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Cuts by Caspase-14 Control the Proteolysis of Filaggrin

Leopold Eckhart[1] and Erwin Tschachler[1,2]

Although mutations in the filaggrin gene (*FLG*) have been shown to be associated with ichthyosis vulgaris and atopic dermatitis, the function and regulation of filaggrin remain incompletely understood. In this issue, Hoste *et al.* report that filaggrin is directly cleaved by caspase-14. Acting in concert with other proteases, caspase-14 controls the breakdown of filaggrin to free amino acids and amino acid derivatives that contribute to the hydration and UVB absorption capacity of the stratum corneum. These findings identify a new layer of complexity in the regulation of epidermal barrier function.

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Processing of filaggrin in the epidermis

Filaggrin became a focus of dermatological research when mutations in its gene (*FLG*) were found to be associated with ichthyosis vulgaris and atopic dermatitis (Irvine and McLean, 2006). Because filaggrin is expressed only in keratinocytes, defects in the epidermal barrier are now considered likely to precede immune activation and allergies in patients with atopic dermatitis (Irvine and McLean, 2006). Although the mechanism of *FLG*-associated barrier defects has not yet been fully elucidated, recent studies have revealed multiple interactions between filaggrin and other proteins that are critical for its physiological function.

Like other members of the S100-fused-type protein family, filaggrin is expressed as a precursor protein consisting of an N-terminal S100 domain and a series of filaggrin repeats. Proteolytic processing yields filaggrin monomers that are important in the aggregation of intermediate filaments during cornification of keratinocytes (Steinert *et al.*, 1981). In the stratum corneum, complete proteolysis of filaggrin leads to the release of free amino acids that function as components of the natural moisturizing factor (NMF) (Scott and Harding, 1986) (Figure 1). Moreover, filaggrin-derived histidine is converted to urocanic acid, which protects the underlying layers of the skin against UVB radiation (Barresi *et al.*, 2011). In addition to proteolysis, protein phosphorylation, dephosphorylation, and deimination control the progressive conversion of profilaggrin to its amino acid constituents (Sandilands *et al.*, 2009). The report by Hoste *et al.* (2011, this issue) uncovers a novel part of the complex processing of filaggrin and highlights the experimental challenges faced by investigators studying biochemical processes in the outermost layers of the skin.

Caspase-14 is a filaggrin-processing enzyme

The cysteine protease caspase-14 was linked to the breakdown of filaggrin in 2007 by Declercq and colleagues, who generated and characterized a caspase-14 knockout mouse model (Denecker *et al.*, 2007). They found that caspase-14 deficiency was associated with the accumulation of incompletely degraded filaggrin fragments within the stratum corneum, a decrease in stratum corneum hydration, increased transepidermal water loss, and sensitivity to UVB photodamage. In an extension of this work, Hoste *et al.* (2011) now provide evidence for the molecular mechanisms underlying the phenotype of caspase-14 knockout mice. The investigators identify direct cleavage sites

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Clinical Implications

- In addition to mutations in the filaggrin gene, defects in the activity of caspase-14 and possibly other proteases can lead to a decrease in the levels of stratum corneum natural moisturizing factor.
- The identification of filaggrin processing intermediates in the stratum corneum may be used in the differential diagnosis of filaggrin-associated skin barrier defects.
- Modifying the microenvironment within the stratum corneum may become a strategy for treating disorders of filaggrin processing.

of caspase-14 within filaggrin monomers *in vitro* and report that lack of caspase-14 leads to a decrease in the concentration of filaggrin-derived components of the NMF and consequently to a decrease in the UVB photoprotectant urocanic acid in the stratum corneum (Hoste *et al.*, 2011).

Caspase-14 is coexpressed with filaggrin in terminally differentiated keratinocytes; it also undergoes proteolytic maturation (Eckhart *et al.*, 2000; Lippens *et al.*, 2000; Figure 1). An as-yet-unknown protease cleaves procaspase-14 to generate a large and a small catalytic subunit that persist in the stratum corneum (Fischer *et al.*, 2004; Hibino *et al.*, 2010). The processing of caspase-14 is essential but not sufficient for catalytic activation (Mikolajczyk *et al.*, 2004). *In vitro* high concentrations of kosmotropic (i.e., structure-stabilizing) salts are required for caspase-14 to be active (Mikolajczyk *et al.*, 2004; Fischer *et al.*, 2004; Hoste *et al.*, 2011). As kosmotropes exert their effects on proteins by competing for water molecules, the *in vitro* requirements of caspase-14 activity may be mimicked by the low water content known to exist close to the skin surface. Indeed, filaggrin is degraded when the hydration level in the outer layers of the stratum corneum decreases (Scott and Harding, 1986). Further studies should define more precisely the microenvironment that allows caspase-14 to cleave filaggrin in the epidermis. It is tempting to speculate that a unique dependency on a distinct milieu allows caspase-14 to act as a sensor for environmental conditions that regulate the degradation of filaggrin.

Hoste *et al.* determined two sites of caspase-14-mediated cleavage of filaggrin *in vitro,* and they suggested the existence of other cleavage sites that could not be identified, largely for technical reasons. Both sites identified in this report conform to caspases' strict requirement for an aspartate residue on the amino-terminal side of the hydrolyzed peptide bond (Mikolajczyk *et al.*, 2004). The substrate specificity of caspases, including caspase-14, also depends on the three amino acid residues that precede the aspartate residue (Mikolajczyk *et al.*, 2004). Intriguingly, filaggrin shows little evolutionary conservation of its amino acid sequence, including the sequence of the caspase-14 cleavage sites identified by Hoste *et al.* (2011). Interspecies differences in the caspase-14 cleavage pattern of filaggrin and the existence of caspase-14 substrates other than filaggrin should be investigated in future studies.

Caspase-14-dependent markers of filaggrin catabolism

The report of Hoste *et al.* (2011) shows that caspase-14 deficiency affects two molecular parameters of filaggrin catabolism: the pattern of filaggrin fragments and the concentrations of NMF components in the stratum corneum. Previously, the NMF content of stratum corneum has been suggested as a marker for *FLG* mutations (Kezic *et al.*, 2008). Hoste *et al.* (2011) now demonstrate that, in addition to the mutation status of filaggrin, the efficiency of filaggrin catabolism is a major determinant of NMF concentrations. Complementary to the decreased concentrations of NMF components, the absence of caspase-14 is also associated with an increase in the abundance of filaggrin degradation intermediates (Hoste *et al.*, 2011). It is conceivable that distinct patterns of filaggrin fragments correlate with different states of stratum corneum homeostasis or even with subsets of human diseases. Interestingly, the level of active caspase-14 has been reported to be reduced in patients with atopic dermatitis (Yamamoto *et al.*, 2011). Unfortunately, the filaggrin fragmentation pattern was not determined in that study. The results of Hoste *et al.* (2011) suggest an unconventional migration behavior of filaggrin fragments in polyacrylamide gel electrophoresis. Therefore, the evaluation of alternative assays and the specific adaption of the analytical methods to the unique properties of filaggrin, including a high content of charged amino acid residues, may be required to define diagnostic characteristics of filaggrin degradation.

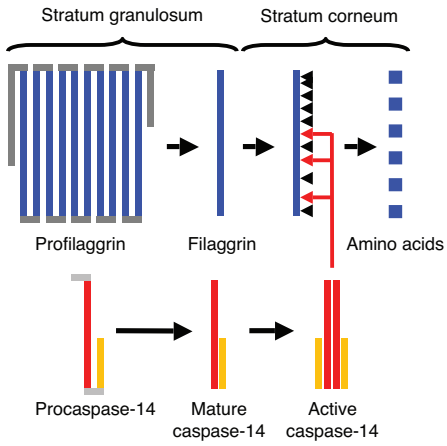

Figure 1. Proteolytic regulation of filaggrin and caspase-14. Filaggrin and caspase-14 are expressed as proproteins that undergo proteolytic maturation in the stratum granulosum. The subunits of caspase-14 (red and orange) require further structural changes, possibly induced by dimerization, to become catalytically active. Filaggrin monomers (blue) are cut at multiple sites by caspase-14 (red arrows) and other proteases (black arrowheads).

It is important to note that Hoste *et al.* (2011) have not proposed that caspase-14 is the only protease that cleaves filaggrin. Rather, their report shows that, in the absence of caspase-14, other enzymes can initiate proteolysis of filaggrin. In addition, proteases such as calpain 1 and bleomycin hydrolase (Kamata *et al.*, 2009) are required to complete the degradation of filaggrin. Therefore, to understand the regulation of filaggrin degradation, it is necessary to determine the interplay of caspase-14 with these other proteases as well as the order in which the proteolytic cuts occur.

Concluding remarks

New evidence demonstrates that not only mutations in the filaggrin gene but also alterations in filaggrin processing may result in skin barrier defects and that caspase-14 takes part in this process. Hoste *et al.* (2011) provide a basis for improving strategies to diagnose filaggrin-associated skin disorders and to modulate caspase-14-dependent barrier function of the stratum corneum.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Demonstration of Epitope Spreading in Bullous Pemphigoid: Results of a Prospective Multicenter Study

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Di Zenzo and colleagues have undertaken a multicenter prospective study to clarify the epitope profile for IgG anti-BP180 and BP230 antibodies in 35 patients with bullous pemphigoid (BP). Both intra- and intermolecular epitope spreading events were observed, in which epitopes shifted exclusively from extracellular to intracellular domains. The presence of IgG antibodies to the BP180 C-terminal domain and BP230, in addition to the BP180-NC16A domain, correlated with disease severity and activity, suggesting specific pathogenic relevance for anti-BP230 antibodies. Epitope spreading was found in both T- and B-cell recognition. IgA anti LAD-1 antibodies are frequently found in patients with BP; these antibodies appear to follow the development of IgG antibodies to BP180 and BP230 by epitope spreading. These observations provide direction for future studies of the pathogenesis of and treatments for BP.

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Epitope spreading plays an important role in the development of autoimmune diseases

Various autoimmune diseases develop and progress via epitope spreading (ES). In ES, inflammation induced by autoimmunity to an initial epitope damages target tissue, which subsequently induces antibodies to secondary epitopes on the same or different antigens (Chan *et al.*, 1998). Intra- or intermolecular ES is not simply an epiphenomenon, because it is important for the development of each disease.

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