

Hair Follicle Chemosensation: TRPM5 Signaling Is Required for Anagen Maintenance



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TO THE EDITOR

Transient receptor potential (TRP) channels comprise a functionally diverse group of ion channels that function as cellular sensors and integrators of a plethora of physical and chemical stimuli; hence, they are considered as central players in chemosensation. Furthermore, TRP channels also act as molecular effectors regulating various cellular processes, such as growth, differentiation, immune response, and hormone secretion, and play crucial roles in skin biology and cutaneous disorders [reviewed in (Caterina and Pang, 2016)]. Several TRP channels, such as TRPV1, TRPV3, and TRPV4, are expressed in the human hair follicle (HF) and regulate HF growth and cycling. Specifically, pharmacological activation of TRP channels induces premature catagen regression in mice and organ-cultured human HFs (Bodó et al., 2005; Borbíró et al., 2011; Szabó et al., 2019). However, the effects of many other chemosensory TRP channels in human HF biology remain to be explored.

One of these is TRPM5, a nonselective cation channel that is activated by a rapid and transient increase in intracellular Ca^{2+} in response to G-protein coupled membrane receptor-mediated phospholipase C/protein kinase C activation, which in turn leads to initiation of downstream pathways (Shigeto et al., 2017). Similar to other TRP ion channels, TRPM5 is activated by a wide range of extra- and intracellular signals, ranging from gustatory agents and odorants to pheromones and acidic pH (Lin et al., 2007; López et al., 2014; Maeda et al., 2017; Talavera et al., 2005). Although TRPM5-expressing chemosensory epithelial cells are sparsely located throughout the

digestive tract, respiratory and olfactory systems, and some other tissues in mice and humans (Wyatt et al., 2017), their presence and function in skin and its appendages remain unknown. Given that we had previously shown human HFs to be regulated by olfactory receptors (Chéret et al., 2018), we have asked whether TRPM5 plays any role in human HF biology.

This study was conducted according to Declaration of Helsinki principles. HF specimens (*Supplementary Table S1*) were obtained from either scalp skin or follicular units of healthy male donors after informed, written patient consent and ethics committee approval (University of Muenster 2015-602-f-S).

By immunofluorescence microscopy, human anagen scalp HF cryosections showed prominent TRPM5 expression in epithelial HF compartments (*Figure 1a* and b), with the strongest TRPM5 immunoreactivity being detected in outer root sheath (ORS) keratinocytes, with weaker signals seen in the hair matrix and inner root sheath (*Figure 1a*). Taken together, this strong epithelial expression pattern suggested that TRPM5 may be involved in regulating the function of HF keratinocytes.

To probe the functional significance of this ion channel, we transfected microdissected human HFs with Accell (Horizon Discovery, United Kingdom) *TRPM5* small interfering RNAs or scrambled oligonucleotides (control) for 72 hours (*Supplementary Figure S1a*). Masson-Fontana histochemistry revealed a minimal to absent melanin clumping in both test and control HFs, suggesting minimal cytotoxicity of the transfection method (*Supplementary Figure S1b*). Although there was only a slight reduction in *TRPM5* transcription levels (*Supplementary Figure S2c*),

TRPM5 small interfering RNA significantly decreased *TRPM5* protein expression in the hair matrix and proximal bulb ORS (*Figure 1c* and d), documenting translational repression and effective *TRPM5* silencing *ex vivo*.

A significantly higher proportion of *TRPM5*-depleted HFs progressed into apoptosis-driven HF regression (catagen) than control HFs, which largely remained in anagen (*Figure 1e*). This effect was associated with a significant reduction in the number of proliferating Ki-67^+ cells in the anagen hair matrix and proximal bulb ORS, whereas the number of apoptotic TUNEL $^+$ cells increased, namely in the proximal ORS (*Figure 1f* and g) (*Supplementary Figure S1d* and e). This suggests that human anagen HFs require constant (tonic) signaling by as yet unknown endogenous *TRPM5* activators to maintain anagen, just as we had previously shown for the olfactory receptor, OR2AT4 (Chéret et al., 2018).

Because HF growth and cycling are tightly controlled by a balance of autocrine and paracrine regulators, we investigated the expression of genes encoding well-documented anagen-promoting (*IGF1*, *LEF1*) or catagen-inducing (*TGFB2*, *SFRP1*) factors (Hawkshaw et al., 2020; Samuelov et al., 2012). By quantitative real-time reverse transcriptase-PCR, transcript levels of *LEF1*, an indicator of WNT activity, and *IGF1*, the key anagen-promoting growth factor (Hawkshaw et al., 2020; Chéret et al., 2018), were significantly downregulated in *TRPM5* small interfering RNA-transfected HFs compared with controls (*Figure 1h*). In contrast, *TRPM5* silencing upregulated transcription of *TGFB2* and *SFRP1* (*Figure 1h*), a key WNT antagonist that promotes catagen in human HFs *ex vivo* (Hawkshaw et al., 2020). This suggests that insufficient *TRPM5* activity profoundly impacts on known key regulators of human HF cycling in a manner that is consistent with the

Abbreviations: HF, hair follicle; ORS, outer root sheath; TPPO, triphenylphosphine oxide; TRP, transient receptor potential

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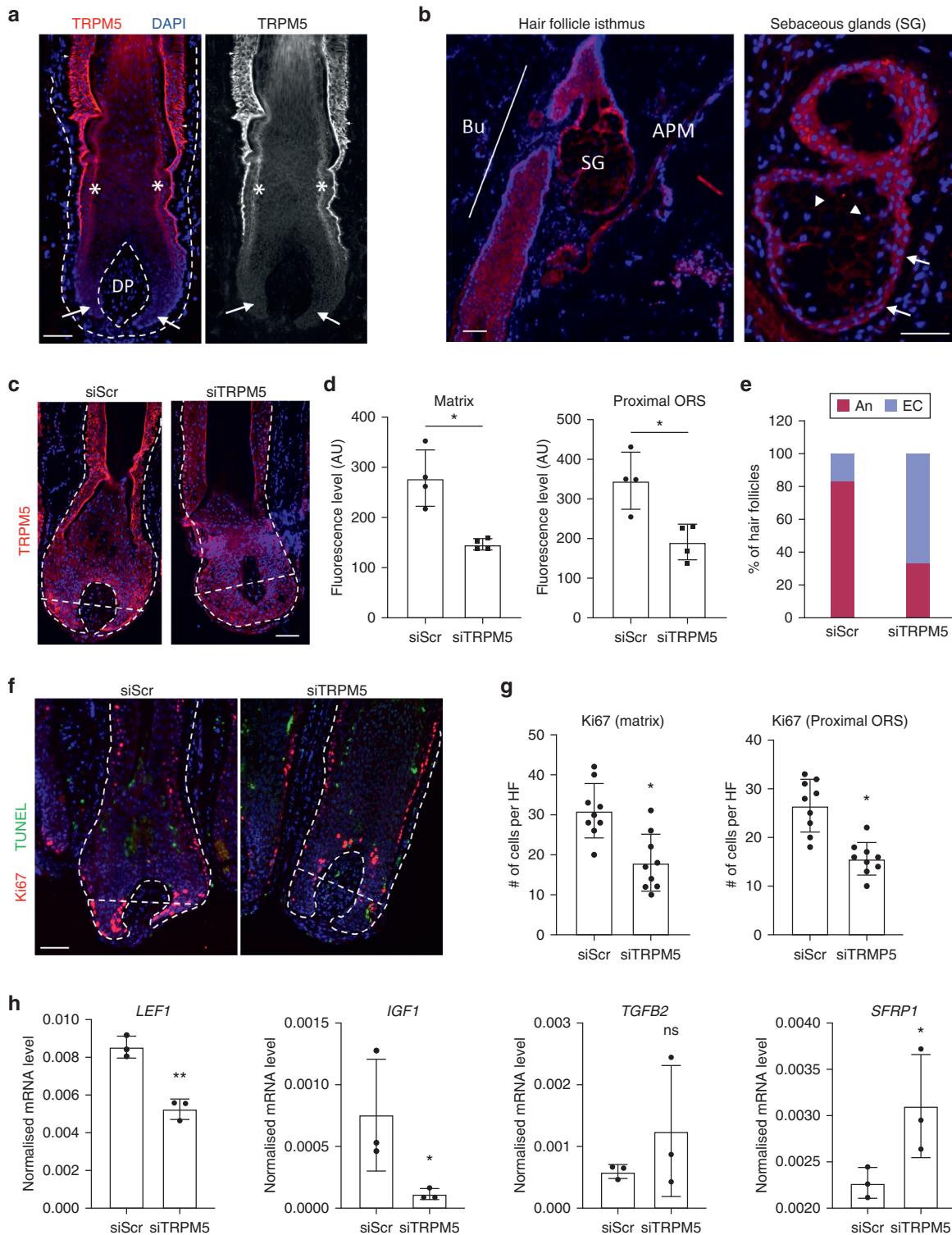


Figure 1. TRPM5 knockdown in human HF organ culture reduces cell proliferation and decreases expression of anagen-promoting genes. (a, b) With immunolabeling, a strong TRPM5 expression is detected in the ORS (arrowheads), with weaker expression in the hair matrix (arrows) and IRS (asterisks), whereas no specific signal was observed in the follicular mesenchyme (DP and connective tissue sheath). Furthermore, TRPM5 immunoreactivity was also detected in the HF isthmus, SGs. Note higher expression of TRPM5 in the basal cells (arrows) of SGs compared with differentiated cells (arrowheads). (c, d) TRPM5 protein expression in TRPM5-depleted human HFs compared with siScr-treated control group as revealed by immunolabeling followed by image analysis. (e) Percentage of HFs in anagen (An) and early catagen (EC) phases of hair cycle in siTRPM5- versus siScr-transfected HFs ($n = 6\text{--}8$ HFs/group, three independent replicates). (f, g) Immunofluorescent detection and quantification of proliferating (Ki-67^+) and apoptotic (TUNEL^+) cells in the hair matrix (below Auber's line) and proximal bulb ORS of siTRPM5- and siScr-treated HFs; (h) HFs were treated with siTRPM5 and siScr for 24 hours and analyzed for gene expression by qRT-PCR ($n = 3$ per group). Mean \pm SD, Mann-Whitney U test, * $P < 0.05$, ** $P < 0.001$. Bar = 100 μm . An, anagen; APM, arrector pili muscle; Bu, bulge; DP, dermal papilla; EC, early catagen; HF, hair follicle; IRS, inner root sheath; ORS, outer root sheath; qRT-PCR, quantitative real-time reverse transcriptase-PCR; SG, sebaceous gland; siScr, scrambled siRNA; siTRPM5, TRPM5 small interfering RNA.

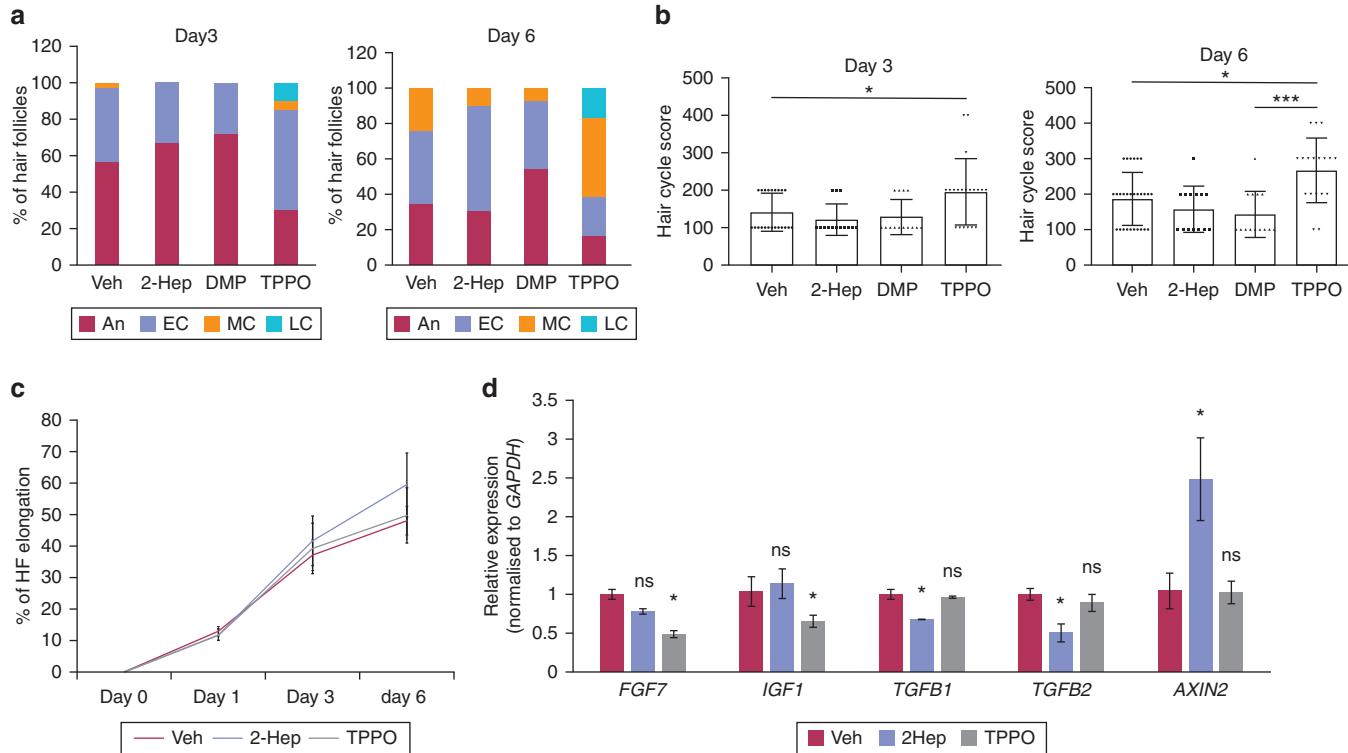


Figure 2. TRPM5 activation promotes hair growth ex vivo. (a, b) Human anagen HF were cultured in the presence of 2-Hep (12.5 μ M), DMP (12.5 μ M), or TPPO (150 μ M), and percentages of HF at different stages of hair cycle and hair cycle score were calculated at day 3 and 6 of the treatments ($n = 6$ –8 HF per group, three independent replicates); for (b), Kruskal–Wallis test and Dunn's multiple comparisons test as post hoc test were performed, * $P < 0.05$, *** $P < 0.001$. (c) HF elongation following 2-Hep and TPPO treatments compared with vehicle control ($n = 6$). (d) HF were treated with 2-Hep (12.5 μ M) and TPPO (150 μ M) for 6 hours and analyzed for gene expression by qRT-PCR ($n = 3$ per group). One-way ANOVA, mean \pm SEM (d) and mean \pm SD (e). Bar = 50 μ m. 2-Hep, 2-heptanone; An, anagen; DMP, 2,5-dimethylpyrazine; EC, early catagen; HF, hair follicle; LC, late catagen; MC, mid-catagen; qRT-PCR, quantitative real-time reverse transcriptase-PCR; TPPO, triphenylphosphine oxide; Veh, vehicle.

catagen-promoting effects of TRPM5 silencing (Figure 1e).

Finally, we manipulated TRPM5 activity using its best-known activators, 2-heptanone and 2,5-dimethylpyrazine, and a selective inhibitor, triphenylphosphine oxide (TPPO) (Supplementary Figure S2a) (López et al., 2014; Palmer et al., 2010). Similar to TRPM5 knockdown, inhibition of TRPM5 activity with TPPO promoted catagen and increased the hair cycle score, whereas TRPM5 activators 2-heptanone and 2,5-dimethylpyrazine maintained HF in anagen at levels similar to or even greater than vehicle controls (Figure 2a and b). We also found a 10–15% increase in hair shaft elongation in both 2-heptanone- and 2,5-dimethylpyrazine-treated HFs compared with the control or TPPO treatments (Figure 2c) (Supplementary Figure S2b). Furthermore, TPPO treatment of HFs downregulated transcription of anagen-promoting factors *FGF7* and *IGF1*, whereas TRPM5 stimulation

by 2-heptanone reduced levels of catagen-inducing factors *TGFB1/2* and increased *AXIN2* expression, suggesting activation of the WNT signaling pathway (Figure 2d).

Taken together, our findings demonstrate that TRPM5 signaling represents an important, previously unreported, therapeutically targetable control of human HF cycling that is required for maintaining anagen and supporting HF keratinocyte proliferation in the ORS and hair matrix. Because the TRPM5 activator 2,5-dimethylpyrazine also stimulates proliferation in the mouse subventricular zone, a brain stem cell niche (Koyama et al., 2013), it is conceivable that TRPM5 signaling maintains proliferative activity of progenitor cells in human HFs as well. This function of TRPM5 is quite unique in comparison to all other previously examined TRP ion channels, which actually inhibit hair matrix keratinocyte proliferation and induce catagen in human HFs

ex vivo (Bodó et al., 2005; Borbíró et al., 2011; Szabó et al., 2019). This encourages one to explore, next, the nature of the unknown endogenous intrafollicular signals and types of G-protein coupled receptors that activate TRPM5 channels (a few plausible candidates are discussed in Supplementary Discussion S1). Collectively, these data highlight TRPM5 as a previously unappreciated promising pharmacological and cosmeceutical target to modulate hair growth and treat hair disorders, such as various forms of alopecia or hirsutism.

Data availability statement

No data sets were used in this study.

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CONFLICT OF INTEREST

The study was mostly executed at Monasterium Laboratory GmbH, Germany. MvL and MA are or were full-time employees of Monasterium Laboratory GmbH. RP is the founder and CEO of Monasterium Laboratory GmbH. TB and AM provide consultancy services to Monasterium Laboratory GmbH. Together with several other findings, the data presented here form part of an extended set of claims in a recent European Patent application (EP20193431.2) submitted by Monasterium Laboratory, Germany.

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AUTHOR CONTRIBUTIONS

Conceptualization: AM, RP, TB; Data Curation: AM, MvL, MA, FJA; Formal Analysis: AM, TB, RP; Funding Acquisition: RP; Methodology: AM, RP, TB; Writing - Original Draft Preparation: AM, BT, RP; Writing - Review and Editing: AM, MvL, MA, FJA, TB, RP

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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.2021.02.747>.

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Real-World Application of a Noninvasive Two-Gene Expression Test for Melanoma Diagnosis



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TO THE EDITOR

A two-gene noninvasive molecular test (pigmented lesion assay [PLA], Derm-Tech, La Jolla, CA) for the diagnosis of melanoma is commercially available in the United States. The test measures

gene expression for *LINC00518* and *PRAME* from stratum corneum samples (Gerami et al., 2017). However, the 2017 validation study and 2018 registry study provided different estimates of the test's diagnostic accuracy (sensitivity of

91–95% and specificity of 53–91%) (Ferris et al., 2018; Gerami et al., 2017). The factors that contribute to different estimates of the PLA's diagnostic accuracy are not well characterized, which has raised concerns about its validity and potential for harm (Beatson and Weinstock, 2019). In this study, we used 2020 registry data (Brouha et al., 2020) to model the real-world application and utility of the PLA.

Abbreviation: PLA, pigmented lesion assay

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