Review

Function of Filaggrin and Caspase-14 in Formation and Maintenance of the Epithelial Barrier

Richard B. Presland

Stratified epithelia line all external and internal body surfaces of mammals and function to protect us from water loss, environmental insults, chemical damage, and infection. This protective barrier is composed of a complex mixture of structural proteins and specialized lipids that together form the anuclear squames of the *stratum corneum*. Filaggrin and caspase-14 are two genes expressed in the granular layer of epidermis and other stratified keratinizing epithelia. Filaggrin is a keratin-binding protein present in the lower layers of the *stratum corneum* which plays a key role in barrier function. Filaggrin is synthesized as a large precursor, profilaggrin, that undergoes specific processing during keratinocyte differentiation to produce filaggrin. In the middle and upper layers of the *stratum corneum*, filaggrin is degraded to free amino acids which have several functions including water adsorption. Recent work has shown that loss-of-function mutations in the filaggrin (FLG) gene cause the dry skin disease ichthyosis vulgaris. Similar FLG variants are strongly associated with the common inflammatory skin disease atopic dermatitis (eczema). Current studies suggest that~50% of European patients with moderate to severe eczema carry one or more FLG null alleles. Caspase-14 is a member of the caspase family of aspartate-specific proteases that is co-expressed with filaggrin within the epidermis. Recent work has shown that it functions both as a profilaggrin protease and protects against UV irradiation. These studies emphasize the importance of proteases in regulating profilaggrin/filaggrin and caspase-14 function, and their common and overlapping roles in epithelial barrier homeostasis. (Dermatol Sinica 27: 1-14, 2009)

Key words: Terminal differentiation, Epithelial barrier, Profilaggrin, Filaggrin, Caspase-14, Ichthyosis Vulgaris, Atopic eczema

Abbreviations

EDC, Epidermal Differentiation Complex; UCA, urocanic acid; NT, N-terminus; kDa, kilodalton; FLG, profilaggrin gene; IV, ichthyosis vulgaris; NMF, Natural Moisturizing Factor

I. INTRODUCTION

Terminal differentiation or "physiological apoptosis" refers to the specialized form of cell death that occurs in the epidermis and other stratified epithelia.[1, 2] Once keratinocytes leave the proliferating basal cell com-

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partment they undergo a program of differentiation in the spinous and granular layers and express a set of differentiation-specific proteins including keratins (primarily K1, K2e, and K10) and many other structural proteins. The non-keratin structural proteins include filaggrin, loricrin, involucrin, small proline-rich proteins (SPRRs), and the late cornified envelope (LCE) proteins.[3, 4] Many of these structural proteins are crosslinked into the cornified cell envelope by transglutaminase enzymes; this insoluble envelope, together with the keratin-containing macrofibrils that fill corneocytes and lipids form the skin barrier that protect mammals from water loss and many kinds of environmental insults. Throughout our lifespan these tissues are constantly being replaced by new cells from the basal layer at a remarkable rate- for example, human epidermis undergoes complete turnover every 28 days and mouse epidermis every 7 days.[5]

This review will focus on the role of filaggrin and caspase-14 in the process of keratinocyte terminal differentiation. While filaggrin and caspase-14 are structurally quite distinct, they share several common biochemical and functional properties. They are both expressed as precursors in the epidermal granular layer which comprises the last living layer in the epidermis and other stratified keratinizing epithelia. Subsequently, both proteins are cleaved by proteases during terminal differentiation. In addition, while both genes are expressed in the granular layer and associate with keratohyalin granules, they carry out their primary functions in the *stratum corneum*. Recent work has revealed that filaggrin is a substrate of caspase-14 linking these proteins in a common biological pathway during keratinocyte terminal differentiation.

II. PROFILAGGRIN/FILAGGRIN

Profilaggrin (gene symbol FLG) is a member of the fused S100 family of S100 Ca^{2+}-binding proteins that share a common N-terminal S100-like Ca^{2+}-binding domain. The fused S100 gene family forms a cluster of 7 genes on human chromosome 1q21, that is part of a larger genomic region termed the Epidermal Differentiation Complex (EDC).[3] The ~1.8 Megabase EDC includes many genes expressed in epidermis and other stratified epithelia such as loricrin, involucrin, the SPRRs and LCE proteins, and many of the small, canonical S100 genes.

Profilaggrin has a relatively simple gene structure consisting of 3 exons. The protein coding region is contained in exons 2 and 3. Exon 2 encodes the N-terminal 46 amino acids of profilaggrin, while exon 3 (12.7 kb) contains the remainder of the protein coding region.[6, 7] Other fused S100 genes such as hornerin and repetin have a similar genomic organization, suggesting they evolved from a single common ancestor.[3] The fused S100 proteins, including profilaggrin, function as structural components of the epithelial barrier, specifically as cornified envelope components and/or as keratin-associated proteins.

1. Profilaggrin expression and proteolytic processing to filaggrin

Profilaggrin is one of the largest proteins found in mammals ranging in size from ~400 kDa in humans to ≥ 800 kDa in rats. It is expressed in the granular layer of keratinizing, stratifying epithelia including epidermis, tongue, and oral gingiva where it localizes to electron-dense keratohyalin granules that gives the granular layer its histologic appearance.[3] Profilaggrin consists of a unique N-terminal domain (subdivided into an S100 Ca^{2+}-binding domain (the A domain) and a basic B domain); a poly-filaggrin region consisting of multiple filaggrin repeats (e.g. between 10 and 12 filaggrin domains

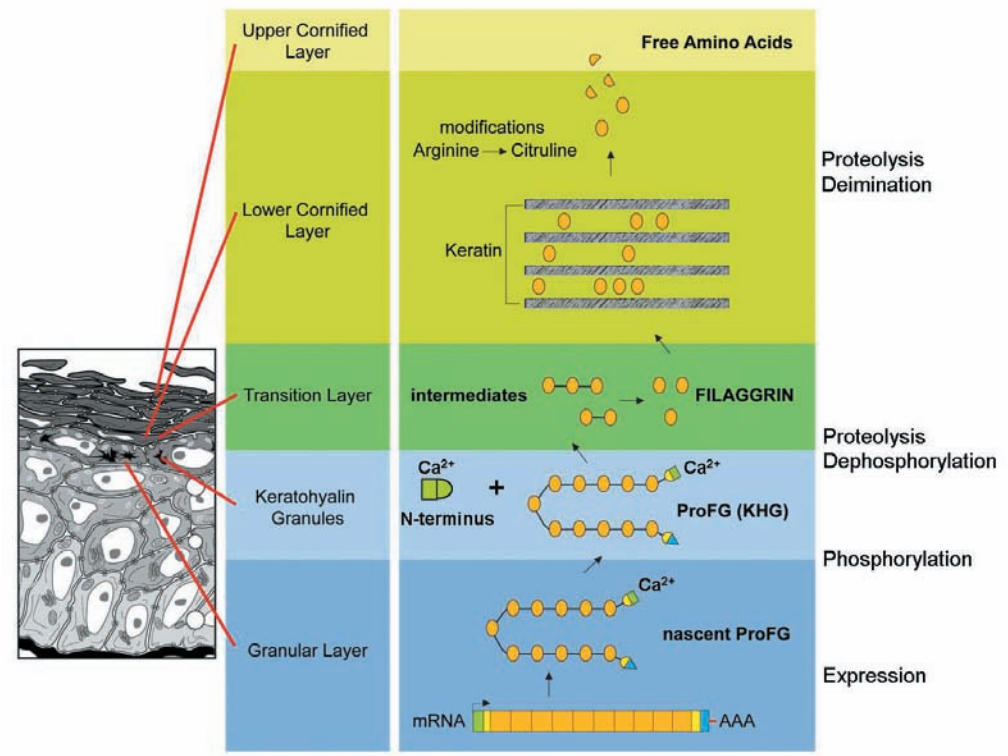

Fig. 1

Expression and processing of profilaggrin during keratinocyte terminal differentiation. Profilaggrin is synthesized and phosphorylated in the granular layer, and is initially stored in keratohyalin granules. During terminal differentiation at the granular to cornified cell transition, profilaggrin is dephosphorylated and cleaved by proteases to filaggrin (orange circles). The N-terminus (green) is cleaved from profilaggrin and associates with other proteins in the cytoplasm and nucleus (see text). Filaggrin aggregates keratin filaments in cornified cells, forming the keratin aggregates (macrofibrils) that are retained in cornified cells until they are sloughed at the tissue surface. Eventually filaggrin is chemically modified and degraded by proteases. The resulting free amino acids carry out various functions in the cornified cells including water retention.

of 324 residues in humans); and a unique C-terminal end domain (Fig. 1, 2). Partial filaggrin domains are also present at each end of the poly-filaggrin region. Profilaggrin is phosphorylated by several serine/threonine protein kinases including casein kinase II;[8, 9] this post-translational modification neutralizes the highly basic (positive) charge of filaggrin, allowing the protein to fold tightly into keratohyalin (Fig. 1). Since phosphorylated filaggrin peptides are unable to interact with keratins, phosphorylation may also function to prevent the premature aggregation of keratins by filaggrin[10, 11] which is detrimental to living cells *in vitro*.[12] Many of the phosphorylation sites identified in rat filaggrin are conserved in the mouse and human proteins.[13, 14]

During terminal differentiation at the granular to cornified cell transition, profilaggrin is rapidly dephosphorylated and cleaved by several endoproteases to generate the primary end-products of profilaggrin processing, filaggrin and the N-terminal domain (Fig. 1). Dephosphorylation appears to involve the activity of several phosphatases including protein phosphatase type 2A (PP2A).[15] PP2A is expressed in the uppermost granular layer of newborn rat skin, consistent with the temporal processing of profilaggrin to filaggrin during *stratum corneum* formation.

Several endoproteases have been implicated in profilaggrin processing, including

calpain I (μ-calpain),[16, 17] a chymotrypsin-like enzyme termed PEP1,[18] furin or a related proprotein convertase (which is specifically involved in cleavage of the N-terminus from profilaggrin),[19] matriptase (MT-SP1),[20] and the serine protease prostatin (CAP1/*Prss8*).[21] Caspase-14 has been shown to cleave profilaggrin at a site within the N-terminal domain as well as at one site within each filaggrin domain.[22] Caspase-14 is important for the final degradation of filaggrin to free amino acids within the *stratum corneum*[23] (see below). Processing of profilaggrin to filaggrin involves several steps. The N-terminus is cleaved and, concurrently, there is processing of the poly-filaggrin sequence, first into intermediates consists of 2-4 filaggrin repeats and finally to mature (single domain) filaggrin (Fig. 1). The poly-filaggrin sequence is cleaved by one or more proteases at a short hydrophobic linker peptide located between each filaggrin domain. In mouse profilaggrin, there are two distinct types of linker sequence that are the targets of distinct proteases (e.g. PEP1 and calpain I), while the human and rat proteins have only one type of linker sequence.

While many proteases have been implicated in profilaggrin processing through the analysis of genetic models or biochemical studies, few have been directly shown to cleave profilaggrin. In some cases, proteases may activate another protease which then cleaves profilaggrin, or alternatively, the protease may degrade a protease inhibitor. One example of a protease inhibitor that plays a vital role in normal keratinocyte differentiation and desquamation is lympho-epithelial Kazal type inhibitor (LEKTI). LEKTI, encoded by the SPINK5 gene, is mutated in the autosomal recessive disorder known as Netherton Syndrome.[24] SPINK5-deficient mice show a range of abnormalities mimicking Netherton Syndrome including abnormal hair, defective desquamation, and increased profilaggrin processing to filaggrin.[25, 26] LEKTI co-localizes to the granular layer with several serine proteases including matriptase and prostatin.[21, 27] Thus, an important aspect of normal terminal differentiation including profilaggrin processing is the balance between proteases and their inhibitors.

2. Filaggrin function in the stratum corneum

The conversion of profilaggrin to filaggrin allows filaggrin to function as a keratin aggregating protein in corneocytes, which results in the bundling of keratin filaments into macrofibrils to generate the keratin pattern of the *stratum corneum*.[28, 29] The formation of macrofibrils allows the keratin proteins to survive the protease-driven remodeling that occurs during keratinocyte terminal differentiation. Once keratins aggregate into macrofibrils they are further stabilized by transglutaminase-mediated crosslinking to other proteins[30, 31] (Fig. 1). In the upper layers of dry epithelia such as the epidermis, filaggrin is degraded to free amino acids, some of which are chemically modified. Glutamine, histidine, and arginine are converted to pyrrolidone carboxylic acid (PCA), urocanic acid (UCA) and citrulline, respectively, which play different roles in the *stratum corneum*.[32] PCA is highly hygroscopic and an important component of Natural Moisturizing Factor (NMF) that functions to keep the *stratum corneum* in a hydrated state.[32, 33] Urocanic acid (UCA), derived from histidine by the action of histidase, has several potential roles in the epidermis including UV photoprotection and as a scavenger of hydroxyl free radicals.[34, 35] *Trans*-UCA is converted by UVB radiation to *cis*-UCA which plays an important role in UVB-mediated immunosuppression.[36] Recent work suggests that the role of *cis*-urocanic acid in UV-mediated immune suppression involves specific changes in gene expression.[37] Lastly, citrulline modi-

fication (deimination) of proteins including filaggrin and keratin 1 is carried out by the enzyme peptidylarginine deiminase (PAD). Deimination of filaggrin in the *stratum corneum* correlates with disruption of the filaggrin-keratin complex and degradation of filaggrin to amino acids.[38, 39]

Several proteases may be involved in filaggrin degradation within the *stratum corneum*; proteases implicated from *in vitro* and *in vivo* studies include caspase-14,[23, 40] *stratum corneum* tryptic and chymotryptic enzymes (SCTE and SCCE, respectively),[26, 41] and cathepsins B and L.[42, 43] In caspase-14 knockout mice, filaggrin processing to free amino acids within the *stratum corneum* is aberrant with an accumulation of low molecular weight filaggrin peptides.[23] Little is known about the control of filaggrin degradation in the *stratum corneum*. Filaggrin proteolysis occurs upon exposure to a low humidity environment, as occurs in mammals *post partum*.[44] This degradation could be prevented by maintaining newborn animals in a high humidity environment. Deimination may represent another way to regulate filaggrin proteolysis since filaggrin degradation occurs after deimination and coincides with the release of filaggrin from the compacted keratin macrofibrils.[38] Some filaggrin escapes the terminal proteolysis pathway by being incorporated into the cornified cell envelope.[30, 45]

3. The profilaggrin N-terminus: an S100-like calcium-binding protein involved in protein-protein interactions

The profilaggrin N-terminus (NT) is a structurally distinct region of profilaggrin that consists of a conserved S100 Ca^{2+}-binding domain (A domain) and a less conserved B domain that contains one or more nuclear localization signals.[46] The profilaggrin NT (molecular weight ~30 kDa) is released from profilaggrin during proteolytic processing and can be found in both the cytoplasmic and nuclear compartments of human and mouse epidermis[47, 48] (Fig. 1). S100 proteins like S100A7 (psoriasin) bind calcium, form homodimers and subsequently associate with other target proteins, thereby regulating their activity and function.[49] Using the yeast two-hybrid system and other protein interaction methods we have shown that the profilaggrin NT forms homodimers *in vivo* and interacts with several other proteins in keratinocytes, including the plasma membrane-associated protein annexin II[50] and apoptosis antagonizing transcription factor (AATF), a gene that has multiple roles in vertebrate development.[51] Further work will be necessary to determine how the profilaggrin NT regulates the function of its target proteins, and what significance these interactions have for terminal differentiation.

4. Filaggrin loss-of-function mutations are associated with Ichthyosis Vulgaris and Atopic Eczema

A. Ichthyosis vulgaris

Ichthyosis vulgaris (IV) is the most common disorder of keratinization with a reported incidence of 0.4% (or 1 in 250 individuals) among English schoolchildren.[52] It is characterized by dry skin, fine superficial scaling on the skin of the trunk and extensor surfaces of the extremities, palm and sole hyperlinearity, keratosis pilaris, and frequently atopy. Histologically, patients usually have a reduced or absent granular layer, and correspondingly both keratohyalin granules and profilaggrin expression were reduced or absent.[53, 54] In the first study linking IV to profilaggrin (FLG) gene mutations, Smith *et al.* demonstrated the presence of loss-of-function FLG mutations in affected individuals from 15 families of European descent.[55] These first two mutations (a nonsense mutation, Arg501Stop, and a small deletion, designated 2282del4) were located in the first

Table. 1 Summary of Prevalent FLG Gene Mutations Associated with Ichthyosis Vulgaris and Atopic Eczema

Mutation Number [a]	*Nature of Mutation*	*Location (Filaggrin Repeat #)*	*Ethnic origin of patients*	*Selected References*
1	1249insG	0 [b]	Singapore Chinese	Chen *et al.*[60]
2	Arg501Stop	1	U.K./European [c]	Smith *et al*;[55] Palmer *et al.*[64] Marenholz *et al.*[71]
3	2282del4	1	U.K./European [c]	Smith *et al*;[55] Palmer *et al.*[64] Marenholz *et al* [71]
4	3321delA	2	Japanese, Chinese	Nomura *et al.*;[59, 67] Sandilands *et al.*[56]
5	3702delG	3	U.K./European	Sandilands *et al.*[56]
6	Glu2422Stop	6	Singapore Chinese	Chen *et al.*[60]
7	Arg2447Stop	7	U.K./European	Sandilands *et al.*;[56] Brown *et al.*[66]
8	Ser2554Stop	7	Japanese	Nomura *et al.*[59, 67]
9	Ser2889Stop	8	Japanese	Nomura *et al.*[67]
10	Ser3247Stop	9	U.K./European	Sandilands *et al.*[56]
11	Ser3296Stop	9	Japanese	Nomura *et al.*[67]

[a] Mutations are numbered from the 5' end of the gene as shown in Fig. 2
[b] Partial filaggrin repeat located upstream of filaggrin domain 1
[c] These mutations were also seen in probands from an American family of European ancestry (Smith *et al.*[55])

filaggrin domain and result in severe truncation of profilaggrin which is expressed at a low level in patient skin.[55] Both homozygous and heterozygous cases were identified with heterozygous patients showing very mild scaling that was less widespread, demonstrating the semidominant nature of this disease. To date a total of 37 different FLG mutations have been identified in IV families from European and Asian populations. The mutations are either nonsense mutations or small insertions or deletions that result in premature stop codons.[56-58] These loss-of-function mutations are located throughout the central polyfilaggrin-encoding portion of the protein (Fig. 2, Table 1). Some mutations, and in particular Arg501Stop and 2282Del4, are very common in European IV patients while others are rare or family-specific.[56] Of the 37 mutations identified to date, 14 occur commonly, that is, they occur in more than one family or study population (the locations of 11 common mutations in the FLG gene/protein are shown in Fig. 2). The FLG mutations found in Japanese and Chinese IV families are, with one or two exceptions, genetically distinct from the mutations of European origin (Table 1).[56, 59, 60]

One of the most significant biological findings with IV is that, while the mutant protein is usually expressed in patient skin (albeit at much lower levels than normal) there is no processing of the truncated profilaggrin peptide to produce filaggrin. This is true even when the mutations occur in the distal (3') half of the gene.[56] This probably explains the observation that severity does not correlate with position of the mutation, i.e. distal mutations appear to be just as penetrant as proximal mutations that are located in the *5'* half of the gene. Thus, all IV cases examined to date are filaggrin-null irrespective of where the mutation is located. It has been proposed that the absence of filaggrin explains the dry, scaly skin that is a hallmark of IV.[53] Consistent with this notion, patients carrying loss-of-function FLG mutations have significantly lower levels of NMF in

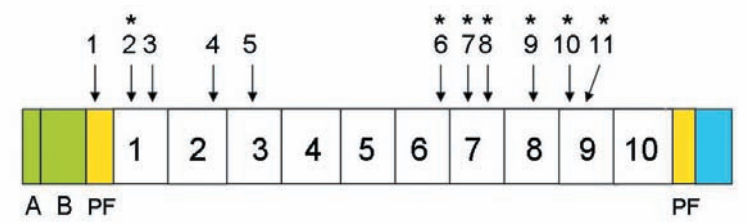

Fig. 2

Structure of human profilaggrin protein showing the location of prevalent mutations associated with ichthyosis vulgaris and atopic eczema. The figure shows a profilaggrin molecule with 10 filaggrin repeats; the number of repeats can vary from 10 to 12.[94] The mutations that are starred represent nonsense mutations; the remainder represent either insertion or deletion mutations, which all result in premature termination. The mutations shown are: 1, 1249insG; 2, Arg501Stop; 3, 2282del4; 4, 3321delA; 5, 3702delG; 6, Glu2422Stop; 7, Arg2447Stop; 8, Ser2554Stop; 9, Ser2889Stop; 10, Ser3247Stop; 11, Ser-3296Stop. The 11 mutations shown here are described further in Table 1. A and B (green regions) represent the N-terminal domains of the protein and PF (yellow regions) indicate the Partial (half) Filaggrin domains located at each end of the poly-filaggrin region. The C-terminal domain is shown in blue.

forearm and palmar skin compared to normal controls.[61] While there is a defect in *stratum corneum* moisturization in IV, keratin filament aggregation in the cornified cells of IV skin is indistinguishable from that of normal skin.[54] One possible explanation is that other filaggrin-like proteins present in epidermis could carry out the keratin filament aggregating function normally performed by filaggrin.[3]

The filaggrin protein deficiency that is a hallmark of human IV is also observed in flaky tail (*ft*) mutant mice. Flaky tail mice show many features in common with IV, including a dry flaky skin (at least in the neonatal period), an absence of normal profilaggrin-containing keratohyalin granules and expression of a truncated profilaggrin peptide that is not processed to filaggrin.[62] Biochemical studies of *ft/ft* homozygous mutant mice point to a mutation in the profilaggrin gene. These results and the IV studies suggest that the unique C-terminal domain is necessary for the normal stability of profilaggrin and its processing to filaggrin.

B. Filaggrin mutations and atopic dermatitis (eczema)

Atopic dermatitis (eczema) is a chronic inflammatory skin disease that involves multiple genetic and environmental components, often manifesting with other allergic conditions including asthma, allergic rhinitis (hay fever) and food allergies. Many genes have been associated with eczema; these genes include components of the epithelial barrier and genes involved in the innate and acquired immune responses.[63] Palmer et al demonstrated that two of the common mutant alleles associated with IV (Arg501Stop and 2282Del4) are very strong predisposing genetic factors in atopic eczema.[64] Indeed, these two mutations are carried by~9% of people of European origin, with the majority of individuals carrying one or more mutant alleles exhibiting atopic eczema. These initial findings have been replicated by a number of studies of European and Asian populations, demonstrating that most of the common FLG null alleles confer a strong genetic susceptibility to atopic eczema.[57, 58, 65] At the genetic level, atopic eczema can be observed in individuals with either one or two FLG null alleles; in contrast, individuals with IV who have just one FLG null allele are extremely mild clinically, or asymptomatic (see previous section). Studies show that atopic eczema patients carrying one of the well characterized FLG null variants (Arg501Stop or 2282Del4) are more likely to have early-onset eczema

that persisted into adulthood. In one study of Irish children, 47% of eczema cases carried FLG mutations showing the strong influence of this gene with regard to disease risk.[56] It has been estimated that an individual carrying one FLG null mutation has a four-fold greater risk of having early-onset persistent eczema, while an individual with two mutant FLG alleles have an~80-fold increased risk of developing eczema compared to an individual with two normal FLG alleles.[66] The distinctive FLG null alleles identified in Japanese populations are also significant predisposing factors for atopic eczema as well as IV.[59, 67]

Individuals who carry one or more FLG alleles often develop asthma in addition to eczema, a phenomenon referred to as 'the atopic march'.[64] Several studies have shown that patients carrying the common European FLG variants have more severe asthma relative to individuals who do not carry FLG mutations. These exacerbations included the need for oral steroids and repeated absences from school.[68] Most studies have demonstrated that asthma occurs in conjunction with a preexisting eczema condition.[64, 69-71] Since filaggrin is not expressed in the bronchial epithelium,[72] it is believed that the association with asthma is a secondary consequence of filaggrin deficiency in the skin. It has been proposed that the barrier defect associated with filaggrin deficiency and eczema leads to penetration of allergens into the skin which in turn leads to induction of a Th2 response resulting in asthma and other atopic manifestations.[73, 74] Concurrently the Th2 cytokines IL-4 and IL-13, which are overexpressed in the skin of atopic dermatitis patients, can downregulate filaggrin expression, potentially further exacerbating both the skin barrier defect and disease progression.[74] This model is consistent with studies showing that allergen exposure can induce a systemic atopic disease in previously healthy mice.[75] Additionally, in atopic eczema there is an increase in *stratum corneum* pH which results in elevated activity of serine proteases and increased colonization by Staphylococcus aureus which further compromises skin integrity and enhances the Th2 response, respectively.[76] More work is needed to better understand why FLG mutations make humans so susceptible to atopic eczema, and why some individuals never get atopic diseases despite the fact that they carry these mutations.[64]

III. CASPASE-14: AN EPITHELIAL-SPECIFIC ENDOPROTEASE THAT FUNCTIONS IN FILAGGRIN PROTEOLYSIS AND UV PROTECTION

Caspase-14 is a recently described member of the caspase family of aspartate-specific proteases that is abundantly expressed in stratified epithelia including the epidermis, keratinizing oral mucosa, and rodent forestomach.[40, 77] It is also expressed in a limited number of non-epithelial tissues including the thymus and the trophoblast cells of the developing human placenta.[23, 78] During mouse embryogenesis, caspase-14 is expressed during stratification and epithelial barrier formation at day 16-18, suggesting an important role in epidermal maturation prior to birth.[79, 80] Besides its restricted tissue distribution, caspase-14 displays some unusual properties compared to most other caspases, namely that it is not activated *in vitro* by apoptotic stimuli such as staurosporine or UVB[81] and that the inactive pro-enzyme is not cleaved by another caspase *in vivo*.[82] Additionally, keratinocytes from caspase-14 null mice can undergo normal apoptosis demonstrating that this protease is dispensable for programmed cell death.[23]

Caspase-14 was originally identified as a member of the caspase family based on both structural and biochemical studies. It has the highest degree of sequence homology

to caspase-1 (ICE) and other short prodomain caspases such as caspase-3.[83] Like other caspases, it is synthesized as an inactive precursor which is subsequently cleaved to generate the large and small subunits that associate to form the active caspase. However, the protease that activates caspase-14 *in vivo* has not been identified. Candidate proteases include a calpain (probably calpain I which also plays a role in profilaggrin processing[82, 84]) or a serine protease with elastase-like activity.[40] In addition, biochemical studies have shown that caspase-14, like other caspases, has an absolute requirement for aspartate (D) at the site of proteolytic cleavage.[84, 85]

In the epidermis, caspase-14 is expressed in the granular layer where it is associated with keratohyalin granules as well as the nucleus and desmosomes, suggesting the possibility of substrates in different cellular compartments.[86] It is present in the *stratum corneum* where it can be isolated in an active form.[87] Remarkably, caspase-14 is the only active caspase present in human or mouse skin under normal conditions.[80, 88] The recent development of caspase-14 deficient mice has demonstrated that this protease has two important functions in keratinocytes, 1) as a profilaggrin protease and 2) to protect against UV radiation and damage.[23] Homozygous null mice lacking caspase-14 were phenotypically normal but showed a mild barrier defect characterized by increased transepidermal water loss. More significantly, profilaggrin processing was abnormal as shown by the accumulation of abnormal low molecular weight (15-20 kDa) peptides smaller than filaggrin.[23] These findings suggest that caspase-14 plays a key role in the terminal degradation of filaggrin to free amino acids. In addition, keratohyalin granules were abnormal, suggesting that caspase-14 may play a role in initial profilaggrin processing as well. *In vitro* studies showed that profilaggrin contains multiple caspase-14 sites, one in the N-terminus and one in the middle of each filaggrin domain.[22] A second major finding of these studies was that caspase-14 deficient mice were very sensitive to UV light, and displayed a reduced UVB filtering capacity characterized by higher levels of cyclobutane pyrimidine dimers after irradiation.[23] The reason for this deficiency is unknown, but one possibility is that because the mutant mice were unable to process filaggrin to free amino acids, they may lack urocanic acid which has a photoprotective function in the *stratum corneum*.

In addition to its role as a profilaggrin protease and in UV protection, caspase-14 also plays a central role in epidermal barrier repair following acute barrier disruption. Caspase-14 is activated during barrier repair in mice after tape stripping, in association with an increase in terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL)-positive cells containing fragmented DNA.[2] The TUNEL-positive cells did not, however, show the classical hallmarks of apoptosis such as caspase-3 activation and PARP cleavage. Caspase-14 deficiency resulted in a slower barrier recovery and poor cornification which correlated with little or no increase in the number of TUNEL-positive cells following barrier disruption.[2] These results suggest that caspase-14 plays a central role in various types of epidermal stress responses, and appears to be essential for limiting subsequent tissue damage and cell death.

To date, no disorders have been identified that involve caspase-14 mutations, although many diseases show altered caspase-14 expression. This altered expression probably relates to perturbations in keratinocyte differentiation rather than specific loss or gain of caspase-14.[40]

IV. CONCLUDING REMARKS AND FUTURE CHALLENGES

Stratified epithelia, and in particular the epidermis, produce a plethora of structural proteins and other molecules which form the protective epithelial barrier. This barrier is constantly being repaired and replaced allowing terrestrial mammals to survive in dry, hostile environments. Filaggrin and caspase-14 represent two epithelial-specific gene products that function as critical players in the formation and maintenance of the epithelial barrier. Filaggrin plays an important role both as a structural component of the barrier and in the hydration of the *stratum corneum* as free amino acids/NMF. The functional importance of filaggrin is underscored by its association with two skin diseases, ichthyosis vulgaris and atopic eczema. Likewise, caspase-14 plays an important protective role against solar irradiation (UVB in particular) and as a profilaggrin protease.

What are some of the remaining research questions and challenges? For filaggrin, we need to understand the mechanism(s) by which FLG mutations lead to atopic eczema, an inflammatory disease with multiple genetic and environmental inputs. Genetic screening of patients for FLG mutations is now possible, with the caveat that different mutations are prevalent in different populations, so genetic testing would have to be tailored based on ethnicity and other considerations.[56] Dermatologists may need to think about filaggrin deficiency in the context of other skin diseases as well, as it has been shown that FLG mutations may exacerbate other conditions such as X-linked ichthyosis[89] and alopecia areata.[90] Variation in the size of the profilaggrin gene (and hence the number of filaggrin domains and corresponding amount of filaggrin produced) may be correlated with dry skin in the general population.[91] Clearly, new therapeutic strategies for eczema aimed in repairing the barrier defect and/or restoring filaggrin expression are worthwhile avenues of future investigation.

Another interesting question is why filaggrin gene mutations are so common in the human population. From an evolutionary standpoint, it suggests a positive selective influence similar to the example of the β-globin gene mutation that causes sickle cell anemia.[92] Indeed, it has been suggested that a partially defective (imperfect) epithelial barrier afforded by filaggrin null alleles might act as a "natural vaccination route", allowing our immune system to be activated as a result of entry of bacterial or viral antigens which might enable humans to more readily fight infections.[56] This is an attractive idea that could be pursued using the appropriate filaggrin knock-down or knockout animal models challenged with various pathogens. Future work on caspase-14 will undoubtedly focus on the mechanism by which it acts as a UV filter. Caspase-14 likely has additional target substrates besides profilaggrin.[93] The use of animal models including caspase-14 knockout mice and the flaky tail mutant mouse will be invaluable for these future efforts.

Acknowledgments

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