induced itch in AD**

1. The expression and the heat-induced channel activities of TRPV3 on keratinocytes derived from patients with AD.
2. The cellular effect of heat stimulation on keratinocytes from AD patients with regard to pruritogens release and the role of TRPV3 in triggering heat-evoked production of pruritogens.
3. The role of TRPV3 in heat-induced itch and underlying molecular mechanisms in a oxazolone-induced AD-like chronic pruritus mice model (Ox-AD mice) by pharmacological intervention and behavioral study.

MATERIALS & METHODS

- Immunohistochemistry was performed using paraffin-embedded skin samples from the lesional skin of patients with AD (n=21), ACD (n=21), psoriasis (n=21) and normal human (n=21).
- Western blot: 1:1000 diluted polyclonal rabbit TRPV3 antibody (Origene, AP11388PU-N) or 1:4000 diluted β-actin.
- Keratinocytes were obtained from the lesional skin of AD patients and normal skin of healthy donors.
- Measurement of $[Ca^{2+}]_i$: Fura-2 loading and fluorescence analyses.
- Knockdown of TRPV3: siRNA directed against TRPV3 (validated silencer select siRNA s46346, respectively; Thermo Fisher Scientific).
- 8 potential keratinocyte-derived pruritogens (TSLP, artemin, NGF, PGE2, substance P, CGRP, ET-1, IL-33) were analyzed by ELISA and qRT-PCR.
- Chronic itch mouse model: Female hairless mice (hr/hr), aged 6–8 weeks old, were treated with 10% oxazolone (Sigma-Aldrich) in acetone on the nape of the neck (100 μl) and each ear (50 μl) on day 0. After 7 days, mice were treated with 0.5 % oxazolone in acetone on the nape of the neck (100 μl) and each ear (50 μl) every other day for additional 10 days. Experiments were performed on day 18.
- Behavioral analysis: Ox-AD mice were acclimated to the experimental room for 30 min and placed in the warmed chamber for 15 minutes for 2 times at 2-hour intervals. 50 μl of TRPV3 antagonists, 17(R)-RvD1 (30 μM, Cayman Chemical #13060), DPTHF (125 μM, Sigma-Aldrich #D187208), or vehicle (NaCl) were injected into the inflamed neck region, 10 minutes prior to each heat stimulation.
- TRPV3 agonists: carvacrol (Sigma-Aldrich, #282197), 2- aminoethoxydiphenyl borate (2-APB, Sigma-Aldrich, #42810).

RESULTS

1. TRPV3 is highly expressed in the epidermis and keratinocytes from lesional skin of patients with AD

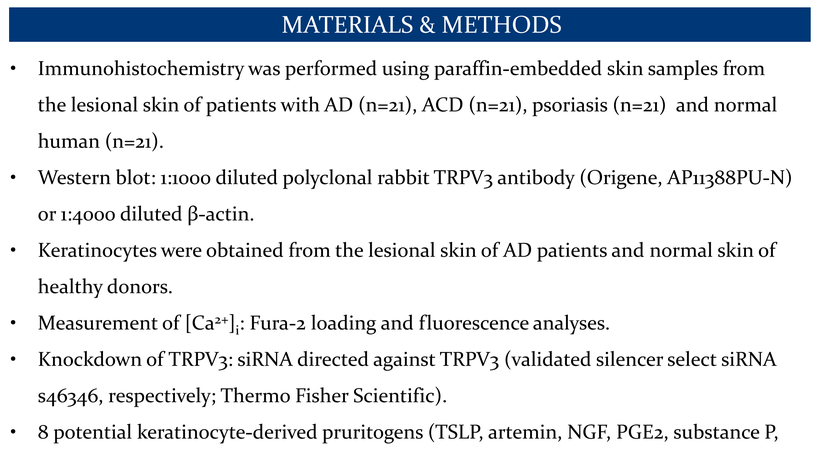


Figure 1. Increased expression of TRPV3 in the epidermal keratinocytes derived from lesional skin of patients with AD. (a) Immunohistochemical analysis of TRPV3 expression in lesional skin of patients with AD, ACD, and psoriasis as well as the skin samples from healthy controls (n=21 in each group). Scale bars, 100 μm. (b) Semiquantitative analysis of TRPV3 immunoreactivity in the epidermis. (c) Western blot for TRPV3 expression in keratinocytes derived from AD patients (n=9) and healthy controls (n=9). β-actin was used as a loading control. #1-7 indicates patient number. Three independent experiments were performed. Data are mean ± SEM. *P < 0.05, **P < 0.01. ACD, allergic contact dermatitis; SEM, standard error of the mean.

2. Keratinocytes from AD patients exhibit enhanced TRPV3 channel activity

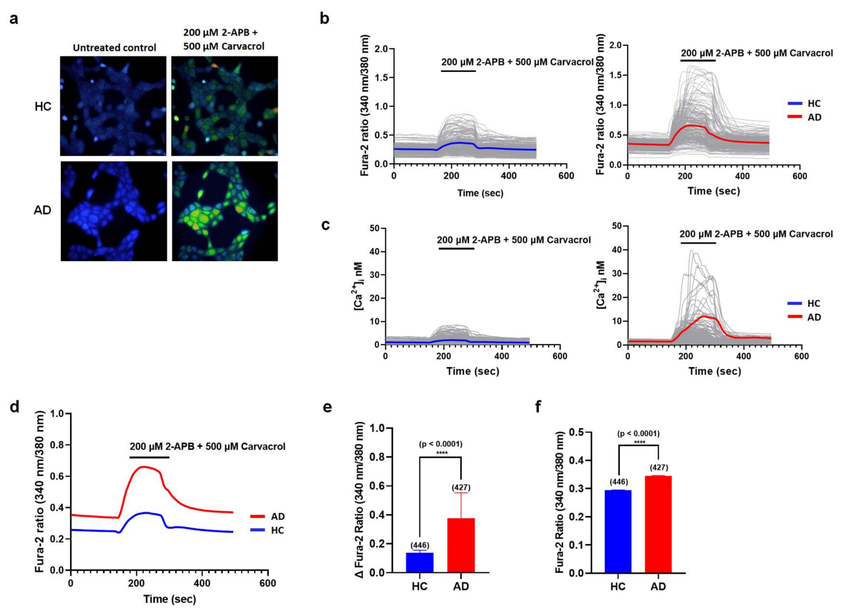


Figure 2. The TRPV3 channel agonists induce greater Ca^{2+} responses in keratinocytes derived from AD patients than those in keratinocyte from healthy controls. (a) Representative fluorescence images of dynamic Fura-2 signaling changes in keratinocytes from patients with AD and healthy controls (HC). (b) The representative traces showing the effect of agonist-induced $[Ca^{2+}]i$ increase in keratinocytes from patients with AD and HC. (c) Calcium calibration to calculate the changes in $[Ca^{2+}]i$ in the keratinocytes from patients with AD and HC. (d, e) Comparative summary of calcium influx induced by chemical agonists in keratinocytes from HC and patients with AD. Each data point represents the mean value of "n" number of cells (in brackets) analyzed from 4-7 replicates from five independent experiments. Error bars represent SEM. HC, healthy controls.

3. Heat-induced TRPV3-mediated Ca^{2+} influx is increased in keratinocytes from AD patients

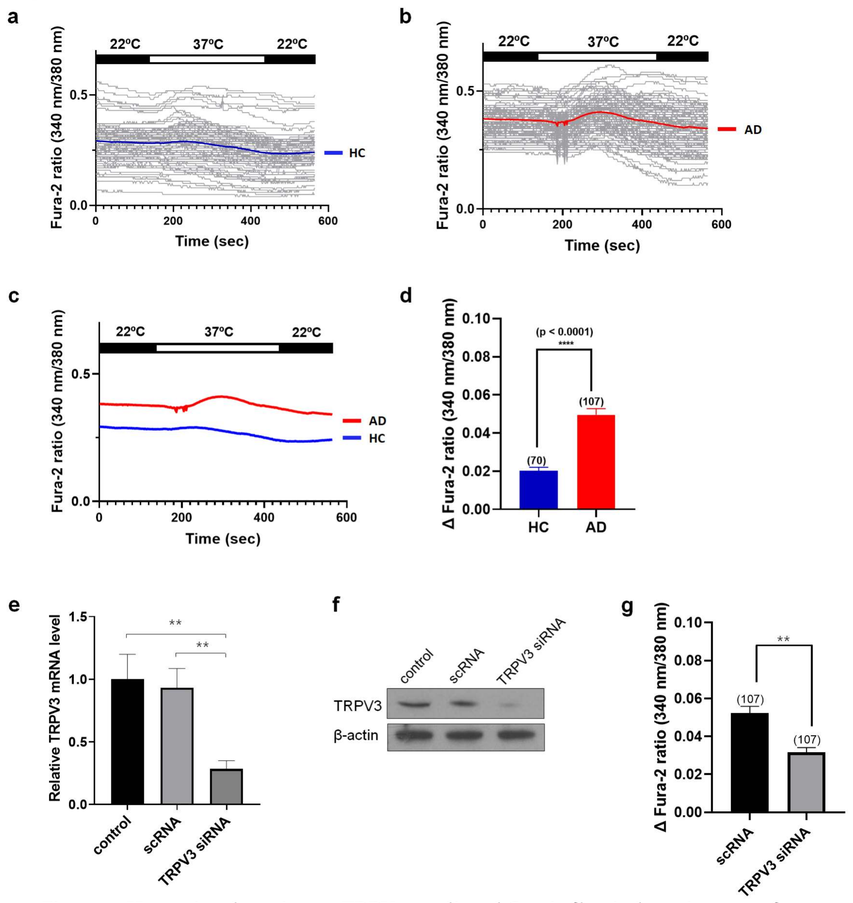
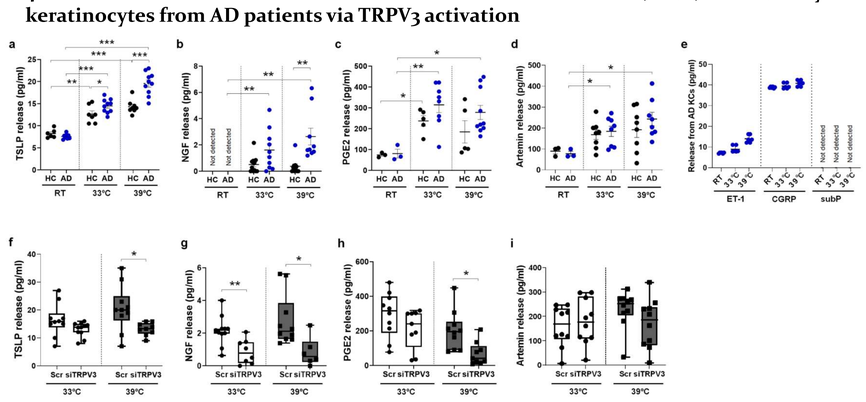


Figure 3. Heat stimulus triggers TRPV3-mediated Ca^{2+} influx in keratinocytes from patients with AD. (a, b) The representative traces show the effect of heat-induced Ca2+ influx in keratinocytes isolated from lesional skin of AD patients (b) and healthy control (HC) skin (a) at 37˚C. (c, d) Comparative summary of calcium influx in the keratinocytes from HC and patients with AD at 37˚C. Fura-2 ratio (340/380 nm) denotes the change of [Ca2+]i. (e-g) Keratinocytes from AD patients were transfected with scrambled (Scr) or TRPV3 siRNA (siTRPV3) and were exposed to increasing temperature from 22˚C to 37˚C. qRT-PCR (e) immunoblotting (f) confirmed the knockdown of TRPV3. (g) Comparative summary of calcium influx in Scr- and siTRPV3-transfected keratinocytes derived from patients with AD at 37˚C. Each data point represents the mean value of "n" number of cells (in brackets) analyzed from 3-4 replicates from three independent experiments. Error bars represent SEM.

4. Innocuous heat stimulus elicits an enhanced secretion of TSLP, NGF, and PGE2 by keratinocytes from AD patients via TRPV3 activation



5. Stimulation of TRPV3 channel with chemical agonists induces the production of TSLP, NGF, PGE2, and IL-33 in normal human keratinocytes

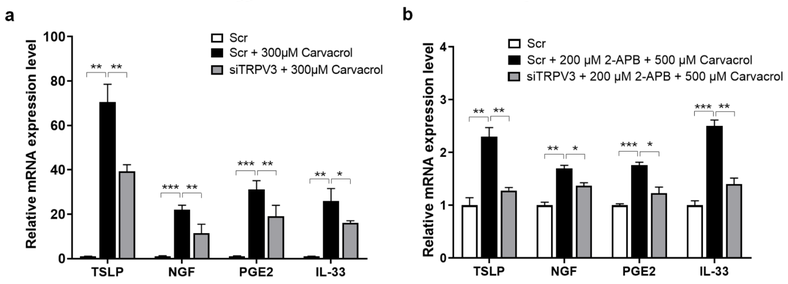


Figure 4. Keratinocytes derived from patients with AD exhibit enhanced release of TSLP, NGF, and PGE2 in response to innocuous heat stimulus by TRPV3 activation. (a-e) Keratinocytes from AD patients and healthy controls were incubated at room temperature (22˚C) or two innocuous warm temperatures (33 and 39˚C) for 24 hours. (f-i) Keratinocytes derived from patients with AD treated with scrambled (Scr) or TRPV3 siRNA (siTRPV3) were incubated at two innocuous warm temperatures (33 and 39˚C) for 24 hours. The culture supernatant was subjected to ELISA for measuring the levels of keratinocyte-derived pruritogens. Three independent experiments were performed. Data are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.0005. HC, healthy control; RT, room temperature; AD KC, keratinocytes from AD patients.

Figure 5. TRPV3 channel activation induces the production of certain pruritogens in normal human keratinocytes. Normal human epidermal keratinocytes transfected with scrambled or TRPV3 siRNA were stimulated with the TRPV3 agonist, carvacrol (300 μM) or TRPV3 agonist cocktail (200 μM 2-APB and 500 μM carvacrol). (a-b) qRT-PCR analysis of mRNA levels of TSLP, NGF, PGE2, and IL-33. (b-e) The culture supernatant was subjected to ELISA for measuring the levels of pruritogens. Data are representative of three independent experiments. Data are mean ± SEM and normalized to the scrambled siRNA-non-treated group. *P < 0.05, **P < 0.01, ***P < 0.0005.

6. TRPV3 is involved in warmth-provoked itch and pruritogens response in oxazolone-induced chronic itch mouse model

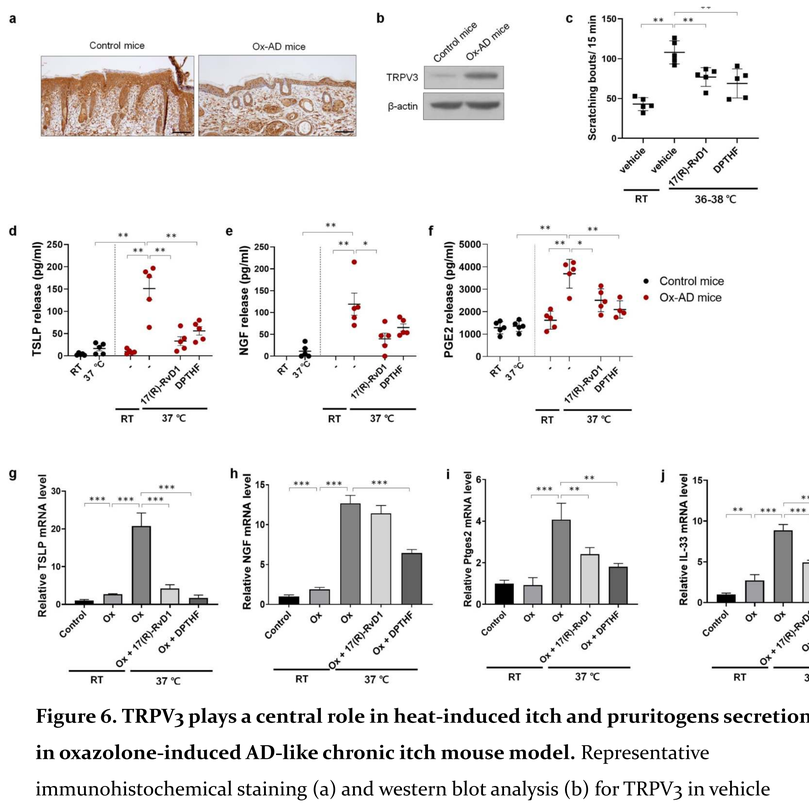


Figure 6. TRPV3 plays a central role in heat-induced itch and pruritogens secretion in oxazolone-induced AD-like chronic itch mouse model. Representative immunohistochemical staining (a) and western blot analysis (b) for TRPV3 in vehicle control and Ox-AD mice. Scale bars, 100 μm. (c) Scratching behavior of vehicle control and TRPV3 antagonist, 17(R)-RvD1 or DPTHF-treated Ox-AD mice during heat stimulation on day 18, n=5 mice per group. Pruritogens secretion in culture supernatant (d-f) and mRNA expression levels of pruritogens (g-j) by the ex vivo epidermis obtained from vehicle control and TRPV3 antagonist, 17(R)-RvD1 or DPTHF-treated Ox-AD mice were quantified by ELISA (d-f) and qRT-PCR (g-j) after 24 hours of incubation at room temperature (22˚C) or 37˚C. qRT-PCR results are normalized to those of vehicle control mice epidermis incubated at room temperature. Three independent experiments were performed. Data are mean ± SEM. *P < 0.05; **P < 0.01, ***P < 0.0005. 17(R)-RvD1, 17(R)-resolvin D1; DPTHF, 2,2-diphenyltetrahydrofuran; i.d.; intradermally; RT, room temperature.

CONCLUSION

1. TRPV3 is highly expressed in lesional epidermis and cultured AD keratinocytes.
2. TRPV3 exhibits enhanced channel activity with or without heat stimuli in AD keratinocytes.
3. Enhanced release of TSLP, bNGF and PGE2 from AD keratinocytes after heat stimuli depends on TRPV3.
4. These results are reproduced in Oxa-AD mice model.
5. Warmth-evoked itch and increase of pruritogens are alleviated by use of TRPV3 antagonists.

→ TRPV3 is highly activated in response to heat in keratinocytes from patients with atopic dermatitis and contributes to warmth-evoked pruritogens release and itch behaviors.