Review Article

Tissue kallikreins: new players in normal and abnormal cell growth?

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Summary

Serine proteases are proteolytic enzymes with an active serine residue in their catalytic site. Kallikreins are a subgroup of the serine protease family and are known to have diverse physiological functions. The human tissue kallikrein gene family has now been fully characterized and includes 15 members, clustered in a 300 kb region on chromosome 19q13.4. In this review, we discuss the common structural features of kallikreins at the DNA, mRNA and protein levels. Kallikreins are secreted as inactive zymogens and are activated by cleavage of an N-terminal peptide. Some kallikreins can undergo autoactivation while others may be activated by other kallikreins or other proteases. Most kallikreins are predicted to have trypsin-like enzymatic activity except for three members which may have chymotrypsin-like activity. Circumstantial evidence suggests that at least some kallikreins may be part of an enzymatic cascade pathway which is activated in aggressive forms of ovarian and probably other cancers. Accumulating evidence suggests potential diagnostic and/or prognostic roles of kallikreins in diverse malignancies. In addition to PSA, many other kallikreins show differential expression in malignancy. For example, hK6, 10 and 11 are promising serological markers for ovarian cancer diagnosis. KLK10 may act as a tumor suppressor. In addition to their diagnostic and prognostic values, kallikreins may also be good therapeutic targets.

Keywords

Kallikreins, tumor markers, prostate cancer, breast cancer, ovarian cancer, serine proteases, prognostic and predictive markers.

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Serine proteases

Proteases are enzymes that cleave proteins by the catalysis of peptide bond hydrolysis. Based on their catalytic mechanisms, they can be classified into 5 main classes: cysteine, aspartate, threonine, serine and metalloproteases. According to the widely used and most comprehensive database of proteases (MEROPS), enzymes of each catalytic type are classified into evolutionarily distinct "clans" and each clan is subdivided into "families" based on sequence homology and order of the catalytic triad (1).

Serine proteases (SP) are a family of enzymes that utilize a uniquely activated serine residue in the substrate-binding pocket to catalytically hydrolyze peptide bonds (2). SP carry out a diverse array of physiological functions, the best known being digestion, blood clotting, fertilization and complement activation (3). They have also been shown to be related to many diseases including cancer, arthritis and emphysema (4-6).

The protein activation catalyzed by serine proteases is an example of "limited proteolysis" because peptide hydrolysis is limited to only one or two particular peptide bonds of the hundreds of peptide bonds in a protein substrate (2). Details of the catalytic procedure have been previously reviewed (7). Serine proteases exhibit preference for hydrolysis of peptide bonds adjacent to a particular class of amino acids. In the trypsin-like group, the protease cleaves peptide bonds following basic amino acids such as arginine or lysine, since it has an aspartate (or glutamate) in the substrate-binding pocket which can form a strong electrostatic bond with these residues. The chymotryp-

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sin-like proteases have a non-polar substrate-binding pocket and thus require an aromatic or bulky non-polar amino acid such as tryptophan, phenylalanine, tyrosine or leucine. On the other hand, the elastase-like enzymes, have bulky amino acids (valine or threonine) in their binding pockets, thus requiring small hydrophobic residues, such as alanine (8).

The human tissue kallikrein family of serine proteases

Kallikreins were named so because they were originally isolated from the pancreas (in Greek, the "kallikreas"). Traditionally, kallikreins were defined as enzymes which can release vasoactive peptides from high molecular weight precursors. In humans and most other species, only one kallikrein, KLK1, fulfills this criterion. More recently, a new structural concept has emerged to describe kallikreins. The concept of a "kallikrein multigene family" was first introduced for mice to refer to these genes (9). This definition was not so much based on the enzymatic function, rather on sequence homology and close chromosomal location.

In humans, there are two classes of kallikreins; plasma kallikrein and tissue kallikreins. The plasma kallikrein is encoded by a 15-exon single gene on chromosome 4q35. This enzyme (a serine protease) releases the vasoactive peptide bradykinin from a high molecular weight precursor synthesized in the liver (10). The human tissue kallikreins are a family of genes localized on chromosome 19, and also encode for serine protease enzymes. The human tissue kallikrein gene family has now been fully characterized with identification of all its 15 members (11). The kallikrein locus organization on chromosome 19q13.4 has been elucidated (12-14) and uniform nomenclature has been established (15). This review is focused on the human tissue kallikrein gene family.

Structure of the human tissue kallikrein genes and proteins

The human kallikrein gene locus on chromosome 19q13.4 consist of a cluster of 15 kallikreins, with no intervening non-kallikrein genes. The average genomic length of kallikrein genes is 5 kb, with most of the size differences attributed to intron lengths. Several structural features are conserved among all kallikreins. All genes are formed of 5 coding exons and most of them have one or more extra 5' untranslated exons. The first coding exon always contains a 5' untranslated region followed by the methionine start codon; the stop codon is located ~156 bp from the beginning of the last coding exon. The intron phases, defined as the position where the intron starts in relation to the last codon of the previous exon, have a conserved pattern of I-II-I-0. The positions of the residues of the catalytic triad of serine proteases are conserved, with the histidine always occurring near the end of the second coding exon, the aspartate in the middle of the third exon and the serine residue at the beginning of the last exon. Kallikrein proteins are synthesized as a pre/pro peptides with a signal peptide of about 17-20 amino acids at the amino terminus, followed by an activation peptide of about 4-9 amino acids (with the exception of hK5 which has a longer peptide), followed by the mature (enzymatically active) protein. The substrate specificity of the kallikrein enzymes is predicted to be mainly trypsin-like (in 12 out of the 15 kallikreins), as indicated by the presence of aspartate or glutamate in the substrate-binding pocket. The specificity is chymotrypsin-like in hK3, hK7 and probably hK9. All proteins contain 10-12 cysteine residues, that will form 5 (in hK1-3 and hK13) or 6 (in the rest) disulphide bonds. The positions of the cysteine residues are also fully conserved. Classical or variant polyadenylation signals have been predicted 10-20 bases away from the poly A tail of all kallikreins. Multiple alignment of kallikrein proteins is shown in Figure 1. For more detailed discussion about the structural aspects of kallikreins, see recent reviews (11, 12, 16).

Three-dimensional structure

In contrast to rodent kallikreins, where the crystal structure has been elucidated for some proteins, hK1 and hK6 are the only human kallikreins whose crystal structure has been determined (17, 18). Most of the discussion in this section is derived from comparative model building of the human kallikrein proteins.

Kallikreins can be roughly divided into two categories, the "classical" kallikreins (hK1-3) and the "new" kallikreins. The new kallikreins appear to be unique in their three-dimensional structure, sharing some features with trypsins and others with the classical kallikreins. Comparative protein models show that the pattern of hydrophobic side chain packing in the protein core is nearly identical in all human kallikreins and the observed differences occur within the solvent-exposed loop segments.

An 11-amino acid residue insertion relative to the trypsin sequences (residues 91-103 in the bovine chymotrypsinogen consensus numbering), known also as "the kallikrein loop", is a unique feature for the three classical kallikreins. This loop is located between the fifth (residues 81-90) and the sixth β-strand (residues 104-108). None of the new human kallikreins contains this loop in its entirety. The hK10 loop is longest, with an 8-residue long insertion relative to the trypsin sequences. It overhangs the substrate-binding groove on the surface of the protease molecule and its length and sequence can directly influence substrate recognition.

The KLK15 gene is particularly interesting, as it lies between two classical glandular kallikrein genes, KLK1 and KLK3 (19), yet the sequence and structure of hK15, with six disulphide bonds and no insertion in the so-called kallikrein loop, clearly place it among the new kallikreins. Moreover, hK15 has an 8-residue insertion (19) that is not found in any other kallikrein. In the three-dimensional structure, the extended loop lies on the opposite side of the active site relative to the kallikrein loop and, although it is more distant to the substrate-

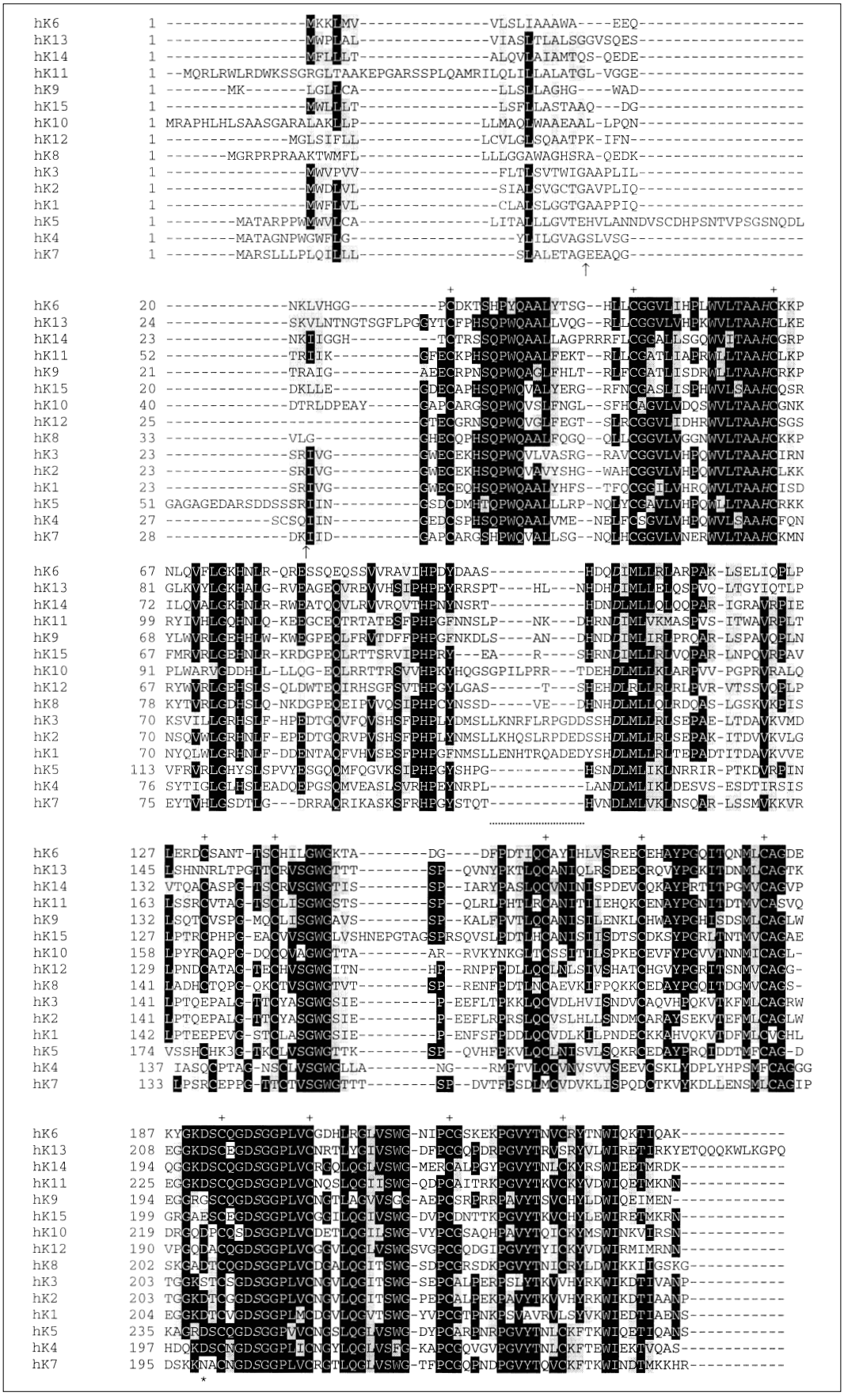

Figure 1: Alignment of the deduced amino acid sequence of the 15 kallikrein proteins. Dashes represent gaps to better align the sequences. The amino acids of the catalytic triad (H, D, S) are shown in italics. Identical amino acids are highlighted in black and similar residues in grey. The site of cleavage of the "pre" and "pro" peptides are indicated by arrows. The cysteine residues are marked by (+) from above. The dotted area represents the kallikrein loop sequence. The asterisk denotes the position of the amino acid of the binding pocket which is crucial for substrate specificity (for trypsin-like enzymes the amino acid is D). For more details, see text.

binding groove, it may also participate in substrate and inhibitor recognition.

Enzymatic activity and substrate-specificity of kallikreins

From the functional point of view, kallikreins are serine proteases. Proteolytic activity of some kallikrein proteins has already been experimentally proven (e.g. hK2-5, and hK7) (20-22). Although experimental evidence is lacking for the rest of them, structural analyses indicate that kallikreins have all the essential criteria to be classified as serine proteases. Phylogenetic analysis also supports the grouping of the kallikrein proteins among the serine protease family of enzymes.

Hydrolysis of peptide bonds starts by the oxygen atom of the hydroxyl group of the serine residue which attacks the carbonyl carbon atom of the susceptible peptide bond. At the same time, the serine transfers a proton to the histidine residue of the catalytic triad, then to the nitrogen atom of the susceptible peptide bond which is then cleaved and released. The other part of the substrate is now covalently bound to the serine by an ester bond. The charge that develops at this stage is partially neutralized by the third (asparate) residue of the catalytic triad. This process is followed by "deacylation" where the histidine draws a proton away from a water molecule and the hydroxyl ion attacks the carbonyl carbon atom of the acyl group that was attached to the serine. The histidine then donates a proton to the oxygen atom of the serine, which will then release the acid component of the substrate.

In addition to the release of vasoactive peptides (as is the case with hK1), kallikreins can also cleave other important molecules, such as growth factors and hormones. Another recently investigated mechanism of action of serine proteases is the activation of the proteinase-activated receptors (PAR). PAR is a novel family of G-protein-coupled receptors which is stimulated by cleavage of their N-termini by a serine protease rather than by ligand-receptor occupancy (23). Activation of these receptors elicits different responses in several tissues. In addition, they switch-on cell-signaling pathways, e.g. the MAP-kinase pathway, leading to cell growth and division (23). The ability of kallikreins to activate PAR needs to be investigated.

Substrate specificity has been experimentally confirmed for some kallikreins. hK3 (PSA) has been shown to have restricted chymotryptic-like activity (24). PSA cleaves lysozyme, insulin and seminogelin I on the carboxy terminal side of certain leucines, tyrosines and phenylalanines (25). hK2 cleaves substrates following single or double arginine residues, confirming its trypsin-like activity (26). hK7 has a chymotrypsin-like primary substrate specificity (27). More recently, hK4 was found to have trypsin-like activity (28). hK15 has a glutamic acid in the substrate binding pocket and showed significant activity against substrates which have an Arg-$_p$NA cleavage site, suggesting a trypsin-like activity (29). further, hK11 has preference to cleave substrates after an arginine residue (30). Multiple alignment of the deduced protein sequences of all 15 kallikreins predicted that 12 out of the 15 kallikreins will have a trypsin-like substrate specificity (as indicated by the presence of an aspartate or glutamate residue in the substrate binding pocket) (11, 12). Two important points are worth mentioning: (1) the activity of a specific protease for a certain type of amino acid only indicates its preference. It might still be able to cleave other substrates, although at a much slower rate. (2) the secondary interaction, outside of $S_1 - S'_1$ region plays an important role in determining the substrate specificity. The differences in substrate specificity of kallikreins have been shown to be dependent on the amino acids located at positions P3-P8 on the C-terminal site of the cleavage site (31). This also explains the inability of hK4 to cleave certain substrates despite having the essential arginine site (28).

Regulation of kallikrein activity

There are different mechanisms for controlling serine protease activity in order to avoid unwanted effects and to allow for spatial and temporal regulation of the proteolytic activity. One mechanism is by synthesizing kallikreins in an inactive "pre-proenzyme" form which will be activated when necessary. The N-terminal extension of the mature enzyme, or the "prosegment" sterically blocks the active site and thus prevents binding of substrates. It is also possibly implicated in folding, stability and intracellular sorting of the zymogen. For more detailed discussion see the recent review by Khan and James (32). All kallikreins are predicted to be synthesized as pre-proenzymes with the N-terminal end formed of the signal peptide followed by the activation peptide. The activation of the zymogen (also called the pro-enzyme) can occur intracellularly, i.e., in the trans-Golgi or the secretory granules, or extracellularly after secretion, and it can be autolytic or dependent on the activity of another enzyme (see below). Interestingly, all of the "proforms" of the kallikrein enzymes, with the exception of hK4, are predicted to be activated by cleavage at the carboxy terminal end of either arginine or lysine (the preferred trypsin cleavage site), indicating that they will need an enzyme with trypsin-like activity for their activation. This observation has been experimentally proven for some kallikreins. hK5 and hK7 can be converted to the active enzyme by trypsin treatment (20, 21) and hK11 can be activated by entrokinase (30). Autoactivation has also been reported among kallikreins. hK2, but not hK3, is capable of autoactivation (33). hK4 is also autoactivated during the refolding process (29) and hK6 is also capable of autoactivation (34). hK13 is also autoactivated upon secretion (G. Sotiropoulou, personal communication).

Proteolytic activation is irreversible. Hence, other means of switching off the activity of these enzymes are needed. One way is the binding of kallikreins to serine protease inhibitors. These are usually poor substrates with strong inhibition and require

hydrolysis of a peptide bond in the inhibitor by the protease. hK3 has been shown to form complexes with many extracellular protease inhibitors such as α_1-antichymotrypsin, α_2-macroglobulin and α1-antitrypsin (35, 36) and hK2 was found to be bound to α_2-antiplasmin, antithrombin III, plasminogen activator inhibitor-1, and α_2 macroglobulin (37). Another mechanism for controlling the activity is by internal cleavage and subsequent degradation. Self-digestion is reported for hK7 (21). Around 30% of hK3 in seminal plasma is inactivated by internal cleavage between lysine 145 and lysine 146 (38), and about 25% of hK2 was found to be internally cleaved between amino acids 145-146 (Arg-Ser) (39).

Interaction between kallikreins

Interactions between serine proteases are common, and substrates of serine proteases are usually other serine proteases that are activated from an inactive precursor (2). The involvement of serine proteases in cascade pathways is well documented. One important example is the blood coagulation cascade. Blood clots are formed by a series of zymogen activations. In this enzymatic cascade, the activated form of one factor catalyzes the activation of the next factor. Very small amounts of the initial factors are sufficient to trigger the cascade because of the catalytic nature of the process. These numerous steps yield a large amplification, thus ensuring a *rapid* and *amplified* response to trauma (40). Signal amplification is an important characteristic of cascades. The cross talk between kallikreins has been recently hypothesized (16, 41) and is supported by circumstantial evidence, including the co-expression of many kallikreins in the same tissue, the ability of some kallikreins to activate each other and the common patterns of hormonal regulation by steroids. Added to this is the parallel pattern of different expression of many kallikreins in diverse malignancies (e.g., the following kallikrein genes are up-regulated in ovarian cancer: KLK4, 5, 6, 7, 8, 10, 11 and the following kallikreins are down-regulated in breast cancer: KLK2, 3, 6, 10, 12, 13, 14).

Recent experimental evidence has shown that hK3 (PSA) can be activated by hK15 (29). hK4 have also recently been shown to activate hK3 much more efficiently compared to hK2 (28). hK5 is predicted to be able to activate hK7 in the skin (20). The activation of hK3 by hK2 is also possible. While Takayama et al., reported that hK2 can activate hK3 (42), Denmeseade et al., reported the opposite (33) and hypothesized that additional proteases may be required, further supporting the proposed hypothesis. It will be interesting to study all possible combinations of interactions among kallikreins, especially those with expression in the same tissues. Bhoola et al. have recently provided strong evidence of the involvement of a "kallikrein cascade" in initiating and maintaining systemic inflammatory responses and immune-modulated disorders (43).

Physiological functions of kallikreins

Little is known about the physiological functions of kallikreins in normal tissues. However, accumulating evidence shows that kallikreins might have diverse functions, depending on the tissue and circumstances of expression. hK1 exerts its biological activity mainly through the release of lysyl-bradykinin (kallidin). It cleaves low-molecular-weight kininogen to produce vasoactive kinin peptides. Intact kinin binds to bradykinin B_2 receptor in target tissues and exerts a broad spectrum of biological effects including vasodilation, blood pressure reduction, smooth muscle relaxation and contraction, pain induction, and inflammation (44). Low renal synthesis and urinary excretion of tissue kallikrein have been repeatedly linked to hypertension in animals and humans (45). It has been also reported that kallikrein cleaves kininogen substrate to produce vasoactive kinin peptides that have been implicated in the proliferation of vascular smooth muscle cells. Abnormality of the tissue kallikrein-kinin system has been implicated in the pathogenesis of hypertension and cardiovascular and renal disorders (46). An hK1 knockout mouse has been recently generated and it was found that mice lacking tissue kallikrein are unable to generate significant levels of kinins in most tissues and develop cardiovascular abnormalities early in adulthood despite normal blood pressure (45).

However, the diverse expression pattern of hK1 has led to the suggestion that the functional role of this enzyme may be specific to different cell types (44). Apart from its kininogenase activity, tissue kallikrein has been implicated in the processing of growth factors and peptide hormones in light of its presence in pituitary, pancreas and other tissues. As summarized by Bhoola et al. (44), hK1 has been shown to cleave pro-insulin, low density lipoprotein, the precursor of atrial natriuretic factor, prorenin, vasoactive intestinal peptide, pro-collagenase and angiotensinogen.

hK2 has been reported to be able to activate the pro-form of PSA (39, 47). Seminal plasma hK2 was found to be able to cleave semenogelin I and semenogelin II but at different cleavage sites and with lower efficiency than PSA (48). Since the amount of hK2 in seminal plasma is much lower than PSA (1 – 5%), the contribution of hK2 in the process of seminal clot liquefaction is expected to be relatively small (22).

Since PSA is present at very high levels in seminal plasma, most studies focused on its biological activity within this fluid. Lilja has shown that PSA rapidly hydrolyzes semenogelin I and semenogelin II, as well as fibronectin, resulting in liquefaction of the seminal plasma clot after ejaculation (49). Several other potential substrates for PSA have been identified, including IGFBP-3, TGF-β, parathyroid hormone-related peptide and plasminogen (50). The physiological relevance of these findings is still not clear.

The mouse and porcine orthologues of hK4 were originally designated "enamel matrix serine protease" because of their

predicted role in the normal teeth development (51). The human KLK4, however, was shown to be highly expressed in the prostate, pointing out to the possibility of having a different function in humans. hK7, and more recently hK5, were found to be highly expressed in the skin, and it is believed that they are involved in skin keratinization and desquamation (52). hK6, hK8 and hK11 are highly expressed in the central nervous system where they are thought to be involved in neural plasticity.

Differential expression of kallikreins in malignancy

The relation between kallikreins and cancer is well established. PSA (hK3) is a valuable marker for prostatic diseases. More recently, human glandular kallikrein (hK2) is being investigated for the same application. A more detailed discussion about hK2 and hK3 as cancer biomarkers can be found elsewhere (22). In addition to hK3 being a marker for prostate cancer diagnosis and monitoring, recent reports suggest the usefulness of hK3 as a marker for breast cancer prognosis (53).

In recent years, reports indicate that other kallikreins might be also related to hormonal malignancies (for instance, breast, prostate, testicular and ovarian cancers). KLK6 (zyme/protease M) was originally isolated by differential display from an ovarian cancer library (54) and hK10 (NES1) was cloned by subtractive hybridization from a breast cancer library (55), and later shown to act as a tumor suppressor (56). hK6 and hK10 are emerging serum diagnostic markers for ovarian cancer (57-60). More recently, hK11 was also shown to be a potential marker for ovarian and prostate cancer (61).Underwood et al. (62) and Magklara et al. (63) have shown that KLK8 (also known as neuropsin, TADG14) is differentially expressed in ovarian cancer. KLK7 is up-regulated in ovarian cancer patients (64), and KLK4 and KLK5 are indicators of poor prognosis of ovarian cancer (65-67). More recently, KLK9 has been shown to be a marker of favorable ovarian cancer prognosis (68). Preliminary reports indicate that hK1 is present in tumors of the breast, lung, stomach, pituitary and uterus (69). A synthetic hK1 inhibitor was recently found to suppress cancer cell invasiveness in human breast cancer cell lines (70).

In Table 1, we summarize published data on measurement of kallikrein genes and proteins in tumor tissue extracts and serum of cancer patients for the purpose of disease diagnosis, monitoring, prognosis or subclassification.

The possible mechanisms of kallikrein involvement in cancer

Several mechanisms can be proposed by which kallikreins could be involved in the pathogenesis of endocrine-related malignancies. Proteolytic enzymes are thought to be involved in tumor progression because of their role in extracellular matrix degradation. Many studies have shown that a variety of proteolytic enzymes are overproduced either by the cancer cells themselves or by the surrounding stromal cells. This overexpression is usually associated with unfavorable clinical prognosis.

Breast, prostate, testicular and ovarian cancers are "hormonal" malignancies. Sex hormones are known to affect the initiation, and/or progression of these malignancies. On the other hand, all kallikreins are under sex steroid hormonal regulation (16, 71-73). Taken together, kallikreins may represent downstream targets by which hormones affect the initiation or progression of such tumors.

Experimental evidence suggests that hK2 and hK4 can activate the pro-form of another serine protease, the urokinase plasminogen activator (uPA) (28, 42). Urokinase activates plasmin (another serine protease) from its inactive form (plasminogen) which is ubiquitously located in the extracellular space, leading to degradation of the extracellular matrix proteins. This might suggest one way of a role of kallikreins in cancer progression and explains the differential expression of several kallikreins in tumors. Plasminogen can also activate the precursor forms of collagenases, thus promoting the degeneration of collagen in the basement membrane surrounding the capillaries and lymph nodes. Another kallikrein, hK7, can degrade the alpha chain of native human fibrinogen (74) and it is also hgypothesized that it is involved in an apoptotic-like mechanism that leads to desquamation of the skin (74). The involvement in growth/apoptotic activities is reported for hK3, which can activate insulin-like growth factor-binding protein (IGFBP-3) (75), and also inactivate by cleavage the amino terminal fragment parathyroid hormone-related protein (PTHrP) (76). Similar findings were observed for some rodent kallikreins (77). Given the parallel expression of kallikreins in the same malignancy, it is possible to hypothesize that kallikreins are involved in a cascade-like reaction which leads to switching on or off of key processes in tumor progression. Another possible mechanism for the involvement of kallikreins in malignancy is the activation of proteinase-activated receptors (PAR), as discussed above.

Bhoola et al. (43), have recently provided strong evidence indicating the presence of hK1 activity in the chemotactically-attracted inflammatory cells of esophageal and renal cancers, suggesting a role for kallikreins in the reaction towards malignancy. Modulation of angiogenic activity is another possible mechanism for kallikrein involvement in cancer. The kinin family of vasoactive peptides, activated by hK1, is believed to regulate the angiogenic process (78). It was recently reported tat immunolabelling for hK1 was intense in the angiogenic endothelial cells derived from mature corpora lutea. Immunoreactivity was lower in non-angiogenic endothelial cells and least in angiogenic endothelial cultures of the regressing corpus luteum (78). Also, hK3 was reported to have anti-angiogenic activities (77).

The elevation of serum concentration of kallikreins in cancer might be due to increased vasculature (angiogenesis) of cancerous tissues and/or the destruction of the glandular architec-

Table 1: The role of kallikreins in cancer diagnosis/prognosis1.

Kallikrein	Sample type	Application	Reference
hK2	Serum and tissue	Diagnosis, prognosis and monitoring of prostate and breast cancer	(79)
hK3 (PSA)	Serum and tissue	Diagnosis, prognosis and monitoring of prostate and breast cancer	(22)
KLK4	Ovarian cancer tissue	Unfavorable prognostic marker	(65)
KLK5	Ovarian cancer tissue	Unfavorable prognostic marker	(66)
	Breast tumor cytosols	Unfavorable prognostic marker	(80)
	Normal and prostate cancer tissues	Lower expression in more aggressive tumors	(81)
	Normal and testicular cancer tissues	Down-regulation in advanced cancer	(82)
hK6	Serum	Diagnosis, prognosis and monitoring of ovarian cancer	(57, 83)
	Breast tumor cytosols	Prognosis: association with hormone receptors	our unpublished data
KLK6	Ovarian cancer mRNA	Overexpression in ovarian cancer	(84)
KLK7	Ovarian cancer mRNA	Overexpression in ovarian cancer	(64)
KLK8	Ovarian cancer mRNA	Marker of favorable prognosis	(63)
	Ovarian cancer mRNA	Higher expression in ovarian cancer	(62)
KLK9	Ovarian cancer mRNA	Marker of favorable prognosis	(68)
KLK9	breast cancer mRNA		(85)
hK10	Serum	Diagnosis and monitoring of ovarian cancer	(59, 60)
	Ovarian cancer cytosols	Prognosis; high levels associated decreased survival	Our unpublished data
hK11	Serum	Diagnosis and prognosis of ovarian cancer	(61)
KLK12	Breast cancer mRNA	down-regulation in breast cancer	(86)
KLK13	Breast cancer mRNA	down-regulation in a subset of breast tumors	(87)
KLK14	Ovarian cancer mRNA	Marker of favorable prognosis	(73)
	Breast cancer mRNA	Down-regulated in breast cancer	(88)
	Normal and testicular cancer mRNA pairs	Down-regulation in cancerous tissue	(88)
	Normal and testicular cancer mRNA pairs	Overexpression in prostate cancer	Our unpublished data
KLK15	Ovarian cancer mRNA	Marker of poor prognosis	Our unpublished data
	Breast cancer mRNA	Marker of favorable prognosis	(85)
	Matched tissue from normal and cancerous tissues	Marker of favorable prognosis	Our unpublished data

1. hK, kallikrein protein; KLK, kallikrein gene

ture of the tissues involved and the subsequent leakage of these proteins into the general circulation. It is possible that the concentration of kallikreins may be increased in serum, due to gene overexpression, as well as to increased diffusion of these molecules into the general circulation.

Therapeutic applications

It is possible that some kallikreins may become valuable therapeutic targets when the biological pathways that are involved are delineated. For example, the enzymatic activity of these serine proteases may initiate (e.g. tumor invasion, activation of hormones, growth factors, other enzymes, receptors or cytokines, amyloid formation) or terminate biological events (e.g. inhibition of angiogenesis, inactivation of growth factors, hormones, enzymes, cytokines or receptors). Once known, these events could be manipulated, for therapeutic purposes, by specific enzyme inhibitors or activators. Another potential therapeutic approach is the cell-specific activation of therapeutic agents. Preliminary reports show potential success by using the PSA promoter to express molecules in a tissue-specific fashion. A third possible therapeutic approach involves immunotherapy and/or development of cancer vaccines. With our increasing knowledge of the hormonal regulation of kallikreins, hormonal activation (or repression) of kallikrein activity could be investigated in the future.

Conclusions

The knowledge on human kallikrein genes is evolving rapidly. Now that the structure of these genes is well-characterized, it will be interesting to study the physiological function of the proteins and their possible connection to pathological processes. Also, interesting is the recent association of kallikrein gene expression with cancer and other diseases and the finding that many circulating kallikreins are biomarkers for cancer. The examination of tissue kallikreins as therapeutic targets (through activation or inhibition) may also be important in selected cases. Clearly, over the next 3-5 years, the physiology and pathobiology of this large family of serine proteases will be more precisely defined.

Non-standard abbreviations

KLK, human kallikrein gene; hK, human kallikrein protein; SP, serine proteases; PSA, prostate specific antigen.

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