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Letter to the Editor

Epidermal overexpression of LRIG1 disturbs development and homeostasis in skin by disrupting the ERBB system

Leucin-rich repeats and immunoglobulin-like domains 1 (LRIG1), one of three members of a transmembrane protein family (LRIG1-3), has attracted attention due to its impact on receptor tyrosine kinases (RTKs) such as the epidermal growth factor receptor (EGFR/ERBB1/HER1) and the other members of the ERBB-receptor family (ERBB2-4/HER2-4) [1]. In human skin, LRIG1 is expressed in the interfollicular epidermis (IFE) and hair follicles (HF) [2], and promotes stem cell (SC) quiescence [3]. A disturbed balance of proliferation and differentiation of HF-SCs can be observed in various skin diseases, including psoriasis and alopecia. Accordingly, LRIG1-deficient mice developed a psoriatic, hyperplastic phenotype, indicating an important function of LRIG1 in the skin [4]. LRIGs are involved in feedback-loop regulation of RTKs in different ways [1]. Since ERBBs inhibit terminal differentiation and promote proliferation of epidermal progenitor cells (PCs) [5], LRIG1 may be an important feedback-regulator of ERBBs in the epidermis, but this has not been shown yet. We addressed this hypothesis by overexpressing LRIG1 skin-specifically in mice using the Tet-Off-system with the keratin 5 (KRT5) promoter. Double transgenic mice (LRIG1-TG) showed open eyes and short, thick whiskers and died within one week after birth (Figs. 1a, S1a). The outside-in barrier was not affected in LRIG1-TG mice, and we found no alteration in bodyweight (Fig. S1b,d). Morphometric studies revealed no differences in epidermal thickness or proliferation of back skin of newborn LRIG1-TG mice and controls (Fig. S1c,e,f). However, HF morphogenesis was severely impaired and HF density was significantly reduced in LRIG1-TG mice (Fig. 1b–d). LRIG1-TG mice showed increased expression of keratin 10 (KRT10), but no obvious differences regarding KRT5 or loricrin (LOR), and ectopic expression of keratin 6 (KRT6) in the IFE (Fig. 1e). These findings point to a delayed HF morphogenesis and a disturbed differentiation of the skin due to LRIG1 overexpression, probably explaining the postnatal lethality. To determine whether LRIG1 affects ERBB-signaling in skin, receptor expression was analyzed by immunofluorescence and Western blots. EGFR and ERBB3 were expressed throughout the IFE, whereas ERBB2 was present in more differentiated epidermal layers in TG and control animals (Fig. S2a). Moreover, ERBB2 phosphorylation and ERBB3 expression were decreased upon LRIG1 overexpression, but the EGFR level was unchanged (Fig. S2b,c). Analysis of ERBB downstream targets revealed a decreased activation of RAC-alpha serine/threonine-protein kinase (AKT) but no changes in mitogen-activated protein kinase 1/2 (MAPK1/2) (Fig. S2b,c). The expression of NOTCH1-receptor, investigated due to its role in embryonic development, was also unchanged (Fig. S1f).

To evaluate the impact of LRIG1 in skin homeostasis, we suppressed LRIG1 overexpression until birth by the application of doxycycline to pregnant mice and thereby obtained skin-specifically LRIG1 overexpressing mice (Fig. S1g,h). LRIG1-TG mice started to lose their hair coat at the age of ten weeks and developed alopecia. After six months, TG animals showed reduced bodyweight and developed hyperkeratosis, malformed and less dense HFs, hyperplasia of sebaceous glands (SGs) and IFE with increased proliferation (Fig. 2a,c–e). Regarding epidermal differentiation, LRIG1-TG mice showed an increase in KRT10 and LOR expression (Fig. 2e). Furthermore, ectopic KRT6 expression occurred in the IFE of TG mice (as in newborns), indicating disturbed balance between differentiation and proliferation during epidermal homeostasis (Fig. 2e). These data demonstrate a crucial impact of LRIG1 overexpression on skin homeostasis. EGFR, ERBB2 and phosphorylated EGFR were significantly reduced in LRIG1-TG mice, resulting in decreased EGFR activation (Fig. 2f,h). The decrease of MAPK1/2 and AKT expression and their phosphorylated forms are in accordance with the downregulation of ERBB-signaling, although no differences in MAPK1/2 or AKT activation were detected (Fig. 2f,g). Evaluation of the effect of LRIG1 overexpression on ERBB-signaling in adult mice revealed a significant downregulation of the EGFR/ERBB2 pathway.

LRIG1-TG mice also showed severe abnormalities in the HF-cycle. While the first catagen-stage at P18 seemed to be unaffected, LRIG1-TG mice showed accelerated transition to anagen at P21 (Fig. S3). The HF-cycle analysis revealed a severe impairment of telogen-anagen-transition in LRIG1-TG mice compared to controls. LRIG1 is known to promote SC quiescence [3], and our data show that its overexpression modulates the transcriptome of HF-SCs. Loss or dysregulation of SCs has been associated with different types of alopecia, including cicatricial alopecia (lichen planopilaris or discoid lupus erythematosus) and non-cicatricial alopecia (as alopecia areata). More recently, taxan-induced effluvium, an acute and diffuse hair loss in humans receiving chemotherapy to treat cancer, was shown to involve damage of HF-SCs and PCs [6]. To our knowledge, there are no reports associating LRIG1 with hair loss in human patients. Nevertheless, LRIG1 overexpression may become a useful model for further studies assessing the involvement of HF-SCs in hair loss.

To address the question whether LRIG1 also has PC function outside its niche we analyzed skin samples of LRIG1-TG and control

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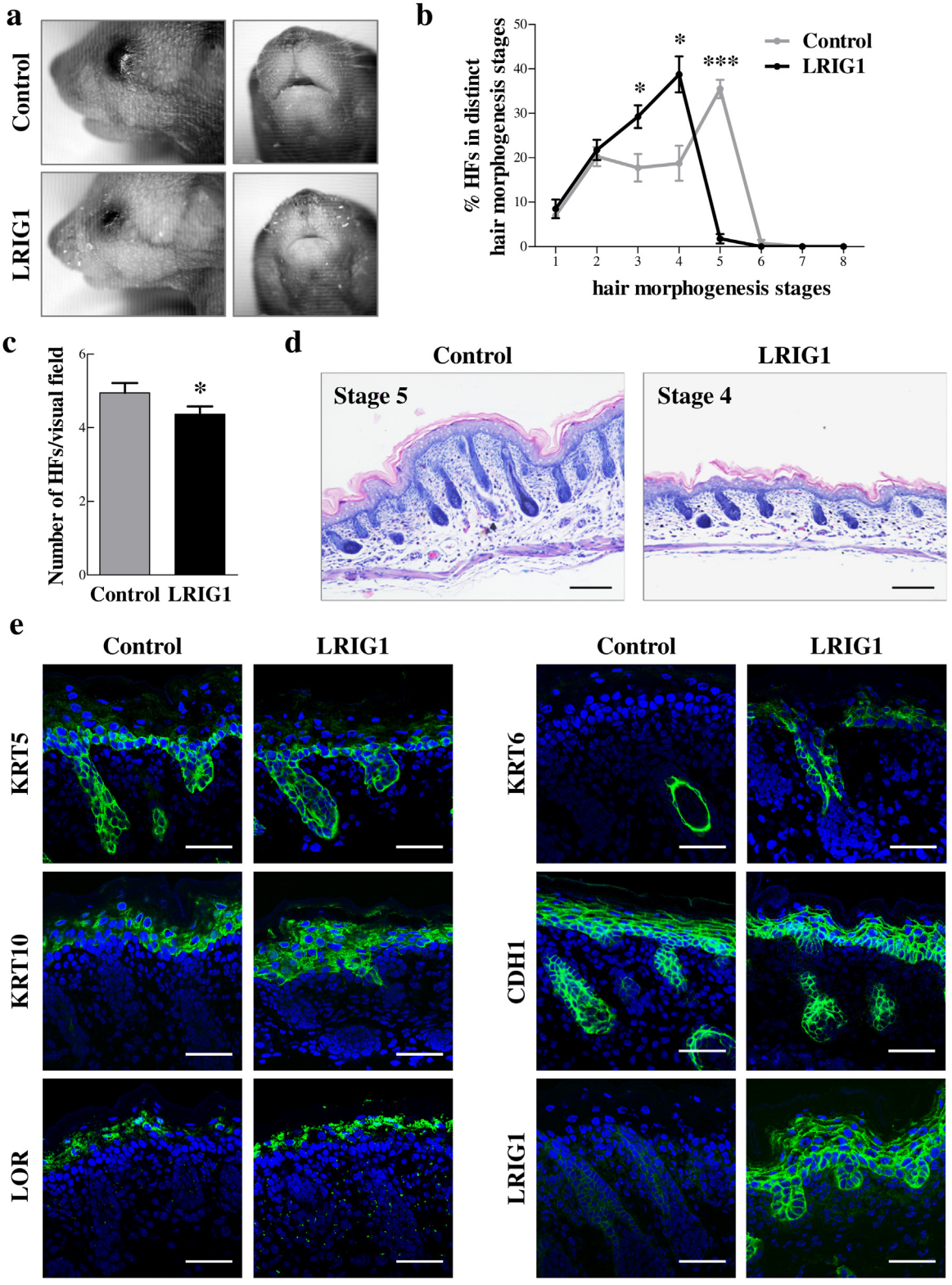

Fig. 1. LRIG1 overexpression was neonatal lethal and resulted in impaired HF morphogenesis and epidermal differentiation. (a) Representative pictures of an eye and whiskers of a newborn LRIG1-TG mouse and a control. (b) Percentage of HFs found in the different hair morphogenesis stages at P0. Control HFs were mainly in morphogenesis stage 5, TG HFs were still in stage 4 at P0. (c) HF density in LRIG1-TG and control mice. (d) Giemsa staining of back skin of LRIG1-TG and control mice at P0 in indicated morphogenesis stages. Scale bars represent 100 μm. (e) Immunofluorescence stainings against keratin 5, 10 and 6 (KRT5, KRT10, KRT6), loricrin (LOR), E-cadherin (CDH1), and LRIG1 (green) on back skin of LRIG1-TG and control mice at P0. Scale bars represent 50 μm. Data in (b) and (c) are means ± SEM and were analyzed by Mann-Whitney *U*-test (n = 4). $^{*}P < 0.05$, $^{***}P < 0.001$.

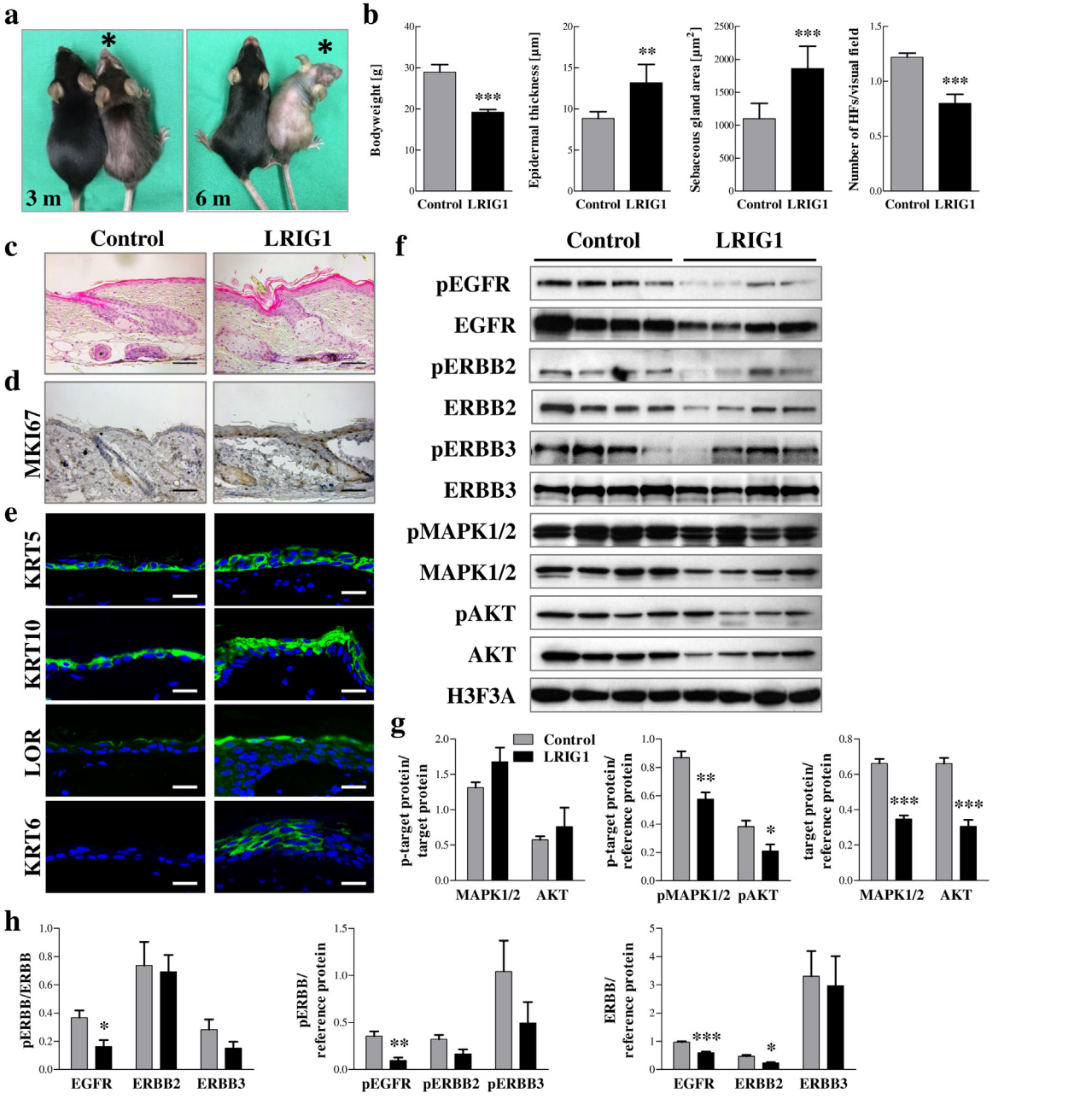

Fig. 2. Postnatal induced LRIG1 overexpression caused alopecia and hyperplasia. (a) Pictures of a three- and six-month-old LRIG1-TG mouse (*) showing alopecia and a control mouse. (c) H&E staining of back skin of six-month-old LRIG1-TG and control mice. (b) Body weight, epidermal thickness, and area of sebaceous glands (SGs) of six-month-old LRIG1-TG mice and controls. HF density in two-month-old LRIG1-TG and control mice. (c) H&E staining of back skin of six-month-old LRIG1-TG and control mice. (d) MKI67-immunohistochemistry on back skin of a six-months-old LRIG1-TG and control mouse. (e) Immunofluorescence on back skin of a LRIG1-TG and control mouse for indicated differentiation markers (green). Cell nuclei are stained with DAPI (blue). (f–h) Western blot and densitometrical analysis of phosphorylated and total ERBB-receptors (ERBB1-3) and their downstream targets MAPK1/2 and AKT in skin samples of six-month-old LRIG1-TG and control mice. H3F3A was used as reference protein. Data are means + SEM and were analyzed by Student's *t*-test or Mann-Whitney *U*-test (n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Scale bars: (c,d) 50 µm, (e) 20 µm.

animals at P60. First, the ERBB-system, important for terminal differentiation and proliferation of PCs in the skin [5], and NOTCH1-signaling, crucial in cell fate decision [7], were studied by Western blot analysis. Contrary to the results of six-month-old animals, the expression of EGFR and ERBB3 was increased but their activation was significantly decreased at P60 (Fig. S4a,b). Furthermore, activation of ERBB2 and the expression of its phosphorylated form were highly increased in LRIG1-TG mice. Consistent with EGFR/ERBB3 downregulation, MAPK1/2 expression and activation was decreased (Fig. S4a,b). Investigations of NOTCH1-signaling revealed increase of cleaved NOTCH1-receptor and activation of its downstream targets like recombining binding protein suppressor of hairless (RBPJ), mastermind-like protein 1 (MAML1), Myc proto-oncogene protein (MYC) and transcription factor HES-1 (HES1) (Fig. S4c,d). Disintegrin and metalloproteinase domain-containing protein 17 (ADAM17), involved in the proteolytic cleavage and activation of NOTCH1 [8], and cyclin-D3 (CCND3), an important regulator of the cell-cycle during G_1/S transition [9], were not affected (Fig. S4c,d). Additionally, proliferation was highly enhanced in LRIG1-TG animals resulting in epidermal hyperplasia (Fig. S4c,d). Further, we used qPCR to investigate epidermal SC marker transcripts in the back skin of LRIG1-TG and control mice at P60 (Fig. S4e). Our data argue for an

increase of SC markers of the bulge and SGs. *Blimp1*, defining a progenitor population in SGs [10], like *Plet1/MTS24* was increased in LRIG1-TG mice compared to control littermates. Transcripts of the bulge SC markers [10] *Cd34* and *Lhx2* were also increased while the transcripts *Lgr6*, *Nfatc1* and *Lgr5* were unchanged (Fig. S4e). In summary, analysis of the early onset of alopecia in LRIG1-TG mice and controls indicated an influence of LRIG1 on ERBB and NOTCH1-signaling, both involved in PC regulation. In addition, LRIG1 overexpression led to an increase of SG and bulge SC markers pointing to an activation of both SC pools.

In conclusion, our gain-of-function model revealed a remarkable impact of LRIG1 on epidermal development and homeostasis, disrupting ERBB-signaling and affecting epidermal SCs. Prenatal overexpression of LRIG1 led to neonatal lethality, probably due to a disturbed epidermal barrier. During adult skin homeostasis, LRIG1 overexpression decreased EGFR activation and caused alopecia with epidermal hyperplasia and altered the HF-cycle. Due to these intriguing findings the LRIG1-TG mouse line might represent a promising model for hair loss and alopecia research.

Declaration of Competing Interest

The authors have declared that no conflict of interest exists.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.11.007.

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