

Proteases: common culprits in human skin disorders

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Recent findings from the clinic and the laboratory have transformed the way proteases and their inhibitors are perceived in the outermost layer of the skin, the epidermis. It now appears that an integrated proteolytic network operates within the epidermis, comprising more than 30 enzymes that carry out a growing list of essential functions. Equally, defective regulation or execution of protease-mediated processes is emerging as a key contributor to diverse human skin pathologies, and in recent years the number of diseases attributable to aberrant proteolytic activity has more than doubled. Here, we survey the different roles of proteases in epidermal homeostasis (from processing enzymes to signalling molecules) and explore the spectrum of rare and common human skin disorders where proteolytic pathways are dysregulated.

The epidermis: structure meets function

The survival of all living organisms depends on maintaining a clear boundary between themselves and the environment. This task is relatively simple for single cell organisms, but for higher order species, more elaborate defensive structures are required to protect both internal and external surfaces. For humans and many other terrestrial animals, the skin establishes a critical border by providing the body with an impenetrable protective covering. This function predominantly relies on the outermost layer of the skin, the epidermis (see [Glossary](#)), which includes hair follicles and their appendages, and is remarkably just a few layers of cells in depth. However, this slender appearance is deceptive. Up close, the epidermis is exquisitely ordered, incredibly dynamic, and immensely complex. In addition to being an effective physical barrier, the epidermis is equipped with biochemical defence systems, sustains an active self-renewal programme that regenerates the entire epidermis every few months, and

works in close partnership with the immune system to defend against external threats.

Structurally, the epidermis is organised into distinct layers, which are visible in cross-sections as a continuum of keratinocytes in all stages of maturation ([Box 1](#)). This stratified arrangement is maintained by the continual production and differentiation of keratinocytes, a process that must be tightly regulated to ensure that the epidermal barrier remains functional as it continues to evolve. Accordingly, the stepwise maturation of keratinocytes is a carefully choreographed routine that is guided by converging signals from a myriad of interconnected pathways [[1](#)]. Proteases and their inhibitors are rapidly emerging as significant contributors throughout this critical operation.

The epidermis is a rich source of proteases that collectively display almost the full repertoire of catalytic chemistries ([Figure 1](#)). Different proteases and inhibitors are found in each layer of the epidermis and reside in various cellular compartments, including within organelles, anchored to (and even inside) membranes, and in the extracellular space. Although all proteases have essentially the same basic function (to cleave peptide bonds), the nature of their dedicated substrates and the biological effects triggered by their processing are exceptionally diverse. Already, these actions are known to include degrading unwanted material, modifying precursor molecules, and activating key cellular pathways, and there are potentially many more protease substrates, inhibitors, and functions that remain to be defined. Each process is vital for epidermal homeostasis and thus must be properly executed and strictly controlled. Indeed, dysregulation of proteolytic pathways is now recognised as the underlying cause of a growing number of human skin pathologies.

Intersecting protease cascades exist within the epidermis

Proteases are typically expressed as inactive precursors, or zymogens, containing an N-terminal pro-peptide that must be removed before the protease can acquire full activity. This is an important checkpoint that separates the activity of a protease from its expression. Consequently, proteases are often organised into activation cascades where individual enzymes lie dormant until their pro-peptide is cleaved. Newly activated proteases are able to amplify the cascade by activating downstream protease targets or execute their intended function.

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Glossary

Acanthosis: abnormal thickening of the living layers of the epidermis (stratum basale, stratum spinosum, and stratum granulosum), generally resulting from keratinocyte hyperproliferation.

Cathelicidin antimicrobial peptide: a component of the biochemical defence system of the skin, cathelicidin is expressed as an 18 kDa inactive precursor that is cleaved by proteases to liberate a 37 amino acid bioactive peptide (LL37). LL37 displays potent antimicrobial and inflammatory activity and is processed further to generate a series of fragments with enhanced antimicrobial and diminished inflammatory activity.

Corneocytes: anuclear, non-viable cellular remnants that are produced during cornification. These subunits possess specialised structural modifications that make them ideal for forming a resilient and impermeable barrier, namely reinforced cell-to-cell connections, a fortified cell membrane, and a robust cytoskeleton.

Cornification (terminal differentiation): the process of programmed cell death initiated at the interface between the granular and cornified layers that transforms keratinocytes into corneocytes.

Cornified envelope: a specialised structure formed just beneath the cell membrane during cornification. The cornified envelope comprises several proteins (including loricrin, involucrin, and small proline-rich proteins) that are crosslinked by transglutaminases and covalently bound to an outer lipid shell.

Desmosomes: important cell–cell adhesive structures that link adjacent cells together via their respective keratin intermediate filament networks. The desmosomal complex contains several proteins, each with a different function: desmosomal cadherins (extracellular cell–cell connection), plakins (anchorage to the keratin cytoskeleton), and armadillo family proteins (adaptor proteins). Modified desmosomes containing specialised components are called corneodesmosomes and are located in the stratum corneum.

Desquamation: the active renewal process in the stratum corneum whereby superficial corneocytes are gradually shed from the surface. This event counterbalances the continual production of keratinocytes in the basal layer and depends on the ability of proteases to degrade desmosomal cadherins.

Epidermis: a self-renewing, stratified epithelium that serves as the outermost layer of the skin. The epidermis primarily comprises keratinocytes, which form an ordered array overlaying the dermis, but also includes hair follicles and their appendages, such as sebaceous glands.

Erythroderma: a prominent feature of several inflammatory skin diseases that is typified by generalised redness of the skin

Filaggrin: an important, multipurpose protein expressed in the upper epidermis. Filaggrin is produced as a large (>400 kDa), histidine-rich phosphoprotein that comprises an N-terminal S100 calcium binding domain followed by 10–12 filaggrin tandem repeats (each 37 kDa). Additionally, the filaggrin repeat series is flanked at either end by additional domains of unknown function. Pro-filaggrin undergoes stepwise proteolytic processing to generate functional subunits involved in keratin aggregation, cornified envelope assembly, stratum corneum acidification and hydration, and protection against UV radiation.

Haploinsufficiency: a situation that arises in diploid organisms when one copy of a gene is defective and the single remaining copy is unable to support the normal function of the gene.

Hyperkeratosis: abnormal thickening of the stratum corneum that can result from keratinocyte hyperproliferation or impaired desquamation.

Ichthyosis: a heterogeneous family of genetic skin disorders characterised by dry, thickened, and scaly or flaky skin.

Inflammasome: a multicomponent protein complex that is assembled in response to detection of damage- or pathogen-associated signals. The inflammasome provides a molecular framework to promote activation of procaspase-1, an important protease for converting latent cytokines, such as pro-IL1 β and pro-IL18, to their respective bioactive forms.

Keratinocyte: the major cell type in the epidermis. The name keratinocyte reflects the dense keratin intermediate filament network that serves as the cytoskeleton within these cells. Keratinocytes arise from multipotent progenitors in the basal layer of the epidermis and undergo a regulated differentiation programme as they migrate towards the surface, eventually undergoing cornification to form corneocytes.

Keratoderma: a term used to describe skin pathologies where hyperkeratosis is a major clinical feature.

Kerato-hyalin granule: a storage body that accumulates in the cytoplasm of keratinocytes in the stratum granulosum and contains proteins such as pro-filaggrin and loricrin.

Lamellar body: an important storage and delivery granule that is synthesised by differentiating keratinocytes and contains pro-barrier lipids, lipid processing enzymes, proteases, and their inhibitors. Lamellar bodies fuse with the cell membrane during cornification to release their contents to the extracellular space.

Monogenic (or Mendelian) disease: a heritable disorder that is caused by mutation of a single gene.

Palmoplantar: related to palms of the hands or soles of the feet.

Papillomatosis: deep projections of the epidermis into the dermis as a consequence of keratinocyte hyperproliferation.

Parakeratosis: the retention of nuclei in corneocytes following terminal differentiation.

Proteases: a structurally diverse family of enzymes whose primary function is to cleave peptide bonds within a peptide or protein. Each protease class uses a different mechanism (involving a unique combination of amino acid residues and/or metal ions) to catalyse this reaction.

Protease inhibitor: a molecule (typically a peptide or protein) that binds to a protease and prevents it from engaging and cleaving its substrates. Most protease inhibitors are competitive inhibitors and function by blocking the active site of the target protease. Different inhibitors are tailored to regulate an entire class of proteases, a subset of several related proteases, or even a single protease, and are capable of reversible or irreversible inhibition.

Proteomic profiling: a method for analysing the full repertoire of proteins present in complex biological samples. Modern proteomic approaches use mass spectrometry-based techniques that can be applied to identify changes in protein abundance or proteolytic cleavage events that coincide with disease.

Pruritus: an itching sensation that prompts scratching.

Pseudoprotease: an inactive protease that is structurally homologous to a conventional protease but lacks catalytic activity. Pseudoproteases have been discovered in the rhomboid family and do not contain a catalytic serine residue. Note that this is distinct from a protease that carries out non-catalytic functions but is capable of catalytic activity.

Stratum corneum: the non-living, outermost layer of the epidermis. The stratum corneum comprises a tightly packed array of corneocytes that are linked together by corneodesmosomes and embedded in an impermeable lipid matrix.

Type 2 T-helper (Th2) cells: a population of T-helper cells that differentiate from naive CD4⁺ T cells and produce proinflammatory and proallergic cytokines, such as IL4, IL5, and IL13. Th2 cells induce antibody class switching in B cells, in turn promoting high serum levels of IgE.

Zymogen: the inactive precursor form of a protease. Protease zymogens contain a regulatory pro-sequence that must be removed by proteolytic cleavage before the protease can acquire full activity.

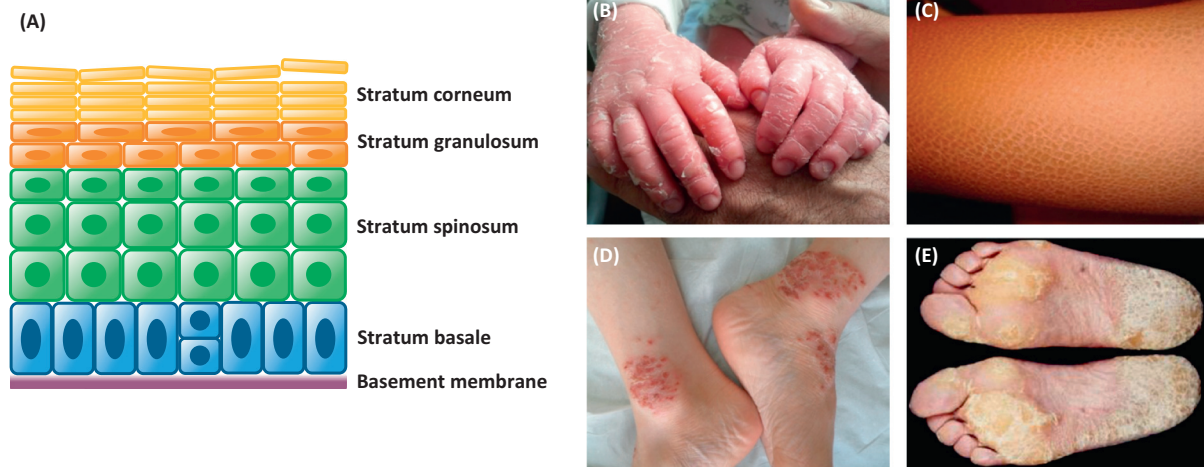
The first proteolytic cascades in the epidermis were identified as clusters of proteases associated with the same physiological process. A kallikrein-related peptidase (KLK) cascade involving KLK5, KLK7, and KLK14 was uncovered by studying activation interactions between KLK proteases linked to desquamation [2,3]. Shortly after, an activation cascade involving membrane-bound serine proteases, matriptase and prostasin, was identified and found to be initiated during terminal differentiation [4]. Activation of transglutaminases involved in crosslinking components of the cornified envelope is controlled by a protease cascade involving legumain, cathepsin L, and cathepsin D [5,6]. Additionally, activation interactions have been identified between matrix metalloproteases (MMPs) involved in wound healing and various serine proteases released by infiltrating immune cells [7].

Recent findings indicate that the degree of cooperation among epidermal proteases is greater than first thought and that the various function-specific cascades may represent different branches of a larger, integrated proteolytic network (Figure 2). This view is supported by *in vivo* proteomic profiling studies, which have revealed that loss of a single protease in the skin has unexpectedly broad effects on global proteolytic activity [8,9]. Indeed, new activation interactions continue to be discovered, such as the recent link between matriptase and the KLK cascade [10]. The matriptase–prostasin cascade has itself been revised following the discovery that prostasin is an essential cofactor for matriptase activation [11]. This suggests that the initially proposed linear cascade is in fact a reciprocal relationship in which non-catalytic binding interactions have a key role as prostasin mutants that lack catalytic activity still support matriptase activation [11]. Cleavage events that remove part of the activation pro-sequence have also surfaced. Meprin- β is able to remove part of the KLK7 pro-sequence to generate an intermediate form that can be activated more

Box 1. Ordered keratinocyte maturation confers the stratified architecture of the epidermis

Keratinocytes arise from a population of multipotent progenitor cells residing at the base of the epidermis and in the hair follicle bulge, and undergo a regulated differentiation programme as they migrate towards the surface (Figure 1A). The switch from proliferation to differentiation is controlled by several signalling cues, such as p63 and Notch, and stimulates changes in the intermediate filament network (keratin 1 and 10 replace keratin 5 and 14) and structures providing cell–cell adhesion. During their outward transit, keratinocytes synthesise the necessary protein and lipid components to construct the epidermal barrier. As cells enter the stratum granulosum and the maturation process approaches its finale (cornification), cellular activity crescendos and the last remaining preparations are made to convert keratinocytes into corneocytes (inert cellular remnants). This transformation is initiated at the interface between

the stratum granulosum and the stratum corneum, and involves a series of dramatic changes both inside and outside the cell. The intracellular space is completely reorganised as organelles are dismantled and the intermediate filament network is condensed into dense keratin macrofibrils. Additionally, the cell membrane is fortified by formation of the cornified envelope, cell-to-cell links are reinforced by insertion of new components into the desmosomal complex, and precursor lipids are secreted from lamellar bodies, processed, and aligned to form a highly impermeable matrix. These changes transform relatively ordinary cells into resilient subunits that form a durable and water-resistant cellular array. Disruption of the ordered keratinocyte differentiation programme occurs in many human skin disorders and impairs the normal structure and function of the epidermis (Figure 1B–E).



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Figure 1. The tiered structure of the epidermis and clinical features of human skin disorders. (A) Schematic representation of the normal structure of the epidermis. The four layers of the epidermis are labelled and each is shown in a different colour (stratum basale, blue; stratum spinosum, green; stratum granulosum, orange; stratum corneum, yellow). Hair follicles and their appendages are also part of the epidermis but are not represented here for simplicity. (B–E) Clinical features of human skin disorders illustrating the range of biological processes affected in different diseases: (B) Netherton syndrome, (C) ichthyosis hypotrichosis syndrome (reproduced, with permission, from [56]), (D) atopic dermatitis, and (E) tylosis oesophageal cancer syndrome (reproduced, with permission, from [43]).

efficiently [12] and KLK7 cleaves procaspase-14 to produce a secondary form that subsequently mediates conventional procaspase-14 activation [13]. These observations add several new layers of complexity to protease activation, indicating that current understanding of the epidermal protease network remains far from complete.

Protease inhibitors: a vital countermeasure in the epidermis

Although proteolytic cascades are important regulatory mechanisms, additional measures that focus on active proteases are needed. This task is performed by a large collection of protease inhibitors, each of which is tailored to target a different subset of proteases (Figure 1). Desquamation is regulated by lympho-epithelial Kazal-type inhibitor (LEKTI), a 15 domain protease inhibitor that is separated into smaller fragments by furin [14], and inhibits KLK5, KLK7, and KLK14 [15]. KLK proteolytic activity is fine-tuned by related inhibitors including serine protease inhibitor Kazal-type (SPINK)6 [16] and SPINK9 [17,18]. Matriptase and prostasin are tightly controlled by the membrane-bound inhibitor, hepatocyte growth factor

activator inhibitor-1 (HAI-1) [19,20], which is proposed to capture either protease into a stable inhibited complex shortly after activation [21]. Elafin and secretory leukocyte protease inhibitor (SLPI) are elastase inhibitors [22,23] that are upregulated following skin injury [24] and act to limit the degree of tissue destruction inflicted by immune cell proteases.

Cystatins are the predominant inhibitors of cysteine proteases in the epidermis. Cystatin A is incorporated into the cornified envelope and inhibits both endogenous (cathepsin L, cathepsin V) and exogenous proteases (dust mite cysteine proteases) [25,26]. By contrast, cystatin M/E seems to be mainly involved in regulating protease-mediated transglutaminase activation [6]. Tissue inhibitors of metalloproteases (TIMPs) are the major inhibitors of MMPs and are predominantly expressed during wound healing [7]. Members of the serpin superfamily complete the complement of epidermal protease inhibitors and provide the ability for irreversible inhibition. Squamous cell carcinoma antigen (SCCA)1 targets cysteine cathepsins [27], whereas SCCA2 inhibits chymotrypsin-like serine proteases [28]. Additionally, SERPINA12 (vaspin) has

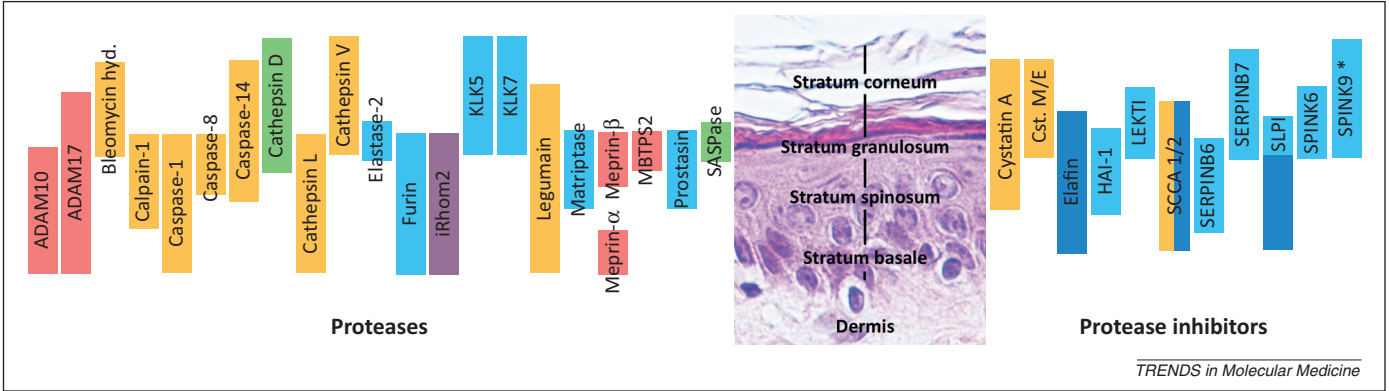


Figure 1. Proteases and their inhibitors are distributed throughout the epidermis. Localisation of key proteases and protease inhibitors produced by keratinocytes in human skin (determined from immunostaining data available in the literature). Proteases are coloured by catalytic family (metalloproteases, red; serine proteases, blue; cysteine proteases, orange; aspartic proteases, green). Purple shading indicates iRhom2, a catalytically inactive member of the rhomboid family. Protease inhibitors are coloured to reflect their target protease class (serine proteases, blue; cysteine proteases, orange). SCCA1/2 is shaded orange and blue to indicate that SCCA1 inhibits cysteine proteases and SCCA2 inhibits serine proteases. Dark blue shading indicates localisation in diseased skin and the asterisk denotes specific expression in palmoplantar epidermis (SPINK9). **Table I** shows the known target proteases for each inhibitor. Abbreviations: ADAM, a disintegrin and metalloprotease; bleomycin hyd., bleomycin hydrolase; cst. M/E, cystatin-M/E; HAI-1, hepatocyte growth factor activator inhibitor-1; KLK, kallikrein-related peptidase; LEKTI, lympho-epithelial Kazal-type inhibitor; MBTPS2, membrane-bound transcription factor protease, site-2; MMP, matrix metalloprotease; SASPase, skin aspartic protease; SCCA, squamous cell carcinoma antigen; SKALP, skin-derived antileukoprotease; SLPI, secretory leukocyte peptidase inhibitor; SPINK, serine protease inhibitor Kazal-type; TIMP, tissue inhibitor of metalloproteases.

Table I. Epidermal protease inhibitors and their known protease targets^a

Inhibitor	Known <i>in vitro</i> protease targets	Refs	Inhibitor	Known <i>in vitro</i> protease targets	Refs
Cystatin A	Cathepsin L, cathepsin V, dust mite cysteine proteases (Derp1, Derf1)	[25,26]	SCCA2 (SERPINB4)	Cathepsin G, mast cell chymase, proteinase 3	[28]
Cystatin M/E	Cathepsin L, cathepsin V, legumain	[26]	SERPINB6	Trypsin, plasmin, cathepsin G, KLK8	[30]
Elafin	Neutrophil elastase, proteinase 3, elastase 2	[22,64]	SLPI	Neutrophil elastase, cathepsin G, elastase 2, KLK7	[23,102]
HAI-1	Matriptase, prostatic	[19,20]	SPINK6	KLK5, KLK13, KLK14	[16]
LEKTI	KLK5, KLK7, KLK14, plasmin, cathepsin G, caspase-14	[15,103]	SPINK9	KLK5	[17,18]
SCCA1 (SERPINB3)	Cathepsin L, cathepsin K, cathepsin S	[27]	TIMP-1	ADAM10 and most MMPs including MMP2, MMP3, MMP9, MMP13	[104,105]

^aAbbreviations: ADAM, a disintegrin and metalloprotease; HAI, hepatocyte growth factor activator inhibitor; KLK, kallikrein-related peptidase; LEKTI, lympho-epithelial Kazal-type inhibitor; MMP, matrix metalloprotease; SCCA, squamous cell carcinoma antigen; SLPI, secretory leukocyte protease inhibitor; SPINK, serine protease inhibitor Kazal-type; TIMP, tissue inhibitor of metalloproteases.

recently been shown to inhibit KLK7 [29] and covalent KLK8/SERPINB6 complexes have been detected in differentiated keratinocytes [30]. Physiological gradients (pH and calcium) that extend throughout the epidermis also modulate proteolytic activity and broadly influence different protease classes.

New mechanisms for protease inhibitor regulation have emerged recently. Certain inhibitors appear to be substrates for non-target proteases and are inactivated once cleaved. This phenomenon has an important role in cutaneous inflammation, as illustrated by MMP2-mediated inactivation of C1 inhibitor, which promotes increased vascular permeability and complement activation [9]. Additionally, potential functions for inhibitors beyond protease regulation have surfaced, such as the proposed role of cystatin A in cornified envelope function and protecting the links between desmosomes and the keratin intermediate filament network [31].

Ordered keratinocyte differentiation relies on proteases and pseudoproteases

The involvement of proteases in epidermal homeostasis can be traced back to the first steps in the keratinocyte maturation process. As keratinocytes withdraw from the

cell cycle and commit to differentiation, proteases begin the task of liberating signalling molecules from the cell surface in order to modulate the activity of key cellular pathways. Membrane-bound ADAMs (a disintegrin and metalloproteases) feature prominently in this process and their ability to select the right ligand at the right time is guided by several layers of regulation [32].

Known substrates for ADAM10 are associated with core signalling pathways in the early stages of differentiation. Ligand binding to the Notch receptor unmasks an ADAM cleavage site within the Notch extracellular domain. ADAM10 predominantly catalyses this cleavage *in vivo* [33], which prompts a second cleavage event, by γ -secretase, to liberate the Notch intracellular domain. This fragment subsequently translocates to the nucleus and interacts with DNA-binding proteins to modulate transcription. In a similar manner, ADAM10 cleaves E-cadherin, an adherens junction component, to stimulate translocation of β -catenin to the nucleus where it activates expression of downstream target genes [34]. ADAM10 also liberates epidermal growth factor (EGF) from the cell surface [35], although this function is shared with other proteases, such as the intramembrane rhomboid serine protease, rhomboid-like 2 (RHBDL2) [36].

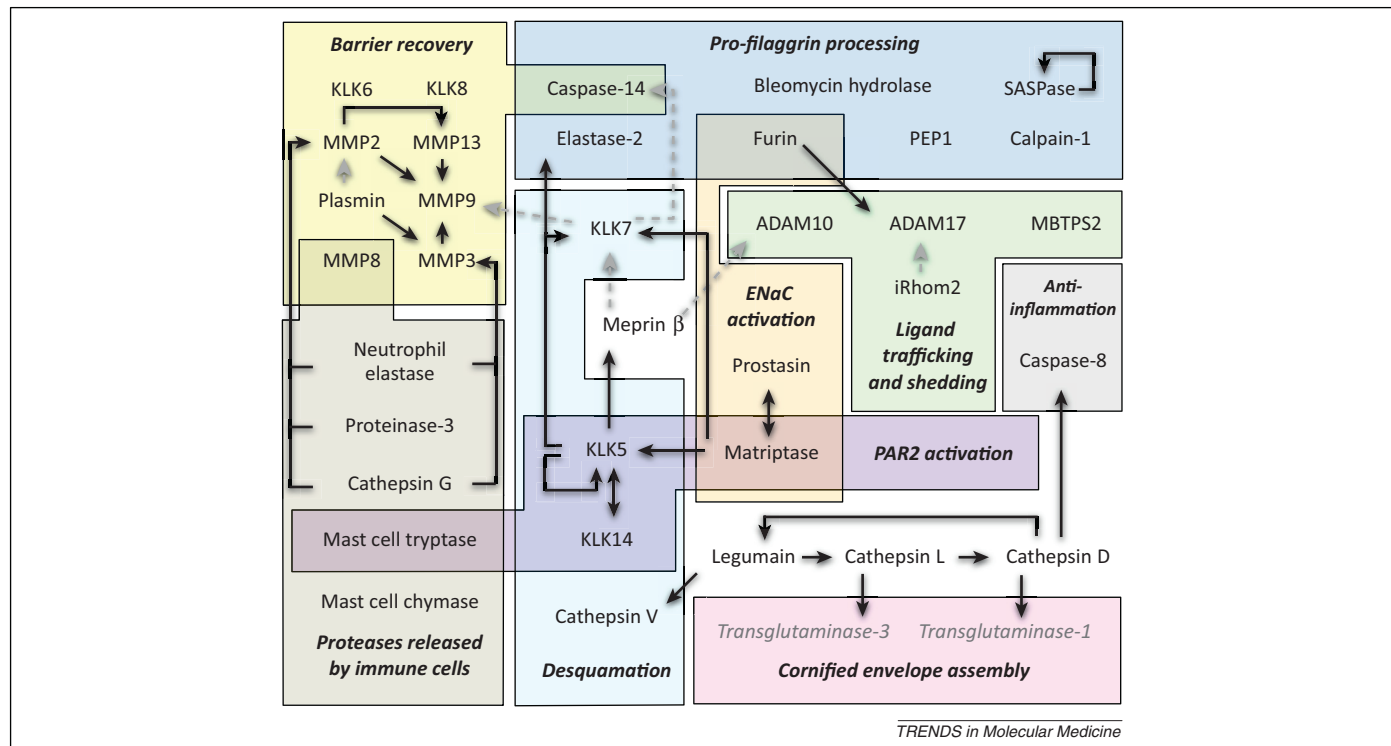


Figure 2. Piecing together the current epidermal protease network. An overview of the epidermal proteolytic network and its convoluted web of activation interactions (shown with arrows). Each box represents a different biological process involved in epidermal homeostasis. Black arrows represent conventional protease activation interactions resulting in conversion of a protease zymogen into a fully active form. Broken silver arrows indicate additional regulatory interactions including cleavage to generate an intermediate form, degradation of a regulatory domain, regulation of protease trafficking, or zymogen cleavage to produce an atypical active form. Transglutaminase-1 and -3 are shown in grey italic lettering to highlight that they are not proteolytic enzymes. Abbreviations: ADAM, a disintegrin and metalloprotease; ENaC, epithelial sodium channel; KLK, kallikrein-related peptidase; LEKTI, lympho-epithelial Kazal-type inhibitor; MBTPS2, membrane-bound transcription factor protease, site-2; MMP, matrix metalloprotease; PAR2, protease activated receptor 2; PEP, pro-filaggrin endopeptidase; RHBDL2, rhomboid-like 2; SASPase, skin aspartic protease.

Conversely, substrates for ADAM17 seem to be predominantly required in the later phases of maturation. These include several EGF receptor ligands such as transforming growth factor- α (TGF- α), heparin-binding EGF, and epiregulin [35,37]. A new regulatory mechanism for ADAM17 and EGF receptor ligands has recently emerged involving iRhom pseudoproteases, which are catalytically inactive members of the rhomboid family. iRhom1 and iRhom2 are both expressed in human skin and coordinately regulate trafficking of EGF and ADAM17 from the endoplasmic reticulum *in vivo* [38,39]. This process is particularly important for ADAM17 because it must reach the next stage of the secretory pathway, the Golgi apparatus, to be activated by furin. Additionally, iRhoms have been shown to influence stimulus-induced shedding of certain ADAM17 substrates *in vitro* [38,40].

The importance of proteases and pseudoproteases during the initial stages of keratinocyte differentiation is evident in the pathogenesis of several recently elucidated monogenic diseases (Table 1). Loss-of-function mutations in *ADAM10* cause reticulate acropigmentation of Kitamura [41], a disorder characterised by recessed, pigmented skin lesions that first appear on the hands and feet before progressing to other sites. These lesions are associated with a reduction in epidermal thickness below the granular layer and melanocyte hyperactivity [41], indicating that ADAM10 haploinsufficiency affects both keratinocytes and melanocytes. Indeed, similar effects are seen in murine models of ADAM10 deficiency. Keratinocyte-specific

deletion of *ADAM10* by conditional knockout causes impaired Notch signalling and markedly reduces the depth of the stratum spinosum [33], whereas ADAM10 haploinsufficiency in *Hairless* mice causes pigmented lesions due to melanocyte hyperproliferation [42].

Additionally, gain-of-function mutations in *RHBDF2* (rhomboid family member 2), encoding iRhom2, have been identified to cause tylosis oesophageal cancer syndrome [43]. Hallmarks of this disease include palmoplantar acanthosis with hyperkeratosis and a significantly elevated lifetime risk of developing oesophageal cancer. Cultured keratinocytes from tylosis patients display inherent tumourigenic potential and show increased proliferation and migration rates, even when exogenous EGF is withdrawn [43]. These effects are proposed to involve enhanced ADAM17 shedding of EGF receptor ligands [44], demonstrating that iRhom pseudoproteases are critical regulators of the EGF signalling axis.

Conversely, ADAM17 deficiency has been shown to cause inflammatory skin and bowel syndrome [45]. Features of this disease include a generalised pustular rash with erythema, scaling, and increased susceptibility to infection. The defective pathway or pathways in this disorder are yet to be elucidated, although impaired liberation of EGF ligands is likely to be involved. Indeed, chemotherapies targeting the EGF receptor often cause cutaneous side effects, including a pustular rash, dry skin, and pruritus [1]. Similar features are seen in keratinocyte-specific ADAM17-deficient mice, which suffer from epidermal

Table 1. The origin and pathogenesis of human skin disorders involving proteases^a

Proliferation	Differentiation	Cornification	Inflammation	Desquamation ^b	Disease	Genetic origin	Pathogenesis
					Tylosis oesophageal cancer syndrome (OMIM 148500)	Gain-of-function mutations in <i>RHBDF2</i> (17q25.1), autosomal dominant	Dysfunctional iRhom2 leads to aberrant ADAM17-mediated EGF receptor ligand shedding, causing palmoplantar keratoderma and elevated risk of oesophageal cancer
					Reticulate acropigmentation of Kitamura (OMIM 179850)	Loss-of-function mutations in <i>ADAM10</i> (15q22), autosomal dominant	ADAM10 haploinsufficiency causes recessed, pigmented lesions, probably involving defective Notch signalling in keratinocytes and melanocytes
					Inflammatory skin and bowel syndrome (OMIM 614328)	Loss-of-function mutations in <i>ADAM17</i> (2p25), autosomal recessive	Absence of functional ADAM17 causes chronic inflammation, presumably linked to defective EGF receptor ligand shedding
				–	Ichthyosis hypotrichosis syndrome (OMIM 610765)	Loss-of-function mutations in <i>ST14</i> (11q24–q25), autosomal recessive	Matriptase hypoactivity results in pronounced scaling, hyperkeratosis due to impaired desquamation, and hair defects
				–	Ichthyosis, follicular atrophoderma, and hypotrichosis (OMIM 602400)		Absence of matriptase prevents prostatic activation, causing scaling and hyperkeratosis (like IHS) but also impaired filaggrin processing and lamellar defects
					Ichthyosis follicularis with atrichia and photophobia (OMIM 308205)	Loss-of-function mutations in <i>MBTPS2</i> (Xp22.12–p22.11), X-linked recessive	MBTPS2 hypoactivity appears to prevent proper liberation of transcription factors from the Golgi membrane, impairing cholesterol biosynthesis and cornification
					Olmsted syndrome, X-linked form		Mutations close to the catalytic zinc-binding site impair MBTPS2 function and disrupt key differentiation signalling pathways
					Nagashima-type palmoplantar keratosis	Loss-of-function mutations in <i>SERPINB7</i> (18q21.3), autosomal recessive	Premature truncation mutations result in absence of functional SERPINB7, an intracellular protease inhibitor, causing hyperkeratosis, barrier defects, and mild inflammation affecting the hands and feet
				–	Papillon-Lefèvre syndrome (OMIM 245000)	Loss-of-function mutations in <i>CTSC</i> (11q14.2), autosomal recessive	Absence of functional cathepsin C causes palmoplantar acanthosis and hyperkeratosis, involving a proliferation/differentiation imbalance and impaired desquamation
				+	Netherton syndrome (OMIM 256500)	Loss-of-function mutations in <i>SPINK5</i> (5q32), autosomal recessive	Absence of LEKTI propagates an uncontrolled KLK cascade, causing stratum corneum detachment, lipid defects, inflammation, and severe allergic manifestations
				+	Acral peeling skin syndrome (OMIM 609796)	Loss-of-function mutations in <i>CSTA</i> (3q21), autosomal recessive	Cystatin A truncation causes superficial peeling associated with defective desmosome/keratin filament anchorage (observed in the stratum corneum)
				+	Exfoliative ichthyosis (OMIM 607936)		Four residue deletion within cystatin A causes superficial peeling associated with defective desmosome/keratin filament anchorage (observed in the lower epidermis)
					Atopic dermatitis	Complex, strongest risk conveyed by loss-of-function mutations in <i>FLG</i> (1q21.3)	Epidermal barrier defects and dysregulation of the immune system trigger sensitisation to allergens, persistent inflammation, pruritus, and atopic manifestations
					Rosacea	Unknown	Dysregulation of the innate immune response causes erythema, vasodilatation, and inflammation. One candidate pathway involves TLR2 and improper cathelicidin processing by KLK proteases

^aAbbreviations: ADAM, a disintegrin and metalloprotease; CSTA, cystatin A; CTSC, cathepsin C; EGF, epidermal growth factor; FLG, filaggrin; IHS, ichthyosis hypotrichosis syndrome; iRhom, inactive rhomboid; KLK, kallikrein-related peptidase; LEKTI, lympho-epithelial Kazal-type inhibitor; MBTPS2, membrane-bound transcription factor protease, site-2; OMIM, online Mendelian inheritance in man; RHBDF2, rhomboid family member 2; SERPINB7, serine protease inhibitor B7; SPINK5, serine protease inhibitor Kazal-type 5; ST14, suppressor of tumorigenesis-14; TLR2, Toll-like receptor 2.

^bPlus (+) or minus (–) signs indicate overactive or impaired desquamation, respectively.

barrier defects and chronic inflammation associated with impaired liberation of TGF- α from the cell surface and low expression of transglutaminase-1 and -3 [37].

Proteases ensure a smooth transition from life to death during cornification

Proteases continue to be heavily involved in the closing stages of the differentiation process as it reaches its finale: the transformation of keratinocytes into corneocytes [46]. This event is a defining moment in the formation of the stratum corneum and lays the foundation for epidermal barrier function. The first clue that caspases were involved in cornification was provided by similarities between terminal differentiation and programmed cell death. However, cornification is distinct from apoptosis because its initiation coincides with activation of caspase-14 but not classical executioner caspases [47]. The important role of caspase-14 in terminal differentiation is evident in *caspase-14* knockout mice, which show delayed cornification, parakeratosis, and incomplete filaggrin processing [48,49]. Caspase-8 also contributes to epidermal homeostasis and its absence causes hyperproliferation in the basal and spinous layers associated with persistent inflammation [50]. This protease has an intriguing regulatory mechanism and can activate cell survival or death pathways depending on its binding partner [51].

Cathepsin D and cathepsin L influence assembly of the cornified envelope by activating transglutaminase-1 and -3. Loss of cathepsin D in mice causes insufficient transglutaminase-1 activation leading to corneocyte structural defects and hyperkeratosis [5]. Conversely, cathepsin L deficiency is primarily associated with hair follicle abnormalities and epidermal hyperplasia in mice. Indeed, cathepsin L has been shown to function as a tumour suppressor in a squamous cell carcinoma murine model by restricting growth factor recycling [52]. Nonetheless, deleting an inhibitor of cathepsin L (cystatin M/E) in mice causes defective cornification, which is propagated by unrestrained cathepsin activity and premature transglutaminase induction, and can only be rescued by cathepsin L knockout [6].

Defects in corneocyte structure, assembly of the lamellar matrix, and filaggrin processing are also evident in the absence of membrane-bound serine proteases, matriptase [53], or prostasin [54]. Although the complete molecular basis for these features remains to be elucidated, the epithelial sodium channel (ENaC) is known to undergo proteolytic processing and is a likely candidate. Channel activation appears to be dependent on furin, which cleaves ENaC at sites within the α and γ subunits. A second cleavage event mediated by proteases, including prostasin and matriptase, is proposed to further increase channel activity. Deleting the α -ENaC subunit causes elevated stratum corneum pH and lipid abnormalities in mice, demonstrating that ENaC is vital for proper cornification [55], and may provide the link between prostasin or matriptase deficiency and lipid defects [53,54]. However, α -ENaC deletion does not alter the expression of differentiation markers or disrupt pro-filaggrin (pro-FLG) processing [55], indicating that other pathways are also affected.

Protease loss-of-function mutations have been identified in a diverse series of human skin disorders characterised by

cornification defects (Table 1). Mutations in *ST14* (suppressor of tumorigenesis-14), which encodes matriptase, cause ichthyosis hypotrichosis syndrome (IHS) [56] and ichthyosis, follicular atrophoderma, and hypotrichosis (IFAH) syndrome [57]. Both recessive diseases involve distinct plate-like scaling and hair defects, whereas lipid abnormalities, impaired filaggrin processing, and absence of active prostasin have only been observed in IFAH to date [56,57]. These differences may reflect the type of *ST14* mutations found in each disease, namely missense mutations in IHS and premature truncations in IFAH, which is significant given that non-catalytic interactions have an important role in regulating matriptase and prostasin activation [11].

Additionally, loss-of-function mutations in *MBTPS2*, encoding membrane-bound transcription factor protease, site-2, have been identified as the cause of ichthyosis follicularis with atrichia and photophobia (IFAP) syndrome [58]. *MBTPS2* is located in the Golgi apparatus and performs the second of two cleavage events to liberate membrane-anchored transcription factors involved in sterol homeostasis and the endoplasmic reticulum stress response. Cells transfected with mutant forms of *MBTPS2* display impaired induction of genes under the control of sterol-responsive elements accompanied by deficient cholesterol biosynthesis compared with cells expressing wild type *MBTPS2* [58]. *MBTPS2* mutations have also been identified in X-linked Olmsted syndrome [59], a severe and debilitating palmoplantar keratoderma, highlighting the essential role of *MBTPS2* in differentiation signalling pathways.

Very recently, loss-of-function mutations in *SERPINB7* have been found to cause Nagashima-type palmoplantar keratosis (NPPK) [60]. This disorder is typified by hyperkeratosis with mild erythema that mainly affects the hands and feet, and increased skin permeability, which causes a white, spongy appearance following water exposure [60]. *SERPINB7* is an intracellular protease inhibitor located in the granular and cornified layers, indicating that one or more intracellular proteases are hyperactive in NPPK. Although its protease targets are currently unknown, the putative sequence of its reactive centre loop suggests that *SERPINB7* inhibits serine proteases.

Proteases unlock the diverse capabilities of filaggrin

One of the critical processes set in motion during terminal differentiation is the stepwise dissection of pro-FLG, a large (>400 kDa) phosphoprotein that contains 10–12 FLG tandem repeats and is packaged into keratohyalin granules. Functional FLG monomers are liberated by successive proteolytic cleavage events (Figure 3) and participate in remarkably diverse functions as their degradation proceeds, such as condensing the intermediate filament network into dense macrofibrils, forming part of the cornified envelope, providing protection against UV radiation, and modulating stratum corneum hydration and pH [61].

Pro-FLG processing must be carefully timed, thus pro-FLG is phosphorylated to prevent premature processing and is dephosphorylated during cornification to allow proteolytic cleavage. The first cleavage sites that enable release of monomeric FLG have been well characterised, but precisely which proteases are involved and in what order

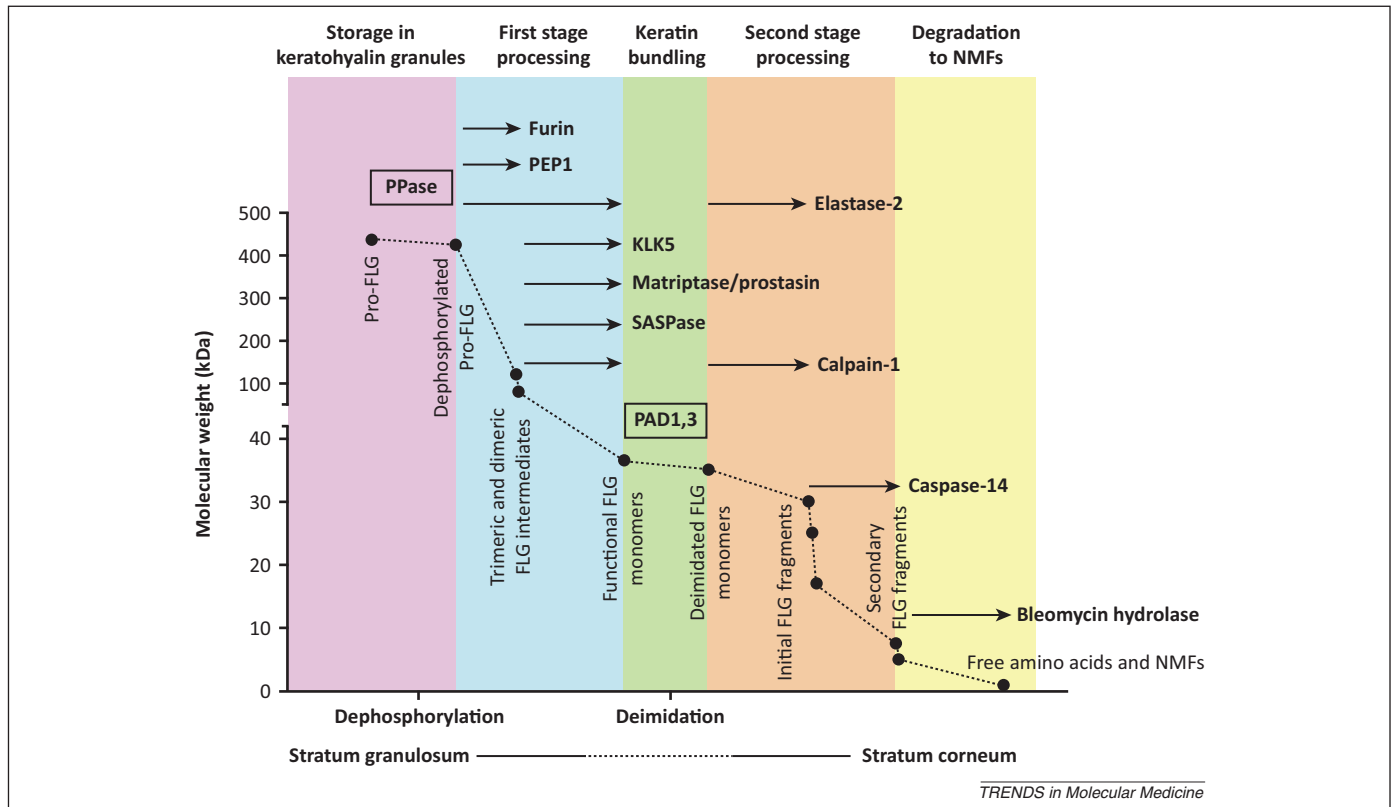


Figure 3. Proteases coordinate the multistage processing of (pro-)filaggrin. The combined involvement of serine, cysteine, and aspartic proteases in the degradation of pro-filaggrin from a high molecular precursor (>400 kDa) to single amino acids. Each processing stage is shown at the top of the graph and is shaded in a different colour on the graph area. The gradual decline in molecular weight is shown on the y-axis and non-proteolytic post-translational modifications are shown on the x-axis with corresponding enzymes shown in boxes, along with the progressive transition from the granular to cornified layer. Abbreviations: FLG, filaggrin; KLK, kallikrein-related peptidase; NMFs, natural moisturising factors; PAD, peptidylarginine deiminase; PEP1, pro-filaggrin endopeptidase-1; PPase, phosphatase; SASPase, skin aspartic protease.

remains unclear. Proteases from the proprotein convertase family, namely furin and paired basic amino acid cleaving enzyme 4 (PACE4), appear to release the N-terminal S100 domain, whereas pro-FLG endopeptidase-1 and calpain-1 were the initial candidates for separating the FLG repeat series. Recent findings indicate that this process is more complex than first thought because accumulation of dimeric and trimeric FLG repeats has been observed following deletion of matrilysin [53], prostasin [54], skin aspartic protease [62], or KLK5 [63] in various *in vivo* and *in vitro* models. A second post-translational modification, deimination, converts FLG arginine residues to citrulline, liberating FLG from assembled macrofibrils by weakening its keratin-binding activity. This event enables the next phase of processing where FLG is completely degraded to generate natural moisturising factors, trans-urocanic acid and pyrrolidone carboxylic acid, which are essential for stratum corneum hydration, acidification, and protection against UV radiation. Caspase-14 [49], elastase-2 [64], calpain-1, and bleomycin hydrolase [65] are all implicated in this process.

FLG mutations and impaired FLG processing attract considerable attention in several skin disorders [61]. The most prevalent of these is atopic dermatitis (AD), a complex multifactorial disease linked to epidermal barrier defects and dysregulation of the immune system. Loss-of-function mutations in FLG are the strongest genetic risk factor for AD [66], and impaired FLG processing has been

identified in AD patients, resulting in lower levels of natural moisturising factors [67]. Altered FLG processing in AD appears to involve several proteases, including caspase-14, which has been found to be strongly down-regulated in lesional AD skin [68], and causes elevated skin dehydration linked to incomplete FLG degradation in *caspase-14* knockout mice [49]. Additionally, impaired FLG processing has been identified in IFAH syndrome where matrilysin is absent and activation of prostasin is prevented [57]. Conversely, FLG degradation is accelerated in Netherton syndrome (NS; see below) where the absence of LEKTI results in hyperactivity of several proteases, including KLK5 and elastase-2 [64,69].

Cutting ties with the corneocyte array drives desquamation

Once the transformation from keratinocyte to corneocyte is complete, proteases assume control of the final event in epidermal homeostasis, desquamation. In this process, corneocytes are actively shed from the surface of the skin to offset the continual proliferation of keratinocytes in the basal layer. Proteases have a critical role in desquamation and gradually degrade the intercellular connections of corneodesmosomes (desmoglein-1, desmocollin-1, and corneodesmosin) to detach superficial corneocytes. Initially, two serine proteases were identified to perform this task, KLK5 and KLK7, and effects of class-specific protease inhibitors on *in vitro* corneocyte release suggested both

proteases worked cooperatively in desquamation. This process is tightly controlled by LEKTI, which functions in a pH-dependent manner to confine corneodesmosome degradation to the upper stratum corneum [15]. Additional KLKs have since been shown to be present in the epidermis [3,70]. Of these, KLK14 efficiently cleaves desmoglein-1 indicating that it may contribute to desquamation, but whether KLK8 is also involved remains unclear. Additionally, the lower pH of the upper stratum corneum lends itself to proteases that prefer acidic conditions, and two such proteases have been shown to colocalise with corneodesmosomes, namely cathepsin D [71] and cathepsin V [72]. Recently, it was reported that stratum corneum acidification (below pH 4, which is ideal for cathepsin D) inhibits corneocyte release [73]. This finding perhaps narrows the candidate desquamation proteases to KLK5, KLK7, KLK14, and cathepsin V.

An imbalance between proteolytic activity and inhibition is a recurring theme in human skin disorders that feature dysregulated desquamation (Table 1). NS is a severe ichthyosiform dermatosis characterised by congenital scaly erythroderma, a characteristic hair shaft defect, and severe atopic manifestations. NS is caused by mutations in *SPINK5*, encoding LEKTI [74], and results in a lack of detectable LEKTI expression. Consequently, proteases normally regulated by LEKTI, including KLK5 and KLK7, are left unopposed in NS, leading to premature degradation of corneodesmosomes and stratum corneum detachment as well as disruption of a range of cellular pathways [69,75,76].

Exfoliative ichthyosis is characterised by dry, scaly skin, and localised peeling at palmoplantar sites, which is aggravated by moisture and trauma. This disease is caused by mutations in *CSTA* (cystatin A), at first suggesting a protease/inhibitor imbalance like in NS. However, ultrastructural analyses have revealed that cell detachment originates in the lower levels of the epidermis, indicating an important role for cystatin A in linking desmosomes to the keratin cytoskeleton [31]. Mutations in *CSTA* have also been found in cases of acral peeling skin syndrome [77], a genetically heterogeneous condition that shows several similarities to exfoliative ichthyosis.

Disorders featuring impaired desquamation have also been identified. In IHS, retention of intact corneodesmosomes has been observed in the upper stratum corneum of patient skin [56], demonstrating that matriptase contributes to activating the KLK proteolytic cascade [10]. Impaired desquamation also features in the most severe congenital ichthyosis, Harlequin ichthyosis. This disease is caused by null mutations in *ABCA12* [adenosine triphosphate (ATP)-binding cassette subfamily A member 12] leading to lamellar body defects and impaired delivery of lipids and proteases during cornification. Reduced expression of KLK5 and cathepsin D has also been observed and probably contributes to the dramatic thickening of the stratum corneum [78]. Finally, palmoplantar acanthosis and hyperkeratosis are prominent features of Papillon-Lefèvre syndrome, a disease caused by loss-of-function mutations in *CTSC* (cathepsin C) [79]. This suggests that cathepsin C is involved in differentiation and desquamation at palmoplantar sites, although whether this is a direct or indirect

contribution remains unknown. Additionally, unstimulated natural killer (NK) cells are defective in Papillon-Lefèvre patients, revealing a role for cathepsin C in activating granzyme B, a protease contained in NK cytotoxic granules, which are released to kill pathogens [80].

Proteases, transmembrane signalling, and the emergency response to barrier disruption

In addition to constructing the epidermal barrier, proteases are at the forefront in responding to barrier disruption (Figure 4). Several molecular reflexes are triggered by a breach in the integrity of the stratum corneum. Firstly, a decline in calcium levels stimulates an immediate burst of lamellar body secretion to re-establish the permeability barrier [81]. Secondly, an increase in pH induces elevated serine protease activity and activation of protease-activated receptor (PAR)2 [82]. Thirdly, recognition of damage- or pathogen-associated signals results in inflammasome assembly and activation of proinflammatory cytokines [83].

PARs are transmembrane G-protein-coupled receptors, which are stimulated by proteolytic cleavage. Release of an N-terminal pro-peptide exposes an attached ligand for the receptor at its new amino terminus, stimulating signal transduction and activation of downstream pathways [84]. PAR2 is directly activated by KLK5, KLK14, matriptase, and mast cell tryptase but can also be disarmed by proteolytic cleavage and removal of the tethered ligand [84]. Additionally, prostasin has been shown to stimulate PAR2 activation *in vivo* and induce persistent inflammation and ichthyosis, although this probably involves an intermediate protease, matriptase [85,86]. In concert with caveolin-1 and caspase-14, PAR2 modulates barrier recovery dynamics by arresting lipid secretion and stimulating cornification in transitional keratinocytes [87,88]. PAR2 is also a prominent mediator of inflammation and pruritus [89] and induces expression of proinflammatory molecules in keratinocytes. In NS, patients suffer from persistent inflammation, pruritus, and severe allergies. Here, uncontrolled proteolytic activity caused by LEKTI deficiency leads to activation of PAR2, resulting in elevated expression of thymic stromal lymphopoietin (TSLP), a pro-type 2 T-helper (Th2) cytokine associated with allergy, as well as inflammatory cytokines including interleukin (IL)8 and tumour necrosis factor- α [90].

Elevated TSLP levels are also found in the skin and serum of AD patients and promote dysregulated T cell activation, which favours the proallergic Th2 pathway. Interestingly, cytokines released by Th2 cells, IL4 and IL13, have been shown to downregulate expression of epidermal barrier components, such as FLG [61], and increase the expression of proteases, including KLK7 [91]. The potential contribution of KLK7 to epidermal barrier defects and inflammation in AD is supported by observations that KLK7-transgenic mice develop skin abnormalities reminiscent of chronic AD lesions [92]. Additionally, LEKTI polymorphisms, such as E420K, have been associated with AD [93]. This substitution alters LEKTI processing by increasing cleavage of the domain 6–7 linker, leading to increased trypsin-like and chymotrypsin-like proteolytic activity in the skin and elevated expression of proallergic cytokines, such as TSLP [93].

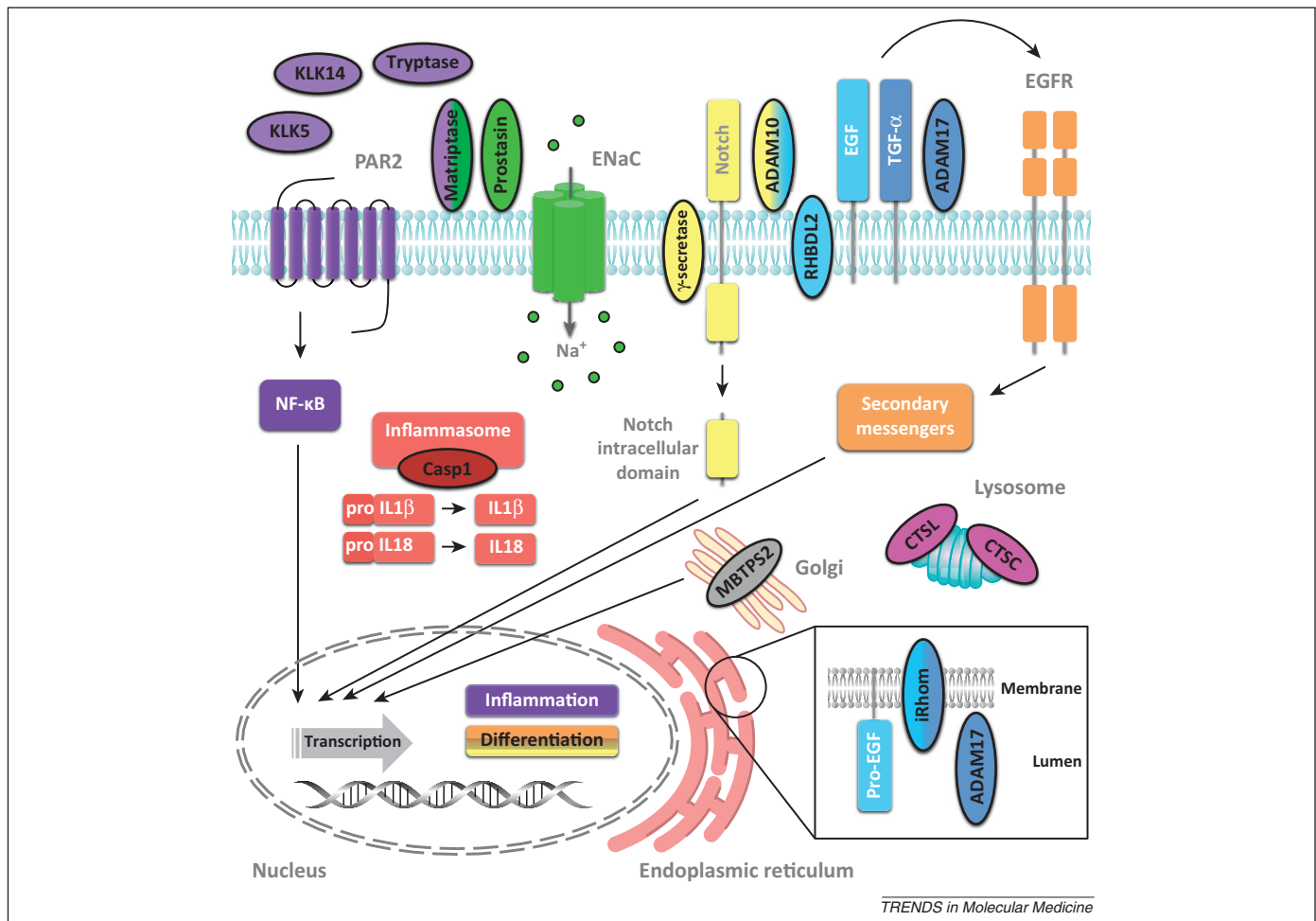


Figure 4. Proteases and pseudoproteases control core signalling and processing pathways throughout the cell. Proteases have major roles in keratinocyte developmental, differentiation, and inflammatory pathways. Proteases and their respective ligands, receptors, or ion channels are represented in the same colour. Activation of PAR2 by KLK5, KLK14, mast cell tryptase, or matriptase results in NF- κ B-mediated expression of proallergic and proinflammatory cytokines. An additional inflammatory signalling pathway in the epidermis involves caspase-1 and the inflammasome, mediating activation of pro-IL1 β and pro-IL18. Ion flux across the cell membrane is influenced by matriptase and prostatic acid phosphatase, which cleave ENaC. Proteases are also involved in activating the Notch and EGF receptor signalling pathways. ADAM10 cleaves the Notch extracellular domain, triggering release of the Notch intracellular domain by γ -secretase, whereas ADAM10 and RHBDL2 are known to release EGF and ADAM17 to release TGF- α to enable stimulation of EGFR. iRhomboid pseudoproteases also influence this process by regulating EGF and ADAM17 trafficking from the endoplasmic reticulum (ER). MBTPS2 liberates key transcription factors involved in regulating sterol biosynthesis and the ER stress response, whereas lysosomal proteases (CTSC and CTSL) have important roles in protein processing and degradation. Abbreviations: ADAM, a disintegrin and metalloprotease; casp1, caspase-1; CTSC, cathepsin C; CTSL, cathepsin L; EGF(R), epidermal growth factor (receptor); ENaC, epithelial sodium channel; IL, interleukin; iRhomb, inactivate rhomboid; KLK, kallikrein-related peptidase; MBTPS2, membrane-bound transcription factor, site-2; NF- κ B, nuclear factor kappa B; PAR, protease-activated receptor; RHBDL2, rhomboid-like 2; TGF- α , transforming growth factor- α .

In addition to inducible inflammatory cytokines, keratinocytes constitutively express IL1 β and IL18, but as inactive precursors that require proteolytic activation. This process is predominantly performed by caspase-1, which itself is normally quiescent and activated in response to damage or infection through assembly of the inflammasome [83]. Other proteases capable of activating pro-IL1 β can be found in the skin, including MMPs, KLK7, neutrophil proteases (neutrophil elastase, proteinase-3, cathepsin G), mast cell enzymes (chymase, cathepsin G), cytotoxic T lymphocyte proteases (granzyme A), and *Staphylococcus aureus* protease [94]. Cathelicidin antimicrobial peptides also undergo proteolytic processing by KLK5, KLK7 [95], and proteinase-3 [96], and aid in defence against pathogens. This procedure generates fragments with differing inflammatory and antimicrobial properties. Defective cathelicidin processing has been identified in rosacea, resulting in accumulation of the LL37 bioactive fragment as a result of increased KLK5 activity [97]. Here, the cause of elevated KLK5 activity is

not the absence of inhibitor but appears to involve increased expression and activation of Toll-like receptor 2 [98].

Once the response to prevent infection has been activated, attention shifts to tissue regeneration. Several proteases are involved in remodelling the extracellular matrix at the site of injury, including plasmin and MMPs [99]. Additionally, MMPs coordinate the immune response by activating and deactivating cytokines and chemokines [7]. Aspartic and serine proteases are also implicated in wound healing. Expression of caspase-8 has been shown to decline immediately surrounding the wound site and is linked to keratinocyte proliferation and inflammation [100]. Additionally, KLK8-deficient mice display delayed recovery to barrier injury, which is proposed to involve KLK6 and PAR2 [101].

Concluding remarks

The growing list of human skin disorders caused by loss of a single protease or inhibitor powerfully demonstrates that

Box 2. Outstanding questions

- How many additional proteases are active in the epidermis and remain to be identified?
- Does each of the protease activation interactions identified *in vitro* occur *in vivo*?
- What is the complete set of targets for each inhibitor and do they have secondary functions not related to protease inhibition?
- To what extent will proteomic profiling strategies uncover new functions for proteases and protease inhibitors in epidermal homeostasis and disease?
- Why are certain skin diseases confined to palmoplantar sites when the defective protease or inhibitor is also expressed in non-palmoplantar epidermis?
- Will therapeutic approaches targeting proteolytic cascades be successful or will individual therapies for prominent downstream protease be necessary?
- How far away from the full complement of protease substrates, pathways, and functions do we currently stand?

proteases and their inhibitors are crucial elements in epidermal homeostasis. Pathogenic mechanisms for each disease reveal that proteases are not only protein degradation and processing enzymes but they also function as key signalling molecules in an expanding set of cellular pathways. Consequently, skin diseases involving aberrant proteolytic activity impact on a broad spectrum of biological processes, including keratinocyte proliferation, differentiation, cornification, and desquamation, as well as hair follicle morphogenesis, melanocyte function, and immune system regulation.

Collectively, these findings highlight the growing potential of proteases as therapeutic targets in human skin pathologies. Indeed, many pharmaceutical companies have active research programmes aimed at translating knowledge on protease-mediated disease pathways into novel therapies. However, epidermal morphogenesis is a complex and tightly ordered process, thus replacing a defective molecule or suppressing an overactive protease is far from simple. Moreover, the full extent of many proteolytic pathways remains to be fully explored (Box 2). With this in mind, molecular replacement strategies must take care to restrict the activity of the substitute protein to the right time and place. Additionally, protease inhibitor engineering approaches should aim for an appropriate level of selectivity in order to minimise interference with unrelated proteolytic pathways. These challenges are surmountable and this rapidly expanding field of research in skin biology holds the promise of new translational developments for novel treatments of rare and more prevalent skin diseases.

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References

- Mascia, F. *et al.* (2012) The black box illuminated: signals and signaling. *J. Invest. Dermatol.* 132, 811–819
- Brattsand, M. *et al.* (2005) A proteolytic cascade of kallikreins in the stratum corneum. *J. Invest. Dermatol.* 124, 198–203
- Borgono, C.A. *et al.* (2007) A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. *J. Biol. Chem.* 282, 3640–3652
- Netzel-Arnett, S. *et al.* (2006) Evidence for a matriptase–prostasin proteolytic cascade regulating terminal epidermal differentiation. *J. Biol. Chem.* 281, 32941–32945
- Egberts, F. *et al.* (2004) Cathepsin D is involved in the regulation of transglutaminase 1 and epidermal differentiation. *J. Cell Sci.* 117, 2295–2307
- Zeeuwen, P.L. *et al.* (2010) The cystatin M/E-cathepsin L balance is essential for tissue homeostasis in epidermis, hair follicles, and cornea. *FASEB J.* 24, 3744–3755
- Martins, V.L. *et al.* (2013) Matrix metalloproteinases and epidermal wound repair. *Cell Tissue Res.* 351, 255–268
- Tholen, S. *et al.* (2013) Deletion of cysteine cathepsins B or L yields differential impacts on murine skin proteome and degradome. *Mol. Cell. Proteomics* 12, 611–625
- Auf dem Keller, U. *et al.* (2013) Systems-level analysis of proteolytic events in increased vascular permeability and complement activation in skin inflammation. *Sci. Signal.* 6, rs2
- Sales, K.U. *et al.* (2010) Matriptase initiates activation of epidermal pro-kallikrein and disease onset in a mouse model of Netherton syndrome. *Nat. Genet.* 42, 676–683
- Friis, S. *et al.* (2013) A matriptase–prostasin reciprocal zymogen activation complex with unique features: prostasin as a non-enzymatic co-factor for matriptase activation. *J. Biol. Chem.* 288, 19028–19039
- Ohler, A. *et al.* (2010) Analyzing the protease web in skin: meprin metalloproteases are activated specifically by KLK4, 5 and 8 vice versa leading to processing of proKLK7 thereby triggering its activation. *Biol. Chem.* 391, 455–460
- Yamamoto, M. *et al.* (2012) Kallikrein-related peptidase-7 regulates caspase-14 maturation during keratinocyte terminal differentiation by generating an intermediate form. *J. Biol. Chem.* 287, 32825–32834
- Fortugno, P. *et al.* (2011) Proteolytic activation cascade of the Netherton syndrome-defective protein, LEKTI, in the epidermis: implications for skin homeostasis. *J. Invest. Dermatol.* 131, 2223–2232
- Deraison, C. *et al.* (2007) LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. *Mol. Biol. Cell* 18, 3607–3619
- Meyer-Hoffert, U. *et al.* (2010) Isolation of SPINK6 in human skin: selective inhibitor of kallikrein-related peptidases. *J. Biol. Chem.* 285, 32174–32181
- Brattsand, M. *et al.* (2009) SPINK9: a selective, skin-specific Kazal-type serine protease inhibitor. *J. Invest. Dermatol.* 129, 1656–1665
- Meyer-Hoffert, U. *et al.* (2009) Identification of lympho-epithelial Kazal-type inhibitor 2 in human skin as a kallikrein-related peptidase 5-specific protease inhibitor. *PLoS ONE* 4, e4372
- Fan, B. *et al.* (2005) Identification of hepatocyte growth factor activator inhibitor-1B as a potential physiological inhibitor of prostasin. *J. Biol. Chem.* 280, 34513–34520
- Lin, C.Y. *et al.* (1999) Purification and characterization of a complex containing matriptase and a Kunitz-type serine protease inhibitor from human milk. *J. Biol. Chem.* 274, 18237–18242
- Chen, Y.W. *et al.* (2010) Regulation of the matriptase–prostasin cell surface proteolytic cascade by hepatocyte growth factor activator inhibitor-1 during epidermal differentiation. *J. Biol. Chem.* 285, 31755–31762
- Wiedow, O. *et al.* (1990) Elafin: an elastase-specific inhibitor of human skin. Purification, characterization, and complete amino acid sequence. *J. Biol. Chem.* 265, 14791–14795
- Wiedow, O. *et al.* (1993) Antileukoprotease in psoriatic scales. *J. Invest. Dermatol.* 101, 305–309
- Roupe, K.M. *et al.* (2010) Injury is a major inducer of epidermal innate immune responses during wound healing. *J. Invest. Dermatol.* 130, 1167–1177
- Kato, T. *et al.* (2005) Cystatin A inhibits IL-8 production by keratinocytes stimulated with Der p 1 and Der f 1: biochemical skin barrier against mite cysteine proteases. *J. Allergy Clin. Immunol.* 116, 169–176
- Cheng, T. *et al.* (2006) Cystatin M/E is a high affinity inhibitor of cathepsin V and cathepsin L by a reactive site that is distinct from the

Review

- legumain-binding site. A novel clue for the role of cystatin M/E in epidermal cornification. *J. Biol. Chem.* 281, 15893–15899
- 27 Schick, C. *et al.* (1998) Cross-class inhibition of the cysteine proteinases cathepsins K, L, and S by the serpin squamous cell carcinoma antigen 1: a kinetic analysis. *Biochemistry* 37, 5258–5266
 - 28 Schick, C. *et al.* (1997) Squamous cell carcinoma antigen 2 is a novel serpin that inhibits the chymotrypsin-like proteinases cathepsin G and mast cell chymase. *J. Biol. Chem.* 272, 1849–1855
 - 29 Heiker, J.T. *et al.* (2013) Vaspin inhibits kallikrein 7 by serpin mechanism. *Cell. Mol. Life Sci.* 70, 2569–2583
 - 30 Scott, F.L. *et al.* (2007) SerpinB6 is an inhibitor of kallikrein-8 in keratinocytes. *J. Biochem.* 142, 435–442
 - 31 Blaydon, D.C. *et al.* (2011) Mutations in *CSTA*, encoding Cystatin A, underlie exfoliative ichthyosis and reveal a role for this protease inhibitor in cell–cell adhesion. *Am. J. Hum. Genet.* 89, 564–571
 - 32 Hartmann, M. *et al.* (2013) Who decides when to cleave an ectodomain? *Trends Biochem. Sci.* 38, 111–120
 - 33 Weber, S. *et al.* (2011) The disintegrin/metalloproteinase Adam10 is essential for epidermal integrity and Notch-mediated signaling. *Development* 138, 495–505
 - 34 Maretzky, T. *et al.* (2005) ADAM10 mediates E-cadherin shedding and regulates epithelial cell–cell adhesion, migration, and β -catenin translocation. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9182–9187
 - 35 Sahin, U. *et al.* (2004) Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands. *J. Cell Biol.* 164, 769–779
 - 36 Adrain, C. *et al.* (2011) Mammalian EGF receptor activation by the rhomboid protease RHBDL2. *EMBO Rep.* 12, 421–427
 - 37 Franzke, C.W. *et al.* (2012) Epidermal ADAM17 maintains the skin barrier by regulating EGFR ligand-dependent terminal keratinocyte differentiation. *J. Exp. Med.* 209, 1105–1119
 - 38 Christova, Y. *et al.* (2013) Mammalian iRhoms have distinct physiological functions including an essential role in TACE regulation. *EMBO Rep.* 14, 884–890
 - 39 Zetl, M. *et al.* (2011) Rhomboid family pseudoproteases use the ER quality control machinery to regulate intercellular signaling. *Cell* 145, 79–91
 - 40 Maretzky, T. *et al.* (2013) iRhomb2 controls the substrate selectivity of stimulated ADAM17-dependent ectodomain shedding. *Proc. Natl. Acad. Sci. U.S.A.* 110, 11433–11438
 - 41 Kono, M. *et al.* (2013) Whole-exome sequencing identifies *ADAM10* mutations as a cause of reticulate acropigmentation of Kitamura, a clinical entity distinct from Dowling–Degos disease. *Hum. Mol. Genet.* 22, 3524–3533
 - 42 Tharmarajah, G. *et al.* (2012) *Adam10* haploinsufficiency causes freckle-like macules in Hairless mice. *Pigment Cell Melanoma Res.* 25, 555–565
 - 43 Blaydon, D.C. *et al.* (2012) *RHBDF2* mutations are associated with tylosis, a familial esophageal cancer syndrome. *Am. J. Hum. Genet.* 90, 340–346
 - 44 Etheridge, S.L. *et al.* (2013) Rhomboid proteins: a role in keratinocyte proliferation and cancer. *Cell Tissue Res.* 351, 301–307
 - 45 Blaydon, D.C. *et al.* (2011) Inflammatory skin and bowel disease linked to *ADAM17* deletion. *N. Engl. J. Med.* 365, 1502–1508
 - 46 Eckhart, L. *et al.* (2013) Cell death by cornification. *Biochim. Biophys. Acta* 1833, 3471–3480
 - 47 Lippens, S. *et al.* (2000) Epidermal differentiation does not involve the pro-apoptotic executioner caspases, but is associated with caspase-14 induction and processing. *Cell Death Differ.* 7, 1218–1224
 - 48 Hoste, E. *et al.* (2013) Caspase-14-deficient mice are more prone to the development of parakeratosis. *J. Invest. Dermatol.* 133, 742–750
 - 49 Hoste, E. *et al.* (2011) Caspase-14 is required for filaggrin degradation to natural moisturizing factors in the skin. *J. Invest. Dermatol.* 131, 2233–2241
 - 50 Kovalenko, A. *et al.* (2009) Caspase-8 deficiency in epidermal keratinocytes triggers an inflammatory skin disease. *J. Exp. Med.* 206, 2161–2177
 - 51 van Raam, B.J. and Salvesen, G.S. (2012) Proliferative versus apoptotic functions of caspase-8 Hetero or homo: the caspase-8 dimer controls cell fate. *Biochim. Biophys. Acta* 1824, 113–122
 - 52 Dennemarker, J. *et al.* (2010) Deficiency for the cysteine protease cathepsin L promotes tumor progression in mouse epidermis. *Oncogene* 29, 1611–1621
 - 53 List, K. *et al.* (2003) Loss of proteolytically processed filaggrin caused by epidermal deletion of Matriptase/MT-SP1. *J. Cell Biol.* 163, 901–910
 - 54 Leyvraz, C. *et al.* (2005) The epidermal barrier function is dependent on the serine protease CAP1/Prss8. *J. Cell Biol.* 170, 487–496
 - 55 Charles, R.P. *et al.* (2008) Postnatal requirement of the epithelial sodium channel for maintenance of epidermal barrier function. *J. Biol. Chem.* 283, 2622–2630
 - 56 Basel-Vanagaite, L. *et al.* (2007) Autosomal recessive ichthyosis with hypotrichosis caused by a mutation in *ST14*, encoding type II transmembrane serine protease matriptase. *Am. J. Hum. Genet.* 80, 467–477
 - 57 Alef, T. *et al.* (2009) Ichthyosis, follicular atrophoderma, and hypotrichosis caused by mutations in *ST14* is associated with impaired profilaggrin processing. *J. Invest. Dermatol.* 129, 862–869
 - 58 Oeffner, F. *et al.* (2009) IFAP syndrome is caused by deficiency in *MBTPS2*, an intramembrane zinc metalloprotease essential for cholesterol homeostasis and ER stress response. *Am. J. Hum. Genet.* 84, 459–467
 - 59 Haghighi, A. *et al.* (2013) A missense mutation in the *MBTPS2* gene underlies the X-linked form of Olmsted syndrome. *J. Invest. Dermatol.* 133, 571–573
 - 60 Kubo, A. *et al.* (2013) Mutations in *SERPINB7*, encoding a member of the serine protease inhibitor superfamily, cause Nagashima-type palmoplantar keratosis. *Am. J. Hum. Genet.* 93, 945–956
 - 61 Irvine, A.D. *et al.* (2011) Filaggrin mutations associated with skin and allergic diseases. *N. Engl. J. Med.* 365, 1315–1327
 - 62 Matsui, T. *et al.* (2011) SASPase regulates stratum corneum hydration through profilaggrin-to-filaggrin processing. *EMBO Mol. Med.* 3, 320–333
 - 63 Sakabe, J. *et al.* (2013) Kallikrein-related peptidase 5 functions in proteolytic processing of profilaggrin in cultured human keratinocytes. *J. Biol. Chem.* 288, 17179–17189
 - 64 Bonnart, C. *et al.* (2010) Elastase 2 is expressed in human and mouse epidermis and impairs skin barrier function in Netherton syndrome through filaggrin and lipid misprocessing. *J. Clin. Invest.* 120, 871–882
 - 65 Kamata, Y. *et al.* (2009) Neutral cysteine protease bleomycin hydrolase is essential for the breakdown of deiminated filaggrin into amino acids. *J. Biol. Chem.* 284, 12829–12836
 - 66 Palmer, C.N. *et al.* (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat. Genet.* 38, 441–446
 - 67 Kezic, S. *et al.* (2011) Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. *Allergy* 66, 934–940
 - 68 Broccardo, C.J. *et al.* (2011) Comparative proteomic profiling of patients with atopic dermatitis based on history of eczema herpeticum infection and *Staphylococcus aureus* colonization. *J. Allergy Clin. Immunol.* 127, 186–193
 - 69 Descargues, P. *et al.* (2005) Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat. Genet.* 37, 56–65
 - 70 Komatsu, N. *et al.* (2005) Multiple tissue kallikrein mRNA and protein expression in normal skin and skin diseases. *Br. J. Dermatol.* 153, 274–281
 - 71 Igarashi, S. *et al.* (2004) Cathepsin D, but not cathepsin E, degrades desmosomes during epidermal desquamation. *Br. J. Dermatol.* 151, 355–361
 - 72 Zeeuwen, P.L. *et al.* (2007) Colocalization of cystatin M/E and cathepsin V in lamellar granules and corneodesmosomes suggests a functional role in epidermal differentiation. *J. Invest. Dermatol.* 127, 120–128
 - 73 Hachem, J.P. *et al.* (2010) Acute acidification of stratum corneum membrane domains using polyhydroxyl acids improves lipid processing and inhibits degradation of corneodesmosomes. *J. Invest. Dermatol.* 130, 500–510
 - 74 Chavanas, S. *et al.* (2000) Mutations in *SPINK5*, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat. Genet.* 25, 141–142
 - 75 Descargues, P. *et al.* (2006) Corneodesmosomal cadherins are preferential targets of stratum corneum trypsin- and chymotrypsin-

- like hyperactivity in Netherton syndrome. *J. Invest. Dermatol.* 126, 1622–1632
- 76 Yang, T. *et al.* (2004) Epidermal detachment, desmosomal dissociation, and destabilization of corneodesmosin in *Spink5*^{−/−} mice. *Genes Dev.* 18, 2354–2358
 - 77 Kronic, A.L. *et al.* (2013) Acral peeling skin syndrome resulting from a homozygous nonsense mutation in the *CSTA* gene encoding cystatin A. *Pediatr. Dermatol.* 30, e87–e88
 - 78 Thomas, A.C. *et al.* (2009) Premature terminal differentiation and a reduction in specific proteases associated with loss of ABCA12 in Harlequin ichthyosis. *Am. J. Pathol.* 174, 970–978
 - 79 Toomes, C. *et al.* (1999) Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. *Nat. Genet.* 23, 421–424
 - 80 Meade, J.L. *et al.* (2006) A family with Papillon-Lefevre syndrome reveals a requirement for cathepsin C in granzyme B activation and NK cell cytolytic activity. *Blood* 107, 3665–3668
 - 81 Menon, G.K. *et al.* (1992) Lamellar body secretory response to barrier disruption. *J. Invest. Dermatol.* 98, 279–289
 - 82 Hachem, J.P. *et al.* (2006) Serine protease signaling of epidermal permeability barrier homeostasis. *J. Invest. Dermatol.* 126, 2074–2086
 - 83 Martinon, F. *et al.* (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL- β . *Mol. Cell* 10, 417–426
 - 84 Ramachandran, R. *et al.* (2012) Targeting proteinase-activated receptors: therapeutic potential and challenges. *Nat. Rev. Drug Discov.* 11, 69–86
 - 85 Camerer, E. *et al.* (2010) Local protease signaling contributes to neural tube closure in the mouse embryo. *Dev. Cell* 18, 25–38
 - 86 Frateschi, S. *et al.* (2011) PAR2 absence completely rescues inflammation and ichthyosis caused by altered CAP1/Prss8 expression in mouse skin. *Nat. Commun.* 2, 161
 - 87 Demerjian, M. *et al.* (2008) Acute modulations in permeability barrier function regulate epidermal cornification: role of caspase-14 and the protease-activated receptor type 2. *Am. J. Pathol.* 172, 86–97
 - 88 Roelandt, T. *et al.* (2009) The “caveolae brake hypothesis” and the epidermal barrier. *J. Invest. Dermatol.* 129, 927–936
 - 89 Steinhoff, M. *et al.* (2003) Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. *J. Neurosci.* 23, 6176–6180
 - 90 Briot, A. *et al.* (2009) Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. *J. Exp. Med.* 206, 1135–1147
 - 91 Morizane, S. *et al.* (2012) TH2 cytokines increase kallikrein 7 expression and function in patients with atopic dermatitis. *J. Allergy Clin. Immunol.* 130, 259–261
 - 92 Hansson, L. *et al.* (2002) Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. *J. Invest. Dermatol.* 118, 444–449
 - 93 Fortugno, P. *et al.* (2012) The 420K LEKTI variant alters LEKTI proteolytic activation and results in protease deregulation: implications for atopic dermatitis. *Hum. Mol. Genet.* 21, 4187–4200
 - 94 Stehlik, C. (2009) Multiple interleukin-1 β -converting enzymes contribute to inflammatory arthritis. *Arthritis Rheum.* 60, 3524–3530
 - 95 Yamasaki, K. *et al.* (2006) Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. *FASEB J.* 20, 2068–2080
 - 96 Sorensen, O.E. *et al.* (2001) Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* 97, 3951–3959
 - 97 Yamasaki, K. *et al.* (2007) Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat. Med.* 13, 975–980
 - 98 Yamasaki, K. *et al.* (2011) TLR2 expression is increased in rosacea and stimulates enhanced serine protease production by keratinocytes. *J. Invest. Dermatol.* 131, 688–697
 - 99 Lund, L.R. *et al.* (1999) Functional overlap between two classes of matrix-degrading proteases in wound healing. *EMBO J.* 18, 4645–4656
 - 100 Lee, P. *et al.* (2009) Dynamic expression of epidermal caspase 8 simulates a wound healing response. *Nature* 458, 519–523
 - 101 Kishibe, M. *et al.* (2012) Kallikrein-related peptidase 8-dependent skin wound healing is associated with upregulation of kallikrein-related peptidase 6 and PAR2. *J. Invest. Dermatol.* 132, 1717–1724
 - 102 Franzke, C.W. *et al.* (1996) Antileukoprotease inhibits stratum corneum chymotryptic enzyme. Evidence for a regulative function in desquamation. *J. Biol. Chem.* 271, 21886–21890
 - 103 Bennett, K. *et al.* (2010) New role for LEKTI in skin barrier formation: label-free quantitative proteomic identification of caspase 14 as a novel target for the protease inhibitor LEKTI. *J. Proteome Res.* 9, 4289–4294
 - 104 Amour, A. *et al.* (2000) The in vitro activity of ADAM-10 is inhibited by TIMP-1 and TIMP-3. *FEBS Lett.* 473, 275–279
 - 105 Olson, M.W. *et al.* (1997) Kinetic analysis of the binding of human matrix metalloproteinase-2 and -9 to tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2. *J. Biol. Chem.* 272, 29975–29983