SMU
Sikkim Manipal University

Manipal
INSPIRED BY LIFE

SMU Medical Journal

ISSN : 2349 – 1604 (Volume – 1, No. 2, July 2014) Review Article

Current Status of LEKTI, a Physiological Inhibitor of Multiple Proteinases in the Skin - A Review

Arumugam Jayakumar*, Venugopal Radjendirane
Department of Experimental Therapeutics
The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77054, USA

*Corresponding author
E-mail: ajayakum@mdanderson.org
Telephone: 0017135939895 (Office)
Cell: 0017132617086.

Manuscript received : 06.05.2014
Manuscript accepted: 01.06.2014

Abstract

Serine Protease Inhibitor Kazal-type 5 *(SPINK5)* gene encodes 3 different Lympho-Epithelial Kazal-Type-Inhibitor (LEKTI) isoforms, which differ in their C-terminal sequence, are organized into longer than 15, 15, and 13 inhibitory domains. Pro-LEKTI is processed intracellular and the bioactive LEKTI fragments are secreted. LEKTI shows a restricted expression pattern in skin, thymus, oral mucosa, vaginal epithelium, Bartholin's glands, pituitary, tonsils, and parathyroid glands. Recombinant full-length LEKTI and rLEKTI fragments inhibit the activity of plasmin, subtilisin A, cathepsin G, neutrophil elastase, trypsin, caspase 14, and kallikreins (KLK) 5, 6, 7, 13, and 14 (involved in skin desquamation and growth hormone processing) to varied extents. Loss-of-function

mutations, polymorphisms, and transcriptional inactivation of the cognate *SPINK5* gene resulting in LEKTI loss or defective LEKTI processing is linked to Netherton syndrome (NS), head and neck squamous cell carcinomas (HNSCC), asthma, and chronic rhinosinusitis. Here, we give a brief review on the published work of LEKTI.

Key words: *SPINK5*, LEKTI, NS, HNSCC, proteinases, KLK, ECM, invasion

Introduction

Proteinases are involved in numerous homeostatic and disease processes (1)**.** Proteinases indeed form a deeply interconnected protease web embedded in every tissue proteome (2). In this network, natural proteinases inhibitors such as the tissue inhibitors of matrix metalloproteases (TIMPs), maspin, elafin, hespin, headpin, SERPINs, SPI, and LEKTI are important control points in proteolytic signaling (3-7). The importance of proteinases and their endogenous inhibitors in the biology of human cancer is also supported by a plethora of data (8-10). Loss-of-function mutations in *SPINK5* gene cause Netherton syndrome (11-17). We and others identified *SPINK5* as one of the genes downregulated in head and neck cancer (18, 19). In this review article, we give a glimpse of the published work on the organization, processing and secretion, and pathophysiological role of LEKTI.

LEKTI Organization

Lympho-epithelial kazal-type-inhibitor (LEKTI) was named by one of the original groups who cloned this protein's gene to reflect the observed pattern of its expression in both epithelial tissue and leukocytes (20). *SPINK5* encodes the LEKTI protein, which consists of 1064 amino acids organized into 15 potential inhibitory domains on the basis of the furin cleavage sites found within the full-length molecule. Each of the *SPINK5* domains is slightly different from the others and this suggests that the protein may have polyvalent action against multiple substrates. At the N-terminal is a secretory signal peptide

sequence consisting of 22 amino acids (21). Two of the 15 LEKTI domains (domains 2 and 15) resemble typical Kazal-type serine proteinase inhibitors; the remaining 13 domains share partial homology to Kazal-type inhibitors but lack one of the three conserved Kazal-type disulfide bridges (22). However, it was later shown that *SPINK5* indeed generates three classes of transcripts encoding three different LEKTI isoforms, which differ in their C-terminal portion (23). Their results discovered that in addition to the previously described 15 domain isoform, *SPINK5* encodes a shorter LEKTI isoform composed of only the first 13 domains, as well as a longer isoform carrying a 30-amino-acid residue insertion between the 13th and 14th inhibitory domains (Figure 1). To investigate the reasons for differences in the folds of the homologous LEKTI domains 1 and 6 (22), Tidow et al. determined the solution structure of a chimeric domain (Dom1PI) from the multidomain Kazal-type serine proteinase inhibitor LEKTI using multidimensional NMR spectroscopy (24) and concluded that the secondary structure of Dom1PI is determined not only by the local protein sequence but also by nonlocal interactions.

LEKTI Processing and Secretion

Initially LEKTI has been shown to be expressed in differentiated primary human keratinocytes (HKs) as two N-glycosylated precursor proteins of 145 and 125 kDa; the latter isoform results from alternative processing of the *SPINK5* pre-mRNA in HKs (25). Later it was demonstrated that in differentiated cultured human keratinocytes *SPINK5* encodes not two but three transcripts due to alternative splicing and all three transcripts are translated into three LEKTI isoforms (longer than 15, 15, and 13). The authors concluded that the alternative processing of the *SPINK5* pre-messenger RNA represents an additional mechanism to further increase the structural and functional diversity of the LEKTI bioactive fragments. We constructed deletion mutants of LEKTI, expressed them in HEK 293T cells, and analyzed their secretion behavior, stability, subcellular distribution, and proteinase inhibitory function. We demonstrated that the

N-terminal signal peptide is required for LEKTI import into the ER and ordered the cleavage products on the 125 kDa pro-LEKTI from the amino- to carboxy-terminal as follows: 37-, 40-, and 60 kDa (26). This arrangement allowed us to suggest two potential furin cleavage sites, one spanning LEKTI residues 352 to 355 and the second one spanning LEKTI residues 678 to 681, which are most likely used during LEKTI intracellular processing *in vivo*. Consistent with our proposal, Fortugno et al. identified three processing LEKTI intermediates and quantified the individual LEKTI fragments in the uppermost epidermis and showed that the ratios between LEKTI polypeptides and active KLK5 are compatible with a fine-tuned inhibition (27). Furthermore, the isolation of three single LEKTI domains (domains 1, 5, and 6) from human blood filtrate (28) and one multiple domain (domains 8-12) from primary epidermal HK conditioned medium (29) further indicated that LEKTI fragments generated intracellular are further cleaved extracellular thus generating a number of potentially bioactive single domain and multiple domain LEKTI active fragments. Consistent with this scheme we recently identified a broad range of protease inhibitors that are cleaved by meprins including LEKTI, implicating meprins in the indirect regulation of KLK activity (30). Interestingly, it was demonstrated a frequent and non-conservative LEKTI variant, E420K, in different atopic dermatitis (AD) populations (31, 32) prevents the formation of the LEKTI fragment D6D9 known to display the strongest inhibitory activity against KLK5-mediated desmoglein-1 (DSG1) degradation (33).

Pathophysiological Role of LEKTI

SPINK5 was identified as the defective gene in Netherton syndrome (11). To date, almost 50 mutations have been identified in the *SPINK5* gene in Netherton's patients, all of which result in premature termination codons implying defective protein expression occurs (17). To study the NS in mouse model, Yang et al. generated a transgenic mouse line with an insertional mutation that inactivated the mouse *SPINK5* ortholog. Their results showed that mutant mice exhibit fragile stratum corneum and perinatal death due

to dehydration (34). Currently, there are no curative treatments for NS. Interestingly, a recent paper showed the development of a HIV-1 based, self-inactivating lentiviral vector to express SPINK5 in keratinocytes as part of an ex-vivo gene therapy strategy for NS (35).

We identified *SPINK5* as one of the genes downregulated in head and neck squamous cell carcinoma (HNSCC) and cloned the cDNA encoding the 125-kDa isoform (18). Subsequently, we purified human recombinant LEKTI (rLEKTI) using a baculovirus/insect cell expression system and examined its inhibitory profile. Our studies discovered that rLEKTI inhibited the serine proteinases plasmin, subtilisin A, cathepsin G, human neutrophil elastase, and trypsin, but not chymotrypsin. Moreover, rLEKTI did not inhibit the cysteine proteinase papain or cathepsin K, L, or S. Further, rLEKTI inhibitory activity was inactivated by treatment with 20 mM DTT, suggesting that disulfide bonds are important to LEKTI function. The inhibition of plasmin, subtilisin A, cathepsin G, elastase, and trypsin by rLEKTI occurred through a noncompetitive-type mechanism, with inhibitory constants (*K*i) of 27 nM ± 5, 49 nM ± 3, 67 nM ± 6, 317 nM ±36, and 849 nM ± 55, respectively (36). In the course of studies aimed at understanding the structure and function of different LEKTI domains, we demonstrated that recombinant LEKTI6-9´ inhibited trypsin and subtilisin A but not plasmin, cathepsin G, or elastase (37). In our subsequent work, we characterized the interaction of two recombinant LEKTI fragments containing three or four intact Kazal domains (domains 6-8 and 9-12) with recombinant rhK5, a trypsin-like protease, and recombinant rhK7, a chymotrypsin-like protease (38). We showed that both fragments inhibited rhK5 similarly in binding and kinetic studies performed at pH 8.0, as well as pH 5.0, the pH of the stratum corneum where both LEKTI and proteases may function. These results established that LEKTI, at least in fragment form, is a potent inhibitor of rhK5 and that this protease may be a target of LEKTI in human skin. In our later studies we extended our studies to some more KLK members and examined their interactions with different

LEKTI fragments (39, 40). Our studies discovered that KLK1 was not inhibited by any serine protease inhibitor tested including LEKTI. However, KLK5, KLK6, KLK13 and KLK14 were potently inhibited by rLEKTI(1-6), rLEKTI(6-9') and rLEKTI(9-12) with K_i values in the range of 2.3-28.4 nM, 6.1-221 nM and 2.7-416 nM for each respective fragment. Only KLK5 was inhibited by rLEKTI(12-15) (Ki = 21.8 nM). No KLK was inhibited by SLPI or elafin. We also found out that apart from KLK13, all KLKs digested the ectodomain of DSG1 within cadherin repeats, Ca^{2+} binding sites or in the juxtamembrane region. These findings may have clinical implications for the treatment of skin disorders in which KLK activity is elevated.

We also assessed the basis for phenotypic variations in patients with "mild", "moderate", and "severe" NS (41). We observed that the magnitude of KLK activation correlated with both the barrier defect and clinical severity, and inversely with residual LEKTI expression and LEKTI co-localizes within the stratum corneum (SC) with kallikreins 5 and 7 and inhibits both KLKs. Collectively Our study indicated that multiple KLKs may participate in desquamation through cleavage of desmoglein 1 and regulation by LEKTI. We also showed that KLKs 5, 6 and 14 is involved in proteolytic processing of hGH in the pituitary and therefore LEKTI indirectly regulates the growth process (42). In an attempt to comprehensively identify candidate protease targets of LEKTI, we employed a label-free quantitative proteomic approach and cells scraped from the elbow and demonstrated that full-length LEKTI and recombinant fragments of LEKTI inhibited caspase 14, dermcidin, and cathepsin G (43, 44). A recent paper showed that epithelial cells transfected with a variant *SPINK5* expression vector produced more IL-6, IL-8 and RANTES compared to non-transfected cells and had no effect on *MUC5AC* transcription suggesting that LEKTI exert its effect in atopic diseases and asthma via a non-protease inhibitory mechanism (45). The development and licensing of several LEKTI monoclonal antibodies by our group now allowed determining the LEKTI protein expression in HNSCC tumor tissues. Using LEKTI mAb 1C11G6, we show here that in specimens of

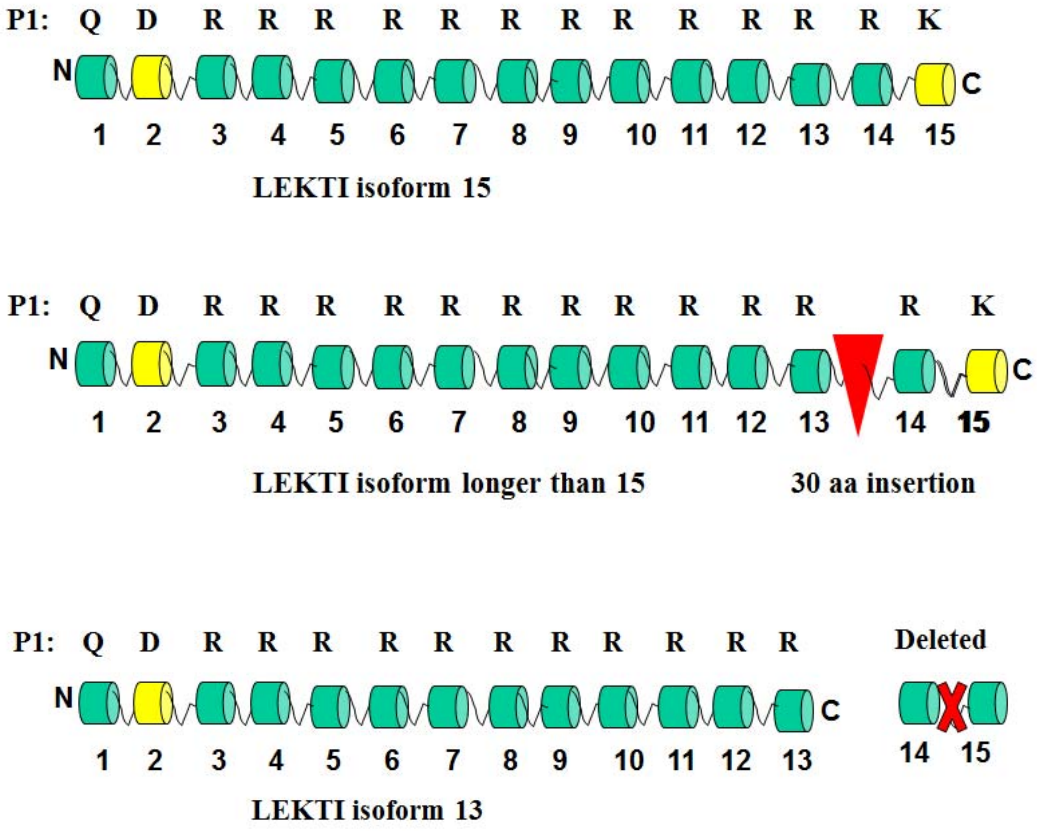

Figure 1: Organization of LEKTI inhibitory domains (Genbank accession no. NP_006837). yellow boxes denote Kazal-type inhibitory domains (3 disulphide bonds); Green boxes represent non-Kazal-type domains (2 disulphide bonds). The identity of the active site P1 residue is indicated above each domain.

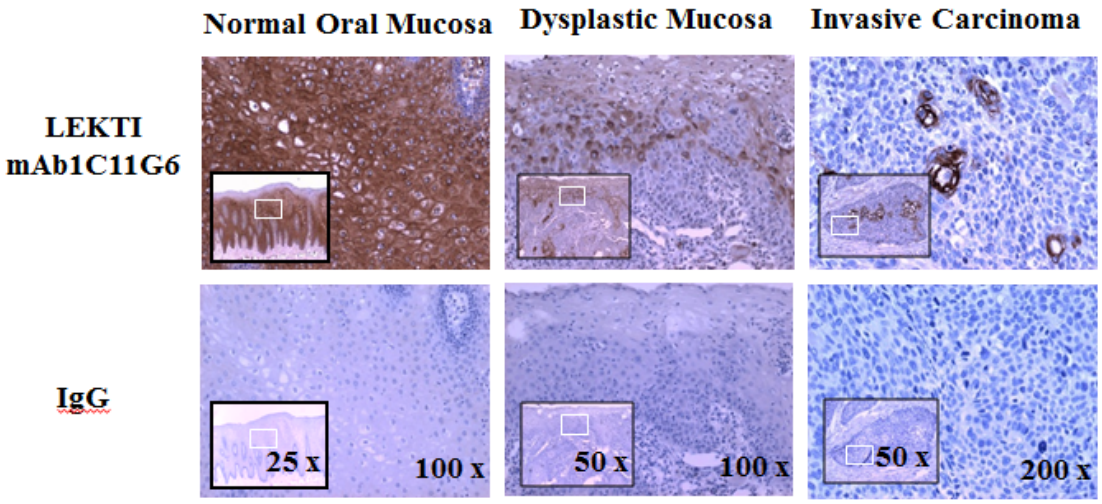

Figure 2: Immunohistochemical analysis of LEKTI in primary tumor specimens of patients with SCC of the oral tongue. Immunostaining was performed in with an automated staining machine (Dako) using an LSAB+ kit. Incubation of the primary antibody was performed with anti-LEKTI diluted at 1:150 for 60 minutes. Slides were analyzed by light microscopy.

histologically normal mucosa, LEKTI-positive staining was present in the cytoplasm of epithelial cells extending above the basal layers (Figure 2). Conversely, in specimens of dysplastic mucosa, LEKTI-positive staining was diminished in all layers of the epithelium. Moreover, in the majority of specimens of invasive carcinoma staining was limited to a few cells scattered within the tumor of nests of more differentiated tumor cells. Our immunohistochemical analysis of LEKTI expression in matched patient specimens confirmed our previous findings of lost or down-regulated LEKTI mRNA transcription in similar specimens (18).

Acknowledgement

The authors are thankful to Dr. Gary L Clayman, Department of Head and Neck Surgery, The University of Texas M.D. Anderson Cancer Center, for the funding and for his encouragement.

References

(1) Matrisian LM. Cancer biology: extracellular proteinases in malignancy. [Review] [10 refs]. Curr Biol 1999;9:R776-R778.

(2) Doucet A, Overall CM. Protease proteomics: revealing protease in vivo functions using systems biology approaches. Mol Aspects Med 2008;29:339-58.

(3) Pemberton PA, Tipton AR, Pavloff N, Smith J, Erickson JR, Mouchabeck ZM, et al. Maspin is an intracellular serpin that partitions into secretory vesicles and is present at the cell surface. Journal of Histochemistry & Cytochemistry 1997;45:1697-706.

(4) Toth M, Bernardo MM, Gervasi DC, Soloway PD, Wang Z, Bigg HF, et al. Tissue inhibitor of metalloproteinase (TIMP)-2 acts synergistically with synthetic matrix metalloproteinase (MMP) inhibitors but not with TIMP-4 to enhance the (Membrane type 1)-MMP-dependent activation of pro-MMP-2. J Biol Chem 2000;275:41415-23.

(5) Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, Gettins PG, et al. The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. [Review] [41 refs]. J Biol Chem 2001;276:33293-6.

(6) Jayakumar A, Kang Y, Frederick MJ, Pak SC, Henderson Y, Holton PR, et al. Inhibition of the cysteine proteinases cathepsins K and L by the serpin headpin (SERPINB13): a kinetic analysis. Archives of Biochemistry & Biophysics 2003;409:367-74.

(7) Roelandt T, Thys B, Heughebaert C, De VA, De PK, Roseeuw D, et al. LEKTI-1 in sickness and in health. Int J Cosmet Sci 2009;31:247-54.

(8) Coussens LM, Werb Z. Matrix metalloproteinases and the development of cancer. [Review] [56 refs]. Chemistry & Biology 1996;3:895-904.

(9) Ortega N, Behonick D, Stickens D, Werb Z. How proteases regulate bone morphogenesis. [Review] [21 refs]. Ann N Y Acad Sci 2003;995:109-16.

(10) Werb Z, Vu TH, Rinkenberger JL, Coussens LM. Matrix-degrading proteases and angiogenesis during development and tumor formation. [Review] [28 refs]. APMIS 1999;107:11-8.

(11) Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat Genet 2000;25:141-2.

(12) Raghunath M, Tontsidou L, Oji V, Aufenvenne K, Schurmeyer-Horst F, Jayakumar A, et al. SPINK5 and Netherton syndrome: novel mutations, demonstration of missing LEKTI, and differential expression of transglutaminases. J Invest Dermatol 2004;123:474-83.

(13) Descargues P, Deraison C, Bonnart C, Kreft M, Kishibe M, Ishida-Yamamoto A, et al. Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat Genet 2005;37:56-65.

(14) Komatsu N, Saijoh K, Jayakumar A, Clayman GL, Tohyama M, Suga Y, et al. Correlation between SPINK5 gene mutations and clinical manifestations in Netherton syndrome patients. J Invest Dermatol 2008;128:1148-59.

(15) Di WL, Hennekam RC, Callard RE, Harper JI. A heterozygous null mutation combined with the G1258A polymorphism of SPINK5 causes impaired LEKTI function and abnormal expression of skin barrier proteins. Br J Dermatol 2009;161:404-12.

(16) Diociaiuti A, Castiglia D, Fortugno P, Bartuli A, Pascucci M, Zambruno G, et al. Lethal Netherton syndrome due to homozygous p.Arg371X mutation in SPINK5. Pediatr Dermatol 2013;30:e65-e67.

(17) D'Alessio M, Fortugno P, Zambruno G, Hovnanian A. Netherton syndrome and its multifaceted defective protein LEKTI. G Ital Dermatol Venereol 2013;148:37-51.

(18) Gonzalez HE, Gujrati M, Frederick M, Henderson Y, Arumugam J, Spring PW, et al. Identification of 9 genes differentially expressed in head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2003;129:754-9.

(19) Shah TM, Patel AK, Bhatt VD, Tripathi AK, Shah S, Shankar V, et al. The landscape of alternative splicing in buccal mucosa squamous cell carcinoma. Oral Oncol 2013;49:604-10.

(20) Magert HJ, Standker L, Kreutzmann P, Zucht HD, Reinecke M, Sommerhoff CP, et al. LEKTI, a novel 15-domain type of human serine proteinase inhibitor. J Biol Chem 1999;274:21499-502.

(21) Walden MF, Kreutzmann PF, Drogemuller KF, John HF, Forssmann WG FAU, Hans-Jurgen M. - Biochemical features, molecular biology and clinical relevance of the human 15-domain serine proteinase inhibitor LEKTI. - Biol Chem 2002 Jul-Aug;383(7-8):1139-41:1139-41.

(22) Lauber TF, Schulz AF, Schweimer KF, Adermann KF, Marx UC. - Homologous proteins with different folds: the three-dimensional structures of domains 1 and 6 of the multiple Kazal-type inhibitor LEKTI. - J Mol Biol 2003 Apr 18;328(1):205-19:205-19.

(23) Tartaglia-Polcini A, Bonnart C, Micheloni A, Cianfarani F, Andre A, Zambruno G, et al. SPINK5, the Defective Gene in Netherton Syndrome, Encodes Multiple LEKTI Isoforms Derived from Alternative Pre-mRNA Processing. J Invest Dermatol 2005;.

(24) Tidow H, Lauber T, Vitzithum K, Sommerhoff CP, Rosch P, Marx UC. The solution structure of a chimeric LEKTI domain reveals a chameleon sequence. Biochemistry (Mosc) 2004;43:11238-47.

(25) Bitoun E, Micheloni A, Lamant L, Bonnart C, Tartaglia-Polcini A, Cobbold C, et al. LEKTI proteolytic processing in human primary keratinocytes, tissue distribution and defective expression in Netherton syndrome. Hum Mol Genet 2003;12:2417-30.

(26) Jayakumar A, Kang Y, Henderson Y, Mitsudo K, Liu X, Briggs K, et al. Consequences of C-terminal domains and N-terminal signal peptide deletions on LEKTI secretion, stability, and subcellular distribution. Archives of Biochemistry & Biophysics 2005;435:89-102.

(27) Fortugno P, Bresciani A, Paolini C, Pazzagli C, El HM, D'Alessio M, et al. Proteolytic activation cascade of the Netherton syndrome-defective protein, LEKTI, in the epidermis: implications for skin homeostasis. J Invest Dermatol 2011;131:2223-32.

(28) Magert HJ, Kreutzmann P, Drogemuller K, Standker L, Adermann K, Walden M, et al. The 15-domain serine proteinase inhibitor LEKTI: biochemical properties, genomic organization, and pathophysiological role. [Review] [59 refs]. Eur J Med Res 2002;7:49-56.

(29) Ahmed A, Kandola P, Ziada G, Parenteau N. Purification and partial amino acid sequence of proteins from human epidermal keratinocyte conditioned medium. J Protein Chem 2001;20:273-8.

(30) Jefferson T, Auf dem KU, Bellac C, Metz VV, Broder C, Hedrich J, et al. The substrate degradome of meprin metalloproteases reveals an unexpected proteolytic link between meprin beta and ADAM10. Cell Mol Life Sci 2013;70:309-33.

(31) Kabesch M, Carr D, Weiland SK, von Mutius E. Association between polymorphisms in serine protease inhibitor, kazal type 5 and asthma phenotypes in a large German population sample. Clin Exp Allergy 2004;34:340-5.

(32) Moffatt MF. SPINK5: a gene for atopic dermatitis and asthma. Clin Exp Allergy 2004;34:325-7.

(33) Fortugno P, Furio L, Teson M, Berretti M, El HM, Zambruno G, et al. The 420K LEKTI variant alters LEKTI proteolytic activation and results in protease deregulation: implications for atopic dermatitis. Hum Mol Genet 2012;21:4187-200.

(34) Yang T, Liang D, Koch PJ, Hohl D, Kheradmand F, Overbeek PA. Epidermal detachment, desmosomal dissociation, and destabilization of corneodesmosin in Spink5-/- mice. Genes Dev 2004;18:2354-8.

(35) Di WL, Larcher F, Semenova E, Talbot GE, Harper JI, Del RM, et al. Ex-vivo gene therapy restores LEKTI activity and corrects the architecture of Netherton syndrome-derived skin grafts. Mol Ther 2011;19:408-16.

(36) Mitsudo K, Jayakumar A, Henderson Y, Frederick MJ, Kang Y, Wang M, et al. Inhibition of serine proteinases plasmin, trypsin, subtilisin A, cathepsin G, and elastase by LEKTI: a kinetic analysis. Biochemistry (Mosc) 2003;42:3874-81.

(37) Jayakumar A, Kang Y, Mitsudo K, Henderson Y, Frederick MJ, Wang M, et al. Expression of LEKTI domains 6-9' in the baculovirus expression system: recombinant LEKTI domains 6-9' inhibit trypsin and subtilisin A. Protein Expression & Purification 2004;35:93-101.

(38) Schechter NM, Choi EJ, Wang ZM, Hanakawa Y, Stanley JR, Kang Y, et al. Inhibition of human kallikreins 5 and 7 by the serine protease inhibitor lympho-epithelial Kazal-type inhibitor (LEKTI). Biol Chem 2005;386:1173-84.

(39) Deraison C, Bonnart C, Lopez F, Besson C, Robinson R, Jayakumar A, et al. LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. Mol Biol Cell 2007;18:3607-19.

(40) Borgono CA, Michael IP, Komatsu N, Jayakumar A, Kapadia R, Clayman GL, et al. A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. J Biol Chem 2007;282:3640-52.

(41) Hachem JP, Wagberg F, Schmuth M, Crumrine D, Lissens W, Jayakumar A, et al. Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome. J Invest Dermatol 2006;126:1609-21.

(42) Komatsu N, Saijoh K, Otsuki N, Kishi T, Micheal IP, Obiezu CV, et al. Proteolytic processing of human growth hormone by multiple tissue kallikreins and regulation by the serine protease inhibitor Kazal-Type5 (SPINK5) protein. Clin Chim Acta 2007;377:228-36.

(43) Bennett K, Callard R, Heywood W, Harper J, Jayakumar A, Clayman GL, et al. New role for LEKTI in skin barrier formation: label-free quantitative proteomic identification of caspase 14 as a novel target for the protease inhibitor LEKTI. J Proteome Res 2010;9:4289-94.

(44) Bennett K, Heywood W, Di WL, Harper J, Clayman GL, Jayakumar A, et al. The identification of a new role for LEKTI in the skin: The use of protein 'bait' arrays to detect defective trafficking of dermcidin in the skin of patients with Netherton syndrome. J Proteomics 2012;75:3925-37.

(45) Birben E, Sackesen C, Turgutoglu N, Kalayci O. The role of SPINK5 in asthma related physiological events in the airway epithelium. Respir Med 2012;106:349-55.

Authors Colounn

Arumugam Jayakumar received his Ph.D. in membrane biochemistry from JNU at New Delhi and moved to US in 1980. He has spent 2 years at NIH, 18 years at Baylor College of Medicine, and since 2000 working as a senior research scientist at M.D. Anderson Cancer Center. He authored or co-authored more than 65 peer-reviewed publications and written 2 book chapters. He has co-built and co-managed an extensive research portfolio funded by NSF/NCI/NIH/HGC, American Academy of Otolaryngology-head and neck surgery, Welch Foundation and Viragh Foundation. He has trained/mentored 30 pre/post-doctoral trainees/junior faculty/clinical fellows/summer students/technicians. He is a co- inventor for 1 issued patent and 2 licensing agreement. He serves as an ad-hoc reviewer for various journals, foreign examiner for Ph.D. theses from India, editorial member for Web Med Central plus, and Future International Journal of Science and Technology (FIJST).