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Clinica Chimica Acta 381 (2007) 78–84
www.elsevier.com/locate/clinchim

Invited critical review

Human tissue kallikreins: A road under construction

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Received 29 January 2007; accepted 13 February 2007
Available online 20 February 2007

Abstract

Background: The human tissue kallikrein gene family, located at chromosome 19q13.4, is the largest contiguous family of proteases in the human genome. The locus encodes all 15 members of the family, 13 of which have been reported as potential biomarkers for several carcinomas and other non-neoplastic diseases. Kallikreins are expressed by a wide range of tissues and implicated in a number of physiological functions, including skin desquamation, semen liquefaction, neural plasticity and the regulation of blood pressure. Kallikrein function is regulated at various levels, including transcription, translation and post-translation. The proteolytic activity of kallikreins is believed to be cascade mediated and may cross-talk with other proteases. These cascades are highly regulated through a series of feedback loops, inhibitors, (auto) degradation and internal cleavage. Uncontrolled proteolytic activity of kallikreins is implicated in a large number of neoplastic and non-neoplastic pathological conditions.
Conclusions: As our understanding of their regulatory and functional mechanisms continues to expand, kallikreins are expected to become novel targets for the design of new therapeutics.

Keywords: Human tissue kallikreins; Serine proteases; PSA; Proteolytic cascades; Biomarkers; Skin desquamation; Semen liquefaction; Tumor growth; Tumor invasion; Angiogenesis

Contents

Abbreviations: AAT, α_1-antitrypsin; ACT, α_1-antichymotrypsin; AD, Alzheimer's disease; AT, anti-thrombin; á$_2$M, α_2 macroglobulin; BKR B2, human bradykinin B2 receptor; CAG, cancer-associated gene; cAMP, cyclic adenosine monophosphate; DHT, dihydrotestosterone; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; EMT, epithelial to mesenchymal transition; EST, expressed sequence tag; FTD, frontotemporal dementia; hCAP, human cathelicidin antimicrobial protein; HRE, hormone response element; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IL, interleukin; JNK, Jun N-terminal kinase; KLK, human tissue kallikrein; LEKTI, lympho-epithelial Kazal-type inhibitor; LLP, low density lipoprotein; LMW, low molecular weight; MAP, mitogen-activated proteins; MBP, myelin basic protein; MMP, matrix metalloprotease; ORF, open reading frame; PAI, plasminogen activator inhibitor; PAP, prostatic acid phosphatase; PAR, protease-activated receptor; PCI, protein C inhibitor; PI, protease inhibitor; PKB, protein kinase B; Pre-ANF, precursor of atrial natriuretic factor; PSA, prostate-specific antigen; PTHrp, parathyroid hormone-related peptide; RT-PCR, reverse transcription-polymerase chain reaction; SAGE, serial analysis of gene expression; TGF, transforming growth factor; uPA, urokinase plasminogen activator; UTR, untranslated region; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide.

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doi:10.1016/j.cca.2007.02.023

1. Introduction

Human tissue kallikreins belong to a subgroup of secreted serine proteases within the S1 family of the clan SA [1]. Until recently, the major interest was given to the three members of the family known as the "classic kallikreins" (KLK1, 2 and 3). In the past decade however, work from our lab and others led to remarkable breakthroughs in the characterization of 12 novel members of the family. According to the new nomenclature system recommended by the Kallikrein subcommittee of HGNC (HUGO Nomenclature Committee), kallikreins 1–15 are denoted as KLKs [2]. To distinguish between proteins and genes, kallikrein proteins are written in standard font, e.g., KLK2, while genes are in italics, e.g., *KLK2*.

2. Genomic organization and protein structure

2.1. Gene structure

Human tissue kallikreins are encoded by the largest contiguous cluster of protease genes in the human genome [3]. So far, fifteen kallikrein genes have been identified as a cluster of approximately 300 kbp on human chromosome 19q13.4 [3,4]. Kallikrein genes range between 4.4 and 10.5 kbp and share common features, including exon/intron organization, conserved intronic intervals and exon length [5,6]. In addition, with the exception of the classic *KLK*s, kallikrein genes contain both 5′ and 3′ untranslated regions (UTRs), with varying length. The locus is confined to the testicular acid phosphatase gene (ACPT) and the cancer-associated gene (CAG) at the centromeric and telomeric ends, respectively [5]. *KLK*s are organized as a tandem array and are transcribed from telomere to centromere, with the exception of *KLK2 and KLK3* [3].

2.2. Phylogeny

Phylogenetic analyses indicate a significant level of homology in the *KLK* locus in mammals, suggesting a conserved function(s) of encoded proteins [4,7]. Evolutionary studies of kallikrein genes in the human, rat and mouse genome, in addition to complementary studies of the genomes of cotton-top tamarin, dog, horse and cow, have revealed a polyphyletic nature of the gene family [7]. However, since gene duplication is common in the *KLK* locus, the actual number of clades is expected to decline as the genomes of more primitive mammals become available. There is accumulating evidence that *KLK* duplication and silencing is species specific, which could explain interspecies variations of the locus size and gene number [5].

2.3. Splice variants

In addition to the fifteen expected full-length mRNAs, kallikrein genes exhibit a large number of transcript variants. To date, approximately 70 alternative splice variants of *KLK*s have been characterized [6]. Variant splicing primarily occurs at the coding regions as a result of exon skipping and consequent exon extension/truncation or intron retention [6,8]. Alternatively, variant transcripts may arise from non-conventional transcription start or polyadenylation sites [5,9–11]. Even though only a few truncated proteins have been identified experimentally [12–14], bioinformatic analyses of open reading frames (ORFs) have revealed several putative protein isoforms [15]. In the majority of cases, the sequence coding for the secretory signal is retained, suggesting that analogous to the full-length kallikreins, the truncated variants are secreted proteins [4]. The clinical utility of certain kallikrein isoforms as cancer biomarkers has been proposed [14,15].

2.4. Protein structure

Kallikrein proteins are expressed as single-chain pre-pro-serine proteases [5]. Each protein contains a signal (pre-) sequence of 16 to 30 amino acids that is cleaved from the N-terminus of the protein prior to secretion [3]. Pro-domains are short peptide sequences, i.e., 37 amino acids in pro-KLK5 and

4–9 amino acids in the remaining KLKs, and are cleaved upon activation [3]. Pro-KLKs, with the exception of pro-KLK4, are believed to be activated by cleavage after arginine (R) or lysine (K), which are preferred trypsin-like cleavage sites [3]. Mature KLKs contain a highly conserved catalytic triad of histidine (H), aspartic acid (D) and serine (S) [3]. Protein folding is believed to be achieved in part through 5–6 disulfide bonds between cystein residues [5].

So far, the protein structure of mature KLK4, KLK1 and both mature and pro-KLK6 have been solved by X-ray crystallography [16–19]. KLK proteins share several common structural features including two interacting β-barrels and α-helices, bridged by the active site and disulfide bonds. KLK1 contains an additional "kallikrein loop" structure, which is believed to play an important role in its substrate and inhibitor specificity [17]. Furthermore, external surface loops surrounding the substrate binding motifs may control enzymatic activity and define enzyme specificity [4].

3. Expression profile

The expression patterns of tissue kallikreins have extensively been studied using various techniques, including northern blot, reverse transcription-PCR (RT-PCR), EST and SAGE analyses, ELISA and immunohistochemical studies. Kallikreins are expressed in varying amounts in different cell types and tissues, both at the mRNA and protein levels [3]. Interestingly, many kallikreins display a striking overlapping pattern of expression. Furthermore, they confer a coordinated pattern of up- or down-regulation in a number of diseases, including several hormone-dependent carcinomas [2].

4. Regulatory mechanisms

4.1. Transcriptional regulation

Kallikrein gene expression is believed to be regulated through hormonal, as well as epigenetic factors such as methylation and histone modification. A large body of evidence indicates that almost all *KLK*s are regulated by steroid hormones [2]. Promoter studies of several kallikrein genes have revealed hormonal response elements (HREs) believed to be involved in the *cis*-regulation of transcription [4]. Given the co-expression pattern of kallikreins, a possible regulatory mechanism through a single locus control region has been suggested [20]. For example, an expression "cassette" of *KLK*s 10, 11, 13 and 14 regulated by dihydrotestosterone (DHT) and norgestrel was recently identified in breast cancer cells [21]. Given that none of these genes contain characterized HREs, an additional role of hormones as trans-acting transcriptional regulators is proposed [20,21].

Transcriptional dysregulation of kallikrein genes are directly associated with several pathological conditions, including certain carcinomas [20]. In addition, several epigenetic abnormalities, including hypermethylation of CpG islands and abnormalities in the chromatin structure of *KLK* locus, have been reported in breast cancer and lymphoblastic leukemia [20,22].

4.2. Regulation of enzymatic activity

Proteases are often activated through highly orchestrated proteolytic cascades [23]. Due to the irreversible nature of proteolytic activation, these cascades are tightly regulated through a series of feedback loops and inhibitors. Proteolytic regulatory mechanisms are critical in preventing deleterious effects due to uncontrolled protease activation.

Analogous to the majority of proteases, kallikreins are believed to exert their physiological functions through regulated proteolytic cascades [24–26]. Kallikreins 5, 14 and 7 are reported to be involved in a proteolytic cascade in the stratum corneum of skin [26]. Uncontrolled activation of these kallikreins is believed to be the major cause of over-desquamation in several skin disorders [27,28]. Additional cascades involving KLKs 5, 2, 11 and 3 may exist in seminal plasma and prostate tissues [4,25,29].

5. Enzyme kinetics

5.1. Substrate specificity

As a subgroup of serine proteases, kallikreins hydrolyze their target substrates through a nucleophilic attack directed by their

Table 1
Specificity, substrates and inhibitors of human tissue kallikreins

Kallikrein	Possible physiologic substrate	Candidate physiologic inhibitors
KLK1	LMW kininogen, pre-ANF, pro-insulin, LLP, prerenin, VIP, procollagenase, angiotensinogen, BKR B2 [1], pro-MMP2, 9, IGFBP3 [4]	Kallistatin, PCI, AAT, placental bikunin [1]
KLK2	Semenogelin I/II, fibronectin, pro-uPA [1,58], IGFBP 2, 3, 4, 5 [58], ADAMTS8, collagen IX-α chain [59]	PCI, PI-6, PAI-1, ATIII, α_2M [1]
KLK3	Semenogelin I/II, fibronectin, laminin, lysozyme, plasminogen, TGF-β, PTHrp [1], IGFP3, 4 [58]	ACT, α_2M, PCI, AAT, ATIII [1]
KLK4	Pro-uPA, PAP [1], enamelin [42]	α_2M, α_2AT, α_2AP [60]
KLK5	Collagen types I, II, III, IV, fibronectin, laminin, plasminogen, LMW kininogen, fibrinogen [35], hCAP18 [41]	α_2M, α_2AP, ATIII [1], LEKTI [28]
KLK6	Fibrinogen, fibronectin, laminin, collagen types I and IV, APP, plasminogen [1], MBP, ionotropic glutamate receptor [61]	ATIII, α_2AP, AAT, ACT [1]
KLK7	IL-1β, corneodesmosin [1], hCAP18 [41], fibrinogen [4]	LEKTI [28], PCI, α_1AT, α_1ACT, kallistatin [62]
KLK8	Fibronectin, gelatin, collagen type IV, fibrinogen and HMW-kininogen, plasminogen activator [63,64]	Antipain, chymostatin, leupeptin [63]
KLK11		PCI [62], APMSF, aprotinin [30]
KLK12		α_2AP, PCI [62], α_2 antiplasmin
KLK13	ECM, plasminogen [1]	α_2M, α_2AP, ACT [1]
KLK14	Collagens I–IV, fibronectin, laminin, kininogen, fibrinogen, plasminogen, vitronectin and IGFBP 2, 3 [31], matrilin4 [4]	α_1AT, α_2AP, antithrombin III and α_1ACT [31]

active serine residues [24]. Analogous to other serine proteases, substrate preference can be predicted based on the amino acid side chain surrounding the active site of the enzyme [5]. The majority of kallikreins contain aspartic or glutamic acid at their S1 position, suggesting their trypsin-like substrate specificity. Kallikreins 3, 7 and 9, with serine, asparagine and glycine S1, respectively, are expected to present a chymotrypsin-like substrate preference [5]. *In vitro* substrate studies indicate a possible ambivalent nature of KLKs 10, 11 and 14 [30–32]. Substrate specificities have been determined experimentally for the majority of kallikreins (Table 1), using diverse techniques including phage display [33], combinatorial libraries [34] and basic kinetic assays.

5.2. Inhibitors

To avoid unwanted substrate catalysis, the proteolytic activity of kallikreins is under intense regulatory mechanisms. Several reports indicate a possible inactivation mechanism in kallikreins 2, 3, 6, 7, 13 and 14 through internal cleavage and subsequent degradation [1]. Degradation may be autolytic or mediated through other proteases. In addition, divalent ions such as zinc have been shown to reversibly inhibit certain kallikreins such as KLKs 2, 3 and 5 [4,35]. Furthermore, a large number of possible endogenous inhibitors have been identified (Table 1). Complex formation between KLKs and some of these inhibitors have been proven *in vivo* [1].

Characteristic to common inhibitory mechanisms in serine proteases, kallikreins are believed to form transient non-covalent complexes with their inhibitors. These intermediate complexes may progress to "inhibitory pathways", resulting in kinetically "trapped" covalent complexes and an irreversible inactivation of the protease. Alternatively, inhibition is prevented through "substrate pathways" and cleavage of inhibitors by the active KLKs [1,36].

6. Signaling pathways

Kallikreins are believed to function partly through cross-talks with various signaling pathways. Signaling through kinins is by far the most studied signaling pathway in kallikreins. KLKs 1, 2 and more recently KLK12 were shown to release active kinins (bradykinin and kallidin) from the kininogens [37,38]. Kinin peptides, in turn, mediate signaling through a number of downstream targets such as prostacyclin-cAMP, nitric oxide cGMP and mitogen-activated protein (MAP) kinases [37]. The kallikrein–kinin system is instrumental in a wide range of processes, including the regulation of blood pressure, sodium homeostasis, inflammation and angiogenesis [37].

In addition to the kinin system, certain kallikreins, e.g., KLKs 2, 4 and 12, can activate the urokinase plasminogen activator (uPA) signaling pathway and consequently convert plasminogen into active plasmin [4,38]. Active plasmin then cleaves several downstream targets, such as fibrin and certain pro-metalloproteases (pro-MMPs) [4].

Lastly, recent reports indicate a possible activation mechanism of the family of protease-activated receptors (PARs) by kallikreins [39]. PARs are members of the G-protein-coupled receptor superfamily and are activated by cleavage of part of their extracellular domains [40]. Kallikreins are believed to act as tethered ligands by cleaving the N-terminus of the receptor. Consequently, the remaining extracellular part of the receptor acts as an agonist, causing physiological responses [39,40].

7. Physiological function

Kallikreins have been implicated in various physiological processes ranging from cellular homeostasis to tissue remodeling. As discussed previously, KLKs 2, 3, 5 and 11 are involved in a proteolytic cascade in seminal plasma. These kallikreins are critical in semen liquefaction through a cascade-mediated processing of semenogelins I and II [1]. In addition, KLK5 has been implicated in the proteolytic cascade in skin, mediating desquamation and possibly certain antimicrobial effects through cathelicidins [26,41].

KLK1 is believed to play an essential role in a large number of processes, including blood pressure regulation, smooth muscle contraction, neutrophil chemotaxis and pain induction, through the kinin signaling pathway [1]. In addition, KLK1 may function independent of the kinin signaling pathway, through growth factors and several other substrates.

Furthermore, based on the expression pattern, substrate recognition and function of orthologous proteins, the probable functions of some of the remaining kallikreins have been suggested. For instance, kallikrein 4 is believed to be involved in enamelogenesis by processing enamelin [42]. KLKs 6,10 and 13 have been implicated in activation of several prohormones in the islands of Langerhans in the pancreas [1]. Lastly, kallikreins 6 and 8 may function in myelination and synaptogenesis in the central system nervous [1,43].

8. Pathobiology

Uncontrolled proteolyses due to over-expression or over-activation of kallikreins have been implicated in a number of pathological conditions in cancer as well as non-cancer disease states.

8.1. Tumor growth regulation

Several kallikreins have been implicated in the regulation of tumor growth mainly through modulating the insulin-like growth factor (IGF) and IGF binding proteins (IGFBPs) [4] . Alternatively, kallikreins may activate several tumor growth factors through the uPA–uPAR and possibly PAR signaling pathways [4]. Recent data suggest a possible synergistic effect of kallikreins in tumor growth. For instance, ectopic co-expression of kallikreins 4, 5, 6 and 7 in ovarian cancer cells, implanted in nude mice, resulted in a significant tumor growth, as compared to the individual KLKs [44].

On the contrary, certain kallikreins are suggested to function as tumor growth suppressors [4]. KLK3 has been proposed to suppress tumor growth via activation of transforming growth factor (TGF) in prostate cancer [4,45]. Despite the correlative

expression pattern and reported cell-culture data, inhibitory effects of these kallikreins have remained controversial.

8.2. Angiogenesis

Kallikreins may promote angiogenesis directly by processing various components of the ECM or indirectly through the kallikrein–kinin and uPA/uPAR signaling pathways. Activation of the kallikrein–kinin system results in the release of active kinin peptides. Active kinin induces angiogenesis through activation of the cAMP, Akt/PKB and VEGF and suppression of the JNK and TGFâ signaling pathways [45]. Alternatively, kallikreins induce angiogenesis by activating several MMPs through the uPA/uPAR signaling pathway. In contrast, certain kallikreins, e.g., KLKs 3, 6 and 13, are reportedly anti-angiogenic. They prevent angiogenesis mainly through plasminogen fragmentation into angiostatin-like components [1,46].

8.3. Invasion

Tumor cells are required to invade their surrounding tissues and gain access to the circulatory system in order to metastasize. Dysregulated kallikrein expression and/or activation have been implicated in several essential steps of tumor invasion. For instance, kallikreins are involved in ECM degradation and remodeling, which are the required steps in tumor invasion. In addition, cancer cells must transform permanently from stationary epithelial cells into migratory mesenchymal cells in order to invade. Recent data indicate that KLKs 3 and 4 are involved in the epithelial to mesenchymal transition (EMT) of prostate cancer cells [4,47,48]. Additional data propose a role of several kallikreins in invasion of cancer cells into bone, through a process of osteoblastic bone metastasis [49,50]. For example, direct injection of KLK3 or KLK3-producing prostate cancer cells into human adult bone implants exhibit increased osteoblast proliferation and reduced osteoclastogenesis in nude mice [50]. Furthermore, murine osteoclast precursor cells treated with KLK3 display a significant KLK3-mediated apoptosis [50]. Kallikreins are proposed to mediate bone remodeling through activation of latent TGFβ2 [49].

8.4. Non-cancer linkage

There is accumulating data linking the aberrant expression and/or proteolytic activity of kallikreins to various non-neoplastic pathologies. For instance, the expression of several kallikreins is significantly elevated in patients with peeling skin syndrome [27]. In addition, uninhibited proteolysis of KLKs 5 and 7 is believed to be the main cause of over-desquamation observed in cases of Netherton syndrome [51–53].

Furthermore, as mentioned previously, kallikreins have been implicated in various physiological processes in the central nervous system and therefore may be involved in certain neurodegenerative disorders [54]. The expression pattern of KLKs 6, 7 and 10 are reportedly altered in patients with Alzheimer's disease (AD) and frontotemporal dementia (FTD) and may serve as diagnostic biomarkers [54,55].

Finally, aberrant kallikrein–kinin signaling may play a major role in a wide range of pathological processes, including inflammation, hypertension and renal diseases [3].

9. Clinical applications

9.1. Biomarkers

KLK3/PSA has received by far the most attention as a valuable tumor marker for screening, diagnosis and monitoring of prostate cancer. However, with the recent development of sensitive immunoassays, there is strong evidence that other kallikreins may serve as alternative or complementary diagnostic and/or prognostic biomarkers.

Studies from our lab and other groups have shown the potential of many kallikreins as biomarkers in a large number of carcinomas, as well as in several non-malignant diseases. So far, thirteen members of the family have been reported as possible serological or tissue tumor biomarkers [2].

9.2. Therapeutic utilities

Given the diverse functional role of kallikreins, this family of enzymes may represent promising novel targets for therapeutic interventions. To date, several therapeutic strategies to regulate the activity of kallikreins have been investigated. For instance, based on the structural features of non-specific endogenous serpins, specific synthetic inhibitors of KLK2 and KLK14 have been constructed [56,57]. Target specificity is achieved by exchange of the reactive center loop of serpins with specific target sequences identified by the phage display technology. In addition, high-throughput compound library screenings to identify agents that confer robust augmentation of kallikrein expression or activity are in progress (our unpublished data).

Lastly, given the tissue specificity of certain kallikreins, particularly KLK3, alternative site-directed therapeutic approaches have been proposed. For instance, cytotoxic agents conjugated to KLK3-specific peptide carriers have been engineered as prodrugs for tumor targeting in prostate cancer [4]. Moreover, several cytoreductive gene therapy strategies have been designed to deliver an exogenous suicide gene through prostate-specific *KLK3* transcriptional regulatory elements [4]. Finally, a number of immunomodulatory gene therapy approaches have been proposed to stimulate KLK3-specific T-cell response in order to augment immune response against prostate tumor cells [4].

10. Conclusion

Even though the value of kallikreins as biomarkers is well established, the functional significance of this family of enzymes has just begun to unravel. Analogous to the majority of proteases, kallikreins are believed to function through highly regulated proteolytic cascades. Dysregulated proteolyses of kallikreins, due to altered expression or uncontrolled activation, have been implicated in various steps of neoplastic development in several carcinomas, as well as many other non-cancer disorders.

Kallikreins may present attractive therapeutic targets due to their critical role in various pathological conditions and their restricted expression profiles. Thus, as our understanding of the functional role of kallikreins evolves, new clinical applications of this important family of enzymes are anticipated.

References

[1] Borgono CA, Michael IP, Diamandis EP. Human tissue kallikreins: physiologic roles and applications in cancer. Mol Cancer Res 2004;2: 257–80.

[2] Lundwall A, Band V, Blaber M, et al. A comprehensive nomenclature for serine proteases with homology to tissue kallikreins. Biol Chem 2006;387:637–41.

[3] Yousef GM, Diamandis EP. The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr Rev 2001;22: 184–204.

[4] Borgono CA, Diamandis EP. The emerging roles of human tissue kallikreins in cancer. Nat Rev Cancer 2004;4:876–90.

[5] Obiezu CV, Diamandis EP. Human tissue kallikrein gene family: applications in cancer. Cancer Lett 2005;224:1–22.

[6] Kurlender L, Borgono C, Michael IP, et al. A survey of alternative transcripts of human tissue kallikrein genes. Biochim Biophys Acta 2005;1755:1–14.

[7] Lundwall A, Clauss A, Olsson AY. Evolution of kallikrein-related peptidases in mammals and identification of a genetic locus encoding potential regulatory inhibitors. Biol Chem 2006;387:243–9.

[8] Michael IP, Kurlender L, Memari N, et al. Intron retention: a common splicing event within the human kallikrein gene family. Clin Chem 2005;51:506–15.

[9] Dong Y, Kaushal A, Brattsand M, Nicklin J, Clements JA. Differential splicing of KLK5 and KLK7 in epithelial ovarian cancer produces novel variants with potential as cancer biomarkers. Clin Cancer Res 2003;9:1710–20.

[10] Liu XF, Essand M, Vasmatzis G, Lee B, Pastan I. Identification of three new alternate human kallikrein 2 transcripts: evidence of long transcript and alternative splicing. Biochem Biophys Res Commun 1999;264:833–9.

[11] Mitsui S, Nakamura T, Okui A, Kominami K, Uemura H, Yamaguchi N. Multiple promoters regulate tissue-specific alternative splicing of the human kallikrein gene, KLK11/hippostasin. FEBS J 2006;273:3678–86.

[12] Heuze N, Olayat S, Gutman N, Zani ML, Courty Y. Molecular cloning and expression of an alternative hKLK3 transcript coding for a variant protein of prostate-specific antigen. Cancer Res 1999;59:2820–4.

[13] Tanaka T, Isono T, Yoshiki T, Yuasa T, Okada Y. A novel form of prostate-specific antigen transcript produced by alternative splicing. Cancer Res 2000;60:56–9.

[14] Dong Y, Bui LT, Odorico DM, et al. Compartmentalized expression of kallikrein 4 (KLK4/hK4) isoforms in prostate cancer: nuclear, cytoplasmic and secreted forms. Endocr Relat Cancer 2005;12:875–89.

[15] Tan OL, Whitbread AK, Clements JA, Dong Y. Kallikrein-related peptidase (KLK) family mRNA variants and protein isoforms in hormone-related cancers: do they have a function? Biol Chem 2006;387: 697–705.

[16] Debela M, Magdolen V, Grimminger V, et al. Crystal structures of human tissue kallikrein 4: activity modulation by a specific zinc binding site. J Mol Biol 2006;362:1094–107.

[17] Laxmikanthan G, Blaber SI, Bernett MJ, Scarisbrick IA, Juliano MA, Blaber M. 1.70 A X-ray structure of human apo kallikrein 1: structural changes upon peptide inhibitor/substrate binding. Proteins 2005;58:802–14.

[18] Bernett MJ, Blaber SI, Scarisbrick IA, Dhanarajan P, Thompson SM, Blaber M. Crystal structure and biochemical characterization of human kallikrein 6 reveals that a trypsin-like kallikrein is expressed in the central nervous system. J Biol Chem 2002;277:24562–70.

[19] Gomis-Ruth FX, Bayes A, Sotiropoulou G, et al. The structure of human prokallikrein 6 reveals a novel activation mechanism for the kallikrein family. J Biol Chem 2002;277:27273–81.

[20] Paliouras M, Diamandis EP. The kallikrein world: an update on the human tissue kallikreins. Biol Chem 2006;387:643–52.

[21] Paliouras M, Diamandis EP. Coordinated steroid hormone-dependent and independent expression of multiple kallikreins in breast cancer cell lines. Breast Cancer Res Treat 2006 (Aug. 8).

[22] Pampalakis G, Sotiropoulou G. Multiple mechanisms underlie the aberrant expression of the human kallikrein 6 gene in breast cancer. Biol Chem 2006;387:773–82.

[23] Amour A, Bird M, Chaudry L, Deadman J, Hayes D, Kay C. General considerations for proteolytic cascades. Biochem Soc Trans 2004;32:15–6.

[24] Yousef GM, Diamandis EP. Human tissue kallikreins: a new enzymatic cascade pathway? Biol Chem 2002;383:1045–57.

[25] Michael IP, Pampalakis G, Mikolajczyk SD, Malm J, Sotiropoulou G, Diamandis EP. Human tissue kallikrein 5 is a member of a proteolytic cascade pathway involved in seminal clot liquefaction and potentially in prostate cancer progression. J Biol Chem 2006;281:12743–50.

[26] Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T. A proteolytic cascade of kallikreins in the stratum corneum. J Invest Dermatol 2005;124:198–203.

[27] Komatsu N, Suga Y, Saijoh K, et al. Elevated human tissue kallikrein levels in the stratum corneum and serum of peeling skin syndrome-type B patients suggests an over-desquamation of corneocytes. J Invest Dermatol 2006;126:2338–42.

[28] Schechter NM, Choi EJ, Wang ZM, 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.

[29] Vaisanen V, Lovgren J, Hellman J, Piironen T, Lilja H, Pettersson K. Characterization and processing of prostate specific antigen (hK3) and human glandular kallikrein (hK2) secreted by LNCaP cells. Prostate Cancer Prostatic Dis 1999;2:91–7.

[30] Luo LY, Shan SJ, Elliott MB, Soosaipillai A, Diamandis EP. Purification and characterization of human kallikrein 11, a candidate prostate and ovarian cancer biomarker, from seminal plasma. Clin Cancer Res 2006;12: 742–50.

[31] Borgono CA, Michael IP, Shaw JL, et al. Expression and functional characterization of the cancer-related serine protease, human tissue kallikrein 14. J Biol Chem 2006.

[32] Debela M, Magdolen V, Schechter N, et al. Specificity profiling of seven human tissue kallikreins reveals individual subsite preferences. J Biol Chem 2006;281:25678–88.

[33] Deperthes D. Phage display substrate: a blind method for determining protease specificity. Biol Chem 2002;383:1107–12.

[34] Harris JL, Backes BJ, Leonetti F, Mahrus S, Ellman JA, Craik CS. Rapid and general profiling of protease specificity by using combinatorial fluorogenic substrate libraries. Proc Natl Acad Sci U S A 2000;97:7754–9.

[35] Michael IP, Sotiropoulou G, Pampalakis G, et al. Biochemical and enzymatic characterization of human kallikrein 5 (hK5), a novel serine protease potentially involved in cancer progression. J Biol Chem 2005;280:14628–35.

[36] Silverman GA, Bird PI, Carrell RW, 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. J Biol Chem 2001;276:33293–6.

[37] Campbell DJ. The kallikrein–kinin system in humans. Clin Exp Pharmacol Physiol 2001;28:1060–5.

[38] Giusti B, Serrati S, Margheri F, et al. The antiangiogenic tissue kallikrein pattern of endothelial cells in systemic sclerosis. Arthritis Rheum 2005;52:3618–28.

[39] Oikonomopoulou K, Hansen KK, Saifeddine M, et al. Proteinase-activated receptors, targets for kallikrein signaling. J Biol Chem 2006;281:32095–112.

[40] Oikonomopoulou K, Hansen KK, Saifeddine M, et al. Proteinase-mediated cell signalling: targeting proteinase-activated receptors (PARs) by kallikreins and more. Biol Chem 2006;387:677–85.

[41] Yamasaki K, Schauber J, Coda A, et al. Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. FASEB J 2006;20:2068–80.

[42] Yamakoshi Y, Hu JC, Fukae M, Yamakoshi F, Simmer JP. How do enamelysin and kallikrein 4 process the 32-kDa enamelin? Eur J Oral Sci 2006;114(Suppl 1):45–51.

[43] Bando Y, Ito S, Nagai Y, et al. Implications of protease M/neurosin in myelination during experimental demyelination and remyelination. Neurosci Lett 2006;405:175–80.
[44] Prezas P, Arlt MJ, Viktorov P, et al. Overexpression of the human tissue kallikrein genes KLK4, 5, 6, and 7 increases the malignant phenotype of ovarian cancer cells. Biol Chem 2006;387:807–11.
[45] Milkiewicz M, Ispanovic E, Doyle JL, Haas TL. Regulators of angiogenesis and strategies for their therapeutic manipulation. Int J Biochem Cell Biol 2006;38:333–57.
[46] Giusti B, Serrati S, Margheri F, et al. The antiangiogenic tissue kallikrein pattern of endothelial cells in systemic sclerosis. Arthritis Rheum 2005;52:3618–28.
[47] Whitbread AK, Veveris-Lowe TL, Lawrence MG, Nicol DL, Clements JA. The role of kallikrein-related peptidases in prostate cancer: potential involvement in an epithelial to mesenchymal transition. Biol Chem 2006;387:707–14.
[48] Veveris-Lowe TL, Lawrence MG, Collard RL, et al. Kallikrein 4 (hK4) and prostate-specific antigen (PSA) are associated with the loss of E-cadherin and an epithelial-mesenchymal transition (EMT)-like effect in prostate cancer cells. Endocr Relat Cancer 2005;12:631–43.
[49] Dallas SL, Zhao S, Cramer SD, Chen Z, Peehl DM, Bonewald LF. Preferential production of latent transforming growth factor beta-2 by primary prostatic epithelial cells and its activation by prostate-specific antigen. J Cell Physiol 2005;202:361–70.
[50] Goya M, Ishii G, Miyamoto S, et al. Prostate-specific antigen induces apoptosis of osteoclast precursors: potential role in osteoblastic bone metastases of prostate cancer. Prostate 2006;66:1573–84.
[51] Komatsu N, Takata M, Otsuki N, et al. Elevated stratum corneum hydrolytic activity in Netherton syndrome suggests an inhibitory regulation of desquamation by SPINK5-derived peptides. J Invest Dermatol 2002;118:436–43.
[52] Descargues P, Deraison C, Bonnart C, et al. Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat Genet 2005;37:56–65.
[53] Egelrud T, Brattsand M, Kreutzmann P, et al. hK5 and hK7, two serine proteinases abundant in human skin, are inhibited by LEKTI domain 6. Br J Dermatol 2005;153:1200–3.
[54] Yousef GM, Kishi T, Diamandis EP. Role of kallikrein enzymes in the central nervous system. Clin Chim Acta 2003;329:1–8.
[55] Diamandis EP, Scorilas A, Kishi T, et al. Altered kallikrein 7 and 10 concentrations in cerebrospinal fluid of patients with Alzheimer's disease and frontotemporal dementia. Clin Biochem 2004;37:230–7.
[56] Cloutier SM, Kundig C, Felber LM, et al. Development of recombinant inhibitors specific to human kallikrein 2 using phage-display selected substrates. Eur J Biochem 2004;271:607–13.
[57] Felber LM, Kundig C, Borgono CA, et al. Mutant recombinant serpins as highly specific inhibitors of human kallikrein 14. FEBS J 2006;273: 2505–14.
[58] Rehault S, Monget P, Mazerbourg S, et al. Insulin-like growth factor binding proteins (IGFBPs) as potential physiological substrates for human kallikreins hK2 and hK3. Eur J Biochem 2001;268:2960–8.
[59] Cloutier SM, Chagas JR, Mach JP, Gygi CM, Leisinger HJ, Deperthes D. Substrate specificity of human kallikrein 2 (hK2) as determined by phage display technology. Eur J Biochem 2002;269:2747–54.
[60] Obiezu CV, Michael IP, Levesque MA, Diamandis EP. Human kallikrein 4: enzymatic activity, inhibition, and degradation of extracellular matrix proteins. Biol Chem 2006;387:749–59.
[61] Angelo PF, Lima AR, Alves FM, et al. Substrate specificity of human kallikrein 6: salt and glycosaminoglycan activation effects. J Biol Chem 2006;281:3116–26.
[62] Luo LY, Jiang W. Inhibition profiles of human tissue kallikreins by serine protease inhibitors. Biol Chem 2006;387:813–6.
[63] Kishi T, Cloutier SM, Kundig C, Deperthes D, Diamandis EP. Activation and enzymatic characterization of recombinant human kallikrein 8. Biol Chem 2006;387:723–31.
[64] Rajapakse S, Ogiwara K, Takano N, Moriyama A, Takahashi T. Biochemical characterization of human kallikrein 8 and its possible involvement in the degradation of extracellular matrix proteins. FEBS Lett 2005;579:6879–84.