Activation of TRPV3 Inhibits Lipogenesis and Stimulates Production of Inflammatory Mediators in Human Sebocytes—A Putative Contributor to Dry Skin Dermatoses

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TO THE EDITOR

TRPV3 was cloned from keratinocytes and described as a thermosensitive member of the transient receptor potential ion channel family. Although its role in thermosensation is currently under debate, it is highly expressed in the epidermis and functions as a nonselective, Ca^{2+}-permeable cation channel (Huang et al., 2011; Nilius and Bíró 2013; Peier et al., 2002). Despite its high abundance in the skin, its genetic deletion causes only moderate, often strain- or sex-dependent, cutaneous phenotypic modifications, such as formation of curly whiskers and wavy hair, defect in epidermal barrier functions, and alterations in epidermal nitrate homeostasis (Cheng et al., 2010; Miyamoto et al., 2011). In contrast, gain-of-function mutations of *TRPV3* result in dramatic cutaneous alterations associated with severely dry skin, dermatitis, and hairless phenotype in both mice and rats (Asakawa et al., 2006; Xiao et al., 2008). Moreover, similar gain-of-function mutations of *TRPV3* were found to play an etiological role in a rare human genodermatosis, Olmsted syndrome, characterized by periorificial hyperkeratosis, hypotrichosis, alopecia, and severe pruritus (He et al., 2015; Lin et al., 2012; Ni et al., 2016). Encouraged by these findings, we verified the role of TRPV3 in human hair growth control by inducing catagen in mechanistic studies (Borbíró et al., 2011) and, most recently, we also described the proinflammatory action of TRPV3 activation in human epidermal keratinocytes (Szöllősi et al., 2018), whereas others reported its role in dry skin-associated itching (Yoshioka et al., 2009). However, the extended inflammatory symptoms and the disrupted lipid barrier found both in rodents and humans suggested that TRPV3-expressing skin cells other than keratinocytes might also be involved in the development of inflammatory skin conditions induced by TRPV3 hyperfunction. Therefore, in this study, we investigated the expression and activation of TRPV3 in human sebocytes, which are important regulators of cutaneous homeostasis (Tóth et al., 2011).

Immunohistochemical analysis showed that, like epidermal keratinocytes, human sebaceous glands (SG) express TRPV3 in situ (Figure 1a). Peripheral undifferentiated cells show stronger immunopositivity than centrally located terminally differentiated cells. We also showed the presence of TRPV3 protein and mRNA transcripts in human SG-derived SZ95 sebocytes (Zouboulis et al., 1999), a widely accepted model cell line to study SG biology in vitro (Figure 1b–d). We observed that TRPV3 expression is decreased in post-confluent, more differentiated cultures compared with the highly proliferating pre-confluent cultures (Figure 1c and d). The synthetic TRPV3 activator 2-APB, as well as the plant-derived carvacrol, evoked marked elevation of the intracellular Ca^{2+} concentration (Figure 1e), suggesting that TRPV3 is, indeed, functionally expressed in human sebocytes. The evoked Ca^{2+} signals were practically abolished in the presence of the general TRP channel blocker ruthenium red but were not affected by either AMG9810 or HC067047, selective antagonists of the closely related channels TRPV1 and TRPV4, respectively (Figure 1f), channels that are also functionally expressed by sebocytes (Oláh et al., 2014; Tóth et al., 2009). Because highly specific TRPV3 activators and inhibitors are not available commercially, we then investigated the effect of RNA interference-based silencing of TRPV3 expression on Ca^{2+} signals. Transfection of the sebocytes with small interfering RNA (siRNA) targeting TRPV3 resulted in a partial, yet marked decrease of the channel expression (see Supplementary Figure S1 online) compared with the scrambled RNA-transfected cells, and significantly suppressed the amplitude and the rate of rise of the agonist-evoked Ca^{2+} signals (Figure 1g and h); these data provided strong evidence for the activation of TRPV3 by the applied compounds. Higher concentrations of the activators reduced the living cell number in 24 hours, but lower concentrations (still able to evoke Ca^{2+} signals) did not influence the viability of sebocytes (see Supplementary Figure S2 online), confirming previous results on Ca^{2+} signaling-induced sebocyte apoptosis (Zouboulis et al., 2017).

Because sebaceous lipids essentially contribute to the epidermal barrier functions, we also investigated the influence of TRPV3 on lipid synthesis in SZ95 cells. Activation of TRPV3 with non-cytotoxic concentrations of 2-APB and carvacrol decreased lipid synthesis during arachidonic acid (AA)-induced differentiation of scrambled RNA-transfected cells used as control. TRPV3 agonists were less effective at suppressing AA-induced lipid synthesis in cells transfected with siRNA targeting TRPV3 (Figure 2a). Moreover, AA was slightly but significantly more effective in TRPV3-silenced cells,

Abbreviations: AA, arachidonic acid; SG, sebaceous gland; siRNA, small interfering RNA

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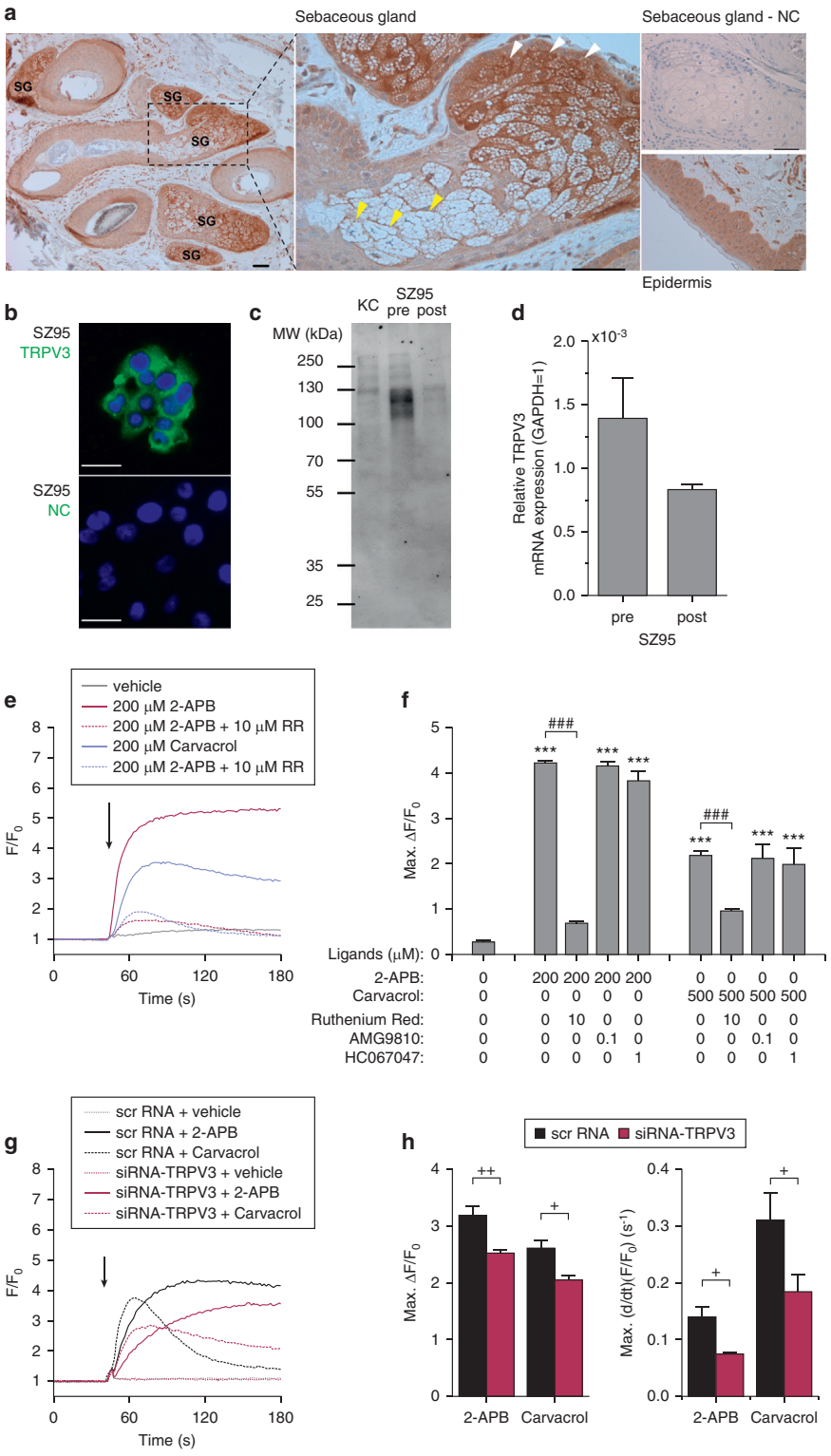

Figure 1. Human sebocytes express functional TRPV3 ion channels. (**a**) Immunohistochemical staining of TRPV3 (3,3′-diaminobenzidine, brown precipitate) on human SGs and epidermis. White and yellow arrowheads indicate undifferentiated and terminally differentiated cells, respectively. Scale bars = 50 µm. (**b**) Immunocytochemical staining of TRPV3 (FITC, green fluorescence) on SZ95 sebocytes. Blue indicates nuclei (DAPI). Scale bar = 25 µm. (**c**) Western blot analysis of protein lysates of human epidermal keratinocytes and SZ95 sebocytes from pre- and post-confluent (pre and post, respectively) cultures followed by immunolabeling with anti-TRPV3 antibody. Molecular weights in kDa are indicated. Expected molecular weight of recombinant TRPV3 is approximately 91 kDa (UniProt ID: Q2M3L1; UniProt, 2006). Multiple bands may refer to uncharacterized posttranslational modifications (e.g., glycosylation) and multimerization of channel subunits. (**d**) Relative expression of TRPV3 transcripts in pre- and post-confluent SZ95 sebocyte cultures. (**e**) Representative Ca^{2+} signals on SZ95 sebocytes evoked by TRPV3 agonists 2-APB and carvacrol in the presence or absence of ruthenium red, applied as indicated. (**f**) Statistical analysis of the amplitudes of the Ca^{2+} signals in various conditions as indicated. n = 6 in each group. $^{***}P < 0.001$ compared with the control and $^{###}P < 0.001$ between the indicated groups as determined by analysis of variance and Bonferroni post hoc test. (**g**) Representative Ca^{2+} signals evoked by TRPV3 agonists 2-APB (200 µmol/L) and carvacrol (500 µmol/L) on human SZ95 sebocytes transfected with scrambled RNA (scrRNA) or siRNA targeting TRPV3 (siRNA-TRPV3). (**h**) Statistical analysis on the amplitude and the rate of rise of the 2-APB– and carvacrol-induced Ca^{2+} signals in sebocytes transfected with scrambled or siRNA targeting TRPV3, as indicated. n = 5 in each group. $^{+}P < 0.05$ and $^{++}P < 0.01$ between the indicated groups, as determined by two-tailed Student *t* test for independent samples. KC, human epidermal keratinocyte; µM, µmol/L; Max., maximal; MW, molecular weight; NC, negative control; RR, ruthenium red; scr, scramble; SG, sebaceous gland; siRNA, small interfering RNA.

suggesting that basal activity of TRPV3 also negatively regulates AA-induced lipid synthesis. The effect of TRPV3 agonists was not restricted to AA-induced lipid synthesis: they also inhibited the lipogenic effect of the endocannabinoid anandamide and of the combination of linoleic acid and testosterone; and they slightly decreased basal lipid synthesis of non-transfected sebocytes, as well (see Supplementary Figure S3 online). Moreover, carvacrol did not induce cellular differentiation because it did not affect cellular granulation either under control conditions or in AA-treated cells. However, carvacrol selectively inhibited the AA-induced accumulation of cellular lipids, the effect of which was prevented by ruthenium red, indicating that indeed TRPV3 mediates the effect (see Supplementary Figure S4a online). Simultaneously, carvacrol down-regulated PPAR-γ and NRIP1, important positive regulators of sebaceous lipid synthesis (Dozsa et al., 2014; Oláh et al., 2014). These results suggest that TRPV3-mediated Ca^{2+} signaling generally inhibits lipid synthesis of sebocytes independently of the activated lipogenic pathways, confirming a recent study of our group on the role of Ca^{2+} signaling in human sebocytes using another

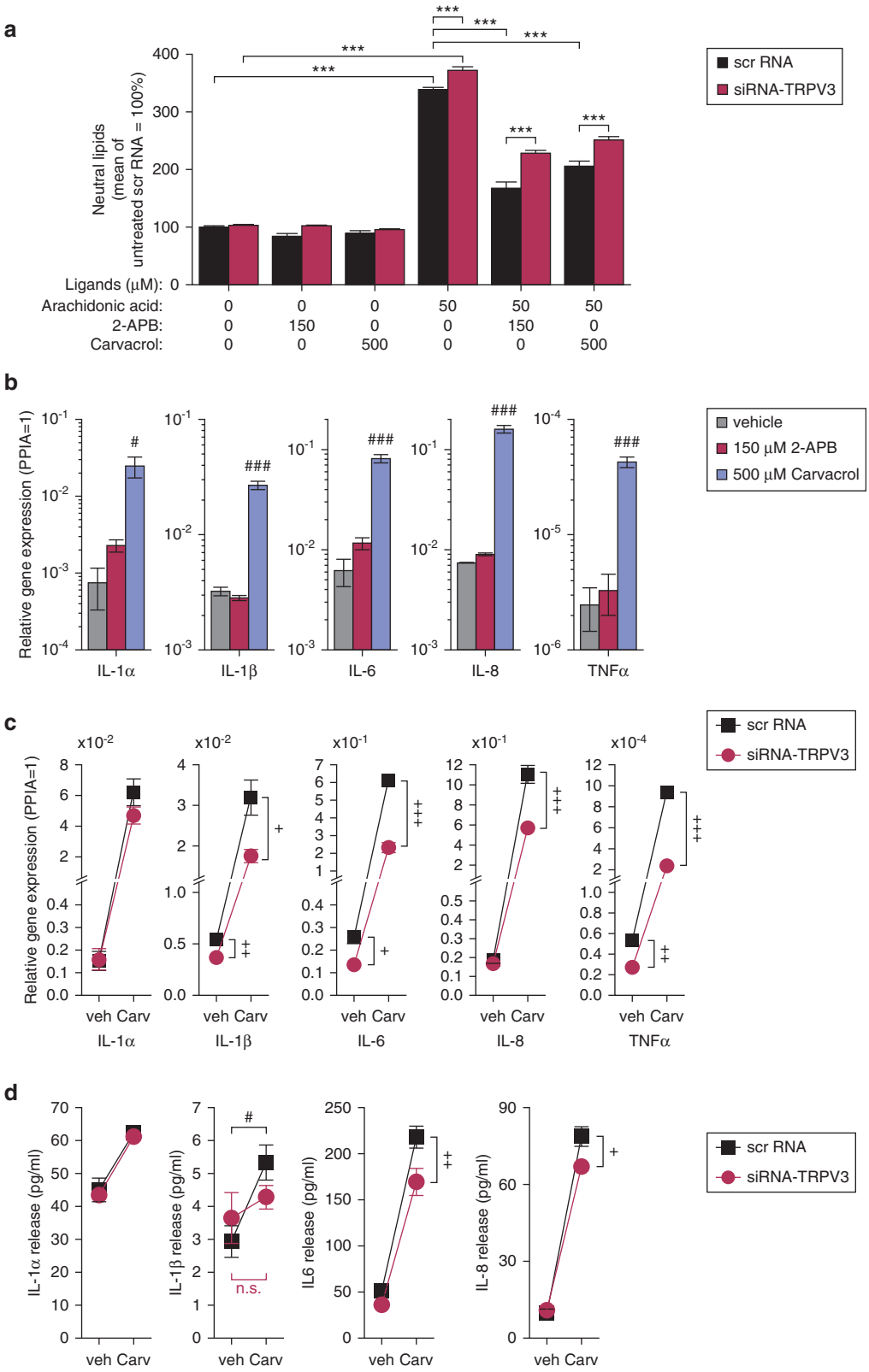

Figure 2. Activation of TRPV3 inhibits lipid synthesis associated with sebaceous differentiation and induces the synthesis and release of proinflammatory cytokines. (**a**) Quantitative Nile Red staining of neutral lipids in scrambled RNA (scr RNA) and TRPV3-targeting siRNA (siRNA-TRPV3) transfected SZ95 sebocytes treated with arachidonic acid and TRPV3 agonists for 24 hours, as indicated. n ≥ 4 in each group. $^{***}P < 0.001$ between the indicated groups as determined by one-way analysis of variance and Bonferroni post hoc test. (**b**) Relative expression of proinflammatory cytokine transcripts in SZ95 sebocytes after a 6-hour–long treatment with vehicle (used as control) or TRPV3 agonists, as indicated. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ compared with the vehicle-treated control using one-way analysis of variance and Dunnett post hoc test. PPIA used as endogenous control reference gene. (**c**) Carvacrol (500 μmol/L, 6 hours) induced changes in relative expression of proinflammatory cytokine genes (determined by quantitative PCR) in scrambled RNA (scr RNA) and TRPV3-specific siRNA (siRNA-TRPV3) transfected SZ95 sebocytes. (**d**) Release of proinflammatory cytokines (determined from supernatants using ELISA) after the same treatments as

experimental setting (Zouboulis et al., 2017). Our data are consistent with our previous findings because TRPV1 and TRPV4 activation by capsaicin and cannabidiol, respectively, inhibited sebaceous lipid synthesis (Oláh et al., 2014; Tóth et al., 2009).

Beyond lipid synthesis, SGs play an important role in the regulation of cutaneous immune functions (Tóth et al., 2011). Therefore, we also assessed the effect of TRPV3 activation on cytokine expression of SZ95 sebocytes. Our findings showed that transcription of several proinflammatory cytokines was unambiguously triggered by the TRPV3 agonist carvacrol within 6 hours (Figure 2b), although during this time, 2-APB was ineffective. To assess the TRPV3 specificity of the carvacrol treatment, we repeated the experiments on TRPV3-silenced SZ95 sebocytes. In this condition, we found a reduced effect of the activator compared with scrambled RNA-transfected cells (Figure 2c), again arguing for the involvement of TRPV3 in mediating the effect of carvacrol. Moreover, we found that not only the expression but also the release of some proinflammatory cytokines was decreased by TRPV3-specific RNA interference in carvacrol-treated sebocytes (Figure 2c).

Taken together, our findings suggest that sebocytes might be involved in the pathogenesis of dry skin-associated inflammatory dermatoses linked to TRPV3 hyperactivity. Furthermore, our preclinical findings introduce TRPV3 as a previously unreported negative regulator of sebaceous lipid synthesis with a marked proinflammatory effect. Further clinical studies are urged to assess the clinical efficacy of TRPV3 inhibitors on the therapeutic management of certain inflammatory skin conditions.

ORCIDs

Attila Oláh: http://orcid.org/0000-0003-4122-5639
Balázs István Tóth: http://orcid.org/0000-0002-4103-4333

CONFLICT OF INTEREST

CCZ owns an international patent on the SZ95 sebaceous gland cell line (WO2000046353). The other authors state no conflict of interest.

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Magdolna Szántó[1,7], Attila Oláh[1,7], Attila Gábor Szöllősi[1], Kinga Fanni Tóth[1], Edit Páyer[1,2], Nóra Czakó[1], Ágnes Pór[3], Ilona Kovács[3], Christos C. Zouboulis[4], Lajos Kemény[5], Tamás Bíró[1,6,8] and Balázs István Tóth[1,8,*]

[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [2]Department of Hematology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [3]Department of Pathology, Gyula Kenézy Hospital, Debrecen, Debrecen, Hungary; [4]Deparments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Theodore Fontane Medical University of Brandenburg, Dessau, Germany; [5]Department of Dermatology and Allergology, Faculty of Medicine, University of Szeged, Szeged, Hungary; and [6]Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[7]These authors contributed equally to this work as first authors.
[8]These authors contributed equally to the work as senior authors.
[*]Corresponding author e-mail: toth.istvan@med.unideb.hu

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.07.015.

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indicated in panel **c**. $^{+}P < 0.05$, $^{++}P < 0.01$ and $^{+++}P < 0.001$ between scrambled RNA and TRPV3-specific siRNA transfected and $^{\#}P < 0.05$ between vehicle- and carvacrol-treated cells as determined by two-tailed Student *t* test for independent samples. Carv, carvacrol; μM, μmol/L; n.s., non-significant; scr, scramble; siRNA, small interfering RNA; veh, vehicle.