Growth Retardation, Loss of Desmosomal Adhesion, and Impaired Tight Junction Function Identify a Unique Role of Plakophilin 1 In Vivo

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Desmosomes mediate strong intercellular adhesion through desmosomal cadherins that interact with intracellular linker proteins including plakophilins (PKPs) 1–3 to anchor the intermediate filaments. PKPs show overlapping but distinct expression patterns in the epidermis. So far, the contribution of individual PKPs in differentially regulating desmosome function is incompletely understood. To resolve the role of PKP1 we ablated the PKP1 gene. Here, we report that PKP1$^{-/-}$ mice were born at the expected mendelian ratio with reduced birth weight, but they otherwise appeared normal immediately after birth. However, their condition rapidly declined, and the mice died within 24 hours, developing fragile skin with lesions in the absence of obvious mechanical trauma. This was accompanied by sparse and small desmosomes. Newborn PKP1$^{-/-}$ mice showed disturbed tight junctions with an impaired inside-out barrier, whereas the outside-in barrier was unaffected. Keratinocytes isolated from these mice showed strongly reduced intercellular cohesion, delayed tight junction formation, and reduced transepithelial resistance and reduced proliferation rates. Our study shows a nonredundant and essential role of PKP1 in desmosome and tight junction function and supports a role of PKP1 in growth control, a function that is crucial in wound healing and epidermal carcinogenesis.

Journal of Investigative Dermatology (2016) **00**, ■–■; doi:10.1016/j.jid.2016.03.021

INTRODUCTION

Desmosomes are cell-cell adhesion structures that provide stability upon mechanical stress to ensure tissue integrity. Although present in all epithelia, they are most abundant in tissues exposed to mechanical stress such as the epidermis and the heart. Desmosomes link neighboring cells through interactions between desmosomal cadherins, desmogleins (DSGs) 1–4, and desmocollins (DSCs) 1–3, which are expressed in a tissue-specific pattern. Their cytoplasmic domains interact with desmoplakin (DSP), plakoglobin (PG), and the three plakophilins (PKPs) that link the desmosome to intermediate filaments (Garrod, 2010; Harmon and Green, 2013; Stappenbeck et al., 1993). Differential expression of DSGs, DSCs, and PKP family members results in the formation of desmosomes of distinct composition (Moll et al., 1997). So far, little is known about how individual members of these gene families regulate desmosome size and function.

Most of the desmosomal plaque proteins are required for cell-cell adhesion and organismal survival (see Supplementary Table S1 online). PG-knockout (KO) embryos died because of severe heart defects with reduced and structurally altered desmosomes (Bierkamp et al., 1996; Ruiz et al., 1996). Although β-catenin, the homologue of PG in adherens junctions (AJs), localized to desmosomes and could substitute for PG in cadherin clustering, it was unable to recruit normal levels of PKP1 and DSP to the plaque (Acehan et al., 2008; Bierkamp et al., 1999). DSP-KO embryos did not survive beyond embryonic day 6.5 and had severe defects in tissue architecture, shaping of the embryo, and in anchoring keratin filaments to desmosomes (Gallicano et al., 1998). In an epidermis-specific DSP-KO mouse, intercellular separations were observed as expected (Vasioukhin et al., 2001). Surprisingly, however, desmosome number was unaltered, but these structures lacked keratin filaments, resulting in a compromised adhesive function.

PKPs were previously considered as nonessential plaque proteins. However, PKP2-KO mice died around day 11.5 of embryonic development because of heart defects, indicating that at least one PKP is required for stable intercellular adhesion (Grossmann et al., 2004). In contrast, ablation of

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Abbreviations: AJ, adherens junction; DSC, desmocollin; DSG, desmoglein; DSP, desmoplakin; EDSFS, ectodermal dysplasia-skin fragility syndrome; IGF1R, IGF1 receptor; KO, knockout; PG, plakoglobin; PKP, plakophilin; TJ, tight junctions; WT, wild-type

Received 30 September 2015; revised 7 March 2016; accepted 8 March 2016; accepted manuscript published online 28 March 2016; corrected proof published online XXX

PKP3 showed only a very mild phenotype, with hair abnormalities and increased inflammation of the skin, which manifested only when mice were not kept in a pathogen-free environment (Sklyarova et al., 2008). Thus, PKP3 is not essential for strong desmosomal cohesion.

An important role for PKP1 in epidermal homeostasis has been reported in humans. Ectodermal dysplasia-skin fragility syndrome (EDSFS, OMIM 604536, see http://www.omim.org/) was first described by McGrath (McGrath et al., 1997) and is characterized by skin fragility with generalized superficial erosions, mostly without blistering and chronic inflammatory plaques and pruritus. In addition, ectodermal abnormalities including alopecia and nail dystrophy were observed (McGrath, 2005; McGrath et al., 1997; McGrath and Mellerio, 2010; Sprecher et al., 2004). Desmosomes in the skin of patients were generally small with altered ultrastructure and perturbed desmosome-keratin interactions.

Mechanistic studies in vitro established PKP1 as a multifunctional protein that either supports strong desmosome adhesion or cell growth depending on its posttranslational modification (Wolf et al., 2013). PKP1 overexpression induced the recruitment of desmosomal proteins to the membrane indicative of increased desmosome formation (Hatzfeld et al., 2000; Kowalczyk et al., 1999; South et al., 2003). In contrast, PKP2 and PKP3 co-localized with known markers of the desmosome when overexpressed but failed to induce desmosomes (Bonne et al., 2003; Chen et al., 2002). Moreover, desmosomes containing an excess of PKP1 were resistant to destabilization by pemphigus antibodies (Tucker et al., 2014), suggesting that PKP1 might facilitate the development of a hyperadhesive state (Garrod, 2010). In addition to a role in desmosomes, both PKP1 and PKP3 play a role in messenger RNA metabolism (Hofmann et al., 2006). We have recently shown that PKP1 regulates protein biosynthesis in an insulin/IGF1-signaling dependent manner (Wolf and Hatzfeld, 2010; Wolf et al., 2010; Wolf et al., 2013).

To resolve the in vivo function of PKP1 in desmosome stability, tissue differentiation, and homeostasis, we have generated PKP1-null mice. Here, we report that homozygous mutant mice die postnatally and show fragile skin with lesions strongly resembling the phenotype observed in human patients. Desmosome number and size were significantly decreased, despite the up-regulation of most desmosomal proteins. Interestingly, PKP1$^{-/-}$ mice have an impaired inside-out barrier caused by disturbed tight junction (TJ) function and a reduced birth weight. Taken together, these data identify PKP1 as a protein essential for organismal growth control and integrity of the epidermis.

RESUTLS

PKP1$^{-/-}$ mice show growth retardation and die postnatally

PKP1$^{-/-}$ mice (see Supplementary Figure S1 online for generation) were born at the expected mendelian ratio of approximately 25%. Macroscopic examination of newborn animals did not show any skin blistering. With the exception of absent whiskers, the skin of mutant mice appeared unaffected. However, during the next hours, PKP1-null mice developed skin lesions in the absence of obvious mechanical trauma (Figure 1a), and the mice died during the first day,

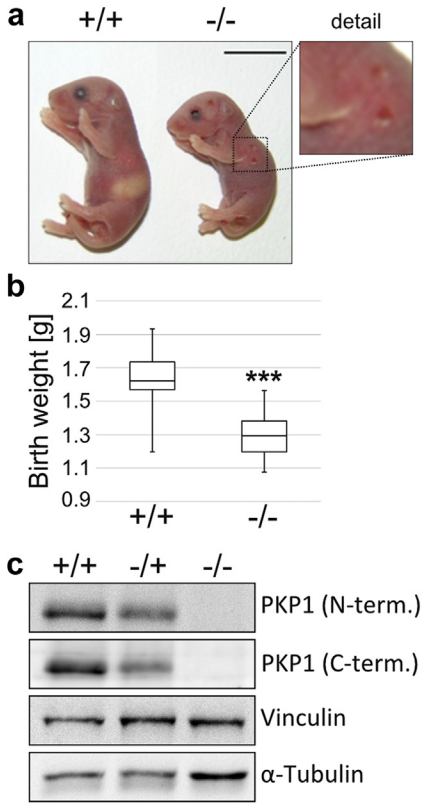

Figure 1. PKP1$^{-/-}$ mice suffer from skin fragility and reduced birth weight. **(a)** Representative PKP1$^{+/+}$ and PKP1$^{-/-}$ newborn pups. Note that the PKP1-KO mouse shows wounds and fragile skin. Scale bar = 1 cm. **(b)** Measurement of birth weight of PKP1-KO mice (n = 23) compared with wild-type littermates (n = 18). $^{***}P < 0.0001$. **(c)** Western blot analysis of total protein from newborn PKP1$^{+/+}$, $^{+/-}$, and $^{-/-}$ dorsal skin. PKP1 was probed with antibodies against N- and C-terminal domains. Vinculin and α-tubulin were used as loading controls. PKP, plakophilin; term., terminal; WT, wild-type.

with an average lifetime of approximately 12 hours. Neonatal lethality with full penetrance indicates an essential role of PKP1 in tissue homeostasis. Surprisingly, mutant mice had significantly lower birth weight than wild-type (WT) mice (Figure 1b), suggesting a role of PKP1 in growth control in vivo. Western blot analyses confirmed the complete absence of PKP1, with no truncated protein fragments found that might be translated from exon 1 or result from alternative start codon usage in exon 3 (Figure 1c and see Supplementary Figure S1d and e).

The epidermis of PKP1$^{-/-}$ mice shows dramatic defects in desmosome formation and mechanical integrity

PKP1$^{-/-}$ epidermis exhibited all stages of terminal differentiation, including the flattened squames of the stratum corneum (Figure 2a and b). Notably, widening of intercellular spaces was observed predominantly in the suprabasal layers, whereas epidermal adhesion in the basal layer and adhesion to the underlying basement membrane appeared normal. Cell separation started in the granular layer, and this layer appeared considerably reduced in thickness in paw skin (Figure 2b). Complete detachment typically occurred between the granular and the spinous layers (Figure 2a). The stratum corneum was often loose and flaky, with diminished cohesion between corneocyte layers, and was reduced in paw skin. Whereas