Transient Receptor Potential Vanilloid 4 is Expressed in Human Hair Follicles and Inhibits Hair Growth in Vitro

DOI:
10.1016/j.jid.2018.11.020

Document Version
Accepted author manuscript

Accepted Manuscript

Transient Receptor Potential Vanilloid 4 is Expressed in Human Hair Follicles and Inhibits Hair Growth in Vitro

Imre Lőrinc Szabó, Erika Herczeg-Lisztes, Andrea Szegedi, Balázs Nemes, Ralf Paus, Tamás Bíró, Attila Gábor Szöllősi

PII: S0022-202X(18)32912-9

DOI: https://doi.org/10.1016/j.jid.2018.11.020

Reference: JID 1704

To appear in: *The Journal of Investigative Dermatology*

Received Date: 26 April 2018

Revised Date: 2 November 2018

Accepted Date: 9 November 2018

Please cite this article as: Szabó IL, Herczeg-Lisztes E, Szegedi A, Nemes B, Paus R, Bíró T, Szöllősi AG, Transient Receptor Potential Vanilloid 4 is Expressed in Human Hair Follicles and Inhibits Hair Growth in Vitro, *The Journal of Investigative Dermatology* (2019), doi: https://doi.org/10.1016/j.jid.2018.11.020.

TRANSIENT RECEPTOR POTENTIAL VANILLOID 4 IS EXPRESSED IN HUMAN HAIR FOLLICLES AND INHIBITS HAIR GROWTH IN VITRO

Imre Lőrinc Szabó[1,2], Erika Herczeg-Lisztes[3], Andrea Szegedi[2, 4], Balázs Nemes[5], Ralf Paus[6,7], Tamás Bíró[3,*], Attila Gábor Szöllősi[1,3,*]

[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[2]Department of Dermatology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[3]Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[4]Division of Dermatological Allergology, Department of Dermatology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[5]Department of Transplantation, Institute of Surgery, Faculty of Medicine, University of Debrecen, Hungary.
[6]Centre for Dermatology Research, The University of Manchester, and NIHR Manchester Biomedical Research Centre, Manchester, UK
[7]Department of Dermatology & Cutaneous Surgery, 19 University of Miami Miller School of Medicine, Miami, FL, USA

* These authors contributed equally to this work
This work was performed in Debrecen, Hajdú-Bihar, Hungary
Correspondence should be addressed to:
Attila Gábor Szöllősi
Department of Immunology, University of Debrecen, Faculty of Medicine H-4002 DEBRECEN Egyetem tér 1. HUNGARY
Office Phone: +36-52-255-575/65104
FAX: +
Email: szollosi.attila@med.unideb.hu

Short title: TRPV4 induces catagen in human hair follicle

Abbreviations: CB, cannabinoid receptors; DP, dermal papilla; ECS, endocannabinoid system; HF, hair follicle; IR, immunoreactivity; IRS, inner root sheath; MK, matrix keratinocyte; MMP, mitochondrial membrane potential; NC, negative control; ORS, outer root sheath; ORSK, outer root sheath keratinocytes; $[Ca^{2+}]_{ic}$, intracellular calcium concentration; TRP, transient receptor potential; TRPV transient receptor potential vanilloid subfamily

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 TRP vanilloid subfamily member 1 (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, Toth et al., 2014). Several members of TRP family (including TRPV1-4, TRP-Melastatin 8, TRP Ankyrin 1) can be activated by discrete temperature ranges from noxious cold to painful heat (Caterina, 2014, Nilius et al., 2007, Toth et al., 2014). Since our 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, Toth et al., 2014), where they regulate many essential functions e.g. epidermal homeostasis, melanogenesis, senescence, maintenance of the epidermal barrier and processes of the hair follicle.

TRPV4, a calcium permeable non-specific 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, as well as 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 (Olah 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 the current study, we aimed to investigate the presence and function of TRPV4 in human HFs, isolated from human skin samples (see Supplementary Materials for details). Human skin samples were obtained following written informed consent from healthy individuals undergoing dermatosurgery, adhering to Helsinki guidelines, and after obtaining Institutional Research Ethics Committee's 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 (IR) in the epithelial compartments of human HFs. Similarly to the expression pattern of TRPV3 and TRPV1 IR can be detected in the outer, and inner root sheath (ORS, IRS) layer of the HF epithelium (Caterina, 2014, Toth et al., 2014), as well as the cortex of the bulbar hair shaft (Figure 1 a-c). TRPV4 expression was also found on ORS keratinocytes (Supplementary figure 1 a). mRNA isolated from microdissected HFs in the anagen VI stage of hair cycle, and primary cultures of human HF derived ORS keratinocytes, also express the TRPV4 transcript (Supplementary figure 1 b). As such we are confident in stating that TRPV4 is expressed in the ORS of the hair follicle both on the mRNA and protein level.

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 1 d) 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 while increasing the number of catagen HFs (Figure 1 e, Supplementary Figure 2a) – 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 MKs demonstrate that TRPV4 activation significantly decreased the ratio of proliferating cells, while increasing the number of apoptotic cells (Figure 1 f, Supplementary Figure 2b). 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. Importantly, all of the observed effects – on elongation, catagen induction, and ratio of apoptotic/proliferating cells – could be blocked by the HC067047 (Supplementary figure 3 a, b, and c respectively), a potent and specific TRPV4 antagonist (Everaerts et al., 2010).

To test the functionality of the ion channel, we performed microfluorimetric Ca^{2+} measurement on human ORS keratinocytes (Figure 2 a-c). GSK1016790A increased the intracellular Ca^{2+} concentration of ORS keratinocytes in a dose dependent manner (Figure 2 a-b), in a similar concentration range as was used to inhibit hair growth, and was also abrogated by the specific antagonist (Supplementary Figure 4a). Activating TRPV4 by temperature stimuli also resulted in calcium influx, which could be partially lowered by HC067047 (Figure 3 c). Based on these results, we conclude that TRPV4 functions as a Ca^{2+} channel on ORS keratinocytes. Since Ca^{2+} influx may reduce ORSKs' viability by inducing apoptotic or necrotic events, we also measured the effect of TRPV4 activation on membrane fragility and mitochondrial membrane potential (MMP) that are known to be early signs of necrosis and apoptosis, respectively. The application of GSK1016790A dramatically reduced MMP of the ORSKs in a concentration dependent manner (Supplementary Figure 4 b). 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.

Our current study added an additional member to the cellular receptors that are known to regulate hair follicle cycling. Following the introduction of TRPV1 and TRPV3 as catagen

inducers in human (Bodo et al., 2005, Borbiro et al., 2011), we demonstrated 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 epithelial cells, especially keratinocytes, and we found that TRPV4 is functionally active in these cells, and participates in responses to thermal stimulus. Since 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 in order 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 Discussion.

.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by Hungarian research grants (NKFIH FK125053, NKFIH K120187, GINOP-2.3.2-15-2016-00050).

Figure legends:

Figure 1. The transient receptor potential vanilloid-4 (TRPV4) is expressed on human organ-cultured hair follicles (HFs), and primary cultures of human HF derived outer root sheath (ORS) keratinocytes and the activation of TRPV4 inhibits human hair shaft elongation in organ-cultured hair follicles (HFs) decreases intrafollicular proliferation, and induces premature catagen regression in organ-cultured HFs:

Immunofluorescence of TRPV4 in organ-cultured human HFs (red) *in situ* **(a-c)** CTS, connective tissue sheath, ORS, outer root sheath, IS, inner root sheath, HsC hairshaft cortex, DP, dermal papilla; MK, matrix keratinocytes. Bars 50 µm. Nuclei were counterstained with 40-6-diamidino-2-phenylindole (DAPI, blue fluorescence). **(d**) Hair shaft elongation curves (18 HFs per group, mean±SEM). *P<0.05 when compared with control. **(e)** Quantitative hair cycle histomorphometry on hematoxylin–eosin-stained sections of HF treated with various conentrations of GSK1016790A or vehicle. Percentage of HF in anagen or early or late catagen state was determined. **(f)** Statistical analysis of number of Ki-67+ and TUNEL+ cells as compared with the number of DAPI+ cells. Calculation is not applicapble for the HF group treated with 1000nm GSK as standardized criteria does not work with late catagen HF – the majority of the HF in that treatment group. *P<0.05 when 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 (4'-6-diamidino-2-phenylindole +, DAPI, blue fluorescence, for representative images see **supplementary figure 2c**).

Figure 2. The outer root sheath keratinocytes express functionally active Ca^{2+} permeable transient receptor potential vanilloid-4 channels, and also respond to thermal stimulus by calcium influx which is mediated by TRPV4 activation:

(a) Representative fluorimetric Ca^{2+}-imaging data recorded on Fluo-4-loaded ORS keratinocytes. The arrow indicates the application of various concentrations of GSK1016790A. **(b)** Statistical analysis of maximal amplitudes of Ca^{2+}-elevations induced by the TRPV4 agonist with or without the application of specific TRPV4 antagonist HC067047. *, # $P<0.05$ when compared with control or without the application HC067047 respectively. **(c)** Fluorimetric Ca^{2+}-imaging data recorded on Fluo-4-loaded ORS keratinocytes. The lower chart indicates the temperature protocol. ORSKs respond to thermal stimulus ($31^{o}C$) with Ca^{2+}-influx, which is lowered by the application of HC067047 (black and red, respectively) Data expressed as mean±SEM, 3 technical repeats. *$P<0.05$ when compared with control.

References

Bodo E, Biro T, Telek A, Czifra G, Griger Z, Toth BI, et al. A hot new twist to hair biology: involvement of vanilloid receptor-1 (VR1/TRPV1) signaling in human hair growth control. The American journal of pathology 2005;166(4):985-98.

Borbiro I, Lisztes E, Toth BI, Czifra G, Olah A, Szollosi AG, et al. Activation of transient receptor potential vanilloid-3 inhibits human hair growth. The Journal of investigative dermatology 2011;131(8):1605-14.

Caterina MJ. Transient receptor potential ion channels as participants in thermosensation and thermoregulation. American journal of physiology Regulatory, integrative and comparative physiology 2007;292(1):R64-76.

Caterina MJ. TRP channel cannabinoid receptors in skin sensation, homeostasis, and inflammation. ACS chemical neuroscience 2014;5(11):1107-16.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997;389(6653):816-24.

Clapham DE. TRP channels as cellular sensors. Nature 2003;426(6966):517-24.

Everaerts W, Zhen X, Ghosh D, Vriens J, Gevaert T, Gilbert JP, et al. Inhibition of the cation channel TRPV4 improves bladder function in mice and rats with cyclophosphamide-induced cystitis. Proc Natl Acad Sci U S A 2010;107(44):19084-9.

Kida N, Sokabe T, Kashio M, Haruna K, Mizuno Y, Suga Y, et al. Importance of transient receptor potential vanilloid 4 (TRPV4) in epidermal barrier function in human skin keratinocytes. Pflugers Archiv : European journal of physiology 2012;463(5):715-25.

Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. Physiological reviews 2007;87(1):165-217.

Olah A, Toth BI, Borbiro I, Sugawara K, Szollosi AG, Czifra G, et al. Cannabidiol exerts sebostatic and antiinflammatory effects on human sebocytes. The Journal of clinical investigation 2014;124(9):3713-24.

Sokabe T, Fukumi-Tominaga T, Yonemura S, Mizuno A, Tominaga M. The TRPV4 channel contributes to intercellular junction formation in keratinocytes. The Journal of biological chemistry 2010;285(24):18749-58.

Sokabe T, Tominaga M. The TRPV4 cation channel: A molecule linking skin temperature and barrier function. Communicative & integrative biology 2010;3(6):619-21.

Toth BI, Olah A, Szollosi AG, Biro T. TRP channels in the skin. British journal of pharmacology 2014;171(10):2568-81.

White JP, Cibelli M, Urban L, Nilius B, McGeown JG, Nagy I. TRPV4: Molecular Conductor of a Diverse Orchestra. Physiological reviews 2016;96(3):911-73.

Willette RN, Bao W, Nerurkar S, Yue TL, Doe CP, Stankus G, et al. Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: Part 2. The Journal of pharmacology and experimental therapeutics 2008;326(2):443-52.

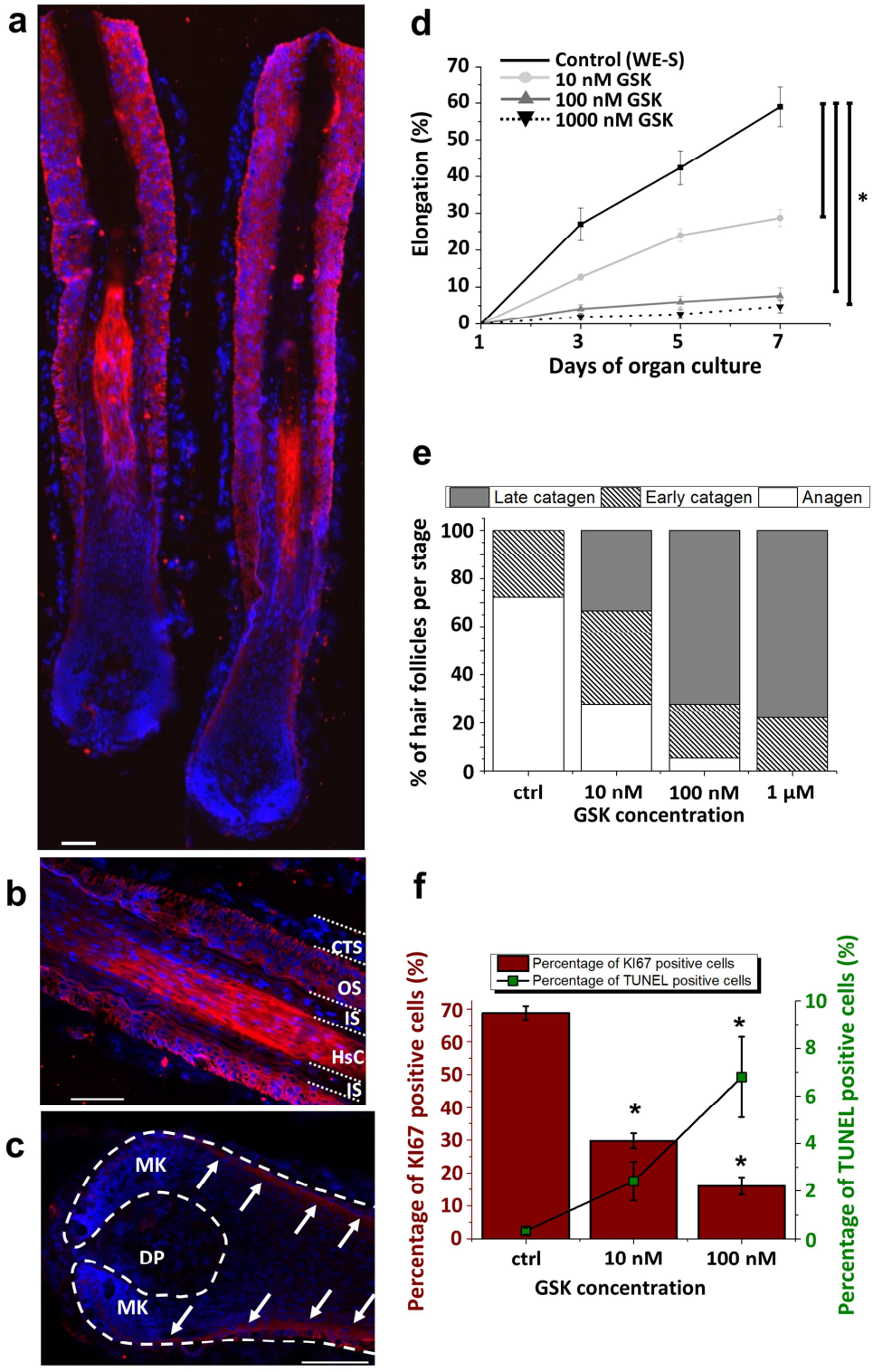
a
b
CTS
OS
IS
HsC
IS
c
MK
DP
MK
d
Control (WE-S)
10 nM GSK
100 nM GSK
1000 nM GSK
Elongation (%)
Days of organ culture
*
e
Late catagen
Early catagen
Anagen
% of hair follicles per stage
ctrl
10 nM
100 nM
1 µM
GSK concentration
f
Percentage of KI67 positive cells
Percentage of TUNEL positive cells
Percentage of KI67 positive cells (%)
Percentage of TUNEL positive cells (%)
ctrl
10 nM
100 nM
GSK concentration

Figure 1.

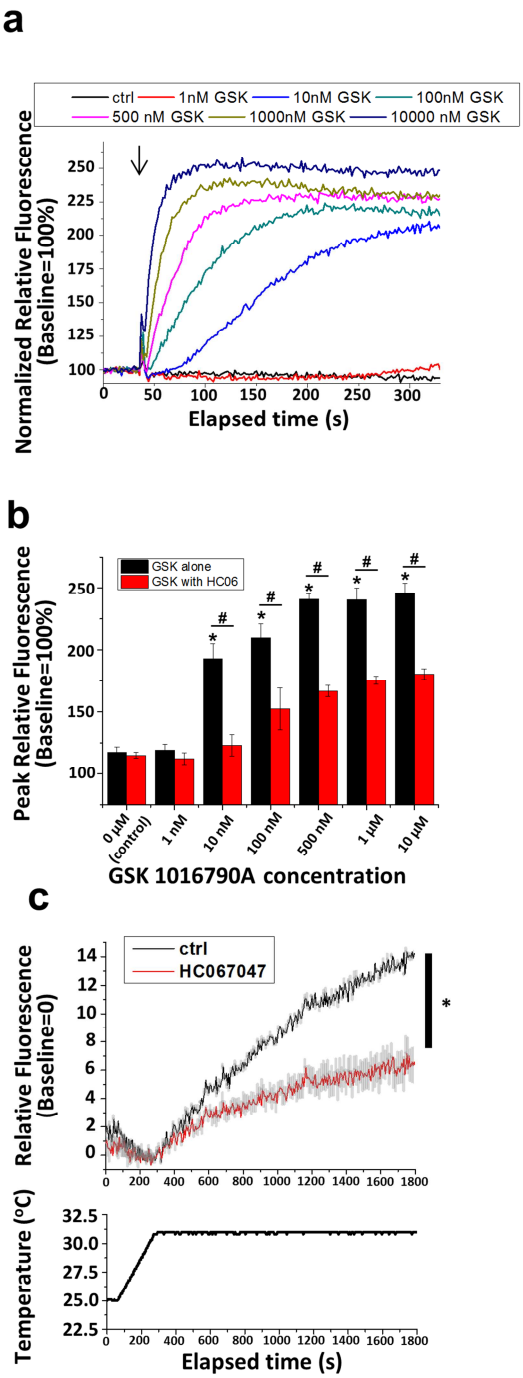
a
ctrl
1nM GSK
10nM GSK
100nM GSK
500 nM GSK
1000nM GSK
10000 nM GSK
Normalized Relative Fluorescence (Baseline=100%)
Elapsed time (s)
b
GSK alone
GSK with HC06
Peak Relative Fluorescence (Baseline=100%)
0 μM (control)
1 nM
10 nM
100 nM
500 nM
1 μM
10 μM
GSK 1016790A concentration
c
ctrl
HC067047
Relative Fluorescence (Baseline=0)
Temperature (°C)
Elapsed time (s)

Figure 2.