Title

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Journal

Journal of Investigative Dermatology, 137(8)

ISSN

0022-202X

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Publication Date

2017-08-01

DOI

10.1016/j.jid.2017.02.985

Overexpression of Transcription Factor Ovol2 in Epidermal Progenitor Cells Results in Skin Blistering

Journal of Investigative Dermatology (2017) **137,** 1805–1808; doi:10.1016/j.jid.2017.02.985

TO THE EDITOR

Functional importance in epidermal development and homeostasis has been shown for numerous transcription factors, yet little is known about their possible involvement in blistering skin diseases. Ovol1 and Ovol2 transcription factors play important roles in epidermal morphogenesis: *Ovol1* ablation expands the suprabasal spinous layers, whereas simultaneous loss of Ovol1 and Ovol2 results in defective cell adhesion and terminal differentiation (Lee et al., 2014; Nair et al., 2006). In these previous studies, we generated *tetracycline responsive element-Ovol2/K5-tTA* bitransgenic (BT) mice, which robustly overexpress Ovol2 in the developing basal layer and produce a thinner embryonic epidermis (Lee et al., 2014). However, how the accumulated developmental defects manifest at birth was not characterized. In the experiments (approved by UC Irvine) below, we obtained evidence that Ovol2 overexpression during embryogenesis causes skin blistering at birth.

We found BT mice to be born at Mendelian ratio, but they are smaller than control littermates, display a translucent skin with severe blistering (Figure 1a, 1b), and die shortly after birth. In contrast, induction of Ovol2 overexpression after birth resulted in no apparent blistering in adult mice (Supplementary Figure S1 online). Although slightly permeable to dye penetration ventrally and at the blistering sites, BT newborns formed an overall functional epidermal permeability barrier (Figure 1c), indicating that they are able to execute a terminal differentiation program. Double staining of basal keratin 14 (K14) and basement membrane protein laminin 332 (laminin 5) suggested basal cell cytolysis (Figure 1d), which was also observed in mice deficient in *Krt5* (K5) or *Krt14* (K14) (Lloyd et al., 1995; Peters et al., 2001). Terminal deoxynucleotidyl transferase dUTP nick end labeling assay revealed the presence of apoptotic cells at blistering sites; however, apoptosis was not remarkable when blistering was not evident (Figure 1e).

To probe the underlying basis of blistering, we performed electron microscopy analysis on E18.5 control and BT epidermis. Morphologically intact BT basal cells contained fewer keratin filament bundles when compared with wild-type counterparts, and their residual keratin filaments were short and disorganized (Figure 1f, 1g). Hemidesmosomes, an adhesion structure that anchors keratin filaments to cell/basement membrane (Figure 1h, 1i), appeared normal in some BT basal cells, but lacked the characteristic inner plate in many others (Figure 1j and data not shown). These abnormal hemidesmosomes also lacked attaching keratin filaments, but instead were adjacent to keratin ring structures previously detected in epidermolysis bullosa simplex keratinocytes (Russell et al., 2004). Thus, Ovol2 overexpression in epidermal basal cells disrupts the basal keratin network and its association with hemidesmosomes.

To gain molecular insights, we compared gene expression in BT and control skin using microarray data derived from E16.5 and E17.5 embryos (Lee et al., 2014). The expression of *Krt5*, *Krt14*, and *Krt15* (K15) was reduced, whereas that of *Krt8* and *Krt18* (encoding simple epithelium K8 and K18) was elevated, in BT embryonic skin (Figure 2a, 2b). The expression of genes encoding K6 and K16, keratins involved in wound healing or hyperproliferative disorders (Ramirez et al., 1998), was also elevated (Figure 2a). In contrast, the expression of genes encoding hemidesmosome-associated proteins (*Dst, Plec1, Col17a1, Itga6,* and *Itgb4*) was largely unaffected (data not shown), except for a slight reduction in *Dst* at E16.5 (1.4-fold; $P = 0.15$). Western blot analysis revealed a significant reduction in K15, and to a lesser extent, K14 proteins (Figure 2c). Immunostaining revealed decreased K5 (Figure 2d) but ectopic K8 (Figure 2e) expression in BT basal cells, and ectopic K6 expression in BT suprabasal layers (Figure 2f). Together, these results show that in the presence of excess Ovol2, embryonic epidermal progenitor cells cannot maintain proper basal keratin expression. The upregulation of simple-epithelia- or repair-associated keratins in BT epidermis may reflect compensatory responses to reduced basal keratin levels.

To ask whether altered keratin expression is a cell-autonomous and self-sufficient effect of Ovol2, we performed RNA-seq analysis to probe Ovol2-induced changes in a heterologous system, MCF10A mammary epithelial cells that normally express K5/K14 (Hong et al., 2015). Ovol2 overexpression resulted in a significant reduction of *KRT5/KRT14* and an increase of *KRT6/KRT16* expression (Figure 2g), recapitulating the changes detected in BT epidermis. This said, MCF10A cells did not upregulate *KRT8/KRT18* or downregulate *KRT15* expression as seen in BT epidermis.

Altered keratin expression in *Ovol2* BT mice is reminiscent of epidermis lacking AP-2α/γ or p63, transcriptional activators of basal keratin expression (Guttormsen et al., 2008; Koster et al., 2006; Wang et al., 2008). We had previously implicated *p63* as a direct target of Ovol2 repression (Lee et al., 2014). Because p63 promotes *AP-2γ*

Abbreviations: BT, bitransgenic; K, keratin

Accepted manuscript published online 27 April 2017; corrected proof published online 27 June 2017

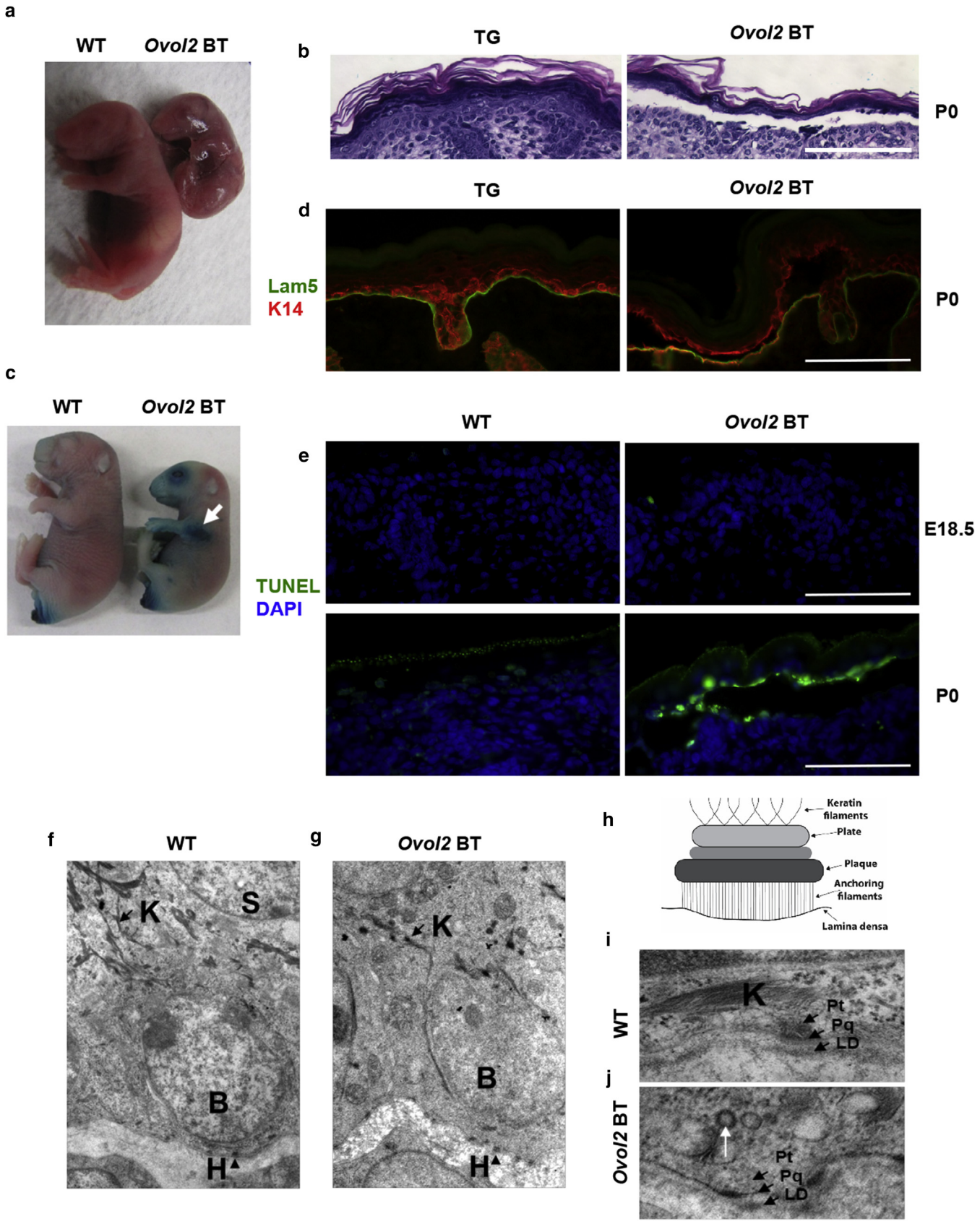
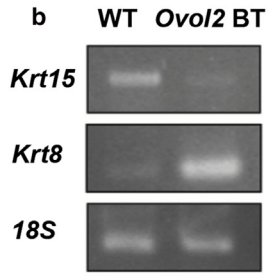
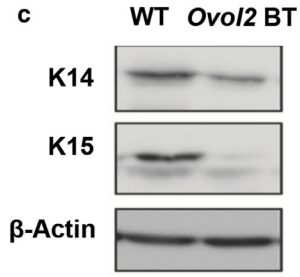


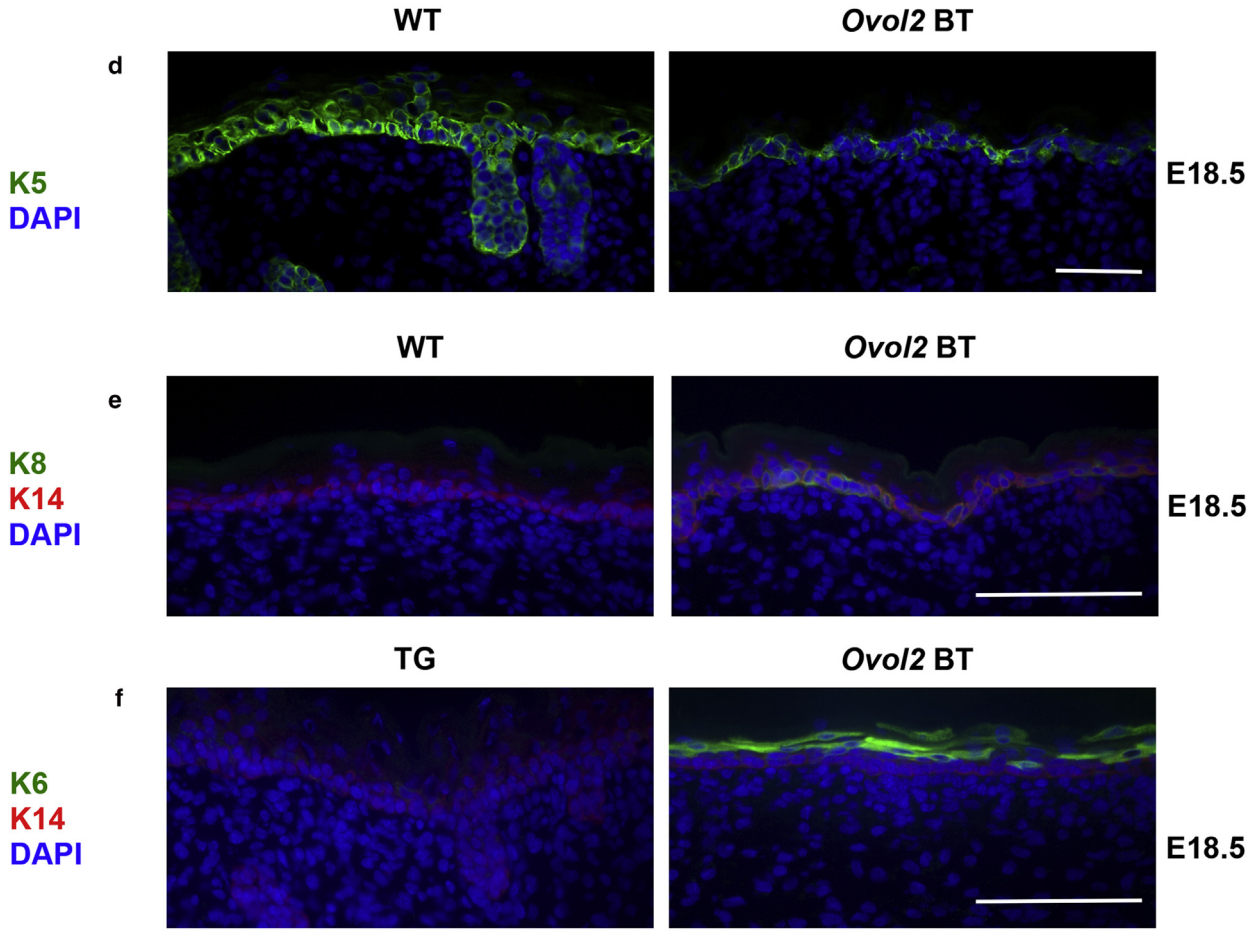
Figure 1. Skin blistering and basal cytolysis in *Ovol2* BT mice. (**a**) *Ovol2* BT newborns display shiny skin with blisters. (**b**) Histologic analysis of newborn skin. (**c**) Barrier assay of newborns. Arrow indicates dye uptake at a blistered site. (**d**) Cytolysis of the basal cells. (**e**) TUNEL-positive cells are present in newborn BT epidermis where blistering occurs. EM images of (**f, i**) WT and (**g, j**) BT epidermal cells. White arrow indicates the keratin ring. (**h**) Diagram of normal hemidesmosomal structure. Scale bar = 50 μm. B, basal cell; BT, bitransgenic; EM, electron microscopy; H, hemidesmosome; K, keratin filaments; LD, lamina densa; Pq, plaque; Pt, inner plate; S, spinous cell; TG, single transgenic control; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; WT, wild-type.

a

Gene Name	E16.5 Fold Change	E17.5 Fold Change
Krt5	-1.83	-2.15
Krt6A	3.44	4.00
Krt6B	20.71	13.57
Krt8	5.09	4.73
Krt14	-1.58	-2.36
Krt15	-2.67	-4.94
Krt16	10.95	3.38
Krt18	2.33	-1.15

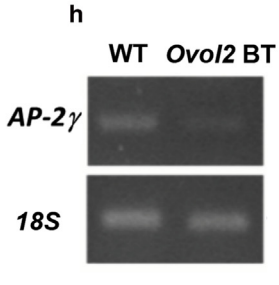






g

Gene Name	Fold Change
KRT5	-13.64
KRT6A	-1.12
KRT6B	12.30
KRT6C	10.90
KRT8	-1.24
KRT14	-3.34
KRT15	4.50
KRT16	23.34
KRT18	-1.10



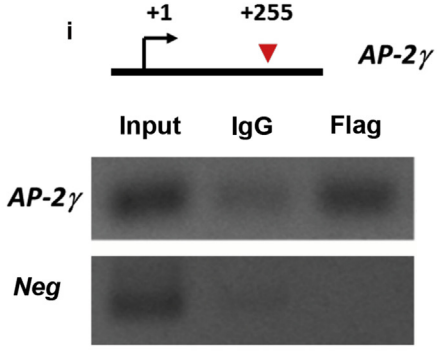


Figure 2. Ovol2 overexpression causes reduced basal keratin expression. (**a**) Microarray and (**b**) RT-PCR analysis revealing altered keratin mRNA levels in embryonic BT skin. $P < 0.002$ in (**a**). (**c**–**f**) Protein expression analysis of E18.5 skin using the indicated antibodies. DAPI stains the nuclei. (**g**) Keratin gene expression in MCF10A cells. (**h**) Semiquantitative RT-PCR revealing the decreased *AP-2γ* mRNA level in BT skin. (**i**) Ovol2 binds to the *AP-2γ* promoter in mouse skin. Scale bar = 50 μm. BT, bitransgenic; Neg, negative control; RT-PCR, reverse transcriptase-PCR; TG, single transgenic control; WT, wild-type.

expression, we wondered whether the level of *AP-2γ* transcripts was decreased in BT skin. This was indeed the case (Figure 2h). Importantly, sequence analysis identified an Ovol2-binding consensus 255 bps downstream of the *AP-2γ* transcription start site (Figure 2i, top). In chromatin immunoprecipitation assay using BT newborn skin, Ovol2 was found to occupy the *AP-2γ* promoter at this predicted site but not a control region (Figure 2i, bottom). This result suggests that *AP-2γ* is also a direct target of Ovol2.

Mutations in *KRT5* and *KRT14* cause fragility of epidermal basal keratinocytes, resulting in epidermolysis bullosa simplex (Coulombe et al., 2009). To our knowledge, this study is the first to show transcription factor overexpression causing epidermolysis bullosa simplex-like skin blistering in a mouse model. Ovol2 regulation of the *p63-AP-2γ*-basal keratin axis provides a possible underlying mechanism. $p63^{-/-}$ mice show compromised epidermal integrity, but this has been attributed to altered cell adhesion (Ihrie et al., 2005; Koster et al., 2007). Skin blistering has not been reported for *AP-2α/AP-2γ* mutant mice (Guttormsen et al., 2008; Wang et al., 2008). The phenotypes of *Ovol2* BT newborns are broader, and occur earlier, than in *Krt5* and *Krt14* null mice (Lloyd et al., 1995; Peters et al., 2001). The more severe consequences of Ovol2 overexpression are likely due to (i) suppression of both *p63* and *AP-2γ* expression; (ii) insufficient compensation from surrogate keratins because of simultaneous reduction of K5, K14, and K15; (iii) additional roles of Ovol2, such as regulating epithelial plasticity and differentiation (Lee et al., 2014).

CONFLICT OF INTEREST

The authors state no conflicts of interest.

ACKNOWLEDGMENTS

This work is dedicated to Dr Qian-Chun Yu, an electron microscopy expert and dear friend who recently passed away. Dr Yu provided invaluable assistance in our electron microscopy analysis. We thank the UCI Genomics High Throughput Facility and Transgenic Mouse Facility for expert service, Julie Segre and Srikala Raghavan for antibodies, and Adam Glick and Satrajit Sinha for *K5-tTA* mice. This work was supported by NIH Grants R01-AR47320, R01-AR068074, and R56-AR064532 (to XD). Briana was supported by the NIH Systems Biology of Development (T32 HD060555-01) and NIH Translational Research in Cancer Genomic Medicine (T32 CA113265) training grants.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2017.02.985.

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Enhanced Proteolytic Activities in Acral Peeling Skin Syndrome: A Role of Transglutaminase 5 in Epidermal Homeostasis

Journal of Investigative Dermatology (2017) **137,** 1808–1811; doi:10.1016/j.jid.2017.03.026

Abbreviations: APSS, acral peeling skin syndrome; KLK, kallikrein-related peptidase; TG5, transglutaminase 5

Accepted manuscript published online 7 April 2017; corrected proof published online 16 June 2017

TO THE EDITOR

Renewal of the stratum corneum is ensured by a finely tuned network of proteases and their endogenous