THE EMERGING ROLES OF HUMAN TISSUE KALLIKREINS IN CANCER

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Abstract | Human tissue kallikreins (hKs), which are encoded by the largest contiguous cluster of protease genes in the human genome, are secreted serine proteases with diverse expression patterns and physiological roles. Although primarily known for their clinical applicability as cancer biomarkers, recent evidence implicates hKs in many cancer-related processes, including cell-growth regulation, angiogenesis, invasion and metastasis. They have been shown to promote or inhibit neoplastic progression, acting individually and/or in cascades with other hKs and proteases, and might represent attractive targets for therapeutic intervention.

SERINE PROTEASES
One of the four mechanistic classes of proteases — enzymes that catalyse the hydrolysis of peptide bonds. They are characterized by a catalytic mechanism by which the hydroxyl group of the active-site serine residue acts as the nucleophile that attacks the peptide bond.

The contribution of extracellular proteolysis to tumour invasion and metastasis, through extracellular-matrix (ECM) degradation, has been recognized for decades. However, this ECM-degrading role for extracellular proteases has substantially evolved and expanded over the past 5 years, with the realization that extracellular matrices are not just simple barriers against tumour invasion. It is now evident that the tumour microenvironment, including the ECM and stromal cells, acts as a crucial modulator of tumour-cell behaviour[1]. The ECM possesses its own inherent structural information and is a reservoir of growth factors and signalling molecules. So, proteases of the extracellular milieu directly regulate and modify the tumour microenvironment and indirectly influence tumour-cell growth or apoptosis, angiogenesis, and invasion and metastasis through irreversible cleavage of ECM and non-ECM components. Such cleavage could expose cryptic binding sites within ECM molecules and thereby alter cell–cell and cell–ECM interactions; generate biologically active ECM fragments; and affect the bioavailability and activity of sequestered growth factors and growth-factor receptors. Consequently, cancer-associated proteolysis in the extracellular space is far more complex than initially anticipated, and ultimately results in tumour-promoting and/or tumour-suppressive effects.

Among the ~500–600 proteases comprising the human degradome[2], only a subset have taken centre stage in extracellular proteolysis during cancer progression[3]. The current dogma asserts that a pericellular cascade initiated by the SERINE-PROTEASE system of urokinase plasminogen activator (uPA), uPA receptor (uPAR) and plasminogen, which results in the activation of latent matrix metalloproteinases (MMPs), is mainly responsible for extracellular proteolysis in cancer. However, this proteolytic network is becoming increasingly complex and many other players have been implicated in modulating the tumour microenvironment, including the tissue-kallikrein family of serine proteases.

Tissue kallikreins have only recently arrived on the cancer proteolysis scene. Numerous experimental results indicate that kallikrein expression and proteolytic activity are dysregulated in tumours, mainly adenocarcinomas, and often associated with patient prognosis. So far, most studies have focussed on the clinical value of kallikreins as serological or tissue tumour markers; for example, hK3 — also known as prostate-specific antigen (PSA) — is used as a marker for prostate cancer. However, emerging data also indicate that kallikreins might be directly involved in neoplastic progression. Kallikreins might exert diverse and often contrasting effects on the tumour and its microenvironment. The activation of members of the uPA–uPAR–MMP proteolytic cascade by several hK-family members further broadens their spectrum of activities during cancer progression. Therefore, hK proteolysis might represent a novel research avenue in cancer initiation and progression.

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Summary

- Human tissue kallikreins (hKs) comprise a subgroup of 15 homologous secreted trypsin or chymotrypsin-like serine proteases, encoded by a tightly clustered multigene family on chromosome 19q13.4.
- *KLK* transcription is modulated by an assortment of stimulatory and inhibitory factors, among which steroid hormones are the best characterized. The proteolytic activity of hKs is regulated in several ways, including zymogen activation; complex formation with endogenous plasma and/or tissue inhibitors, such as α_2-macroglobulin and serpins; inhibition by inorganic ions; and inactivation through internal (auto)fragmentation.
- hKs are primarily expressed within the glandular epithelia of many organs and implicated in a range of normal physiological functions. New proteomic technologies could facilitate the identification of putative *in vivo* substrates and/or the substrate specificity for many of the newer, relatively uncharacterized hKs.
- Kallikrein genes/proteins are aberrantly expressed in many cancer types and their expression is often associated with patient prognosis.
- So far, experimental evidence indicates that hKs might promote or inhibit cancer-cell growth, angiogenesis, invasion and metastasis by proteolytic processing of growth-factor-binding proteins, activation of growth factors and other proteases, release of angiogenic or anti-angiogenic factors, and degradation of extracellular-matrix components. hKs are also implicated in the development of osteoblastic bone metastasis in prostate cancer.
- The initial claim to fame of hKs is mainly attributed to the clinical impact of prostate-specific antigen as a biomarker for screening, diagnosis, staging and monitoring of prostate cancer. Recent reports indicate that many other kallikrein genes/proteins might prove to be promising tissue and/or serological cancer markers.
- Exploitation and modulation of hK protease activity are attractive therapeutic approaches. hKs have been used in the activation of prodrugs and in the development of cancer vaccines, whereas hK promoters have been used for the specific delivery of toxic genes to tumour cells. Highly specific inhibitors of hK activity have also been developed and might represent promising agents for cancer treatment.

ORTHOLOGUE
Homologous genes in different species that are derived from a common ancestral gene following speciation. Orthologues usually retain the same function in the course of evolution.

LOCUS CONTROL REGIONS
A class of *cis*-acting regulatory elements that regulate chromatin and the expression of linked genes over distances as long as 100 kb or more in a tissue- and copy-number-specific manner in a wide spectrum of mammalian gene families.

SERPIN
A superfamily of serine-protease inhibitors. Most serpins are inhibitory and share a unique mechanism of inhibition in which they undergo a profound conformational change to trap their target protease in an irreversible complex, but differ in their specificity towards different serine proteases.

Historical overview

hKs comprise a family of 15 homologous single-chain, secreted serine endopeptidases of ~25–30 kDa, with ORTHOLOGUES present in species from at least six mammalian orders. The first member of this protease subclass was identified in the 1930s as an abundant protein in the pancreas — 'kallikreas' in Greek — and so was named tissue kallikrein. During the 1970s, the search for male-specific antigens in semen, for forensic purposes, led to the discovery of hK3/PSA[4], the most well-characterized kallikrein so far and the most valuable biomarker in clinical medicine for prostate cancer diagnosis and monitoring in high-risk populations in the United States. Cloning of the tissue kallikrein and *KLK3*/*PSA* genes, together with that of another novel kallikrein, human glandular kallikrein (*KLK2*), and their colocalization to chromosomal region 19q13.4 (REF. 5) ensued about a decade later. During the 1990s, another 12 serine-protease genes were discovered and assigned to the kallikrein family based on localization to 19q13.4, as well as sequence and structural similarities to the first 3 kallikrein genes[6–8] (FIG. 1; BOXES 1,2).

Regulation of kallikrein expression and activity

Several transcriptional and post-translational mechanisms are in place to regulate kallikrein expression and proteolytic activity, respectively. *KLK* expression is modulated by numerous stimulatory and inhibitory factors that influence many signalling pathways. The most extensively studied regulators of *KLK* transcription are sex-steroid hormones. Sex-steroid hormones have an important role in the development of many organs and endocrine-related tumours. *KLK2* and *KLK3* are model androgen-regulated genes expressed almost exclusively in the prostate. Androgen–androgen receptor complexes bind to several functional androgen response elements (AREs) within the proximal promoter and enhancer regions of *KLK2* and *KLK3* and stimulate their transcription[9]. By contrast, other kallikrein genes, including *KLK5* and *KLK6*, are more responsive to oestrogens. Expression of all other *KLK* genes is also affected by androgenic and/or oestrogenic stimulation. Given the frequent co-expression of subgroups of kallikreins in tissues, transcription of *KLK* genes might be coordinately regulated in a tissue-specific manner by *cis*-acting LOCUS CONTROL REGIONS, a mechanism that has also been implicated in controlling the salivary-gland-specific expression of the rat *Klk* gene family[10]. Other regulatory elements involved in modulating *KLK* expression act independently of and/or modify steroid-hormone-receptor signalling.

As protease action is always irreversible, four post-translational regulatory mechanisms normally serve to prevent unwanted hK proteolysis and maintain tissue integrity: zymogen activation, endogenous inhibitors, inactivation through internal cleavage and allosteric regulation. Analogous to other proteolytic enzymes, all hKs are first translated as inactive or minimally active zymogens (pro-enzymes) containing an amino-terminal propeptide domain of 4–9 amino acids that maintains their latency (FIG. 1c). Activation of hK zymogens is generally accomplished by limited hydrolysis of the propeptide, which induces a conformational change in the active site and substrate-specificity pocket, thereby creating the active hK. Processing of pro-hK zymogens can occur intra- or extracellularly and typically requires a trypsin-like activity for removal of the propeptide, exclusive of hK4. *In vitro* studies indicate that some hKs are autoactivated, such as hK2 (REF. 11), hK6 (REF. 12) and hK13 (REF. 13), and/or activated by other hKs. Pro-hK3 is activated by hK2 (REF. 14), hK4 (REF. 15) and hK15 (REF. 16); pro-hK7 is activated by hK5 (REF. 17) and other serine proteases, including trypsin[18], as well as metalloproteinases[19].

Zymogen activation is irreversible. Once activated, hKs are tightly controlled by endogenous inhibitors, mainly α_2-macroglobulin (α_2M) and SERPINS in fluids and tissues. Several hKs, such as hK2 (REF. 20), can form a complex with the broad-specificity inhibitor α_2M in serum. Proteases are lured to a particularly exposed peptide region (the 'bait' region) on α_2M and cleave it, resulting in a large conformational change that 'traps' the protease in a non-covalent complex. This does not lead to inhibition of protease activity, but does prevent hKs from interacting with large substrates or inhibitors by steric hinderance. As with other serine proteases of the S1 family, hKs interact with serpins through their reactive-site loops by two pathways: the first is the 'inhibitory' pathway, which results in the formation of a covalent complex and induces deformation and irreversible inactivation of the

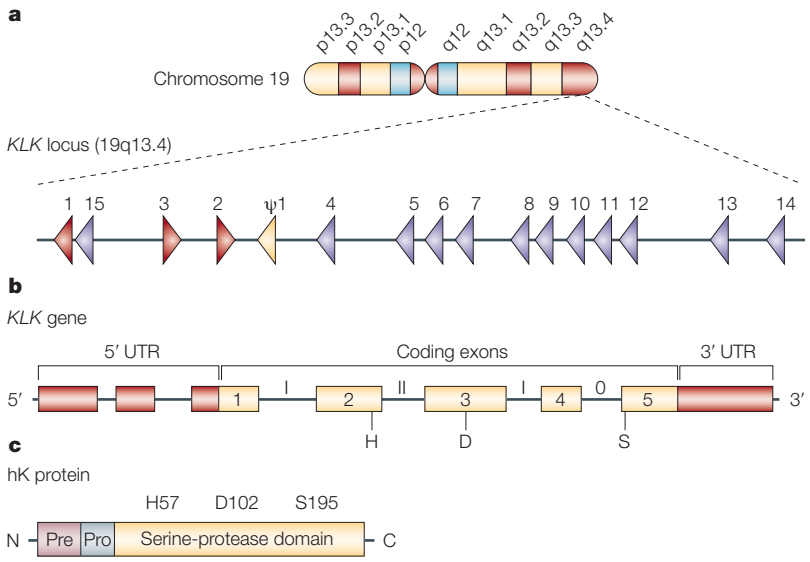


Figure 1 | **Kallikrein locus, gene and protein characteristics. a** | The *KLK* locus spans ~300 kb on the long arm of chromosome 19 in cytogenic region 13.4. The 15 *KLK* genes are tightly clustered in a tandem array without intervention by non-*KLK* genes. With the exception of *KLK2* and *KLK3*, the direction of gene transcription is from telomere to centromere. The 'classical' *KLK* genes (*KLK1*, *KLK2* and *KLK3*/*PSA*) are represented by red arrowheads, *KLK4–KLK15* by purple arrowheads and the *ΨKLK1* processed pseudogene by a yellow arrowhead. **b** | *KLK* genes range from 4–10 kb in length and all consist of 5 coding exons and 4 intervening introns with a conserved intron phase pattern (I, II, I, 0). Coding-exon (yellow boxes) lengths are similar, if not identical, among *KLK* genes, but intron lengths vary considerably. The positions of the codons for the active-site catalytic residues are highly conserved, with the histidine (H) codon near the end of coding-exon 2, aspartic acid (D) codon in the middle of coding exon 3 and serine (S) codon near the start of coding-exon 5. Most *KLK* genes, exclusive of the 'classical' *KLK* genes, also have one or two non-coding exons (red boxes) in the 5′ untranslated region (UTR). The 3′ UTR typically varies in length. **c** | hK proteins are single-chain, secreted serine proteases. They are synthesized as pre-proenzymes containing an amino-terminal signal sequence (Pre) that directs them to the endoplasmic reticulum for secretion, a propeptide (Pro) that maintains them as inactive precursors (zymogens) and a serine-protease domain responsible for catalytic activity.

hK (for example, hK3 and α_1-antichymotrypsin[21]); and/or the second is the 'substrate' pathway, in which the hK cleaves the serpin, yielding an active hK and an inactive serpin (for example, hK6 and α_2-antiplasmin[12]). Ultimately, hK–α_2M and hK–serpin complexes are cleared from the circulation primarily by binding to the α_2M receptor/low-density lipoprotein receptor-related protein followed by endocytosis. hKs complexed to serpins are also susceptible to degradation by circulating proteases, because of their partially denatured structure. Furthermore, antileukoprotease (secretory leukocyte protease inhibitor), elafin (skin-derived antileukoprotease) and inhibitory peptides derived from the serine protease inhibitor Kazal type 5 (REF. 22) might regulate hK activity in the epidermal layers of the skin.

hKs can also lose activity through internal cleavage. Degraded forms of hK2 and hK3 have been identified in seminal plasma and/or prostatic tissues[23]. Although not certain at present, evidence indicates the proteases that are responsible for internal proteolytic processing of hK2 and hK3 probably possess trypsin-like specificity. Similarly, recombinant hK6 (REFS 12,24), hK7 (REF. 25) and hK13 (REF. 13) are fragmented *in vitro*, probably by an autoproteolytic mechanism. So, some hKs, such as hK6 (REF. 24), modulate their own activity by auto-activation followed by auto-inactivation.

Divalent cations, in particular zinc, have been shown to reversibly inhibit both hK2 (REF. 26) and hK3 (REF. 27) following binding at two allosteric sites. This regulatory mechanism is thought to occur in the prostate, which accumulates the highest levels of zinc in the body, and is probably reversed within the seminal plasma, where zinc concentrations are far lower. Also, zinc levels are markedly decreased in prostate cancer tissues compared with normal surrounding tissues, which might result in increased hK activity within prostatic tumours.

Box 1 | **Features of the kallikrein family**

According to the official nomenclature, kallikrein gene and protein symbols are denoted *KLK* and hK, respectively[6]. The *KLK* locus (FIG. 1a) is now recognized as the largest contiguous cluster of protease genes of any catalytic type within the entire human genome[2]. The kallikrein family is defined by several common features, including chromosomal localization, genomic organization and protease structure[143–145] (FIG. 1b,c). Kallikreins generally share 30–50% sequence similarity at the nucleotide and amino-acid levels[144]. The 'classical' kallikreins (*KLK1*, *KLK2* and *KLK3*) are by far the most homologous, exhibiting 73–84% nucleotide and 61–77% amino-acid sequence similarity[146]. Each *KLK* gene can generate at least two distinct transcripts that can encode structurally and functionally unique proteins, creating additional levels of transcriptomic, proteomic and degradomic complexity within this family. In addition to the conserved His57, Asp102, Ser195 catalytic triad that characterizes serine proteases, each hK protein also contains either 10–12 conserved cysteine residues that form five (in hK1, hK2, hK3 and hK13) or six (in hK4–hK12 and hK15) disulphide bonds. hKs possess the archetypal tertiary structure of chymotrypsin-like serine proteases, which consist of two juxtaposed β-barrels and two α-helices, with the active-site catalytic triad bridging the barrels, as confirmed by the X-ray crystallographic structures for hK1 and hK6 (REFS 96,147,148). The specificity of hKs and chymotrypsin-like serine proteases is usually defined by the P1-S1 interaction (Schechter and Berger nomenclature[149]) and, in particular, by the amino acid at position 189 of the S1 subsite, located six amino acids amino-terminal of catalytic Ser195. Twelve hKs have a predicted or experimentally verified trypsin-like specificity due to the presence of acidic residues Asp189 (hK1, 2, 4, 5, 6, 8, 10, 11, 12, 13, 14) or Glu189 (hK15) within the S1 subsite, which help to accommodate arginine or lysine at the P1 position. By contrast, hK3, hK7 and hK9 have Ser189, Asn189 and Gly189, respectively, accomodating bulky non-polar amino acids such as tyrosine or phenylalanine, and conferring a chymotrypsin-like specificity. Secondary interactions outside the S1 subsite mediated by the six highly variable surface loops surrounding the active-site catalytic triad have a role in diversifying hK substrate specificities[150,151].

Box 2 | **The kallikrein transcriptome and cancer**

It is now clear that gene number is only one way of increasing protein diversity and that the transcriptome can markedly expand the coding capacity of the genome. The transcriptome harbours a plethora of alternatively processed mRNA transcripts derived from ALTERNATIVE PRE-mRNA SPLICING**, alternative promoter/transcriptional start sites and/or variant polyadenylation signal usage, which might encode numerous functionally distinct proteins. Alternative pre-mRNA splicing is considered the most significant source of variant transcripts/proteins, as 35–74% of all human genes are alternatively spliced and ~70–88% of alternative-splicing events alter the encoded protein[152,153].**

So far, the *KLK* transcriptome has been found to comprise 82 mRNAs encoding up to 56 putative protein isoforms. All *KLK* genes possess at least two transcripts as a result of alternative splicing, promoters/transcription start sites, polyadenylation signals and combinations thereof. Interestingly, one *KLK4* transcript codes for an intracellular form of the hK4 protein primarily localized to the nucleus, in contrast to the extracellular localization and function of other hKs[154]. Emerging data indicate that the *KLK* transcriptome is altered during neoplastic progression and harbours several cancer-specific mRNAs. Three *KLK4* (REF. 33), three *KLK5* (REFS 155–157), one *KLK7* (REF. 155) and two *KLK8* (REF. 41) alternative transcripts are overexpressed in ovarian tumours and/or cell lines compared with normal ovaries. The *KLK11* gene has two transcript variants denoted brain type and prostate type[158], both of which are upregulated in prostate carcinoma[159]. *KLK13* possesses at least five splice variants exclusively expressed in the normal testis, but absent in the adjacent cancerous tissues[160].

Given the upregulation of alternative *KLK* transcripts in tumours, and the likely secretion of their encoded protein isoforms, which would facilitate their detection by biopsy or in body fluids, respectively, the alternative *KLK* transcriptome might constitute a new generation of cancer markers within the *KLK* family.

Kallikreins in normal physiology

Kallikreins are often co-expressed in the skin, breast, prostate, pancreas and brain, primarily by secretory epithelial cells, from which they enter bodily fluids such as sweat, milk, saliva, seminal plasma, cerebrospinal fluid or pericellular spaces. As such, they are implicated in a vast range of normal and pathological processes, where they act independently and/or as part of one or more proteolytic cascades (BOX 3). However, the physiological roles and *in vivo* targets of many hK-family members remain generally ill-defined and have only been delineated for hK1 and hK3/PSA so far. Potential substrates and functions for the remaining hKs have been identified by *in vitro* experimentation and/or extrapolated from the known actions of their orthologues. Most recently, degradomics tools, including biological and chemical combinatorial peptide-based specificity-profiling technologies such as phage display[28] and fluorogenic substrate libraries[29], respectively, have been used to determine the preferred consensus sequence, substrate specificity and even candidate physiological targets of hK2 (REF. 30), hK3 (REF. 31) and hK14 (C.A.B. and E.P.D, unpublished observations). For example, biochemical characterization of hK2 by substrate phage display revealed its strict cleavage consensus sequence and three putative targets[30] (TABLE 1).

Kallikreins in cancer

Accumulating evidence indicates that the *KLK* family is dysregulated in cancer. Numerous studies report aberrant amounts of kallikrein transcripts and/or proteins in cancer cells, particularly adenocarcinomas derived from steroid-hormone-regulated tissues, as compared with their normal, benign and/or pre-malignant tissue counterparts (TABLE 2). The most striking example is the concurrent upregulation of 12 *KLK* genes in ovarian carcinoma. Overexpression of *KLK2*, *KLK3*, *KLK4*, *KLK5*, *KLK6*, *KLK7*, *KLK8*, *KLK10*, *KLK11*, *KLK13*, *KLK14* and *KLK15* transcripts and/or proteins in ovarian carcinoma tissues, cell lines and/or serum and tumour ascites fluid has been demonstrated by several experimental approaches, including *in-silico*-, RT-PCR-, northern-blot-, microarray-, immunohistochemical- and ELISA-based technologies[32–52]. In contrast to their upregulation in ovarian cancer, kallikrein genes and proteins are generally downregulated in breast[53–61], prostate[62–68] and testicular[60,69,70] tumours. In addition to steroid-hormone-regulated cancers, kallikreins are dysregulated in several other tumour types, including lung adenocarinomas[71], pancreatic cancer[72,73], head and neck squamous-cell carcinoma[74] and acute lymphoblastic leukaemia[75].

Although many hKs are overexpressed in cancerous tissues, it is not known whether this also reflects an increase in proteolytic activity. This is due to the heterogeneity of hK forms that exist in the extracellular milieu of tissues together with the active enzymes, including inactive pro-hKs, hKs sequestered in inhibitor complexes, hKs inactivated by internal cleavage and even variant hK isoforms encoded by alternative *KLK* transcripts. However, a recent study has shown that most (80–90%) of hK3 within the interstitial fluid of prostatic tumours is primarily uncomplexed to inhibitors and enzymatically active[76], indicating that hK expression might correlate with hK activity in tumours.

Kallikreins in neoplastic progression

Extensive correlative clinical data linking increased kallikrein expression with patient prognosis strongly indicates that hKs are implicated in tumour progression (TABLE 2). Although most reports have linked high kallikrein expression with poor patient prognosis, a few studies have also acknowledged kallikreins as favourable prognostic indicators. This apparent paradox might be attributed to the dual role that hKs have during tumour progression as stimulatory and inhibitory factors that modulate tumour cells and their microenvironment in an autocrine and paracrine fashion in different tissues or under different steroid-hormone balances. Recent studies indicate that hK-mediated pericellular proteolysis in the extracellular space might be important in many facets of cancer development, including regulation of tumour-cell growth, angiogenesis, invasion and metastasis. Whether hKs exert cancer-promoting or cancer-inhibiting activities might ultimately depend on the tissue type and tumour microenvironment. As most data have been based on *in vitro* biochemical and cell-culture systems, more direct evidence is needed to determine the roles of hKs in cancer biology.

Kallikreins and regulation of tumour growth. Increasing evidence indicates that several hKs might participate early in neoplastic progression by directly or indirectly promoting or inhibiting cancer-cell proliferation. hKs

ALTERNATIVE PRE-mRNA SPLICING
The process through which different combinations of exons within a single pre-mRNA are joined together to produce two or more distinct mature mRNAs. This is the most common mechanism for producing functionally diverse proteins from a single gene.

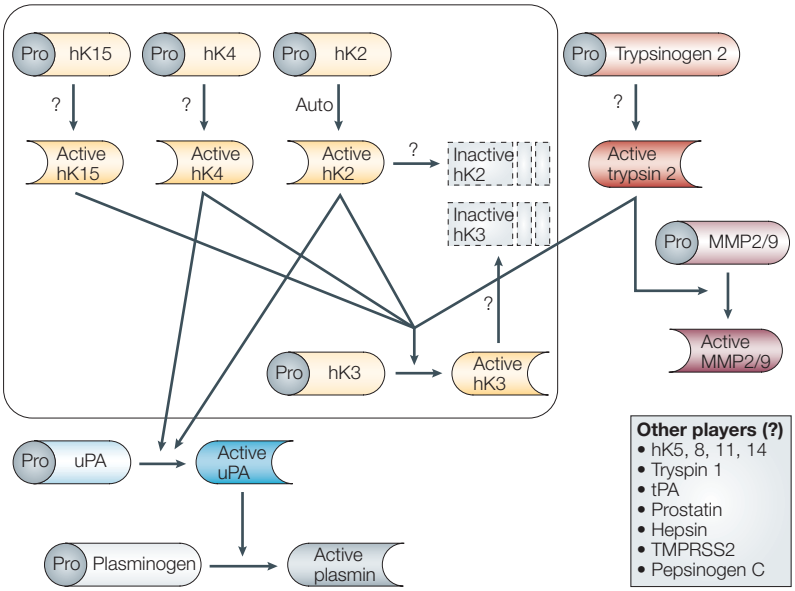
ELISA
(Enzyme-linked immunosorbent assay.) A serological assay in which bound antigen is detected by antibodies linked to an enzyme, the activity of which can be assayed for the quantitative determination of the antigen–antibody interaction.

Box 3 | **Evidence for human tissue kallikrein cascades**

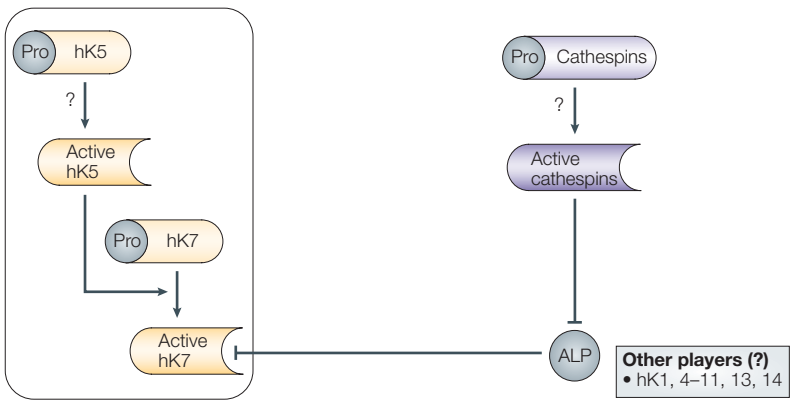
Proteases are initially synthesized as inactive zymogens, which generally require limited (auto)proteolysis of their propeptide domains for conversion to their active forms. This type of activation mechanism can give rise to 'proteolytic cascades' of sequential latent zymogen activation, in which the activated form of one enzyme catalyses the processing of the following zymogen. Such cascades, involving serine proteases and those of other catalytic classes, are well-characterized in the digestive system and the coagulation–fibrinolysis pathways. Several lines of evidence indicate that human tissue kallikreins (hKs) exert their normal and pathological functions cooperatively through proteolytic cascades. All *KLK* genes colocalize to 19q13.4, are coordinately regulated, co-expressed in various tissues and bodily fluids, and concurrently up- or downregulated during tumour progression, and some hKs autoactivate and/or activate other hK zymogens.

Based on current knowledge, hK cascades might contribute to a range of bodily processes in specific tissues, including extracellular-matrix remodelling and the regulation of cellular proliferation, differentiation and apoptosis in the normal and neoplastic prostate tissues, and the regulation of the dissolution of the seminal clot shortly after ejaculation in seminal plasma (see figure, part a); and the regulation of skin desquamation (see figure, part b). It is likely that such cascades are operating in other organs, such as the pancreas and pituitary, and participate in hormone processing or in signal-transduction pathways involving cell-surface receptors such as protease-activated receptors. MMP, matrix metalloproteinase; TMPRSS2, transmembrane protease serine 2; uPA, urokinase plasminogen activator.

a Prostatic/seminal plasma cascade



b Skin desquamation cascade



are able to modulate the bioavailability and activity of latent growth factors, and, conversely, suppress the oncogenicity of tumours (FIG. 2a).

Several reports indicate that hK2 and hK3 might represent important regulators of the insulin-like growth factor (IGF) axis in prostate carcinogenesis. IGFs (IGF1 and IGF2) are important mitogenic peptides involved in regulating normal and malignant cellular proliferation, differentiation, apoptosis and transformation. Before IGFs act, they must first be released from IGF-binding proteins (IGFBPs), a family of six proteins that antagonize the binding of IGFs to the IGF1 receptor (IGF1R). IGFBPs also possess intrinsic bioactivity, independent of IGFs and IGF1R signalling, presumably mediated following binding to a receptor. For example, IGFBP3 induces apoptosis and inhibits the growth of prostate cancer cell lines[77]. hK1, hK2 and hK3 are IGFBP proteases that collectively degrade IGFBP2, IGFBP3, IGFBP4 and IGFBP5, abrogating their inherent tumour-suppressive effects and decreasing their affinity for IGF1, thereby increasing the bioavailability of IGF1, which, in turn, stimulates the growth of normal, stromal and malignant prostate cells *in vitro*[78–80]. The *in vivo* interaction between hKs and IGFBPs probably occurs within the tumour microenvironment of the prostate, when the boundaries separating the stromal and epithelial compartments have been compromised, as well as at metastatic sites. Therefore, certain hKs might indirectly modulate prostatic tumour and stromal-cell growth and survival.

In addition to IGFBP proteolysis, hK3 is also able to stimulate the production of reactive oxygen species (ROS) in prostate cancer cells, independent of its serine-protease activity, an effect that is probably mediated following binding of hK3 to a cell-surface receptor[81]. As ROS can cause DNA damage — which can induce apoptosis or lead to the activation of proto-oncogenes and inactivation of tumour-suppressor genes, and can cause oxidative injury to proteins and tissues — hK3, through ROS, might indirectly contribute to tumorigenesis and/or tumour progression.

Lastly, hK2 and hK4 can activate single-chain uPA[15,82] and hK2 can inactivate plasminogen activator inhibitor 1 (REF. 83), leading to activation of the uPA system. *In vivo*, uPA is bound to its cell-surface receptor, uPAR, and converts plasminogen to plasmin, leading to pericellular ECM degradation and the release and/or activation of tumour growth factors. As the IGF–IGFBP and uPA–uPAR systems operate in many organs, their involvement in cancers other than the prostate is likely.

hKs themselves might act as growth factors through activation of protease-activated receptor (PAR) signalling. PARs are a small family of four G-protein-coupled receptors that are activated by serine protease (for example, thrombin and trypsin) cleavage of an extracellular N-terminal segment, unmasking the cryptic ligand, which then interacts with an extracellular loop to transmit intracellular signals. PARs are expressed by a range of tumour cells and cells of the tumour microenvironment, including endothelial cells and macrophages[84]. Tumour-derived serine

Table 1 | **Potential kallikrein substrates during tumour progression**

Kallikrein	Substrate(s)	Role in tumour progression	References
hK1	Pro-MMP2, pro-MMP9	Activation of latent MMPs resulting in ECM degradation; role in angiogenesis, invasion and metastasis	98–100
	IGFBP3	Release of IGFs, resulting in tumour-cell and osteoblast proliferation and survival; role in tumour-growth regulation	79
hK2	Pro-hK3	Activation of pro-hK3	14,18,161
	IGFBP2, IGFBP3, IGFBP4, IGFBP5	Release of IGFs, resulting in tumour-cell and osteoblast proliferation and survival; role in tumour-growth regulation and osteoblastic responses in prostate cancer bone metastases	79
	Pro-uPA	Activation of pro-uPA, leading to plasmin activation and ECM degradation; role in angiogenesis, invasion and metastasis	82
	Fibronectin	ECM degradation; role in angiogenesis, invasion and metastasis	92
	ADAMTS8 precursor, cadherin-related tumour suppressor homologue precursor, collagen IX α-chain	Putative substrates identified by phage display	30
hK3	IGFBP3, IGFBP4	Release of IGFs, resulting in tumour-cell and osteoblast proliferation and survival; role in tumour-growth regulation and osteoblastic responses in prostate cancer bone metastases	78,79
	Latent TGFβ	Activation of latent TGFβ, resulting in the proliferation of osteoblasts as well as tumour suppression (growth inhibition, apoptosis) or progression (induction of EMT, angiogenesis)	101
	Fibronectin, laminin, gelatin, fibrinogen	ECM degradation; role in angiogenesis, invasion and metastasis	93–95
	Matrigel (laminin, collagen type IV, heparan sulphate proteoglycans, entactin and nidogen)	ECM degradation; role in angiogenesis, invasion and metastasis	110
	Lys-plasminogen	Generation of anti-angiogenic, angiostatin-like fragments; role in inhibition of angiogenesis	105
	PTHrP	Cleavage and inactivation of PTHrP; role in inhibiting bone resorption mediated by PTHrP	115,116
hK4	Pro-hK3	Activation of pro-hK3	15
	Pro-uPA	Activation of pro-uPA, leading to plasmin activation and ECM degradation; role in angiogenesis, invasion and metastasis	15
hK6	Fibrinogen, collagen I and IV, laminin and fibronectin	ECM degradation; role in tumour angiogenesis, invasion and metastasis	12,96
	Plasminogen	Generation of anti-angiogenic, angiostatin-like fragments; role in inhibition of angiogenesis	24
hK7	Fibrinogen	ECM degradation; role in angiogenesis, invasion and metastasis	97
hK13	Plasminogen	Generation of anti-angiogenic, angiostatin-like fragments; role in inhibition of angiogenesis	13
hK14	Matrilin-4, laminin α1-chain, laminin α5-chain, collagen IV α1-chain, collagen XII α3-chain	Putative substrates identified by phage display	C.A.B. and E.P.D, unpublished observations
hK15	Pro-hK3	Activation of pro-hK3	16
	Pro-uPA	Activation of pro-uPA, leading to plasmin activation and ECM degradation; role in angiogenesis, invasion and metastasis	16

ADAMTS8, a disintegrin and metalloprotease with thrombospondin type 1 motif 8; ECM, extracellular matrix; hK, human tissue kallikrein; IGF, insulin-like growth factor 1; IGFBP3, insulin-like growth factor binding protein 3; pro-MMP2, pro-matrix metalloprotease 2; pro-uPA, pro-urokinase plasminogen activator; PTHrP, parathyroid-hormone-related protein.

proteases such as trypsin might act in an autocrine fashion to stimulate cancer-cell growth through PARs[85]. The potential role of hK-mediated PAR signalling in tumour progression should be explored.

Certain hKs might also negatively regulate tumour-cell growth. Tumour-suppressive actions have been assigned to both hK3 and hK10. hK3 is able to inhibit the growth of the oestrogen-receptor-positive MCF-7 breast cancer cell line by stimulating the conversion of the potent oestrogen oestradiol to the less potent oestrone[86]. This effect was not observed with the oestrogen-receptor-negative breast cancer cell line MDA-MB-231, indicating that hK3 is a negative regulator of hormone-dependent breast carcinomas. These results correlate well with previous findings indicating that hK3 expression is lowest in advanced-stage breast cancer tissues[53] and that patients with hK3-positive breast tumours have a better prognosis[87]. The reported ability of hK3 to activate latent transforming growth factor-β (TGFβ), a suppressor of growth and inducer of apoptosis in many normal and neoplastic cells[88], further supports a tumour-inhibiting role for hK3. hK10 is also considered a putative tumour suppressor, by virtue of its downregulation at the mRNA level in several cancer types (that is, breast, prostate and testicular cancer, and acute lymphoblastic leukaemia)[55–57,70,75]. Furthermore, transfection of *KLK10* into the breast cancer cell line MDA-MB-231 reduced its anchorage-independent growth, and tumour formation of nude mice inoculated with this *KLK10*-transfected cell line was significantly inhibited[56]. Therefore, *KLK10* is able to suppress the tumorigenic phenotype *in vitro* and *in vivo*.

However, some studies report that hK3, whether enzymatically inactive or active, has no stimulatory or inhibitory effect on the growth of prostate cancer cell lines *in vitro*, calling into question the previous findings[89,90].

Kallikreins and angiogenesis. Angiogenesis, defined as the sprouting of new blood vessels from pre-existing vasculature, is crucial for tumour growth, invasion and metastasis. Attainment of the angiogenic phenotype is a multistep process involving the endothelium and the tumour microenvironment and depends on the balance between pro- and anti-angiogenic growth factors. The overexpression of pro-angiogenic stimuli and/or loss of inhibitory regulators is thought to 'switch on' or activate the angiogenic process[91]. hKs might contribute to this process by modulating the angiogenic switch and facilitating endothelial-cell proliferation, migration and capillary-tube formation through direct or indirect ECM degradation (FIG. 2b).

Kallikreins can promote angiogenesis. Several *in vitro* studies show that hKs support angiogenesis by either directly and/or indirectly disrupting ECM barriers. hK2 (REFS 30,92), hK3 (REFS 93–95), hK6 (REFS 12,96), hK7 (REF. 97) and hK14 (C.A.B. and E.P.D, unpublished observations) directly catalyse the hydrolysis of a distinct and partially overlapping set of ECM proteins (TABLE 1), which might enable both endothelial-cell as well as tumour-cell migration and invasion. Several hKs might also indirectly influence ECM degradation. For instance, hK1 activates pro-MMP2 and pro-MMP9, two type IV collagenases that degrade collagen IV and other constituents of basement membranes[98–100]. Activation of the uPA–uPAR system by hK2 and hK4 (REFS 15,82,83) can also result in the degradation of a wide spectrum of ECM components through plasmin, accompanied by the liberation and/or activation of sequestered pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF) and the activation of pro-MMPs. So, hKs participate in a proteolytic cascade that regulates tumour angiogenesis. hK3 might directly promote angiogenesis by activating the pro-angiogenic growth factor TGFβ[88,101]. Furthermore, hK1 is expressed in angiogenic endothelial cells[102], and hK1-generated kinins — multifunctional biologically active peptides released from low-molecular-weight kininogen — stimulate angiogenesis[103]. hKs might also induce proliferation of endothelial cells through PAR activation, as previously shown for trypsin[104].

In addition to their permissive effects on angiogenesis, hKs can also antagonize this process. hK3, hK6 and hK13 generate angiostatin-like fragments from plasminogen *in vitro*[13,24,105]. Angiostatin is a potent inhibitor of endothelial-cell proliferation and angiogenesis *in vivo*. Similarly, purified angiostatin-like fragments derived from hK3-mediated proteolysis inhibited the proliferation and tubular formation of endothelial cells in *in vitro* morphogenesis assays[105]. The potential anti-angiogenic effects of the hK6 and hK13-derived angiostatin-like fragments have not been further characterized. Furthermore, hK3, independent of its serine-protease activity, is able to act as an endogenous anti-angiogenic protein by blocking fibroblast growth factor 2 (FGF2)- and/or VEGF-stimulated endothelial-cell proliferation, migration and invasion *in vitro*, and by inhibiting angiogenesis and tumour metastasis *in vivo*[89,106]. These findings are further substantiated by a clinical study showing a negative correlation between hK3 expression and mean vessel density, a measure of angiogenesis *in situ*, in prostate tumours[107] and might collectively help to explain the relatively indolent course of prostate carcinomas. Lastly, hK9 was found to preferentially home to angiogenic endothelial cells within pre-malignant dysplastic skin lesions through the circulation, and might be implicated in the vascular changes that occur during the progression of normal skin to dysplasia and neoplasia[108].

Kallikreins in invasion and metastasis. hKs probably participate in many stages of the metastatic cascade by facilitating tumour-cell detachment; by enabling invasion through multiple ECM barriers, independently and in collaboration with other extracellular proteases; and, potentially, by contributing to the metastatic spread of prostate cancer cells to bone (FIG. 2c).

For tumour cells to detach, invade local tissues and metastasize, they typically undergo an epithelial-to-mesenchymal transition (EMT) through which they attain a malignant phenotype. EMT is characterized by

extensive changes in the expression of adhesion molecules, including the loss of E-cadherin-mediated cell–cell adhesion and *de novo* expression of mesenchymal cadherins such as N-cadherin. Loss of E-cadherin facilitates tumour-cell detachment and N-cadherin promotes cell motility and migration. As TGFβ has been found to induce EMT, hK3 might be indirectly implicated in promoting the invasive phenotype and tumour-cell detachment, through its activation of the latent TGFβ complex[88,101].

Extracellular proteolysis is crucial for dissolution of the many ECM barriers encountered during tumour-cell invasion. As discussed in the previous section, numerous *in vitro* studies have indicated that hKs are directly and indirectly involved in the degradation of ECM proteins, including collagens, laminins and glycoproteins such as fibronectin. Further experimental evidence for the role of hKs in metastasis has been demonstrated by *in vitro* Matrigel invasion assays[95,109,110]. The invasion of LNCaP prostate cancer cells through Matrigel was shown to be attenuated by hK3-neutralizing antibodies[95,110] as well as by zinc[110], whereas invasion of MDA-MB-231 breast cancer cells into Matrigel was suppressed by a synthetic hK1 inhibitor[109]. In addition, the synthetic hK1 inhibitor was able to inhibit invasion of breast cancer cells in a novel *ex vivo* assay of cancer-cell invasion in explanted rat lungs[109]. Conversely, in an experimental *in vivo* metastasis assay, mice treated with hK3 had a significantly lower number of surface lung metastases compared with controls, indicating that hK3 inhibits the metastatic process[89]. This anti-metastatic effect of hK3 might be attributable to its anti-angiogenic activity, which is independent of its serine-protease action. Whether hK3 functions as an anti- or pro-metastatic protein *in vivo* might depend on the tumour type and on factors present in the tumour microenvironment. Moreover, hKs might be able to promote or inhibit cancer-cell invasion through PAR signalling, in a manner akin to thrombin[111,112].

Several lines of evidence indicate that hKs have a role in the formation of osteoblastic (bone-forming) metastases that occur in ~90% of prostate cancer cases (FIG. 2d). The selective metastasis of prostate cancer cells to bone is influenced by a range of soluble and insoluble factors present in the microenvironments of the primary tumour, circulation and bone, including chemotactic factors, as well as the preferential adherence of prostate cancer cells to bone-marrow endothelial cells (BMECs). A recent study indicates that hK3 is an important mediator of prostate cancer cell–bone endothelium interactions, as an hK3 antibody attenuated prostate cancer cell adhesion to BMECs and prostate cancer cells with reduced hK3 production, due to knockdown of *KLK3* mRNA by RNA interference, showed low adhesive ability[113]. Although the mechanism is unknown, hK3 might mediate cell–cell adhesion on the surface of prostate cancer cells through direct or indirect interactions with a receptor or substrate on BMEC surfaces[113]. Metastatic prostate cancer cells can also secrete growth factors, such as TGFβ and IGFs, which alter the normal bone-remodelling balance between osteoblasts, osteoclasts and constituents of the bone matrix to stimulate bone formation. Several reports indicate that hKs are involved in this osteoblastic response at metastatic sites. hK3 can induce proliferation, activation and/or detachment of cultured osteoblasts *in vitro*[101,114] and *in vivo*[114], which might be mediated indirectly, by activation of latent TGFβ[101], or directly, by modulation of cell-surface receptors[101] or by an autonomous mechanism that is independent of the growth factors produced by prostate cancer cells[114]. Several hKs increase IGF bioavailability by degrading IGFBPs *in vitro*[78,79]. Furthermore, hK3 has been shown to cleave and inactivate parathyroid-hormone-related protein[115,116], a potent osteoclast activator that is implicated in osteolytic bone metastases. Therefore, hKs might stimulate bone formation and inhibit bone resorption, thereby contributing to the pathogenesis of metastatic prostate cancer in bone.

Kallikrein regulation in tumours

Several mechanisms might account for the differential expression of kallikrein genes during tumorigenesis. As chromosome 19q13 is altered in a range of tumours, genetic aberrations within the *KLK* locus might be one mechanism for altered *KLK* expression in tumours. For instance, amplification of the *KLK6* gene might be partially responsible for its increased expression in ovarian tumours[117]. Other genetic changes, such as intragenic mutations, have not been identified within the two kallikrein genes examined so far — *KLK3* (REFS 118,119) and *KLK10* (REF. 55) — and have not been extensively studied otherwise.

Transcriptional alterations due to GENETIC POLYMORPHISMS, epigenetic modifications or alterations in *trans*-acting transcription factors might be the most likely determinants of aberrant *KLK* expression in tumours. A range of genetic polymorphisms, primarily single-nucleotide polymorphisms (SNPs), exist within *KLK* coding and promoter/enhancer regions. These polymorphisms affect transcriptional regulation and/or confer increased susceptibility or resistance to cancer. With respect to *KLK3*, patients with breast cancer who are homozygous for the G allele at position –158 within the proximal promoter region show higher hK3 tumour concentrations and an increased overall survival than those homozygous for the A allele[120]. Several studies have also reported a link between the SNPs at –158 and –252 and *KLK3* expression, serum hK3 levels and prostate cancer risk[121,122], whereas others have not[123,124]. One likely explanation for these disparate findings has been proposed by Cramer *et al.*, who report that most of the polymorphisms within the *KLK3* promoter and enhancer regions are in strong LINKAGE DISEQUILIBRIUM[124]. Additional confounders, such as ethnicity and lifestyle factors might also contribute to these inconsistent results[123]. Furthermore, the T allele of a non-synonymous SNP (C→T) located within exon 3 of *KLK10* that changes the amino acid from alanine to serine is associated with a higher prostate cancer risk[125].

GENETIC POLYMORPHISMS
Normal variant forms of a particular gene (that is, alleles) that are present in the population at a frequency of 1% or greater. Single-nucleotide polymorphisms are variations of a single base-pair position within a DNA sequence and are the most common form of genetic variation in human DNA.

LINKAGE DISEQUILIBRIUM
When alleles at two or more different genetic loci occur in gametes more frequently in the population than expected given the known allele frequencies and recombination fraction between the two loci. This indicates that the loci are tightly linked; that is, sufficiently close together on the same chromosome to be co-inherited 50% of the time.

Table 2 | **Possible clinical implications of kallikrein expression in human cancers**

Tumour	hK	Localization	Expression	Clinical relevance
ALL	10	NA	↓ expression due to ↑ methylation in ALL cell lines[75]	Unfavourable prognosis[75]
Breast	1	Cancer cells[162,163]	ND	ND
Breast	3	Cancer cells[164]; serum[165]	↓ expression in malignant tumour tissue[53]	Favourable prognosis[54,87,166]; predictive of response to tamoxifen[166]
Breast	5	Serum[35]	↓ expression in malignant tumours[61]; ↑ levels in serum of cancer patients[35]	Diagnosis[35]; unfavourable prognosis[167]
Breast	6	ND	↓ expression in metastatic tumours; ↑ expression in primary tumours[36]; ↓ expression in malignant tumours[61]	ND
Breast	7	ND	ND	Unfavourable prognosis[168]
Breast	8	ND	↓ expression in malignant tumours[61]	ND
Breast	9	ND	ND	Favourable prognosis[169]
Breast	10	ND	↓ expression in malignant tumours and cell lines[55,57,61]	Predictive of response to tamoxifen[170]
Breast	12	ND	↓ expression in malignant tumours[58]	ND
Breast	13	ND	↓ expression in malignant tumours and cell lines[59]	Favourable prognosis[171]
Breast	14	Cancer cells[48]; serum	↓ expression in malignant tumours and cell lines[60]; ↑ levels in serum[48] of cancer patients	Diagnosis[48]; unfavourable prognosis[172]
Breast	15	ND	ND	Favourable prognosis[173]
Cervix	8	Cancer cells[174]	↑ expression in cancer cell lines and primary tumour cultures[174]	ND
Colon	1	ND	↓ expression in malignant tumours[73]	ND
Colon	6, 8, 10	ND	↑ expression in malignant tumours[73]	ND
Lung	11	ND	↑ expression in a subgroup (cluster C2) of neuroendocrine tumours[71]	Unfavourable prognosis — worst outcome for C2 tumour subtype[71]
Ovary	2,3	ND	↑ expression in malignant tumours[52]	ND
Ovary	4	Cancer cells[33]	↑ expression in malignant tumours and cell lines[32,33]	Unfavourable prognosis[32]
Ovary	5	Serum[50]; ascites[50]	↑ expression in malignant tumours and cell lines[34,35,50,155,175]; ↑ levels in serum and ascites fluid in cancer patients[50]	Diagnosis[50]; unfavourable prognosis[34,176]
Ovary	6	Cancer cells[37,117]; serum[38,131]	↑ expression in malignant tumours and tumours of low malignant potential[36,37,50–52,117,175,177]; ↑ levels in serum of cancer patients[38,13]	Diagnosis[38,131]; unfavourable prognosis[131,178]; monitoring[38,131]
Ovary	7	ND	↑ expression in malignant tumours and cell lines[39,50,52,155,175]	Unfavourable prognosis[179]
Ovary	8	Cancer cells[40,180]; serum and ascites[42]	↑ expression in malignant tumours[40–42,50,52,175,180]; ↑ levels in serum of cancer patients[42]	Diagnosis[42]; favourable prognosis[41,42,180]; monitoring[42]
Ovary	9	Cancer cells[181]	ND	Favourable prognosis[181]
Ovary	10	Cancer cells[43]; serum[44,45]	↑ expression in malignant tumours[43,50–52,177]; ↑ levels in serum of cancer patients[44,45]	Diagnosis[44,45]; unfavourable prognosis[43,45]; monitoring[44]
Ovary	11	Cancer cells[46]	↑ expression in malignant tumours[50,52,182]; ↑ levels in serum of cancer patients[46]	Diagnosis[46]; favourable prognosis[183]; unfavourable prognosis[182]
Ovary	13	Ascites[47]	↑ expression in malignant tumours[47,52]	Favourable prognosis[184]
Ovary	14	Cancer cells[48]	↓ expression in malignant tumours[60]; ↑ expression in malignant tumours[48,50]; ↑ levels in serum of cancer patients[48]	Diagnosis[48]; favourable prognosis[185]
Ovary	15	ND	↑ expression in malignant tumours[49]	Unfavourable prognosis[49]
Pancreas	1	Cancer cells, fibroblasts, neutrophils and lymphocytes[109]	ND	ND
Pancreas	6	ND	↑ expression in malignant tumours[73]	ND
Pancreas	10	ND	↑ expression in malignant tumours[72,73]	ND
Prostate	2	Cancer cells[186]	↓ expression in malignant tumours[62]	Diagnosis[23]
Prostate	3	Cancer cells[65]	↓ expression in malignant tumours[62,65]	Population screening, diagnosis, prognosis,monitoring[23]
Prostate	4	Cancer cells[154,187–189]	↑ expression in malignant tumours[154]	ND
Prostate	5	ND	↓ expression in malignant tumours[66]	Favourable prognosis[66]
Prostate	6	ND	↓ expression in malignant tumours[68]	ND
Prostate	10	ND	↓ expression in malignant tumours[56,67,68]	ND
Prostate	11	ND	↑ expression in malignant tumours[159]; ↑ levels in serum of cancer patients[46]	Diagnosis[46,130]; favourable prognosis[159]
Prostate	13	Cancer cells[47]	↓ expression in malignant tumours[68]	ND
Prostate	14	Cancer cells[190]	↓ expression in malignant tumours[60]; ↑ expression in malignant tumours[191]	Unfavourable prognosis[191]
Prostate	15	ND	↑ expression in malignant tumours[192,193]	Unfavourable prognosis[192,193]
Head and neck SCC	10	ND	↑ expression in group 1 tumour subtype[74]	Unfavourable prognosis — worst outcome for group 1 tumour subtype[74]
Testicular	5	ND	↑ expression in malignant tumours[69]	Favourable prognosis[69]
Testicular	10	Cancer cells[70]	↓ expression in malignant tumours[70]	ND
Testicular	14	ND	↓ expression in malignant tumours[60]	ND

ALL, acute lymphoblastic leukaemia; hK, human tissue kallikrein; NA, not applicable; ND, not determined; SCC, squamous-cell carcinoma.

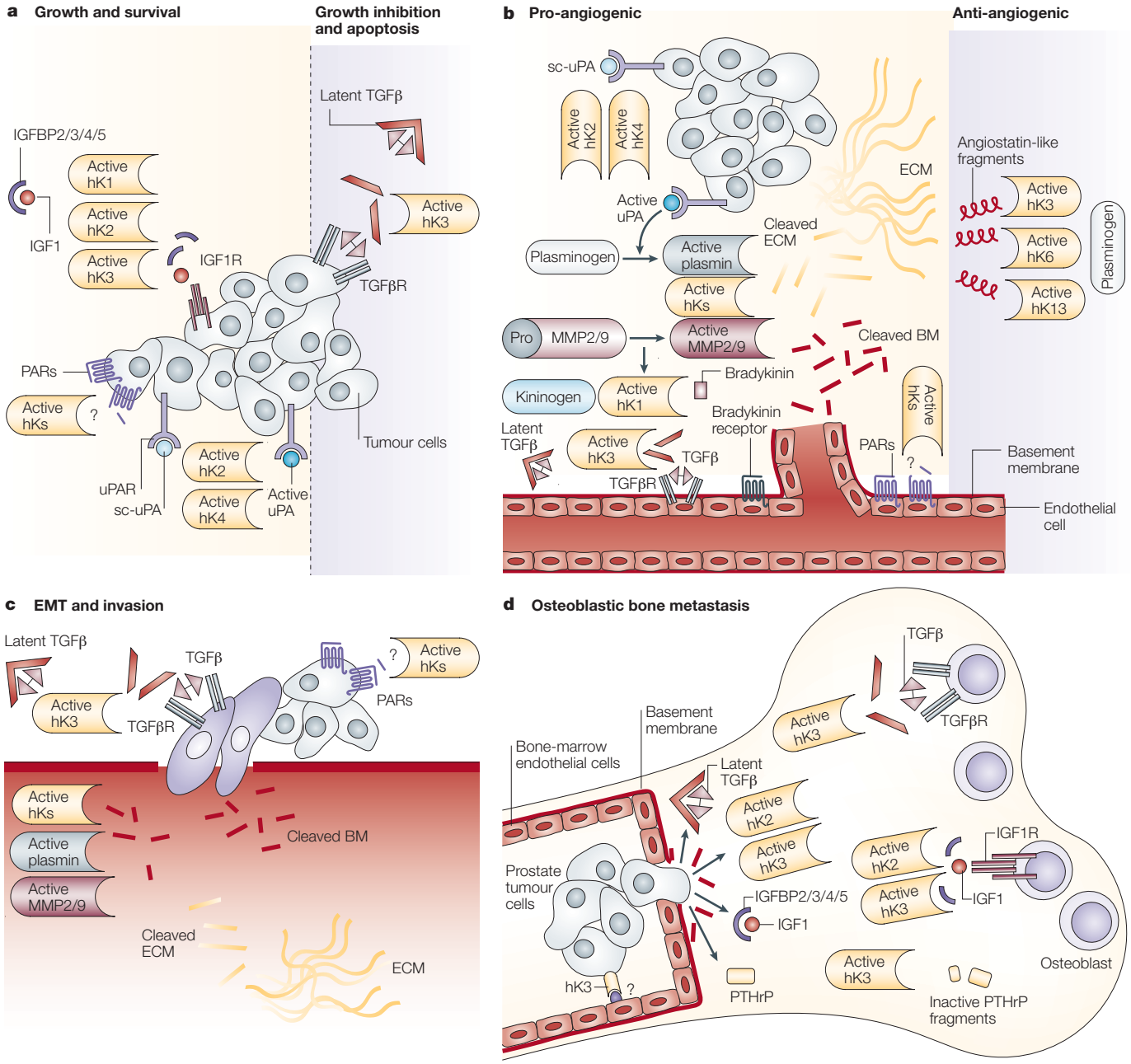


Figure 2 | **Putative roles of human tissue kallikreins in tumour development**. **a** | Human tissue kallikrein 1 (hK1), hK2 and hK3 might stimulate the growth and survival of tumour cells by degrading insulin-like growth factor binding proteins (IGFBP2, 3, 4 and 5), thereby liberating the mitogenic growth factor insulin-like growth factor 1 (IGF1), which binds to its cell-surface receptor (IGF1R) and induces cell proliferation and prevents apoptosis. hK2 and hK4 also activate the urokinase plasminogen activator–urokinase plasminogen activator receptor (uPA–uPAR) system, leading to release and/or activation of latent growth factors from the extracellular matrix (ECM). hKs could directly stimulate tumour-cell growth through protease-activated receptors (PARs). Conversely, hK3 could also suppress tumour growth by releasing transforming growth factor-β (TGFβ) from its latent complex, allowing it to bind to its receptor (TGFβR). **b** | hKs might promote angiogenesis by independently cleaving structural components of the subendothelial basement membrane (BM) and ECM. hKs also participate in a pericellular proteolytic cascade in which they activate the uPA–uPAR system (leading to plasmin activation) and matrix metalloproteinases (MMPs) to further promote ECM degradation, facilitating endothelial invasion and migration. hK3 activates latent TGFβ and hK1 releases bradykinin from kininogen, stimulating the angiogenic response through the bradykinin receptor. hKs might be implicated in PAR signalling that induces endothelial-cell proliferation. Conversely, hK3, hK6 and hK13 might also inhibit angiogenesis by generating angiostatin-like fragments from plasminogen. **c** | hK3 could indirectly promote epithelial-to-mesenchymal transition (EMT) by liberating TGFβ from its latent complex. Other hKs might directly and indirectly regulate invasion by dissolution of ECM barriers. hKs could activate PAR signalling, which can have a stimulatory or inhibitory effect on tumour-cell invasion. **d** | hKs could promote the formation of osteoblastic bone metastases in prostate cancer. hK3 might be involved in the preferential adherence of prostate cancer cells to the bone-marrow endothelium. Prostate tumours produce hKs , TGFβ and IGF1, which directly stimulate osteoblastic activity and subsequent bone formation. hK3 activates latent TGFβ and releases IGFs from IGFBPs. hK3 also inactivates parathyroid-hormone-related protein (PTHrP), an osteoclastic stimulator. sc-uPA, single-chain uPA

Similar to other tumour-suppressor genes, epigenetic silencing of the putative kallikrein tumour suppresor *KLK10* through DNA methylation is one mechanism responsible for its tumour-specific loss and/or downregulation in breast[126], prostate and ovarian carcinomas (C.A.B. and E.P.D, unpublished observations) and in acute lymphoblastic leukaemia[75]. Oncogenesis-associated DNA methylation usually occurs in CpG ISLANDS within promoters. Even though the *KLK10* proximal promoter lacks canonical CpG islands, methylation of individual CpG dinucleotides and CC(A/T)GG motifs in this region can still interfere with *KLK10* transcription in tumours[75,126].

Trans-acting regulatory factors might also have a role in modulating *KLK* transcription during tumorigenesis. Differential levels of several androgen-receptor co-regulatory activators and repressors, and associated proteins, have been observed among breast cancer cell lines displaying variable levels of the androgen-regulated *KLK2* and *KLK3* genes[127]. This indicates that under- or overexpression of these *trans*-acting factors might modulate the transcriptional activity of *KLK2* and *KLK3* during breast cancer tumorigenesis. Among the coactivators examined, SRC1 mRNA correlated with secreted hK3 protein levels in *KLK3*-positive cells, implicating SRC1 in the androgen-regulated transcription of *KLK3* (REF. 127). Furthermore, a defect or loss of *trans*-acting factors might partially account for the tumour-specific loss of *KLK10* mRNA expression in a subset of breast carcinomas, in addition to epigenetic silencing through hypermethylation[126].

A large and compelling body of epidemiological and experimental evidence implicates steroid hormones in the aetiology of hormone-responsive cancers, including breast, ovarian and prostate carcinomas. Steroid hormones regulate the expression of numerous growth factors, which, in turn, can mediate growth and differentiation signals. As *KLK* transcription is also controlled by steroid hormones, kallikreins might also represent downstream targets through which hormones drive tumour progression.

Clinical applications of kallikreins

Kallikreins as cancer biomarkers. Biological tumour markers are endogenous molecules (for example, DNA, mRNA and proteins) that can be measured in bodily fluids or tissues to indicate the presence of malignancy, evaluate cancer risk, assess prognosis and monitor response to therapy, to improve patient management and disease outcomes. Accumulating reports indicate that the tissue-kallikrein family will prove to be a rich source of tumour biomarkers, particularly for hormone-dependent malignancies, including prostate, breast, ovarian and testicular carcinomas (TABLE 2).

Among all the biomarkers identified so far, hK3/PSA has had the greatest impact in clinical medicine, for the screening, diagnosis, staging and monitoring of prostate cancer[128]. Several attempts have been made over the years to further enhance the clinical use of total serum hK3, given its lack of desired SENSITIVITY and SPECIFICITY at the conventional minimum threshold of 4 ng/mL for prostate cancer detection. These include the development of decision algorithms, such as hK3 velocity, density and age-specific reference ranges, and the measurement of different molecular forms of hK3, such as free hK3 (pro-hK3, N-terminally clipped forms of pro-hK3, active hK3, fragmented hK3, isoforms encoded by alternative *KLK3* transcripts), free/total hK3 ratio and hK3 complexed to inhibitors, for example, α_1-antichymotrypsin (α_1-ACT)[23]. In addition to hK3, serum hK2 (REF. 129) and hK11 (REFS 46,130) might also function as complementary biomarkers to hK3 for prostatic diseases. With respect to ovarian cancer, serum hK5, hK6, hK8, hK10, hK11 and hK14 represent potential biomarkers because of their increased levels in the serum of a subgroup of patients[35,38,42,44–46,48,131]. Notably, pre-surgical serum hK6 and hK10 levels can increase the diagnostic sensitivity of cancer antigen 125 (CA-125) in patients with early-stage (I/II) ovarian cancer and are associated with poor patient prognosis[45,131]. Therefore, serum hK6 and hK10 might complement CA-125, increasing the prospect of detecting ovarian cancer at an early, curable stage. In addition to their clinical value as serological biomarkers, kallikrein mRNA and protein expression within tumours is often associated with patient prognosis and might also predict a likely response to therapy, as listed in TABLE 2.

Unfortunately, no single marker or parameter can clearly detect or predict disease progression or outcome of individual cancer patients. The current trend is to merge the diagnostic/prognostic information provided by individual biomarkers into single-valued indices through multivariate algorithms, such as artificial neural networks. It is tempting to speculate that the combination of a subset of classical and/or variant kallikreins into a multiparametric panel might provide superior diagnostic/prognostic information than that of the single analytes alone.

Therapeutic uses of hK proteins. Given the roles of hKs in tumour progression, this subfamily of serine proteases might represent a promising new and untapped source of potential targets for the development of novel cancer therapeutics. So far, several therapeutic modalities that either inhibit or harness the activity and tissue specificity of hKs have been explored.

On the basis of numerous reports that incriminate hKs as factors that promote tumour growth through their proteolytic activity, the design of hK inhibitors as putative therapeutic agents for cancer treatment is under development. Cloutier *et al.* have recently described a novel method of generating highly specific serpins to hK2 by modifying their reactive-site loops[132]. In this approach, the reactive-site-loop cleavage site of α_1-ACT was replaced by phage display-selected pentapeptide substrates previously identified for hK2 (REF. 30), resulting in the production of several recombinant α_1-ACT inhibitors, some of which displayed unique reactivity to hK2, but not to other proteases. Given that serpins are endogenous proteins, their modified recombinant forms

CpG ISLANDS
Short stretches of DNA with an increased density of CpG dinucleotides relative to the bulk genome. Unmethylated CpG islands are positioned at the 5′ ends of many human genes. Aberrant methylation of CpG islands can cause gene silencing and contributes to carcinogenesis.

SENSITIVITY
Represents the number of patients who are positive for a test result (true positives) divided by the total number of patients with the disease (true positive plus false negatives).

SPECIFICITY
Represents the number of healthy individuals with a negative result (true negatives) divided by the total number of healthy individuals (true negative plus false positives).

are likely to be less toxic, less immunogenic and have lower clearance rates than small chemical inhibitors and might have therapeutic value in the future.

Other therapeutic strategies exploit hK activity and/or tissue specificity, including the development of hK3-activated prodrugs, delivery of toxic genes under control of *KLK3* promoter/enhancer elements and active immunotherapy using hK-based vaccines. As hK3 is highly expressed in the diseased prostate and its enzymatic activity is largely uninhibited within prostate tumour microenvironments[76,133], several latent 'prodrugs' — cytotoxic agents (for example, doxorubicin, vinblastine and thapsigargin) coupled to a peptide carrier through an hK3-cleavable bond — have been engineered[134–137] to target prostatic tumours. Indeed, preclinical studies indicate that these prodrugs are selectively toxic to hK3-producing prostate cancer cells *in vitro* and inhibit tumour growth in animals bearing hK3-producing LNCaP tumour xenografts *in vivo*, with relatively low or no host toxicity and mortality[134,136,137].

The specificity of hK3 for diseased prostate tissue has also been exploited in the development of cytoreductive gene-therapy approaches for the selective destruction of prostate tumours. The most common strategy has involved the construction of viral (for example, adenoviral) or non-viral (for example, liposomal) vectors containing a suicide gene encoding a prodrug-activating enzyme under the control of prostate-specific *KLK3* promoter and/or enhancer elements. Preclinical investigations have shown that the prostate-specific *KLK3* promoter/enhancer is able to selectively deliver and stimulate expression of suicide genes within hK3-producing prostate cancer cells, resulting in subsequent activation of a given prodrug followed by prostate cancer cell death *in vitro* and inhibition of prostate tumour growth in xenografted mice *in vivo*[138,139].

Another potential anticancer strategy involves the development of hK3-based vaccines for active immunotherapy against prostate tumours. Phase I clinical trials have been conducted to evaluate vaccines consisting of recombinant vaccinia viruses expressing hK3 (REF. 140), *KLK3*-mRNA-transfected dendritic cells[141] and hK3-pulsed dendritic cells[142] in men with metastatic prostate cancer and/or in men with prostate cancer who have undergone prostatectomy and who are in biochemical relapse. These vaccines were able to stimulate hK3-specific T-cell responses without severe toxicity or serious adverse effects.

Perspectives

The hK family of serine proteases harbours many new players in cancer proteolysis, the roles of which are just emerging. Kallikrein expression is dysregulated in diverse cancer types. Numerous *in vitro* and *in vivo* studies have indicated that hKs act on ECM and non-ECM (for example, growth factors, other proteases and receptors) proteins within the tumour microenvironment, exerting stimulatory or inhibitory effects on many phases of tumour progression. Several lines of evidence indicate that hKs participate in important proteolytic networks (cascades) in collaboration with proteases of serine and other catalytic classes. Based on the diverse expression patterns of hKs and putative *in vitro* substrates, hKs probably orchestrate a wide range of normal physiological processes as well. So far, most putative hK targets and mechanisms have been discovered within *in vitro* systems, which might or might not reflect their *in vivo* functions. One of the greatest challenges for the future of hK research is the identification of biologically relevant substrates and definitive *in vivo* roles in physiological and pathological processes. To this end, future investigations will involve combinatorial substrate libraries, phage-display technologies and proteomics-based strategies.

Additional future prospects include the continued evaluation of kallikreins as tumour markers for diagnosis, prognosis and monitoring of patients with cancer and as targets for anticancer therapies. With respect to biomarker development, the best approach might be to incorporate certain kallikrein genes/proteins and/or their alternative mRNA/protein isoforms into useful multiparametric panels with other potential tumour markers. Results of preclinical and clinical investigations of hK-based anitneoplastic treatments indicate that hKs represent promising therapeutic targets. Most antitumour strategies have focused on one kallikrein, hK3, so far. Future directions for cancer therapy will use other hKs as well. In addition to hKs, many other proteases such as MMPs, uPA, plasmin and prostasin have already been identified as attractive targets for the development of anticancer therapies. In summary, the functional and clinical implications of hK proteolysis and crosstalk with other cancer-associated proteases and other molecules/pathways in the onset and/or progression of malignant disease will certainly be a key focus of future cancer research.

1. Liotta, L. A. & Kohn, E. C. The microenvironment of the tumour–host interface. *Nature* **411**, 375–379 (2001).
2. Puente, X. S., Sanchez, L. M., Overall, C. M. & Lopez-Otin, C. Human and mouse proteases: a comparative genomic approach. *Nature Rev. Genet.* **4**, 544–558 (2003).
The first study to compare the protease repertoire (degradome) within human and mouse genomes and to report that the *KLK* locus is the largest cluster of contiguous protease genes within the human genome.
3. Werb, Z. ECM and cell surface proteolysis: regulating cellular ecology. *Cell* **91**, 439–442 (1997).
4. Hara, M., Koyanagi, Y., Inoue, T. & Fukuyama, T. [Some physico-chemical characteristics of "-seminoprotein", an antigenic component specific for human seminal plasma. Forensic immunological study of body fluids and secretion. VII.] *Nippon Hoigaku Zasshi* **25**, 322–324 (1971).
5. Riegman, P. H. *et al.* The prostate-specific antigen gene and the human glandular kallikrein-1 gene are tandemly located on chromosome 19. *FEBS Lett.* **247**, 123–126 (1989).
6. Diamandis, E. P. *et al.* New nomenclature for the human tissue kallikrein gene family. *Clin. Chem.* **46**, 1855–1858 (2000).
This paper describes the current official nomenclature used to denote human kallikrein genes and proteins and the rationale behind its use.
7. Yousef, G. M., Chang, A., Scorilas, A. & Diamandis, E. P. Genomic organization of the human kallikrein gene family on chromosome 19q13.3-q13.4. *Biochem. Biophys. Res. Commun.* **276**, 125–133 (2000).
The first report to describe the topology and organization of the extended human kallikrein locus on 19q13.4; later confirmed by others (reference 8).
8. Harvey, T. J. *et al.* Tissue-specific expression patterns and fine mapping of the human kallikrein (KLK) locus on proximal 19q13.4. *J. Biol. Chem.* **275**, 37397–37406 (2000).
9. Cleutjens, K. B., van Eekelen, C. C., van der Korput, H. A., Brinkmann, A. O. & Trapman, J. Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. *J. Biol. Chem.* **271**, 6379–6388 (1996).

10. Smith, M. S., Lechago, J., Wines, D. R., MacDonald, R. J. & Hammer, R. E. Tissue-specific expression of kallikrein family transgenes in mice and rats. *DNA Cell Biol.* **11**, 345–358 (1992).
11. Mikolajczyk, S. D. *et al.* Ala217 is important for the catalytic function and autoactivation of prostate-specific human kallikrein 2. *Eur. J. Biochem.* **246**, 440–446 (1997).
12. Magklara, A. *et al.* Characterization of the enzymatic activity of human kallikrein 6: autoactivation, substrate specificity, and regulation by inhibitors. *Biochem. Biophys. Res. Commun.* **307**, 948–955 (2003).
13. Sotiropoulou, G. *et al.* Emerging interest in the kallikrein gene family for understanding and diagnosing cancer. *Oncol. Res.* **13**, 381–391 (2003).
14. Lovgren, J., Rajakoski, K., Karp, M., Lundwall, A. & Lilja, H. Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2. *Biochem. Biophys. Res. Commun.* **238**, 549–555 (1997).
15. Takayama, T. K., McMullen, B. A., Nelson, P. S., Matsumura, M. & Fujikawa, K. 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 (2001).
16. Takayama, T. K., Carter, C. A. & Deng, T. Activation of prostate-specific antigen precursor (pro-PSA) by prostin, a novel human prostatic serine protease identified by degenerate PCR. *Biochemistry* **40**, 1679–1687 (2001).
17. Caubet, C. *et al.* 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 (2004).
The first report to demonstrate that an hK cascade might regulate skin desquamation.
18. Takayama, T. K., Fujikawa, K. & Davie, E. W. Characterization of the precursor of prostate-specific antigen. Activation by trypsin and by human glandular kallikrein. *J. Biol. Chem.* **272**, 21582–21588 (1997).
19. Takada, Y., Skidgel, R. A. & Erdos, E. G. Purification of human urinary prokallikrein. Identification of the site of activation by the metalloproteinase thermolysin. *Biochem. J.* **232**, 851–858 (1985).
20. Heeb, M. J. & Espana, F. α2-macroglobulin and C1-inactivator are plasma inhibitors of human glandular kallikrein. *Blood Cells Mol. Dis.* **24**, 412–419 (1998).
21. Christensson, A., Laurell, C. B. & Lilja, H. Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. *Eur. J. Biochem.* **194**, 755–763 (1990).
22. Komatsu, N. *et al.* Expression and localization of tissue kallikrein mRNAs in human epidermis and appendages. *J. Invest. Dermatol.* **121**, 542–549 (2003).
23. Rittenhouse, H. G., Finlay, J. A., Mikolajczyk, S. D. & Partin, A. W. 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 (1998).
An extensive review on hk2 and hK3/PSA that describes their discovery, isolation, biochemical characteristics and clinical applications.
24. Bayes, A. *et al.* Human kallikrein 6 activity is regulated via an autoproteolytic mechanism of activation/inactivation. *Biol. Chem.* **385**, 517–524 (2004).
25. Hansson, L. *et al.* Cloning, expression, and characterization of stratum corneum chymotryptic enzyme. A skin-specific human serine proteinase. *J. Biol. Chem.* **269**, 19420–19426 (1994).
26. Lovgren, J., Airas, K. & Lilja, H. Enzymatic action of human glandular kallikrein 2 (hK2). Substrate specificity and regulation by Zn^{2+} and extracellular protease inhibitors. *Eur. J. Biochem.* **262**, 781–789 (1999).
27. Malm, J., Hellman, J., Hogg, P. & Lilja, H. Enzymatic action of prostate-specific antigen (PSA or hK3): substrate specificity and regulation by Zn^{2+}, a tight-binding inhibitor. *Prostate* **45**, 132–139 (2000).
28. Deperthes, D. Phage display substrate: a blind method for determining protease specificity. *Biol. Chem.* **383**, 1107–1112 (2002).
A comprehensive review describing the feasibility of using phage display for the identification of protease specificity and putative substrates.
29. Harris, J. L. *et al.* Rapid and general profiling of protease specificity by using combinatorial fluorogenic substrate libraries. *Proc. Natl Acad. Sci. USA* **97**, 7754–7759 (2000).
This paper describes the preparation and use of exhaustive fluorogenic tetrapeptide substrate libraries to identify the N-terminal substrate specificities of proteases.
30. Cloutier, S. M. *et al.* Substrate specificity of human kallikrein 2 (hK2) as determined by phage display technology. *Eur. J. Biochem.* **269**, 2747–2754 (2002).
31. Coombs, G. S. *et al.* Substrate specificity of prostate-specific antigen (PSA). *Chem. Biol.* **5**, 475–488 (1998).
32. Obiezu, C. V. *et al.* Higher human kallikrein gene 4 (klk4) expression indicates poor prognosis of ovarian cancer patients. *Clin. Cancer Res.* **7**, 2380–2386 (2001).
33. Dong, Y. *et al.* Human kallikrein 4 (KLK4) is highly expressed in serous ovarian carcinomas. *Clin. Cancer Res.* **7**, 2363–2371 (2001).
34. Kim, H. *et al.* Human kallikrein gene 5 (*KLK5*) expression is an indicator of poor prognosis in ovarian cancer. *Br. J. Cancer* **84**, 643–650 (2001).
35. Yousef, G. M. *et al.* Human kallikrein 5: a potential novel serum biomarker for breast and ovarian cancer. *Cancer Res.* **63**, 3958–3965 (2003).
36. Anisowicz, A., Sotiropoulou, G., Stenman, G., Mok, S. C. & Sager, R. A novel protease homolog differentially expressed in breast and ovarian cancer. *Mol. Med.* **2**, 624–636 (1996).
37. Tanimoto, H., Underwood, L. J., Shigemasa, K., Parmley, T. H. & O'Brien, T. J. Increased expression of protease M in ovarian tumors. *Tumour Biol.* **22**, 11–18 (2001).
38. Diamandis, E. P., Yousef, G. M., Soosaipillai, A. R. & Bunting, P. Human kallikrein 6 (zyme/protease M/neurosin): a new serum biomarker of ovarian carcinoma. *Clin. Biochem.* **33**, 579–583 (2000).
This study was among the first to show that a member of the extended kallikrein family might be a promising cancer biomarker.
39. Tanimoto, H. *et al.* The stratum corneum chymotryptic enzyme that mediates shedding and desquamation of skin cells is highly overexpressed in ovarian tumor cells. *Cancer* **86**, 2074–2082 (1999).
40. Underwood, L. J. *et al.* Cloning of tumor-associated differentially expressed gene-14, a novel serine protease overexpressed by ovarian carcinoma. *Cancer Res.* **59**, 4435–4439 (1999).
41. Magklara, A. *et al.* The human KLK8 (neuropsin/ovasin) gene: identification of two novel splice variants and its prognostic value in ovarian cancer. *Clin. Cancer Res.* **7**, 806–811 (2001).
42. Kishi, T. *et al.* Human kallikrein 8, a novel biomarker for ovarian carcinoma. *Cancer Res.* **63**, 2771–2774 (2003).
43. Luo, L. Y. *et al.* Prognostic value of human kallikrein 10 expression in epithelial ovarian carcinoma. *Clin. Cancer Res.* **7**, 2372–2379 (2001).
44. Luo, L., Bunting, P., Scorilas, A. & Diamandis, E. P. Human kallikrein 10: a novel tumor marker for ovarian carcinoma? *Clin. Chim. Acta* **306**, 111–118 (2001).
45. Luo, L. Y. *et al.* The serum concentration of human kallikrein 10 represents a novel biomarker for ovarian cancer diagnosis and prognosis. *Cancer Res.* **63**, 807–811 (2003).
46. Diamandis, E. P. *et al.* Human kallikrein 11: a new biomarker of prostate and ovarian carcinoma. *Cancer Res.* **62**, 295–300 (2002).
47. Kapadia, C. *et al.* Human kallikrein 13: production and purification of recombinant protein and monoclonal and polyclonal antibodies, and development of a sensitive and specific immunofluorometric assay. *Clin. Chem.* **49**, 77–86 (2003).
48. Borgono, C. A. *et al.* Human kallikrein 14: a new potential biomarker for ovarian and breast cancer. *Cancer Res.* **63**, 9032–9041 (2003).
49. Yousef, G. M. *et al.* Prognostic value of the human kallikrein gene 15 expression in ovarian cancer. *J. Clin. Oncol.* **21**, 3119–3126 (2003).
50. Yousef, G. M. *et al.* Parallel overexpression of seven kallikrein genes in ovarian cancer. *Cancer Res.* **63**, 2223–2227 (2003).
51. Welsh, J. B. *et al.* Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. *Proc. Natl Acad. Sci. USA* **100**, 3410–3415 (2003).
52. Adib, T. R. *et al.* Predicting biomarkers for ovarian cancer using gene-expression microarrays. *Br. J. Cancer* **90**, 686–692 (2004).
53. Yu, H. *et al.* Prostate specific antigen in breast cancer, benign breast disease and normal breast tissue. *Breast Cancer Res. Treat.* **40**, 171–178 (1996).
54. Yu, H., Levesque, M. A., Clark, G. M. & Diamandis, E. P. Prognostic value of prostate-specific antigen for women with breast cancer: a large United States cohort study. *Clin. Cancer Res.* **4**, 1489–1497 (1998).
55. Liu, X. L., Wazer, D. E., Watanabe, K. & Band, V. Identification of a novel serine protease-like gene, the expression of which is down-regulated during breast cancer progression. *Cancer Res.* **56**, 3371–3379 (1996).
56. Goyal, J. *et al.* The role for NES1 serine protease as a novel tumor suppressor. *Cancer Res.* **58**, 4782–4786 (1998).
57. Dhar, S. *et al.* Analysis of normal epithelial cell specific-1 (NES1)/Kallikrein 10 mRNA expression by *in situ* hybridization, a novel marker for breast cancer. *Clin. Cancer Res.* **7**, 3393–3398 (2001).
58. Yousef, G. M., Magklara, A. & Diamandis, E. P. KLK12 is a novel serine protease and a new member of the human kallikrein gene family-differential expression in breast cancer. *Genomics* **69**, 331–341 (2000).
59. Yousef, G. M., Chang, A. & Diamandis, E. P. Identification and characterization of KLK-L4, a new kallikrein-like gene that appears to be down-regulated in breast cancer tissues. *J. Biol. Chem.* **275**, 11891–11898 (2000).
60. Yousef, G. M. *et al.* Cloning of a new member of the human kallikrein gene family, *KLK14*, which is down-regulated in different malignancies. *Cancer Res.* **61**, 3425–3431 (2001).
61. Yousef, G. M. *et al.* Kallikrein gene downregulation in breast cancer. *Br. J. Cancer* **90**, 167–172 (2004).
62. Magklara, A. *et al.* Decreased concentrations of prostate-specific antigen and human glandular kallikrein 2 in malignant versus nonmalignant prostatic tissue. *Urology* **56**, 527–532 (2000).
63. Abrahamsson, P. A., Lilja, H., Falkmer, S. & Wadstrom, L. B. Immunohistochemical distribution of the three predominant secretory proteins in the parenchyma of hyperplastic and neoplastic prostate glands. *Prostate* **12**, 39–46 (1988).
64. Pretlow, T. G. *et al.* Tissue concentrations of prostate-specific antigen in prostatic carcinoma and benign prostatic hyperplasia. *Int. J. Cancer* **49**, 645–649 (1991).
65. Hakalahti, L. *et al.* Evaluation of *PAP* and *PSA* gene expression in prostatic hyperplasia and prostatic carcinoma using northern-blot analyses, *in situ* hybridization and immunohistochemical stainings with monoclonal and bispecific antibodies. *Int. J. Cancer* **55**, 590–597 (1993).
66. Yousef, G. M. *et al.* Down-regulation of the human kallikrein gene 5 (*KLK5*) in prostate cancer tissues. *Prostate* **51**, 126–132 (2002).
67. Luo, L. Y. & Diamandis, E. P. Down-regulation of the normal epithelial cell–specific 1 (*NES1*) gene is associated with unfavorable outcome of prostate cancer. *Clin. Biochem.* **33**, 237 (2000).
68. Petraki, C. D. *et al.* Immunohistochemical localization of human kallikreins 6, 10 and 13 in benign and malignant prostatic tissues. *Prostate Cancer Prostatic Dis.* **6**, 223–227 (2003).
69. Yousef, G. M. *et al.* Differential expression of Kallikrein gene 5 in cancerous and normal testicular tissues. *Urology* **60**, 714–718 (2002).
70. Luo, L. Y., Rajpert-De Meyts, E. R., Jung, K. & Diamandis, E. P. Expression of the normal epithelial cell-specific 1 (NES1; KLK10) candidate tumour suppressor gene in normal and malignant testicular tissue. *Br. J. Cancer* **85**, 220–224 (2001).
71. Bhattacharjee, A. *et al.* Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proc. Natl Acad. Sci. USA* **98**, 13790–13795 (2001).
72. Iacobuzio-Donahue, C. A. *et al.* Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive characterization and comparison of the transcription profiles obtained from three major technologies. *Cancer Res.* **63**, 8614–8622 (2003).
73. Yousef, G. M. *et al. In-silico* analysis of kallikrein gene expression in pancreatic and colon cancers. *AntiCancer Res.* **24**, 43–51 (2004).
74. Chung, C. H. *et al.* Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. *Cancer Cell* **5**, 489–500 (2004).
75. Roman-Gomez, J. *et al.* The normal epithelial cell-specific 1 (*NES1*) gene, a candidate tumor suppressor gene on chromosome 19q13.3-4, is downregulated by hypermethylation in acute lymphoblastic leukemia. *Leukemia* **18**, 362–365 (2004).
76. Denmeade, S. R., Sokoll, L. J., Chan, D. W., Khan, S. R. & Isaacs, J. T. Concentration of enzymatically active prostate-specific antigen (PSA) in the extracellular fluid of primary human prostate cancers and human prostate cancer xenograft models. *Prostate* **48**, 1–6 (2001).
77. Rajah, R., Valentinis, B. & Cohen, P. Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-β1 on programmed cell death through a p53- and IGF-independent mechanism. *J. Biol. Chem.* **272**, 12181–12188 (1997).
78. Cohen, P. *et al.* Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. *J. Clin. Endocrinol. Metab.* **75**, 1046–1053 (1992).
One of the first reports to show that kallikreins might be involved in tumour progression by modulating tumour-cell growth *in vitro* and *in vivo* through growth factors.
79. Rehault, S. *et al.* Insulin-like growth factor binding proteins (IGFBPs) as potential physiological substrates for human

kallikreins hK2 and hK3. *Eur. J. Biochem.* **268**, 2960–2968 (2001).
80. Sutkowski, D. M. *et al.* Growth regulation of prostatic stromal cells by prostate-specific antigen. *J. Natl Cancer Inst.* **91**, 1663–1669 (1999).
81. Sun, X. Y., Donald, S. P. & Phang, J. M. Testosterone and prostate specific antigen stimulate generation of reactive oxygen species in prostate cancer cells. *Carcinogenesis* **22**, 1775–1780 (2001).
82. Frenette, G., Tremblay, R. R., Lazure, C. & Dube, J. Y. Prostatic kallikrein hK2, but not prostate-specific antigen (hK3), activates single-chain urokinase-type plasminogen activator. *Int. J. Cancer* **71**, 897–899 (1997).
83. Mikolajczyk, S. D., Millar, L. S., Kumar, A. & Saedi, M. S. Prostatic human kallikrein 2 inactivates and complexes with plasminogen activator inhibitor-1. *Int. J. Cancer* **81**, 438–442 (1999).
84. D'Andrea, M. R., Derian, C. K., Santulli, R. J. & Andrade-Gordon, P. Differential expression of protease-activated receptors-1 and-2 in stromal fibroblasts of normal, benign, and malignant human tissues. *Am. J. Pathol.* **158**, 2031–2041 (2001).
85. Ohta, T. *et al.* Protease-activated receptor-2 expression and the role of trypsin in cell proliferation in human pancreatic cancers. *Int. J. Oncol.* **23**, 61–66 (2003).
86. Lai, L. C., Erbas, H., Lennard, T. W. & Peaston, R. T. Prostate-specific antigen in breast cyst fluid: possible role of prostate-specific antigen in hormone-dependent breast cancer. *Int. J. Cancer* **66**, 743–746 (1996).
87. Yu, H. *et al.* Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. *Cancer Res.* **55**, 2104–2110 (1995).
88. Derynck, R., Akhurst, R. J. & Balmain, A. TGF-β signaling in tumor suppression and cancer progression. *Nature Genet.* **29**, 117–129 (2001).
89. Fortier, A. H., Nelson, B. J., Grella, D. K. & Holaday, J. W. Antiangiogenic activity of prostate-specific antigen. *J. Natl Cancer Inst.* **91**, 1635–1640 (1999).
The first paper to illustrate that a kallikrein family member might be involved in the regulation of angiogenesis *in vivo*.
90. Denmeade, S. R., Litvinov, I., Sokoll, L. J., Lilja, H. & Isaacs, J. T. 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 (2003).
91. Hanahan, D. & Folkman, J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* **86**, 353–364 (1996).
92. Deperthes, D. *et al.* Potential involvement of kallikrein hK2 in the hydrolysis of the human seminal vesicle proteins after ejaculation. *J. Androl.* **17**, 659–665 (1996).
93. Lilja, H. A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. *J. Clin. Invest.* **76**, 1899–1903 (1985).
94. Watt, K. W., Lee, P. J., M'Timkulu, T., Chan, W. P. & Loor, R. Human prostate-specific antigen: structural and functional similarity with serine proteases. *Proc. Natl Acad. Sci. USA* **83**, 3166–3170 (1986).
95. Webber, M. M., Waghray, A. & Bello, D. Prostate-specific antigen, a serine protease, facilitates human prostate cancer cell invasion. *Clin. Cancer Res.* **1**, 1089–1094 (1995).
96. Bernett, M. J. *et al.* 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 (2002).
Together with references 147 and 148, this paper describes the three-dimensional trypsin-like fold of human kallikreins as determined by X-ray cystallography.
97. Barrett, A. D., Rawlings, N. D. & Woessner, J. F. (eds) *Handbook of Proteolytic Enzymes.* 1556–1558 (Elsevier Academic, London, 2004).
A comprehensive textbook that categorizes and describes all known proteases.
98. Tschesche, H., Michaelis, J., Kohnert, U., Fedrowitz, J. & Oberhoff, R. Tissue kallikrein effectively activates latent matrix degrading metalloenzymes. *Adv. Exp. Med. Biol.* **247A**, 545–548 (1989).
99. Desrivieres, S. *et al.* Activation of the 92 kDa type IV collagenase by tissue kallikrein. *J. Cell Physiol.* **157**, 587–593 (1993).
100. Menashi, S. *et al.* Regulation of 92-kDa gelatinase B activity in the extracellular matrix by tissue kallikrein. *Ann. NY Acad. Sci.* **732**, 466–468 (1994).
101. Killian, C. S., Corral, D. A., Kawinski, E. & Constantine, R. I. Mitogenic response of osteoblast cells to prostate-specific antigen suggests an activation of latent TGF-β and a proteolytic modulation of cell adhesion receptors. *Biochem. Biophys. Res. Commun.* **192**, 940–947 (1993).
102. Plendl, J. *et al.* Expression of tissue kallikrein and kinin receptors in angiogenic microvascular endothelial cells. *Biol. Chem.* **381**, 1103–1115 (2000).
103. Emanueli, C. *et al.* Local delivery of human tissue kallikrein gene accelerates spontaneous angiogenesis in mouse model of hindlimb ischemia. *Circulation* **103**, 125–132 (2001).
104. Jin, E. *et al.* Protease-activated receptor (PAR)-1 and PAR-2 participate in the cell growth of alveolar capillary endothelium in primary lung adenocarcinomas. *Cancer* **97**, 703–713 (2003).
105. Heidtmann, H. H. *et al.* Generation of angiostatin-like fragments from plasminogen by prostate-specific antigen. *Br. J. Cancer* **81**, 1269–1273 (1999).
106. Fortier, A. H. *et al.* Recombinant prostate specific antigen inhibits angiogenesis *in vitro* and *in vivo*. *Prostate* **56**, 212–219 (2003).
107. Papadopoulos, I., Sivridis, E., Giatromanolaki, A. & Koukourakis, M. I. Tumor angiogenesis is associated with MUC1 overexpression and loss of prostate-specific antigen expression in prostate cancer. *Clin. Cancer Res.* **7**, 1533–1538 (2001).
108. Hoffman, J. A. *et al.* Progressive vascular changes in a transgenic mouse model of squamous cell carcinoma. *Cancer Cell* **4**, 383–391 (2003).
109. Wolf, W. C., Evans, D. M., Chao, L. & Chao, J. A synthetic tissue kallikrein inhibitor suppresses cancer cell invasiveness. *Am. J. Pathol.* **159**, 1797–1805 (2001).
110. Ishii, K. *et al.* Evidence that the prostate-specific antigen (PSA)/Zn^{2+} axis may play a role in human prostate cancer cell invasion. *Cancer Lett.* **207**, 79–87 (2004).
111. Henrikson, K. P., Salazar, S. L., Fenton, J. W. & Pentecost, B. T. Role of thrombin receptor in breast cancer invasiveness. *Br. J. Cancer* **79**, 401–406 (1999).
112. Kamath, L., Meydani, A., Foss, F. & Kuliopulos, A. Signaling from protease-activated receptor-1 inhibits migration and invasion of breast cancer cells. *Cancer Res.* **61**, 5933–5940 (2001).
113. Romanov, V. I., Whyard, T., Adler, H. L., Waltzer, W. C. & Zucker, S. Prostate cancer cell adhesion to bone marrow endothelium: the role of prostate-specific antigen. *Cancer Res.* **64**, 2083–2089 (2004).
114. Yonou, H. *et al.* Prostate-specific antigen induces osteoplastic changes by an autonomous mechanism. *Biochem. Biophys. Res. Commun.* **289**, 1082–1087 (2001).
115. Iwamura, M., Hellman, J., Cockett, A. T., Lilja, H. & Gershagen, S. Alteration of the hormonal bioactivity of parathyroid hormone-related protein (PTHrP) as a result of limited proteolysis by prostate-specific antigen. *Urology* **48**, 317–325 (1996).
116. Cramer, S. D., Chen, Z. & Peehl, D. M. Prostate specific antigen cleaves parathyroid hormone-related protein in the PTH-like domain: inactivation of PTHrP-stimulated cAMP accumulation in mouse osteoblasts. *J. Urol.* **156**, 526–531 (1996).
117. Ni, X. *et al.* Characterisation of human kallikrein 6/protease M expression in ovarian cancer. *Br. J. Cancer* **91**, 725–731 (2004).
118. Majumdar, S. & Diamandis, E. P. The promoter and the enhancer region of the KLK 3 (prostate specific antigen) gene is frequently mutated in breast tumours and in breast carcinoma cell lines. *Br. J. Cancer* **79**, 1594–1602 (1999).
119. Tsuyuki, D., Grass, L. & Diamandis, E. P. Frequent detection of mutations in the 5′ flanking region of the prostate-specific antigen gene in female breast cancer. *Eur. J. Cancer* **33**, 1851–1854 (1997).
120. Bharaj, B. *et al.* Breast cancer prognostic significance of a single nucleotide polymorphism in the proximal androgen response element of the prostate specific antigen gene promoter. *Breast Cancer Res. Treat.* **61**, 111–119 (2000).
121. Xue, W. *et al.* Susceptibility to prostate cancer: interaction between genotypes at the androgen receptor and prostate-specific antigen loci. *Cancer Res.* **60**, 839–841 (2000).
122. Chiang, C. H., Chen, K. K., Chang, L. S. & Hong, C. J. The impact of polymorphism on prostate specific antigen gene on the risk, tumor volume and pathological stage of prostate cancer. *J. Urol.* **171**, 1529–1532 (2004).
123. Wang, L. Z. *et al.* Polymorphisms in prostate-specific antigen (*PSA*) gene, risk of prostate cancer, and serum PSA levels in Japanese population. *Cancer Lett.* **202**, 53–59 (2003).
124. Cramer, S. D. *et al.* Association between genetic polymorphisms in the prostate-specific antigen gene promoter and serum prostate-specific antigen levels. *J. Natl. Cancer Inst.* **95**, 1044–1053 (2003).
125. Bharaj, B. B., Luo, L. Y., Jung, K., Stephan, C. & Diamandis, E. P. Identification of single nucleotide polymorphisms in the human kallikrein 10 (*KLK10*) gene and their association with prostate, breast, testicular, and ovarian cancers. *Prostate* **51**, 35–41 (2002).
126. Li, B. *et al.* CpG methylation as a basis for breast tumor-specific loss of NES1/kallikrein 10 expression. *Cancer Res.* **61**, 8014–8021 (2001).
The first study to propose an epigenetic mechanism for dysregulated kallikrein expression during tumorigenesis.
127. Magklara, A., Brown, T. J. & Diamandis, E. P. 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 (2002).
128. Stamey, T. A. *et al.* Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. *N. Engl. J. Med.* **317**, 909–916 (1987).
129. Stenman, U. H. New ultrasensitive assays facilitate studies on the role of human glandular kallikrein (hK2) as a marker for prostatic disease. *Clin. Chem.* **45**, 753–754 (1999).
130. Nakamura, T. *et al.* The usefulness of serum human kallikrein 11 for discriminating between prostate cancer and benign prostatic hyperplasia. *Cancer Res.* **63**, 6543–6546 (2003).
131. Diamandis, E. P. *et al.* Human kallikrein 6 (hK6): a new potential serum biomarker for diagnosis and prognosis of ovarian carcinoma. *J. Clin. Oncol.* **21**, 1035–1043 (2003).
132. Cloutier, S. M. *et al.* Development of recombinant inhibitors specific to human kallikrein 2 using phage-display selected substrates. *Eur. J. Biochem.* **271**, 607–613 (2004).
This paper describes a novel approach for designing kallikrein-specific inhibitors, which might be useful in anticancer therapies.
133. Denmeade, S. R. *et al.* Specific and efficient peptide substrates for assaying the proteolytic activity of prostate-specific antigen. *Cancer Res.* **57**, 4924–4930 (1997).
134. Denmeade, S. R. *et al.* Enzymatic activation of a doxorubicin-peptide prodrug by prostate-specific antigen. *Cancer Res.* **58**, 2537–2540 (1998).
135. DiPaola, R. S. *et al.* Characterization of a novel prostate-specific antigen-activated peptide-doxorubicin conjugate in patients with prostate cancer. *J. Clin. Oncol.* **20**, 1874–1879 (2002).
136. DeFeo-Jones, D. *et al.* A prostate-specific antigen (PSA)-activated vinblastine prodrug selectively kills PSA-secreting cells *in vivo*. *Mol. Cancer Ther.* **1**, 451–459 (2002).
137. Denmeade, S. R. *et al.* Prostate-specific antigen-activated thapsigargin prodrug as targeted therapy for prostate cancer. *J. Natl. Cancer Inst.* **95**, 990–1000 (2003).
138. Latham, J. P., Searle, P. F., Mautner, V. & James, N. D. Prostate-specific antigen promoter/enhancer driven gene therapy for prostate cancer: construction and testing of a tissue-specific adenovirus vector. *Cancer Res.* **60**, 334–341 (2000).
139. Suzuki, S. *et al.* Liposome-mediated gene therapy using HSV-TK/ganciclovir under the control of human PSA promoter in prostate cancer cells. *Urol. Int.* **67**, 216–223 (2001).
140. Eder, J. P. *et al.* A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. *Clin. Cancer Res.* **6**, 1632–1638 (2000).
141. Heiser, A. *et al.* Human dendritic cells transfected with renal tumor RNA stimulate polyclonal T-cell responses against antigens expressed by primary and metastatic tumors. *Cancer Res.* **61**, 3388–3393 (2001).
142. Barrou, B. *et al.* Vaccination of prostatectomized prostate cancer patients in biochemical relapse, with autologous dendritic cells pulsed with recombinant human PSA. *Cancer Immunol. Immunother.* **53**, 453–460 (2004).
143. Diamandis, E. P., Yousef, G. M., Luo, L. Y., Magklara, A. & Obiezu, C. V. The new human kallikrein gene family: implications in carcinogenesis. *Trends Endocrinol. Metab.* **11**, 54–60 (2000).
144. Yousef, G. M. & Diamandis, E. P. The new human tissue kallikrein gene family: structure, function, and association to disease. *Endocr. Rev.* **22**, 184–204 (2001).
A comprehensive review on the genomic characteristics and clinical usefulness of the extended human kallikrein family.
145. Clements, J. A., Willemsen, N. M., Myers, S. A. & Dong, Y. The tissue kallikrein family of serine proteases: functional roles in human disease and potential as clinical biomarkers. *Crit. Rev. Clin. Lab. Sci.* **41**, 265–312 (2004).
146. Henttu, P. & Vihko, P. cDNA coding for the entire human prostate specific antigen shows high homologies to the human tissue kallikrein genes. *Biochem. Biophys. Res. Commun.* **160**, 903–910 (1989).
147. Katz, B. A., Liu, B., Barnes, M. & Springman, E. B. Crystal structure of recombinant human tissue kallikrein at 2.0 A resolution. *Protein Sci.* **7**, 875–885 (1998).
148. Gomis-Ruth, F. X. *et al.* The structure of human prokallikrein 6 reveals a novel activation mechanism for the kallikrein family. *J. Biol. Chem.* **277**, 27273–27281 (2002).

149. Schechter, I. & Berger, A. On the size of the active site in proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **27**, 157–162 (1967).
150. Brillard-Bourdet, M., Moreau, T. & Gauthier, F. Substrate specificity of tissue kallikreins: importance of an extended interaction site. *Biochim. Biophys. Acta* **1246**, 47–52 (1995).
151. Oka, T. *et al.* Role of loop structures of neuropsin in the activity of serine protease and regulated secretion. *J. Biol. Chem.* **277**, 14724–14730 (2002).
152. Modrek, B. & Lee, C. A genomic view of alternative splicing. *Nature Genet.* **30**, 13–19 (2002).
153. Johnson, J. M. *et al.* Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. *Science* **302**, 2141–2144 (2003).
One of the latest studies to investigate the frequency of alternative pre-mRNA splicing within the human genome.
154. Xi, Z. *et al.* Kallikrein 4 is a predominantly nuclear protein and is overexpressed in prostate cancer. *Cancer Res.* **64**, 2365–2370 (2004).
155. Dong, Y., Kaushal, A., Brattsand, M., Nicklin, J. & Clements, J. A. Differential Splicing of KLK5 and KLK7 in epithelial ovarian cancer produces novel variants with potential as cancer biomarkers. *Clin. Cancer Res.* **9**, 1710–1720 (2003).
156. Kurlender, L. *et al.* Differential expression of a human kallikrein 5 (KLK5) splice variant in ovarian and prostate cancer. *Tumor Biol.* **25**, 149–156 (2004).
157. Yousef, G. M. *et al.* The kallikrein gene 5 (*KLK5*) splice variant 2 is a new biomarker for breast and ovarian cancer. *Tumor Biol.* (in the press).
158. Mitsui, S. *et al.* A novel isoform of a kallikrein-like protease, TLSP/hippostasin, (PRSS20), is expressed in the human brain and prostate. *Biochem. Biophys. Res. Commun.* **272**, 205–211 (2000).
159. Nakamura, T. *et al.* Quantitative analysis of hippostasin/*KLK11* gene expression in cancerous and noncancerous prostatic tissues. *Urology* **61**, 1042–1046 (2003).
160. Chang, A., Yousef, G. M., Jung, K., Meyts, E. R. & Diamanids, E. P. Identification and molecular characterization of five novel kallikrein gene 13 (*KLK13*;*KLK-L4*) splice variants: differential expression in human testis and testicular cancer. *AntiCancer Res.* **21**, 3147–3152 (2001).
161. Kumar, A., Mikolajczyk, S. D., Goel, A. S., Millar, L. S. & Saedi, M. S. 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 (1997).
162. Rehbock, J., Buchinger, P., Hermann, A. & Figueroa, C. Identification of immunoreactive tissue kallikrein in human ductal breast carcinomas. *J. Cancer Res. Clin. Oncol.* **121**, 64–68 (1995).
163. Hermann, A., Buchinger, P. & Rehbock, J. Visualization of tissue kallikrein in human breast carcinoma by two-dimensional western blotting and immunohistochemistry. *Biol. Chem. Hoppe Seyler* **376**, 365–370 (1995).
164. Howarth, D. J., Aronson, I. B. & Diamandis, E. P. Immunohistochemical localization of prostate-specific antigen in benign and malignant breast tissues. *Br. J. Cancer* **75**, 1646–1651 (1997).
165. Yu, H. & Diamandis, E. P. Measurement of serum prostate specific antigen levels in women and in prostatectomized men with an ultrasensitive immunoassay technique. *J. Urol.* **153**, 1004–1008 (1995).
166. Foekens, J. A. *et al.* Expression of prostate-specific antigen (PSA) correlates with poor response to tamoxifen therapy in recurrent breast cancer. *Br. J. Cancer* **79**, 888–894 (1999).
167. Yousef, G. M. *et al.* Human kallikrein gene 5 (*KLK5*) expression by quantitative PCR: an independent indicator of poor prognosis in breast cancer. *Clin. Chem.* **48**, 1241–1250 (2002).
168. Talieri, M., Diamandis, E. P., Gourgiotis, D., Mathioudaki, K. & Scorilas, A. 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 (2004).
169. Yousef, G. *et al.* The prognostic value of the human kallikrein gene 9 (*KLK9*) in breast cancer. *Breast Cancer Res. Treat.* **78**, 149–158 (2003).
170. Luo, L. Y., Diamandis, E. P., Look, M. P., Soosaipillai, A. P. & Foekens, J. A. Higher expression of human kallikrein 10 in breast cancer tissue predicts tamoxifen resistance. *Br. J. Cancer* **86**, 1790–1796 (2002).
171. Chang, A. *et al.* Human kallikrein gene 13 (*KLK13*) expression by quantitative RT-PCR: an independent indicator of favourable prognosis in breast cancer. *Br. J. Cancer* **86**, 1457–1464 (2002).
172. Yousef, G. M. *et al.* Quantitative analysis of human kallikrein gene 14 expression in breast tumours indicates association with poor prognosis. *Br. J. Cancer* **87**, 1287–1293 (2002).
173. Yousef, G. M. *et al.* The androgen-regulated gene human kallikrein 15 (*KLK15*) is an independent and favourable prognostic marker for breast cancer. *Br. J. Cancer* **87**, 1294–1300 (2002).
174. Cane, S. *et al.* The novel serine protease tumor-associated differentially expressed gene-14 (*KLK8*/Neuropsin/Ovasin) is highly overexpressed in cervical cancer. *Am. J. Obstet. Gynecol.* **190**, 60–66 (2004).
175. Hibbs, K. *et al.* Differential gene expression in ovarian carcinoma: identification of potential biomarkers. *Am. J. Pathol.* **165**, 397–414 (2004).
176. Diamandis, E. P. *et al.* Immunofluorometric quantification of human kallikrein 5 expression in ovarian cancer cytosols and its association with unfavorable patient prognosis. *Tumour. Biol.* **24**, 299–309 (2003).
177. Lu, K. H. *et al.* Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. *Clin. Cancer Res.* **10**, 3291–3300 (2004).
178. Hoffman, B. R. *et al.* Immunofluorometric quantitation and histochemical localisation of kallikrein 6 protein in ovarian cancer tissue: a new independent unfavourable prognostic biomarker. *Br. J. Cancer* **87**, 763–771 (2002).
179. Kyriakopoulou, L. G. *et al.* Prognostic value of quantitatively assessed KLK7 expression in ovarian cancer. *Clin. Biochem.* **36**, 135–143 (2003).
180. Shigemasa, K. *et al.* Human kallikrein 8 (hK8/TADG-14) expression is associated with an early clinical stage and favorable prognosis in ovarian cancer. *Oncol. Rep.* **11**, 1153–1159 (2004).
181. Yousef, G. M. *et al.* Quantitative expression of the human kallikrein gene 9 (KLK9) in ovarian cancer: a new independent and favorable prognostic marker. *Cancer Res.* **61**, 7811–7818 (2001).
182. Shigemasa, K., Gu, L., Tanimoto, H., O'Brien, T. J. & Ohama, K. Human kallikrein gene 11 (*KLK11*) mRNA overexpression is associated with poor prognosis in patients with epithelial ovarian cancer. *Clin. Cancer Res.* **10**, 2766–2770 (2004).
183. Borgono, C. A. *et al.* Favorable prognostic value of tissue human kallikrein 11 (hK11) in patients with ovarian carcinoma. *Int. J. Cancer* **106**, 605–610 (2003).
184. Scorilas, A. *et al.* Human kallikrein 13 protein in ovarian cancer cytosols: a new favorable prognostic marker. *J. Clin. Oncol.* **22**, 678–685 (2004).
185. Yousef, G. M. *et al.* Steroid hormone regulation and prognostic value of the human kallikrein gene 14 in ovarian cancer. *Am. J. Clin. Pathol.* **119**, 346–355 (2003).
186. Darson, M. F. *et al.* Human glandular kallikrein 2 (hK2) expression in prostatic intraepithelial neoplasia and adenocarcinoma: a novel prostate cancer marker. *Urology* **49**, 857–862 (1997).
187. Nelson, P. S. *et al.* Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression. *Proc. Natl Acad. Sci. USA* **96**, 3114–3119 (1999).
This is one of several papers to report the cloning of a novel kallikrein gene (*KLK4*) in addition to *KLK1, KLK2* and *KLK3*, and one of the first indications of an extended human kallikrein gene family.
188. Day, C. H. *et al.* Characterization of KLK4 expression and detection of KLK4-specific antibody in prostate cancer patient sera. *Oncogene* **21**, 7114–7120 (2002).
189. Obiezu, C. V. *et al.* Detection of human kallikrein 4 in healthy and cancerous prostatic tissues by immunofluorometry and immunohistochemistry. *Clin. Chem.* **48**, 1232–1240 (2002).
190. Hooper, J. D. *et al.* Identification and characterization of *klk14*, a novel kallikrein serine protease gene located on human chromosome 19q13.4 and expressed in prostate and skeletal muscle. *Genomics* **73**, 117–122 (2001).
191. Yousef, G. M. *et al.* Differential expression of the human kallikrein gene 14 (*KLK14*) in normal and cancerous prostatic tissues. *Prostate* **56**, 287–292 (2003).
192. Yousef, G. M., Scorilas, A., Jung, K., Ashworth, L. K. & Diamandis, E. P. Molecular cloning of the human kallikrein 15 gene (*KLK15*). Up-regulation in prostate cancer. *J. Biol. Chem.* **276**, 53–61 (2001).
193. Stephan, C. *et al.* Quantitative analysis of kallikrein 15 gene expression in prostate tissue. *J. Urol.* **169**, 361–364 (2003).

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Competing interests statement

The authors declare no competing financial interests.

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