Kallikrein-Related Peptidase 8 (KLK8): The Structure and Function in the Epidermis

Mari Kishibe*
Department of Dermatology, Asahikawa Medical University, Japan

Abstract

Human kallikrein-related peptidases (KLKs) have been emerging as important factors in pathophysiology of skin inflammatory diseases. Among trypsin-like KLKs, KLK8 is abundantly found in the stratum corneum and in sweat: KLK8 expression is increased in inflammatory skin diseases and skin tumors. However, its function in normal and disease skin remains relatively unexplored. Several previous experiments using human samples and Klk8 knockout (Klk8-/-) mice have shown that KLK8 might be associated with skin barrier integrity and inflammation in response to external stimuli and/or wounding.

ABBREVIATIONS

KLKs: kallikrein-related Peptidases; hKLK8: Human KLK8; mKlk8: Mouse Klk8; UTR: Untranslated Region; DSG1: Desmoglein 1; DSC1: Desmocollin 1; CDSN: Corneodesmosin; PAR2: Protease-Activated Receptor 2; TPA: 12-o-tetradecanoyl-phorbol ester; UV: Ultra-Violet; Klk8-/-: Klk8 knockout; WT: Wild-Type; NGF: Nerve Growth Factor

INTRODUCTION

Human tissue kallikreins (hKLKs) comprise a subgroup of 15 serine proteases encoded by a tightly clustered multigene family on chromosome 19q13.4 [1-3]. According to the comprehensive nomenclature, KLK1 is a tissue kallikrein and the other 14 KLKs (KLK2- KLK15) are kallikrein-related peptidases [4]. They are encoded by five coding exons. The first exon encodes the 5'-Untranslated Region (UTR), the start codon, and the signal sequence [5]. The second, third, and last exons contain the histidine, aspartate and serine resides of the catalytic triad. The last exon contains the terminal codon and 3'UTR. Epidermal keratinocytes express at least nine hKLKs [6], and among these proteases, KLK7 has chymotrypsin-like activity and other KLKs, including KLK8, have been shown to have a trypsin-like activity.

An important role of hKLKs has been implicated in skin diseases characterized by abnormal barrier functions, such as atopic dermatitis [7], psoriasis vulgaris [8], acne rosacea [9], and Netherton syndrome [10]. In previous reports, KLK5 and KLK7 have drawn attention as key players in the epidermal barrier, functioning through the cleavage of adhesion molecules of the corneodesmosome [11], the maturation of antimicrobial peptides [12], and the activation of protease-activated receptor 2 [13-15].

Among several KLKs expressed in the epidermis, KLK8 has unique properties. Although the expression of KLK8 is detected in the epidermis, the function is currently unclear. This review will summarize the previous reports regarding epidermal KLK8, including the results obtained from Klk8 knockout mice experiments.

The structure of kallikrein-related peptidase 8 (KLK8) and its expression in skin

Human KLK8 (hKLK8), also known as neutropsin, ovasin, and tumor-associated differentially expressed gene-14, was originally cloned from skin cDNA as the homologue of mouse Klk8 (mKlk8) [16]. hKLK8 cDNA and the predicted amino acid sequence have 72% sequence identity to mKlk8 and key amino acid residues for the enzyme activity were conserved between human and mouse [16,17]. Since the activation motif of human KLK8 differs from that of the mouse Klk8, endogenous activators of KLK8 seem to differ between species [18]. Although hKLK8 has similar enzymatic activity to mKlk8, we must keep in mind the differences between human and mouse species to avoid misinterpretation [18].

Until now, six splicing variants of KLK8 have been identified only in human (Figure 1). The longer form (type 2) of KLK8 is generated by alternative splicing [19]. This human specific isoform is thought to contribute to learning and memory in the brain [20]. The short form splice variants, type 3 and type 4, are detected abundantly in many tissues [21]. KLK8 type 3 mRNA encodes a truncated form of the KLK8 protein. The KLK8 type 4 variant lacks exons 3-5. Since it is formed by deletion of whole exons, it encodes an incomplete signal peptide and catalytic triad of serine proteases. Therefore, the type 4 variant is not likely to be secreted with protease activity. The type 5 isoform is another shorter form lacking an exon in the 5' coding region, and type 6 has no serine protease activity [22]. Among six isoforms of KLK8, two isoforms (type 3 and type 4) are detected in normal skin [6].
and four variants (types 1-4) are detected in psoriasis skin [our unpublished data]. However, the functional significance of these splicing variants in skin remains unknown.

According to previous reports, epidermal keratinocytes express at least 9 KLKs; KLK1, KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK13, and KLK14 [6]. There are various reports on the expression of KLKs in the epidermis. Among trypsin-like KLKs, KLK8 is relatively highly expressed in the stratum corneum [23]. It is also found in sweat glands, sebaceous glands, and hair follicles [24]. Sweat contains a high proportion of KLK8 [23].

**The activation of KLK8 in the proteolytic activation cascade**

KLK activity is controlled by complex regulatory mechanisms, involving their proteolytic activation cascade, other proteases such as matrix metalloproteinase, their inhibitors, and/or cationic ions.

KLKs are synthesized as pre-pro-enzymes, and then transported separately by lamellar granules in the stratum granulosum [25]. After secretion into the stratum granulosum and stratum corneum interface, pre-KLKs are activated via removing signal peptides by other proteases and/or their activation cascade [26]. KLK5 is thought to initiate the cascade reaction through auto activation and processing of pro-KLK7 and pro-KLK14 in the human epidermis. The activated form of KLK14 then activates pro-KLK5, resulting in positive feedback [26]. The role of KLK8 in the activation cascade is relatively unknown. KLK8 could be activated by KLK5, and activated KLK8 processes pro-KLK1 and pro-KLK11 in vitro [18].

Recently, the metalloprotease meprin was found to interact with KLKs in their activation cascade. Like KLKs, meprin is released as a zymogen and acts in the intercellular space. A recent report has shown that KLK8 and other KLKs (KLK5 and KLK4) are able to activate meprin β, which is expressed in the granular layer [27]. Meprin β has the ability to trigger desquamation through the activation of pro-KLK7, which is enhanced by the presence of trypsin, and induces the activation of IL-1β and IL-18 which act as growth factors [27]. Although the optimal pH of KLK8 is 8.5, the activity is weakly retained at pH 5.0 [18], which means KLK8 has activity not only at elevated pH of the skin surface often seen in inflammatory skin diseases but also even at the normal skin surface. Therefore, KLK8 has the potential to be involved in desquamation and proliferation of keratinocytes through the activation of other proteases.

Although the activity of several KLKs is regulated by serine protease inhibitors of Kazal-type (SPINK)/Lympho-Epithelial Kazal-type-related Inhibitor (LEKTI), they have no inhibitory potential on KLK8 [18,28-30]. KLK8 is regulated more strongly by chymotrypsin-like enzyme inhibitors [18]. A recent report reveals that the activity of KLK8 is inhibited by proteinase inhibitor 6/PI-6 (serpinB6) in epidermal keratinocytes [31]. SerpinB6 and its mouse homolog SPI3/serpinb6a are co-localized with human KLK8 and mouse Klk8 in the epidermis [31]. In monocytes and neutrophils, serpinB6 inhibits chymotryptic serine proteinase cathepsin G to protect against leakage of lysosomal content during stress and inflammation [32]. Since KLKs generally act in the intercellular space after processing, whether SerpinB6 is able to inhibit aberrant intracellular activity of KLK8 in abnormal skin conditions such as inflammation or wounds remains to be elucidated.

**KLK8 in epidermal barrier formation**

According to previous reports, KLK5 and KLK7 play critical roles in the epidermal permeability barrier function in skin diseases that are characterized by skin barrier disruption. Both KLKs have been shown to be involved in skin desquamation by degrading desmosome and/or corneodesmosome component proteins such as Desmoglein 1 (DSG1), Desmocollin 1 (DSC1) and
Corneodesmosin (CDSN) in vitro [11]. Moreover, KLK1, KLK6 and KLK14 are able to cleave DSG1 [33]. According to previous in vitro experiments, KLK8 has the potential to cleave fibronectin [34,35], collagen IV [34], and L1 adhesion molecule [36], but does not degrade DSG1, DSC1, and CDSN, suggesting that KLK8 is not able to disrupt desmosomes or corneodesmosome directly.

KLK5 and KLK7 control the innate antimicrobial activity by formation of antimicrobial peptides cleaved from cathelicidin/LL-37 [12]. KLK8 is able to process synthetic LL-37 peptide in vitro, leading to production of small fragments including active antimicrobial peptides KS-30, LL-29, and LL-23, suggesting that KLK8 has the potential to enhance antimicrobial activity in the skin and sweat [18].

**KLK8 and protease-activated receptor 2 (PAR2)**

PARs, G-protein-coupled seven transmembrane domain receptors expressed by many cell types, are activated by cleavage of the extracellular N-terminal domain of the receptor, releasing a small peptide, which activates the receptor as a tethered ligand [37]. PAR2 is expressed in epidermal keratinocytes and is activated by KLK5, KLK6, KLK7 and KLK14 in vitro [38,39]. The activation of PAR2 by KLKs has received much attention in studying the regulation of keratinocyte proliferation and differentiation [40,41], epidermal barrier homeostasis [13], inflammation [14,15] and pruritus [42].

Although hKLK8 is able to cleave the synthetic peptides containing tethered ligand sequences of human PAR2, it is unable to trigger both calcium signaling and MAPK signaling through PAR2 [43]. In contrast to human KLK8, rat KLK8 can activate calcium signaling via PAR2 [43]. This difference between species is thought to be due to minor sequence differences of KLK8 between human and rat due to different glycosylation of recombinant human and rat PAR2 expressed in kidney-derived cells [43]. Our group previously suggested that KLK8 might be associated with PAR2 activation and upregulation of mKlk6 during cutaneous wound healing [44]. Therefore, at this time, it is unclear whether hKLK8 can activate PAR2 through activation of other proteases or impair the PAR2 signaling response triggered by other proteases through cleavage of its tethered ligand. However, we need to consider the differences between species regarding KLKs-mediated activation of PAR2 to interpret in vivo data.

**KLK8 and inflammatory skin diseases**

Previous reports have shown that KLK8 is upregulated in inflammatory skin diseases such as atopic dermatitis, lichen planus, and psoriasis, as well as in skin tumors [7,8,45]. Therefore, it has been speculated that KLK8 may influence the proliferation and inflammatory responses of keratinocytes.

Although there are structural differences between human KLK8 and mouse Klk8, the studies using Klk8 knockout (Klk8-/-) mice have contributed to the understanding of the role of KLK8 in the epidermis [46]. The expression of Klk8 in adult mice is lower compared to embryonic mice [46]; however, it is markedly increased in some pathological conditions induced by topical 12-o-Tetradecanoyl-Phorbol Ester (TPA), Ultra-Violet (UV) irradiation, or skin wounding [47-49].

The skins of Klk8-/- mice show no remarkable abnormality between that of Wild-Type (WT) without external stimuli [50]. UV irradiation induces acanthosis along with the increase of Klk8 mRNA expression in WT mice. The stratum corneum of Klk8-/- mice is significantly thicker than that of WT mice after UV irradiation, and the epidermis of Klk8-KO mice exhibit delayed recovery from UVB-irradiation, suggesting that Klk8 might be associated with desquamation and/or skin homeostasis [48].

Until imiquimod-induced skin inflammation is established as a good mouse psoriasis model, topical application of TPA had been used to induce the phenotype similar to psoriatic skin because of remarkable acanthosis accompanied by inflammation [51]. Our group found that mKlk8 mRNA and protein were increased in TPA-mediated psoriasis-like inflammatory skin of WT mice along with the upregulation of mKlk6 and mKlk7 [49]. On the other hand, the TPA-induced increase in mKlk6 and mKlk7 expression is greatly suppressed in Klk8-/- mice, suggesting that Klk8 is involved in the induction of other KLKs in response to external stimuli. The number of layers of the stratum corneum in Klk8-/- mice was significantly higher compared to WT mice. This was associated with the abnormal degradation of corneodesmosomal component proteins (DG51 and CDSN) in Klk8-/- mice [49]. These findings indicate that Klk8 is involved in desquamation and skin inflammation in cooperation with other KLKs. We further suggested that cutaneous wound healing was significantly delayed in Klk8-/- mice, while the mKlk6 induction and the PAR2 activation were dampened in these mice. This suggests that Klk8 is involved in cutaneous wound healing associated with mKlk6 and PAR2 activation [44].

The close relationship between KLK8 and Nerve Growth Factor (NGF) has been indicated recently. The increased expression of NGF is observed in atopic dermatitis and psoriasis [52,53]. In atopic dermatitis, the level of NGF in the stratum corneum is correlated with the severity of itching and eruptions [52]. Higher levels of NGF produced by keratinocytes in psoriatic skin can act as a mitogen and activate T cells. Blocking NGF signaling has been shown to be therapeutically effective in psoriasis [53]. A recent report has shown that NGF mRNA was lower in the epidermis of Klk8-/- mice compared to wild type mice [54]. Interestingly, Klk8 is expressed at lower levels in the epidermis of NGF-p75 knockout mice which lack the low affinity receptor of NGF. Moreover, the enhancement of Klk8 expression induced by sodium lauryl sulphate, a detergent which causes irritation and inflammation of the skin, is significantly impaired in NGF-p75 knockout mice [54]. Although the mechanism is still unclear, the interaction between KLK8 and NGF might contribute to disease severity in inflammatory skin diseases.

Recently, the expression levels of KLKs in serum and synovial fluids of psoriasis patients with or without arthritis were assessed [55]. This report suggests that KLK6 and KLK8, but not the other seven KLKs, are elevated to high levels in both psoriatic arthritis synovial fluids and psoriatic plaques [55]. Importantly, only serum KLK8 levels are significantly associated with psoriatic disease in correlation with the clinical skin severity of psoriasis [55]. Since the serum level of KLK8 has no correlation with joint symptoms, it is not a biomarker of psoriatic arthritis; however, it could be a marker of disease severity in psoriasis.
KLK8 and skin tumors

The expression of KLK8 has been investigated mainly in non-skin tumors. Overexpressed KLK8 is observed in cervical cancer [56], ovarian cancer [57,58], and oral squamous cell carcinoma [59]. KLK8 is known as a prospective biomarker of ovarian cancer [57,58]. Overexpressed type 1 and type 2 KLK8 mRNAs in lung cancer cells have been shown to have protective effects through the proteolysis of extracellular fibronectin [60]. KLK8-mediated degradation of fibronectin suppresses integrin signaling, and decreases lung cancer cell motility through inhibition of actin polymerization [60]. On the other hand, alternative transcriptional variants (type 4) of KLK8 are indicated as unfavorable markers of lung cancer [22].

In malignant skin tumors, KLK8 is upregulated in squamous cell carcinoma, which show severe hyperkeratosis [45]. However, the expression patterns of alternatively spliced forms of KLK8 and their role in skin tumor pathology remain to be fully elucidated. Further experiments are required.

CONCLUSION

The function of hKLK8 in epidermis is summarized in Figure 2. KLK8 appears to contribute little to normal skin homeostasis; however, it seems to be associated with inflammation and skin barrier recovery in response to external stimuli and/or wounding. Aberrant expression or activity of KLK8 might be involved in the pathophysiology of inflammatory skin diseases and skin tumors. Moreover, there is a possibility that KLK8 is associated with skin appendage diseases since its expression is observed in sweat and skin appendages. However, the function of KLK8 in the epidermis largely remains to be determined. Although further studies are required, elucidating the pathophysiological role of KLK8 associated with complex proteolytic cascades in inflammatory skin diseases and skin tumors will contribute to develop new treatment strategies.

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