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Abstract

Desquamation in mammalian skin is a well balanced process of producing corneocytes and shedding them from the surface of the skin. The corneodesmosome, which is a modified desmosome, is the main adhesive structure in the cornified cell layer. The major extracellular constituents of corneodesmosomes are desmoglein 1, desmocollin 1 and corneodesmosin. Proteases involved in the degradation of corneodesmosomes and their inhibitors are secreted from lamellar granules in the granular cell layer. Genetic defects in corneodesmosin and protease inhibitors result in accelerated desquamation and severe barrier impairment. Abnormalities in transportation and secretion of lamellar granules underlie ichthyosis seen in certain human diseases.

Introduction

Skin is the largest organ in the body and its main function is to cover and protect our body. The protective skin functions reside largely in the stratum corneum of the epidermis, the most superficial layer of the skin.[1,2] For a long time, the 'bricks and mortar' model proposed by Elias[3] has been used to understand the basic structure and function of stratum corneum. In this model, bricks are the cornified cells and the mortar is the intercellular lipid, which provides both barrier and cohesive functions. However, a component missing in this model has been drawing more attention recently because of its role as the most crucial cell adhesive structure of the stratum corneum. That structure is the corneodesmosome (or corneosome).[4,5] Strictly controlled degradation of corneodesmosomes is the key factor for a steady rate of desquamation (the shedding of dead corneocytes).[6] Accelerated corneodesmosome degradation results in barrier defects and delayed degradation leads to hyperkeratosis (ichthyosis). Interestingly, major players in the formation and degradation of corneodesmosomes are provided by lamellar granules (LGs) which are secreted from the granular cells of the epidermis. It has recently been revealed that abnormal transportation and/or secretion of LGs could result in ichthyosis. In this review, we give a synopsis of the current research findings regarding the formation and degradation of corneodesmosomes, transportation/secretion of LGs, as well as the pathological mechanisms of some diseases related to corneodesmosomes and LGs.

Corneodesmosomes

Found in the stratum corneum, the corneodesmosome is a modified form of desmosome which differs ultrastructurally (Figs. 1A, 1B, 1C, 2).[4,7] There is an electron dense mid-line structure in the extracellular parts (desmoglea) of desmosomes. When desmosomes are transformed into corneodesmosomes between the stratum granulosum and the stratum corneum, desmoglea loses its tri-lamellar structure and becomes homogeneously electron dense. On the cytoplasmic side,

the attachment plaque (desmosomal plaque) becomes incorporated into the cornified cell envelopes in corneodesmosomes. Keratin filaments are connected to the attachment plaque in desmosomes, whereas this association is no longer visible in corneodesmosomes in the cornified cells.

Desmosomes are composed of several cytoplasmic and transmembrane proteins.[8,9] The latter are members of the cadherins families known as desmogleins and desmocollins. Desmoglein 1 and desmocollin 1 constitute extracellular parts of corneodesmosomes as well.[10-13] In corneodesmosomes there is a unique extracellular component known as corneodesmosin. Cleavage of desmoglein 1, desmocollin 1 and corneodesmosin in the stratum corneum by kallikreins and cathepsins is a key step in desquamation (see below).

Corneodesmosin is a 52-56 kDa glycoprotein produced by keratinocytes.[11,14] It is stored and secreted by LGs. After the secretion from the apical cell surface of granular cells, corneodesmosin is localized in the extracellular structures of corneodesmosomes and covalently crosslinked to the cornified cell envelopes. This coincides with the morphological transformation of desmosomes into corneodesmosomes. In vitro studies suggest that corneodesmosin mediates homophilic binding to counterparts on adjacent corneocytes.[15] During corneocyte maturation, corneodesmosin is progressively proteolyzed. Its actual function as an adhesive molecule has recently been revealed by generation of corneodesmosin knockout mice (see below). Corneodesmosin is cleaved by kallikrein-related peptidases (KLKs) and cathepsins.[16-18]

Lamellar granules (LGs)

LGs (or lamellar bodies, Odland bodies, membrane coating granules, and keratinosomes) are membrane bound cytoplasmic organelles found in the spinous and granular cells of the epidermis (Fig. 1A, 1D).[19,20] In transmission electron microscopy pictures, LGs appear as round or oblong granules of 300 – 400 nm in length and 100 – 150 nm in width. They show characteristic ordered,

lamellate internal structures. In the granular layer, lamellar granules are fused with the plasma membrane and secrete their contents into the intercellular space. LGs contain various molecules, including lipids, proteases, protease inhibitors, anti-microbial peptides and corneodesmosomal proteins.

Elias et al. used lipase cytochemistry techniques to show that LGs are continuous with tubular structures of trans-Golgi network (TGN).[21] Norlen also suggested that TGN and LGs are continuous membrane structures.[22] Our observation supported the notion that LGs are continuous with TGN.[23,24] We have also shown that the LG-molecules are expressed and transported sequentially and separately. Interestingly, KLKs, lymphocyte-epithelial Kazal-type related inhibitor (LEKTI), cathepsins, cystatins, and corneodesmosin involved in regulation of corneocyte desquamation are all LG-molecules.

Transportation mechanisms for lamellar granules

Very little is known about the mechanisms for maturation, transportation, and secretion of LGs. A decline in cation (calcium and potassium) gradients across the epidermis stimulates the initial secretion of LGs that occurs in response to barrier disruption.[25] We have suggested that Rab11 may be involved in LG transportation.[26] Studies of two recently identified genetic human diseases have shown that SNARE molecules are involved in LG transportation and secretion (see below). Sando et al. suggested that caveolins may play a role in LG assembly, trafficking, and/or fusion.[27] Roelandt et al. proposed the ‘caveolae brake hypothesis’ where caveolin-1 delivery to the apical plasma membrane of the outermost stratum granulosum arrests LG secretion and induces cornification.[28]

Desquamation enzymes

A number of different proteases of the serine, cysteine, or aspartic protease families have been

identified in the differentiated keratinocytes.[29] Among these, KLKs and cathepsins are two groups of proteases implicated in desmosome degradation. KLKs constitute a family of 15 (chymo)trypsin-like serine proteases (KLK1-15) and function through proteolytic cascades.[30-33] In the skin, at least eight KLKs, including KLK5 (stratum corneum tryptic enzyme) and KLK7 (stratum corneum chymotryptic enzyme) are expressed and secreted to the extracellular space at the transition point between the granular and cornified layers.[34] KLK14 is unique in its high expression in the plantar epidermis.[35] Because KLK5 can activate itself as well as other KLKs, KLK5 is considered to be the initiator of KLK cascades.[30,36] It is assumed that KLK5 is autoactivated in the stratum granulosum, but its activity is quenched by immediate binding of fragments of LEKTI, a KLK inhibitor in the skin. Dissociation of the KLK5-LEKTI complexes and release of active KLK5 enzyme occurs as it diffuses into the stratum corneum. Then, KLK5 activates KLK7 and KLK14.[30] Active KLK5, KLK7, and KLK14 can digest corneodesmosomal components.[33] KLK7 directly cleaves desmocollin 1 and corneodesmosin, but is unable to degrade desmoglein 1. KLK5 induces degradation of all three components. Various factors controlling proteolytic degradation of corneodesmosomes by KLKs include protease inhibitors (see below), relative humidity, and pH gradient.[37-39]

Two cysteine proteases, cathepsin V (also called cathepsin L2 or stratum corneum thiol protease) and cathepsin L-like enzyme[17], and one aspartic acid protease known as cathepsin D are all involved in corneodesmosomal degradation.[18,40] Cathepsin V is an LG-molecule localized at desmosomes after secretion.[41] Cathepsin D is also an LG-protein,[24] but cathepsin L is not an LG-protein, even though it is localized in the cytosol.[41]

Desquamation enzyme inhibitors

Wide varieties of protease inhibitors are also present in the epidermis and are implicated in the regulation of desquamation-associated proteolysis. LEKTI is a 15-domain serine protease

inhibitor encoded by the *SPINK5* gene.[42] It is expressed in the granular layer of the epidermis and transported by LGs into the extracellular space.[23] Its fragments inhibit epidermal KLK5, -7, and -14 forming a tight binding complex.[37] A model in which pH controls KLK activities by regulating their interaction with LEKTI has been proposed.[37] According to this model, in the deep stratum corneum, neutral pH allows strong interaction between LEKTI and its KLK targets, thus preventing corneodesmosomal cleavage. As the pH acidifies moving upward, LEKTI and KLK5 dissociate, allowing proteases to progressively degrade its corneodesmosomal targets.

LEKTI2 (SPINK9) is a recently identified KLK5-specific inhibitor strongly expressed in the palmo-planter epidermis.[43,44] Two other serine protease inhibitors, thought to be involved in desquamation control, are skin-derived anti-leukoproteases (SKALP), also known as elafin, and secretory leukocyte protease inhibitor (SLPI).[45] Both inhibitors have the ability to effectively reduce desquamation in vitro. In particular, SLPI is a potent inhibitor of KLK7. Cystatin M/E is a cysteine protease inhibitor.[41,46] It inhibits cathepsin V involved in desquamation (see above). Cystatin M/E is highly expressed in the epidermis and it is secreted by LGs. After secretion, it co-localizes with cathepsin V on (corneo) desmosomes. Alpha-2 macroglobulin-like 1 (A2ML1) is a novel epidermal pan-protease inhibitor expressed in the granular layer and secreted by LGs.[47] It can bind KLK7 and may also bind cathepsin L2 and cathepsin L-like enzyme. This evidence suggests that A2ML1 may play a role in controlling the desquamation process. Cholesterol sulphate acts as a potent inhibitor of serine proteases.[48] Zn^{2+} is also a very potent inhibitor of different KLKs, including KLK5 and KLK7.[49,50]

Abnormalities in desquamation enzyme inhibitors

Netherton syndrome is a rare autosomal recessive disease characterized by severe ichthyosis, hair-shaft defects (bamboo hair) and atopic features caused by mutations in the *SPINK5* gene encoding LEKTI (Table 1). Insufficient LEKTI activity results in increased proteolytic activity

of KLKs.[51] Clinical manifestations correlate with *SPINK5* gene mutations.[52] In normal skin, LEKTI-derived inhibitors prevent corneodesmosomes from being destroyed immediately after the secretion of KLKs. In Netherton syndrome, as soon as KLKs are released, desmosomal components undergo proteolytic digestion and premature desquamation.[13,51] A null mutation in the *cystatin M/E* gene in mice results in abnormalities in cornification and desquamation as well as neonatal lethality (Table 1).[53,46]

Abnormalities of desquamation enzymes

Transgenic mice which express excessive KLK7, showed a scaly skin phenotype with epidermal hyperplasia, dermal inflammation, and severe pruritus (Table 1).[54] Mice deficient in cathepsin D showed impaired stratum corneum morphology as well.[55]

Abnormal corneodesmosomes

What would happen if corneodesmosomal adhesion molecules were abnormal? In the case of desmoglein 1, there is a disease caused by dominant mutations in the gene, namely striate palmoplantar keratoderma.[56] Electron microscopy has revealed significantly reduced numbers of desmosomes in the suprabasal layers and decreased desmosome size, but no abnormalities in stratum corneum structure or in the desquamation process have been reported.[57] Desmocollin 1 knockout mice have been generated and the mice developed acantholysis and hyperproliferation of the epidermis.[58] The stratum corneum was thickened probably due to hyperproliferation.

Until very recently, it was unknown what would happen if the corneodesmosin gene was missing. Two independent groups produced corneodesmosin knockout mice.[59,60] The mutation was lethal and the mice died within several hours of birth. In the skin, early detachment of the stratum corneum and structurally abnormal corneodesmosomes were found. The desmoglea was not electron dense when compared with that of wild type mice.[59] This clearly demonstrates that

corneodesmosin is a crucial molecule for corneocyte adhesion.

Abnormal LG transportation and secretion

There are two diseases with ichthyosis characterized by abnormal LG transportation. The first one is a new and rare autosomal recessive disease called CEDNIK syndrome.[61] CEDNIK stands for *ce*rebral *d*ysgenesis, *n*europathy, *i*chthyosis, and palmoplantar *k*eratoderma, all of which are characteristics of this disease. CEDNIK is caused by a loss-of-function mutation in the *SNAP29* gene coding a SNARE protein. SNARE proteins mediate membrane fusion between vesicles and target membranes, and the SNAP29 molecule is involved in intracellular trafficking steps between Golgi apparatus, the TGN and the plasma membrane. A large family of SNARE proteins consists of two types; v-SNARE located on vesicular membranes and t-SNARE on the target membranes.[62] Within the t-SNARE protein type, there is a syntaxin family and a SNAP-25 family of which SNAP29 is a member.

In order to see pathological mechanisms of ichthyosis in CEDNIK syndrome and to gain some insight into the functions of SNAP29 in the epidermis, we examined skin samples from patients using electron microscopy. There were countless clear vesicles or abnormal granules in the spinous, granular and cornified cells. Immunoelectron microscopy revealed that abnormal granules in CEDNIK cornified cells contain un-secreted LG-molecules such as glucosylceramides and KLK7. From these observations, we concluded that SNAP29 may be involved in maturation, transportation and secretion of LGs.

The other disease belong to this category is also a rare autosomal recessive disorder known as ARC syndrome.[63,64] In addition to *a*rthrogryposis, *r*enal dysfunction and *c*holestatic jaundice, patients develop severe ichthyosis. The nature of this ichthyosis has not yet been elucidated. We, therefore, looked at skin specimens and found abnormalities in LG secretion. LGs in the spinous and granular cell appeared normal, but a number of entombed LG-like

structures were found in the stratum corneum. ARC syndrome is caused by a loss-of-function mutation in VPS33B. VPS33B is a protein which regulates SNARE protein-mediated membrane fusion. It binds to t-SNARE, determining the specificity of this membrane fusion process. In ARC syndrome, ichthyosis is likely to be caused by abnormal secretion of LGs and delayed desquamation. These data suggest that VPS33B may regulate transportation and/or secretion of LGs.

Conclusion

We have reviewed factors involved in the process of desquamation and their abnormalities. This knowledge is essential to develop effective strategies to correct abnormal desquamation processes in various skin diseases including ichthyosis and keratoderma.

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References

1. Madison KC (2003) Barrier function of the skin: `La Raison d'etre' of the epidermis. J Invest Dermatol 121: 231-241
2. Elias PM (2005) Stratum corneum defensive functions: an integrated view. J Invest Dermatol 125: 183-200
3. Elias PM (1983) Epidermal lipids, barrier function, and desquamation. J Invest Dermatol 80 Suppl: 44s-49s
4. Raknerud N (1975) The ultrastructure of the interfollicular epidermis of the hairless (hr/hr) mouse. III. Desmosomal transformation during keratinization. J Ultrastr Res 52: 32-51
5. Chapman SJ, Walsh A, Jackson SM, Freidmann PS (1991) Lipids, proteins and corneocyte adhesion. Arch Dermatol Res 283: 167-173
6. Egelrud T (2000) Desquamation in the stratum corneum. Acta Derm Venereol Sup 208: 44-45
7. Chapman SJ, Walsh A (1990) Desmosomes, corneosomes and desquamation. An ultrastructural study of adult pig epidermis. Arch Dermatol Res 282: 304-310
8. Green KJ, Simpson CL (2007) Desmosomes: new perspectives on a classic. J Invest Dermatol 127: 2499-2515
9. Garrod D, Chidgey M (2008) Desmosome structure, composition and function. Biochim Biophys Acta 1778: 572-587
10. Suzuki Y, Koyama J, Moro O, Horii I, Kikuchi K, Tanida M, Tagami H (1996) The role of two endogeneous proteases of the stratum corneum in degradation of desmoglein-1 and their reduced activity in the skin of ichthyotic patients. Br J Dermatol 134: 460-464
11. Simon M, Montézin M, Guerrin M, Durieux J-J, Serre G (1997) Characterization and purification of human corneodesmosin, an epidermal basic glycoprotein associated with corneocyte-specific modified desmosomes. J Biol Chem 272: 31770-31776
12. Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, Egelrud T, Simon M, Serre G (2004) Degradation of Corneodesmosome Proteins by Two Serine Proteases of the Kallikrein Family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. J Invest Dermatol 122: 1235-1244
13. Descargues P, Deraison C, Prost C, Fraitag S, Mazereeuw-Hautier J, D'Alessio M, Ishida-Yamamoto A, Bodemer C, Zambruno G, Hovnanian A (2006) Corneodesmosomal cadherins are preferential targets of stratum corneum trypsin- and chymotrypsin-like hyperactivity in Netherton syndrome. J Invest Dermatol 126: 1622-1632
14. Serre G, Mils V, Haftek M, Vincent C, Croute F, Reano A, Ouhayoun J-P, Bettinger S, Soleihavoup J-P (1991) Identification of late differentiation antigens of human cornified epithelia, expressed in re-organized

desmosomes and bound to cross-linked envelope. J Invest Dermatol 97: 1061-1072

15. Jonca N, Guerrin M, Hadjiolova K, Caubet C, Gallinaro H, Simon M, Serre G (2002) Corneodesmosin, a component of epidermal corneocyte desmosomes, displays homophilic adhesive properties. J Biol Chem 277: 5024-5029
16. Simon M, Jonca N, Guerrin M, Haftek M, Bernard D, Caubet C, Egelrud T, Schmidt R, Serre G (2001) Refined characterization of corneodesmosin proteolysis during terminal differentiation of human epidermis and its relationship to desquamation. J Biol Chem 276: 20292-20299
17. Bernard D, Mehul B, Thomas-Collignon A, Simonetti L, Remy V, Bernard MA, Schmidt R (2003) Analysis of proteins with caseinolytic activity in a human stratum corneum extract revealed a yet unidentified cysteine protease and identified the so-called "stratum corneum thiol protease" as cathepsin L2. J Invest Dermatol 120: 592-600
18. Igarashi S, Takizawa T, Yasuda Y, Uchiwa H, Hayashi S, Brysk H, Robinson JM, Yamamoto K, Brysk MM, Horikoshi T (2004) Cathepsin D, but not cathepsin E, degrades desmosomes during epidermal desquamation. Br J Dermatol 151: 355-361
19. Odland GF, Holbrook K (1981) The lamellar granules of epidermis. Curr Probl Derm 9: 29-49
20. Madison KC, Sando GN, Howard EJ, True CA, Gilbert D, Swartzendruber DC, Wertz PW (1998) Lamellar granule biogenesis: a role for ceramide glucosyltransferase, lysosomal enzyme transport, and the Golgi. J Invest Dermatol Symposium Proceedings 3: 80-86
21. Elias PM, Cullander C, Mauro T, Rassner U, Komuves L, Brown BE, Menon GK (1998) The secretory granular cell: the outermost granular cell as a specialized secretory cell. J Invest Dermatol Symp Proc 3: 87-100
22. Norlen L (2001) Skin barrier formation: the membrane folding model. J Invest Dermatol 117: 823-829
23. Ishida-Yamamoto A, Deraison C, Bonnart C, Bitoun E, Robinson R, O'Brien TJ, Wakamatsu K, Ohtsubo S, Takahashi H, Hashimoto Y, Dopping-Hepenstal PJ, McGrath JA, Iizuka H, Richard G, Hovnanian A (2005) LEKTI is localized in lamellar granules, separated from KLK5 and KLK7, and is secreted in the extracellular spaces of the superficial stratum granulosum. J Invest Dermatol 124: 360-366
24. Ishida-Yamamoto A, Simon M, Kishibe M, Miyauchi Y, Takahashi H, Yoshida S, O'Brien TJ, Serre G, Iizuka H (2004) Epidermal lamellar granules transport different cargoes as distinct aggregates. J Invest Dermatol 122: 1137-1144
25. Menon GK, Price LF, Bommannan B, Elias PM, Feingold KR (1994) Selective obliteretion of the epidermal

calcium gradient lead to enhanced lamellar body secretion. J Invest Dermatol 102: 789-795

26. Ishida-Yamamoto A, Kishibe M, Takahashi H, Iizuka H (2007) Rab11 is associated with epidermal lamellar granules. J Invest Dermatol 127: 2166-2170
27. Sando GN, Zhu H, Weis JM, Richman JT, Wertz PW, Madison KC (2003) Caveolin expression and localization in human keratinocytes suggest a role in lamellar granule biogenesis. J Invest Dermatol 120: 531-541
28. Roelandt T, Giddelo C, Heughebaert C, Denecker G, Hupe M, Crumrine D, Kusuma A, Haftek M, Roseeuw D, Declercq W, Feingold KR, Elias PM, Hachem JP (2009) The "Caveolae Brake Hypothesis" and the Epidermal Barrier. J Invest Dermatol 129: 927-936
29. Ovaere P, Lippens S, Vandenabeele P, Declercq W (2009) The emerging roles of serine protease cascades in the epidermis. Trends Biochem Sci 34: 453-463
30. Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T (2005) A proteolytic cascade of kallikreins in the stratum corneum. J Invest Dermatol 124: 198-203
31. Lundwall A, Brattsand M (2008) Kallikrein-related peptidases. Cell Mol Life Sci 65: 2019-2038
32. Sotiropoulou G, Pampalakis G, Diamandis EP (2009) Functional roles of human kallikrein-related peptidases. J Biol Chem 284: 32989-32994
33. Borgono CA, Michael IP, Komatsu N, Jayakumar A, Kapadia R, Clayman GL, Sotiropoulou G, Diamandis EP (2007) A Potential Role for Multiple Tissue Kallikrein Serine Proteases in Epidermal Desquamation. J Biol Chem 282: 3640-3652
34. Komatsu N, Tsai B, Sidiropoulos M, Saijoh K, Levesque MA, Takehara K, Diamandis EP (2006) Quantification of eight tissue kallikreins in the stratum corneum and sweat. J Invest Dermatol 126: 925-929
35. Stefansson K, Brattsand M, Ny A, Glas B, Egelrud T (2006) Kallikrein-related peptidase 14 may be a major contributor to trypsin-like proteolytic activity in human stratum corneum. Biol Chem 387: 761-768
36. Michael IP, Pampalakis G, Mikolajczyk SD, Malm J, Sotiropoulou G, Diamandis EP (2006) Human tissue kallikrein 5 is a member of a proteolytic cascade pathway involved in seminal clot liquefaction and potentially in prostate cancer progression. J Biol Chem 281: 12743-12750
37. Deraison C, Bonnart C, Lopez F, Besson C, Robinson R, Jayakumar A, Wagberg F, Brattsand M, Hachem JP, Leonardsson G, Hovnanian A (2007) LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. Mol Biol Cell 18: 3607-3619
38. Watkinson A, Harding C, Moore A, Coan P (2001) Water modulation of stratum corneum chymotryptic enzyme activity and desquamation. Arch Dermatol Res 293: 470-476

39. Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM (2003) pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. J Invest Dermatol 121: 345-353

40. Horikoshi, Igarashi, Uchiwa, Brysk (1999) Role of endogenous cathepsin D-like and chymotrypsin-like proteolysis in human epidermal desquamation. Br J Dermatol 141: 453-459

41. Zeeuwen PL, Ishida-Yamamoto A, van Vlijmen-Willems IM, Cheng T, Bergers M, Iizuka H, Schalkwijk J (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

42. Magert H-J, Standker L, Kreutzmann P, Zucht H-D, Reinecke M, Sommerhoff CP, Fritz H, Gorssmann W (1999) LEKTI, a novel 15-domain type of human serine proteinase inhibitor. J Biol Chem 274: 21499-21502

43. Brattsand M, Stefansson K, Hubiche T, Nilsson SK, Egelrud T (2009) SPINK9: a selective, skin-specific Kazal-type serine protease inhibitor. J Invest Dermatol 129: 1656-1665

44. Meyer-Hoffert U, Wu Z, Schroder JM (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

45. Franzke C-W, Baici A, Bartels J, Christophers E, Wiedow O (1996) Antileukoprotease inhibits stratum corneum chymotryptic enzyme. Evidence for a regulative function in desquamation. J Biol Chem 271: 21886-21890

46. Zeeuwen PL, Cheng T, Schalkwijk J (2009) The biology of cystatin M/E and its cognate target proteases. J Invest Dermatol 129: 1327-1338

47. Galliano MF, Toulza E, Gallinaro H, Jonca N, Ishida-Yamamoto A, Serre G, Guerrin M (2006) A novel protease inhibitor of the alpha2-macroglobulin family expressed in the human epidermis. J Biol Chem 281: 5780-5789

48. Elias PM, Crumrine D, Rassner U, Hachem JP, Menon GK, Man W, Choy MH, Leypoldt L, Feingold KR, Williams ML (2004) Basis for abnormal desquamation and permeability barrier dysfunction in RXLI. J Invest Dermatol 122: 314-319

49. Debela M, Hess P, Magdolen V, Schechter NM, Steiner T, Huber R, Bode W, Goettig P (2007) Chymotryptic specificity determinants in the 1.0 A structure of the zinc-inhibited human tissue kallikrein 7. Proc Natl Acad Sci U S A 104: 16086-16091

50. Debela M, Goettig P, Magdolen V, Huber R, Schechter NM, Bode W (2007) Structural basis of the zinc inhibition of human tissue kallikrein 5. J Mol Biol 373: 1017-1031

51. Descargues P, Deraison C, Bonnart C, Kreft M, Kishibe M, Ishida-Yamamoto A, Elias P, Barrandon Y,

Zambruno G, Sonnenberg A, Hovnanian A (2005) Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat Genet 37: 56-65

52. Komatsu N, Saijoh K, Jayakumar A, Clayman GL, Tohyama M, Suga Y, Mizuno Y, Tsukamoto K, Taniuchi K, Takehara K, Diamandis EP (2008) Correlation between SPINK5 gene mutations and clinical manifestations in Netherton syndrome patients. J Invest Dermatol 128: 1148-1159
53. Zeeuwen PLJM, van Vlijmen-Willems IMJJ, Hendriks W, Merkx GFM, Schalkwijk J (2002) A null mutation in the cystatin M/E gene of ichq mice causes juvenile lethality and defects in epidermal cornification. Hum Mol Genet 11: 2867-2875
54. Hansson L, Backman A, Ny A, Edlund M, Ekholm E, Ekstrand Hammarstrom B, Tornell J, Wallbrandt P, Wennbo H, Egelrud T (2002) Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. J Invest Dermatol 118: 444-449
55. Egberts F, Heinrich M, Jensen J-M, Winoto-Morbach S, Pfeiffer S, Wickel M, Schunck M, Steude J, Saftig P, Proksch E, Schutze S (2004) Cathepsin D is involved in the regulation of transglutaminase 1 and epidermal differentiation. J Cell Sci 117: 2295-2307
56. Rickman L, Simrak D, Stevens HP, Hunt DM, King IA, Bryant SP, Eady RAJ, Leigh IM, Arnemann J, Magee AI, Kelsel DP, Buxton RS (1999) N-terminal deletion in a desmosomal cadherin causes the autosomal dominant skin disease striate palmoplantar keratoderma. Human Mol Genet 8: 971-976
57. Wan H, Dopping-Hepenstal PJC, Gratian MJ, Stone MG, Zhu G, Purkis PE, South AP, Keane F, Armstrong DKB, Buxton RS, McGrath JA, Eady RAJ (2004) Striate palmoplantar keratoderma arising from desmoplakin and desmoglein 1 mutations is associated with contrasting perturbations of desmosomes and the keratin filament network. Br J Dermatol 150: 878-891
58. Chidgey M, Brakebusch C, Gustafsson E, Cruchley A, Hail C, Kirk S, Merritt A, North A, Tselepis C, Hewitt J, Byrne C, Fassler R, Garrod D. (2001) Mice lacking desmocollin 1 show epidermal fragility accompanied by barrier defects and abnormal differentiation. J Cell Biol 155: 821-832
59. Matsumoto M, Zhou Y, Matsuo S, Nakanishi H, Hirose K, Oura H, Arase S, Ishida-Yamamoto A, Bando Y, Izumi K, Kiyonari H, Oshima N, Nakayama R, Matsushima A, Hirota F, Mouri Y, Kuroda N, Sano S, Chaplin DD (2008) Targeted deletion of the murine corneodesmosin gene delineates its essential role in skin and hair physiology. Proc Natl Acad Sci U S A 105: 6720-6724
60. Leclerc EA, Huchenq A, Mattiuzzo NR, Metzger D, Chambon P, Ghyselinck NB, Serre G, Jonca N, Guerrin M (2009) Corneodesmosin gene ablation induces lethal skin-barrier disruption and hair-follicle degeneration

related to desmosome dysfunction. J Cell Sci 122: 2699-2709

61. Sprecher E, Ishida-Yamamoto A, Mizrahi-Koren M, Rapaport D, Goldsher D, Indelman M, Topaz O, Chefetz I, Keren H, O'Brien T J, Bercovich D, Shalev S, Geiger D, Bergman R, Horowitz M, Mandel H (2005) A mutation in SNAP29, coding for a SNARE protein involved in intracellular trafficking, causes a novel neurocutaneous syndrome characterized by cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma. Am J Hum Genet 77: 242-251
62. Hong W (2005) SNAREs and traffic. Biochim Biophys Acta 1744: 493-517
63. Hershkovitz D, Mandel H, Ishida-Yamamoto A, Chefetz I, Hino B, Luder A, Indelman M, Bergman R, Sprecher E (2008) Defective lamellar granule secretion in arthrogryposis, renal dysfunction, and cholestasis syndrome caused by a mutation in VPS33B. Arch Dermatol 144: 334-340
64. Jang JY, Kim KM, Kim GH, Yu E, Lee JJ, Park YS, Yoo HW (2009) Clinical characteristics and VPS33B mutations in patients with ARC syndrome. J Pediatr Gastroenterol Nutr 48: 348-354

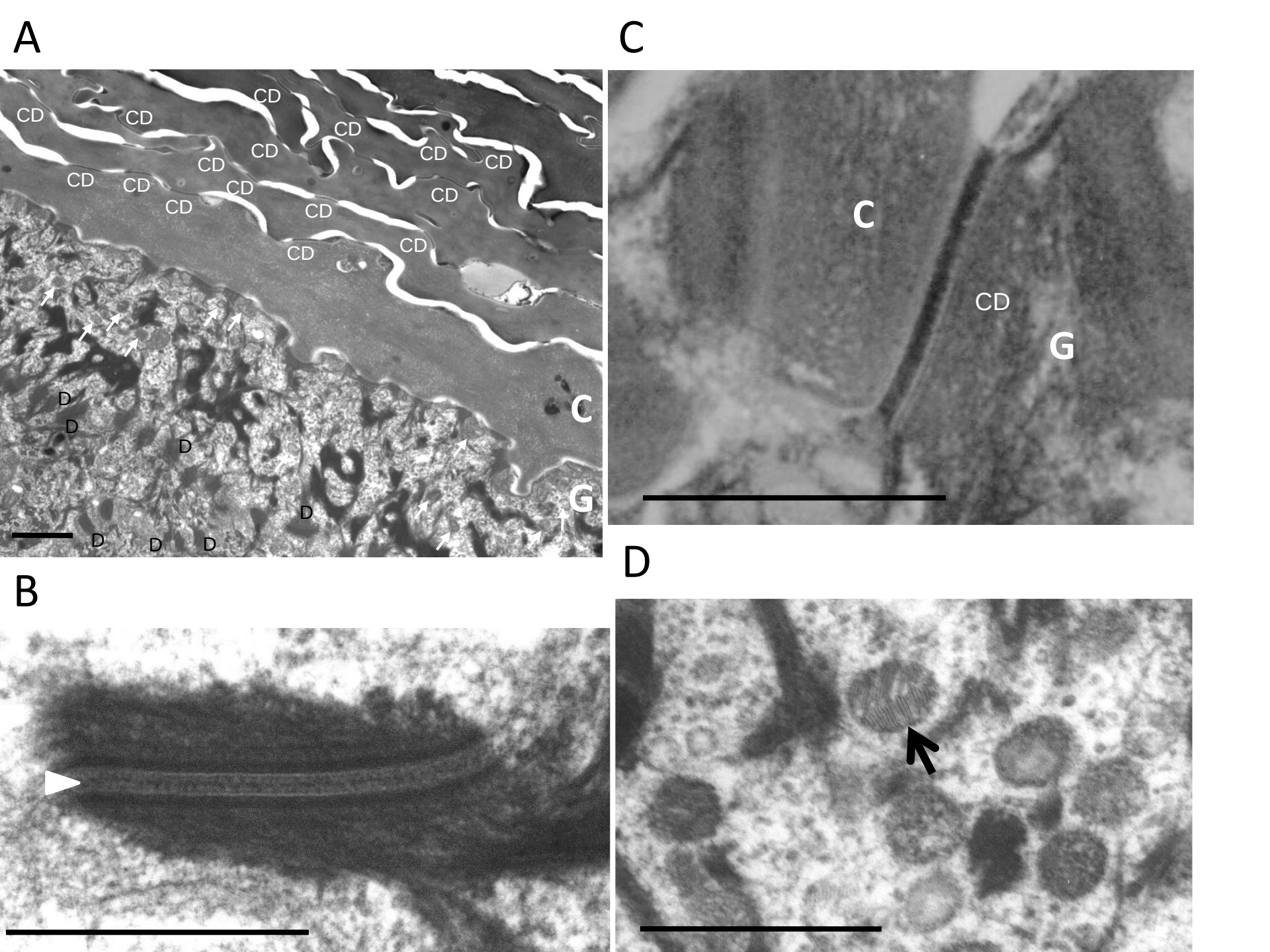
Figure legends.

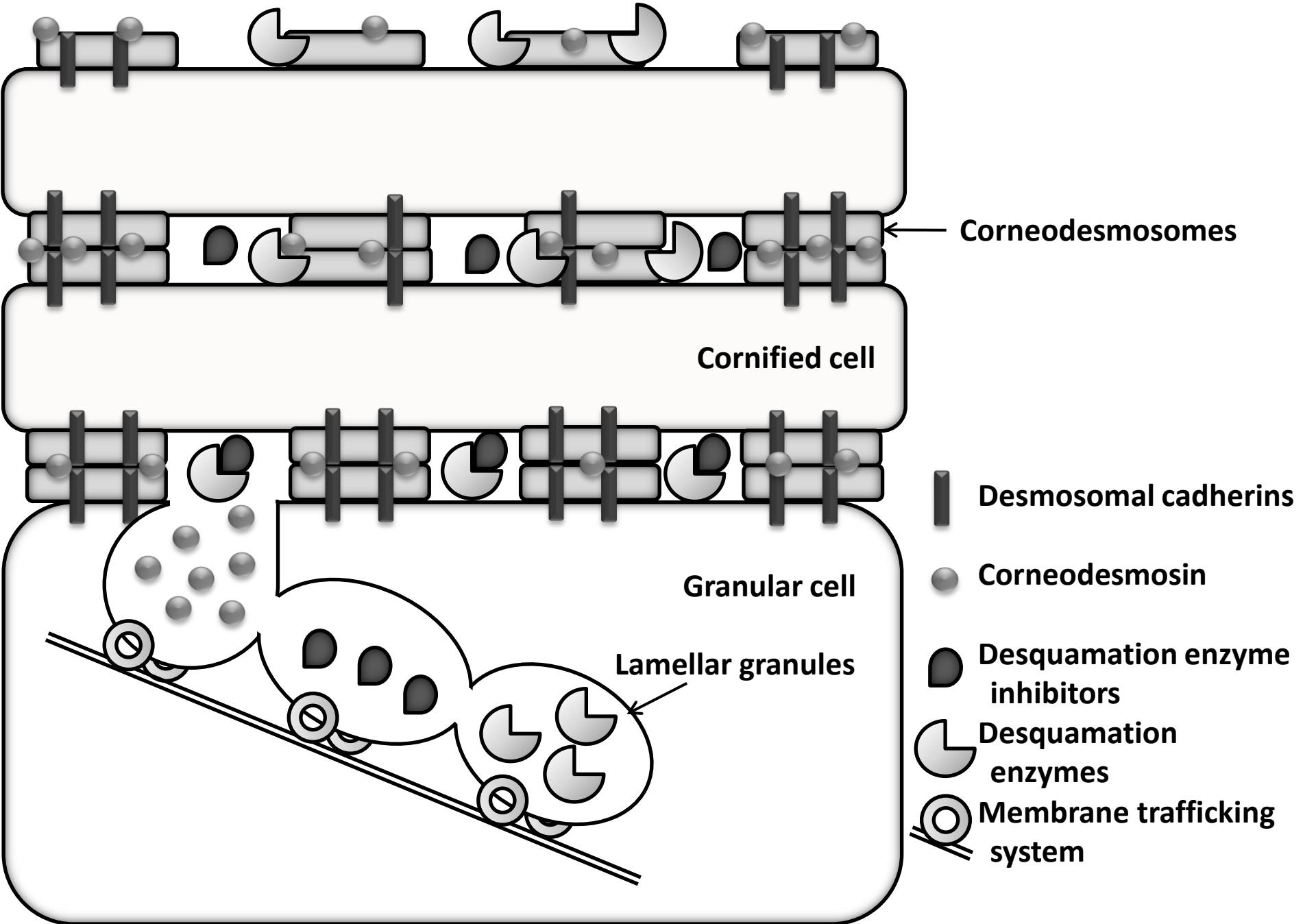
Fig. 1. (A) Ultrastructure of normal human epidermis. White arrows, lamellar granules. D, desmosomes, CD, corneodesmosomes, C, cornified cell. G, granular cell. *Bar* 1 μm. Higher magnification views of a desmosome (B), a corneodesmosome (C) and lamellar granules (D). Electron dense mid-line structure of the desmosome (arrowhead) and the laminated internal structure of lamellar granules (black arrow) are indicated. *Bars* 500 nm.

Fig. 2. A schematic model to show key members involved in the desquamation process. Lamellar granules containing corneodesmosin, desquamation enzymes and their inhibitors are transported via a membrane trafficking system and are secreted from the apical surface of granular cells. Desquamation enzymes released from inhibition by their inhibitors degrade corneodesmosomal components in the stratum corneum.

Table 1. Proteases, protease inhibitors, and diseases

Proteases	Diseases	References
KLK7 overexpression	Chronic itchy dermatitis in mice	54
Cathepsin D deficiency	Ichthyosiform skin in mice	55
Protease inhibitors		
LEKTI deficiency	Netherton syndrome	51, 52
Cystatin M/E deficiency	*ichq* mice	53


A
CD
C
G
D
B
C
CD
G
D


Corneodesmosomes
Cornified cell
Granular cell
Lamellar granules
Desmosomal cadherins
Corneodesmosin
Desquamation enzyme inhibitors
Desquamation enzymes
Membrane trafficking system