The stratum corneum and disease

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1. Introduction

The epidermis resides in the outermost layer of the skin. It plays an essential role in protecting the mammalian body from the penetration of external pathogens (an inside-to-outside barrier) and the loss of body fluids (an inside-to-outside barrier). The structural integrity of the epidermis is therefore essential in maintaining homeostasis of the mammalian body.

However, suppose genetic alterations, environmental factors or the combination thereof impair the integrity. In that case, it allows colonisation of microbes in the stratum corneum (SC), subsequent invasion of microbes or allergens and trans-epidermal water loss. These conditions lead to bacterial skin infections and allergic skin diseases, which are developed primarily or secondary to genodermatoses. Also, inflammatory changes in the skin disrupt the cutaneous barrier function.

This lecture aims to explain the primary SC components, which are associated with the skin barrier, and diseases related to aberrant cutaneous barrier functions in animals.

2. The main SC components related to skin barrier function

In the SC, corneocytes are embedded in the extracellular lipids and act as the “bricks” and “mortar” of the skin barrier.

Corneocytes are enucleated, terminally differentiated keratinocytes and the major cellular constituents in the SC. Corneocytes are differentiated from keratinocytes in the stratum granulosum (SG) through the cornification process and cover the viable epidermis. In superficial SC, corneocytes are shed from the SC surface through desquamation [1]. The extracellular lipids in the SC are a mixture of ceramides, cholesterol and free fatty acids generated in granular cells. Ceramides are the major constituents of the SC extracellular lipids in dogs [2].

2.1 Corneodesmosomes

Corneocytes are tightly linked to each other by corneodesmosomes, which contain extracellular components such as desmoglein (Dsg) 1, desmocollin (Dsc) 1, and corneodesmosins. The intercellular components of corneodesmosomes, such as plakophilins, envoplakin and periplakin, link the transmembrane constituents of corneodesmosomes and keratin filaments [3].

2.2 Filaggrin and profilaggrin

In the cytoplasm of corneocytes, filaggrin (FLG) binds to keratin intermediate filaments to form keratin bundles, which provide mechanical strength [1]. The keratin bundles also collapse the corneocytes, resulting in their flattened shape. FLG is produced as the precursor protein profilaggrin, which contains 10-12 repeats of FLG monomers in humans [4], whereas it contains 4-6 repeats in dogs [5]. It is stored in the SG and is found in keratohyalin granules. The role of FLG in the SC barrier function has been well studied using FLG-deficient mice or flaky tail mice, the latter of which have a spontaneous nonsense mutation in the FLG gene [6-8].

During cornification, profilaggrin is secreted into the cytoplasm and cleaved by proteases to generate FLG monomers. Channel-activating serine protease (CAP) 1 and skin-specific retroviral-like aspartic protease (SASPase) play crucial roles in the cleavage of profilaggrin into filaggrin monomers [9, 10]. Mice lacking those enzymes exhibited impaired cutaneous barrier function due to disturbed corneocyte morphogenesis and/or FLG processing.

In the superficial SC, FLG monomers dissociate from keratin filaments and are subsequently degraded to urocanic acid (UCA) and pyrrolidine carboxylic acid (PCA) by caspase-14, calpain-1 and bleomycin hydrolase [11, 12]. UCA decreases skin surface pH, and mice lacking FLG showed an increase in the skin surface pH [13]. There is *in vitro* evidence showing that UCA reduces the expression of co-stimulatory molecules on monocyte-derived dendritic cells and increases their ability to induce a regulatory T-cell phenotype in mixed lymphocyte reactions [14]. Indeed, epidermal Langerhans cells (LCs) expressing CD11c and CD83 increased in FLG-deficient mice [14], suggesting that the lack of UCA due to FLG deficiency may be associated with immune dysregulation in the skin. Meanwhile, PCA acts as a natural moisturising factor, which may have water-holding capacity in the SC [3].

Immunological disturbances can also downregulate FLG expression. Th2 cytokines such as IL-4, IL-13 and IL-31, as well as IL-33, which is stored in keratinocytes, downregulate FLG expression [3, 15, 16].

2.3 Cornified envelope

The cornified envelope (CE) resides beneath the plasma membrane of corneocytes, providing mechanical strength to the cell periphery. In the early stage of CE formation, envoplakin, periplakin, and involucrin accumulate beneath the plasma membrane and are crosslinked by transglutaminase (TG) 1 and TG5 [17]. Subsequently, loricrin and small proline-rich (SPR) protein families are repeatedly crosslinked by TG3 to reinforce the CE. Loricrin and SPR protein crosslink onto the involucrin scaffold via TG1 and TG5 [18]. Among the CE constituents, involucrin acts as a scaffold for the CE, while loricrin forms the majority of the CE proteins [3]. The CE proteins are thought to have compensatory effects, as single-knockout mice of genes encoding the CE proteins did not exhibit obvious skin phenotypes [19-21]. In contrast, TG1-deficient mice showed neonatal death owing to increased trans-epidermal water loss and severe dehydration [22]. Similar to FLG, expressions of loriculin and involucrin can be downregulated by Th2 cytokines [16].

2.4 Extracellular lipids

In the SC, precursor lipids of ceramides are produced and packed into lamellar granules. It has been reported that two transmembrane lipid transporters, ATP-binding cassette subfamily A member 12 (ABCA12) and transmembrane protein 79/mattrin (Tmem/Matt), play essential roles in the transportation of lamellar granule contents. ABCA12-deficient mice exhibited severe fatal skin barrier defects with accumulation of intracellular lipids in keratinocytes [23], while Tmem-deficient mice exhibited spontaneous dermatitis with defective skin barrier [24]. Glucosylceramide and sphingomyelin are ceramide precursors recognised in lamellar granules [1].

During cornification, the contents of lamellar granules are secreted to the SC-SG boundary. Glucosylceramide and sphingomyelin are metabolised into free extractable ceramides by β-glucocerebrosidase and sphingomyelinase, respectively [1]. β-glucocerebrosidase deficiency in mice led to lipid barrier defects in the skin [25]. It has been reported that sustained SC chymotryptic enzyme activity, resulting from a prolonged increase in skin pH, leads to the degradation of ceramide-producing enzymes [26], suggesting that these enzymes are potential substrates for KLKs. Free extractable ceramides in canine SC can be divided into 11 groups according to their sphingoid and fatty acid structures [27]. Among the ceramide groups, esterified ω-hydroxyceramides are converted into non-esterified ω-hydroxyceramides by 12R-lipoxygenase (12R-LOX), epidermal lipoxygenase-3 (eLOX3) and hydrolase. The ω-hydroxyceramides crosslink onto the involucrin scaffold via TG1. The lipid-involucrin crosslink forms protein-bound ceramides that anchor the extracellular lipid lamellae, consisting of free, extractable ceramides, to the CE [27, 28]. In addition to the suspected primary role of ceramides in the SC barrier function, Th2 cytokines can downregulate ceramide production [3]. Moreover, interferon-γ appeared to have an adverse effect on the SC structure and function, possibly by decreasing ceramides' long-chain fatty acids [29, 30]. These findings suggest that the skin inflammation leads to a decrease in the SC ceramides.

3. Diseases with stratum corneum abnormalities

3.1 Corneodesmosomes and diseases

There is immunofluorescence evidence showing that fluorescent intensities of corneodesmosin and claudin-1 are reduced in experimental AD dogs with house dust mite sensitisation [31]. Genodermatoses caused by mutations in genes encoding corneodesmosomal proteins have not been reported in the veterinary literature.

3.2 FLG and diseases

To date, FLG gene mutations in canine genodermatosis or other skin diseases have not been reported. Previous studies demonstrated a possible association of altered FLG transcription or protein expression in canine AD. Quantitative real-time PCR analysis revealed that FLG gene transcription in clinically non-lesional skin decreased in the subgroup of West Highland White Terriers with AD [32]. Immunostaining analysis revealed the absence of C-terminal FLG expression in a small subset of dogs with spontaneous AD [33]. In contrast, another study reported that FLG gene transcription appeared to increase in the skin of dogs

with spontaneous and experimentally induced AD, as determined by quantitative real-time PCR analysis [34, 35]. Immunostaining intensities of FLG-metabolising enzymes such as calpain-1, caspase-14 and matriptase increased in the skin obtained from an experimental canine model of AD [36], suggesting abnormal catabolism of FLG in canine atopic skin. To date, there is no consensus among researchers regarding abnormal FLG expression in CAD.

Recently, a heterozygous variant in the ASPRV1 gene, which encodes SASPase and results in altered FLG expression, was identified in a German Shepherd dog with ichthyosis [37]. Moreover, a single point mutation in the suppressor of tumorigenicity 14 gene (ST14), which encodes matriptase, causes naked foal syndrome in Akhal-Teke horses [38]. Horses with this disease are born hairless and often die within days after birth, possibly due to a cutaneous barrier defect.

3.3 Cornified envelope and diseases

LINE-1 insertion in the TG1 gene has been reported to cause lamellar ichthyosis in the Jack Russell Terrier dog [39]. In addition, it was reported that transcription of the involucrin gene was upregulated in dogs with spontaneous AD [34]; however, its clinical and biological significance remains to be further elucidated.

3.4 Extracellular lipids and diseases

Accumulating evidence indicates that mutations in lipid transporters or lipid metabolism enzymes are recognised in genodermatoses in animals. A single point mutation in the *ABCA12* gene causes ichthyosis fetalis in Chianina cattle [40]. *PNPLA1* mutation was recognised in golden retrievers and their crossbreed with autosomal recessive congenital ichthyosis (ARCI) [41, 42]. It has been reported that PNPLA1 deficiency in mice results in a defect in omega-*O*-acylceramide synthesis [43], suggesting that the gene is involved in ceramide metabolism in the skin. A splice acceptor site mutation in the *SLC27A4* gene and deficiency of NIPAL4 have been identified as causes of ARCI in Great Danes and American Bulldogs, respectively [44-46]. NIPAL4, also referred to as ichthyin, localises to keratins and desmosomes in the epidermis and is considered to play a role in the epidermal lipid metabolism [46].

A decrease in SC ceramides and alterations in ceramide profiles have been reported in veterinary literature. It has been reported that esterified or non-esterified ω-hydroxyceramides in the SC decrease in canine AD [27, 47], suggesting that the reduction of free extractable and protein-bound ceramide classes with long carbon chains is associated with this disease. In addition, non-hydroxy acylceramides have also been shown to be decreased in the SC of dogs with spontaneous AD [27, 47].

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