



Review Article

LEKTI-1 in sickness and in health

T. Roelandt*, B. Thys†, C. Heughebaert*, A. De Vroede*, K. De Paepe‡, D. Roseeuw*, B. Rombaut† and J.-P. Hachem*

*Department of Dermatology, Universitair Ziekenhuis, Brussels, †Department of Pharmaceutical Biotechnology and Molecular Biology (Dermo-Cosmetology and Pharmacognosy), Vrije Universiteit Brussel, Brussels, Belgium and ‡fayf

Received 2 February 2009, Accepted 25 March 2009

Keywords: LEKTI, serine protease, serine protease inhibitors, SPINKS, stratum corneum

Synopsis

The stratum corneum (SC) is a biosensor that mediates responses to a variety of exogenous insults through various signalling mechanisms, including the activation of SC serine proteases (SP) kallikrein cascade. The SPINK5 gene encodes an SP inhibitor, the lympho-epithelial-Kazal-type-1 inhibitor (LEKTI-1), which in turn will buffer the excess of SP cascade initiation, key in the maintenance of permeability barrier homeostasis. We demonstrate that LEKTI processing can occur within the SC after secretion from stratum granulosum keratinocytes at least partially by klk7, an SC-specific chymotryptic SP. Unlike the recently described LEKTI-2, neither recombinant full-length LEKTI-1 nor recombinant LEKTI-1 fragments exhibit antimicrobial activity. Finally, we discuss the pathophysiological implications of LEKTI-1 in skin biology as well as its contribution to the pathogenesis of Netherton Syndrome and its potential involvement in atopic dermatitis.

Résumé

A la manière d'un biosenseur, le stratum corneum (SC) agit comme une sentinelle pour détecter les agressions externes et avertir les couches épidermiques sous jacentes de toute modification environn-

ementale. Cette fonction est en partie assurée par l'activation en cascade des sérines protéases de la famille des kallikréines (klk). Le gène SPINK5 encode un inhibiteur de protéase connu sous le nom Lympho-Epithelial-Kazal-Type-1 Inhibitor (LEKTI-1). Ce dernier inhibe la cascade protéolytique et participe de ce fait à la réponse homéostatique déclenchée par les kallikréines. Nous démontrons aussi que le remaniement post-transcriptionnel de LEKTI-1 peut avoir lieu dans le SC et ceci par l'action de klk7, une protéase à activité chymotryptique propre au SC. Contrairement à LEKTI-2 décrite récemment, nous n'avons pas pu démontrer une activité anti-microbienne propre à LEKTI-1 et ses fragments. Nous discutons vers la fin de cette revue l'implication de LEKTI-1 dans la fonction biologique de la peau et nous décrivons sa contribution dans la pathogenèse du syndrome de Netherton et de la dermatite atopique.

Introduction

The essential function of the epidermis is to provide an efficient barrier against water loss from the desiccating environment and to protect our inner system from the ingress of xenobiotics [1]. The stratum corneum (SC), the outermost layer of the epidermis, largely contributes to the maintenance of the integrity of the epidermal permeability barrier [2]. It consists of metabolically inactive keratinocytes (i.e. corneocytes) surrounded by a lipid matrix and interconnected by specialized forms of desmosomes (i.e. corneodesmosomes, CD)

Correspondence: Jean-Pierre Hachem, M.D., Ph.D., Universitair Ziekenhuis-Vrije Universiteit Brussel, Dienst Dermatologie, Laarbeeklaan 101, 1090 Brussel, Belgium. Tel.: +32 2 476 3114; fax: +32 2 477 6800; e-mail: jeanpierre.hachem@uzbrussel.be

[3]. SC formation results from the terminal differentiation of the highly differentiated stratum granulosum (SG) cells [4]. At the junction between the SG and the SC layer occurs the polar secretion of lamellar bodies (LB) containing both precursor lipids as well as the necessary enzymes for the processing of these precursors into mature lipid matrix constituents [5]. Prior to secretion, LB assembly takes place within the different compartments of the trans-golgi network resulting in the differential packaging of LB with diverse contents [6]. Besides the above-mentioned lipid metabolites, LB deliver into the extracellular space of the SC proteotically active enzymes such as serine proteases (SP) from the kallikrein (klk) family as well as their inhibitor (SPI), the lympho-epithelial-Kazal-type-1 inhibitor (LEKTI-1) [7]. LEKTI-1 is transported by specific intracellular LB cargoes until its secretion in the extracellular space, between granular and cornified cells [7, 8].

Lympho-epithelial-Kazal-type-1 inhibitor belongs to the Kazal family of SPI and it is encoded by the SPINK5 gene [9]. LEKTI-1 is expressed in keratinizing and non-keratinizing epithelia, thymus, tonsils, parathyroid glands, hair follicles and trachea [9, 10]. In the epidermis, it was reported to be present in the SG [10], but we also found that it co-localizes with klk7 to the membrane domains of normal SC [8]. Several types of SPI have been found in human SC. Four of these, secretory leucocyte protease inhibitor (SLPI) [11], elafin (SKALP) [12, 13], plasminogen activator inhibitor type 2 [14] and cystatin [15] are normally cross-linked to the cornified envelope (CE). The consequent sequestration within the CE could render those inhibitors unavailable to secreted 'free' proteases. This means that LEKTI-1 may constitute the major SPI within the SC. Whereas Kazal SPI family members generally contain 3–7 tandem kazal domains, LEKTI-1 exhibits as many as 15 potential SPI domains (D1–D15) separated by 14 spacing segments [16, 17]. D2 and D15 resemble typical Kazal-type SPI, as suggested by their primary structure, with a characteristic pattern of six cysteine residues. The remaining 13 domains share high homology with Kazal-type inhibitors but lack one of the three conserved typical disulphide bridges [17].

In this review, we address the functions of LEKTI-1 in the epidermis as well as its contribution to the maintenance of permeability barrier homeostasis. We demonstrate that LEKTI-1 is

activated/degraded by klk7 consistent with the co-expression pattern of both proteins. We also addressed the potential antimicrobial role of LEKTI-1 in the SC. We finally provide preliminary data assessing the potential implications of LEKTI-1 in the pathogenesis of atopic dermatitis (AD).

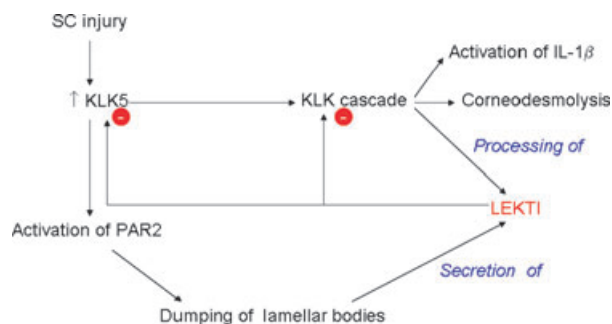
LEKTI-1 and epidermal homeostasis

LEKTI-1/pH-dependent desquamation

Desquamation of the SC is the result of a tightly regulated multistep proteolytic event. To control the activity of the SP in desquamation, active protease inhibitors in the SC must be present. CD, the modified desmosome of the SC, regulate SC cohesion and consequently contribute to the integrity of the skin barrier [18]. Processes leading to normal desquamation include the adequate proteolytic degradation of the CD unit largely constituted of cadherins, namely, desmoglein 1 (dsg1) and desmocollin 1 [19]. Epidermis-specific SP present in the SC tryptic enzyme (klk5), SC chymotryptic enzyme (SCCE, klk7) and klk14 are involved in this process [20–22]. Integrity/Cohesion is a pH-dependent SC function inversely related to rates of corneocyte shedding (desquamation), a process regulated by klk cascade of activation [18, 19, 23]. As both SPs exhibit neutral-to-alkaline pH optima, they are likely to be active in the lower SC (= stratum compactum) [19]. Hence, these enzymes exhibit some residual activity at an acidic pH to sustain sufficient but slow rate of proteolysis [19] that maintains gradual desquamation rates in the outer 'acid mantled' SC (= stratum disjunctum). Although its acidic surface pH was discovered over a century ago, the SC pH gradient ranges from 4.5 to 5.0 in the outer SC approaching neutrality in the lower SC [24]. However, the SG–SC interface again appears to become more acidic, suggesting that the epidermis possesses a biphasic pH gradient [25]. Consequently, differential acidification within the SC levels may contribute to the regulation of SP activity and could influence key SC functions in discrete locations within the SC. Indeed, increasing SC pH activates SP of the SC leading to permeability barrier abrogation and loss of SC integrity/cohesion [18, 23].

Besides the regulatory effect of SC pH on SP activity (Fig. 1) LEKTI may be implicated in other

Figure 1 Serine protease and lympho-epithelial-Kazal-type-1 inhibitor (LEKTI-1) response to stratum corneum (SC) injury. (PAR2: protease-activated receptor 2 see Hachem *et al.*[46]). IL, interleukin.



functions. The full-length recombinant LEKTI-1 (rLEKTI) protein has been shown to inhibit trypsin, subtilisin A, plasmin, cathepsin G and neutrophil elastase, but not chymotrypsin [26]. We also demonstrated that rLEKTI-1 inhibits both klk5 and 7 [8]. In addition, the single domain D6 was shown to be a potent inhibitor of trypsin, klk5 and klk7, whereas D15 was not effective against these two kallikreins [27]. Taken together, these results demonstrate that the different LEKTI-1 sub-domains exert diverse inhibitory profiles. In addition, the pH gradient by itself controls the interaction strength between LEKTI-1 and klk5, allowing the controlled release of active SP in the superficial SC layers. The highest inhibitory capacity of LEKTI-1 D8–D11 fragment against klk5 and 7 was obtained at neutral pH value [28]. However, acidification decreases the strength of binding between LEKTI-1 D8–D11 fragment and both klk 5 and 8 [28] consistent with the possibility that during the passage from the deep (pH 7.5) to the superficial SC (pH 4.5), klk5 and klk7 gradually detach from LEKTI. The strong binding between LEKTI-1 fragment and klk at neutral pH prevents premature desquamation within deep SC where the pH is neutral. Although restricted, acidic pH levels of the upper SC and within the acidic microdomains of SG–SC interface, allow sufficient activity for klk5 and 7 to degrade corneodesmosomal components leading to a tightly regulated desquamation process [19].

LEKTI-1 fragments processing: intracellular vs. extracellular

Lympho-epithelial-Kazal-type-1 inhibitor is expressed as three precursors [29]: (i) the full-length isoform of 15 domains (145 kDa), (ii) a shorter LEKTI-1 isoform (125 kDa) composed of the first 13 domains and (iii) a longer isoform (148 kDa) carrying a 30-amino acid residue insertion between the thirteenth and the fourteenth

inhibitory domains. LEKTI-1 precursors were found to be rapidly transformed into proteolytic fragments in the post-endoplasmic reticulum compartment [10, 30] signifying that processing of LEKTI-1 precedes its secretion into the extracellular space between the SC and the SG. Furin is reported to be involved in the intracellular processing of LEKTI-1 as furin-deficient chinese hamster ovary cells are unable to process LEKTI-1 [10, 28]. However, we demonstrated that full-length LEKTI-1 is also present in SC extract, which suggests an alternative pathway for the extracellular processing of secreted LEKTI-1 [8]. To gain further insight into LEKTI-1 processing, we addressed whether LEKTI-1 fragmentation could occur in the presence of klk7. Both enzyme and inhibitor were incubated at 37°C for 10, 30 min and 3 h and were further subjected to western immunoblotting using LEKTI-1 monoclonal antibody for the detection of full-length LEKTI-1 and LEKTI-1 fragments. Klk7 produces a time-dependent proteolysis of LEKTI-1 generating sub-domains very similar in molecular weight to those observed in both cultured human keratinocytes and foreskin epidermis [10]. LEKTI-1 bands of 125 and 100 kDa were detected when LEKTI-1 was incubated alone for 3 h at 37°C (Fig. 2). After 10 min of incubation with klk7, the presence of a much higher band (>125) was detected, which disappears in function of time (i.e. at 3 h of incubation; Fig. 2), suggesting that it may correspond to the complex generated by klk7 + LEKTI. Compared with LEKTI-1 alone, incubation with klk7 produces LEKTI-1 fragments with molecular weights ranging from 20 to 80 kDa. LEKTI-1 fragments of 20 kDa were reported in human epidermis and the extracellular fraction of cultured human keratinocytes using the D1–D6 specific antibodies indicating that these LEKTI-1 N-terminal fragments are secreted [10]. The appearance of a 20-kDa band when klk7 and LEKTI-1 were co-incubated suggests that

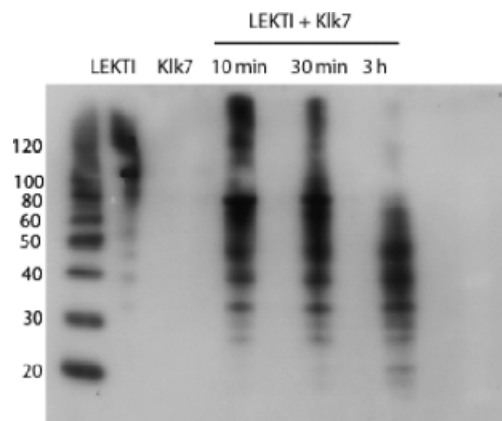


Figure 2 KLK7 degrades/processes full-length lympho-epithelial-Kazal-type-1 inhibitor (LEKTI-1) *in vitro*. Lane 1: ladder, lane 2: recombinant LEKTI (rLEKTI-1) alone, lane 3: klk7 and lanes 4 to 6 LEKTI-1 + klk7.

extracellular processing of LEKTI-1 may occur following secretion. Proteolytic fragments of 31, 37 and ≈ 65 kDa were progressively detected in function of time by incubation of LEKTI-1 with klk7. These fragments may correspond to the previously detected fragments in human epidermis and in the conditioned medium of cultured human keratinocytes.

Even though LEKTI-1 precursors could not be detected in the extracts of whole epidermis [10], we were still able to demonstrate that full-length LEKTI-1 is present in the SC and consequently (at least partly) secreted under its precursor form [8]. The co-localization of LEKTI-1 and klk7 within the membrane domains of the SC and the current data showing klk7-dependent processing of LEKTI-1 *in vitro* clearly suggest that both proteins interact extracellularly [8]. LEKTI-1 inhibits klk7, but klk7 in turn would process/degrade LEKTI-1 to produce active/inactive fragments. Altogether, these results also highlight the heterogeneity in the molecular weights of LEKTI-1 proteolytic fragments producing both high-molecular weight proteolytic fragments and several small LEKTI-1 fragments.

Antimicrobial defense

Human skin is constantly exposed to a variety of potentially dangerous microorganisms. Yet, the epidermis is highly resistant to infectious agents. Innate immunity plays an important role in epidermal antimicrobial barrier [31]. It consists of antigen-non-specific defense mechanisms, an initial

response to eliminate microbes and prevent infection. The SC is dry, acidic and has a temperature lower than 37°C . These conditions are not in favour of bacterial growth but still sustain a resident normal flora, which in turn inhibits the development of harmful pathogens [31, 32]. In addition, epithelial cells produce a variety of antimicrobial proteins such as defensins, cathelicidins and SPI that kill microbes [33]. SLPI is a low-molecular weight SPI and exhibits a broad-spectrum antibiotic activity that includes anti-retroviral, bactericidal and antifungal activity [11]. A recently described antimicrobial peptide derived from human LEKTI-2 has been reported to possess anti-*Escherichia Coli* activity and was found to be encoded by SPINK9 [34]. We therefore asked the question whether LEKTI-1 exhibits an antimicrobial activity against the most common pathogens, namely *Staphylococcus aureus* and *Candida albicans*. Either full-length LEKTI-1 alone or different rLEKTI-1 domains (1–6, 9–12; 9–15) were incubated with either *S. aureus* or *C. albicans* and microbial growth inhibition was assessed in function of time (Fig. 3). Neither full-length LEKTI-1 nor different rLEKTI-1 domains demonstrated any antimicrobial profile. However, an antimicrobial activity could not be totally excluded because we only addressed two pathogens (i.e. *S. aureus* and *C. albicans*). Therefore, additional screening experiments may be required to address other microbial species (i.e. Gram-negative bacteria). On the other hand, only residues of LEKTI-2 exhibit a colicidal effects, whereas full-length LEKTI-2 was found to be inactive against *E. coli*. It could be therefore hypothesized that only LEKTI-1 residues generated by klk7-dependent degradation/activation may produce antimicrobial peptides. Additional experiments are required to rule out this eventuality.

LEKTI-1 and skin diseases

Netherton syndrome

The importance of LEKTI-1 for maintaining epidermal homeostasis could be clearly illustrated by the clinical findings from SPINK5 mutations in patients with Netherton syndrome (NS) [8]. NS is an autosomal recessive disorder with a broad spectrum of phenotype severity correlating with the residual expression of LEKTI-1 [8, 35] (Fig. 4). When LEKTI-1 is mutated, failure to thrive, infections and hypernatraemic dehydration may

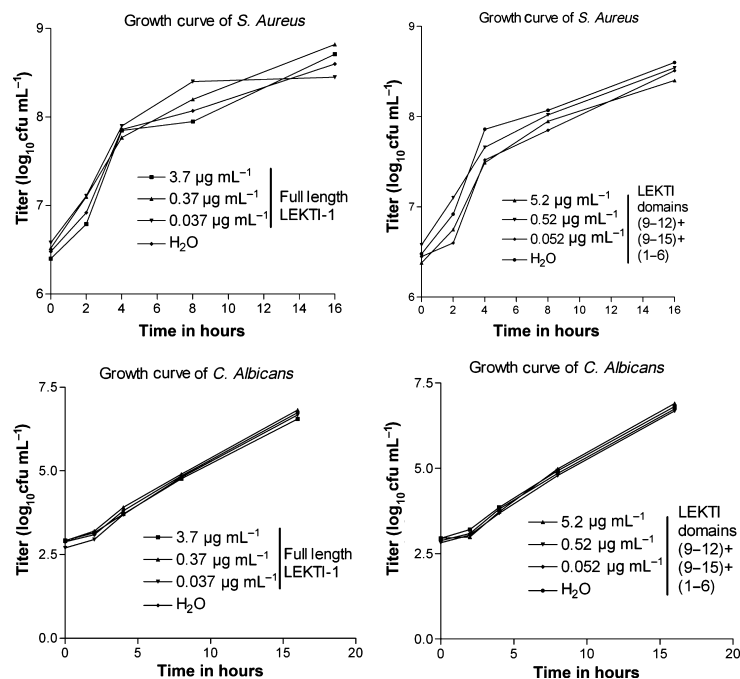


Figure 3 Antimicrobial activity of lympho-epithelial-Kazal-type-1 inhibitor (LEKTI-1).

result in a high postnatal mortality. Patients, who survive this critical period, develop AD-like eruption with increased IgE, scaling, erythrodermic ichthyosis [36]. Hair shaft defects (trichorrhexis invaginata or 'bamboo hair') is also observed in NS patients and when associated with erythrodermic ichthyosis, it constitutes a pathognomonic hallmark of the disease [37]. We have also demonstrated that the phenotypic variations seen in NS correlate with the magnitude of SP activation, which is in turn inversely correlated with residual LEKTI-1 expression [8]. Unrestricted SP activity in NS provokes the degradation of *dsg1* and *dsc1*, both normally expressed in the suprabasal layers of human epidermis [8]. Unlike pemphigus vulgaris, an auto-immune disorder resulting from the production of auto-antibodies directed against *dsg1*, Nicholski test remains negative in patients with NS and epidermal blister formation has never been reported. A compensatory mechanism leading to the expression of both *dsg3* and *dsc3* in the outer epidermis (normally present in the lower epidermis) may compensate for the loss of *dsg1/dsc1* and consequently prevent epidermal blistering [8]. Hypersecretion of LB is also a typical feature of NS and constitutes another compensatory feature consequent to the profound permeability barrier abnormality [8, 36].

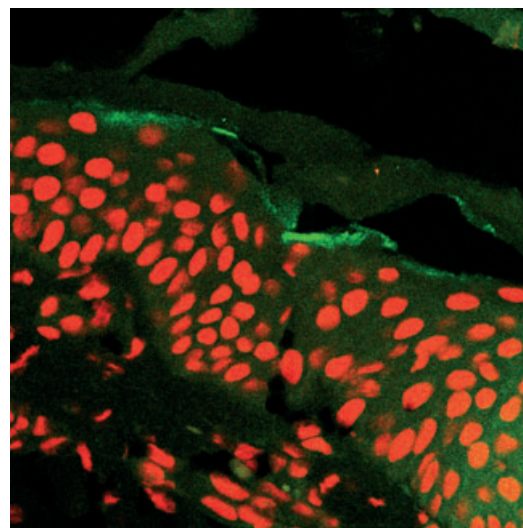


Figure 4 Residual lympho-epithelial-Kazal-type-1 inhibitor (LEKTI-1) expression in a patient with Netherton syndrome.

Atopic dermatitis

Because NS includes AD features and because AD and NS are characterized by skin barrier dysfunction [38, 39] and elevated serum IgE levels, it is considered that non-syndromic AD might also involve variation in *SPINK5* gene. Walley *et al.*

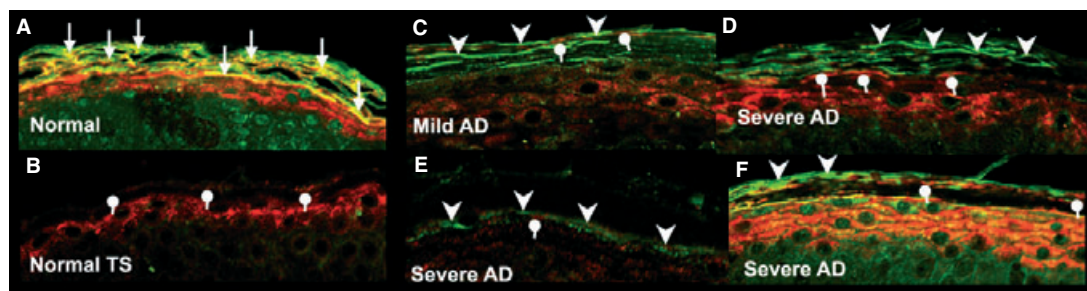


Figure 5 Co-expression of KLK7 and lympho-epithelial-Kazal-type inhibitor (LEKTI) was performed on sections from normal epidermis prior to (A) and following cellophane-induced tape stripping (TS) of the SC (B) and mild to severe non-involved atopic individuals (C–F).

[40] found that a single nucleotide polymorphism Glu420Lys variant is associated with AD and atopy. Kato *et al.* [41] also showed six other gene polymorphisms in Japanese patients associated with AD. These two studies strongly suggest that SPINK5 gene encoding for LEKTI-1 plays a role in the pathogenesis of AD. Interestingly, a U.K. case-control study found an insertion in the 3'-untranslated region of the *klk7* gene associated with AD, suggesting an additional SPI/SPI imbalance as potentially responsible mechanism in the onset of AD [42]. On the other hand, the two common null mutations in the filaggrin (FLG) gene are now widely recognized as strong risk factors for AD [43]. However, gene-gene interaction analysis between FLG mutations and both polymorphisms in *klk7* and SPINK5 genes showed no correlation with AD manifestations [44].

We therefore addressed protein-protein interaction on skin sections from a non-atopic human volunteer (prior to and following permeability barrier abrogation by tape stripping) and from non-involved skin biopsies taken from patients with mild to severe AD (Fig. 5). Double immunostaining was performed for *klk7* (green) and LEKTI (red). Under basal conditions, areas of LEKTI-SCCE co-expression (yellow areas corresponding to the accumulation of red – LEKTI – and green – *klk7*; shown by arrows) are observed within the SC and at the SC-SG junction (the SC-SC interface delineated by dots in Fig. 5). Following tape-stripping (TS)-induced barrier abrogation, the immunostaining for both *klk7* and LEKTI is decreased, whereas the yellow co-expression areas completely disappear. Similarly, the yellow co-expression areas are hardly observed within the SC of patients with mild to severe AD. Mainly, the green staining for *klk7* predominates. Knowing that LEKTI is

possessed and further degraded in the presence of active *klk7* (Fig. 2), tape stripping-induced activation of the SP cascade may be responsible for LEKTI consumption following TS (i.e. normal human volunteer), whereas increased level/activity of *klk* in AD [45] may result in similar over-utilization of LEKTI resources. How mutations in the FLG gene contribute to the activation of the *klk* cascade and/or the decrease in the expression of LEKTI still needs to be determined.

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