Caspase-14-Deficient Mice Are More Prone to the Development of Parakeratosis

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Caspase-14 is an important protease in the proper formation of a fully functional skin barrier. Newborn mice that are deficient in caspase-14 exhibit increased transepidermal water loss and are highly sensitive to UVB-induced photodamage. Decreased caspase-14 expression and incomplete caspase-14 processing in lesional psoriatic parakeratotic stratum corneum has been reported previously. In this study, we show that caspase-14-deficient skin frequently displays incompletely cornified cells in the transitional zone between the granular and the cornified layers, pointing to a delay in cornification. We also demonstrate that after challenge of epidermal permeability barrier function by repetitive acetone treatment, a higher incidence of large parakeratotic plaques was observed in caspase-14-deficient skin. Furthermore, caspase-14-deficient mice are more prone than control mice to the development of parakeratosis upon induction of psoriasis-like dermatitis by imiquimod treatment. These results show that lack of caspase-14 expression predisposes to the development of parakeratosis and that caspase-14 has an important role in keratinocyte terminal differentiation and the maintenance of normal stratum corneum, especially in conditions causing epidermal hyperproliferation.

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INTRODUCTION

Skin barrier function is crucial for terrestrial mammalian life, as it protects the body against water loss. The epidermis mainly consists of keratinocytes, which differentiate to produce a functional skin barrier. An impaired balance between keratinocyte proliferation and differentiation causes a variety of skin disorders, including psoriasis, dermatitis, and skin cancer. Recently, it has become clear that defects in the epidermal barrier can underlie the development of inflammatory skin diseases (Proksch *et al.*, 2008). The epidermal permeability barrier function is pivotally maintained by two components of the stratum corneum (SC), namely the cornified envelopes (CEs), which are the end products of keratinocytes undergoing terminal differentiation, and the lipid-enriched extracellular matrix enshrouding these CEs.

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Abbreviations: CE, cornified envelope; IMQ, imiquimod; KO, knockout; SC, stratum corneum; TEWL, transepidermal water loss; WT, wild type

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Caspases are cysteinyl aspartate–specific proteases that exert their functions during apoptosis and inflammation (Lamkanfi *et al.*, 2003). Recently, it has become clear that caspases also have important functions in maintaining epidermal homeostasis. Caspase-14 is the sole caspase that is fully processed during physiological keratinocyte terminal differentiation (Fischer *et al.*, 2004; Raymond *et al.*, 2007). Its expression is constrained to the suprabasal layers of the epidermis and the thymic Hassall's bodies in mice and humans (Eckhart *et al.*, 2000; Lippens *et al.*, 2000), and to the forestomach of rodents (Lippens *et al.*, 2003; Denecker *et al.*, 2007). These tissues contain cornified epithelial cells and express additional terminal differentiation markers such as filaggrin and loricrin (Jarnik *et al.*, 1996). Caspase-14-deficient mice show defects in skin barrier function reflected in a higher transepidermal water loss (TEWL) and increased sensitivity to UVB-induced photodamage. Identification of (pro)filaggrin as the first, and currently only, *in vivo* substrate of caspase-14 provides further evidence that caspase-14 has a crucial role in epidermal barrier function (Denecker *et al.*, 2007; Hoste *et al.*, 2011). We have previously shown that caspase-14 expression is significantly downregulated in parakeratotic plaques of psoriatic patients (Lippens *et al.*, 2000). Treatment of these patients with vitamin D_3 analogs or with epigallocatechin-3-gallate, two inducers of caspase-14 expression, resulted in clinical improvement accompanied by increased caspase-14 expression levels (Lippens *et al.*, 2004; Hsu *et al.*, 2007). Furthermore, in parakeratotic human SC, caspase-14 is present partly in its zymogen form (Fischer *et al.*, 2004; Raymond

et al., 2007), whereas in normal SC, caspase-14 is completely processed and uniquely present in its activated state. The importance of caspases in skin homeostasis has been reinforced by the finding that mice lacking epidermal caspase-8 exhibit a strong inflammatory skin phenotype (Kovalenko *et al.*, 2009; Lee *et al.*, 2009). However, mechanistically, caspase-8 and caspase-14 are acting at different levels. A low level of caspase-8 activity is required to prevent RIPK1 (receptor interacting kinase)- and RIK3-mediated necrotic cell death that leads to skin inflammation (Kaiser *et al.*, 2011), whereas caspase-14 is required for filaggrin degradation during keratinocyte cornification (Denecker *et al.*, 2007; Hoste *et al.*, 2011).

Psoriasis is a common inflammatory skin disorder manifested in a fine, silvery scaling on the affected skin regions and characterized by excessive keratinocyte growth, decreased barrier function (Ghadially *et al.*, 1996), infiltration of neutrophils and mononuclear leukocytes (T cells and dendritic cells), and defective differentiation in the lesional epidermis, resulting in parakeratotic plaque formation (reviewed in Bowcock and Krueger, 2005; Lowes *et al.*, 2007). Parakeratosis is defined as incomplete cornification in which nuclei and DNA are still observed in the SC. It occurs in several dermatological disorders, but the molecular mechanisms leading to parakeratotic plaque formation are largely unknown (Brady, 2004). Although more insight into epidermal DNA catabolism has been gained during recent years, the process of nuclear degradation during cornification is still poorly understood (reviewed in Eckhart *et al.*, 2012). Recently, it has been shown that repeated topical application of imiquimod (IMQ) on mouse skin results in an inflammatory cutaneous response resembling psoriasis (van der Fits *et al.*, 2009; Swindell *et al.*, 2011). Patients with a strong predisposition to psoriasis show exacerbation or relapse of psoriatic features upon IMQ treatment. IMQ binds Toll-like receptor-7 and -8, which are present on antigen-presenting cells, and upon binding of their natural or synthetic ligands they activate the innate immune system to produce several proinflammatory cytokines such as IFN-γ, tumor necrosis factor, IL-6, and IL-12 (Wagner *et al.*, 1999). As in psoriasis patients, the IL-23/IL-17 axis is of key importance in IMQ-induced psoriasis-like dermatitis in mice (van der Fits *et al.*, 2009).

Here, we describe spontaneous ultrastructural abnormalities in caspase-14-deficient corneocytes pointing to defective or delayed keratinocyte differentiation. We also show that caspase-14$^{-/-}$ mice are more prone to the development of parakeratosis upon treatment with IMQ to induce psoriasis-like dermatitis. These data indicate the importance of caspase-14 for correct keratinocyte cornification, especially under challenging conditions that trigger epidermal hyperproliferation.

RESULTS

Electron microscopic analysis reveals ultrastructural abnormalities in caspase-14$^{-/-}$ skin

Caspase-14$^{-/-}$ mice exhibit a defect in the epidermal permeability barrier, and neonates display a shiny and hyperlichenified skin (Denecker *et al.*, 2007). As this phenotype is most prominent in caspase-14 neonates between P1.5 and P5.5, a detailed electron microscopic analysis was performed on the skin of newborn wild-type (WT) and caspase-14$^{-/-}$ mice at P3.5 and on adult skin at the age of 9 weeks. At P3.5, caspase-14-deficient skin frequently contains cells resembling transitional cells, characterized by disorganized nonhomogeneous intracellular content at the stratum granulosum/SC interface (Figure 1a). In some cases, such cells are found up to the fourth cornified layer (SC4). Similar but less frequent incompletely cornified cells were observed in adult caspase-14-deficient epidermis (Figure 1a). These cells were far less abundant in WT counterparts. The occurrence of such cells points to a delay in cornification, although no structural differences between CEs in WT versus caspase-14$^{-/-}$ skin were apparent (Figure 1b). We assessed whether the observed structural differences were due to defects in DNA degradation in caspase-14$^{-/-}$ skin by performing TUNEL staining, but TUNEL-positive cells were scarce in both WT and caspase-14$^{-/-}$ epidermis (data not shown). In addition, by using Hoechst staining in combination with differential interference contrast microscopy, we were unable to detect the remaining DNA in the cornifying layers in both genotypes (Supplementary Figure S1 online). No differences were observed in extracellular lamellar structures between WT and caspase-14$^{-/-}$ epidermis (data not shown). The structural differences observed in caspase-14-deficient skin did not affect the resistance of the skin to tape strip–induced physical damage compared with WT mice, as evaluated by measuring TEWL after repeated tape stripping (Supplementary Figure S2 online).

Epidermal caspase-14 deficiency facilitates the formation of parakeratotic plaques upon topical acetone treatment

Upon repetitive topical treatment with acetone, the epidermis tries to compensate for the loss in the cutaneous permeability barrier by increasing the formation of corneocytes (hyperkeratosis) (Denda *et al.*, 1996). It has been shown that repeated topical application of acetone removes all stainable neutral lipids from the cornified layers, thereby disrupting the skin barrier. This results in an increase in TEWL and causes a hyperproliferative response (Menon *et al.*, 1985). We challenged the skin of WT and caspase-14$^{-/-}$ mice by repetitive acetone wipes two times a day for 5 consecutive days to test whether more pronounced cornification defects would be present in caspase-14-deficient skin compared with WT skin. Analysis of hematoxylin–eosin-stained skin sections demonstrated that repeated acetone treatment induced parakeratotic plaque formation in the skin of both WT and caspase-14$^{-/-}$ mice (Figure 2). A similar rise of TEWL and hyperproliferation was observed in the two genotypes upon acetone treatment (Figure 2b, Supplementary Figure S3 online). Interestingly, the percentage of parakeratotic SC induced by acetone treatment was higher in caspase-14$^{-/-}$ skin compared with WT skin (Figure 2c). The same trend was observed when counting parakeratotic plaques larger than 100 μm (Figure 2d). Our results indicate that caspase-14 is important for the maintenance of normal keratinocyte terminal differentiation and *SC* architecture under skin barrier–challenging conditions.