TO THE EDITOR

Transient receptor potential (TRP) ion channels form a family of 28 known members that have versatile functions (Caterina, 2007; Clapham, 2003). The prototypic TRP channel TRPV1 (Caterina et al., 1997), was found to be a molecular integrator of noxious stimuli on peripheral sensory nerves. Since then, TRP receptors have been shown to be functionally expressed in practically all organs of the human body, including the skin, where they regulate essential processes (Caterina, 2014; Nilius et al., 2007; Tóth et al., 2014). Several members of the TRP family (including TRPV1–4, TRP-melastatin 8, and TRP ankyrin 1) can be activated by discrete temperature ranges from noxious cold to painful heat (Caterina, 2014; Nilius et al., 2007; Tóth et al., 2014). Because skin—as the outermost layer of our body—also serves as a thermal barrier and is often met with temperature challenges, in the past decade a huge effort was invested to determine the presence and function of TRP channels in the skin. These studies have proven that multiple TRP channels are expressed in human skin (Caterina, 2014; Tóth et al., 2014), where they regulate many essential functions such as epidermal homeostasis, melanogenesis, senescence, maintenance of the epidermal barrier, and processes of the hair follicle.

TRPV4, a calcium-permeable, nonspecific cation channel usually discussed with TRPV3 based on the similar range of thermosensation and expression pattern, is also activated by a wide array of different stimuli, including UV light, low pH, and osmotic and mechanical triggers (White et al., 2016). TRPV4 interacts with intercellular adhesion proteins and thereby contributes to epidermal integrity and barrier functions (Kida et al., 2012; Sokabe et al., 2010; Sokabe and Tominaga, 2010). In the pilosebaceous unit, TRPV4 is expressed on sebaceous gland cells, where its activation results in suppressed proliferation of sebocytes and a strong lipostatic effect (Oláh et al., 2014).

Although TRPV4 seems to play a crucial role in the epidermis and sebaceous glands, to the best of our knowledge nothing is known about its presence and function in the HF. Therefore, in this study, we investigated the presence and function of TRPV4 in human HFs isolated from human skin samples (see Supplementary Materials online for details). Human skin samples were obtained after we received written informed consent from healthy individuals undergoing dermatosurgery, in accordance with Declaration of Helsinki guidelines, and after we obtained institutional research ethics committee permission.

Initially, we confirmed the expression of TRPV4 on intact HFs in the anagen VI stage of the hair cycle, where we detected TRPV4-specific immunoreactivity in the epithelial compartments of human HFs. Similarly to the expression patterns of TRPV3 and TRPV1, immunoreactivity can be detected in the outer (ORS) and inner root sheath layer of the HF epithelium (Caterina, 2014; Tóth et al., 2014), as well as the cortex of the bulbar hair shaft (Figure 1a–c). TRPV4 expression was also found on ORS keratinocytes (see Supplementary Figure S1a online). mRNA isolated from microdissected HFs in the anagen VI stage of the hair cycle and primary cultures of human HF-derived ORS keratinocytes also express the TRPV4 transcript (see Supplementary Figure S1b). As such, we are confident in stating that TRPV4 is expressed in the ORS of the hair follicle both at the mRNA and protein levels.

To determine the effect of TRPV4 activation on hair cycle and hair growth in vitro, human HFs were treated with the highly selective, synthetic TRPV4 agonist GSK1016790A (Willette et al., 2008). This resulted in significantly decreased hair shaft elongation in a concentration-dependent manner (Figure 1d), suggesting that TRPV4, similarly to TRPV1 and TRPV3, dose-dependently inhibits hair shaft elongation in vitro. GSK1016790A treatment also decreased the ratio of anagen HFs and increased the number of catagen HFs (Figure 1e, and see Supplementary Figure S2a online), showing that TRPV4 activation induces premature catagen regression. Quantitative analysis of Ki67/TUNEL-positive cells (which mark proliferating and apoptotic cells, respectively) in the area of matrix keratinocytes showed that TRPV4 activation significantly decreased the ratio of proliferating cells and increased the number of apoptotic cells (Figure 1f, and see Supplementary Figure S2b). Besides being a specific feature of catagen HFs, this finding strongly supports the overall picture of the inhibitory effect of TRPV4 activation on hair growth. All of the observed effects—on elongation, catagen induction, and ratio of apoptotic/proliferating cells—could be blocked by HC067047 (see Supplementary Figure S3a–c online), a potent and specific TRPV4 antagonist (Everaerts et al., 2010).

To test the functionality of the ion channel, we performed microfluorimetric Ca2+ measurement on human ORS keratinocytes (Figure 2a–c). GSK1016790A increased the intracellular Ca2+ concentration of ORS keratinocytes in a dose-dependent manner.

Abbreviations: HF, hair follicle; ORS, outer root sheath; ORSK, outer root sheath keratinocyte; TRP, transient receptor potential

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TRPV4 is expressed on human organ-cultured HFs, and primary cultures of human HF-derived ORS keratinocytes and the activation of TRPV4 inhibits human hair shaft elongation in organ-cultured HFs decreases intrafollicular proliferation and induces premature catagen regression. (a–c) Immunofluorescence of TRPV4 in organ-cultured human HFs (red) in situ. Scale bars = 50 μm. Nuclei were counterstained with DAPI (blue fluorescence). (d) Hair shaft elongation curves (18 HFs per group, mean ± standard error of the mean). *P < 0.05 compared with control. (e) Quantitative hair cycle histomorphometry on hematoxylin and eosin-stained sections of HF treated with various concentrations of GSK1016790A or vehicle. Percentages of HF in anagen or early or late catagen state were determined. (f) Statistical analysis of the numbers of Ki-67+ and TUNEL+ cells compared with the number of DAPI+ cells. Calculation is not applicable for the HF group treated with 1,000 nm GSK, because standardized criteria do not work with late-catagen HFs—the majority of the HFs in that treatment group. *P < 0.05 compared with control. These calculations were based on co-immunolabeling of proliferating (Ki-67+, red fluorescence) and apoptotic (TUNEL+, green fluorescence) cells, along with nuclei (DAPI+, blue fluorescence). For representative images, see Supplementary Figure S2c). ctrl, control; CTS, connective tissue sheath; DAPI, 4′,6-diamidino-2-phenylindole; DP, dermal papilla; GSK, GSK1016790A; HF, hair follicle; HSCh, hair shaft cortex; IS, inner root sheath; M, mol/L; MK, matrix keratinocytes; ORS, outer root sheath; WE-S, Williams E Medium.
(Figure 2a and b), in a similar concentration range as was used to inhibit hair growth, and was also abrogated by the specific antagonist (see Supplementary Figure S4a). Activating TRPV4 by temperature stimuli also resulted in calcium influx, which could be partially lowered by HC067047 (Figure 2c). Based on these results, we conclude that TRPV4 functions as a Ca\(^{2+}\) channel on ORS keratinocytes. Because Ca\(^{2+}\) influx may reduce the viability of ORSKs by inducing apoptotic or necrotic events, we also measured the effect of TRPV4 activation on membrane fragility and mitochondrial membrane potential, which are known to be early signs of necrosis and apoptosis, respectively. The application of GSK1016790A dramatically reduced mitochondrial membrane potential of the ORSKs in a concentration-dependent manner (see Supplementary Figure S4b). These results collectively suggest that TRPV4 activation induces apoptotic processes exclusively, without any signs of necrosis, as an early response to the elevation of intracellular Ca\(^{2+}\) concentration after TRPV4 channel activation.

This study added an additional member to the cellular receptors that are known to regulate hair follicle cycling. After the introduction of TRPV1 and TRPV3 as catagen inducers in humans (Bodó et al., 2005; Borbíro et al., 2011), we showed that TRPV4 activation also leads to a dramatic decrease in hair shaft elongation, along with morphological and structural changes characterizing catagen HFs. Calcium homeostasis is fundamental in

![Graph](https://www.jidonline.org/1387)
epithelial cells, especially keratinocytes, and we found that TRPV4 is functionally active in these cells and participates in responses to thermal stimuli. Because TRPV4 can be activated by various stimuli besides temperature, including pH, osmolarity, pressure, and UV light (White et al., 2016), further investigation could be promising to characterize the channel's involvement in other physiological or pathological processes. For a more in-depth look at the role of this channel in the context of dermal and epidermal function, please see the Supplementary Materials.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
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REFERENCES


Measurement Properties of the Hospital Anxiety and Depression Scale Used in Atopic Dermatitis in Adults

Jl Silverberg et al.
HADS in Adult AD

TO THE EDITOR
Atopic dermatitis (AD) is associated with increased prevalence and severity of anxiety and depression (Silverberg et al., 2018b, c; Yu and Silverberg, 2015). Adults with AD, particularly moderate to severe AD, may benefit from screening for and treatment of anxiety and depression in clinical practice. Recent guidelines recommend that depression and other neuropsychiatric conditions be addressed in treatment/management plans (level of evidence: I, II; strength of recommendation: C) (Eichenfield et al., 2014). The emotional impact of AD should be considered when deciding to use systemic therapy (Simpson et al., 2017). There is insufficient evidence to recommend specific assessments for this purpose. One potential assessment is the Hospital Anxiety and Depression Scale (HADS). HADS is composed of 14 questions, with domains for anxiety (HADS-A) and depression (HADS-D) (Zigmond and Snait, 1983). The

Abbreviations: AD, atopic dermatitis; DIF, differential item functioning; HADS, Hospital Anxiety and Depression Scale; HADS-A, Hospital Anxiety and Depression Scale—Anxiety score; HADS-D, Hospital Anxiety and Depression Scale—Depression score; MH, mental health; PO-SCORAD, Patient-Oriented Scoring Atopic Dermatitis; POEM, Patient-Oriented Eczema Measure

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