MOLECULAR ONCOLOGY 1 (2007) 269–287

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

Review

New insights into the functional mechanisms and clinical applications of the kallikrein-related peptidase family

Nashmil Emami[a,b], *Eleftherios P. Diamandis*[a,b,*]

[a]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario M5G 1L5, Canada*
[b]*Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada*

ARTICLE INFO

Article history:
Received 13 July 2007
Received in revised form
4 September 2007
Accepted 7 September 2007
Available online 15 September 2007

Keywords:
Kallikrein-related peptidases
PSA
Proteolytic cascades
Skin desquamation

ABSTRACT

The Kallikrein-related peptidase (KLK) family consists of fifteen conserved serine proteases that form the largest contiguous cluster of proteases in the human genome. While primarily recognized for their clinical utilities as potential disease biomarkers, new compelling evidence suggests that this family plays a significant role in various physiological processes, including skin desquamation, semen liquefaction, neural plasticity, and body fluid homeostasis. KLK activation is believed to be mediated through highly organized proteolytic cascades, regulated through a series of feedback loops, inhibitors, auto-degradation and internal cleavages. Gene expression is mainly hormone-dependent, even though transcriptional epigenetic regulation has also been reported. These regulatory mechanisms are integrated with various signaling pathways to mediate multiple functions. Dysregulation of these pathways has been implicated in a large number of neoplastic and non-neoplastic pathological conditions. This review highlights our current knowledge of structural/

* *Corresponding author*. Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario M5G 1X5, Canada. Tel.: +1 416 586 8443; fax: +1 416 619 5521.
E-mail address: ediamandis@mtsinai.on.ca (E.P. Diamandis).

Abbreviations: ACC, 7-amino-4-carbamoylmethylcoumarin; ACT, anti-chymotrypsin; AD, Alzheimer's disease; ADAMTS8, ADAM metallopeptidase with thrombospondin type 1 motif 8; ANF, atrial natriuretic factor; AI, amelogenesis imperfecta; AP, anti plasmin; APMSF, 4-amidino-phenyl-methane-sulfonyl fluoride; AT, antitrypsin; bFGF, basic fibroblast growth factor; B2R, human bradykinin B2 receptor; CAG, cancer-associated gene; cdk, cyclin-dependent kinase 7; CDSN, corneodesmosin; CNS, central nervous system; DSC, desmocollin; DSG, desmoglein; ECM, extracellular matrix; FN, fibronectin; FTD, frontotemporal dementia; FRET, fluorescence resonance energy transfer; HEK, human embryonic kidney; hCAP, human cathelicidin; hGH, human growth hormone; HRE, hormone response element; hsp, heat shock protein; IGF, insulin-like growth factor; IGFBP, IGF binding protein; IFN, interferon; IL, interleukin; KD, kallidin; KLK, kallikrein-related peptidase; KNRK, Kirsten virus-transformed normal rat kidney; LEKTI, lympho-epithelial Kazal-type inhibitor; LLP, low density lipoprotein; LK, low molecular weight kininogen; LMW, low molecular weight; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; MMP, matrix metalloproteinase; NS, Netherton syndrome; OLG, oligodendrocyte; PAI, plasminogen activator inhibitor; PAP, poly A polymerase; PAR, protease activated receptor; PCI, protein C inhibitor; PI, protease inhibitor; PKA, protein kinase A; PKC, protein kinase C; PS-SCL, positional scanning synthetic combinatorial library; PTHrp, parathyroid hormone-related peptide; SC, stratum corneum; Sg, seminogelin; siRNA, small interfering RNA; SLPI, secretory leukocyte protease inhibitor; SNP, single nucleotide polymorphism; SPINK, serine protease inhibitor Kazal-1; TGF, tumour growth factor; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; UTR, untranslated region; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide.

doi:10.1016/j.molonc.2007.09.003

Semen liquefaction
Tumour growth
Tumour invasion
Angiogenesis
uPA signaling
PARs

phylogenetic features, functional role and regulatory/signaling mechanisms of this important family of enzymes.

1. Introduction

Proteases (also known as peptidases) are a major group of enzymes participating in multitude of physiological processes, including coagulation, apoptosis, tissue remodeling, and immune responses (Barrett et al., 2004). Depending on the cleavage site, proteases are classified as exo- and endo-peptidases (Barrett et al., 2004). Endopeptidases (or proteinases) cleave their target proteins internally, whereas exopeptidases sequentially remove amino acids from either the N or C-terminus (Barrett et al., 2004). Based on the amino acid residue present at the active site, endopeptidases can further be grouped into four major classes of serine-, cysteine-, aspartic-, and metallo-proteinases (Barrett et al., 2004).

Serine proteases exhibit diverse functions in digestion, coagulation, and cellular and humoral immunity (Rawlings and Barrett, 1994). Based on their evolutionary relationships, serine proteases can further be grouped into eleven clans (Rawlings and Barrett, 1994). The trypsin/chymotrypsin-like (clan SA) enzymes are considered as the main clan in the subfamily of the serine proteases (Rawlings and Barrett, 1994).

Kallikrein-related peptidases belong to a subgroup of secreted serine proteases within the S1 family of clan SA (Borgono et al., 2004). So far, fifteen members of the family (KLK1-15) have been identified, most of which have been reported as potential prognostic and/or diagnostic tumour biomarkers.

The initial work in the kallikrein-related peptidase research was devoted to the discovery and characterization of the three members of the family known as the ''classic'' kallikrein-related peptidases (KLK1, 2, and 3), during 1930s–1980s (Borgono et al., 2004). Subsequent work from our laboratory and others has eventually led to the characterization of twelve novel members in the past decade.

According to the official nomenclature system recommended by the Kallikrein subcommittee of HGNC (HUGO Nomenclature Committee) in 2006, kallikrein-related peptidases are denoted as KLKs (Lundwall et al., 2006a). To distinguish between proteins and genes, proteins are written in standard font, e.g. KLK2, while genes are in italics, e.g. *KLK2*.

2. Gene organization and protein structure

KLKs are encoded by a cluster of strikingly similar genes with varying length, ranging from 4.4 to 10.5 kbp (Obiezu and Diamandis, 2005). The size difference is mainly attributed to intron length, which varies significantly between the genes. Some of the common features shared among *KLK*s include exon/intron organization, number and length of exonic regions, intron phase, and conserved translational start and stop sites, as well as the catalytic triad codons (Borgono et al., 2004; Yousef and Diamandis, 2001). Each gene consists of 5 coding exons, separated by 4 introns with the highly conserved GT-AG splice junction pattern. Furthermore, with the exception of the ''classic'' *KLK*s that lack 5′ untranslated exons, KLKs contain both 5′ and 3′ UTRs. The 3′ UTR contains either the canonical (AATAAA) or a variant polyadenylation site distal to the stop codon (Fig. 1) (Borgono et al., 2004).

KLK proteins are secreted serine endoproteases, expressed as single chain preproenzymes of approximately 30–40 kDa (Fig. 1) (Obiezu and Diamandis, 2005). The signal (pre-) sequence is 16–30 amino acids in length and is cleaved from the N-terminus of the protein prior to secretion (Yousef and Diamandis, 2001). Enzyme activation may subsequently occur through limited proteolysis targeted to the peptide bond between basic and hydrophobic residues of the ''pro''- sequence (Gomis-Ruth et al., 2002) (Table 1). Characteristic to serine proteases, KLKs contain a catalytic serine residue at their active site cleft. Along with the active serine, histidine and aspartic acid residues of the catalytic triad, serve as a charge relay system (Obiezu and Diamandis, 2005; Gomis-Ruth et al., 2002).

KLKs share a high level of amino acid identity in areas flanking the catalytic triad (Obiezu and Diamandis, 2005). The overall sequence similarity, however, is estimated at a lower level (40%–80%) with highest sequence similarity between the ''classic'' KLKs (Obiezu and Diamandis, 2005).

So far, the 3D structure of mature KLK1, KLK4, and both mature and pro-KLK6 have been determined by X-ray crystallography (Debela et al., 2006a; Laxmikanthan et al., 2005; Bernett et al., 2002; Gomis-Ruth et al., 2002). As a subgroup of the trypsin/chymotrypsin-like serine proteases, these KLKs are folded into two hydrophobically interacting domains of six-stranded β-barrels and an α-helix. The catalytic triad is located at the interface between the two domains, as a shallow depression on the frontal surface (Debela et al., 2006a; Gomis-Ruth et al., 2002; Bernett et al., 2002). KLK1 contains an additional ''kallikrein loop'', unique to the ''classic'' KLKs (Laxmikanthan et al., 2005). The loop consists of 11 amino acids, inserted at position 95 (Laxmikanthan et al., 2005). Given its close proximity to the active site, the kallikrein loop is believed to affect the substrate accessibility of the enzyme (Laxmikanthan et al., 2005). Substrate/inhibitor binding may also be determined by diverse external loops surrounding the substrate binding sites (Bernett et al., 2002).

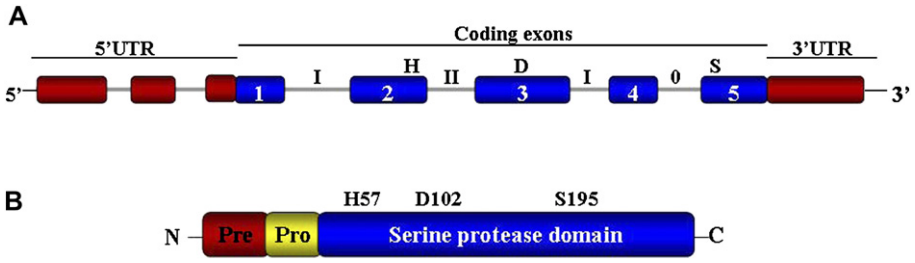

Figure 1 – Schematic representation of a kallikrein gene and protein. A) Kallikrein genes consist of 5 coding exons (blue boxes) of similar length and 4 introns (gray bar) with varying size. Red boxes represent the 5′ and 3′ UTRs. Roman numbers show intron phase. B) Kallikrein proteins are expressed as pre-proenzymes. The amino-terminal pre- (signal) sequence guides the enzyme to the endoplasmic reticulum for secretion. Proenzymes are activated extracellularly upon cleavage of the pro-domain. H57, D102, and S195 are the amino acids of the catalytic triad.

3. Phylogenetic evolution of the locus

The *KLK* locus resides on the long arm of chromosome 19, at position q13.4 and is confounded centromerically by the testicular acid phosphatase gene (ACTP) and telomerically by the cancer-associated gene (CAG) and *Siglec-9*, a member of the sialic acid-binding Ig-like lectin family (Borgono et al., 2004; Yousef et al., 2001a; Foussias et al., 2000). In human, *KLK* are organized as the largest uninterrupted tandem array of protease genes and are transcribed from telomere to centromere, with the exception of *KLK2* and *KLK3* (Fig. 2) (Obiezu and Diamandis, 2005).

Phylogenetic and comparative analyses of the *KLK* locus have revealed a significant level of locus similarity among mammals, suggesting a conserved function(s) of the encoded proteins (Lundwall et al., 2006b; Elliott et al., 2006). Experimental and *in-silico* identification of mammalian *KLKs* in human, rat, mouse, pig, dog, chimpanzee, and opossum, as well as comparative studies of the genome of horse and cow have revealed a polyphyletic nature of the gene family (Elliott et al., 2006; Fernando et al., 2007; Lundwall et al., 2006b). Further phylogenetic studies have suggested five main subfamilies with shared recent ancestry, namely *KLK*4, 5, 14; *KLK*9, 11, 15; *KLK*10, 12; *KLK*6, 13; and *KLK*8, 1, 2, 3 (Elliott et al., 2006). No *KLK* was found in the non-mammalian species examined thus far (Elliott et al., 2006).

Bayesian phylogenetic analyses of the *KLK* locus of the genome of human, chimpanzee, mouse, rat, dog, pig, and opossum indicate that these species carry at least one copy of the *KLK*4–15 (Elliott et al., 2006). Interestingly, ''classic'' *KLK*s exhibit the most variability in the number of gene copies, with the highest number of duplications in rodents (Elliott et al., 2006). Given that the number of gene copies are similar in marsupial species, the majority of duplication events probably date back to 125–175 million years ago, prior to the marsupial-placental divergence (Elliott et al., 2006). Despite major progress in understanding of the phylogenetic changes of the *KLK* family, evolutionary processes of *KLK*4/*KLK*5 has mainly remained a mystery. *KLK*4 is reportedly missing in the mono-delphine genome (Elliott et al., 2006). However, whether the gene was deleted or was duplicated post marsupial lineage divergence is still not clear. Given that *KLK*4 is highly varies among other mammalian species supports the latter hypothesis (Elliott et al., 2006). Lastly, the phylogenetic tree constructed from both individual genes and concatenated *KLK*s suggest a tandem duplication mechanism of sister taxa, including *KLK*9/*KLK*11 and *KLK*10/*KLK*12 (Elliott et al., 2006).

The predicted number of clades is expected to decline as the genome of more primitive mammals becomes available. The rapidly accumulating phylogenetic data are expected to provide new clues on the biological significance of the family.

Table 1 – Activation motifs of human tissue kallikreins

KLK	Activation motif[a]
proKLK1	I-Q-S-R↓I-V-G
proKLK2	I-Q-S-R↓I-V-G (Kumar et al., 1996)
proKLK3	I-L-S-R↓I-V-G (Lovgren et al., 1997)
proKLK4	S-C-S-Q↓I-I-N
proKLK5	S-S-S-R↓I-I-N (Brattsand and Egelrud, 1999)
proKLK6	E-Q-N-K↓L-V-H (Gomis-Ruth et al., 2002)
proKLK7	Q-G-D-K↓I-I-D (Hansson et al., 1994)
proKLK8	Q-E-D-K↓V-L-G (Shimizu et al., 1998)
proKLK9	A-D-T-R↓A-I-G
proKLK10	N-D-T-R↓L-D-P
proKLK11	G-E-T-R↓I-I-K (Luo et al., 2006)
proKLK12	A-T-P-K↓I-F-N (Memari et al., 2007)
proKLK13	E-S-S-K↓V-L-N
proKLK14	D-E-N-K↓I-I-G (Brattsand et al., 2005)
proKLK15	D-G-D-K↓L-L-E

a Activation sites are shown by arrows. Note that, with the exception of KLK4, all KLKs are activated upon cleavage after arginine or lysine amino acid residues.

4. Substrate specificity

Based on their substrate binding pocket, KLKs are broadly grouped into two clans of chymotrypsin-like and trypsin-like serine proteases (Yousef and Diamandis, 2001). Experimental and *in-silico* analyses indicate that the majority of KLKs, namely KLK1, 2, 4–6, 8, 10–15, contain an asparatic or glutamic acid residue at the base of their substrate pocket, enabling them to cleave peptide bonds following a positively-charged amino acid residue (Yousef and Diamandis, 2001). The remaining KLKs, i.e. KLK3, 7, and 9, contain a hydrophobic

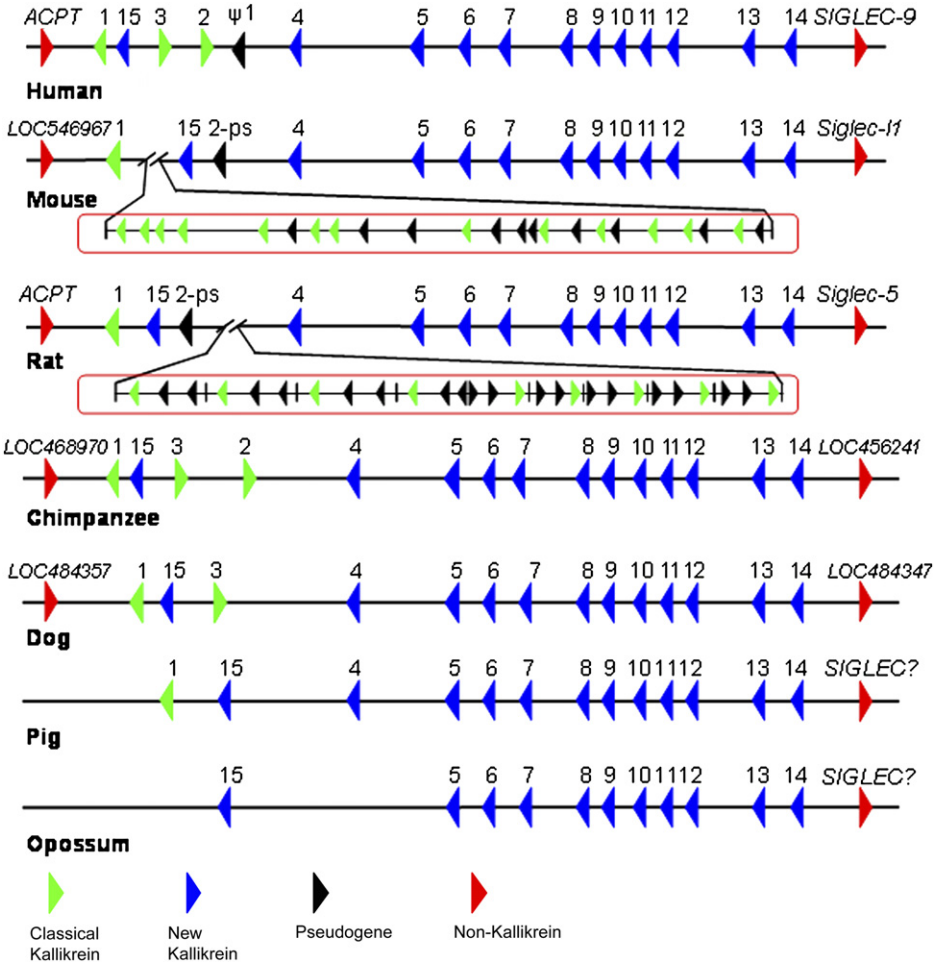

Figure 2 – Kallikrein locus conservation. Arrowheads indicate the approximate location of genes and their transcription direction. Green, "classical" kallikreins, Blue, newly discovered kallikreins, Black, pseudogenes, Red, non-kallikreins. Figure is not to scale (modified from Elliott et al., 2006).

pocket mainly suited for cleavage of substrate scissile bonds with bulky hydrophobic amino acids such as phenylalanine, tryptophan and tyrosine (Yousef and Diamandis, 2001).

Substrate specificities of a large number of KLKs have been determined experimentally, using diverse techniques such as phage display, combinatorial libraries, fluorescence resonance energy transfer (FRET) peptide libraries, and kinetic assays (Deperthes, 2002; Magklara et al., 2003; Harris et al., 2000; Angelo et al., 2006). Substrate selection through the phage display is carried out, using a library of random nucleotide sequences coding all possible combinations of amino acids. These sequences are expressed at the phage surface. Recombinant phages are then fused to a ligand and immobilized on an affinity support through a receptor. Phages expressing desired substrates are released by proteolysis with the protease of interest. Selectivity is improved by multiple rounds of selection. Finally, fragments cleaved by the protease are identified by sequencing phage DNA (Deperthes, 2002). So far, phage display technology has been utilized in substrate recognition of KLK2 and 14 (Cloutier et al., 2002; Felber et al., 2006). KLK14 exhibits both trypsin and chymotrypsin-like activity, which has further been confirmed using kinetic assays (Felber et al., 2006; Borgono et al., 2006b).

Alternatively, positional scanning synthetic combinatorial libraries (PS-SCLs) can be employed to determine substrate recognition (Thornberry et al., 1997). The newly modified PS-SCL screening approach, using ACC (7-amino-4-carbamoyl methylcoumarin) as the fluorogenic leaving group, has emerged as an alternative approach for rapid substrate profiling (Harris et al., 2000). In this approach, a library comprised of 4 sublibraries of fixed P1-4 positions, each containing the twenty canonical amino acids, is constructed. The three remaining positions of each sublibrary contain an equimolar mixture of amino acids (Debela et al., 2006b). This approach has been used to verify substrate preference of KLK3–7 and 10–11 (Debela et al., 2006b). Interestingly, KLK10 and 11 were shown to have a dual chymotrypsin- and trypsin-like substrate specificities (Debela et al., 2006b). Similarly, a library of FRET peptides has been utilized in KLK6 substrate identification (Angelo et al., 2006).

Lastly, potential endogenous substrates of several KLKs have been identified, using fluorogenic or colourimetric conjugated, or full-length substrates (Table 2).

Table 2 – Specificity, substrates and inhibitors of human tissue kallikreins

Kallikrein	S1 amino acid	Substrate specificity	Candidate physiologic substrate	Candidate physiologic inhibitors
KLK1	Asp	Trypsin-like	LMW kininogen, pre ANF, pro-insulin, LLP, Prerenin, VIP, procollagenase, angiotensinogen, B2R (Borgono et al., 2004), Pro-MMP2, 9, IGFBP3 (Borgono and Diamandis, 2004)	Kallistatin, PCI, α_1AT, placental bikunin (Borgono et al., 2004)
KLK2	Asp	Trypsin-like	Seminogelin I/II, fibronectin, pro-uPA (Borgono et al., 2004; Rehault et al., 2001), IGFBP 2, 3, 4, 5 (Rehault et al., 2001), ADAMTS8, collagen IX-α chain (Cloutier et al., 2002)	PCI, PI-6, PAI-1, ATIII, α_2M (Borgono et al., 2004)
KLK3	Ser	Chymotrypsin-like	Seminogelin I/II, fibronectin, laminin, lysozyme, plasminogen, TGF-β, PTHrp (Borgono et al., 2004), IGFP3, 4 (Rehault et al., 2001)	ACT, α_2M, PCI, α_1AT, ATIII (Borgono et al., 2004)
KLK4	Asp	Trypsin-like	Pro-uPA, PAP (Borgono et al., 2004), enamelin (Yamakoshi et al., 2006)	α_2M, α_2AT, α_2AP (Obiezu et al., 2006)
KLK5	Asp	Trypsin-like	Collagens type I, II, III, IV, fibronectin, laminin, plasminogen, LMW kininogen, fibrinogen (Michael et al., 2005), hCAP18 (Yamasaki et al., 2006), CDSN, DSG1, DSC1 (Caubet et al., 2004)	α_2M, α_2AP, ATIII (Borgono et al., 2004), LEKTI (Schechter et al., 2005)
KLK6	Asp	Trypsin-like	Fibrinogen, fibronectin, laminin, collagen types I and IV, APP, plasminogen (Borgono et al., 2004), MBP, ionotropic glutamate receptor (Angelo et al., 2006)	ATIII, α_2AP, α_1AT, ACT (Borgono et al., 2004)
KLK7	Asn	Chymotrypsin-like	IL-1β, corneodesmosin (Borgono et al., 2004), hCAP18 (Yamasaki et al., 2006), fibrinogen (Borgono and Diamandis, 2004), CDSN, DSC1 (Caubet et al., 2004)	LEKI (Schechter et al., 2005), PCI, α_1AT, α_1 ACT, kallistatin (Luo and Jiang, 2006)
KLK8	Asp	Trypsin-like	Fibronectin, gelatin, collagen type IV, fibrinogen, and HMW- kininogen, plasminogen activator (Kishi et al., 2006; Rajapakse et al., 2005)	Antipain, chymostatin, leupeptin (Kishi et al., 2006)
KLK9	Gly	Chymotrypsin-like		
KLK10	Asp	Trypsin/Chymotrypsin-like		
KLK11	Asp	Trypsin/Chymotrypsin-like		PCI (Luo and Jiang, 2006), APMSF, Aprotinin (Luo et al., 2006)
KLK12	Asp	Trypsin-like		α_2 AP, PCI (Luo and Jiang, 2006), α_2 AT (submitted for publication)
KLK13	Asp	Trypsin-like	ECM, plasminogen (Borgono et al., 2004)	α_2M, α_2AP, ACT (Borgono et al., 2004)
KLK14	Asp	Trypsin/Chymotrypsin-like	Collagens I–IV, fibronectin, laminin, kininogen, fibrinogen, plasminogen, vitronectin and IGFBP 2, 3 (Borgono et al., 2006b), matrilin4 (Borgono and Diamandis, 2004)	α_1-AT, α_2-AP, AT III and α_1-ACT (Borgono et al., 2006b)
KLK15	Glu	Trypsin-like		

Please see ''abbreviation'' for full names of proteins.

5. Physiological functions

KLKs are expressed in diverse cell populations and have been implicated in a wide range of physiological processes. Due to their early discovery, functional roles of the ''classical'' KLKs have long been studied. KLK1 is expressed in a large number of tissues, including kidney, blood vessels, central nervous system, pancreas, gut, salivary and sweat glands, spleen, adrenal and neutrophils, suggesting a paracrine nature of the enzyme (Moreau et al., 2005). KLK1 has also has been detected in plasma, possibly originating from exocrine glands (Moreau et al., 2005). KLK1 primarily functions through the release of kallidin (KD) mainly from the low molecular weight kininogen (LK) (Moreau et al., 2005). The kinin-mediated signaling pathway of KLK1 has been implicated in a number of processes, including regulation of blood pressure, smooth muscle contraction, neutrophil chemotaxis, pain induction, vascular permeability, electrolyte balance, and inflammation (Borgono et al., 2004). Additional functions associated with KLK1 include processing of growth factors and peptide hormones, increased nitric oxide formation, and reduced oxidative stress

(Borgono et al., 2004; Yao et al., 2006). Recent evidence suggest that KLK1 may also function independent of kininogens (Biyashev et al., 2006).

The remaining "classic" KLKs, KLK2 and 3, have extensively been examined due to their restricted expression mainly in prostate and seminal plasma. Activated KLK2 and 3 are believed to contribute to seminal clot liquefaction through hydrolysis of seminal vesicle proteins, i.e. seminogelin I and II, and fibronectin that are essential for sperm motility (Malm et al., 2000).

Other members of the KLK family have also been suggested to play a role in semen liquefaction. For instance, recent evidence has implicated KLK5 in a proteolytic cascade in seminal plasma (Michael et al., 2006). Similarly, KLK11 has been detected at a relatively high level (2–37 μg/mL) in seminal plasma, 40% of which were found to be active and might potentially be involved in semen liquefaction (Luo et al., 2006).

Furthermore, recent studies provide compelling evidence that KLKs may play an essential role in the normal physiology of skin. KLK5 and 7 were originally isolated and cloned from the stratum corneum (SC), the outermost layer of skin (Hansson et al., 1994; Brattsand and Egelrud, 1999). Subsequent substrate analysis suggested that these KLKs might be involved in skin desquamation through processing of main adhesive proteins of the extracellular corneodesmosomes, i.e. corneodesmosin (CDSN), desmoglein 1 (DSG1), and desmocollin 1 (DSC1) (Caubet et al., 2004). KLK5 was shown to cleave all three components, while KLK7 was able to digest only CDSN and DSC1 (Caubet et al., 2004). Further immunohistochemical studies revealed the subcellular localization of KLK7 in lamellar bodies in the stratum granulosum and its subsequent transport to the extracellular space of SC, supporting the proposed role of KLK7 in desquamation (Sondell et al., 1995). Additional *in-vitro* studies suggested an activation mechanism of KLK7 through a proteolytic cascade, involving KLK5, 7, and 14 (Brattsand et al., 2005). In addition to KLK5 and 7, varying levels of KLKs 1, 6, 8, 10,11, and 13 have been reported in SC (Komatsu et al., 2005a; Borgono et al., 2006a). KLK1, 5, 6, and 14 are believed to be involved in skin desquamation through processing of DSG1 (Borgono et al., 2006a). In particular, KLK14 has been suggested to play a major role in skin remodeling as it contributes to approximately half of the total trypsin-like proteolytic activity in the SC layer (Stefansson et al., 2006). Similarly, based on the reported features of KLK8 knockout mouse, KLK8 may play an overlapping function in skin desquamation through processing of DSG1 and CDSN (Kishibe et al., 2006).

Recent data has shown an additional antimicrobial function of KLKs in skin through the regulation of cathelicidin peptides (Yamasaki et al., 2006). KLK5 and 7 were found to efficiently cleave cathelicidin precursor (hCAP18) to its mature form (LL-37), *in-vitro* (Yamasaki et al., 2006). Moreover, mice lacking the serine protease inhibitor LEKTI exhibit an increased antimicrobial activity in skin, further supporting the hypothesized function of KLKs in skin immune defense (Fig. 3) (Yamasaki et al., 2006).

Several reports suggest a possible role of KLKs in the processing of hormones. For instance, a large number of KLKs (KLK5–8 and 10–14) are reportedly expressed in the pituitary gland, some of which co-localize with the human growth hormone (hGH) (Komatsu et al., 2007). With the exception of KLK10-12, these KLKs were shown to cleave hGH, *in-vitro* (Komatsu et al., 2007). Similarly, various immunohistochemical reports suggest that KLK1, 6, 10, and 13 are strongly expressed in the islets of Langerhans and may regulate prohormone activation of insulin, glucagon, somatostatin, and pancreatic polypeptide (Petraki et al., 2001, 2002, 2003; Pinkus et al., 1983).

Furthermore, accumulating data suggest a potential role of KLKs in the central nervous system (CNS). So far, the main

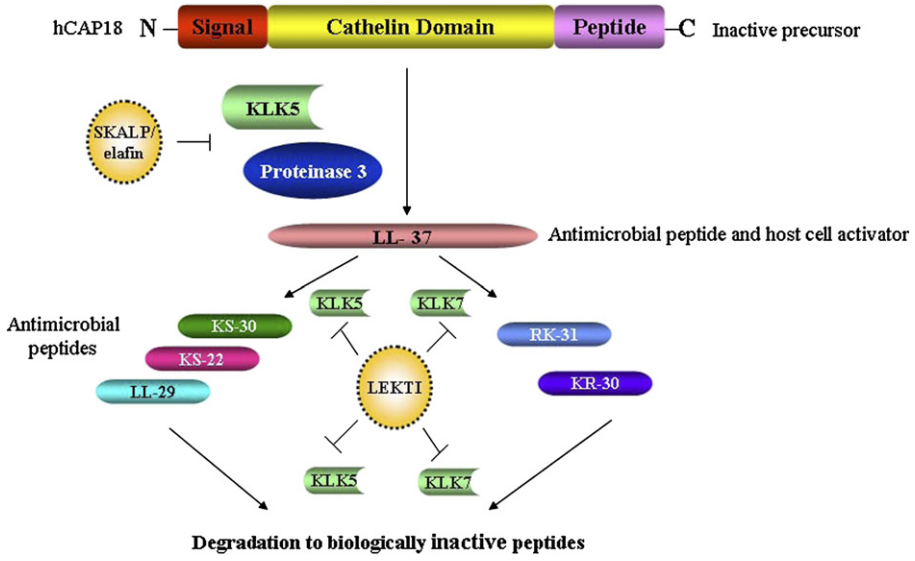

Figure 3 – Antimicrobial function of kallikreins. KLK5 cleaves the inactive cathelicidin precursor (hCAP18) to the host cell activator LL-37. Along with KLK7, KLK5 further breaks down the LL-37 peptide to various antimicrobial peptides, namely KS-30, KS-22, LL-29, RK-31, and KR-30. These peptides are consequently degraded to biologically inactive peptides. KLK-mediated antimicrobial effect of cathelicidin is tightly regulated by a number of serine protease inhibitors, such as elafin and LEKTI (modified from Yamasaki et al., 2006).

focus has been on KLK6 and 8, as they show a distinct expression pattern in the CNS of adult human (Yousef et al., 2003a). KLK6 was reportedly expressed in the peripheral nerves, choroid plexus epithelium, and some neuroendocrine cells of the CNS (Petraki et al., 2001). Similarly, KLK8 is preferentially expressed in adult CNS, particularly in the brain (Yousef et al., 2003a; Tarttelin et al., 2003). Despite the convincing expression data in human, most of our current knowledge of KLK physiology in the CNS comes from the work done on rodents. It is reasoned that since these KLKs exhibit a high level of similarity (>70%) to their rodent orthologs, the function of these proteins is more likely conserved (Terayama et al., 2004; Borgono et al., 2004). There is accumulating data that rodent KLK6 and 8 are critical in neural and brain development. For instance, KLK8$^{-/-}$ mice exhibit a severe loss of long-term potentiation (LTP), required for the hippocampus-associated memory formation (Tamura et al., 2006).

KLK8 is believed to be involved in LTP by modifying the morphology of excitatory synapses by changing their adhesiveness (Tamura et al., 2006). Analogous to KLK8, the mouse ortholog of KLK6 has been suggested to play a major role in CNS development through the maintenance of myelination in oligodendrocytes (OLGs) (Bando et al., 2006). Similarly, the rat ortholog of KLK6 has been implicated in the regulation of CNS demyelination (Angelo et al., 2006).

Lastly, a putative function for KLK4 has recently been proposed, based on the expression profile, mutational pattern, and substrate specificity of the protein. KLK4 was originally purified and cloned from porcine enamel extract and was designated as enamel matrix serine protease 1 (EMSP1) (Simmer et al., 1998). Subsequent mutational analysis of individuals with amelogenesis imperfecta (AI) has revealed a mutation in the KLK4 gene, suggesting a possible role of KLK4 in enamel formation (Hart et al., 2003, 2004; Stephanopoulos et al., 2005; Kim et al., 2006; Wright et al., 2006). Using purified porcine proteins, it was further shown that KLK4 cleaves the 32-kDa fragment of enamelin, normally accumulating in the deeper layers of enamel (Yamakoshi et al., 2006).

6. Cancer pathobiology

Accumulating evidence indicates that the KLK family is dysregulated in cancer. Notably, KLKs exhibit a differential expression pattern and confer a coordinated pattern of up- or down-regulation (Borgono and Diamandis, 2004). Given their distinct expression profile in various malignancies, particularly in endocrine-related carcinomas, the KLK family was shown to represent a rich source of tumour biomarkers. KLK3/PSA has received by far the most attention as a valuable tumour marker for screening, diagnosis, and monitoring of prostate cancer (Paliouras et al., 2007). In addition to KLK3, KLK2, 5, 11, 14, and 15 might function as complementary biomarkers in diagnosis/prognosis of prostate cancer (Stephan et al., 2003; Kurlender et al., 2004; Borgono and Diamandis, 2004). Similarly, KLK3, 5, 7, 9, 14, 15 represent potential biomarkers for breast cancer (Yu et al., 1998; Borgono et al., 2003; Yousef et al., 2003b,c; Stephan et al., 2003; Talieri et al., 2004). Finally, KLK5-11, 13, and 14 have been suggested as tumour biomarkers for ovarian cancer (Yousef et al., 2003b; Diamandis et al., 2003; Kyriakopoulou et al., 2003; Kishi et al., 2003; Yousef et al., 2001b; Luo et al., 2003; Scorilas et al., 2004; Borgono et al., 2003). In addition to these endocrine-related cancers, emerging reports suggest the utility of KLK biomarkers in other carcinomas, including the non-hormonal malignant mesothelioma and intracranial malignancies (Prezas et al., 2006; Davidson et al., 2007).

Despite significant progress in understanding the biomarker utility of the KLK family, their pathophysiology in cancer remains insufficiently understood. Emerging evidence indicates a possible role of the KLK family in diverse cancer-related processes, including tumour growth, angiogenesis, invasion, and metastasis.

6.1. *Tumour growth*

There is emerging evidence that KLKs might participate in early neoplastic progression by regulating tumour cell proliferation (Borgono and Diamandis, 2004). KLK- mediated tumour growth is believed to be modulated mainly through insulin-like growth factors (IGFs) (Borgono and Diamandis, 2004). IGF proteins are the key components of growth during embryonic, fetal, and postnatal development (Rosenfeld, 2003). They are primarily bound to their respective binding proteins (IGFBPs), which are believed to increase the half-life of the IGFs and their delivery to target tissues (Yakar et al., 1999). There is some evidence that high level of IGFBP3, a binding partner of IGF1, can prevent tumour development (Samani et al., 2006). KLK2, and 3 are believed to cleave a number of IGFBPs and as a result, may indirectly be involved in tumour growth (Rehault et al., 2001). In spite of strong *in-vitro* evidence, the ability of these KLKs to digest circulating IGBFB proteins and their correlation with *in-vivo* tumour growth is still debatable (Koistinen et al., 2002; Peternac et al., 2006). Based on the substrate specificity of the remaining KLKs, KLK4, 5, and 14 are also suggested as potential upstream regulators of IGFBPs (Borgono et al., 2006b; Michael et al., 2005; Matsumura et al., 2005).

Conversely, KLK3 and 10 have been implicated in tumour growth suppression. Correlative clinical studies indicate that these KLKs are downregulated in several advanced-stage malignancies (Borgono and Diamandis, 2004). However, gene expression seems to vary among different cancers. For example, while KLK10 has been reported to be downregulated in breast and prostate cancers, it is upregulated in ovarian, pancreatic, and colon carcinomas (Yousef et al., 2005). Similarly, KLK3 is reportedly downregulated in progressive breast and prostate cancer (Yu et al., 1998; Stege et al., 2000). Accordingly, KLK3 was shown to induce the expression of putative tumour-suppressor genes, including IFN-δ, and suppress tumor growth promoters, such as uPA, VEGF, and Pim-1 oncogene, in the PC-3M prostate cancer cell line treated with free KLK3 purified from seminal plasma (Bindukumar et al., 2005). The reported ability of KLK3 to reduce tumour load in PC-3M tumor-bearing nude mice (Bindukumar et al., 2005), further supports a tumour suppressor function of KLK3. Similarly, anchorage- independent growth and tumour formation in nude mice is reportedly suppressed in MDA-MB-231 breast cancer cell implants, stably expressing KLK10 (Goyal et al., 1998). Despite

the evidence in favour of the tumour-inhibiting role of these KLKs, their pathological function *in-vivo* is still controversial. For instance, one study reports that KLK3 has no inhibitory effect on the growth of prostate cancer cell lines PC3, DU145, stably expressing pro-KLK3 (Denmeade et al., 2003).

6.2. *Angiogenesis*

Tumour angiogenesis, characterized by the formation of new blood vessels from existing vasculature, is a critical process in tumour maintenance and metastasis (Fidler, 2000). Angiogenesis in neoplasms often begins with an imbalance in angiogenic molecules favouring pro-factors (Fidler, 2000). There is compelling evidence that KLKs may stimulate the process by facilitating blood vessel remodeling and modulating the balance between angiogenic activators and inhibitors (Borgono and Diamandis, 2004).

Angiogenesis can further progress when the basement membrane of existing blood vessels and their surrounding ECM is degraded, allowing endothelial cells to migrate into the stroma of the neighbouring tissue (Fidler, 2000). The invading cells then proliferate and coalesce into new capillary tubes. KLKs are believed to be directly involved in the process of endothelial cell invasion and migration by processing ECM components (Table 2). KLKs may also mediate ECM remodeling indirectly through the MMPs, uPA, and kinin signaling pathways. For instance, KLK1 activates pro-MMP1, 2, and 9 (Desrivieres et al., 1993; Menashi et al., 1994; Saunders et al., 2005). Furthermore, KLK2, 4, and 12 signal through the uPA system, which results in plasmin formation (Takayama et al., 2001; Giusti et al., 2005; Wang, 2001; Frenette et al., 1997). Plasmin, in turn, degrades a large number of ECM proteins, including fibronectin, laminin, proteoglycans, and fibrin, and activates latent collagenases (Sidenius and Blasi, 2003). Likewise, KLK1 and 12 are expressed by endothelial cells and are believed to function through the kinin signaling pathway (Emanueli et al., 2001; Giusti et al., 2005). Active kinin promotes angiogenesis by upregulation of bFGF or stimulation of VEGF formation (Colman, 2006).

In contrast, certain KLKs could suppress angiogenesis. For example, KLK3, either purified from the seminal plasma or expressed recombinantly, was shown to prevent angiogenesis *in-vitro* (Fortier et al., 1999, 2003). The anti-angiogenic effect of recombinant KLK3 was further demonstrated *in-vivo*, using matrigel plug assay in wild type mice (Fortier et al., 2003). Interestingly, the antagonistic function of KLK3 was independent of its enzymatic activity (Fortier et al., 2003). KLK3 is believed to prevent vasculature formation through angiostatin-like components, potent inhibitors of endothelial cell proliferation and angiogenesis (Heidtmann et al., 1999). In addition to KLK3, *in-vitro* data indicate that KLK5, 6, and 13 can potentially generate angiostatin-like fragments from plasminogen (Michael et al., 2005; Emanueli et al., 2001; Bayes et al., 2004; Sotiropoulou et al., 2003).

6.3. *Invasion and metastasis*

Having independent blood supply, primary tumour cells gain the propensity to leave their stromal niche, invade the surrounding tissue, enter the vascular system and ultimately colonize distant organs (Christofori, 2006). Cell motility is primarily achieved by the conversion of the newly metastasized epithelial cells to a migratory mesenchymal morphology, a process known as epithelial to mesenchymal transition (EMT) (Christofori, 2006). These cells consequently break down the basement membrane, connective tissue and the surrounding ECM of the neighbouring normal epithelial cells in order to infiltrate the underlying interstitial stroma.

In a recent study, KLK3 and 4 have been reported to be involved in phenotypical changes that could be indicative of EMT (Veveris-Lowe et al., 2005). Stable expression of these KLKs in the prostate cell line PC-3 resulted in an increase in cell invasiveness (Veveris-Lowe et al., 2005). Transfected cells reportedly acquired mesenchymal characteristics and lost certain morphological features unique to epithelial cells (Veveris-Lowe et al., 2005). Predominantly, a significant loss of E-cadherin, a member of the calcium-dependent cell-cell adhesion molecules, and expression of the mesenchymal molecule vimentin were observed (Veveris-Lowe et al., 2005). Accordingly, the observation that suppressing KLK3 attenuates invasion in the LNCaP prostate cancer cells (Webber et al., 1995; Ishii et al., 2004) is consistent with the proposed function of KLK3 in EMT. However, a reduced number of surface lung metastases was found in mice treated with KLK3, suggesting that KLK3 inhibits tumour metastasis (Fortier et al., 1999). Whether the metastatic role of KLK3 is cell-specific or is counterbalanced by its anti-angiogenic effect *in-vivo* needs to be further investigated.

In addition to their proposed function in EMT, KLKs have been implicated in metastatic dissemination through ECM remodeling. As mentioned previously, numerous *in-vitro* substrate studies have indicated a direct or indirect involvement of KLKs in degradation of various ECM proteins, including fibronectin, collagen, laminin, and proteoglycans. Furthermore, KLKs might participate in tissue degradation through the uPA and MMP signaling pathways. As discussed above, plasmin, a key player in ECM degradation, can be activated from plasminogen by the uPA- uPA receptor (uPAR) complex. It has been postulated that KLK4 can mediate the tumour-related plasminogen activation by activating the proenzyme of uPA or cleaving its cell-surface receptor (Takayama et al., 2001; Beaufort et al., 2006).

Lastly, recent findings indicate a possible role of several KLKs in bone metastasis. Bone metastasis in cancer is broadly divided in two categories, osteoclastic and osteoblastic, based on the type of activated precursor cells. Even though these classes are non-exclusive, one often predominates the other, depending on the neoplastic origin of the tumour (Sohara et al., 2005).

Osteoblastic bone metastasis has the highest prevalence in prostate cancer and is characterized by increased osteoblast proliferation and reduced osteoclastic activity (Goya et al., 2006; Sohara et al., 2005). Several lines of evidence indicate that KLK3 can induce osteoblastic proliferation and osteoclast apoptosis *in-vitro* and *in-vivo* (Goya et al., 2006; Yonou et al., 2001). Direct injection of KLK3 or KLK3- producing prostate cancer cells into nude mice implanted with human adult bone exhibit a marked increase of osteoblast proliferation, accompanied by osteoclast apoptosis (Goya et al., 2006; Yonou et al., 2001). These results were further verified *in-vitro*, using

murine bone precursor cells (Goya et al., 2006; Yonou et al., 2001). Accordingly, KLK3 suppression either through neutralizing antibodies or siRNA, abrogated prostate cancer cell adhesion to bone marrow endothelial cells (Romanov et al., 2004). Although the functional mechanism of KLK3-induced bone metastasis is not fully understood, an autonomous function independent of tumour growth factors has been proposed (Yonou et al., 2001). However there are several reports indicating a possible signaling through latent TGFβ or other cell surface receptors (Yonou et al., 2001; Killian et al., 1993).

7. Non-malignant disorders

As discussed previously, KLKs play an important role in SC desquamation and are critical in the maintenance of skin barrier function. Desquamation is a complex biological event, exquisitely regulated through a series of biological checks and balances. Imbalances in the proteolytic activity of KLKs, either as a result of gene over-expression or dysregulated activity, is considered as one of the main etiological factors in a number of skin disorders, including chronic itchy dermatitis, peeling skin syndrome, psoriasis, atopic dermatitis, and Netherton syndrome (Komatsu et al., 2005b; Hachem et al., 2006; Komatsu et al., 2006; Hansson et al., 2002; Ekholm and Egelrud, 1999).

Clinical studies indicate that the expression of multiple KLKs is significantly up-regulated in psoriasis, atopic dermatitis, peeling skin syndrome type-B, and chronic lesions of atopic dermatitis (Hansson et al., 2002; Komatsu et al., 2005b, 2006). Furthermore, mutational analyses in patients with Netherton syndrome (NS), an autosomal recessive skin disorder, have identified several frame shifts and non-sense mutations in the SPINK5 gene encoding for LEKTI (Komatsu et al., 2002; Chavanas et al., 2000; Sprecher et al., 2001). Such genetic defects lead to truncation of the protein and loss of inhibitory domains (Chavanas et al., 2000; Sprecher et al., 2001). As mentioned previously, LEKTI is a serine protease inhibitor shown to repress the proteolytic activity of several KLKs, including KLK5, 6, 7, 13, and 14 (Egelrud et al., 2005; Borgono et al., 2006a). As expected, a reduced level of LEKTI domains and uninhibited serine protease activity of KLKs have been observed in the SC of NS patients, as well as the disease model, namely *spink5*$^{-/-}$ mice (Komatsu et al., 2002; Descargues et al., 2005, 2006; Hachem et al., 2006).

According to clinical data and their putative physiological functions, several KLKs have been implicated in a number of other disorders, including oral and maxillofacial and neurodegenerative disorders. For instance, the expression of KLK6, 7, and 10 are reportedly altered in patients with Alzheimer's disease (AD) and frontotemporal dementia (FTD), which may have some utility as diagnostic biomarkers (Yousef et al., 2003a; Diamandis et al., 2004).

Lastly, aberrant KLK-kinin signaling and their role in a wide range of pathological processes, including inflammation, hypertension, and renal diseases have extensively been investigated (for reviews see Refs. Moreau et al., 2005; Sharma, 2006; Chao and Chao, 2005).

8. Signaling mechanisms

Emerging evidence suggests that KLKs function partly through cross-talk with other signal transduction pathways. Signaling through active kinins, uPA, protease activated receptors (PARs), and MMPs have so far been examined (Emami and Diamandis, 2007) (Fig. 4). KLK signaling through kinins is one of the most well-characterized signaling pathways studied

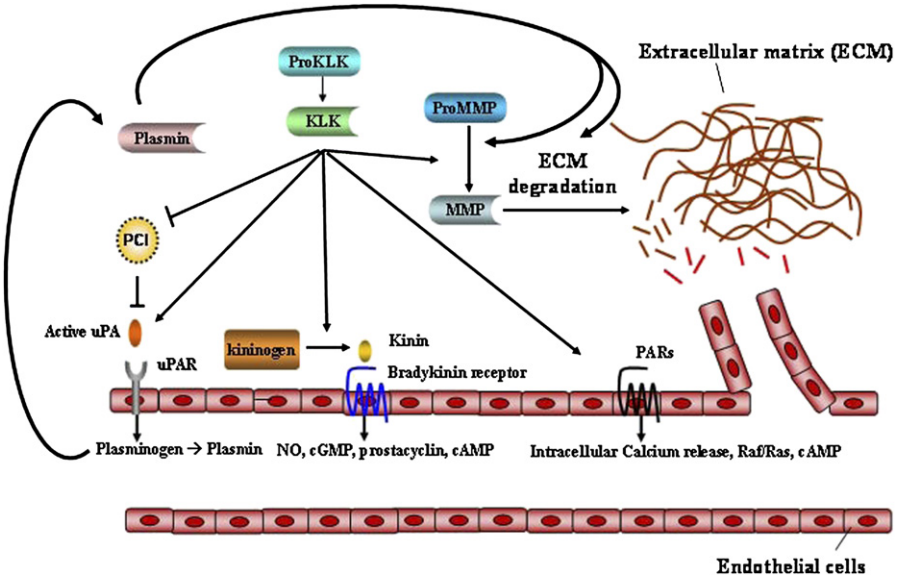

Figure 4 – Schematic representation of kallikrein signaling pathways. Kallikreins mediate uPA activation and subsequent conversion of plasminogen into active plasmin and cleavage of several downstream targets, such as fibrin and MMPs. Kallikreins cleave kininogen to kinin and induce a number of downstream targets including cAMP, NO, prostacyclin and cAMP. Kallikreins cleave and activate PARs at their extracellular N-termini. Active PARs signal mainly through calcium signaling, Raf/Ras activation and cAMP inhibition. Kallikreins participate in ECM remodeling directly and/or indirectly through MMPs. For more information, please refer to our non-standard abbreviations.

thus far. KLK1, 2, and more recently 12, were shown to release active kinins (Lys-bradykinin or kallidin) from the kininogens, in particular the low molecular weight kininogens (LK) (Campbell, 2001; Giusti et al., 2005). Subsequently, active kallidin mediates signaling mainly through two types of G-protein-coupled receptors (GPCRs), designated as B1 and B2 (Moreau et al., 2005). The binding of kinin peptides to their respective receptors activates a number of downstream targets such as nitric oxide (NO), cGMP, prostacyclin and cAMP, which in turn induce a wide range of biological processes involved in angiogenesis, vasodilatation, smooth muscle contraction/relaxation, inflammation and pain (Chao et al., 2006).

In addition to the kinin system, certain KLKs, e.g. KLK2 and 4, can cross-talk with the uPA-uPAR signaling pathway. KLK2 was shown to cleave and activate the single chain uPA at Lys^{158} (Frenette et al., 1997). Further studies indicate an alternative route through complexing and inactivation of PAI-1, the main inhibitor of uPA in tissues (Mikolajczyk et al., 1999). Similarly, as mentioned previously, active chimeric KLK4 was found to activate uPA, *in-vitro* (Takayama et al., 2001). Plasminogen activation through the uPA/uPAR signaling has been implicated in a broad spectrum of biological effects, including cleavage of various ECM components and MMP activation. MMP activation has also been suggested to occur directly through several KLKs (Borgono and Diamandis, 2004).

Lastly, emerging evidence suggests that KLKs can activate and signal through several members of the PAR family (Oikonomopoulou et al., 2006). PARs are members of the seven transmembrane GPCR superfamily and are activated by serine proteases (Dery et al., 1998). Activation is achieved via cleavage of a portion of the extracellular N-terminus of the receptor, resulting in formation of a tethered ligand and activation of the cleaved receptor (Dery et al., 1998). The PAR family functions through the recruitment of several different heterotrimeric G proteins. The activation of Gq results in Ca^{2+} mobilization and PKC activation through its α-subunit (Dery et al., 1998), whereas signaling through G_i can suppress cAMP formation through adenylyl cyclase suppression (Dery et al., 1998). Alternatively, the $\beta\gamma$-complex of G_i can induce a number of tyrosine kinases and subsequent activation of the MAP kinases (Dery et al., 1998).

In a recent study, KLK5, 6, and 14 were shown to cleave the activation domain of PARs 1, 2, and 4, *in-vitro* (Oikonomopoulou et al., 2006). KLK14-mediated cleavage of PAR2 was further confirmed in rat PAR2-expressing KNRK (Kirsten virus-transformed normal rat kidney) cells (Oikonomopoulou et al., 2006). As mentioned above, PARs have been implicated in Ca^{2+} mobilization. The Ca^{2+}-mediated signaling was shown to be modulated in KNRK cells by all three KLKs, with the highest level in KLK14 (Oikonomopoulou et al., 2006). A similar result was observed in the HEK (human embryonic kidney) cells transfected with PAR1, PAR2, or both (Oikonomopoulou et al., 2006).

As well, KLK14 was found to have a preference towards PAR2, as determined by pre-desensitization of receptors (Oikonomopoulou et al., 2006). PAR1 and 2 activation was further confirmed *in-vivo*, as a distinct rat/mouse aorta ring relaxation was observed upon KLK treatment (Oikonomopoulou et al., 2006). Additional data indicates a possible negative regulatory feedback system through which KLK14 deactivates PAR1. Lastly, analogous to PAR2, PAR4 was found to be activated by KLK4 in the rat platelet cells lacking other PARs and PAR4-transfected HEK cells (Oikonomopoulou et al., 2006).

9. Proteolytic activation cascades

Proteolytic processes are often mediated by highly orchestrated cascades, through which protease enzymes function coordinately to ensure a stepwise activation (Amour et al., 2004). These cascades can be organized into three main consecutive phases of initiation, propagation, and execution (Amour et al., 2004). Upon stimulation, ''initiator'' zymogens (pro-enzymes) are self-activated by autocatalysis. Active initiators then convert downstream ''propagator'' proteases, which, in turn, catalyse the processing of the following ''executor'' zymogens. Active executers then elicit proper signals in order to repair or block adverse effects of the stimuli. Such cascades result in a rapid and highly controlled amplification of active proteases and physiologically safe proteolysis. Proteolytic cascades have extensively been examined in a large number of protease families and are well characterized in vital physiological processes, such as coagulation-fibrinolysis, caspase-mediated apoptosis, and the complement system in both innate and acquired immunity (Schenone et al., 2004; Chowdhury et al., 2006; Kemper and Atkinson, 2007).

There is accumulating evidence suggesting that KLKs exert their physiological function through highly regulated proteolytic cascades. All KLKs, with the exception of KLK4, contain a pro-peptide with lysine or arginine in their C-termini, suggesting their activation by trypsin-like proteases (Table 1). However, as mentioned previously, some of the KLKs are chymotrypsin-like and thus require other trypsin-like proteases for their activation (Table 2). These data are suggestive of a network consisting of multiple KLKs, acting as initiators, propagators, and executors. Moreover, the majority of KLKs exhibit common regulatory mechanisms (through steroids) and dysregulation patterns in various pathological conditions, which further supports the proposed cascade-mediated activation mechanism (Yousef and Diamandis, 2002).

Experimental data demonstrating proteolytic cascades with KLKs are emerging. For instance, KLK2, 3, and 5 were recently shown to participate in a proteolytic cascade in seminal plasma (Fig. 5A) (Michael et al., 2006; Kumar et al., 1997; Vaisanen et al., 1999). These KLKs have previously been identified in seminal plasma and prostate tissues (Lovgren et al., 1999b; Rittenhouse et al., 1998; Wang et al., 1979). KLK5 was shown to autoactivate and in turn, activate pro-KLK3 (Michael et al., 2006). Activated KLK3, consequently, sends negative feedbacks to deactivate KLK5 (Michael et al., 2006). Similarly, active KLK2 has been reported to cleave and activate pro-KLK3, *in-vitro* (Kumar et al., 1997; Vaisanen et al., 1999). However, one study reports that active KLK2 is unable to cleave the pro-peptide sequence of KLK3 (Denmeade et al., 2001), calling into question the previous findings.

Additional evidence supporting proteolytic cascades of KLKs comes from the *in-vitro* work done with KLK5, 7, and 14 (Brattsand et al., 2005). KLK5 was shown to autoactivate and activate KLK7 and 14. Activated KLK14 is believed to in turn

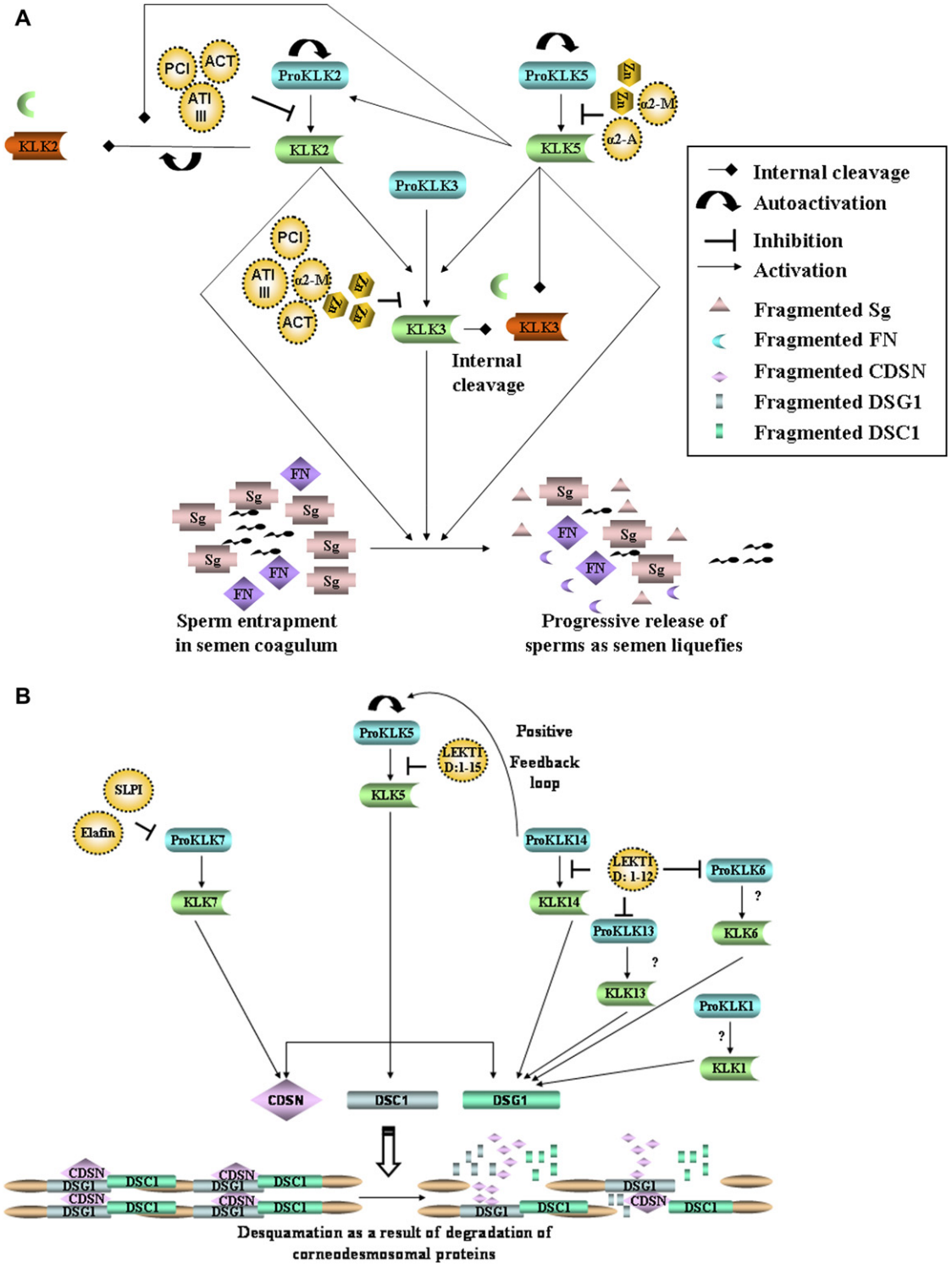

Figure 5 – Schematic presentation of A) Kallikrein cascade in seminal plasma. KLK2 and 5 autoactivate and, in turn, activate pro-KLK3. Activated KLK3 acts as an executor protease in the liquefaction of seminal clot and release of spermatozoa through processing of Sgl I/Sgl II and FN. The cascade is regulated by a number of negative and positive feedback loops, including internal cleavages, auto degradation, Zn^{2+}, and endogenous inhibitors. B) Kallikrein cascade in skin. KLK5 autoactivates and activates KLK14 and 7, which, along with active KLK1, 6, and 13 function in skin desquamation through degradation of the corneodesmosomal proteins, i.e. desmogelin1 (DSG1), desmocollin1 (DSC1), and corneodesmosin (CDSN). Desquamation is regulated by of various serine protease inhibitors, such as SLPI, elafin, and certain LEKTI domains. For more definitions, please refer to our non-standard abbreviations.

send positive feedbacks to amplify the KLK5 activation (Fig. 5B). Given their coexpression pattern in the SC of skin, this cascade has been associated with the desquamatory function of KLKs (Brattsand et al., 2005).

10. Regulatory mechanisms

10.1. *Transcriptional regulation*

Hormone-dependent control of gene expression is a complex mechanism and is essential in a large number of physiological processes. Transcriptional regulation by steroid hormones is one of the best characterized model systems for studying the hormonal regulation of transcription (Beato, 1989). Gene regulation by steroid hormones is initiated by receptor complexing of the hormone and release of the Hsp90 protein bound to the receptor (Rousseau, 1984; Picard, 2006). Hsp90 is a ubiquitous chaperone protein regulating the activity of various signaling proteins, including steroid hormone receptors (Picard, 2006; Zhao and Houry, 2005). The receptor complexes dimerize upon release of Hsp90 and bind to the cis-acting sequences of the target gene to induce gene expression (Beato, 1989). Such transcriptional events are further controlled through phosphorylation of receptors by general transcription factor-related kinases, e.g. cdk7 within TFIIH or other signaling pathways, e.g. MAPKs, Akt, PKA, and PKC (Rochette-Egly, 2003).

Accumulating evidence indicates that the majority of *KLK*s are under steroid hormone regulation. *KLK* expression was shown to be significantly induced in steroid-treated cell lines both at the mRNA and protein levels (Yousef and Diamandis, 2001). Subsequent *in-silico* and deletion analysis identified a number of hormone response elements (HREs) distal to transcriptional start sites of several *KLK*s. For example, three androgen response elements have been identified in the KLK3 promoter. Two of these regulatory regions, identified at −170 to −400 bp of KLK3 promoter regions, were shown to act cooperatively (Cleutjens et al., 1996). A SNP (single nucleotide polymorphism) located at the first regulatory region was further shown to associate with receptor binding and KLK3 expression (Lai et al., 2006). In addition, a more potent enhancer, located at ∼ − 4000 bp, was reported and verified using DNAseI-hypersensitivity assay (Schuur et al., 1996; Cleutjens et al., 1997). Similarly, two androgen regulatory regions, at position −170 bp and −3000 bp, have been determined experimentally in KLK2 (Murtha et al., 1993; Yu et al., 1999). Further studies suggest that additional regulatory factors are indirectly involved in the transcriptional regulation of these *KLK*s. For instance, a Fos-containing protein complex was found to bind distal to the AREs of *KLK2* and *KLK3* promoters and regulate androgen-mediated gene expression (Sun et al., 1997). As well, a number of co-regulatory factors, e.g. SRC-1, AIB1, ARA24, ARA54, FHL2, and PDEF, have been identified in varying amount in several breast cancer cell lines (Magklara et al., 2002). It has been postulated that these factors modulate the expression of KLK2 and 3, in cooperation with androgen receptors (Magklara et al., 2002). In addition, androgen-mediated gene expression in *KLK3* was found to significantly be potentiated, through a novel regulatory element (Wang et al., 2003). In contrast, a negative cis-acting regulatory region, recruiting both the p65 component of the NF-κB and androgen receptor, was identified in the promoter region of KLK3 (Cinar et al., 2004). Similarly, androgen-mediated *KLK3* expression was found to be significantly reduced in prostate cell lines with constitutively active Ras/MAPK pathway (Bakin et al., 2003), suggesting the regulatory function of receptor phosphorylation in *KLK* expression.

Recent reports demonstrated a synergistic hormonal regulation in *KLK*s, suggesting a control mechanism through single locus regions (Paliouras and Diamandis, 2006b). For instance *KLK*10, 11, 13, and 14 were found to be coordinately regulated by dihydrotestosterone (DHT) and norgestrel in several breast cancer cells (Paliouras and Diamandis, 2006b). Interestingly, none of these genes contain characterized HREs, suggesting an indirect function of steroid hormones as trans-acting transcriptional regulators of *KLK* expression (Paliouras and Diamandis, 2006a,b).

Alternatively, *KLK* expression can be regulated through epigenetic factors, in particular DNA methylation. For instance, KLK10 downregulation has been associated with the hypermethylation of CpG islands in breast cancer and lymphoblastic leukemia (Li et al., 2001; Sidiropoulos et al., 2005). A similar regulatory mechanism has been reported for the *KLK6* gene (Pampalakis and Sotiropoulou, 2006).

10.2. *Regulation of proteolytic activity*

Due to the irreversible nature of proteolytic activation, proteases often remain as inactive zymogens in quiescent state. Activation is often triggered by an external stimulus and, as mentioned previously, mediated through proteolytic cascades. These cascades are tightly regulated through a series of highly orchestrated feedback loops and inhibitors, to prevent deleterious effects due to uncontrolled protease activation. Dysregulated proteolysis has been implicated in several pathological conditions, such as amyloidogenesis in Alzheimer's disease, intravascular coagulation in sepsis and neoplastic progression in various carcinomas (Amour et al., 2004; Sidera et al., 2004; Borg, 2004).

For KLKs, protease activity is believed to be regulated at the level of zymogen activation and/or, later on, through endogenous inhibitors. Several *in-vitro* studies suggest that KLK activation is regulated through various regulatory feedback loops. For instance, as mentioned previously, KLK5 activation was shown to be positively regulated by active KLK14, while negatively controlled by active KLK 3 (Brattsand et al., 2005; Michael et al., 2006). In addition, several reports indicate a possible inactivation mechanism in KLK2, 6, 7, 13, and 14 through internal cleavages and subsequent degradation (Hansson et al., 1994; Lovgren et al., 1999a; Sotiropoulou et al., 2003). Degradation may be autolytic or mediated through other proteases. Furthermore, divalent ions such as zinc have been shown to reversibly inhibit certain KLKs, including KLK2, 3, and 5 (Borgono and Diamandis, 2004; Michael et al., 2005). These control mechanisms are of essential importance *in-vivo*, as they assure an adequate physiological response.

Alternatively, protease activity can be regulated through endogenous inhibitors.

The inhibitory mechanism of the serine protease inhibitors has been characterized in detail. Proteases are shown to form transient non-covalent complexes with respective inhibitors. The complex can consequently progress to the ''inhibitory pathway'' through a molecular ''trap'' mechanism. Alternatively, inhibition is prevented through the ''substrate pathway'' whereby the protease cleaves and therefore inactivates the inhibitor (Silverman et al., 2001).

A large number of potential endogenous KLK inhibitors have been identified *in-vitro* (Table 2). Complex formation of some of these inhibitors has been proven *in-vivo*. For instance, the majority of serum KLK3 (70%–90%) was found to complex with anti-chymotrypsin (ACT), a member of the serpin family (Stenman et al., 1991).

Most of the recognized inhibitors exhibit a relatively low level of specificity. For instance, protein C inhibitor (PCI) was found to efficiently inhibit KLK1, 2, 3, 5, 7, 8, 11, 13, and 14 (Espana et al., 1995; Luo and Jiang, 2006). The most specific inhibitor identified so far is kallistatin, an inhibitor of KLK1 and 7 (Chen et al., 2000b; Luo and Jiang, 2006). An amino acid residue, Phe387, has been shown to be essential in the specificity of the inhibitory function of the protein by retaining the hydrophobicity required for the optimal interaction with KLK1 (Chen et al., 2000a). Further structural analysis has revealed a secondary binding site between the H helix and the C2 sheet possibly facilitating complex formation (Chen et al., 2000a).

Alternatively, inhibition specificity can be achieved *in-vivo* by directing the inhibitor to the location of its protease target. This mechanism was demonstrated for the KLK inhibitor LEKTI. Immunohistochemical analysis has shown a temporal compartmentalization of LEKTI and its target KLKs, e.g. KLK5 and 7, in the lamellar granule (LG) of normal skin. It has been suggested that in normal physiological state, LG transports LEKTI earlier to prevent unwanted proteolytic activity of KLKs and a premature corneocyte desquamation (Ishida-Yamamoto et al., 2005). LEKTI contains 15 inhibitory domains, two of which, namely domains 2 and 5, contain three sulfide bonds characteristic of Kazal-type domains (Magert et al., 2002). Despite of the difference in sulfide bonds, domain 6 was shown to consist of two helices and a β-hairpin, found in Kazal-type domains (Lauber et al., 2003). Further kinetic analysis of domains 1–6, 6–9, 9–12, and 12–15 showed a strong inhibition of KLK5, 6, 13, and 14 by the three former regions (Borgono et al., 2006a). As well, domain 6 of LEKTI was found to inhibit KLK7 (Egelrud et al., 2005).

11. Conclusion

In recent years, there has been substantial progress in understanding the functional mechanism, regulation, and physiological/pathbiological role of the KLK family. New findings have provided evidence for an ever-increasing complex regulatory mechanism, which controls KLK function prior to translation and/or post-translation, through highly organized proteolytic cascades. As well, evidence for biological mechanisms underlying KLK dysregulation in diverse pathological conditions, including cancer, as well as, many other non-cancer disorders are emerging. Identification and further characterization of these pathways may have important clinical applications and could lead to the discovery of novel targets for therapeutic interventions.

REFERENCES

Amour, A., Bird, M., Chaudry, L., Deadman, J., Hayes, D., Kay, C., 2004. General considerations for proteolytic cascades. Biochem. Soc. Trans. 32, 15–16.

Angelo, P.F., Lima, A.R., Alves, F.M., Blaber, S.I., Scarisbrick, I.A., Blaber, M., Juliano, L., Juliano, M.A., 2006. Substrate specificity of human kallikrein 6: salt and glycosaminoglycan activation effects. J. Biol. Chem. 281, 3116–3126.

Bakin, R.E., Gioeli, D., Sikes, R.A., Bissonette, E.A., Weber, M.J., 2003. Constitutive activation of the Ras/mitogen-activated protein kinase signaling pathway promotes androgen hypersensitivity in LNCaP prostate cancer cells. Cancer Res. 63, 1981–1989.

Bando, Y., Ito, S., Nagai, Y., Terayama, R., Kishibe, M., Jiang, Y.P., Mitrovic, B., Takahashi, T., Yoshida, S., 2006. Implications of protease M/neurosin in myelination during experimental demyelination and remyelination. Neurosci. Lett. 405, 175–180.

Barrett, A.J., Rawlings, N.D., Woessner, J.F., 2004. Handbook of Proteolytic Enzymes. Academic Press Inc, San Diego.

Bayes, A., Tsetsenis, T., Ventura, S., Vendrell, J., Aviles, F.X., Sotiropoulou, G., 2004. Human kallikrein 6 activity is regulated via an autoproteolytic mechanism of activation/inactivation. Biol. Chem. 385, 517–524.

Beato, M., 1989. Gene regulation by steroid hormones. Cell 56, 335–344.

Beaufort, N., Debela, M., Creutzburg, S., Kellermann, J., Bode, W., Schmitt, M., Pidard, D., Magdolen, V., 2006. Interplay of human tissue kallikrein 4 (hK4) with the plasminogen activation system: hK4 regulates the structure and functions of the urokinase-type plasminogen activator receptor (uPAR). Biol. Chem. 387, 217–222.

Bernett, M.J., Blaber, S.I., Scarisbrick, I.A., Dhanarajan, P., Thompson, S.M., Blaber, M., 2002. 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. 277, 24562–24570.

Bindukumar, B., Schwartz, S.A., Nair, M.P., Aalinkeel, R., Kawinski, E., Chadha, K.C., 2005. Prostate-specific antigen modulates the expression of genes involved in prostate tumor growth. Neoplasia 7, 241–252.

Biyashev, D., Tan, F., Chen, Z., Zhang, K., Deddish, P.A., Erdos, E.G., Hecquet, C., 2006. Kallikrein activates bradykinin B2 receptors in absence of kininogen. Am. J. Physiol Heart Circ. Physiol 290, H1244–H1250.

Borg, T.K., 2004. It's the matrix! ECM, proteases, and cancer. Am. J. Pathol. 164, 1141–1142.

Borgono, C.A., Diamandis, E.P., 2004. The emerging roles of human tissue kallikreins in cancer. Nat. Rev. Cancer 4, 876–890.

Borgono, C.A., Grass, L., Soosaipillai, A., Yousef, G.M., Petraki, C.D., Howarth, D.H., Fracchioli, S., Katsaros, D., Diamandis, E.P., 2003. Human kallikrein 14: a new potential biomarker for ovarian and breast cancer. Cancer Res. 63, 9032–9041.

Borgono, C.A., Michael, I.P., Diamandis, E.P., 2004. Human tissue kallikreins: physiologic roles and applications in cancer. Mol. Cancer Res. 2, 257–280.

Borgono, C.A., Michael, I.P., Komatsu, N., Jayakumar, A., Kapadia, R., Clayman, G.L., Sotiropoulou, G., Diamandis, E.P., 2006a. A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. J. Biol. Chem. 282, 3640–3652.

Borgono, C.A., Michael, I.P., Shaw, J.L., Luo, L.Y., Ghosh, M.C., Soosaipillai, A., Grass, L., Katsaros, D., Diamandis, E.P., 2006b. Expression and functional characterization of the cancer-related serine protease, human tissue kallikrein 14. J. Biol. Chem.

Brattsand, M., Egelrud, T., 1999. Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. J. Biol. Chem. 274, 30033–30040.

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

Campbell, D.J., 2001. The kallikrein-kinin system in humans. Clin. Exp. Pharmacol. Physiol. 28, 1060–1065.

Caubet, C., Jonca, N., Brattsand, M., Guerrin, M., Bernard, D., Schmidt, R., Egelrud, T., Simon, M., Serre, G., 2004. Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. J. Invest. Dermatol. 122, 1235–1244.

Chao, J., Chao, L., 2005. Kallikrein-kinin in stroke, cardiovascular and renal disease. Exp. Physiol. 90, 291–298.

Chao, J., Bledsoe, G., Yin, H., Chao, L., 2006. The tissue kallikrein-kinin system protects against cardiovascular and renal diseases and ischemic stroke independently of blood pressure reduction. Biol. Chem. 387, 665–675.

Chavanas, S., Bodemer, C., Rochat, A., Hamel-Teillac, D., Ali, M., Irvine, A.D., Bonafe, J.L., Wilkinson, J., Taieb, A., Barrandon, Y., Harper, J.I., de Prost, Y., Hovnanian, A., 2000. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat. Genet. 25, 141–142.

Chen, V.C., Chao, L., Chao, J., 2000a. Reactive-site specificity of human kallistatin toward tissue kallikrein probed by site-directed mutagenesis. Biochim. Biophys. Acta 1479, 237–246.

Chen, V.C., Chao, L., Chao, J., 2000b. Roles of the P1, P2, and P3 residues in determining inhibitory specificity of kallistatin toward human tissue kallikrein. J. Biol. Chem. 275, 38457–38466.

Chowdhury, I., Tharakan, B., Bhat, G.K., 2006. Current concepts in apoptosis: the physiological suicide program revisited. Cell Mol. Biol. Lett. 11, 506–525.

Christofori, G., 2006. New signals from the invasive front. Nature 441, 444–450.

Cinar, B., Yeung, F., Konaka, H., Mayo, M.W., Freeman, M.R., Zhau, H.E., Chung, L.W., 2004. Identification of a negative regulatory cis-element in the enhancer core region of the prostate-specific antigen promoter: implications for intersection of androgen receptor and nuclear factor-kappaB signalling in prostate cancer cells. Biochem. J. 379, 421–431.

Cleutjens, K.B., van Eekelen, C.C., van der Korput, H.A., Brinkmann, A.O., Trapman, J., 1996. Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J. Biol. Chem. 271, 6379–6388.

Cleutjens, K.B., van der Korput, H.A., van Eekelen, C.C., van Rooij, H.C., Faber, P.W., Trapman, J., 1997. An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate-specific antigen promoter. Mol. Endocrinol. 11, 148–161.

Cloutier, S.M., Chagas, J.R., Mach, J.P., Gygi, C.M., Leisinger, H.J., Deperthes, D., 2002. Substrate specificity of human kallikrein 2 (hK2) as determined by phage display technology. Eur. J. Biochem. 269, 2747–2754.

Colman, R.W., 2006. Regulation of angiogenesis by the kallikrein-kinin system. Curr. Pharm. Des 12, 2599–2607.

Davidson, B., Xi, Z., Saatcioglu, F., 2007. Kallikrein 4 is expressed in malignant mesothelioma—further evidence for the histogenetic link between mesothelial and epithelial cells. Diagn. Cytopathol. 35, 80–84.

Debela, M., Magdolen, V., Grimminger, V., Sommerhoff, C., Messerschmidt, A., Huber, R., Friedrich, R., Bode, W., Goettig, P., 2006a. Crystal structures of human tissue kallikrein 4: activity modulation by a specific zinc binding site. J. Mol. Biol. 362, 1094–1107.

Debela, M., Magdolen, V., Schechter, N., Valachova, M., Lottspeich, F., Craik, C.S., Choe, Y., Bode, W., Goettig, P., 2006b. Specificity profiling of seven human tissue kallikreins reveals individual subsite preferences. J. Biol. Chem. 281, 25678–25688.

Denmeade, S.R., Lovgren, J., Khan, S.R., Lilja, H., Isaacs, J.T., 2001. Activation of latent protease function of pro-hK2, but not pro-PSA, involves autoprocessing. Prostate 48, 122–126.

Denmeade, S.R., Litvinov, I., Sokoll, L.J., Lilja, H., Isaacs, J.T., 2003. Prostate-specific antigen (PSA) protein does not affect growth of prostate cancer cells in vitro or prostate cancer xenografts in vivo. Prostate 56, 45–53.

Deperthes, D., 2002. Phage display substrate: a blind method for determining protease specificity. Biol. Chem. 383, 1107–1112.

Dery, O., Corvera, C.U., Steinhoff, M., Bunnett, N.W., 1998. Proteinase-activated receptors: novel mechanisms of signaling by serine proteases. Am.. J. Physiol 274, C1429–C1452.

Descargues, P., Deraison, C., Bonnart, C., Kreft, M., Kishibe, M., Ishida-Yamamoto, A., Elias, P., Barrandon, Y., Zambruno, G., Sonnenberg, A., Hovnanian, A., 2005. Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat. Genet. 37, 56–65.

Descargues, P., Deraison, C., Prost, C., Fraitag, S., Mazereeuw-Hautier, J., D'Alessio, M., Ishida-Yamamoto, A., Bodemer, C., Zambruno, G., Hovnanian, A., 2006. Corneodesmosomal cadherins are preferential targets of stratum corneum trypsin- and chymotrypsin-like hyperactivity in Netherton syndrome. J. Invest. Dermatol. 126, 1622–1632.

Desrivieres, S., Lu, H., Peyri, N., Soria, C., Legrand, Y., Menashi, S., 1993. Activation of the 92 kDa type IV collagenase by tissue kallikrein. J. Cell Physiol. 157, 587–593.

Diamandis, E.P., Scorilas, A., Fracchioli, S., Van Gramberen, M., De Bruijn, H., Henrik, A., Soosaipillai, A., Grass, L., Yousef, G.M., Stenman, U.H., Massobrio, M., Van Der Zee, A.G., Vergote, I., Katsaros, D., 2003. Human kallikrein 6 (hK6): a new potential serum biomarker for diagnosis and prognosis of ovarian carcinoma. J. Clin. Oncol. 21, 1035–1043.

Diamandis, E.P., Scorilas, A., Kishi, T., Blennow, K., Luo, L.Y., Soosaipillai, A., Rademaker, A.W., Sjogren, M., 2004. Altered kallikrein 7 and 10 concentrations in cerebrospinal fluid of patients with Alzheimer's disease and frontotemporal dementia. Clin. Biochem. 37, 230–237.

Egelrud, T., Brattsand, M., Kreutzmann, P., Walden, M., Vitzithum, K., Marx, U.C., Forssmann, W.G., Magert, H.J., 2005. hK5 and hK7, two serine proteinases abundant in human skin, are inhibited by LEKTI domain 6. Br. J. Dermatol. 153, 1200–1203.

Ekholm, E., Egelrud, T., 1999. Stratum corneum chymotryptic enzyme in psoriasis. Arch. Dermatol. Res. 291, 195–200.

Elliott, M.B., Irwin, D.M., Diamandis, E.P., 2006. In silico identification and Bayesian phylogenetic analysis of multiple new mammalian kallikrein gene families. Genomics 88, 591–599.

Emami, N., Diamandis, E.P., 2007. Human tissue kallikreins: a road under construction. Clin. Chim. Acta 381, 78–84.

Emanueli, C., Minasi, A., Zacheo, A., Chao, J., Chao, L., Salis, M.B., Straino, S., Tozzi, M.G., Smith, R., Gaspa, L., Bianchini, G., Stillo, F., Capogrossi, M.C., Madeddu, P., 2001. Local delivery of human tissue kallikrein gene accelerates spontaneous angiogenesis in mouse model of hindlimb ischemia. Circulation 103, 125–132.

Espana, F., Fink, E., Sanchez-Cuenca, J., Gilabert, J., Estelles, A., Witzgall, K., 1995. Complexes of tissue kallikrein with protein

C inhibitor in human semen and urine. Eur. J. Biochem. 234, 641–649.
Felber, L.M., Kundig, C., Borgono, C.A., Chagas, J.R., Tasinato, A., Jichlinski, P., Gygi, C.M., Leisinger, H.J., Diamandis, E.P., Deperthes, D., Cloutier, S.M., 2006. Mutant recombinant serpins as highly specific inhibitors of human kallikrein 14. FEBS J. 273, 2505–2514.
Fernando, S.C., Najar, F.Z., Guo, X., Zhou, L., Fu, Y., Geisert, R.D., Roe, B.A., Desilva, U., 2007. Porcine kallikrein gene family: genomic structure, mapping, and differential expression analysis. Genomics 89 (3), 429–438.
Fidler, I.J., 2000. Angiogenesis and cancer metastasis. Cancer J. 6 (Suppl. 2), S134–S141.
Fortier, A.H., Nelson, B.J., Grella, D.K., Holaday, J.W., 1999. Antiangiogenic activity of prostate-specific antigen. J. Natl. Cancer Inst. 91, 1635–1640.
Fortier, A.H., Holaday, J.W., Liang, H., Dey, C., Grella, D.K., Holland-Linn, J., Vu, H., Plum, S.M., Nelson, B.J., 2003. Recombinant prostate specific antigen inhibits angiogenesis in vitro and in vivo. Prostate 56, 212–219.
Foussias, G., Yousef, G.M., Diamandis, E.P., 2000. Identification and molecular characterization of a novel member of the siglec family (SIGLEC9). Genomics 67, 171–178.
Frenette, G., Tremblay, R.R., Lazure, C., Dube, J.Y., 1997. Prostatic kallikrein hK2, but not prostate-specific antigen (hK3), activates single-chain urokinase-type plasminogen activator. Int. J. Cancer 71, 897–899.
Giusti, B., Serrati, S., Margheri, F., Papucci, L., Rossi, L., Poggi, F., Magi, A., Del Rosso, A., Cinelli, M., Guiducci, S., Kahaleh, B., Matucci-Cerinic, M., Abbate, R., Fibbi, G., Del Rosso, M., 2005. The antiangiogenic tissue kallikrein pattern of endothelial cells in systemic sclerosis. Arthritis Rheum. 52, 3618–3628.
Gomis-Ruth, F.X., Bayes, A., Sotiropoulou, G., Pampalakis, G., Tsetsenis, T., Villegas, V., Aviles, F.X., Coll, M., 2002. The structure of human prokallikrein 6 reveals a novel activation mechanism for the kallikrein family. J. Biol. Chem. 277, 27273–27281.
Goya, M., Ishii, G., Miyamoto, S., Hasebe, T., Nagai, K., Yonou, H., Hatano, T., Ogawa, Y., Ochiai, A., 2006. Prostate-specific antigen induces apoptosis of osteoclast precursors: potential role in osteoblastic bone metastases of prostate cancer. Prostate 66, 1573–1584.
Goyal, J., Smith, K.M., Cowan, J.M., Wazer, D.E., Lee, S.W., Band, V., 1998. The role for NES1 serine protease as a novel tumor suppressor. Cancer Res. 58, 4782–4786.
Hachem, J.P., Wagberg, F., Schmuth, M., Crumrine, D., Lissens, W., Jayakumar, A., Houben, E., Mauro, T.M., Leonardsson, G., Brattsand, M., Egelrud, T., Roseeuw, D., Clayman, G.L., Feingold, K.R., Williams, M.L., Elias, P.M., 2006. Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome. J. Invest. Dermatol. 126, 1609–1621.
Hansson, L., Backman, A., Ny, A., Edlund, M., Ekholm, E., Ekstrand, H.B., Tornell, J., Wallbrandt, P., Wennbo, H., Egelrud, T., 2002. Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. J. Invest. Dermatol. 118, 444–449.
Hansson, L., Stromqvist, M., Backman, A., Wallbrandt, P., Carlstein, A., Egelrud, T., 1994. Cloning, expression, and characterization of stratum corneum chymotryptic enzyme. A skin-specific human serine proteinase. J. Biol. Chem. 269, 19420–19426.
Harris, J.L., Backes, B.J., Leonetti, F., Mahrus, S., Ellman, J.A., Craik, C.S., 2000. Rapid and general profiling of protease specificity by using combinatorial fluorogenic substrate libraries. Proc. Natl. Acad. Sci. USA 97, 7754–7759.
Hart, P.S., Wright, J.T., Savage, M., Kang, G., Bensen, J.T., Gorry, M.C., Hart, T.C., 2003. Exclusion of candidate genes in two families with autosomal dominant hypocalcified amelogenesis imperfecta. Eur.. J. Oral Sci. 111, 326–331.
Hart, P.S., Hart, T.C., Michalec, M.D., Ryu, O.H., Simmons, D., Hong, S., Wright, J.T., 2004. Mutation in kallikrein 4 causes autosomal recessive hypomaturation amelogenesis imperfecta. J. Med. Genet. 41, 545–549.
Heidtmann, H.H., Nettelbeck, D.M., Mingels, A., Jager, R., Welker, H.G., Kontermann, R.E., 1999. Generation of angiostatin-like fragments from plasminogen by prostate-specific antigen. Br.. J. Cancer 81, 1269–1273.
Ishida-Yamamoto, A., Deraison, C., Bonnart, C., Bitoun, E., Robinson, R., O'Brien, T.J., Wakamatsu, K., Ohtsubo, S., Takahashi, H., Hashimoto, Y., Dopping-Hepenstal, P.J., McGrath, J.A., Iizuka, H., Richard, G., Hovnanian, A., 2005. LEKTI is localized in lamellar granules, separated from KLK5 and KLK7, and is secreted in the extracellular spaces of the superficial stratum granulosum. J. Invest. Dermatol. 124, 360–366.
Ishii, K., Otsuka, T., Iguchi, K., Usui, S., Yamamoto, H., Sugimura, Y., Yoshikawa, K., Hayward, S.W., Hirano, K., 2004. Evidence that the prostate-specific antigen (PSA)/Zn2+ axis may play a role in human prostate cancer cell invasion. Cancer Lett. 207, 79–87.
Kemper, C., Atkinson, J.P., 2007. T-cell regulation: with complements from innate immunity. Nat. Rev. Immunol. 7, 9–18.
Killian, C.S., Corral, D.A., Kawinski, E., Constantine, R.I., 1993. Mitogenic response of osteoblast cells to prostate-specific antigen suggests an activation of latent TGF-beta and a proteolytic modulation of cell adhesion receptors. Biochem. Biophys. Res. Commun. 192, 940–947.
Kim, J.W., Simmer, J.P., Lin, B.P., Seymen, F., Bartlett, J.D., Hu, J.C., 2006. Mutational analysis of candidate genes in 24 amelogenesis imperfecta families. Eur. J. Oral Sci. 114 (Suppl. 1), 3–12.
Kishi, T., Grass, L., Soosaipillai, A., Scorilas, A., Harbeck, N., Schmalfeldt, B., Dorn, J., Mysliwiec, M., Schmitt, M., Diamandis, E.P., 2003. Human kallikrein 8, a novel biomarker for ovarian carcinoma. Cancer Res. 63, 2771–2774.
Kishi, T., Cloutier, S.M., Kundig, C., Deperthes, D., Diamandis, E.P., 2006. Activation and enzymatic characterization of recombinant human kallikrein 8. Biol. Chem. 387, 723–731.
Kishibe, M., Bando, Y., Terayama, R., Namikawa, K., Takahashi, H., Hashimoto, Y., Ishida-Yamamoto, A., Jiang, Y.P., Mitrovic, B., Perez, D., Iizuka, H., Yoshida, S., 2006. Kallikrein 8 is involved in skin desquamation in cooperation with other kallikreins. J. Biol. Chem. 282, 5834–5841.
Koistinen, H., Paju, A., Koistinen, R., Finne, P., Lovgren, J., Wu, P., Seppala, M., Stenman, U.H., 2002. Prostate-specific antigen and other prostate-derived proteases cleave IGFBP-3, but prostate cancer is not associated with proteolytically cleaved circulating IGFBP-3. Prostate 50, 112–118.
Komatsu, N., Takata, M., Otsuki, N., Ohka, R., Amano, O., Takehara, K., Saijoh, K., 2002. Elevated stratum corneum hydrolytic activity in Netherton syndrome suggests an inhibitory regulation of desquamation by SPINK5-derived peptides. J. Invest. Dermatol. 118, 436–443.
Komatsu, N., Saijoh, K., Sidiropoulos, M., Tsai, B., Levesque, M.A., Elliott, M.B., Takehara, K., Diamandis, E.P., 2005a. Quantification of human tissue kallikreins in the stratum corneum: dependence on age and gender. J. Invest. Dermatol. 125, 1182–1189.
Komatsu, N., Saijoh, K., Toyama, T., Ohka, R., Otsuki, N., Hussack, G., Takehara, K., Diamandis, E.P., 2005b. Multiple tissue kallikrein mRNA and protein expression in normal skin and skin diseases. Br. J. Dermatol. 153, 274–281.
Komatsu, N., Suga, Y., Saijoh, K., Liu, A.C., Khan, S., Mizuno, Y., Ikeda, S., Wu, H.K., Jayakumar, A., Clayman, G.L., Shirasaki, F.,

Takehara, K., Diamandis, E.P., 2006. 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. 126, 2338–2342.

Komatsu, N., Saijoh, K., Otsuki, N., Kishi, T., Micheal, I.P., Obiezu, C.V., Borgono, C.A., Takehara, K., Jayakumar, A., Wu, H.K., Clayman, G.L., Diamandis, E.P., 2007. Proteolytic processing of human growth hormone by multiple tissue kallikreins and regulation by the serine protease inhibitor Kazal-Type5 (SPINK5) protein. Clin. Chim. Acta 377, 228–236.

Kumar, A., Goel, A.S., Hill, T.M., Mikolajczyk, S.D., Millar, L.S., Kuus-Reichel, K., Saedi, M.S., 1996. Expression of human glandular kallikrein, hK2, in mammalian cells. Cancer Res. 56, 5397–5402.

Kumar, A., Mikolajczyk, S.D., Goel, A.S., Millar, L.S., Saedi, M.S., 1997. Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2. Cancer Res. 57, 3111–3114.

Kurlender, L., Yousef, G.M., Memari, N., Robb, J.D., Michael, I.P., Borgono, C., Katsaros, D., Stephan, C., Jung, K., Diamandis, E.P., 2004. Differential expression of a human kallikrein 5 (KLK5) splice variant in ovarian and prostate cancer. Tumour. Biol. 25, 149–156.

Kyriakopoulou, L.G., Yousef, G.M., Scorilas, A., Katsaros, D., Massobrio, M., Fracchioli, S., Diamandis, E.P., 2003. Prognostic value of quantitatively assessed KLK7 expression in ovarian cancer. Clin. Biochem 36, 135–143.

Lai, J., Kedda, M.A., Hinze, K., Smith, R.L., Yaxley, J., Spurdle, A.B., Morris, C.P., Harris, J., Clements, J.A., 2006. PSA/KLK3 AREI promoter polymorphism alters androgen receptor binding and is associated with prostate cancer susceptibility. Carcinogenesis 28, 1032–1039.

Lauber, T., Schulz, A., Schweimer, K., Adermann, K., Marx, U.C., 2003. Homologous proteins with different folds: the three-dimensional structures of domains 1 and 6 of the multiple Kazal-type inhibitor LEKTI. J. Mol. Biol. 328, 205–219.

Laxmikanthan, G., Blaber, S.I., Bernett, M.J., Scarisbrick, I.A., Juliano, M.A., Blaber, M., 2005. 1.70 A X-ray structure of human apo kallikrein 1: structural changes upon peptide inhibitor/substrate binding. Proteins 58, 802–814.

Li, B., Goyal, J., Dhar, S., Dimri, G., Evron, E., Sukumar, S., Wazer, D.E., Band, V., 2001. CpG methylation as a basis for breast tumor-specific loss of NES1/kallikrein 10 expression. Cancer Res. 61, 8014–8021.

Lovgren, J., Rajakoski, K., Karp, M., Lundwall, A., Lilja, H., 1997. Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2. Biochem. Biophys. Res. Commun 238, 549–555.

Lovgren, J., Airas, K., Lilja, H., 1999a. Enzymatic action of human glandular kallikrein 2 (hK2). Substrate specificity and regulation by Zn2+ and extracellular protease inhibitors. Eur. J. Biochem. 262, 781–789.

Lovgren, J., Valtonen-Andre, C., Marsal, K., Lilja, H., Lundwall, A., 1999b. Measurement of prostate-specific antigen and human glandular kallikrein 2 in different body fluids. J. Androl 20, 348–355.

Lundwall, A., Band, V., Blaber, M., Clements, J.A., Courty, Y., Diamandis, E.P., Fritz, H., Lilja, H., Malm, J., Maltais, L.J., Olsson, A.Y., Petraki, C., Scorilas, A., Sotiropoulou, G., Stenman, U.H., Stephan, C., Talieri, M., Yousef, G.M., 2006a. A comprehensive nomenclature for serine proteases with homology to tissue kallikreins. Biol. Chem. 387, 637–641.

Lundwall, A., Clauss, A., Olsson, A.Y., 2006b. Evolution of kallikrein-related peptidases in mammals and identification of a genetic locus encoding potential regulatory inhibitors. Biol. Chem. 387, 243–249.

Luo, L.Y., Jiang, W., 2006. Inhibition profiles of human tissue kallikreins by serine protease inhibitors. Biol. Chem. 387, 813–816.

Luo, L.Y., Katsaros, D., Scorilas, A., Fracchioli, S., Bellino, R., Van Gramberen, M., De Bruijn, H., Henrik, A., Stenman, U.H., Massobrio, M., Van Der Zee, A.G., Vergote, I., Diamandis, E.P., 2003. The serum concentration of human kallikrein 10 represents a novel biomarker for ovarian cancer diagnosis and prognosis. Cancer Res. 63, 807–811.

Luo, L.Y., Shan, S.J., Elliott, M.B., Soosaipillai, A., Diamandis, E.P., 2006. Purification and characterization of human kallikrein 11, a candidate prostate and ovarian cancer biomarker, from seminal plasma. Clin. Cancer Res. 12, 742–750.

Magert, H.J., Kreutzmann, P., Standker, L., Walden, M., Drogemuller, K., Forssmann, W.G., 2002. LEKTI: a multidomain serine proteinase inhibitor with pathophysiological relevance. Int. J. Biochem. Cell Biol. 34, 573–576.

Magklara, A., Brown, T.J., Diamandis, E.P., 2002. Characterization of androgen receptor and nuclear receptor co-regulator expression in human breast cancer cell lines exhibiting differential regulation of kallikreins 2 and 3. Int. J. Cancer 100, 507–514.

Magklara, A., Mellati, A.A., Wasney, G.A., Little, S.P., Sotiropoulou, G., Becker, G.W., Diamandis, E.P., 2003. Characterization of the enzymatic activity of human kallikrein 6: autoactivation, substrate specificity, and regulation by inhibitors. Biochem. Biophys. Res. Commun. 307, 948–955.

Malm, J., Hellman, J., Hogg, P., Lilja, H., 2000. Enzymatic action of prostate-specific antigen (PSA or hK3): substrate specificity and regulation by Zn(2+), a tight-binding inhibitor. Prostate 45, 132–139.

Matsumura, M., Bhatt, A.S., Andress, D., Clegg, N., Takayama, T.K., Craik, C.S., Nelson, P.S., 2005. Substrates of the prostate-specific serine protease prostase/KLK4 defined by positional-scanning peptide libraries. Prostate 62, 1–13.

Memari, N., Jiang, W., Diamandis, E.P., Luo, L.Y., 2007. Enzymatic properties of human kallikrein-related peptidase 12 (KLK12). Biol. Chem. 388, 427–435.

Menashi, S., Fridman, R., Desrivieres, S., Lu, H., Legrand, Y., Soria, C., 1994. Regulation of 92-kDa gelatinase B activity in the extracellular matrix by tissue kallikrein. Ann.. N.Y. Acad. Sci. 732, 466–468.

Michael, I.P., Sotiropoulou, G., Pampalakis, G., Magklara, A., Ghosh, M., Wasney, G., Diamandis, E.P., 2005. Biochemical and enzymatic characterization of human kallikrein 5 (hK5), a novel serine protease potentially involved in cancer progression. J. Biol. Chem. 280, 14628–14635.

Michael, I.P., Pampalakis, G., Mikolajczyk, S.D., Malm, J., Sotiropoulou, G., Diamandis, E.P., 2006. 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. 281, 12743–12750.

Mikolajczyk, S.D., Millar, L.S., Kumar, A., Saedi, M.S., 1999. Prostatic human kallikrein 2 inactivates and complexes with plasminogen activator inhibitor-1. Int. J. Cancer 81, 438–442.

Moreau, M.E., Garbacki, N., Molinaro, G., Brown, N.J., Marceau, F., Adam, A., 2005. The kallikrein-kinin system: current and future pharmacological targets. J. Pharmacol. Sci. 99, 6–38.

Murtha, P., Tindall, D.J., Young, C.Y., 1993. Androgen induction of a human prostate-specific kallikrein, hKLK2: characterization of an androgen response element in the 5′ promoter region of the gene. Biochemistry 32, 6459–6464.

Obiezu, C.V., Diamandis, E.P., 2005. Human tissue kallikrein gene family: applications in cancer. Cancer Lett. 224, 1–22.

Obiezu, C.V., Michael, I.P., Levesque, M.A., Diamandis, E.P., 2006. Human kallikrein 4: enzymatic activity, inhibition, and degradation of extracellular matrix proteins. Biol. Chem. 387, 749–759.

Oikonomopoulou, K., Hansen, K.K., Saifeddine, M., Tea, I., Blaber, M., Blaber, S.I., Scarisbrick, I., Andrade-Gordon, P., Cottrell, G.S., Bunnett, N.W., Diamandis, E.P., Hollenberg, M.D., 2006. Proteinase-activated receptors, targets for kallikrein signaling. J. Biol. Chem. 281, 32095–32112.

Paliouras, M., Diamandis, E.P., 2006a. Coordinated steroid hormone-dependent and independent expression of multiple kallikreins in breast cancer cell lines. Breast Cancer Res. Treat. 102, 7–19.

Paliouras, M., Diamandis, E.P., 2006b. The kallikrein world: an update on the human tissue kallikreins. Biol. Chem. 387, 643–652.

Paliouras, M., Borgono, C., Diamandis, E.P., 2007. Human tissue kallikreins: the cancer biomarker family. Cancer Lett. 249, 61–79.

Pampalakis, G., Sotiropoulou, G., 2006. Multiple mechanisms underlie the aberrant expression of the human kallikrein 6 gene in breast cancer. Biol. Chem. 387, 773–782.

Peternac, D., Klima, I., Cecchini, M.G., Studer, U.E., Thalmann, G.N., 2006. Prostate specific antigen expression does not necessarily correlate with prostate cancer cell growth. J. Urol. 176, 354–360.

Petraki, C.D., Karavana, V.N., Skoufogiannis, P.T., Little, S.P., Howarth, D.J., Yousef, G.M., Diamandis, E.P., 2001. The spectrum of human kallikrein 6 (zyme/protease M/neurosin) expression in human tissues as assessed by immunohistochemistry. J. Histochem. Cytochem. 49, 1431–1441.

Petraki, C.D., Karavana, V.N., Luo, L.Y., Diamandis, E.P., 2002. Human kallikrein 10 expression in normal tissues by immunohistochemistry. J. Histochem. Cytochem. 50, 1247–1261.

Petraki, C.D., Karavana, V.N., Diamandis, E.P., 2003. Human kallikrein 13 expression in normal tissues: an immunohistochemical study. J. Histochem. Cytochem. 51, 493–501.

Picard, D., 2006. Chaperoning steroid hormone action. Trends Endocrinol. Metab 17, 229–235.

Pinkus, G.S., Maier, M., Seldin, D.C., ole-MoiYoi, O.K., Austen, K.F., Spragg, J., 1983. Immunohistochemical localization of glandular kallikrein in the endocrine and exocrine human pancreas. J. Histochem. Cytochem. 31, 1279–1288.

Prezas, P., Scorilas, A., Yfanti, C., Viktorov, P., Agnanti, N., Diamandis, E., Talieri, M., 2006. The role of human tissue kallikreins 7 and 8 in intracranial malignancies. Biol. Chem. 387, 1607–1612.

Rajapakse, S., Ogiwara, K., Takano, N., Moriyama, A., Takahashi, T., 2005. Biochemical characterization of human kallikrein 8 and its possible involvement in the degradation of extracellular matrix proteins. FEBS Lett. 579, 6879–6884.

Rawlings, N.D., Barrett, A.J., 1994. Families of serine peptidases. Methods Enzymol. 244, 19–61.

Rehault, S., Monget, P., Mazerbourg, S., Tremblay, R., Gutman, N., Gauthier, F., Moreau, T., 2001. Insulin-like growth factor binding proteins (IGFBPs) as potential physiological substrates for human kallikreins hK2 and hK3. Eur. J. Biochem. 268, 2960–2968.

Rittenhouse, H.G., Finlay, J.A., Mikolajczyk, S.D., Partin, A.W., 1998. Human Kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. Crit. Rev. Clin. Lab Sci. 35, 275–368.

Rochette-Egly, C., 2003. Nuclear receptors: integration of multiple signalling pathways through phosphorylation. Cell Signal. 15, 355–366.

Romanov, V.I., Whyard, T., Adler, H.L., Waltzer, W.C., Zucker, S., 2004. Prostate cancer cell adhesion to bone marrow endothelium: the role of prostate-specific antigen. Cancer Res. 64, 2083–2089.

Rosenfeld, R.G., 2003. Insulin-like growth factors and the basis of growth. N. Engl. J. Med. 349, 2184–2186.

Rousseau, G.G., 1984. Control of gene expression by glucocorticoid hormones. Biochem. J. 224, 1–12.

Samani, A.A., Yakar, S., Leroith, D., Brodt, P., 2006. The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocr. Rev. 28, 20–47.

Saunders, W.B., Bayless, K.J., Davis, G.E., 2005. MMP-1 activation by serine proteases and MMP-10 induces human capillary tubular network collapse and regression in 3D collagen matrices. J. Cell Sci. 118, 2325–2340.

Schechter, N.M., Choi, E.J., Wang, Z.M., Hanakawa, Y., Stanley, J.R., Kang, Y., Clayman, G.L., Jayakumar, A., 2005. Inhibition of human kallikreins 5 and 7 by the serine protease inhibitor lympho-epithelial Kazal-type inhibitor (LEKTI). Biol. Chem. 386, 1173–1184.

Schenone, M., Furie, B.C., Furie, B., 2004. The blood coagulation cascade. Curr. Opin. Hematol 11, 272–277.

Schuur, E.R., Henderson, G.A., Kmetec, L.A., Miller, J.D., Lamparski, H.G., Henderson, D.R., 1996. Prostate-specific antigen expression is regulated by an upstream enhancer. J. Biol. Chem. 271, 7043–7051.

Scorilas, A., Borgono, C.A., Harbeck, N., Dorn, J., Schmalfeldt, B., Schmitt, M., Diamandis, E.P., 2004. Human kallikrein 13 protein in ovarian cancer cytosols: a new favorable prognostic marker. J. Clin. Oncol. 22, 678–685.

Sharma, J.N., 2006. Role of tissue kallikrein-kininogen-kinin pathways in the cardiovascular system. Arch. Med. Res. 37, 299–306.

Shimizu, C., Yoshida, S., Shibata, M., Kato, K., Momota, Y., Matsumoto, K., Shiosaka, T., Midorikawa, R., Kamachi, T., Kawabe, A., Shiosaka, S., 1998. Characterization of recombinant and brain neuropsin, a plasticity-related serine protease. J. Biol. Chem. 273, 11189–11196.

Sidenius, N., Blasi, F., 2003. The urokinase plasminogen activator system in cancer: recent advances and implication for prognosis and therapy. Cancer Metastasis Rev. 22, 205–222.

Sidera, C., Parsons, R., Austen, B., 2004. Proteolytic cascade in the amyloidogenesis of Alzheimer's disease. Biochem. Soc. Trans. 32, 33–36.

Sidiropoulos, M., Pampalakis, G., Sotiropoulou, G., Katsaros, D., Diamandis, E.P., 2005. Downregulation of human kallikrein 10 (KLK10/NES1) by CpG island hypermethylation in breast, ovarian and prostate cancers. Tumour. Biol. 26, 324–336.

Silverman, G.A., Bird, P.I., Carrell, R.W., Church, F.C., Coughlin, P.B., Gettins, P.G., Irving, J.A., Lomas, D.A., Luke, C.J., Moyer, R.W., Pemberton, P.A., Remold-O'Donnell, E., Salvesen, G.S., Travis, J., Whisstock, J.C., 2001. 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. 276, 33293–33296.

Simmer, J.P., Fukae, M., Tanabe, T., Yamakoshi, Y., Uchida, T., Xue, J., Margolis, H.C., Shimizu, M., DeHart, B.C., Hu, C.C., Bartlett, J.D., 1998. Purification, characterization, and cloning of enamel matrix serine proteinase 1. J. Dent. Res. 77, 377–386.

Sohara, Y., Shimada, H., DeClerck, Y.A., 2005. Mechanisms of bone invasion and metastasis in human neuroblastoma. Cancer Lett. 228, 203–209.

Sondell, B., Thornell, L.E., Egelrud, T., 1995. Evidence that stratum corneum chymotryptic enzyme is transported to the stratum corneum extracellular space via lamellar bodies. J. Invest. Dermatol. 104, 819–823.

Sotiropoulou, G., Rogakos, V., Tsetsenis, T., Pampalakis, G., Zafiropoulos, N., Simillides, G., Yiotakis, A., Diamandis, E.P., 2003. Emerging interest in the kallikrein gene family for understanding and diagnosing cancer. Oncol. Res. 13, 381–391.

Sprecher, E., Chavanas, S., DiGiovanna, J.J., Amin, S., Nielsen, K., Prendiville, J.S., Silverman, R., Esterly, N.B., Spraker, M.K., Guelig, E., de Luna, M.L., Williams, M.L., Buehler, B., Siegfried, E.C., Van Maldergem, L., Pfendner, E., Bale, S.J., Uitto, J., Hovnanian, A., Richard, G., 2001. The spectrum of pathogenic mutations in SPINK5 in 19 families with Netherton syndrome: implications for mutation detection and first case of prenatal diagnosis. J. Invest. Dermatol. 117, 179–187.

Stefansson, K., Brattsand, M., Ny, A., Glas, B., Egelrud, T., 2006. Kallikrein-related peptidase 14 may be a major contributor to trypsin-like proteolytic activity in human stratum corneum. Biol. Chem. 387, 761–768.

Stege, R., Grande, M., Carlstrom, K., Tribukait, B., Pousette, A., 2000. Prognostic significance of tissue prostate-specific antigen in endocrine-treated prostate carcinomas. Clin. Cancer Res. 6, 160–165.

Stenman, U.H., Leinonen, J., Alfthan, H., Rannikko, S., Tuhkanen, K., Alfthan, O., 1991. A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. Cancer Res. 51, 222–226.

Stephan, C., Yousef, G.M., Scorilas, A., Jung, K., Jung, M., Kristiansen, G., Hauptmann, S., Bharaj, B.S., Nakamura, T., Loening, S.A., Diamandis, E.P., 2003. Quantitative analysis of kallikrein 15 gene expression in prostate tissue. J. Urol. 169, 361–364.

Stephanopoulos, G., Garefalaki, M.E., Lyroudia, K., 2005. Genes and related proteins involved in amelogenesis imperfecta. J. Dent. Res. 84, 1117–1126.

Sun, Z., Pan, J., Balk, S.P., 1997. Androgen receptor-associated protein complex binds upstream of the androgen-responsive elements in the promoters of human prostate-specific antigen and kallikrein 2 genes. Nucleic Acids Res. 25, 3318–3325.

Takayama, T.K., McMullen, B.A., Nelson, P.S., Matsumura, M., Fujikawa, K., 2001. Characterization of hK4 (prostase), a prostate-specific serine protease: activation of the precursor of prostate specific antigen (pro-PSA) and single-chain urokinase-type plasminogen activator and degradation of prostatic acid phosphatase. Biochemistry 40, 15341–15348.

Talieri, M., Diamandis, E.P., Gourgiotis, D., Mathioudaki, K., Scorilas, A., 2004. Expression analysis of the human kallikrein 7 (KLK7) in breast tumors: a new potential biomarker for prognosis of breast carcinoma. Thromb. Haemost. 91, 180–186.

Tamura, H., Ishikawa, Y., Hino, N., Maeda, M., Yoshida, S., Kaku, S., Shiosaka, S., 2006. Neuropsin is essential for early processes of memory acquisition and Schaffer collateral long-term potentiation in adult mouse hippocampus in vivo. J. Physiol. 570, 541–551.

Tarttelin, E.E., Bellingham, J., Hankins, M.W., Foster, R.G., Lucas, R.J., 2003. Neuropsin (Opn5): a novel opsin identified in mammalian neural tissue. FEBS Lett. 554, 410–416.

Terayama, R., Bando, Y., Takahashi, T., Yoshida, S., 2004. Differential expression of neuropsin and protease M/neurosin in oligodendrocytes after injury to the spinal cord. Glia 48, 91–101.

Thornberry, N.A., Rano, T.A., Peterson, E.P., Rasper, D.M., Timkey, T., Garcia-Calvo, M., Houtzager, V.M., Nordstrom, P.A., Roy, S., Vaillancourt, J.P., Chapman, K.T., Nicholson, D.W., 1997. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. J. Biol. Chem. 272, 17907–17911.

Vaisanen, V., Lovgren, J., Hellman, J., Piironen, T., Lilja, H., Pettersson, K., 1999. Characterization and processing of prostate specific antigen (hK3) and human glandular kallikrein (hK2) secreted by LNCaP cells. Prostate Cancer Prostatic. Dis. 2, 91–97.

Veveris-Lowe, T.L., Lawrence, M.G., Collard, R.L., Bui, L., Herington, A.C., Nicol, D.L., Clements, J.A., 2005. 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 12, 631–643.

Wang, Y., 2001. The role and regulation of urokinase-type plasminogen activator receptor gene expression in cancer invasion and metastasis. Med. Res. Rev. 21, 146–170.

Wang, M.C., Valenzuela, L.A., Murphy, G.P., Chu, T.M., 1979. Purification of a human prostate specific antigen. Invest. Urol 17, 159–163.

Wang, C., Yeung, F., Liu, P.C., Attar, R.M., Geng, J., Chung, L.W., Gottardis, M., Kao, C., 2003. Identification of a novel transcription factor, GAGATA-binding protein, involved in androgen-mediated expression of prostate-specific antigen. J. Biol. Chem. 278, 32423–32430.

Webber, M.M., Waghray, A., Bello, D., 1995. Prostate-specific antigen, a serine protease, facilitates human prostate cancer cell invasion. Clin. Cancer Res. 1, 1089–1094.

Wright, J.T., Daly, B., Simmons, D., Hong, S., Hart, S.P., Hart, T.C., Atsawasuwan, P., Yamauchi, M., 2006. Human enamel phenotype associated with amelogenesis imperfecta and a kallikrein-4 (g.2142G > A) proteinase mutation. Eur. J. Oral Sci. 114 (Suppl. 1), 13–17.

Yakar, S., Liu, J.L., Stannard, B., Butler, A., Accili, D., Sauer, B., Leroith, D., 1999. Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc. Natl. Acad. Sci. USA 96, 7324–7329.

Yamakoshi, Y., Hu, J.C., Fukae, M., Yamakoshi, F., Simmer, J.P., 2006. How do enamelysin and kallikrein 4 process the 32-kDa enamelin? Eur.. J. Oral Sci. 114 (Suppl. 1), 45–51.

Yamasaki, K., Schauber, J., Coda, A., Lin, H., Dorschner, R.A., Schechter, N.M., Bonnart, C., Descargues, P., Hovnanian, A., Gallo, R.L., 2006. Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. FASEB J. 20, 2068–2080.

Yao, Y.Y., Yin, H., Shen, B., Chao, L., Chao, J., 2006. Tissue kallikrein infusion prevents cardiomyocyte apoptosis, inflammation and ventricular remodeling after myocardial infarction. Regul. Pept. 140, 12–20.

Yonou, H., Aoyagi, Y., Kanomata, N., Kamijo, T., Oda, T., Yokose, T., Hasebe, T., Nagai, K., Hatano, T., Ogawa, Y., Ochiai, A., 2001. Prostate-specific antigen induces osteoplastic changes by an autonomous mechanism. Biochem. Biophys. Res. Commun. 289, 1082–1087.

Yousef, G.M., Diamandis, E.P., 2001. The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr. Rev. 22, 184–204.

Yousef, G.M., Diamandis, E.P., 2002. Human tissue kallikreins: a new enzymatic cascade pathway? Biol. Chem. 383, 1045–1057.

Yousef, G.M., Diamandis, M., Jung, K., Diamandis, E.P., 2001a. Molecular cloning of a novel human acid phosphatase gene (ACPT) that is highly expressed in the testis. Genomics 74, 385–395.

Yousef, G.M., Kyriakopoulou, L.G., Scorilas, A., Fracchioli, S., Ghiringhello, B., Zarghooni, M., Chang, A., Diamandis, M., Giardina, G., Hartwick, W.J., Richiardi, G., Massobrio, M., Diamandis, E.P., Katsaros, D., 2001b. Quantitative expression of the human kallikrein gene 9 (KLK9) in ovarian cancer: a new independent and favorable prognostic marker. Cancer Res. 61, 7811–7818.

Yousef, G.M., Kishi, T., Diamandis, E.P., 2003a. Role of kallikrein enzymes in the central nervous system. Clin. Chim. Acta 329, 1–8.

Yousef, G.M., Polymeris, M.E., Grass, L., Soosaipillai, A., Chan, P.C., Scorilas, A., Borgono, C., Harbeck, N.,

Schmalfeldt, B., Dorn, J., Schmitt, M., Diamandis, E.P., 2003b. Human kallikrein 5: a potential novel serum biomarker for breast and ovarian cancer. Cancer Res. 63, 3958–3965.

Yousef, G.M., Scorilas, A., Nakamura, T., Ellatif, M.A., Ponzone, R., Biglia, N., Maggiorotto, F., Roagna, R., Sismondi, P., Diamandis, E.P., 2003c. The prognostic value of the human kallikrein gene 9 (KLK9) in breast cancer. Breast Cancer Res. Treat. 78, 149–158.

Yousef, G.M., White, N.M., Michael, I.P., Cho, J.C., Robb, J.D., Kurlender, L., Khan, S., Diamandis, E.P., 2005. Identification of new splice variants and differential expression of the human kallikrein 10 gene, a candidate cancer biomarker. Tumour. Biol. 26, 227–235.

Yu, H., Levesque, M.A., Clark, G.M., Diamandis, E.P., 1998. Prognostic value of prostate-specific antigen for women with breast cancer: a large United States cohort study. Clin. Cancer Res. 4, 1489–1497.

Yu, D.C., Sakamoto, G.T., Henderson, D.R., 1999. Identification of the transcriptional regulatory sequences of human kallikrein 2 and their use in the construction of calydon virus 764, an attenuated replication competent adenovirus for prostate cancer therapy. Cancer Res. 59, 1498–1504.

Zhao, R., Houry, W.A., 2005. Hsp90: a chaperone for protein folding and gene regulation. Biochem. Cell Biol. 83, 703–710.