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TRP Channel Regulates EGFR Signaling in Hair Morphogenesis and Skin Barrier Formation

Xiping Cheng,[1,8] Jie Jin,[2,3,8] Lily Hu,[1] Dongbiao Shen,[1] Xian-ping Dong,[1] Mohammad A. Samie,[1] Jayne Knoff,[1] Brian Eisinger,[1] Mei-ling Liu,[1] Susan M. Huang,[4] Michael J. Caterina,[4] Peter Dempsey,[5] Lowell Evan Michael,[6] Andrzej A. Dlugosz,[6] Nancy C. Andrews,[2,7] David E. Clapham,[3,*] and Haoxing Xu[1,3,*]

[1]The Department of Molecular, Cellular, and Developmental Biology, the University of Michigan, 3089 Natural Science Building (Kraus), 830 North University, Ann Arbor, MI 48109, USA

[2]Division of Hematology and Oncology, Children's Hospital Boston, Karp Family Building 8-125A, Boston, MA 02115, USA

[3]The Department of Cardiology, Children's Hospital Boston, Manton Center for Orphan Disease, Enders 1350, 320 Longwood Avenue, Boston, MA 02115, USA

[4]Department of Biological Chemistry and Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21025, USA

[5]The Department of Pediatrics and Communicable Diseases and Department of Molecular & Integrative Physiology, the University of Michigan, 1500 East Medical Center Drive, Room D3252, Ann Arbor, MI 48109, USA

[6]The Department of Dermatology and Comprehensive Cancer Center, the University of Michigan, 1500 East Medical Center Drive, Ann Arbor, MI 48109, USA

[7]Department of Pediatrics and Department of Pharmacology and Cancer Biology, Duke University School of Medicine, DUMC 2927, Durham, NC 27710, USA

[8]These authors contributed equally to this work

*Correspondence: dclapham@enders.tch.harvard.edu (D.E.C.), haoxingx@umich.edu (H.X.)

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SUMMARY

A plethora of growth factors regulate keratinocyte proliferation and differentiation that control hair morphogenesis and skin barrier formation. Wavy hair phenotypes in mice result from naturally occurring loss-of-function mutations in the genes for TGF-α and EGFR. Conversely, excessive activities of TGF-α/EGFR result in hairless phenotypes and skin cancers. Unexpectedly, we found that mice lacking the *Trpv3* gene also exhibit wavy hair coat and curly whiskers. Here we show that keratinocyte TRPV3, a member of the *t*ransient *r*eceptor *p*otential (TRP) family of Ca^{2+}-permeant channels, forms a signaling complex with TGF-α/EGFR. Activation of EGFR leads to increased TRPV3 channel activity, which in turn stimulates TGF-α release. TRPV3 is also required for the formation of the skin barrier by regulating the activities of transglutaminases, a family of Ca^{2+}-dependent crosslinking enzymes essential for keratinocyte cornification. Our results show that a TRP channel plays a role in regulating growth factor signaling by direct complex formation.

INTRODUCTION

Skin and its appendages provide a protective barrier essential for animal survival. Hair morphogenesis and epidermal development are orchestrated by an array of cytokines and growth factors (Fuchs and Raghavan, 2002). Signaling by these diffusible molecules provides spatially and temporally controlled cellular programs for keratinocyte proliferation, differentiation, migration, and finally, terminal differentiation and cornification. TGF-α and epidermal growth factor (EGF) are related autocrine/paracrine growth factors that activate the EGF receptor (EGFR; ErbB1) to regulate the balance between keratinocyte proliferation and differentiation (Schneider et al., 2008). Defective TGF-α/EGFR signaling leads to abnormal hair morphogenesis, manifested by the "wavy hair" and "curly whiskers" phenotypes of spontaneous loss-of-function mouse mutations in TGF-α (named *waved-1* or *wa1*) and in EGFR (named *waved-2* or *wa2*), respectively (Ballaro et al., 2005; Luetteke et al., 1993, 1994; Mann et al., 1993; Murillas et al., 1995; Schneider et al., 2008; Sibilia and Wagner, 1995; Threadgill et al., 1995). Excessive activities of TGF-α/EGFR cause a hairless phenotype and skin cancers (Ferby et al., 2006; Schneider et al., 2008). The mechanisms by which TGF-α/EGFR signaling determines cell fate (proliferation versus differentiation) of follicular and interfollicular (epidermal) keratinocytes are not completely understood.

Accumulated evidence suggests that both negative and positive feedback mechanisms coexist in the TGF-α/EGFR signaling axis. EGF binding triggers rapid degradation of the EGFR through endocytic pathways but also leads to further production and release/shedding of TGF-α/EGF (Coffey et al., 1987; Peschon et al., 1998). This unique autoinduction mechanism may contribute to the effects of TGF-α/EGF on keratinocyte terminal differentiation (Peus et al., 1997; Sakai et al., 1994;

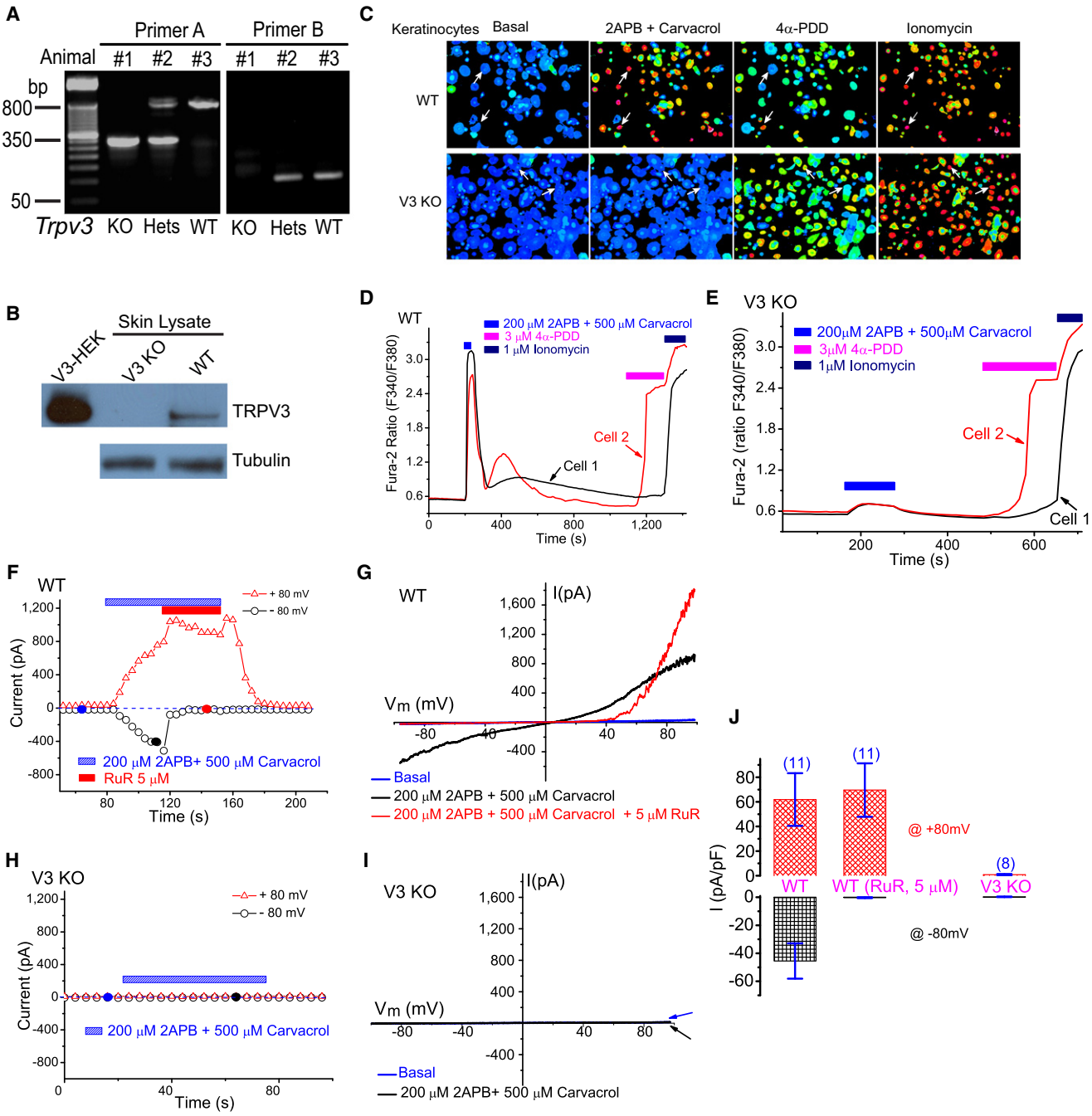

Figure 1. Targeted Deletion of Mouse *Trpv3* Abolishes the Response of Keratinocytes to TRPV3 Activators

(A) PCR genotyping of wild-type (WT), V3 KO, and heterozygous (Hets) mice. Two sets of primers were used as described in Experimental Procedures. PCR products for primer set A: WT 800 bp, KO 300 bp. For primer set B: WT 130 bp, KO no product.

(B) Lack of TRPV3 protein expression in the skin of V3 KO mice. TRPV3 was immunoprecipitated and immunoblotted using a TRPV3-specific monoclonal antibody. Cell lysates from HEK293T cells expressing recombinant mouse TRPV3 (V3-HEK) were used as positive controls. γ-Tubulin served as a loading control for skin lysates.

(C–E) Lack of agonist-induced V3-like Ca^{2+} response in V3 KO primary keratinocytes. (C) TRPV3 agonist cocktail (200 μM 2-APB + 500 μM Carvacrol) induced large increases of $[Ca^{2+}]_i$ in primary cultured keratinocytes isolated from WT ($V3^{+/+}$) but not V3 KO ($V3^{-/-}$) mice. Whereas more than 80% of WT keratinocytes responded strongly to the V3 agonist cocktail, negligible responses were observed for V3 KO keratinocytes. Positive controls: ~60%–80% keratinocytes from both genotypes (WT and V3 KO) responded to 4α-PDD (3 μM; agonist of TRPV4). All cells responded to ionomycin (1 μM). (D) Ca^{2+} responses of two representative WT cells from (C) (arrows; upper panels). One cell responded to both TRPV3 and TRPV4 agonists whereas the other one only responded to the V3 agonist cocktail. (E) Ca^{2+} responses of two representative V3 KO cells from (C) (arrows; lower panels). One cell responded to the TRPV4 agonist (cell 2); neither cell responded significantly (<0.1 fura-2 ratio) to the V3 agonist cocktail.