Skin Fragility and Impaired Desmosomal Adhesion in Mice Lacking All Keratins

Janina Bär[1], Vinod Kumar[1], Wera Roth[2], Nicole Schwarz[3], Miriam Richter[1], Rudolf E. Leube[3] and Thomas M. Magin[1]

Keratins perform major structural and regulatory functions in epithelia. Owing to redundancy, their respective contribution to epidermal integrity, adhesion, and cell junction formation has not been addressed in full. Unexpectedly, the constitutive deletion of type II keratins in mice was embryonic lethal ~E9.5 without extensive tissue damage. This prompted us to analyze keratin functions in skin where keratins are best characterized. Here, we compare the mosaic and complete deletion of all type II keratins in mouse skin, with distinct consequences on epidermal integrity, adhesion, and organismal survival. Mosaic knockout (KO) mice survived ~12 days while global KO mice died perinatally because of extensive epidermal damage. Coinciding with absence of keratins, epidermal fragility, inflammation, increased epidermal thickness, and increased proliferation were noted in both strains of mice, accompanied by significantly smaller desmosomes. Decreased desmosome size was due to accumulation of desmosomal proteins in the cytoplasm, causing intercellular adhesion defects resulting in intercellular splits. Mixing different ratios of wild-type and KO keratinocytes revealed that ~60% of keratin-expressing cells were sufficient to maintain epithelial sheets under stress. Our data reveal a major contribution of keratins to the maintenance of desmosomal adhesion and epidermal integrity with relevance for the treatment of epidermolysis bullosa simplex and other keratinopathies.

Journal of Investigative Dermatology (2014) **134,** 1012–1022; doi:10.1038/jid.2013.416; published online 14 November 2013

INTRODUCTION

The mammalian epidermis is a stratified epithelium that protects the body against mechanical injury, dehydration, and infections. The family of keratin proteins that forms the major cytoskeleton of all epithelia is believed to contribute largely to these specialized keratinocyte functions by forming protein interactions in a context-dependent manner, in particular to desmosomal, hemidesmosomal, and cornified envelope proteins (Jones and Green, 1991; Kouklis *et al.*, 1994; Candi *et al.*, 1998) Keratin genes are located in two clusters on mouse chromosome 11 (type I) and 15 (type II) and are coordinately transcribed to allow formation of keratin intermediate filaments (KIFs) from heterodimers of a type I and a type II protein (Hesse *et al.*, 2004; Rogers *et al.*, 2004; Schweizer *et al.*, 2006; Kurokawa *et al.*, 2011). Keratin expression is tightly linked to specialized epidermal functions; whereas proliferating basal cells express K5, K14, and K15 (Nelson and Sun, 1983; Lloyd *et al.*, 1995; Porter *et al.*, 2000), the switch to terminal differentiation is accompanied by K1, K2e, and K10 expression in suprabasal cells, whereas disruption of epidermal homeostasis and tissue repair result in transient expression of K6, K16 and K17 (Fuchs and Green, 1980; Byrne *et al.*, 1994; Freedberg *et al.*, 2001). The notion that KIFs confer mechanical stability to epithelia upon physical trauma (Coulombe *et al.*, 1991) is substantiated by several keratin knockout (KO) mouse models and human keratinopathies including epidermolysis bullosa simplex (EBS) and epidermolytic hyperkeratosis resulting from missense mutations in keratin genes *KRT5, KRT14, KRT1,* and *KRT10,* respectively (Bonifas *et al.*, 1991; Coulombe *et al.*, 1991; Fuchs *et al.*, 1992; Lane *et al.*, 1992; Rothnagel *et al.*, 1992; Lloyd *et al.*, 1995; Peters *et al.*, 2001; Reichelt and Magin, 2002; Jack Fu *et al.*, 2013). EBS is a hereditable skin blistering disorder in which ruptures occur in the subnuclear cytoplasm of basal cells (Haneke and Anton-Lamprecht, 1982) causing fragility of the basal cell compartment upon mechanical trauma. Most cases of EBS are due to dominantly acting mutations in genes encoding K5 or K14 (Bonifas *et al.*, 1991; Coulombe *et al.*, 1991; Lane *et al.*, 1992) and differ in the severity of the phenotype, depending on the site of mutation. Homozygous recessive cases of EBS have also been reported where a premature termination codon mutation in KRT14

[1]*Translational Center for Regenerative Medicine and Institute of Biology, University of Leipzig, Leipzig, Germany;* [2]*German Center for Neurodegenerative Diseases, Bonn, Germany and* [3]*Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Aachen, Germany*

Correspondence: Thomas M. Magin, Translational Centre for Regenerative Medicine (TRM) and Institute of Biology, Cell and Developmental Biology, University of Leipzig, Philipp-Rosenthal-Straße 55, Leipzig D-04103, Germany. E-mail: thomas.magin@trm.uni-leipzig.de

Abbreviations: DP, desmoplakin; EBS, epidermolysis bullosa simplex; HD, hemidesmosome; KIF, keratin intermediate filament; KO, knockout; KtyII, keratin type II gene cluster; PM, plasma membrane; WT, wild-type

Received 20 June 2013; revised 27 August 2013; accepted 12 September 2013; accepted article preview online 11 October 2013; published online 14 November 2013

gene resulted in the lack of K14 (Rugg *et al.*, 1994). In the latter case, the extent of blistering was comparable to the severity of dominant-negative mutations (McLean and Moore, 2011) suggesting that the degree of cytoskeletal damage positively correlated to the extent of skin integrity. Similarly, mutations in the suprabasal keratins K1 and K10 cause widespread blistering and erosions due to continuous lysis of suprabasal keratinocytes accompanied by hyperkeratosis in humans (Rothnagel *et al.*, 1992) and in corresponding mouse models (Arin and Roop, 2001). In contrast, constitutive deletion of K1 or K10 had only moderate effect on skin integrity, because of compensatory suprabasal K5/K14 expression in the latter model (Reichelt and Magin, 2002; Roth *et al.*, 2012).

Altogether these findings raised the question of how much keratin IF are needed to maintain epithelial stability. Previously, we reported that the deletion of all keratins in cultured keratinocytes led to fragility of epithelial sheets when exposed to mechanical stress but re-expression of K5/K14 reaching ~13% of wild-type (WT) levels rescued sheet integrity (Kroger *et al.*, 2013). We had shown that improved intercellular adhesion was mediated by a keratin-dependent stabilization of desmosomes. However, owing to keratin redundancy, the respective contribution of keratins to desmosome formation and maintenance was difficult to analyze in single keratin KO mice (Hesse *et al.*, 2000; Roth *et al.*, 2012). Combined deletion of K1 and K10 in mice was accompanied by formation of smaller desmosomes (Wallace *et al.*, 2012). To further dissect the role of keratins in intercellular adhesion formation, skin integrity and stress resistance, we here present a comparative analysis of two strains of mice in which the entire keratin protein family was deleted either completely or in a mosaic pattern, using Cre-mediated genome engineering. We show that complete absence of keratins permits epidermal morphogenesis and stratification but strongly impairs desmosomal adhesion and causes cytolysis in basal and suprabasal layers combining major phenotypes of EBS and epidermolytic hyperkeratosis in one mouse model. This results in perinatal lethality. In contrast, mice with mosaic patches of keratin-free and keratin-expressing epidermis survive ~12 days, defining the respective contribution of keratins to skin integrity.

RESULTS

Mosaic and total deletion of type II keratin genes in skin cause distinct epidermal fragility

We have previously shown that ubiquitous deletion of the keratin type II gene cluster (KtyII) (Supplementary Figure S1a online) in mice resulted in loss of the entire keratin protein family. Resulting embryos died before onset of epidermal development ~E9.5 due to severe growth defects and, surprisingly, embryonic epithelia remained intact without keratins (Vijayaraj *et al.*, 2009; Kroger *et al.*, 2011). To investigate keratin function during epidermal morphogenesis, we devised two different KO strategies allowing formation of KIFs in simple epithelia but not in skin (Figure 1a, Supplementary Figure S1a and b online). In the first mouse model, epidermal-specific deletion of type II keratin genes was performed, using a K14-Cre variant that deletes in a mosaic pattern (Huelsken *et al.*, 2001), resulting in patches of normal and keratin-deficient epidermis. Thus, WT and keratin-depleted skin become comparable in the same individual. The second mouse model aimed to analyze the consequences of total keratin deficiency in skin. To overcome embryo mortality, a genetic rescue experiment was performed mating KtyII$^{-/-}$ to transgenic mice expressing murine K8 controlled by its own promoter. The skin of K8 transgenic mice appeared normal without overt phenotype. Mating these mice to KtyII$^{-/-}$ mice enabled KIF formation between transgenic K8 and endogenous simple epithelial keratins 19 and 20 encoded by the KtyI gene locus (Zhou *et al.*, 2003; Hesse *et al.*, 2004; Moll *et al.*, 2008). As K8 expression is confined to simple and glandular epithelia (Wu *et al.*, 1982; Byrne *et al.*, 1994; Moll *et al.*, 2008), these KIFs maintain morphogenesis of internal epithelia and rescue embryo development until birth (Kumar *et al.*, in preparation). In contrast, K8 is not expressed in epidermal keratinocytes that allows analysis of epidermal development in a keratin-free background. K14-Cre KO mice are referred to as KtyII$_m^{-/-}$ (mosaic KO) and were born alive at approximate Mendelian ratio, showing no overt phenotypic abnormalities at birth (Supplementary Figure S2a online). From postnatal day 5 onward, they developed a hyperkeratotic, scaly, unelastic skin (Figure 1b) with skin lesions restricted to limb and neck folds, regions exposed to natural mechanical strain. The skin phenotype, accompanied by weight loss (Figure 1c) worsened and the mice died at ~8–12 days of age (Supplementary Figure S2b online). Next, histology and immunofluorescence of dorsal skin of 8-day-old mutant and control mice were performed. The former revealed acanthotic and hyperkeratotic epidermis patches (Figure 1f) along with neighboring unaffected areas (Figure 1g) that were similar to WT skin (Figure 1e). Affected skin patches showed enlarged intercellular spaces, cytolysis at the dermo-epidermal interface, and throughout all epidermal layers (Figure 1f and h). Unlike K1/K10 double-deficient mice where nuclei were lost prematurely (Wallace *et al.*, 2012), loss of all keratins caused accumulation of parakeratotic keratinocytes in the stratum corneum of hyperkeratotic skin patches (Figure 1f). The basis for the mosaic distribution of skin lesions became evident upon antibody staining for several keratins at postnatal day 8, demonstrating patches of keratin-free and keratin-expressing epidermis (Figure 2 a–e). In those epidermal patches, K5 and K14 remained expressed in basal and suprabasal epidermis, whereas neighboring patches were keratin-free following cre-mediated deletion. Western blot analysis of whole skin lysates of KtyII$_m^{-/-}$ mice showed insignificantly altered amounts of K5, whereas K14 was increased (Supplementary Figure S2c and d online). In contrast to K5, K1 was reduced by 60% (Figure 2b, Supplementary Figure S2c and d online), whereas the amount of K10 was increased although it could not be detected by immunofluorescence in spinous and granular keratinocytes (Figure 2c; Supplementary Figure S2c and d online). In unaffected, histologically normal KtyII$_m^{-/-}$ skin patches, the deletion of keratins had occurred only in a minor number of keratinocytes (Figure 2e) whereas affected skin patches contained a large number keratin-free keratinocytes (Figure 2a–d).