Kallikreins: Essential epidermal messengers for regulation of the skin microenvironment during homeostasis, repair and disease

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https://doi.org/10.1016/j.mbplus.2019.100019

Abstract

As the outermost layer of the skin, the epidermis is playing a major role in organism homeostasis providing the first barrier against external aggressions. Although considered as an extracellular matrix (ECM)-poor subtissue, the epidermal microenvironment is a key regulator of skin homeostasis and functionality. Among the proteins essential for upholding the epidermal microenvironment are the members of the kallikrein (KLK) family composed of 15 secreted serine proteases. Most of the members of these epithelial-specific proteins are present in skin and regulate skin desquamation and inflammation. However, although epidermal products, the consequences of KLK activities are not confined to the epidermis but widespread in the skin. In this review starting with the location and proteolytic activation cascade of KLKs, we present KLKs involvement in skin homeostasis, regeneration and pathology. KLKs have a large variety of substrates including ECM proteins, and evidence suggests that they are involved in the different steps of skin wound healing as discussed here. KLKs are also used as prognosis/diagnosis markers for many cancer types and we are focusing later on KLKs in cutaneous cancers, although their pathogenicity remains to be fully elucidated. Dysregulation of the KLK cascade is directly responsible for skin diseases with heavy inflammatory aspects, highlighting their involvement in skin immune homeostasis. Future studies will be needed to support the therapeutic potential of adjusting KLK activities for treatment of inflammatory skin diseases and wound healing pathologies.

Introduction

Skin is the largest organs. It prevents against external aggressions such as pathogens or chemical challenges. The epidermis is the outside most layer of the skin and by its physical, chemical and immunological characteristics it majorly contributes to the skin barrier properties. It is histologically composed of four different layers. Proliferating and non-proliferating keratinocytes attached to the epidermal basement membrane constitute the basal layer. Keratinocytes progressively differentiate through the spinous and granular layers of the epidermis until they become corneocytes in the cornified layer of the epidermis (Fig. 1A). During these events, keratinocytes acquire specific features and notably modify expression of some proteins such as the epidermal-specific kallikrein (KLK) proteases.

Tissue kallikrein-related peptidases comprise a family of 15 extracellular proteases containing the human tissue kallikrein (KLK1) and the kallikrein-related peptidases (KLK2 to KLK15). KLKs are serine proteases exhibiting trypsin- or chymotrypsin-like activities and sharing important structural and functional properties. They are encoded by 15 different genes each containing five exons and being organized in a tandem cluster, on chromosome 19 in human [1]. All KLKs possess high sequence homology both at the DNA and protein level. In a similar way to other proteases, KLKs are first translated as pre-pro-polypeptides. They are

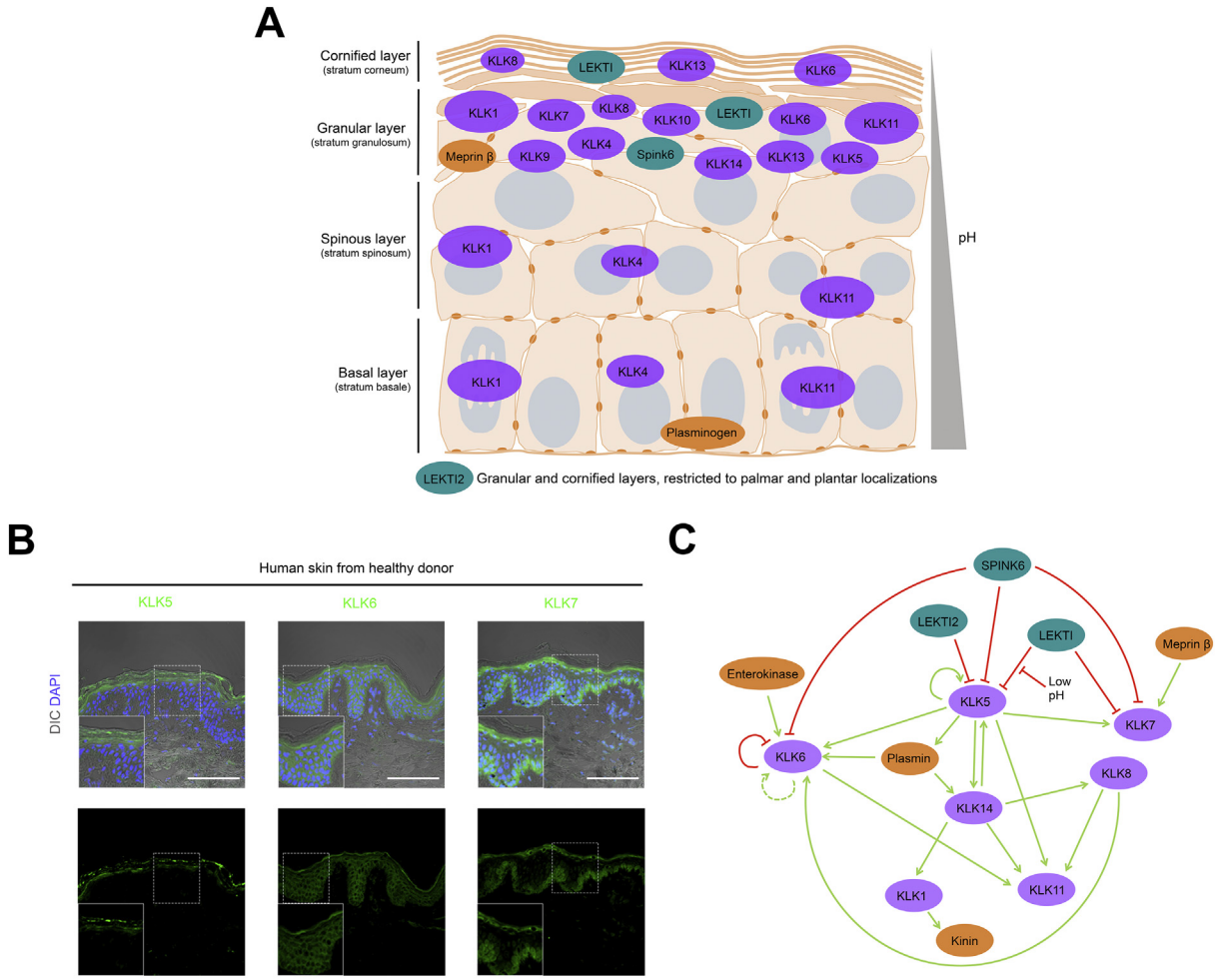


Fig. 1. Kallikreins in the epidermis and their proteolytic cascade. (A) Schematic representation of the KLKs' localization in the epidermis according to the literature (purple). Natural KLK inhibitors are shown in green and KLK activators are denoted in orange. (B) Immunofluorescent staining of KLK5 (R&D systems, AF1108), KLK6 (Abcam, 190924) and KLK7 (Abcam, 96710) in normal human skin. Nuclei were counterstained with DAPI. Differential Interference Contrast (DIC) was used to visualize the non-nucleated cells in the upper epidermis. Scale bars: 100 µm. (C) Putative KLK proteolytic cascade activation in skin. Green arrows show activation by proteolytic cleavage of the proKLK. Red arrows show inhibition by physical interactions for LEKTI proteins or autolysis for KLK6.

then secreted as pro-enzymes that have to be extracellularly cleaved to become activated. Notably, KLKs have numerous putative extracellular matrix (ECM) substrates. In addition, KLKs also show activity toward cell surface molecules or other enzymes. Collectively, this allows them to regulate the epidermal microenvironment.

Several KLKs are expressed in the epidermis of the skin and they are crucially involved in the regulation of skin desquamation and inflammation. Dysregulation of KLK expression is associated with many inflammatory diseases and cancer types. Specifically for skin, abnormal activation of the KLK proteolytic cascade is reported in Netherton syndrome (NS), atopic dermatitis (AD) and psoriasis highlighting pro-inflammatory roles of KLKs. However, KLKs fulfill other less explored functions in skin particularly during skin homeostasis and wound healing. Cutaneous repair is a major challenge for many organisms. It requires synchronized efforts of the epidermis and the underlying dermis through cell proliferation and migration, secretion of diffusible factors, and ECM production to achieve the restoration of a functional tissue. KLKs being epidermal products with the ability to regulate inflammation and ECM remodeling could thus conduct an epidermal injury response to the dermal compartment during skin wound healing.

In skin, the dermis is an ECM-rich structure composed of a highly-specialized and intricately built ECM [2]. Naturally, this is the most explored skin ECM structure. The epidermis on the other hand is an ECM-poor subtissue. However, despite this, its microenvironment is essential for the regulation of epidermal functionality and thus wider the homeostasis of the entire skin and, by extension, organism's physiology and life. In this review, we highlight this concept by focusing in on KLKs as epidermal-specific regulators of the epidermal and the dermal microenvironment as well as downstream consequences on skin quality. First, we detail the expression of KLKs in homeostatic skin and KLK cascade activation in healthy skin. Next, we described KLKs role in skin desquamation and inflammation and their emerging roles during cutaneous repair. Finally, we will review the involvement of KLKs in skin diseases and cancers.

KLKs expression in the homeostatic skin

Among the 15 members of the KLK family, 11 have been reported to be expressed in skin with a pattern restricted to the epidermis and to the skin appendages. Gene expression analyses have revealed that KLK1 and KLK11 are the highest expressed KLKs in the skin. KLK4, KLK5, KLK6, KLK7 and KLK13 are moderately expressed, while KLK8 mRNA is sparsely detected under homeostatic conditions [3]. In terms of spatial expression, KLK6, KLK9, KLK10, KLK13 and KLK14 mRNAs appear to be strictly restricted to the stratum granulosum in healthy epidermis, while KLK1, KLK4 and KLK11 mRNAs are also found in the stratum spinosum and the basal layer of keratinocytes (Fig. 1A). Most of the KLK genes expressed in the epidermis are also expressed in skin appendages [3]. At the protein level, KLKs generally show a broader epidermal distribution. KLK5 is present in the stratum granulosum and stratum corneum while KLK6 is found in the whole epidermis and only enriched in the upper stratum granulosum (Fig. 1B). KLK7 is detected both in the basal layer of the epidermis and in the upper stratum granulosum but almost undetectable in areas in between (Fig. 1B). In addition, KLK8 and KLK13 have been observed in the stratum corneum and stratum granulosum as well as in the appendages in healthy skin [4,5]. Thus, the distribution of proteins is wider than gene expression indicating diffusion of KLKs in the epidermis. Of all KLKs, KLK14 seems to be most specific for skin with a low abundance in other tissues but relatively high levels in skin – 250 ng/g of total protein [6,7].

The KLK cascade activation in healthy skin

KLKs operate as a proteolytic cascade and, in skin, KLK5 is believed to be the main activator of the cascade. KLK5 is able to autoactivate and its activity in skin is restricted from the deeper stratum corneum to the limit of the stratum granulosum (Fig. 1B). When activated, KLK5 can, through a proteolytic cleavage, convert both proKLK7, which can also be activated by meprin β, and proKLK14 to active forms [8,9] (Fig. 1C). Active KLK14 is then able to activate newly produced proKLK5, thus creating a positive feedback loop. All these processes are closely dependent on the environmental pH as demonstrated *in vitro* by Brattsand and collaborators [8]. KLK5 is also able to activate proKLK6, however, the main KLK6 activators particularly in wounded skin are enterokinase and plasmin [10]. In addition, KLK6 undergo autoactivation but its self-activation seems negligible when compared to that of external proteases, at least *in vitro* [10,11] (Fig. 1C). Deeper in the KLK activation web, KLK5, KLK6 and KLK14 activate proKLK11 that is highly expressed in skin [12,13], however its function remains elusive. Following activation, KLK14 can convert both proKLK1 and proKLK8 to active enzymes [14,15].

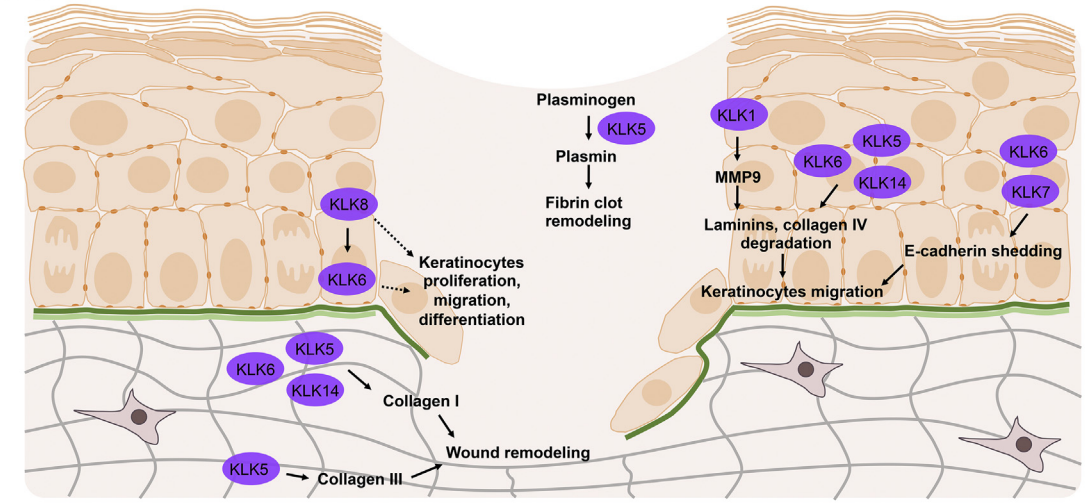
Once KLKs are converted to mature molecules, their activities are regulated by endogenous serine protease inhibitors belonging to the lympho-epithelial Kazal-type inhibitor (LEKTI) protein family (Fig. 1C). *SPINK5* encodes for the LEKTI protein, an endogenous inhibitor of KLKs. *SPINK5* is expressed in the stratum granulosum and in the outermost layer of the stratum spinosum (Fig. 1C). KLK5 activity is regulated by LEKTI but also partially by LEKTI2, encoded by the *SPINK9* gene, in palms and soles [16,17]. More recently SPINK6 was also described as a broad-spectrum inhibitor of KLKs including KLK5, KLK6 and KLK7, but not KLK8 and KLK14 [18].

As reported a long time ago, the skin pH varies between 4 and 7 and depends on the individual, the age and the anatomical site [19]. However, a more acidic pH is found at the outside most layer of the epidermis. A lower pH is vital for the skin barrier function, protecting against microorganisms. The KLK5 - LEKTI interaction is pH dependent and active KLK5 is released from the complex concomitantly with pH decrease, explaining the locally restricted activity of KLK5 [20].

KLKs role in healthy skin

In a healthy skin the outermost layer of the epidermis is regularly shed or peeled in a process referred to as skin desquamation. KLKs are involved

A



B

		Targeted protein	KLK involved	Mechanism	References
Core Matrisome	ECM glycoproteins	Fibrinogen	KLK6, KLK14	Proteolytic cleavage	Magklara et al., 2003; Rajapakse et al., 2007
		Fibronectin	KLK5, KLK6, KLK7, KLK14	Proteolytic cleavage	Rajapakse et al., 2006; Ghost et al., 2004; Rajapakse et al., 2007; Ramani et al., 2008
		Laminin	KLK5, KLK6, KLK14	Proteolytic cleavage	Michael et al., 2005; Magklara et al., 2003; Borgono et al., 2007
		Vitronectin	KLK6, KLK14	Proteolytic cleavage	Ghost et al., 2004; Borgono et al., 2007
	Collagens	Collagen I	KLK5, KLK6, KLK14	Proteolytic cleavage	Michael et al., 2005; Magklara et al., 2003; Rajapakse et al., 2007
		Collagen III	KLK5	Proteolytic cleavage	Michael et al., 2005
		Collagen IV	KLK5, KLK6, KLK14	Proteolytic cleavage	Michael et al., 2005 Magklara et al., 2003, Rajapakse et al., 2007; Borgono et al., 2007
Matrisome-associated	ECM regulators	MMP2, MMP9	KLK1	Proteolytic cleavage	Kyriakides et al., 2009; Desrivieres et al., 1993
Adhesome		Kininogen 1	KLK1	Proteolytic cleavage	Henderson et al., 1994
		E-cadherin	KLK6, KLK7	Proteolytic cleavage	Klucky et al., 2007 ; Johnson et al., 2007
		Integrin α5β1	KLK5, KLK6, KLK7	Unknown (transcription)	Loessner et al., 2012
		Integrin αVβ3	KLK5, KLK6, KLK7	Unknown (transcription)	Loessner et al., 2012
Epidermal micro-environment		Corneodesmosin	KLK5, KLK7	Proteolytic cleavage	Caubet et al., 2004, Simon et al., 2001
		Desmocollin 1	KLK5, KLK7	Proteolytic cleavage	Caubet et al., 2004, Simon et al., 2001
		Desmoglein 1	KLK5, KLK6, KLK13, KLK14	Proteolytic cleavage	Caubet et al., 2004, Borgono et al., 2007; Williams et al. 2017

Fig. 2. KLKs in wound healing and interactions with extracellular matrix proteins. (A) Schematic representation of suggested direct or indirect roles that KLKs play during the different steps of cutaneous wound healing. (B) Summary of the KLK substrates reported in the literature that belongs to the matrisome, adhesome or epidermal extracellular space.

in the skin desquamation by degradation of corneodesmosmes that are junctional structures allowing the adhesion of corneocytes. Corneodesmosmes are composed by one extracellular protein, corneodesmosin (CDSN), and two transmembrane proteins, desmoglein 1 (DSG1) and desmocollin 1 (DSC1). Degradation of these proteins by KLKs allows for proper skin desquamation and is required for skin homeostasis. Indeed, deficient desquamation is responsible for several diseases including Harlequin Ichthyosis [21]. KLK5 degrades CDSN, DSG1 and DSC1. CDSN and DSC1 but not DSG1 can also be efficiently cleaved by KLK7 [22]. KLK5 and KLK7 exhibit similar proteolytic efficacy on CDSN and DSC1 at neutral (7.2) or acidic (5.6) pH, while cleavage of DSG1 by KLK5 is only observed at acidic pH [22,23]. *In vitro* studies also demonstrated that both KLK6, KLK13 and KLK14 are able to degrade DSG1 at an optimal pH of 7.6 [24,25]. This suggests that - depending on the substrate - KLKs could be active in a quite wide range of pHs. In *Klk8* deficient mice the skin displays a hyperkeratosis phenotype resulting from a defective DSG1 and CDSN cleavage. It suggests that Klk8 actively

contributes to corneocytes shedding [5]. However, since modifications of the Klk8 level subsequently affect Klk6 and Klk7 gene and protein expression, as well as protease activity, it remains unclear whether Klk8 directly cleaves DSG1 and CDSN or acts through the KLK cascade. In addition, *Klk8*$^{-/-}$ mouse skin also shows less cell proliferation in the epidermis accompanied with an increased number of cell layers in the stratum corneum [5], suggesting that some KLKs are involved in regulation of keratinocyte proliferation. In accordance with this observation, Klucky and collaborators showed that overexpression of KLK6 in human keratinocytes increases proliferation and migration [26].

Based on genetic diseases linked to dysregulated KLK activity (see below) and genetic mouse models, it is clear that KLKs are important contributors of the skin barrier function [27]. However, relatively limited knowledge exists on their functions in healthy skin. Antimicrobial peptides are playing important roles in human innate immunity preventing against infections. LL-37 is the unique antimicrobial peptide derived from cathelicidin in human. LL-37 is found in normal skin and it has broad-spectrum activity. KLK5 and KLK7 control the availability of LL-37 in skin by processing the immature cathelicidin into its active form LL-37 [28]. Furthermore, LL-37 is further processed into smaller peptides with alternate physiological activity. Eissa and collaborators showed that KLK5 and KLK8 are at least *in vitro*, able to produce such bioactive peptides [15].

Additionally, KLK5 mediates cleavage of profilaggrin - another important player of the skin barrier function [29]. Filaggrin is responsible for the aggregation of keratin intermediate filaments during epidermal differentiation. It contributes to the mechanically robust envelop of corneocytes and it participates both to water retention in skin and to the establishment of acidic pH of the external most layer of the epidermis. In 2013, Sakabe and coworkers showed that profilaggrin and KLK5 colocalize in the stratum corneum and identified KLK5 as a potent actor of filaggrin maturation in skin [29]. A more recent study demonstrated that KLK6, KLK13 and KLK14 are also able to process profilaggrin [25].

To sum up, KLKs are epidermal-specific proteases that in homeostatic healthy skin promote skin desquamation by direct cleavage of transmembrane and extracellular epidermal proteins and regulate innate immunity.

KLKs in wound healing

Cutaneous wound healing is a complex multistep process that requires coordinated actions of skin cells to rebuild the damaged tissue. In mammals, wound healing is divided in four overlapping phases: (1) hemostasis and fibril clot formation; (2) inflammation phase; (3) proliferation phase and (4) wound remodeling. Both the epidermis and the dermis contribute to cutaneous repair through cell migration and proliferation. These events are crucially regulated through ECM protein secretion, deposition, maturation, arrangement and degradation. During wound healing KLKs are released from the damaged epidermis into the wound microenvironment. In addition, KLKs are small molecules that are able to diffuse through the blood circulation, as it was shown in cancers and skin diseases (see below), and thus they could reach the wound bed. The drastic pH decrease observed during skin repair [30] would reduce KLK interactions with endogenous inhibitors thus increasing KLK activity during wound healing. KLKs appear to be involved in all four phases of wound healing.

KLKs in the fibrin clot formation and inflammatory phase of wound healing

Plasminogen is a multidomain glycoprotein that when processed to plasmin, is involved in hemostasis by digestion of blood plasma-derived proteins including the fibrin clot. While the main activators of plasminogen are tissue plasminogen activator (tPA) and urokinase type plasminogen activator (uPA), KLK5 is also able to generate plasmin [31]. It indicates a role of this protease in the remodeling of the fibrin clot during wound healing (Fig. 2A). Interestingly, plasmin is also able to convert proKLK6 and proKLK14 into active proteases [12], extending and adding complexity to the KLKs-plasmin web in wound repair.

KLK1 is involved in cutaneous healing through the kallikrein-kinin system. KLK1 is responsible for the cleavage of kininogen into kinin [32]. When liberated, kinin peptides interact with their specific receptors – B1 and B2 and contribute to the inflammatory phase of wound healing. Indeed, mice with single or combined deficiency of these receptors exhibit a delayed skin wound healing process accompanied by a reduced infiltration of leukocytes at the injured site at day 2 and 3 post wounding [33].

KLKs and keratinocyte migration

KLK1-mediated generation of kinin and subsequent B2 receptor activation also determine the final wound quality by contributing to re-epithelialization, cell proliferation and myofibroblast conversion of fibroblasts [33]. In addition, KLK1 has been reported to accelerate skin wound healing *in vivo* in rats in an EGFR-dependent way [34]. *In vitro* analyses suggest that KLK1 induces keratinocyte migration through cleavage of the protease activated receptor 1 (PAR1) receptor. Once activated, PAR1 induces EGFR transactivation by MMPs ultimately leading to intracellular Src and ERK signaling pathway in keratinocytes [34].

Some years ago, in an in-depth study by Kishibe and collaborators aimed at deciphering the role of KLK8 during wound healing [35] (Fig. 2A). The authors showed that Klk8 is progressively upregulated during the first days of wound healing and its expression peaks at seven days post wounding. Wound closure was significantly delayed in *Klk8*$^{-/-}$ mice compared to wild-type controls, due to defective keratinocyte proliferation, differentiation and migration. In *Klk8*$^{-/-}$ mice, Klk6 expression was dramatically reduced during wound healing suggesting that Klk8 could induce *Klk6* mRNA expression and, subsequently activate proKlk6 through proteolytic cleavage. Interestingly, and importantly, this study showed that KLKs act independently of the inflammatory response during wound healing, as the level of several inflammatory cytokines was not affected in *Klk8*$^{-/-}$ mice compared to wild-type controls. In addition, the authors showed that *Klk7* mRNA levels were five-fold upregulated at five days post wounding revealing that Klks are also regulated at the transcriptional level during wound healing. Similarly, *Klk6* mRNA is highly upregulated during skin wound healing in mice between one and five days post wounding with a peak of a 40-fold increase at three days post wounding [35].

Keratinocyte migration is dependent on E-cadherin – a transmembrane protein required for the establishment of cell-cell junctions in the epidermis [36]. The E-cadherin level drastically decreases at one day post wounding throughout the epidermis, facilitating keratinocyte migration to cover the site of injury. KLK6 upregulation promotes E-cadherin shedding, presumably through ADAM10 activation. In line with this, overexpression of KLK6 under the control of a ubiquitous promotor in mice significantly decreases cell membrane-associated E-cadherin on keratinocytes. It also accelerates re-epithelialization of skin wounds by increasing keratinocyte proliferation [26]. These data align nicely with the observation of decreased re-epithelialization of Klk8 deficient mouse wounds, which show a reduction of Klk6 levels [35]. Some reports also provide evidence that KLK7 is able to directly mediate E-cadherin shedding *in vitro*, suggesting that it could contribute to keratinocyte migration during wound healing [37] (Fig. 2A).

In addition to the aforementioned studies, numerous *in vitro* studies suggest that KLKs could play a role in keratinocyte migration during wound healing. During re-epithelialization, migrating keratinocytes are in close contact to the provisional ECM that is mainly composed of fibrin, plasma fibronectin and vitronectin. Several KLKs are able to cleave fibrinogen, a fibrin precursor, fibronectin and vitronectin (Fig. 2B), indicating KLK contribution to the provisional ECM remodeling during cutaneous repair. Additional studies point to that during re-epithelialization, keratinocytes migrate on the collagen I-rich dermal matrix rather than through the fibrin clot, suggesting a potential role of collagen I in wound re-epithelialization [38,39]. Collagen I has been described as a substrate for KLK5, KLK6 and KLK14 hinting that KLKs could facilitate keratinocyte migration over the dermal collagen I matrix during wound healing, as it was already reported for matrix metalloproteinases 1 (MMP1) [40] (Fig. 2A, B). Following this, later during wound healing keratinocyte migration depends on laminins and collagen IV [39]. MMP9 – a zinc-containing endopeptidase, is produced by migrating keratinocytes and promotes cell migration over the wound through degradation of laminins and collagen IV [41,42]. MMP9 is secreted as an inactive pro-enzyme that needs to be activated by proteolytic cleavage and KLK1 participates to its activation [43] (Fig. 2B). In addition, as KLK5, KLK6 and KLK14 are able to directly degrade both laminins and collagen IV *in vitro*, they could also influence keratinocyte migration (Fig. 2A).

Another mechanism by which KLKs may regulate migration is through changes of abundance of ECM receptors at the cell surface. The main cell receptors of fibronectin and vitronectin are integrin α5β1 and αVβ3, respectively. Overexpression of KLK5, KLK6 or KLK7 in ovarian cancer cell lines leads to a downregulation of integrins α5β1 and αVβ3. It thus reduces cell adhesion and promoting cell invasion and metastasis suggesting that KLKs could interfere with keratinocyte migration [44] (Fig. 2B). As these RGD-binding integrins are also essential for deposition of a fibronectin matrix [45], which is a main constituent of the provisional wound ECM [46], KLK-mediated reduction of integrin α5β1 and αVβ3 abundance may alter the deposition of the provisional wound ECM. Furthermore, KLK activity in the wound is likely not limited to the epidermis and extends to the wound bed. This may impact the deposition of a mesenchymal fibronectin matrix, which acts as a scaffold for collagen fibril and fibrillin microfibril formation and arrangement [47].

In addition to the interfollicular epidermis, skin appendages also largely contribute to cutaneous healing and in particular to re-epithelialization. As an example, genetic tracking of Keratin 15-positive cells – a marker of hair follicle bulge cells, shows that these cells migrate centrally during wound healing and participate in re-epithelialization, suggesting epidermal stem cell plasticity [48]. However, most cells do not stay longer than a few weeks in the epidermis, showing that they are only transiently recruited for acute wound repair. In addition, hair follicle stem cells from the bulge also reform sebaceous glands or new hair follicles during skin repair [49]. Some studies suggest that sweat glands contribute both to their own self renewal and to epidermis repair [50]. Many KLKs are detected in hair follicles, sebaceous glands and eccrine sweat glands. In addition, endogenous KLK inhibitors such as SPINK5 and SPINK6 are also found

in these structures [3,18]. Furthermore, KLK6, KLK8 and KLK13 are also highly expressed in the sensory nerves [4]. However, at the moment limited information has been reported on the function of specific KLKs in skin appendages, but given their important role in the skin, it is likely that KLK are actively involved in the homeostasis of these structures. None of the existing genetic mouse models of KLK deficiency or overexpression exhibit an overt hair phenotype, suggesting a rather subtle or redundant role of KLKs in skin appendages. Further studies should carefully and in detail investigate KLK involvement in skin appendage homeostasis and the consequences of these involvements on skin healing.

KLKs and wound remodeling

Collagen I and collagen III are the most abundant collagens in skin and play critical roles during wound healing contributing to tissue architecture maintenance and strength. Following the above, KLK activity in the wound bed could impact the deposition of collagen fibrils and thus the ECM of the transient scar. In addition, and better founded, collagen I has been shown to be a potential substrate for KLK5, KLK6 and KLK14. *In vitro*, KLK5 is also able to degrade collagen III (Fig. 2A, B). To simplify, collagen III is considered as a regenerative/wounding molecule as it is the first collagen upregulated in the granulation tissue between three to five days post-wounding when fibroblasts progressively invade the wound. Progressively collagen III is degraded during the later phases of wound healing and replaced by collagen I in the mature scar [2].

KLKs in diseases

KLKs in cancer

Cancer can, in many aspects, be viewed as an extended wound healing pathology [51]. KLKs are dysregulated in multiple types of cancers, such as prostate or ovarian cancer and could potentially be used as diagnostic and prognostic markers [52]. In this context, KLKs dysregulation could either be detected directly in the affected tissue or in sera, as KLKs are able to diffuse through tissues to reach the blood circulation. However, only a limited number of studies have aimed to decipher the precise functions of KLKs in cancer initiation and progression [7,37,44,53–59]. Squamous cell carcinoma (SCC) is a heterogenous entity of epidermally-driven cancers that develop at different sites of the body and results from malignant transformation of epithelial tissues.

Elevated abundance of KLK6 was reported in cutaneous SCC (cSCC) [26] suggesting an involvement of KLK6 in carcinogenesis. Indeed it was shown that KLK6 promotes cell proliferation, migration and invasion partially through E-cadherin shedding as previously discussed [26]. In accordance with these observations, inactivation of *Klk6* in mice leads to resistance to tumor development and growth after chemical carcinogenesis and is associated with reduced inflammation [57]. However, contradictorily, low KLK6 expression was reported as an unfavorable risk factor that induces epithelial-to-mesenchymal transition (EMT) and ultimately promotes tumor progression [60]. Findings from studies of oral SCC (oSCC) may consolidate these observations. Here, KLK6 plays a differential role either associated with malignant transformation of keratinocytes when overexpressed, or participating to the EMT mediated by the oncoprotein ΔNp63 when reduced [59].

Similarly to $Klk6^{-/-}$ mice, knockout of *Klk5* in mice leads to tumor-formation resistance but by a different mechanism. In this case, the inflammatory status of $Klk5^{-/-}$ mice is comparable to controls but, rather animals show increased keratinocytes apoptosis [61]. Supporting a role in cancer progression, KLK5 is overexpressed in malignant oSCC cells leading to an increased DSG1 cleavage and consequently a loss of cell-cell adhesion promoting metastatic dissemination [62]. KLK4 has also been reported to be increased in oSCC and could promote EMT through activation of the PI3K/AKT pathway [55]. As a consequence, KLK4 downregulation by shRNA decreases cell migration and invasion [55]. Furthermore, KLK13 downregulation in oSCC cells promotes invasiveness and metastasis suggesting a protective role of this protease [58]. Recently, a study provided insights to the role of KLKs in melanomas showing that KLK7 expression leads a more malignant phenotype by acting on cell adhesion [56]. These examples illustrate that KLKs play dual roles in cancer in a stage- and context-dependent manner.

KLKs in skin diseases

Dysregulation of KLKs is clearly associated with three inflammatory diseases of the skin: Netherton syndrome (NS), atopic dermatitis (AD) and psoriaris.

NS is a severe autosomal recessive skin disorder characterized by severe desquamation, erythrodermia and severe atopy associated with chronic skin inflammation. It could also be associated with extracutaneous manifestations such as asthma, growth delay or hypernatremic dehydration due to defective water absorption by the damaged skin. NS is caused by mutations in the *SPINK5* gene encoding the LEKTI protein – a KLK5 inhibitor. Lack of LEKTI leads to uncontrolled activation of

KLK5 and the downstream proteases KLK7, KLK14 and elastase 2, and ultimately, impairs the skin physical barrier [63,64]. In NS, enhanced KLK5 activity increases cleavage and activation of PAR2 protein – a G-protein coupled receptor expressed by keratinocytes and other cell types, as shown *in vitro*. This leads to an increased production of NFkβ-dependent cytokines: Thymic Stromal Lymphopoietin (TSLP), IL-8 and TNFα [65]. The pathway results in an enhanced inflammatory response and in the appearance of the atopic-dermatitis-like lesions observed in NS patients. However, KLK5 overexpression also acts through a PAR2 independent pathway to promote the secretion of proinflammatory cytokines such as TSLP, IL-10 and IL-8 [66]. Recently, KLK6 was described as an additional player of the NS. Genetic inactivation of *Klk6* in *Spink5*$^{-/-}$ mouse that reproduces the NS phenotype, reduces skin inflammation. However, the skin barrier defects of the double knockout mice were similar to that of the *Spink*5$^{-/-}$ mouse [67,68]. In addition, transgenic expression of *KLK5* in mice recapitulates both cutaneous and extracutaneous features of NS [63]. Interestingly, in this study the human KLK5 was specifically overexpressed in the granular layer of the mouse epidermis and leads to an increased inflammation in the dermal part of the skin showing that dysregulation of the epidermal specific proteases affects the whole skin. In addition, inhibition of *Klk5* or *Klk7* in *Spink5*$^{-/-}$ mice ameliorates the NS phenotype and notably the quality of the epidermis and the skin inflammation [69,70]. These studies have encouraged the development of KLK5 inhibitors as a major target therapeutic target for NS.

AD is a common inflammatory skin disease that affects around 20% of people at some point during their lives. It is a multifactorial disease involving genetic factors, mutations in the gene encoding filaggrin, and environmental factors such as bacterial colonization of the skin [71]. AD patients exhibit red, itchy and swollen skin associated with hyperkeratosis. KLK7 is the main KLK involved in AD pathogenesis (reviewed in [72]). Indeed, mice overexpressing the human KLK7 reproduce AD lesions both in terms of epidermal thickness, hyperkeratosis, pruritus and dermal inflammation [73]. While KLK7 is overexpressed in AD, its activity seems to be reduced explaining the hyperkeratosis observed in patients. Indeed, in AD lesions KLK7 secretion in the extracellular space is impaired and LEKTI, a KLK5 and KLK7 inhibitor, is overexpressed leading to an insufficient degradation of corneodesmosomes [74]. In AD patients, levels of KLK7 in the sera are correlated with the IL-4 amount – a cytokine that induces the differentiation of Th2 lymphocytes. Conversely, Th2 cells secrete IL-4 and IL-13 that in turn increase KLK7 expression thus creating an inflammatory positive feedback loop in AD lesions [75]. Finally, AD skin shows high bacterial colonization with *Staphylococcus aureus* leading to KLK6, KLK13 and KLK14 upregulation, suggesting that other KLKs could be involved in the disease [25].

Psoriaris vulgaris is a chronic autoimmune skin disorder characterized by patches of AD-like lesions. Similarly to AD, psoriasis is multifactorial although immune defects play a large role. KLK6 and KLK8 are highly upregulated in psoriatic lesions and KLK8 amount in the sera correlates with the disease severity [76]. Involvement of KLK8 was confirmed in a murine model of psoriasis showing that Klk8 contributes to the formation of skin lesions including dermal inflammation. Klk8 also induces IL-36, a cytokine that contributes to inflammation in psoriasis [77,78]. In addition, Klk6 is also upregulated in psoriasiform epidermis in mice and participates to the development of the psoriatic lesions in a PAR2-independent manner while its mechanism of action remains to be elucidated [79].

Collectively, these diseases highlight the role of KLKs and their activities as master regulators of skin fitness and inflammation.

Outlook

In healthy skin, KLKs regulate desquamation and innate immunity and KLK cascade dysregulation leads to severe skin disorders. However, through their capacity to influence inflammation and cleave ECM proteins, KLKs are also emerging as new actors of cutaneous repair. While their roles in skin diseases or cancers are now appreciated, their involvement in physiological and pathological skin wound healing repair remains to be elucidated. Unresolved inflammation and dysregulation of ECM deposition and remodeling are responsible for inefficient tissue repair and fibrosis [80]. So far, most focus of understanding these diseases in skin has been on the dermis and fibroblasts. From fibrotic diseases in other organs it is clear that the stressed epithelium plays a crucial role in disease initiation and perpetuation [81,82]. Recent studies have alluded to also keratinocytes playing such part in the setting of scleroderma [83]. For the skin, given the ability of KLKs to influence dermal inflammation and potential direct proteolytic activities on the dermal ECM, KLKs could be mediators converting an epidermal stress response to pathological remodeling of the dermal ECM. In other words, the involvement of individual KLKs and the KLK protease web should be carefully and systematically assessed in acute wound healing as well as wound healing pathologies, including chronic wounds and fibrosis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The work by AN related to the topics discussed in the review is supported by grants from the German Research Foundation (DFG) grant numbers: NY-90 2/1, NY-90 3/2, NY 90-5/1, SFB850 project B11 and SFB1160 project B03.

Received 18 October 2019;
Received in revised form 8 November 2019;
Accepted 14 November 2019
Available online 21 November 2019

Keywords:
Kallikrein;
Epidermis;
Epidermal microenvironment;
Inflammation;
Diseases;
Wound healing

Abbreviations used:
AD, atopic dermatitis; CDSN, corneodesmosin; DSC1, desmocollin 1; DSG1, desmoglein 1; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; KLKs, kallikreins; LEKTI, lympho-epithelial Kazal-type inhibitor; NS, Netherton syndrome; PAR1/2, protease activated-receptor 1/2; SCC, squamous cell carcinoma; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator.

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