FUNCTIONAL ROLES OF HUMAN KALLIKREIN-RELATED PEPTIDASES

Georgia Sotiropoulou1*, Georgios Pampalakis1 and Eleftherios P. Diamandis2,3,4

1Department of Pharmacy, University of Patras, Rion, 26500 Rion-Patras, Greece
2Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 3Department of Clinical Biochemistry, University Health Network and 4Department of Laboratory Medicine and Pathobiology, University of Toronto, ON, Canada

Running Head: Kallikrein-Related Peptidases: Pleiotropic Proteins

*Author to whom correspondence should be addressed at the Department of Pharmacy, University of Patras, Rion-Patras 26500, Greece.
Tel.: +30-2610-969939, Tel./FAX: +30-2610-969940, E-mail: gdsotiro@upatras.gr

Kallikrein-related peptidases constitute a single family of 15 (chymo)trypsin-like proteases (KLK1-15) with pleiotropic physiological roles. Aberrant regulation of KLKs has been associated with diverse diseases, such as hypertension, renal dysfunction, skin disorders, inflammation, neurodegeneration and cancer. Recent studies suggested that co-ordinated activation and regulation of KLK activity is achieved via a complex network of interactions, referred to as “KLK activome”. However, it remains to be validated whether these hypothetical KLK activation cascade pathways are operative in vivo. In addition, KLKs have emerged as versatile signaling molecules. In summary, KLKs represent attractive biomarkers for clinical applications and potential therapeutic targets for common human pathologies.

The KLK family

Kallikrein-related peptidases (KLKs) constitute a single family of 15 highly conserved trypsin- or chymotrypsin-like serine proteases encoded by the largest uninterrupted cluster of protease-encoding genes (KLK1-15) in the human genome. KLK3 or PSA (prostate-specific antigen) is the most widely known KLK due to its application in diagnosis and monitoring of prostate cancer (2, 3). KLK genes share multiple similar structural features, including exon/ intron organization, conserved intronic intervals, and exon lengths (2). All KLK genes encode for single-chain pre-pro-enzymes with lengths varying between 244 and 293 amino acid residues and about 40% protein identity among each other. Based on KLK concentrations in human tissues and biological fluids, KLK abundance is classified as highly restricted in the prostate (KLK2 and 3), restricted in certain tissues (KLK5, 6, 7, 8, 13) and broad (KLK1, 4, 9, 10, 11, 12, 14, and 15). Multiple KLKs are often co-expressed in normal tissues and usually they are coordinately deregulated in disease states pointing to common mechanisms of regulation. Indeed, the expression of most KLK genes is regulated by nuclear receptor signaling, while KLK zymogen activation is thought to proceed via complex proteolytic cascades that lead to sequential activation of multiple KLK enzymes that, in turn, regulate important normal and pathobiological processes, such as semen liquefaction, skin desquamation, innate immunity, neurodegeneration, degradation and remodeling of extracellular matrix (ECM). In addition to KLK3/PSA, certain KLKs are aberrantly expressed in different types of cancer and provide novel tumour markers (mRNA, protein, genomic DNA methylation) for cancer diagnosis, prognosis and monitoring (4). In addition, KLK6 has been suggested as a potential marker for Alzheimer’s disease (2).

Due to their significant roles in common human pathologies, KLKs are currently under study as potential therapeutic targets. Prostate-specific expression of KLK3/PSA has been exploited for PSA-targeting therapeutic strategies that include PSA-loaded antigen-presenting cells and PSA-vaccines for prostate cancer (reviewed in ref 5). In addition, PSA-activated prodrugs have been designed for treatment of prostate cancer based on the fact that serum PSA is mostly enzymatically inactive, while in the prostate gland it is found in its active form (6). Notably, administration of recombinant KLK6 in mice with...
Experimental autoimmune encephalomyelitis (EAE) resulted in the production of anti-KLK6 antibodies that inhibited its enzymatic activity, attenuated the severity of symptoms and delayed the course of disease progression (7). Finally, a synthetic KLK1 inhibitor was shown to suppress breast cancer cell invasiveness, suggesting that KLK activity could be targeted for anticancer therapies (8).

Regulation of KLKs–The KLK Activome

Regulation of KLK activity occurs at multiple levels that involve genomic aberrations (mutations, gene amplifications or rearrangements), transcriptional, post-transcriptional and/or post-translational mechanisms. More specifically, it was shown that multiple KLK genes exhibit copy number variations in ovarian tumours (9). Usage of alternative promoters was described for the synthesis of multiple transcripts by the KLK6 (10) and KLK11 (11) genes. Based on extended variations in their 5' untranslated regions, additional KLK genes may also be transcribed via multiple promoters. Single nucleotide polymorphisms (SNPs) correlated with differential KLK expression levels, while KLK3/PSA was identified as a candidate susceptibility gene for prostate cancer (12). In addition, it is well-established that transcription of KLKs in various tissues is under the control of steroid hormones (2) and vitamin D receptor signaling (13, 14). In addition, we and others have shown that DNA methylation and possibly other epigenetic mechanisms lead to silencing of certain KLK genes in cancer cells (2, 13). KLK activity is further regulated by the production of multiple alternatively spliced transcript variants that mostly encode for inactive KLK isoforms (15).

KLK proteins are synthesized as inactive prepro-forms that are proteolytically processed to secreted inactive pro-forms via the removal of their N-terminal secretion signal peptide. Subsequently, pro-KLKs are activated to mature peptidases by specific proteolytic removal of their N-terminal pro-peptide either via autocatalytic activity or by another KLK or by other endopeptidases. The term “KLK activome” was introduced to describe the serial activation of KLK zymogens by other mature KLKs (16). Our present understanding of activation profiles and the completed “KLK activome” is based on in vitro proteolytic cleavage of KLK propeptides and activation of recombinant pro-KLK proteins, and it is hypothesized to involve a complex network of activation events (autolytic, reciprocal cros activations, reverse activations), as depicted in Figure 1.

Following activation, mature KLK enzymes are amenable to inactivation by endogenous inhibitors as for example the kallistatin, which is a specific inhibitor of KLK1, and the LEKTI (Lymphoepithelial Kazal Type Inhibitor) inhibitor encoded by SPINK5 (Serine Protease Inhibitor Kazal-type 5). LEKTI is a secreted serpin that requires proteolytic cleavage for generation of bioactive LEKTI fragments that act as specific inhibitors of serine proteases, including certain KLKs (17). Inhibition of KLK activity by serine protease inhibitors (serpins) occurs through an irreversible suicide substrate mechanism which is referred to as the “inhibitory pathway”. Upon contact with the serpin, the KLK enzyme forms an initial Michaelis-like enzyme-inhibitor complex that involves the interaction of residues that flank the serpin’s scissile bond (P1-P1'). Upon nucleophilic attack of the active site Ser to the carbonyl backbone of the P1 residue, an acyl intermediate is formed. Subsequently, the reaction can proceed via two possible pathways: either the cleaved and, thus, inactive serpin is removed from the complex and active KLK is released (“non-inhibitory or substrate pathway”), or the acyl intermediate remains kinetically trapped as a stable covalent complex with the serpin (“inhibitory pathway”). KLK-inhibitor complexes have been identified in vivo, such as the KLK3-α2-macroglobulin and KLK3-α1-antichymotrypsin (2). In addition, autocatalytic inactivation via internal cleavage has been shown for KLK6 and other KLKs (2), while inactivation may also occur through internal cleavage by other proteases as demonstrated in vitro for the deactivation of KLK11 by plasmin (18). An interesting feature of these enzymes is that activation of some KLK zymogens by another KLK is followed by subsequent internal cleavage and inactivation by the same or a different mature KLK, as was
demonstrated for the serial activation of pro-
KLK3/PSA by KLK5, and its subsequent inactivation upon prolonged incubation (19). Zinc ions (Zn$^{2+}$) and pH are also very important reversible inhibitors of KLK enzymatic activities and are considered important regulators of KLK functions, as discussed below.

Structure and activity

A number of recent studies described the resolution of crystal structures for KLK1 (20), KLK3 (21), KLK4, 5, and 7 (reviewed in ref 22), KLK6 (23) and pro-KLK6 (24), which will facilitate the detailed description of their substrate specificity. Common structural features of KLKs were revealed, such as the two interacting β-barrels and α-helices bridged by the active site. Protein folding is facilitated by 5 or 6 disulphide bonds. Among KLKs, only KLK1, KLK2 and KLK3/PSA contain the characteristic kallikrein loop of 9-11 amino acid residues located prior to active site Asp, which confers specificity for kininogenase activity, namely the ability to release kinin from kinogen. Notably, KLK3/PSA is not able to cleave kininogen. Removal of the pro-peptide results in the formation of a salt-bridge between the α-ammonium group of Ile/Leu$^{16}$ and the carboxylate of Asp$^{194}$ side chain that is important for conformational rigidity of the active protease (22). An important characteristic of KLK3 is that two different conformations can be adapted by the 11 amino acid kallikrein loop, which can acquire either a closed or an open conformation that leads to either mature intact KLK3 with no enzymatic activity or enzymatically active KLK3, respectively. Conversion of inactive (closed) to active (open) KLK3 conformation can be achieved by high salt concentration or by monoclonal antibodies that capture and stabilize active KLK3, as modelled recently (21).

As mentioned, each KLK contains a signal peptide (pre) of 16 to 30 amino acid residues that is cleaved prior to secretion, leaving the pro-KLK form. For KLK4, an isoform that lacks the pre-peptide and localizes in the nucleus was identified in prostate cancer cells (25). Activation of pro-KLKs entails the removal of an N-terminal pro-peptide of 4-9 amino acids with the exception of KLK5 that carries a 37 amino acid long activation peptide. The cleavage site includes a P1 Arg or Lys, except for KLK4 that has Gln at P1 position. Accordingly, serine proteinases with trypsin-like activity are required for activation of all other pro-KLKs, while MMP20 was suggested as the endogenous activator of KLK4 based on in vitro proteolysis data (26). The activity of KLKs is trypsin-like (KLK1, 2, 4, 5, 6, 8, 12, 13, and 15), chymotrypsin-like (KLK3, 7, and 9) or mixed-type (KLK11 and 14). Phage display, combinatorial libraries and kinetic analyses were employed in order to characterize the substrate specificity of different KLKs. Paradoxically, KLK10/NES1 is considered to lack protease activity (16), which may be due Ser substitution of Gly$^{193}$ (chymotrypsin numbering) in KLK10/NES1. With very few exceptions, Gly$^{193}$ in the oxyanion hole is highly conserved in serine proteinases and its role is the stabilization of the oxyanion-intermediate during hydrolysis of the peptide bond. It should be noted, however, that using a library of small peptide substrates Debela et al. reported activity for KLK10 with ambivalent specificity (27). This controversy is presently not resolved due to the lack of the 3D structure and of known physiological substrates and could be due to a very restricted specificity of the KLK10/NES1 tumour suppressor. However, it is possible that KLK10/NES1 and probably other members of the KLK family exert biological roles independent of their serine protease activity, as demonstrated for KLK3/PSA that produces reactive oxygen radicals in prostate cancer cells independent of its proteolytic activity (28).

Regulatory Cascades and Functional Roles of KLKs

It is well-established that KLK1 can cleave low molecular weight kininogen (LMWK) to release kinin, which mediates signaling by a number of downstream targets. In addition, KLK1 can cleave pro-insulin, low-density lipoprotein, pro-renin, precursor of atrial natriuretic factor and other factors (29). Recently, it was shown that KLKs, especially KLK1, exert a protective role against lupus and anti-glomerular basement membrane-specific antibody-induced nephritis in mice and
humans (30). Furthermore, human systemic lupus erythematosus and spontaneous lupus nephritis were found to be associated with SNPs located on KLK1 and KLK3 promoters (30). The observation that KLK1−/− mice showed reduced ability for renal Ca2+ re-absorption, led to the hypothesis that KLK1 could be another physiologic regulator of Ca2+ homeostasis (31). Interestingly, KLK1−/− mice have normal blood pressure but they are characterized by cardiac and vascular abnormalities (32).

In vitro studies showed that KLK2, 4 and 12 are able to activate the pro-urokinase plasminogen activator (pro-uPA) to plasmin, which activates the uPA-uPAR (uPA receptor)-MMP proteolytic pathways known to be involved in the degradation and remodeling of ECM. The direct crosstalk between KLKs and MMPs is also indicated by the in vitro activation of pro-MMP2 and pro-MMP9 by KLK1 (2) and of pro-KLK4 by MMP20 protease that is important for amelogenesis, namely, the formation of tooth enamel (26).

Recent studies in vitro and in mice implicate KLK6 in inflammation of the central nervous system (CNS) and in multiple sclerosis (MS). Consistently, KLK6 is abundantly expressed at sites of demyelination in the EAE mouse model of MS, as well as in lesions detected in the brains of human patients (7). Efficient proteolytic cleavage of myelin basic protein in vitro supports a role of KLK6 in demyelination and/or remyelination (23). A potential role of KLK6 in the physiological degradation of α-synuclein and in the pathogenesis of Parkinson’s disease and other synucleinopathies was suggested based on findings with cultured cells showing that KLK6 degrades α-synuclein and co-localizes with pathological inclusions such as Lewy bodies and glial cytoplasmic inclusions (33). This was also shown by immunofluorescence visualization in sections of postmortem human brains and by co-immunoprecipitation experiments using extracts of mouse brains. Involvement of KLK6 in synucleinopathies is further sustained by an in vitro study showing that KLK6 is localized in mitochondria and, upon cellular stress, it is released into the cytoplasm, where limited proteolysis of α-synuclein by KLK6 activity yields fragmented α-synucleins that inhibit polymerization by reducing the amount of monomer, thus, preventing the formation of aggregates, a hallmark of these pathologies (33). On the other hand, KLK8−/− mice are predisposed to global seizures pointing to anti-epileptogenic activity of KLK8 (34). Importantly, KLK8−/− mice exhibit attenuated demyelination and oligodendrocyte death in EAE model (35). In addition, the proteolytic activities of KLK3, 6 and 13 in vitro were shown to produce angiotatin-like peptides with known antiangiogenic activity by limited proteolysis of plasminogen at specific internal sites (2).

Accumulating evidence suggests that KLKs are activators of protease-activated receptors (PARs), known members of the G-protein-coupled receptor superfamily that are activated by partial proteolytic cleavage of their extracellular domains (36). These data are corroborated by observations in a mouse model of Netherton syndrome (NS), where KLK5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression. Interestingly, uncontrolled KLK5 activity due to lack of KLK5 inhibition by LEKTI was shown to trigger a proinflammatary and proallergic microenvironment in NS epidermis independently of the environment and the adaptive immune system. This illustrates the crucial role of protease signaling in skin inflammation (37). Importantly, KLKs are known to cleave insulin-like growth factor binding proteins (IGFBPs) leading to increased availability of insulin-like growth factors (IGFs) that bind and activate their corresponding receptors and, in turn, can modulate cell survival, mitogenesis, and differentiation. In addition, KLK3 activates latent TGFβ by cleaving the TGFβ-binding protein. Activation of TGFβ in semen exerts an embryotrophic function, while it prevents growth of early-stage prostate tumours (2).

The large number of KLK genes, their co-ordinated regulation and tissue co-expression patterns led to the hypothesis that the encoded proteins could participate in proteolytic cascades, currently postulated to be involved in semen liquefaction, skin desquamation, neurodegeneration, and tumour-promoting or inhibiting effects (Figure 1). Because KLK5 can activate itself, as well as pro-KLK2, 3, 6, 7, 11, 12
and 14, KLK5 is considered the initiator of putative KLK cascades (19). As mentioned, evidence for the operation of KLK activation cascades mainly comes from in vitro proteolysis of recombinant pro-KLKS (2, 16, 18, 38). It should be noted that activation of KLK zymogens may also involve other proteases, such as specific MMPs and uPA (2).

It is well-established that KLK3/PSA is the physiological enzyme responsible for the resolution of the seminal clot by digestion of semenogelins I and II (SgI and SgII). However, KLK3 is secreted from the prostate as an inactive zymogen that requires activation. Recently, it was demonstrated by in vitro proteolysis that KLKs secreted in the prostatic fluid can participate in a hypothetical cascade that leads to activation of pro-KLK3. Since it was shown that KLK5 is able to autoactivate and also activate several other pro-KLKS (2, 3, 6, 7, 11, 12 and 14) it is speculated that KLK5 could be the key molecule for the initiation of the postulated prostate cascade. Importantly, prostatic fluid contains significantly elevated concentrations of Zn\(^{2+}\) (~2 mM) that were shown to block the enzymatic activities of most KLKs in vitro. During ejaculation, the prostatic fluid mixes with epididymal fluid that contains spermatozoa and with seminal vesicle fluid that contains SgI and SgII. The SgI and SgII along with fibronectin form the seminal clot that entraps spermatozoa. Redistribution of Zn\(^{2+}\) to SgI and SgII, which have high affinity for Zn\(^{2+}\), is expected to activate the KLK cascade that will eventually lead to activation of KLK3/PSA and digestion of the seminal clot. In prostate cancer, reduced concentrations of Zn\(^{2+}\) were measured in prostate lumen due to the established down-regulation of zinc transporter proteins. Presumably, low levels of Zn\(^{2+}\) cause activation of KLKs in the prostatic tissue and loss of prostate tissue architecture due to KLK-mediated degradation of the ECM (19, 39). Moreover, the prostatic KLK cascade may play significant role(s) in bone metastasis of prostate cancer. Interactions between tumour cells and bone cells (osteoblasts) are critical for the establishment of metastatic tumours associated with drug resistance and high mortality. In particular, KLK4 is considered to mediate bone metastasis, since in vitro experiments demonstrated that the enzymatic activity of KLK4 is required for increased migration of prostate cancer cells against osteoblast-secreted factors. Further, KLK4-expressing prostate cancer cells showed enhanced attachment on bone-matrix proteins (40). Also, KLK4 could promote prostate cancer metastasis by activating pro-KLK3 to mature KLK3/PSA and pro-uPA to uPA associated with invasion due to extensive degradation of ECM (2).

In skin, KLK activities are mainly regulated by LEKTI inhibitor in combination with changes in microenvironmental pH, as shown by in vitro studies (17) and in SPINK5\(^{-/-}\) mice, an established animal model of Netherton syndrome (NS). The NS is a severe form of ichthyosis (e. g. enhanced desquamation) caused by mutations in SPINK5 and lack of LEKTI inhibitor resulting in increased proteolytic activities of KLK5 and 7 (41). It has been demonstrated in vitro that LEKTI binding and inactivation of KLKs is reversed by a decrease in pH to the range 4.5 to 5.5 (17). It is known that the upper skin layer (stratum corneum) maintains a pH in this range. On the other hand, KLK5, 7 and 14 along with LEKTI are produced in the lower skin layer (stratum granulosum), where the pH is almost neutral. Based on these data, a hypothetical KLK cascade involved in skin desquamation has been proposed (38). It is postulated that KLK5 is the upstream initiator of this cascade. KLK5 displays enzymatic activity both in acidic pH (4.5-5.5), which is found in the stratum corneum due to “acid mantle”, as well as in neutral pH values of the stratum granulosum. It is assumed that KLK5 autoactivates in the stratum granulosum but its activity is quenched by immediate binding of LEKTI fragments. Dissociation of the KLK5-LEKTI complexes and release of active KLK5 enzyme occurs as it diffuses into the stratum corneum that maintains an acidic pH. Then, KLK5 activates KLK7 and 14, while active KLK14 augments KLK5 activity in a feedback loop as shown in vitro using recombinant enzymes (17). Active KLK5, 7 and 14 can digest the corneocyte binding proteins desmoglein, desmocollin and corneodesmosin that leads to skin desquamation (38). A detailed model of the skin desquamation cascade is presented in Figure 1.
Based on the fact that several KLKs (5, 6, 7, 8, 10, 11, 12, and 13) are present in human cervico-vaginal fluid at exceptionally high concentrations (range: 0.5-3 mg/l), it was hypothesized that KLK activities may participate in desquamation of vaginal epithelial cells, reminiscent of the skin desquamation process (42). In addition, KLKs could be involved in the proteolytic release and processing of antimicrobial peptides found in vaginal fluid. Indeed, it was shown in vitro that KLK5 can activate the α1-defensin precursor to its mature form (42). Thus, KLK5 proteolytic activity at an epithelial interface may be linked to mechanisms underlying the regulation of innate immunity defense. This hypothesis is supported further by the fact that, in human skin, KLK5 and 7 were shown to control the activation of cathelicidin precursor protein hCAP18 and also influence further processing to smaller peptides with increased antimicrobial activities (43). Nonetheless, it is known that cathelicidin peptides provide an important mechanism for prevention of infection against a wide variety of microbial pathogens (43). The importance of KLKs to antimicrobial activity in vivo is supported by the finding that epidermal extracts from SPINK5-/- mice display significantly increased antimicrobial activity that was shown to be due to KLK-mediated processing of cathelicidin (43).

Recent functional studies show that aberrant regulation of KLKs interferes with different stages of cancer growth and progression, including tumour growth, de-differentiation, angiogenesis and metastasis. It was shown that KLK10/NES1 acts as a tumour suppressor in breast (44) and gastric cancers (45). The mechanism(s) underlying the tumour suppressor function of KLK10/NES1 have not been described. Recently, it was shown that when KLK6 is expressed at physiological concentrations, it dramatically inhibits growth of primary breast tumours (46). Also, a number of studies implicate certain KLKs in epithelial-to-mesenchymal transitions (EMT) that represent a critical step in the process of increased motility, invasion and metastasis of tumour cells. In prostate cancer cells, expression of KLK3 and 4 results in loss of E-cadherin and induction of expression of the mesenchymal marker vimentin that represents a hallmark of EMT (47). Nonetheless, in transgenic mice over-expressing KLK6 it was shown that KLK6 is implicated in E-cadherin shedding in epidermal keratinocytes (48), while KLK7 was shown to directly induce E-cadherin shedding in vitro (49). Contrary, re-expression of KLK6 results in marked reduction of vimentin expression in metastatic breast tumour cells (46). Overall, existing evidence points to dual roles of KLKs in cancer, as also described for other proteases (reviewed in ref 50). The function of KLKs may vary in different tissues, tumour types and cancer stage. Interestingly, KLK function likely depends on subcellular localization, as shown for a KLK4 nuclear isoform, and on the concentration and/or activity levels. In this respect, it was found that the tumour protective effect(s) of KLK6 are restricted to normal concentrations of the protein, while marked over-expression of KLK6, also observed in a subset of breast tumours, seems to be associated with enhanced tumour growth (46) probably via PAR signaling, since this concentration range corresponds to KLK6 affinity for PAR2 (36). Tumour-associated constitutive over-expression of KLK6 was recently associated with hyperproliferation of cancer cells in NSCLC (non-small cell lung cancer). It was suggested that increased expression of KLK6 led to accelerated cell cycles, between the G1 and S phase, associated with decreased p21, increased cyclin E and enhanced synthesis of c-Myc (51). Consistent with KLK roles as cell cycle regulators, KLK4 was reported as a proliferative factor when over-expressed in prostate cancer cells where it was shown to affect the expression of cell cycle-related genes (25). Nonetheless, a recent study showed that in colon cancer cells, KLK6 is up-regulated via the K-RAS pathway and this increased expression of KLK6 was correlated with enhanced migration and invasion of tumour cells (52). On the other hand, it was shown recently that KLK6 can evoke intracellular Ca\textsuperscript{2+} flux via PAR1 signaling in cultured neurons and via PAR1 and PAR2 in cultured astrocytes. In addition, depending on the cell type, KLK6 promotes or inhibits AKT activation and also signals in a bradykinin 2 (B2) receptor-dependent manner to regulate CNS physiologic
function and dysfunction (53). However, these findings should be interpreted with caution, since the levels of active KLK6 used in these experiments were orders of magnitude higher than those found physiologically. Cumulatively, recent advances revealed interesting and unexpected roles of KLKs however, these are currently only partially characterized.

Conclusions and Outlook

Kallikrein-related peptidases represent a major proteolytic system operating in many tissues but its biological roles are still not well-defined. An increasing number of studies implicate aberrant regulation of KLKs in common human diseases and point to their clinical applicability as disease biomarkers but also as attractive targets for therapeutic intervention. In recent years, KLK serine proteases emerged as important players in the vast landscape of normal and disease-associated proteolysis. Several lines of data indicate that KLKs act individually and/or in complex networks or “KLK cascades” that may also involve crosstalk(s) with other serine or metalloproteases. It is currently known that KLKs activate signaling via the kallikrein-kinin system, protease-activated receptors, urokinase plasminogen activator and by processing of TGFβ and IGFBPs. Most of this data were derived in vitro and their significance in vivo awaits validation. Delineation of the complete “KLK activome” in vivo and identification of endogenous substrates of KLK enzymes will allow for the detailed description of the versatile functional roles of KLKs in physiological and pathological processes. In addition, the identification of potent specific and selective inhibitors of KLKs mainly through high-throughput screening platforms and substrate-guided design (54) will aid the development of novel KLK activity-modulating agents and the discovery of presently unidentified pathways mediated by KLKs in vivo. It should be mentioned that current therapeutic approaches include the development of synthetic inhibitors and “site-directed” strategies, such as the KLK-targeted activation of prodrugs and the cytoreductive and immunomodulatory gene therapy. In conclusion, the KLK field represents a largely unexploited area which will likely grow considerably over the next decade.

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Footnotes

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Keywords: human kallikrein-related peptidases, regulatory cascades, semen liquefaction, epidermal desquamation, neurodegeneration, cancer, epithelial-to-mesenchymal transition, biomarkers.

Figure Legend

Fig. 1. Regulatory KLK cascades in normal physiology and disease states. Proteolytic activities produced by zymogen activation via the “KLK activome” are involved in processes of skin desquamation, innate immunity, hypertension, semen liquefaction, neurodegeneration, and tumour-promoting or inhibiting effects. Notably, certain KLKs exert pleiotropic functions by activating molecules involved in multiple processes, e.g. cathelicidin that is involved in skin desquamation and innate immunity. It should be noted that only those KLKs that have been shown to activate recombinant pro-KLKs were included in the depicted “KLK activome”.
Desquamation
KLK5, 7 and 14 digestion of cell adhesion molecules

Activation of cascade by pH gradient

pH=7
Inhibition of cascade

Defensin-1 alpha (active)
LL-37
KLK5
KLK7
KLK14
Desquamation

KLK3, 7 and 14 digestion of cell adhesion molecules

Activation of cascade

pH=4-6
Activation of cascade

KLK5
KLK14
corneocytes
keratinocytes

Anti-tumor effect
Release of angiostatin peptides
Activation of PARs
Latent TGFβ
Decline of ECM
Induction of apoptosis
Invasion and metastasis

Activation of PARs
Release of IGFs
Digestion of ECM
Activation of TGFβ
Release of angiostatin peptides

KLK3 digestion of Sgi1 and Sgi2
Semen liquefaction cascade
Activation by Zn²⁺ redistribution to Sgs

KLK3, 7 and 14 digestion of cell adhesion molecules

Activation of cascade

pH=7
Inhibition of cascade

Defensin-1 alpha (active)
LL-37
Smaller antimicrobial peptides
Defensin-1 alpha (active)

Further processing
Smaller antimicrobial peptides

KLK ACTIVOME

KLK3 digestion of Sgi1 and Sgi2
Semen liquefaction cascade
Activation by Zn²⁺ redistribution to Sgs

Parkinson’s inhibition of α-synuclein aggregation
LeWy body

Multiple sclerosis
epitope spreading
MBP
Axon
Oligodendrocyte B1 (2) receptor
KLK1
BK
kininogen

KLK6
KLK4
KLK5
KLK 13
KLK14
proKLK5
KLK5
Auto proKLK3
proKLK14
KLK14
proKLK2
KLK2
Auto proKLK1
proKLK7
KLK7
Auto proKLK11
proKLK11
KLK15
proKLK4
KLK4
proKLK6
KLK6
Auto proKLK12
KLK12
Auto

Induction of apoptosis
Invasion and metastasis

All KLKs

KLK ACTIVOME

KLK3 digestion of Sgi1 and Sgi2
Semen liquefaction cascade
Activation by Zn²⁺ redistribution to Sgs

Parkinson’s inhibition of α-synuclein aggregation
LeWy body

Multiple sclerosis
epitope spreading
MBP
Axon
Oligodendrocyte B1 (2) receptor
KLK1
BK
kininogen

KLK6
KLK4
KLK5
KLK 13
KLK14
proKLK5
KLK5
Auto proKLK3
proKLK14
KLK14
proKLK2
KLK2
Auto proKLK1
proKLK7
KLK7
Auto proKLK11
proKLK11
KLK15
proKLK4
KLK4
proKLK6
KLK6
Auto proKLK12
KLK12
Auto
Functional roles of human kallikrein-related peptidases
Georgia Sotiropoulou, Georgios Pampalakis and Eleftherios P. Diamandis

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