Clin Chem Lab Med 2012;50(2):211–233 © 2012 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/CCLM.2011.750

Review

The physiology and pathobiology of human kallikrein-related peptidase 6 (*KLK6*)

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Abstract

The human kallikrein-related peptidase 6 (*KLK6*) gene belongs to the 15-member kallikrein (*KLK*) gene family mapping to chromosome 19q13.3–13.4. Encoding for an enzyme with trypsin-like properties, *KLK6* can degrade components of the extracellular matrix. The successful utilisation of another *KLK* member (KLK3/PSA) for prostate cancer diagnosis has led many to evaluate *KLK6* as a potential biomarker for other cancer and diseased states. The observed dysregulated expression in cancers, neurodegenerative diseases and skin conditions has led to the discovery that *KLK6* participates in other cellular pathways including inflammation, receptor activation and regulation of apoptosis. Moreover, the improvements in high-throughput genomics have not only enabled the identification of sequence polymorphisms, but of transcript variants, whose functional significances have yet to be realised. This comprehensive review will summarise the current findings of KLK6 pathophysiology and discuss its potential as a viable biomarker.

Keywords: biomarker; cancer; genomic instability; neurodegenerative disease; serine protease; single nucleotide polymorphism (SNP); variant transcript.

Introduction

The past decade has seen an unprecedented explosion of bioinformatic data due to technical advances in high-throughput surveys of normal and diseased genomes, transcriptomes, proteomes and metabolomes. However, few, if any, biomarkers have made their way to the clinic. Meanwhile, some FDA-approved biomarkers are not used in standard clinical practice and labelled as "analyte-specific reagents (ASRs)", recommended for research purposes only (1). Multiple biomarkers for disease diagnosis and personalised therapy are the way of the future, but many still hope to find simple biomarkers that rival the likes of prostate specific antigen (*PSA*) for prostate cancer (2). *PSA*, also known as kallikrein 3 (*KLK3*), belongs to a unique family of 15 tissue kallikrein and kallikrein-related peptidase genes (*KLK*s) (3–5). Following the identification of all human *KLK* family members in the late 1990s (6, 7), many investigators, including our group, have actively pursued the potential for other *KLK* family members as putative biomarkers (3, 4). The highly restrictive expression of *KLK3/PSA* and *KLK2* to the prostate is unmatched to any other *KLK* member. However, a number of *KLK* genes and proteins have shown promise in enhancing prognostic and predictive medicine, among them *KLK6*. In this review, we will summarise new findings regarding this *KLK* family member and discuss its pattern of expression in normal and diseased tissues; the mechanisms regulating its expression; recent developments in its physiological functions, and implications for diagnostics, personalised medicine and therapeutics.

The kallikrein gene family: overview

There are currently several excellent and comprehensive reviews on the kallikrein (*KLK*) gene family (3–5, 8, 9) and the highlights are briefly summarised here. The *KLK*s, which include the tissue kallikrein gene (*KLK1*) and kallikrein-related peptidase genes (*KLK2-KLK15*), encode for secreated serine proteases with trypsin- or chymotrypsin-like activities (6, 10). In normal physiology, the *KLK* genes are expressed in various tissues (11) and are involved in proteolytic cascades (12–16). The KLK proteins also cleave a number of substrates including matrix metalloproteases (MMPs), insulin-like growth factor binding proteins (IGF2BPs), latent transforming growth factor β (TGFβ), fibronectins and collagens [reviewed by Borgono and Diamandis (3)]. More recently, the *KLK*s have also been implicated in tumourigenic events (17, 18) and postulated to play a role in the pathogenesis of Alzheimer's disease (AD) (19, 20). It is apparent, therefore, that *KLK*s play diverse roles in physiology and pathobiology.

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Received August 19, 2011; accepted September 21, 2011; previously published online November 3, 2011

The 15 protein-encoding gene members share common features, including the presence of five coding exons, and the position of the start and stop codons (6, 7). Variability between the members exists in the number of 5′ untranslated exons (6, 7). In addition, to maintaining similar exon sizes, the catalytic triad (His, Asp and Ser) is conserved (5, 21). Each gene encodes a single-chain prepro-enzyme, which is subsequently processed to an enzymatically inactive proKLK. The proKLK is secreted after removal of the amino-terminal signal peptide and later activated into its mature peptidase by the proteolytic cleavage of the amino-terminal propeptide (5). The entire family maps to an approximately 300 kb region at chromosome 19q13.3–13.4 (6, 7) (Figure 1) in humans. In addition, to the 15 protein-encoding members, there is at least one KLK pseudogene located between *KLKs 2* and *4* (23, 24), though recent studies predict that there may be up to four in this region, under stages of sequence divergence (25). One of the most distinguishing characteristics of the human *KLK* gene family is the fact that all members localise to the same chromosomal region in a contiguous manner, unlike other trypsin and trypsin-like proteases, whose gene-family members map to other genomic loci (22, 26). Recently, an in-depth evolutionary study of the *KLK* gene family by Pavolpoulou et al. (22) shed new light as to the timing of gene-duplicating events, showing the recurrent theme of in situ duplication across various phyla. Previous studies suggested the emergence of the *KLK* genes to approximately 150 million years ago (mya), however the addition of reptilian, avian and amphibian genomes now implicate the emergence of the *KLK* genes to an amazing 330 mya (22). Utilizing sequence, protein and tyrpsin core homologies, several groups (7, 9, 22) have confirmed the overall relatedness between *KLK1*, *KLK2* and *KLK3*; *KLK4* and *KLK5*; *KLK6*, *KLK13* and *KLK14*; *KLK9* and *KLK11*; and between *KLK10* and *KLK12*, which are summarised in Figure 1. Interestingly, both *KLK2* and *KLK3* are the only gene members which are transcribed in the opposite direction to the rest of the locus (9, 27). This suggests that they arose later in *KLK* evolution (22), likely as a selective force favouring these *KLK* for roles in reproductive physiology (28).

KLK expression can be generally described as restrictive (3, 4, 9, 27), with a distinct group of members showing tissue-restrictive expression; while another group shows a relatively broader pattern of expression (7, 11). In the human *KLK* cluster, a specific preponderance for prostate-specific expression is seen among *KLK15*, *KLK3* (*PSA*), *KLK2* and *KLK4* (9, 11, 27). Varying and co-ordinate expression of the other *KLK* members has been documented in the normal tissues of the pancreas, lung, heart, central nervous system; as well as endocrine-regulated tissues, such as the thyroid and breast (11). These patterns of expression are recapitulated in the neoplastic and disease states of these tissues, where numerous studies have reported the observed over-expression of KLKs (3, 19, 27, 29).

Diverse mechanisms influencing gene or protein expression have been investigated including copy-number (30–32) and methylation (33–38). However, the vast majority of work has shown the influence of hormones on KLK expression (3, 39–42). Hormone response elements (HRSs), showing sensitivity to androgens, have been identified in the proximal promoter and enhancer regions of *KLK2* and *KLK3* (43). Interestingly, only the classical *KLK*s (*KLK1*, *KLK2* and *KLK3*) possess defined TATA boxes within their promoter sequences, with the others possessing TATA-like sequences (9, 17, 44, 45). It is still unknown whether a locus control region (LCR) or defined promoter elements are the primary means of regulating transcription (3, 22, 46). However, elegant studies by Kroon et al. (46), utilizing transgenic mice expressing rat KLK genes, demonstrated in this model system, that gene expression was directed by transcriptional enhancers rather than a LCR. The advent of high-throughput sequencing has also enabled the identification of single nucleotide polymorphisms (SNPs) for each family member (47). Several studies are now investigating the significance of such variations in the context of disease (48–55). Post-transcriptionally, microRNAs (mRNAs) (56, 57) have been shown to be important

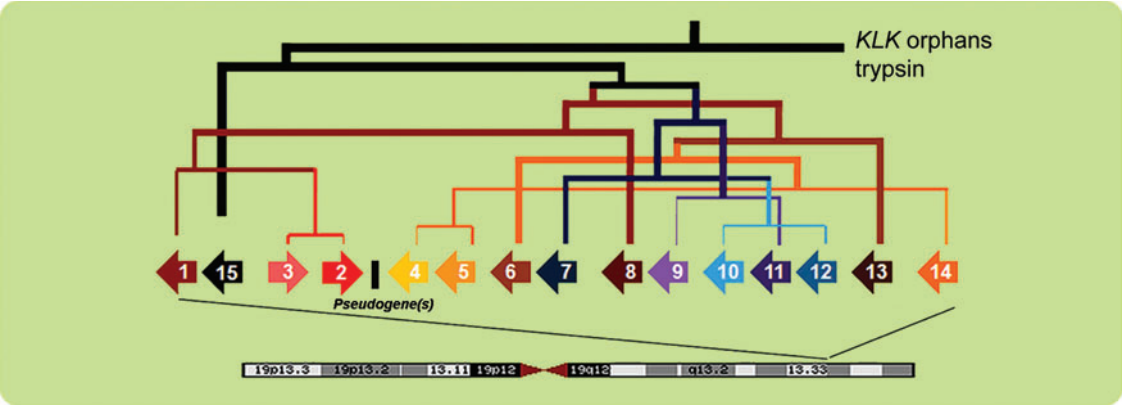

Figure 1 The human kallikrein locus at chromosome 19q13.3/q13.4.
Shown is the organisation and relative phylogenetic relationship of the human *KLK* locus, the transcriptional orientations and relative relationship (not drawn to scale) of the *KLK* gene family members based on the studies by Harvey et al. (7), Pavlopoulou et al. (22), and Yousef et al. (9).

players in the regulation of protein synthesis (58–65). Post-translationally, activated KLK proteins are controlled by endogenous inhibitors, primarily α2-macroglobulin (α2M) and serpins; and may also lose activity through internal cleavage (3). Amazingly, despite the similarities between the family members, they are sufficiently unique to encompass the gamut of normal and abnormal biological processes.

Kallikrein 6: historical perspectives and genetics

Kallikrein 6 (*KLK6*), the HUGO Gene Nomenclature Committee (HGNC) Database – approved name and symbol, has previously been identified by a number of aliases, including *PRSS9*, *Bssp*, *Neurosin*, *serine protease 9*, *Protease M*, *serine protease 19*, *PRSS18*, *SP59* and *ZYME* (http://www.genenames.org/index.html). Although the classical *KLKs* (*KLK1*, *2* and *3*) were already known, *KLK6* was the first of the expanded members to be identified. Originally recognised as *Protease M*, through a cDNA screen of primary and metastatic breast cancer cell lines (66), KLK6 was shown to be down-regulated in some metastatic breast cancer cell lines, but shown to possess high transcript levels in primary breast and ovarian cancer cell lines as well as primary tumours (66). A year later, Yamashiro et al. (67) cloned the same cDNA from the COLO201 human colon adenocarcinoma cell line and named it *Neurosin*. Shortly thereafter several groups, including ours, reported the differential expression of KLK6 in various tissues and disease states (described in detail below).

With seven exons and six introns, *KLK6* spans approximately 11.0 kb (NCBI Reference Sequence: NG_011825.1), mapping between *KLK5* and *KLK7* (7, 9, 68, 69) (Figures 1 and 2A). The first two exons are untranslated, with the remaining five exons and position of the catalytic triad consistent with the other family members. To date, no classical TATA boxes or CAAT sequences have been identified for *KLK6* (9), however TATA-like (17, 44, 45) and initiator-like sequences (9) have been found. All splice junctions are conserved in *KLK6*, with an AATAAA polyadenylation signal 14 bp away from the poly-A tail. Simple repeat elements in addition to di- and tri-nucleotide repeats occur in the polymorphic intronic regions (UCSC Genome Browser: http://genome.ucsc.edu/) (Figure 2A). Moreover, no mutations in the coding exons of *KLK6* have been reported within the Sanger's Catalogue of Somatic Mutations in Cancer (COSMIC) (http://www.sanger.ac.uk/perl/genetics/CGP/cosmic), International Cancer Genome Consortium (http://www.icgc.org/), nor by sequencing of the coding regions of primary cancers and controls (70). Sequence and protein comparisons show that *KLK6* has greatest homology to *KLK13* (9, 22, 68). Likely evolving from an ancestral trypsin-like-*KLK* gene, *KLK6*, together with *KLK14*, *KLK5*, *KLK10* and *KLK15*, represents one of the earliest *KLKs* to come on scene (22).

The technical advances in deep sequencing, Copy Number Variations (CNVs) (77) and Single Nucleotide Polymorphisms (SNPs) (78) now form the basis for many Genome-Wide Association studies (GWAs). Defined as common variations at a single nucleotide position, where the least common allele is present in at least 1% of a given population (79), SNPs occur approximately one in every 150–300 bp in the human genome (80–82). To investigate the frequency of SNPs within the *KLK* locus, Goard et al. (47) surveyed the SNPs across the locus using an in-house, custom-designed software tool termed "ParSNPs" and "LocusAnnotator", which mined the National Center for Biotechnology Information (NCBI) dbSNP database. As of February 2007, the entire *KLK* locus contained 1856 polymorphisms as annotated in dbSNP (47). Focusing on the functional class annotations and accounting for redundancy due to context-dependent functional classes, *KLK6* possessed a total of 23 validated polymorphisms, increasing the number from 22 previously reported by Yousef et al. (69). Within the locus analysed by Goard et al. (47) and Yousef et al. (69), currently over 110 SNPs on the positive and negative strands spanning the gene have been identified by the NCBI (Figure 2A) and continue to change as more genomic sequences become available. Six SNPs (of the positive strand) have been localised to coding or splice sites and include: rs77760094; rs111672933; rs1701950; rs113724718; rs61469141; rs111738447 (Table 1). Two synonomous SNPs are found in exon 6, resulting in no change in the amino acid. However, the four remaining SNPs have a missense functional class, resulting in an amino acid change, whose significance requires investigation. For many of these SNPs as well as those detected throughout the gene, validation of the frequency has still yet to be determined.

KLK6 mRNA

KLK6 possesses a number of alternative transcripts encoding for the full-length KLK6 protein [reviewed by Kurlender et al. (83) and Yousef et al. (69)], in addition to those predicted to produce truncated forms (http://www.ncbi.nlm.nih.gov/genbank/) (Table 2 and Figure 2B). The KLK6 "classical transcript" (also called Isoform A; NM_002774.3; GenBank Accession U62801) was originally detected in a normal mammary myoepithelial cell strain (66), then from the human colon adenocarcioma cell line (COLO 201) (67) and later from Alzheimer's disease brain tissues (71). The longest reported classic transcript cDNA clone spans 1526 nucleotides (66) and includes the five coding exons and two 5′ untranslated exons, yielding a predicted 244 amino acid protein (Figure 2B and C).

RNA expression of KLK6 in normal human tissues has been confirmed (7, 11, 66–69, 71–73, 86, 87) (Figure 2B), with tissues of the central nervous system (CNS) unequivocally showing the highest expression of RNA transcripts, followed closely by tissues of the breast, kidney and uterus; salivary gland, thymus, spleen, thyroid and testis. Cells of the ovary, lung, liver, bone marrow and pituitary show comparatively the least amount of KLK6 mRNA.

The classical transcript utilises the promoter (P1) located in exon 1, while transcriptional variants (AY318867 and AY318869) utilise a second promoter (P2) starting in a 20 bp region within the first intron of *KLK6* (73), thus lacking exon 1 (Figure 2B). A third promoter (P3) has also been identified in intron 2 and contains no untranslated exons (72, 73). Variant transcript 1 (AY318869) has a size of 1517 bp, while

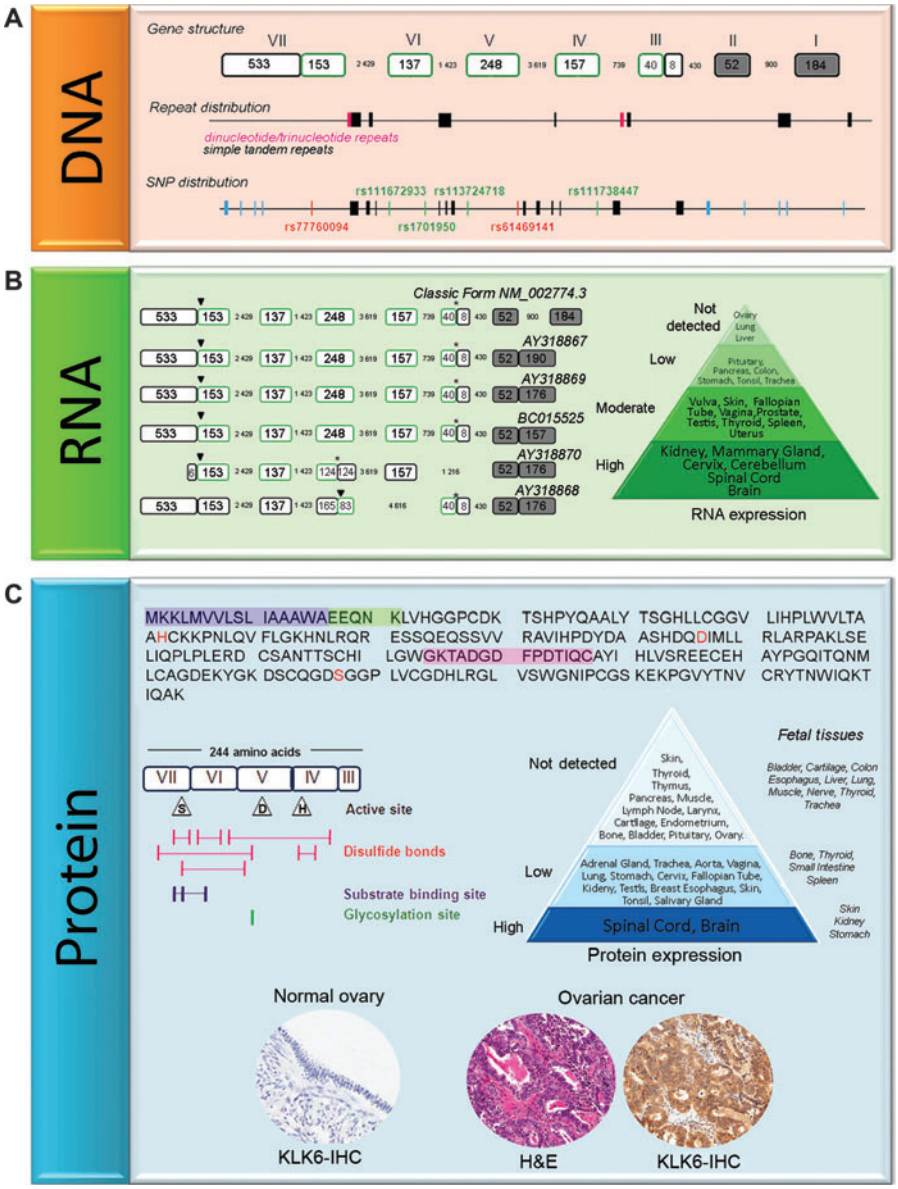

Figure 2 DNA, RNA and protein properties of kallikrein 6.
(A) Genomic organisation of KLK6. Shown are the seven exons (I–VII) with untranslated exons shaded in grey and coding exons in green. The sizes of each exon and intron are indicated. The distribution of both simple tandem-, di- and trinucleotide repeats are also shown below. Relative SNP distribution across the gene is shown, with six exon-coding SNPs described in Table 1 shown here in green and red. Green SNPs indicate those identified as missense or synonomous, near splice sites; while red SNPs indicate those located in coding exons. Blue SNPs indicate those located in non-coding regions and introns. (B) Organisation of KLK6 RNA transcripts. Shown are a number of identified RNA transcripts as described in the text and in Table 2. The asterisk (*) indicates the transcriptional start site and the downward arrowhead indicates the stop site. Predicted untranslated exons are shaded in grey, predicted coding exons are shown in green. (Right) Shown is the relative expression of KLK6 RNA in normal adult human tissues based on several studies (7, 11, 16, 66–68, 70–74). Human adult brain shows the greatest KLK6 transcript expression among all tissues in the human body as compared to ovary, lung and liver which barely shows any expression or expression at the level of detection for PCR or northern analysis. (C) Organisation of KLK6 protein. Shown is the amino acid sequence of the full-length KLK transcript. Amino acids shaded in blue indicates the signal peptide sequence; green indicates the propeptide sequence; fusia indicates the autolysis loop and amino acids coloured in red are those in the catalytic triad. (Lower Left) Summary of other protein features including disulphide bonds (red), substrate binding sites (blue) and a glycosylation site (green). The amino acids of the catalytic triad are indicated in triangles. (Lower Right) Shown is the relative expression of KLK6 protein detected in both normal adult and foetal tissues as detected by ELISA (11, 75, 76). Similar to RNA findings, normal adult brain shows the highest KLK6 protein expression. The majority of tissues show low to non-detectable levels when compared to the adult brain. In foetal tissues, stomach shows the greatest expression followed by kidney and skin. (Bottom) KLK6-specific immunohistochemical analyses of normal ovarian surface epithelium and ovarian carcinoma. Normal ovarian surface epithelium shows very low/barely detectable levels of KLK6, in contrast to a representative ovarian cancer showing strong immunoreactivity to KLK6 antibodies.

Table 1 Single nucleotide polymorphisms (SNPs) identified in coding exons of KLK6.

SNP ID	Location	Description
rs111738447	Exon 4	Missense C → T Leu (L) → Phe (F)
rs61469141	Exon 5	Missense C→ T Arg (R) → Trp (W)
rs113724718	Exon 5	Missense A →G Asp (D) →Gly (G)
rs1701950	Exon 6	Synonomous G →C Leu (L) → Leu (L)
rs111672933	Exon 6	Synonomous T →C Cys (C) → Cys (C)
rs77760094	Exon 7	Missense C →T Thr (T) →Met (M)

transcript variant 2 (AY318867) has a size of 1503 bp (72, 73). In addition to transcriptional variants of the classical form, splice variants have also been identified (73, 83). Splice variant 2 (AY318870), at 929 bp, lacks exon 3 which contains the classical transcript translational start site ($A^{246}GT$) (73). This is in contrast to splice variant 3 (AY318868), at 820 bp, which lacks exon 4. Such splice variants are predicted to yield a 137 amino acid and 44 amino acid protein product for splice variant 2 and 3, respectively; however it has not as yet been determined whether these mRNAs are translated. Additional unpublished variants by Kurlender et al. (83): AY279383 and AY457039; Pampalakis and Sotiropoulou (Unpublished data): AY457039; Strausberg et al. (Unpublished data): BC015525, Wakamatsu et al. (Unpublished data): AK314897 have also been reported. Furthermore, in a detailed in silico analysis of the KLK6 transcript using 185 expressed sequence tag (EST) clones, Yousef et al. (69) identified a number of additional splice variants derived from the libraries of testicular and ovarian tissues; as well as from cell lines from gastric cancers, myeloma, colon adenocarcinoma and tumours of the head and neck. However, these splice variants need to be confirmed experimentally.

Table 2 Summary of KLK6 RNA transcripts.

mRNAs	Length in bp	Aliases	Comments	Reference/submitted by
Full length				
NM_002774.3	1527	244aa	Encodes 244 amino acid protein	(66, 67)
U62801	1506		Lacks exon 2	(66)
AF013988	1451		Lacks exon 2	(71)
D78203	1419		Lacks exon 2	(71)
AF149289.1	1506			(68)
Transcript variants				
AY318867	1517	Variant 1[a]	Encodes 244 amino acid protein Lacks exon 1	(73)
AY318869	1503	Variant 2	Encodes 244 amino acid protein Lacks exon 1	(73)
BC015525	1500	Variant 2	Encodes 244 amino acid protein Lacks exon 1	(84)
Splice variants				
AY318870	929	Variant 2	Lacks exon 3 Lacks exon 3 and 7b	(73)
AY279383	1355	Variant 1	Lacks exon 4 Encodes 132aa Lacks exon 3 and 4	(83) Kurlender et al. (Unpublished data)[b]
AY318868	820	Variant 3/Variant 1	Lacks exon 1 and 4	(73)
AY457039	378	KLK6 variant 4 – alternatively spliced	Lacks exon 5, 6 and 7b	Pampalakis and Sotiropoulou (Unpublished data)[b]
DQ223012	842	Transcript variant 3 alternatively spliced	Lacks exon 2 And 7b	(33)
Other transcripts and unpublished synthetic clones				
AK314897	884		Lacks exon 2 and 7b	Wakamatsu et al. (Unpublished data)[b]
DQ893845	775		Lacks exon 1, 2 and 7b	Rolfs et al. (Unpublished data)[b]
DQ893548	775		Lacks exon 1, 2 and 7b	Rolfs et al. (Unpublished data)[b]
AY927548	586			(85)
AY891558	735			Hines et al. (Unpublished data)[b]
AY888953.1	735			Hines et al. (Unpublished data)[b]
BT006852	735			Kalnine et al. (Unpublished data)[b]

[a]Although named Variant 1, it is not similar to AY279383, while AY318868 is actually the same as AY279383. [b]L. Kurlender et al. can be accessed at http://www.ncbi.nlm.nih.gov/nuccore/AY279383, A. Wakamatsu et al. at http://www.ncbi.nlm.nih.gov/nuccore/AK314897, N. Kalnine et al. at http://www.ncbi.nlm.nih.gov/nucleotide/30582542, G. Pampalakis and G. Sotiropoulou at http://www.ncbi.nlm.nih.gov/nuccore/AY457039, A. Rolfs et al. at http://www.ncbi.nlm.nih.gov/nuccore/DQ893845; http://www.ncbi.nlm.nih.gov/nuccore/DQ893548 and L. Hines et al. at http://www.ncbi.nlm.nih.gov/nuccore/AY891558; http://www.ncbi.nlm.nih.gov/nuccore/AY888953.1.

Table 3 KLK6 transcript heterogeneity.

Normal tissue	Multiple transcript detection by primer-specific PCR					
	Classic	AY318869/ AY318867	AY318868 (lacks Exon 4)	AY279383	AY318870	Reference
Embryonic liver	Not detected	◦	–	–	–	(73)
Breast	••	••••	••••	–	–	(73)
Spleen	•	••••	–	–	–	(73)
	Not detected	••••				(72)
Skeletal muscle	◦	•	Not detected		–	(73)
Thymus	◦	••	Not detected	–	–	(73)
	Not detected					(72)
Trachea		•	–		–	(73)
Uterus	Not detected	••••	–	–	–	(73)
Placenta	Not detected	••	–	–	–	(73)
Adrenal gland	◦	◦	Not detected	–	–	(73)
Brain	••••••	•	–	–	–	(70, 72, 73)
Spinal cord	••••••••	••••••••	••••	–	–	(73)
Prostate	–	–	◦	–	–	(73)
Salivary gland	–	–	••••••	–	–	(73)
Ovary	Not detected	Not detected	–	–	–	(70)
Kidney	Not detected	•••	–	–	–	(72)
Peripheral nerve	Not detected	•••	–	–	–	(72)
Liver	Not detected	◦	–	–	–	(72)
Pancreas	Not detected	◦	–	–	–	(72)
Submandibular gland	Not detected	◦	–	–	–	(72)
Diseased tissue						
Ovarian carcinoma tumours	◦	•••••	–	◦	–	(70)
Cell lines						
76N	•••••	–	•	◦	–	(73)
21PT	•••••	–	•••	•••	–	(73)
MDA-MB-468	•••••	–	••	Not detected	–	(73)

– Indicates not tested; ◦ indicates positivity at barely detectable levels; • indicates positivity.

The ability to generate multiple mRNAs from a given gene increases protein diversity and is a reflection of the inherent biological complexities and specificities of different cell types (88). The significance of these KLK6 variant transcripts has yet to be determined (89), however it is evident that there is the propensity for some tissues to express more than one transcript (69, 83, 72, 73) (Table 3). Pampalakis et al. (73) reported the presence of transcript heterogeneity; with tissues of the CNS overwhelmingly expressing the classical transcript over the variants AY318869, AY318867 and AY279383. In contrast, breast, spleen, uterus and skeletal muscle predominantly expressed the variants AY318869 and AY318867, with either very little or non-detectable levels of the classical transcript. Similar studies by Christophi et al. (72) and others (90, 91) comparing murine and human KLK6 transcripts confirmed the observation of tissue-specific expression of KLK6 variants mediated by alternative promoter usage (88).

The presence of these transcripts still needs to be comprehensively investigated in both normal and diseased states. However, in keeping with the notion of heterogeneity of mRNA transcripts, several breast cancer cell lines have shown the concomitant expression of full length transcripts (i.e., classic and AY318869/AY318867) with splice variant transcripts AY318868 and AY279383 (73). Additionally, primary ovarian carcinomas also exhibit transcript heterogeneity (70). In transcript-specific PCR, ovarian cancers were shown to express the classic transcript, alternative transcript (AY318869) and, in a small number of cases, the splice variant for AY279383. The alternative transcript, AY318869, comprised the major species of ovarian cancer-derived KLK6 mRNA (70). With the exception of these studies, the majority of expression analyses do not specify the KLK6 transcript being detected. Therefore, the significance of variant transcripts has not been fully appreciated. However, with improvements in RNA sequencing (92), the full extent of RNA expression and heterogeneity may be fully appreciated.

Increased KLK6 mRNA expression has been identified in ovarian carcinomas (11, 32, 66, 70, 93–96), breast (66, 97), uterine (98), pancreatic (99), colorectal (75, 100, 101), gastric (102), skin (74) and urinary bladder cancers (103). In contrast to various cancers, the decrease of KLK6 mRNA has been identified in the brain tissues of patients with Alzheimer's and Parkinson's disease (PD) (20, 104). The physiological implications of these findings will be discussed later.

KLK6 protein

KLK6 encodes a single-chain enzymatically inactive preproenzyme of 244 amino acids (66–68, 71) (Figure 2C). This prepro-enzyme is subsequently processed to an inactive proKLK through the removal of the predicted 16 amino acid signal peptide [Ala (A) ↓ Glu (E)] and passed through the endoplasmic reticulum. The proKLK is secreated to the extracellular space and becomes converted into its activated mature peptidase by the proteolytic cleavage of the five amino acid (Lys (K) ↓ Leu (L)) activation peptide (68, 105). The catalytic triad, which characterises serine proteases, is conserved in KLK6 (i.e., His^{62}, Asp^{106} and Ser^{197}) (68). Furthermore, the presence of Asp at position 191 suggests that KLK6 has trypsin-like activity. KLK6 also possesses six disulphide bonds, one glycosylation site (Figure 2C) and 12 cysteine residues, with 27 of the 29 "invariant" amino acids near the active site of serine proteases also conserved (68, 105).

The three-dimensional crystal structure for mature, active recombinant KLK6 (1LO6), at a resolution of 1.75-Å, was first reported by Bernett et al. (106); who also reviewed its biochemical properties. Later, the pro-form was further detailed by Gomis-Rüth et al. (107). Briefly, with an oblate ellipsoid shape of approximately 35–50 Å, KLK6 is folded into two adjacent six-stranded β-barrels, interconnected by three trans-domain segments (105–107). Unlike the classical *KLK*s, KLK6 lacks the "kallikrein loop", a sequence of up to 11 amino acids inserted between the sixth and seventh β-sheets (106, 107). However, the existing loop is similar in structure to trypsin and chymotrypsin. The KLK6 S1 binding pocket includes the catalytic triad and also shows structural similarity to trypsin, with sufficient differences in amino acid sequence to give KLK6 a higher catalytic efficiency towards substrates with an Arg rather than a Lys residue at the P1 position (105, 106). Similarly, at the P2 position, KLK6 prefers Arg over Lys. However, no strong preference is seen at the P3 and P4 sites (105). The active site cleft centres on the Ser catlytic residue of the triad (107). One glycosylation site, located relatively far from the active site, has been identified; which is in contrast to the other KLKs where the glycosylation site is located within the kallikrein loop (106).

The activation of KLK6 is likely to involve proteinases endowed with trypsin-like properties, thus the *KLK*s may be able to activate themselves or each other (68, 105). The activation of KLK6 by enterokinase, plasmin and KLK5 (108–110) has been reported, as well as by KLKs 4, 11 and 14 (13, 108, 109, 111), urokinase plasminogen activator (uPA) (110) and by glycosaminoglycans and kosmotropic salts (112). While the reports from recombinant pro-KLK6 studies (71) suggest that KLK6 can undergo autoactivation, this has yet to be entirely resolved due to issues raised regarding experimental host protease contamination and whether the observed autoactivation is a transient step leading to KLK6 inactivation (i.e., negative feedback inhibition vs. positive feedback activation) (108). However, improvements in the production of purified pro-KLK6 have helped to better elucidate such mechanisms (108, 113, 114). Indeed, the study by Bayes et al. (113) addressed this issue through the additional specific expression of a proKLK6 carrying a mutation in the active site (S197A), finding that endogenous proteases do not appear to recognise and cleave the propeptide. Generally, the majority of in vitro studies agree that KLK6 can autoproteolytically cleave at the internal Arg^{76}-Glu^{77} peptide bond (105, 106, 108).

KLK6 has also recently been shown to be differentially glycosylated (115). Kuzmanov et al. (115) characterised and compared the N-glycosylation status of KLK6 in cerebrospinal fluid and KLK6 derived from ovarian cancer ascites. Mobility gel shift western analysis coupled to glycosidase digestion revealed a difference in molecular weight between the two isoforms due to a modification of the ovarian derived KLK6 by α2-6-linked sialic acid. Site-directed mutagenesis confirmed the single N-glycosylation site (106).

Critical to understanding the physiological role of *KLK6* is discovering its substrates (Table 4). Magklara et al. (114) demonstrated the degradation of casein, collagen type I, collagen type IV, fibrinogen as well as synthetic peptides corresponding to the N-terminal region of amyloid precursor protein (APP) by KLK6; while the chymotrypsin substrate AAPF-AMC was not cleaved; again confirming the lack of chymotrypsin-like activity for KLK6 (116). Furthermore, in a comprehensive in vitro study, Yoon et al. (109) demonstrated the ability for KLK6 to activate pro-KLKs 1, 2, 3, 5, 9 and 11.

Due to the successful utilisation of KLK3/PSA for prostate cancer diagnostics (2), the differential expression of KLK6 protein has been actively pursued, aided by improvements in the production of recombinant proteins and enhancements in the specificity and sensitivity of resultant KLK6 antibodies (32, 121, 76). KLK6 expression has been assayed in many normal tissues and fluids primarily by enzyme-linked immunosorbent assay (ELISA) (11, 76) or immunohistochemistry (IHC) (122) (Figure 2C). Development of antibodies and a sensitive immunofluorometric assay by our group facilitated a method for quantifying KLK6 proteins in biological fluids including nipple aspirate, breast cyst, cerebral spinal and ascites fluids, in addition to the milk of lactating women (76). Interrogation of adult and foetal tissues, and various biological fluids by Shaw and Diamandis (11) reported that the highest expression of KLK6 is found in the cell lysate of adult brain and in the CSF by ELISA. Adult spinal cord showed the second greatest KLK6 expression, at roughly 50% that of the brain. All other adult tissues possessed significantly lower concentrations (<10% of the brain). In contrast, foetal stomach showed the greatest expression of KLK6 among all foetal tissues tested, but still less than 50% of the adult brain; followed by foetal kidney and skin. Although not tested by Shaw and Diamandis, KLK6 has not been detected in fetal brain (90).

By immunohistochemistry, Petraki et al. (122) used monoclonal and polyclonal antibodies (71) against full-length KLK6, to assess its expression and localisation in over 40 different tissue types. Briefly, tissues of the central and peripheral nervous system showed a range of immunoreactivity; with intense staining in the peripheral nerves, but weak nerve cell staining within the CNS; strong staining in the epithelium of the choroid plexus; and moderate

Table 4 Summary of tested substrates for KLK6.

	Reference
Degraded by KLK6	
Pro-KLK1	(109)
Pro-KLK2	(109)
Pro-KLK3	(109)
Pro-KLK5	(109)
Pro-KLK6	(108, 113, 114)
Pro-KLK9	(109)
Pro-KLK11	(109)
Plasminogen	(113)
Casein	(114)
Collagen type I	(114)
Collagen type II	(116)
Collagen type III	(116)
Collagen type IV	(114)
Fibrinogen	(114)
Amyloid precursor protein (synthetic)	(112, 114)
Laminin	(106, 116)
Fibronectin	(106, 116)
Vitronectin	(116)
Protease-activated receptor 2	(117, 118)
α-synuclein	(119)
Human growth hormone	(87)
Desmoglein 1	(120)
Myelin basic protein (synthetic)	(112)
Ionotropic glutamate receptor (synthetic)	(112)
Phe-Ser-Arg-AMC	(114, 116)
Val-Pro-Arg-AMC	(114)
Asp-Pro-Arg-AMC	(114, 116)
Gln-Gly-Arg-AMC	(114)
Pro-Phe-Arg-AMC	(114, 116)
Val-Pro-Arg-AMC	(114, 116)
Val-Leu-Lys-AMC	(114, 116)
Glu-Gly-Arg-AMC	(116)
Gly-Gly-Arg-AMC	(116)
Not degraded by KLK6	
AAPF-AMC	(114)
Glu-Lys-Lys-AMC	(114, 116)
Ala-Ala-Pro-Phe-AMC	(114, 116)
Pro-KLK4	(109)
Pro-KLK7	(109)
Pro-KLK8	(109)
Pro-KLK10	(109)
Pro-KLK12	(109)
Pro-KLK13	(109)
Pro-KLK14	(109)
Pro-KLK15	(109)

AMC, minomethylcoumarin.

expression among Purkinje, stellate and glial cells. Granular cells were negative for KLK6 expression. Although not as intense in its immunoreactivity as seen in the peripheral nerves and choroid plexus, the cytoplasm of the breast and enometrial epithelium was moderately positive for KLK6. Prostate columnar cells showed strong diffuse staining, in contrast to basal cells, which failed to show any immunoreactivity. Various tissues in the gastrointestinal tract also showed positivity of expression, including the glandular epithelium of the large bowel and duct epithelium of the oesophagus and anus, particularly the neuroendocrine cells; and islets of Langerhans in the pancreas. Negative immunoreactivity was seen in hepatocytes and acinar cells of the exocrine pancreas. Among the other tissues studied, strong positivity was seen in the Hassall's corpuscles of the thymus and cells of the anterior pituitary; while moderate-to-weak expression could be seen among the cells of the respiratory tract, adrenal cells and mesenchymal tissues. Others have similarly reported the lack or barely detectable levels of KLK6 in the normal epithelium of the ovary (32, 123, 124), low reactivity in the epithelium of the bronchi (125, 126), moderate expression in glial cells (126, 127) and salivary glands (128).

For most normal tissues and biological fluids analysed, there is general concordance between RNA and protein levels. However, due to the issues of transcript heterogeneity, it is unclear whether the mRNA detected represents translatable protein. As well, IHC analyses has also shown the variable KLK6 expression of specific cell types or structures (i.e., stroma vs. secretory cells or glandular epithelium) within a given organ (122, 124, 129), which has implications for quantifications derived from bulk tissue extracted lysates. At the protein level, expression is influenced by the presence of inhibitors as well as its stability in fluids. Nevertheless, bulk extracted brain tissue and CNS fluids maintain the highest KLK6 mRNA and protein expression in the adult; while most other tissues that expressed KLK6 mRNA also showed some degree of protein expression. However, the change in KLK6 protein levels in disease tissues and biological fluids have been identified in ovarian cancers (30–32, 68, 70, 86, 76, 124, 130–136) (Figure 2C), renal cancers (137), gliomas (127), lung carcinomas (125, 126), salivary gland tumours (128), pancreatic ductal carcinomas (99) and uterine cancers (98) by ELISA and/or IHC. In the case of AD and PD, decreased KLK6 has been observed (20, 138–140); while skin disorders have also reported changes in KLK6 levels (141, 142).

Regulation of KLK6 expression and activity

The biology of an organism relies on the regulation of a gene at the DNA, mRNA and protein levels, but also relies on the inter-play between these factors and its tissue-specific microenvironment. A number of genomic features that influence KLK6 expression have been alluded to above and shown in Figure 3 and include the finding that no overt sequence mutations in the coding regions of *KLK6* have been found to date that results in its aberrant expression. However, the presence of SNPs within both coding and non-coding regions of the gene offers the possibility for uncovering genotype-phenotype associations as more genome sequences become available.

In the case of cancers, genomic instability is a frequent feature, resulting in gene dosage changes that could affect its expression (143, 144). Thus, the relationship between *KLK6* copy number and transcriptional or protein over-expression has been investigated. Indeed, copy number gains of *KLK6* have been identified in ovarian cancers showing over-expression of *KLK6* mRNA or protein (30–32).

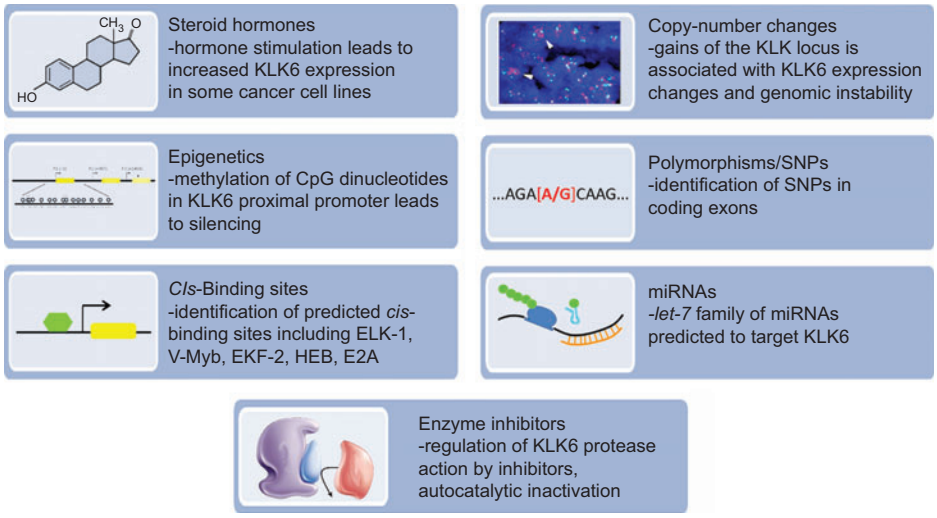

Figure 3 Mechanisms of regulation of KLK6 expression.
Shown is a summary of the various modes of regulation identified for KLK6 as described in the text.

To determine whether only *KLK6* is preferentially gained/amplified, we investigated the copy-number status of *KLKs 3*, *4*, *6* and *13* in a number of breast, prostate and ovarian cell lines and ovarian primary tumours using both *KLK*-specific and whole-*KLK* locus fluorescence in situ hybridisation (FISH) (31). Interestingly, we discovered that all *KLK*s tested showed the same copy number change within the same metaphase spread. Since those *KLK* used in the study span the entire locus, it was determined that the entire locus was involved in copy-number imbalances, rather than individual members. Indeed this appears to be the case based on the copy-number data generated by Beroukhim et al. (145) of over 260 cancer cell lines. Thus, whilst copy-number likely contributes to over-expression in the case of cancers, it is clearly not the only mechanism; as we have also shown that ovarian cancers with one or two copies of the locus can result in protein over-expression comparable with those showing extra copies (30). Furthermore, the differential KLK6 expression observed in diseases, such as AD, where there are no associated changes in gene dosage, implicates alternative mechanisms.

Due to the extensive work on *KLK3/PSA* and its responsiveness to androgens (146, 147), the effect by hormones on gene expression has also been examined for *KLK6*. Several studies have documented the concomitant dose-dependent increase in *KLK6* gene by the in vitro exposure of cell lines to androgens and oestrogens (40, 68). However, others (70) have shown minimal effects on KLK6 expression upon stimulation. Interestingly, significant hormone responses were detected in breast cancer cell lines (40, 68), while ovarian cancer cell lines showed non-significant responses (70), suggesting that factors, such as cell-type specificity plays a role in regulation. To date, no oestrogen response elements (EREs) have been identified within 6 kb upstream of the proximal *KLK6* promoter (40), which is consistent with previous observations that classical hormone response elements (HREs) have not been identified (9). This implicates a more sophisticated integration of mechanisms that probably include the coordinate binding of transcription factors or by hormone-dependent *trans*-activating factors. To further demonstrate the need for more studies, a *cis*-acting mechanism of regulation has also been proposed for *KLK6* and the other non-classical KLK gene members (3, 72) based on the mechanism in salivary-gland expression of the rat *Klk* family (148). As such, Christophi et al. (72) examined a region of 500 bp upstream from the start site of the human and mouse *KLK6* genes and found 10 *cis*-acting regulatory sequences which were common to both, and were of interest in terms of inflammatory responses in the CNS. Some of the candidate genes associated with these *cis*-acting regulatory elements include; *v-Myb*, Ets-family member *ELF-2* (*NERF1*), sterol regulatory element binding protein 1 and 2, and *ELK-1*. Later, similar integrative in silico studies in breast cancer cell lines also identified the putative *ELK-1* binding site, in addition to an E-box and *AP-1* binding site and several Sp-1 sites (33). More recently, the in silico comparative analysis of mammalian *KLK6* genes (149) enabled the identification of additional putative transcription factor binding sites including *HEB*, *E2A*, *PU.1*, hepatocyte nuclear factor 4 (*HNF4*) and liver x receptor (*LXR*). Finally, *KLK6* has also been identified as a target for the vitamin D analogue, EB1089, in studies where KLK6 expression was induced upon treatment with EB1089, in squamous carcinomas (150), colon cancer (151) and the T47D breast cancer cell line (152). Further examination of the *KLK6* proximal promoter indicated predicted multiple vitamin D response-element consensus sequences (152).

Epigenetic mechanisms have also been extensively examined by Pampalakis and colleagues (17, 33, 152). Sequence analysis (33) found no CpG islands, defined by the parameters of a length of more than 200 bp, and with a C+G content more than 50% or with an observed/expected ratio >0.6;

either upstream or within exon-intron sequences of *KLK6*. Experimentally, treatment of breast cancer cell lines with 5-aza-2′deoxycytidine (5-aza-dC) resulted in the induction of *KLK6* gene and protein expression offering the possibility that non-CpG island cytosines are involved in the regulation of transcription. Indeed, these findings were confirmed (17, 33, 149), where specific CpG dinucleotides were subjected to methylation in breast cancer cell lines, resulting in the loss of KLK6 expression. Interestingly, the in silico comparison of these CpGs across other mammalian KLK6 orthologues showed conservation of these dinucleotides (149). Fifteen CpG dinucleotides located within the human P1 transcriptional start site of the classic transcript were analysed. In breast cancer cell lines lacking KLK6 expression, CpG dinucleotides at positions –72, –64, –56, –53, –35, +3 and +14 were completely methylated. Similarly, those cell lines showing KLK6 expression possessed unmethylated CpG dinucleotides. To investigate the role of chromatin structure, treatment of breast cancer cell lines with a histone deacetylase (HDAC) inhibitor, Trichostatin A (TSA) was also able to induce KLK6 expression, though not to the level demonstrated by 5-aza-dC (33). Further studies (17) implicate the formation of transcriptional repression complexes, through the recruitment of MeCP2 and the localised deacetylation of histone H4 of the *KLK6* proximal promoter.

As discussed above, *KLK6* possesses several mRNA variants and promoter sites, however the significance and frequency of such variants has not been thoroughly investigated; nor has it been determined whether a functional protein results or what physiological role such isoforms may play (89). However, the translation of RNA to protein provides another level for gene regulation. In recent years, microRNAs (miRNAs) have been implicated in contributing to disease and malignancies (153). miRNAs represent a class of non-coding RNAs that range in size from 19 to 25 nucleotides, originally described in *Caenorhabditis elegans* (57). The mechanism of regulation is mediated through degrees of complementarity to the target mRNA 3′ untranslated region (UTR). Perfect complementarity results in the cleavage and degradation of the target mRNA; whereas less than perfect pairing represses the translation process (56). In silico analyses of publicly accessible databases show that a number of miRNAs are predicted to target KLK6, among them the members of the *hsa-let-7* family of miRNAs (58). Experimentally, Chow et al. (58) demonstrated the decrease of KLK6 expression upon transient transfection of *hsa-let-7f* to the breast cancer cell line MDA-MB-468. Our own preliminary work (Bayani et al. unpublished) in primary ovarian carcinomas and cell lines shows the differential expression of miRNAs predicted to target *KLK6*. KLK6-over-expressing cell lines showed a decrease of miRNAs predicted to target the KLK6 3′ UTR in contrast to KLK6-non-expressing/low expressing cell lines which showed relatively higher levels of miRNAs. These preliminary miRNA findings for *KLK6* as well as for other *KLK*s (62, 63, 154) will continue to provide important insight as to the fine-tuning of protein expression.

The influences of oncogenic mutations in other genes have recently been investigated by Henkhaus et al. (101), who suggested that the up-regulation of KLK6 mRNA in colorectal cancer cell lines are *K-RAS*-dependent. Following the stable transfection with mutant *K-RAS* into the colon cancer cell line $Caco_2$, expression microarray analyses detected an 11-fold increase in KLK6 expression in the *K-RAS* mutant line over the parental line (155, 156). Through the use of pharmacological inhibitors of pathways downstream of K-RAS, it was demonstrated that the PI3K and p42/44 MAPK pathways contribute to the induction of KLK6 in mutant K-RAS-expressing colon cancer cells. Consistent with previous studies, this increase in KLK6 expression was associated with increased cell migration, which was reversed in the presence of siRNAs or KLK6-specific antibodies. This same group (157) also reported the Caveolin-1 (CAV-1)-mediated expression of KLK6 in colon cancer, showing the concomitant decrease in KLK6 mRNA and secreated protein expression when HCT116 cells were stably transfected with a CAV-1 anti-sense vector. Sucrose gradient fractionation revealed that KLK6 is localised to CAV-1 containing membrane fractions only when CAV-1 was expressed. However, immuoprecipitation analyses showed that KLK6 was not directly associated with CAV-1. When mutant *SRC* was transfected to be constitutively expressed and insensitive to negative regulation, KLK6 secretion was also reduced. Finally, investigation into the downstream pathways showed that pharmacological inhibition of AKT led to reduced KLK6 expression and protein secretion and may be the likely mechanism by which CAV-1 influences KLK6 gene expression.

Post-translationally, several mechanisms are in place to ensure that enzyme activity is controlled. The inactive KLK6 zymogen requires the hydrolysis of the activation peptide, resulting in a conformational change of the enzyme active site for substrate specificity. Thus, the activation of KLK6, by the aforementioned activators, plays a critical role in both normal and disease physiology. Conversely, the inactivation of KLK6 may be facilitated by autocatalysis, as well as through the actions of protease inhibitors. It has been previously mentioned that KLK6 possesses autocatalyic abilities (105, 106, 108). Using purified recombinant pro-KLK6 and synthetic peptides, Blaber et al. (108) demonstrated that the internal KLK6 autolysis loop sequences were a far more efficient substrate target for KLK6 than the KLK6 pro-sequences. However, while KLK6 was found to activate itself, the level of activation was similar to the detection limit of the assay (i.e., <1%) and significantly less than the activation imparted by enterokinase, plasmin and KLK5 (108). As is the case with many proteases, once an enzyme has been activated, the process is irreversible. Thus, activated KLKs may be inhibited by endogenous serine-protease inhibitors called serpins. Serpins act to form complexes, preventing the interaction of the active protease with its substrate (8). A number of inhibitors of KLK6 include, protein-C inhibitor, aprotonin, soybean trypsin inhibitor (SBTI), PMSF, leupeptin, antipain, antithrombin III (ATIII), α_2-antiplasmin (α_2AP), α_1-antitrypsin (AAT) (114, 158–161) and α_1-antichymotrypsin (ACT), which was identified in vivo in cerebral spinal fluid to complex with KLK6 (161).

Role of KLK6 in disease

The expression of the *KLK* genes in a variety of tissues (11) and their proteolytic abilities enable them to participate in a variety of physiological and pathobiological processes (3, 5). The observed over-expression or under-expression of KLKs have helped to elucidate their role in these cellular processes when the normal balance of their expression is shifted. In the following sections, we present the experimental evidence linking KLK6 to numerous cellular processes and consider the integrative effect this could have in the context of diseases, such as cancer or neurodegeneration, which are summarised in Figure 4.

Inflammation, immunity and skin pathophysiology

A number of KLK members have been implicated in the normal physiological functioning of the skin, which is tightly regulated for innate immunity and inflammation [reviewed by Sotiropoulou and Pampalakis (162)]. A large body of work has shown the role of KLKs in skin desquamation, thus forming the basis for their study in various skin disorders (16, 120, 163). Psoriasis and atopic dermatitis are both chronic inflammatory skin diseases characterised by abnormal keratinocyte proliferation and differentiation. In studies by Kishibe et al. (15) that compared WT and Klk8$^{-/-}$ mice in a model for psoriasis, Klk8 was found to be an important factor in inducing the expression of Klk6 and Klk7 after 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment. These findings are consistent with earlier observations by Komatsu et al. (163) showing the increased expression in psoriatic and atopic dermatitis patients of KLK6, KLK8 and KLK13 in the stratum corneum, stratum granulosum, sebaceous glands, eccrine sweat glands, hair follicles and nerves. This increase in proteins, as detected by IHC, was associated with increased mRNA expression, detected by in situ hybridisation. More specifically, elevated KLKs were identified in the upper epidermis of psoriasis patients; while, in atopic dermatitis patients, KLK expression was diffusely expressed through to the lower epidermis. The same group (142) later investigated the presence of elevated KLK levels in the serum and stratum corneum of psoriasis patients. KLK6, KLK10 and KLK13 levels were significantly elevated, even in the non-lesional stratum corneum of these

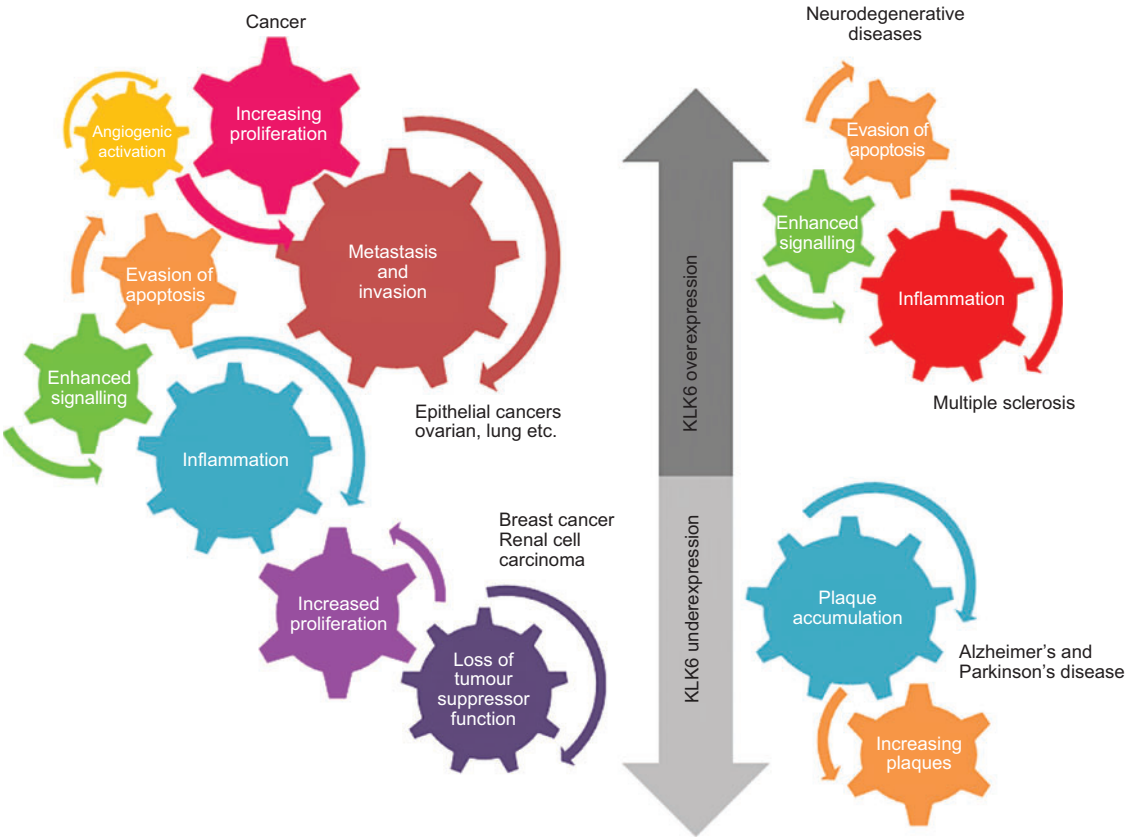

Figure 4 Consequences of dysregulated KLK 6 expression in cancer and neurodegenerative diseases.
Experimental evidence discussed in the text implicates KLK6 in many diverse molecular pathways. In cancers, the increase of KLK expression can be oncogenic, leading to increases in inflammation, enhanced signalling and evasion of apoptosis; which can drive cancer progression to enhance pathways involved with cellular proliferation, invasion, and metastasis. However, studies in breast cancer indicate a tumour suppressor role for KLK6. Loss of KLK6 expression in breast, and possibly renal cancers, results in the loss of this tumour suppressive function, leading to cancer progression. For multiple sclerosis, experimental evidence suggests that increased KLK6 levels are associated with inflammation, which can also enhance signalling and evasion of apoptosis for inflammatory cells with sustained signalling. Loss of KLK6 in the case of AD and PD leads to a shift in the normal turnover of neural proteins, leading to increased accumulation of plaque-forming fragments.

patients. Serum KLK6, KLK8, KLK10 and KLK13 levels in patients with untreated psoriasis significantly correlated with Psoriasis Area and Severity Index (PASI) score (164). Moreover, patients with erythrodermic psoriasis exhibited significantly higher serum KLK levels than normal subjects or patients with psoriasis vulgaris or arthropathic psoriasis. Similar to psoriasis and atopic dermatitis, elevated KLK expression, including KLK6, has been identified in Peeling Skin Syndrome Type B, a congenital skin disease associated with continual skin peeling and ichthyotic erythroderma (165), implicating a prominent role for the KLKs in the hyperactive desquamation associated with such skin disorders.

Furthermore, in vitro evidence shows that while KLK7 can directly induce E-cadherin shedding (166) leading to enhanced keratinocyte proliferation, migration and wound-healing associated epithelialisation, KLK6 (74) can also participate in the process. This was supported by in vivo evidence (74), through the creation of transgenic mice expressing a Klk6-Myc/His fusion protein, showing a highly significant increase in the percentage of proliferating cell nuclear antigen (PCNA)-positive keratinocytes following wounding. Work by Scarisbrick and colleagues (91, 138, 167) suggests that KLK6 drives the dysregulated inflammatory processes in the CNS, as shown by its up-regulation in activated immune cells. Indeed, Klk6 shows expression in $CD4^+$ and $CD8^+$ T-cells, as well as macrophages (168, 169). More recently, this group (170) examined whether KLK6 alters immune cell survival. Over-expression of recombinant KLK6 in mouse splenocytes showed no significant change in splenocyte proliferation after 24 or 72 h, however a dose-dependent reduction in the number of cells positive for markers of cell death was observed. These observations were strengthened when these cells were treated with the death-inducing agents dexamethosone, staurosporine or Fas ligand. KLK6-treated Jurkat cells also showed similar survival phenotypes and led to the conclusion that KLK6 can differentially regulate Bcl-2 signalling through the up-regulation of the pro-survival protein, Bcl-XL; and inhibition of the pro-apoptotic Bcl-2 family member protein, Bim. Together these findings strongly endow KLK6 with pro-survival activities.

Tumourigenesis

We already mentioned the observed over-expression of KLK6 transcript and protein in a variety of cancers, such as ovarian carcinomas (11, 30–32, 66, 68, 70, 76, 86, 93–96, 124, 130–136), breast (66, 97), uterine (98), pancreatic (99), colorectal (75, 100, 101), gastric (102), skin (74), urinary bladder cancers (103), gliomas (127), lung (125, 126) and salivary gland tumours (128). Focus is now being diverted to revealing the details of KLK6's role in promoting tumourigenesis.

The majority of investigations regarding the consequences of KLK6 over-epxression have been linked to functions supported by in vitro studies demonstrating the ability of KLK6 to degrade fibrinogen and various collagens, which constitute the basement membrane (114, 116). These findings have significant implications for tissue remodelling, tumour invasion and migration. Indeed, several studies have shown that the decrease of KLK6 protein in cancer cell line models, either through the use of inhibitors or siRNAs (75, 101–103), results in the concomitant reduction in invasive potential and cellular proliferation. The KLK6-siRNA treatment of colon cancer cell lines by Kim et al. (75) showed a variable decrease in cell proliferation, although some cell lines remained similar to controls. Down-regulation of matrix metalloprotease-1 (MMP-1) and MMP-12 were also observed in some cell lines treated with siKLK6. Although a study by Prezas et al. (136) showed an increase in tumour burden and invasive potential in ovarian cancers transfected with an expression vector inducing KLKs 4–7, it is unclear what level of contribution to the observed tumourigenicity each member made. The ectopic Klk6 expression, in mouse keratinocyte cell lines by Klucky et al. (74), induced spindle-like morphology and enhanced proliferation, migration and invasive capacity. Furthermore, a reduction of E-cadherin protein levels was also detected in the cell membrane and of β-catenin nuclear translocation in Klk6-expressing mouse keratinocytes and human HEK293 cells transfected with a KLK6 expression plasmid. These observed changes in E-cadherin were also seen by the strong inhibition of the E-cadherin promoter upon exogenous KLK6-GFP over-expression in transfected colon carcinoma cell lines (75), strongly suggesting that the secretion of KLK6 contributes to the down-regulation of E-cadherin. Cell-adhesion defects were also observed in the presence of KLK6, which were rescued in the presence of inhibitor of metalloproteinase (TIMP)-1 and TIMP-3. Other studies have made similar associations between KLK6 expression and aberrant expression of adhesion and communication molecules. In a study by Rezze et al. (171), IHC analyses of the adhesion and communication molecules connexin 43, desmocollin 3, cytokeratin 5 together with KLK6 and KLK7 in a melanoma progression model were investigated and it was found that the expression of KLK6 and KLK7 may be responsible for the loss of cell-cell adhesion.

Cell adhesion defects not only characterise morphological changes, but mark alterations in other molecular pathways including apopotosis and angiogenesis. In keeping with findings by Scarisbrick et al. (170) describing that the over-expression of KLK6 in hematopoietic cells leads to the activation of pro-survival pathways, Kim et al. (75) found that suppression of KLK6 was associated with the activation of caspase-8 and caspase-3, as well as the up-regulation of $p21^{cip}$. Anoikis, defined as the process by which an anchorage-dependent cell undergoes programmed cell death as a result of detachment from the surrounding extracellular matrix (172, 173), is another affected mechanism by which cancers progress to metastatic disease. In a study by Kupferman et al. (174), anoikis-resistant oral squamous carcinoma cell lines were generated under detached growth conditions. cDNA expression profiling identified a 6.2-fold increase in KLK6, among others, in anoikis-resistant cell lines compared to anoikis-sensitive cells under detached conditions. Based on these findings, this novel association of KLK6 in anti-apoptotic pathways should be further investigated. Cancer progression also involves the formation of additional vasculature. Aimes et al. (175) identified the expression of genes, including KLK6,

in endothelial cells which appear to be regulated, in part, by the nature of the substratum associated with these cells.

The role of KLK6 in intracellular signalling has also been actively studied in the context of protease-activated receptors (PARs) (117, 176, 178, 179). PARs 1–4 are members of the G-protein-coupled family of receptors which are subject to activation through the proteolytic cleavage of the receptor N-terminal sequence to reveal an activating tethered ligand (180, 181). These receptors have been implicated in various physiological roles, including platelet activation, the modulation of endothelial and vascular smooth muscle cell function, inflammatory responses to joint injury and tumour cell growth and metastasis [reviewed by Oikonomopoulou et al. (182)]. Extensive in vitro work performed by Oikonomopoulou et al. (117, 178, 179) demonstrated the ability of KLK6, as well as other KLK members to cleave the synthetic peptides representing the cleavage-activation sequences of PAR1, PAR2 and PAR4 and either activating or disarming the receptor. Calcium signalling was activated by KLKs 5, 6 and 14 in rat PAR2-expressing K-RAS transformed kidney (KNRK) cells, while calcium signalling in HEK cells co-expressing human PAR1 and PAR2 was also triggered by KLK6 through PAR2. Although the majority of work points to pathways associated with inflammation, cancer investigators are increasingly aware of the link and high degree of cross-talk with inflammation (183–185). Thus, activation of PARs by KLK6 in the context of carcinogenesis would feed into known oncogenic signalling pathways, such as PI3K and RAS (186); recapitulating recent findings for KLK4 in signalling through PARs 1 and 2 in prostate cancer (187–189). More compelling evidence comes from Scarisbrick et al. (170), who demonstrated the lack in ability of KLK6 to promote survival of splenic T-cells derived from PAR1-deficient mice.

Due to the associated over-expression in virtually most epithelial cancers, the vast majority of evidence supports an oncogenic role for KLK6. However, the original cloning of KLK6 (66) implicated KLK6 as a putative class II tumour suppressor gene due to its loss of expression in metastatic breast cancer. Work performed by Pampalakis et al. (17), already discussed in this review, demonstrated the inactivation of KLK6 in metastatic breast cancer due to epigenetic events. The functional consequences of such inactivation were further investigated to show that KLK6 may have a protective role against breast cancer progression by inhibiting the epithelial-to-mesenchymal transition (EMT). Indeed, stable transfection of the non-KLK6 expressing breast cancer cell line, MDA-MB-231, with a preproKLK6 construct resulted in a reduction of cellular proliferation, motility and anchorage-independent growth in soft agar. Subsequent proteomic analyses of these transfected lines revealed the down-regulation of the mesenchymal marker, vimentin; and up-regulation of the epithelial markers cytokeratin 8, cytokeratin 19 and calreticulin; indicating a transition back to a more epithelial phenotype. When implanted into the mammary fat-pads of SCID mice, KLK6-expressing MDA-MB-231 cells showed significant inhibition of tumour growth compared to mock-transfected and parental cell lines. Interestingly, when clones expressing KLK6 at near-physiological conditions for breast tissues (i.e., 1–30 μg/L) were compared to those grossly over-expressing KLK6 (i.e., >100–400 μg/L), tumours resulting from grossly KLK6-over-expressing clones exhibited similarities in size and frequency to parental and mock transfected lines by the study endpoint. Additionally, in renal cell carcinomas, a decrease in KLK6 expression has also been identified in comparison to normal kidney (62, 137, 190), but found to be generally higher in high-grade tumours as compared to low-grade tumours. These findings stress the functional importance of physiological levels and the balance of such expression, since data derived from the breast cancer studies suggest that over-expression resulted in tumour formation consistent with the parental line.

Neurodegeneration

The highest expression of KLK6 in normal tissues is in the CNS. The distribution of KLK6 protein expression within the different cell types and regions of the brain varies. The highest protein expression is seen in the adult spinal cord, brain stem, cerebral cortex, choroid plexus and hypothalamus (90, 104, 122). Neurons and glial cells show positivity for KLK expression (90, 104, 191), with weaker staining of Purkinje cells and stellate cells (122). Particular interest in the role of KLK6 in the brain comes from its identification (as *Zyme*) by Little et al. (71). As such, its role in both normal and diseased brains has been actively pursued.

Alzheimer's disease, the most common form of dementia (192, 193), is characterised by the accumulation of amyloid β (Aβ), a proteolytic fragment of the amyloid precursor protein (APP) (194). Little et al. (71) observed in vitro that co-expression of *KLK6* cDNA with *APP* cDNA in HEK cells resulted in an abundance of truncated amyloidogenic fragments, implicating a role for KLK6 in the normal turnover of such proteins in the brain. Indeed, compelling evidence shows the ability for KLK6 to cleave APP at three sites: one at the amino acid outside of the amino terminal end of the Aβ sequence and the other two located within the Aβ sequence (71, 112, 114). Moreover, the proteotyic abilities of KLK6 against components of the extracellular matrix and perineuronal net appears to help maintain the clearance and turnover of the neural microenvironment (106, 195). These observations are in keeping with observations by IHC in normal and AD brain sections showing the decreased expression of KLK6 in AD brains over controls (20, 104). Similarly, KLK6 levels in cerebrospinal fluid, blood and tissues from AD patients revealed a similar trend with an average 2-fold decrease of KLK6 in brain tissue extracts compared to controls (139, 140, 191). Menendez-Gonzalez et al. (139) measured KLK6 plasma levels in 228 healthy individuals and 447 patients with cognitive symptoms (including AD, mild cognitive impairment, dementia with Lewy bodies or Parkinson's-dementia, frontotemporal dementia, Huntington's disease, primary progressive aphasia, corticobasal degeneration, Creutzfeldt-Jakob's disease or pseudodementia). KLK6 was found to increase with age in healthy individuals, but was decreased in AD patients, consistent with previous findings in brain extracts (140, 196) and CSF (191). Furthermore, levels

of KLK6 in plasma differed significantly between AD and Vascular Dementia (VaD) patients, Pseudodementia patients and the control group, with no significant differences among the other cognitive-impaired symptoms with AD. Closer investigation by Ashby et al. (20) reported similar findings of decreased KLK6 protein and RNA expression in AD patients and the increase of KLK6 in patients with VaD. Interestingly, positive immunoreactivity was seen in only the normal endothelial cells lining blood vessels of the temporal cortex, white matter and leptomeninges; and a lack of co-localisation between KLK6 and plaques or neurofibrillary tangles. When KLK6 protein levels were adjusted for endothelial cell density, AD patients showed a significant decrease in the frontal cortex, although not in the temporal cortex, when compared to controls. Among VaD patients, however, a significant 2-fold increase was found in the frontal cortex, but not in the temporal cortex, when compared to controls. This curious finding of increased KLK6 expression in the blood vessels of the frontal cortex of VaD patients warrants further investigation, as there may be relevant connections to KLK6-implicated roles in inflammation and angiogenesis.

Like AD, (PD) is also a neurodegenerative disorder but is characterised by the accumulation of insoluble α-synuclein (α-synuclein) (197). The various reports to date in PD are less consistent than those in AD indicating the need for more studies to resolve such contradictions. Iwata et al. (119) observed the in vitro degradation of α-synuclein in HEK-293 cells, which was dose-dependent when serine protease inhibitors and KLK inhibitors were used. It's subcellular localisation in the HEK-293 model was identified with mitochondrial and microsomal fractions, rather than nuclear or cytosolic fractions, which was confirmed by co-localisation of KLK6 with cytochrome C. Interestingly, under stress conditions induced by UV irradiation, KLK6 was released from the mitochondria into the cytosol. siRNA experiments also showed the increased expression of intact α-synuclein in SH-SY5Y cells. Moreover, KLK6-mediated degradation of α-synuclein also resulted in the inhibition of α-synuclein polymerisation. When proteolytic activity was tested against mutant strains of α-synuclein, KLK6 showed less efficient degradation. The resistance to degradation of mutant or modified forms of α-synuclein was similarly confirmed by Kasai et al. (198), showing that phosphorylated α-synuclein was more resistant to degradation than non-phosphorylated α-synuclein. Moreover, the α-synuclein A30P-mutant, which has been linked to familial PD (199), was more resistant to degradation than the wild-type and other α-synuclein mutants. Interestingly, this is in contradiction to findings by Iwata et al. (119), who reported similar rates of digestion in the type α-synuclein and the A30P mutant, with the A53T for showing greatest resistance to degradation. Other inconsistencies are reflected by the in vitro studies by Tatebe et al. (200), who demonstrated that prepro-KLK6 localised to the endoplasmic reticulum, rather than localisation to the mitochondria as reported by Iwata et al. (119) using the same cell line system. As well, proteolytic activity of KLK6 was limited to extracellular KLK6 and not intracellular KLK6. Co-transfection of KLK6 and α-synuclein into HEK-293 cells showed no cleavage of intracellular α-synuclein, contrary to findings by Iwata et al. (119), who suggested that α-synuclein was degraded intracellularly by KLK6. As these issues continue to be resolved, these studies suggest that the failure of KLK6 to degrade α-synuclein contributes to the pathogenesis of PD, possibly through the disrupted trafficking of KLK6 (200).

Unlike AD and PD, KLK6 levels in patients with Multiple Sclerosis (MS) are elevated (91, 138, 167, 170, 201) and linked to inflammatory pathways. Indeed, several studies by Scarisbrick and colleagues (91, 138, 167, 170) as well as Hebb et al. (201) have documented significant increases in serum-KLK6 in patients with MS, as well as those experiencing a secondary progressive disease course. MS is a neurodegenerative disorder whereby the myelin sheath, that protects nerve cells, is damaged. Damage to the myelin sheath is driven primarily by inflammation (202). We have already eluded to the role of KLK6 in inflammation above, and many of the KLK6-related studies undertaken in MS point to dysregulated inflammatory pathways. Animal models of MS have also shown increased KLK6 levels from infiltrating immune cells at the site of CNS inflammation (72, 138, 167, 169). The KLK6 link to inflammation is further strengthened by the ability of KLK6 to activate PARs, which have key roles in driving the inflammatory process (117, 170, 176–179, 203). Vandell et al. (203) corroborated the intracellular calcium flux by PAR1 in neurons, and by PAR1 and PAR2 in astrocytes, but also demonstrated an altered activation state of MAPK and AKT. The prolonged survival of inflammatory cells imparted by KLK6, and through altered expression of Bcl-2-family members (170), reveals a novel mechanism in signalling cascades within the CNS. In addition, to its contribution to sustained activation of inflammatory signalling, KLK6 also degrades myelin proteins (106, 169) and has been detected in elevated levels in inflammatory demyelinating lesions of both viral and autoimmune MS models, as well as in MS pathogenic lesions (72, 168, 169).

With strong evidence to support the hypothesis that KLK6 plays a role in the homeostasis of the CNS, KLK6 has been investigated in mouse models of spinal cord injury. Terayama et al. (204) observed that, after injury to the mouse spinal cord, KLK6 mRNA expression was induced in the white matter in the area immediately adjacent to the lesion which peaked 4 days post-injury and declined over 14 days. In the white and gray matter surrounding the lesion, enhanced expression of KLK6 mRNA was observed to peak at 4 days and persisted over 14 days. Furthermore, Bando et al. (205) examined experimental oligodendrocytic demyelination and remyelination in mice, showing that KLK6 mRNA and protein were reduced during demyelination and increased during remyelination. The observed association of myelin basic protein (MBP) with KLK6, in cultured primary oligodendrocytes, was strengthened by the inhibition of KLK6 using RNAi that resulted in the concomitant reduction of MBP mRNA.

Clinical utility

The use of KLK3/PSA as a diagnostic and prognostic marker in prostate cancer has led to the evaluation of KLK6 as

a putative biomarker for many of the neoplasms and diseased states already mentioned in this review. Since KLK6 is secreted, it has great potential as a biomarker that can be evaluated in patient specimens obtained by less invasive means, such as blood or urine. The ability to detect KLK6 in blood and other biological fluids has already been discussed. To date, the majority of clinical investigations have been performed retrospectively from tissue specimens, with a handful also looking at KLK6 levels from paired biological fluids.

In cancer, an overwhelming number of studies show the overall increase in KLK6 mRNA and protein expression when compared to their respective normal tissues, providing the basis for its evaluation across clinical parameters. The vast majority of KLK6 cancer-related studies encompass ovarian cancer (30, 70, 94–96, 131, 132, 134, 206). A heterogeneous disease, ovarian cancer is classified under four major subtypes (serous, endometrioid, mucinous and clear cell) (207). KLK6 appears to be over-expressed across all sub-types (32, 95, 96, 133), with serous and undifferentiated cancers generally showing greater percentages of cases with KLK6 positivity (70, 131). Currently, the carbohydrate antigen, CA-125, is routinely used as a biomaker of ovarian carcinoma, but suffers from the lack of sensitivity for early stage cancers; and from the lack of specificity since other physiological conditions can contribute to elevated CA-125 levels. However, the high sensitivity and specificity for patients with advanced stage disease makes CA-125 a more appropriate marker for ovarian cancer recurrence detection and monitoring therapy (208, 209). Nonetheless, a small percentage of cancers fail to exhibit elevated CA-125 levels (209). The utility of KLK6, in this case, was demonstrated by Rosen et al. (94), who showed that of the approximately 22% of ovarian cancers which were negative for CA-125 expression, all were positive for other markers including KLK6. Along these lines, several studies have consistently shown that the combination of KLK6 with CA-125 enhances their individual diagnostic power (95, 131, 132, 206). White et al. (95) evaluated the RNA expression of 106 sporadic cancers, showing an overall improved sensitivity from 82% to 93% for the detection of early staged cancer when KLK6 or KLK13 was combined with CA-125. The negative predictive value increased from 27% to 50% when CA-125 was combined with either KLK. These findings are consistent with KLK6 RNA expression studies by Shan et al. (70) who also showed that KLK6 expression was significantly associated with late stage (stage III/IV) disease, higher tumour grade, sub-optimal debulking and serous and undifferentiated histotypes. Furthermore, univariate Cox regression showed that KLK6-positive patients showed an increased risk of relapse and death, in contrast to KLK6-negative patients. Progression-free survival (PFS) and overall survival (OS) continued to be significant with positive KLK6 expression when treated as a continuous variable (after logarithmic transformation). Kaplan-Meier analysis revealed a significant association between KLK6 expression status and both progression-free survival (PFS) and overall survival (OS); which was in contrast to those generated based on CA-125 alone. Tissue KLK6 protein levels in tumour specimens by Hoffman et al. (131) also showed that patients with KLK6-positive tissues were more likely to suffer from progressive disease and die from their cancers; and this finding was recapitulated in survival curves. The value of KLK6 RNA levels to tumour recurrence was also investigated by White et al. (96), showing that the expression levels of KLK6 and KLK13 were significantly increased in invasive cancers as compared to normal ovarian tissues. The high expression was also shown to be a poor prognostic indicator and indicative of a shorter recurrence-free survival. On multivariate analysis, patients with high KLK6 had a 3-fold chance of recurring than those patients with lower KLK6 expression. In contrast to RNA or protein levels, only three studies to date have addressed the role of copy number in ovarian carcinomas. Bayani et al. (30, 31) and others (32) have demonstrated that the *KLK* locus is subject to copy-number changes. In our recent study (30), we showed that the *KLK* locus experiences high genomic instability and copy-number heterogeneity which was significantly correlated to tumour grade. However, there was no strong correlation between copy-number and level of immunoreactivity for KLK6 by IHC. Kaplan-Meier curves indicated a trend for better OS and PFS for those patients who had a net deletion (one copy) of the locus, over those with normal copies (two copies) or those with extra copies of the locus. At the protein level, post-translation modifications of KLK6 have been recently investigated by Kuzmanov et al. (115). Comparison between CSF-derived KLK6 and ovarian cancer ascites-derived KLK6 showed modification of the ovarian-derived KLK6 isoform with α2-6-linked sialic acid. Although requiring further investigation, the characterisation and detection of cancer-specific isoforms could greatly improve cancer detection and monitoring. Due to these studies, KLK6 has been included in large ovarian cancer biomarker validation efforts by the Early Detection Research network (EDRN) and Specialised Programs of Research Excellence (SPORE) (210, 211), of which our laboratory participated. Consistent with the previously described studies, KLK6 generally performed well, ranking among the top 10–20 (of 49 markers). Specifically, KLK6 ranked 9th across all cases and general population controls in terms of sensitivity (95% confidence interval) and 15th in Area Under the Curve (AUC – 95% confidence interval). However, for early staged cases and general population controls, KLK6 ranked 29th and 28th for sensitivity (95% confidence interval) and AUC (95% confidence interval), respectively. The results of these studies clearly indicate the need for identifying novel biomarkers, to be utilised individually and in panels, for both early detection and monitoring, since none of the biomarkers tested performed any better than CA-125 alone.

For other cancers, evaluation of KLK6 against clinical outcome and histopathological data has also shown similar results. In colon cancer, a comprehensive study of 143 cancers by Kim et al. (75) showed significantly elevated transcript and protein levels between cancerous and paired non-cancerous colon tissues. Histologically, KLK6 was strongly expressed in adenocarcinomas, but not in the normal mucosa or in premalignant dysplastic lesions. In sera, patients with cancer also showed an increase in KLK6 concentration as compared to normal controls. When compared by stage, there was no difference between the levels of expression. Kaplan-Meier curves

showed a significant correlation between high KLK6 expression and shorter OS and PFS as well as greater risk of recurrence. KLK6 was significantly associated with lymph node status, tumour metastasis and Duke's stage. Similarly, Ogawa et al. (100) showed that the significant over-expression of KLK6 transcripts was associated with serosal invasion, liver metastasis, as well as advanced Dukes stage. KLK6 transcript levels have also been detected in the circulating tumour cells from the peripheral blood of colorectal cancers (212), however the clinical utility of this method still requires rigorous investigation. Among gastric cancers, Nagahara et al. (102) showed that elevated KLK6 transcript levels were significantly associated with lymphatic invasion and tumour grade, as well as significantly lower survival rate, as compared to those with lower KLK6 transcript expression. In pancreatic ductal adenocarcinomas, statistical analysis showed that co-expression of KLK6 and KLK10 correlated with an R1-resection status and worse outcome for OS (99). For renal cell carcinomas (RCC), Petraki et al. (190) performed a comprehensive survey of both normal kidney and RCCs, correlating these to tumour size, histological type, histological malignancy according to the Fuhrman four-grade scale, mitotic index, pathological stage and disease survival. KLK6 protein expression by IHC was decreased in cancers as compared to the normal kidney. Highly malignant RCCs showed greater expression than much lower malignant RCCs and the data were statistically significant. A strong positive correlation with both Robson and TNM (2002) staging was seen for KLK6. Kaplan-Meier and univariate Cox regression showed that KLK6 expression was negatively correlated with disease-specific survival. Gabril et al. (137) reported detailed histopatholgical findings of KLK6 expression among the various renal cell carcinoma sub-types. Among clear cell subtypes, low nuclear grade tumours showed granular cytoplasmic and membranous staining with some evidence of nuclear immunoreactivity. The papillary sub-type showed strong diffuse cytoplasmic expression with focal apical accentuation. In contrast, oncocytomas and chromophobe RCCs showed no expression of KLK6 in tumour cells, making KLK6 a potential biomarker for distinguishing the different sub-types of RCC. The observations by IHC were confirmed by White et al. (62), who evaluated the level of KLK6 transcript across the various sub-types of RCCs. KLK6 transcripts showed an overall decrease across the tumours as compared to paired normal.

Finally, the clinical utility KLK6 has great potential for neurological disorders, such as AD, PD and MS, as already discussed in length above. Thus, more intensive investigation is required to unlock the biological relevance of this protease and translate this to meaningful information that can benefit the patient.

Translational and therapeutic applications

In light of the diverse and putative roles to which KLK6 contributes, it is apparent that re-establishing normal physiological levels, targeting up- or down-stream pathways, molecules or substrates could be effective in managing the disease course. It is critical, therefore, to continue investigating all facets of this protease. We have already discussed the possibility that some identified RNA species may not produce a protein; or that proteins produced may not be functional. It is conceivable that such non-functional isoforms could act to sequester partner molecules, such as inhibitors, increasing the potential for active enzyme isoforms to access their substrates. Furthermore, transcript heterogeneity detected in both normal and diseased specimens should be investigated, as the importance of such variants are becoming realised due to advances in high-throughput genome technologies. Clearly, the observed over- and under-expression of KLK6 has biological and clinical implications, however very little information exists regarding the proportion of immunoreactive enzyme that can actively participate in enzymatic reactions. With this in mind, Oikonomopoulou et al. (213) developed a serine proteinase-targeted-activity-based-probe-coupled-to-antibody capture assay based on previous studies (214). This enabled the quantification of the proportion of enzymatically active KLK6 from CSF and ascites fluid from ovarian cancer patients, in addition to supernatants from cancer cell lines. This approach enabled the monitoring of pro-KLK6 conversion to its active enzyme species demonstrating up to 5% of immunoreactive KLK6 detected in clinical samples represents active enzyme. It can be envisioned that improvements in such technology could help determine and aid in the modulation to physiologically relevant levels, which can have profound effects on many down-stream cellular processes (Figure 4). Similarly, we have seen that the modulation of KLK6 levels in vitro using siRNA technologies and alike result in biological consequences. Therefore, advancements in targeted gene therapy and delivery (215, 216) could be applied to enhance expression in specific tissues. However, most work to date focuses on identifying the substrates and inhibitors for KLK6 as well as the other KLKs (114, 158–161). No doubt screening for inhibitors using small molecule and pharmacological libraries are currently underway and, once identified and comprehensively tested, these will have profound effects on all aspects of normal and disease physiology and implications on their treatments. Indeed, several patent documents can be easily found within the public domain describing the potential utility of KLK6 as a biomarker for many of the disease states mentioned in this review, making the development of such inhibitors a valuable asset.

Concluding remarks

Since the discovery and characterisation of the human *KLK* locus just over a decade ago, we are still uncovering the similarity and diversity of the *KLK* family members. In this review we attempted to describe the newest findings of one of these members. KLK6 has shown to support both oncogenic and protective functions; participate in cascade signalling; activate signal transduction pathways; participate in angiogenesis, apoptosis and inflammatory pathways. The variability of its substrates, including components of the basement membrane and extraceullular matrix, could play important roles on the tissue microenvironment as it relates to normal physiological

homeostasis; or in disease progression and metastasis. The observed over- or under-expression of either the RNA or protein across different cancers and diseases qualifies KLK6 as a potentially important clinical biomarker. As we move towards personalised medicine, it is evident that the greatest strength of this (or other) putative biomarker will be realised in combination with other molecules. With continued improvements in the technologies for studying molecular biology, there is no doubt that new roles for KLK6 will be elucidated.

Acknowledgments

The authors wish to acknowledge the efforts and contributions of all the past and present members of the Diamandis Laboratory as well as those of our many collaborators. The authors regret the omission of any studies to this body of work.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. Nat Rev Cancer 2005;5:845–56.
2. Lilja H, Ulmert D, Vickers AJ. Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. Nat Rev Cancer 2008;8:268–78.
3. Borgono CA, Diamandis EP. The emerging roles of human tissue kallikreins in cancer. Nat Rev Cancer 2004;4:876–90.
4. Clements JA. Reflections on the tissue kallikrein and kallikrein-related peptidase family – from mice to men – what have we learnt in the last two decades? Biol Chem 2008;389:1447–54.
5. Sotiropoulou G, Pampalakis G, Diamandis EP. Functional roles of human kallikrein-related peptidases. J Biol Chem 2009;284:32989–94.
6. Yousef GM, Chang A, Scorilas A, Diamandis EP. Genomic organization of the human kallikrein gene family on chromosome 19q13.3-q13.4. Biochem Biophys Res Commun 2000;276:125–33.
7. Harvey TJ, Hooper JD, Myers SA, Stephenson SA, Ashworth LK, Clements JA. Tissue-specific expression patterns and fine mapping of the human kallikrein (klk) locus on proximal 19q13.4. J Biol Chem 2000;275:37397–406.
8. Borgono CA, Michael IP, Diamandis EP. Human tissue kallikreins: physiologic roles and applications in cancer. Mol Cancer Res 2004;2:257–80.
9. Yousef GM, Diamandis EP. The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr Rev 2001;22:184–204.
10. Diamandis EP, Yousef GM, Luo LY, Magklara A, Obiezu CV. The new human kallikrein gene family: Implications in carcinogenesis. Trends Endocrinol Metab 2000;11:54–60.
11. Shaw JL, Diamandis EP. Distribution of 15 human kallikreins in tissues and biological fluids. Clin Chem 2007;53:1423–32.
12. Emami N, Diamandis EP. Human kallikrein-related peptidase 14 (klk14) is a new activator component of the klk proteolytic cascade. Possible function in seminal plasma and skin. J Biol Chem 2008;283:3031–41.
13. Michael IP, Pampalakis G, Mikolajczyk SD, Malm J, Sotiropoulou G, Diamandis EP. Human tissue kallikrein 5 is a member of a proteolytic cascade pathway involved in seminal clot liquefaction and potentially in prostate cancer progression. J Biol Chem 2006;281:12743–50.
14. Eissa A, Amodeo V, Smith CR, Diamandis EP. Kallikrein-related peptidase-8 (klk8) is an active serine protease in human epidermis and sweat and is involved in a skin barrier proteolytic cascade. J Biol Chem 2010;286:687–706.
15. Kishibe M, Bando Y, Terayama R, Namikawa K, Takahashi H, Hashimoto Y, et al. Kallikrein 8 is involved in skin desquamation in cooperation with other kallikreins. J Biol Chem 2007;282:5834–41.
16. Komatsu N, Takata M, Otsuki N, Toyama T, Ohka R, Takehara K, et al. Expression and localization of tissue kallikrein mrnas in human epidermis and appendages. J Invest Dermatol 2003;121:542–9.
17. Pampalakis G, Prosnikli E, Agalioti T, Vlahou A, Zoumpourlis V, Sotiropoulou G. A tumor-protective role for human kallikrein-related peptidase 6 in breast cancer mediated by inhibition of epithelial-to-mesenchymal transition. Cancer Res 2009;69:3779–87.
18. Mo L, Zhang J, Shi J, Xuan Q, Yang X, Qin M, et al. Human kallikrein 7 induces epithelial-mesenchymal transition-like changes in prostate carcinoma cells: a role in prostate cancer invasion and progression. Anticancer Res 2010;30:3413–20.
19. Yousef GM, Kishi T, Diamandis EP. Role of kallikrein enzymes in the central nervous system. Clin Chim Acta 2003;329:1–8.
20. Ashby EL, Kehoe PG, Love S. Kallikrein-related peptidase 6 in alzheimer's disease and vascular dementia. Brain Res 2010:1–10.
21. Schmidt AE, Ogawa T, Gailani D, Bajaj SP. Structural role of gly (193) in serine proteases: investigations of a g555e (gly193 in chymotrypsin) mutant of blood coagulation factor xi. J Biol Chem 2004;279:29485–92.
22. Pavlopoulou A, Pampalakis G, Michalopoulos I, Sotiropoulou G. Evolutionary history of tissue kallikreins. Plos One 2010;5:e13781. doi:10.1371/journal.pone.0013781.
23. Yousef GM, Borgono CA, Michael IP, Diamandis EP. Cloning of a kallikrein pseudogene. Clin Biochem 2004;37:961–7.
24. Gan L, Lee I, Smith R, Argonza-Barrett R, Lei H, McCuaig J, et al. Sequencing and expression analysis of the serine protease gene cluster located in chromosome 19q13 region. Gene 2000;257:119–30.
25. Lu W, Zhou D, Glusman G, Utleg AG, White JT, Nelson PS, et al. Klk31p is a novel androgen regulated and transcribed pseudogene of kallikreins that is expressed at lower levels in prostate cancer cells than in normal prostate cells. Prostate 2006;66:936–44.
26. Puente XS, Lopez-Otin C. A genomic analysis of rat proteases and protease inhibitors. Genome Res 2004;14:609–22.
27. Clements JA, Willemsen NM, Myers SA, Dong Y. The tissue kallikrein family of serine proteases: functional roles in human disease and potential as clinical biomarkers. Crit Rev Clin Lab Sci 2004;41:265–312.
28. Lundwall A, Brattsand M. Kallikrein-related peptidases. Cell Mol Life Sci 2008;65:2019–38.
29. Sotiropoulou G, Rogakos V, Tsetsenis T, Pampalakis G, Zafiropoulos N, Simillides G, et al. Emerging interest in the kallikrein gene family for understanding and diagnosing cancer. Oncol Res 2003;13:381–91.

30. Bayani J, Marrano P, Graham C, Zheng Y, Li L, Katsaros D, et al. Genomic instability and copy-number heterogeneity of chromosome 19q, including the kallikrein locus, in ovarian carcinomas. Mol Oncol 2011;5:48–60.
31. Bayani J, Paliouras M, Planque C, Shan SJ, Graham C, Squire JA, et al. Impact of cytogenetic and genomic aberrations of the kallikrein locus in ovarian cancer. Mol Oncol 2008;2:250–60.
32. Ni X, Zhang W, Huang KC, Wang Y, Ng SK, Mok SC, et al. Characterisation of human kallikrein 6/protease m expression in ovarian cancer. Br J Cancer 2004;91:725–31.
33. Pampalakis G, Sotiropoulou G. Multiple mechanisms underlie the aberrant expression of the human kallikrein 6 gene in breast cancer. Biol Chem 2006;387:773–82.
34. Huang W, Zhong J, Wu LY, Yu LF, Tian XL, Zhang YF, et al. Downregulation and cpg island hypermethylation of nes1/hk10 gene in the pathogenesis of human gastric cancer. Cancer Lett 2007;251:78–85.
35. Sidiropoulos M, Pampalakis G, Sotiropoulou G, Katsaros D, Diamandis EP. Downregulation of human kallikrein 10 (klk10/nes1) by cpg island hypermethylation in breast, ovarian and prostate cancers. Tumour Biol 2005;26:324–36.
36. Cho NY, Kim JH, Moon KC, Kang GH. Genomic hypomethylation and cpg island hypermethylation in prostatic intraepithelial neoplasm. Virchows Arch 2009;454:17–23.
37. Li B, Goyal J, Dhar S, Dimri G, Evron E, Sukumar S, et al. Cpg methylation as a basis for breast tumor-specific loss of nes1/kallikrein 10 expression. Cancer Res 2001;61:8014–21.
38. Lu CY, Hsieh SY, Lu YJ, Wu CS, Chen LC, Lo SJ, et al. Aberrant DNA methylation profile and frequent methylation of klk10 and oxgr1 genes in hepatocellular carcinoma. Genes Chromosomes Cancer 2009;48:1057–68.
39. Lawrence MG, Lai J, Clements JA. Kallikreins on steroids: structure, function, and hormonal regulation of prostate-specific antigen and the extended kallikrein locus. Endocr Rev 2010;31:407–46.
40. Paliouras M, Diamandis EP. Coordinated steroid hormone-dependent and independent expression of multiple kallikreins in breast cancer cell lines. Breast Cancer Res Treat 2007;102:7–18.
41. Zarghami N, Grass L, Diamandis EP. Steroid hormone regulation of prostate-specific antigen gene expression in breast cancer. Br J Cancer 1997;75:579–88.
42. Myers SA, Clements JA. Kallikrein 4 (klk4), a new member of the human kallikrein gene family is up-regulated by estrogen and progesterone in the human endometrial cancer cell line, kle. J Clin Endocrinol Metab 2001;86:2323–6.
43. Cleutjens KB, van Eekelen CC, van der Korput HA, Brinkmann AO, Trapman J. Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J Biol Chem 1996;271:6379–88.
44. Dong Y, Kaushal A, Brattsand M, Nicklin J, Clements JA. Differential splicing of klk5 and klk7 in epithelial ovarian cancer produces novel variants with potential as cancer biomarkers. Clin Cancer Res 2003;9:1710–20.
45. Dong Y, Matigian N, Harvey TJ, Samaratunga H, Hooper JD, Clements JA. Tissue-specific promoter utilisation of the kallikrein-related peptidase genes, klk5 and klk7, and cellular localisation of the encoded proteins suggest roles in exocrine pancreatic function. Biol Chem 2008;389:99–109.
46. Kroon E, MacDonald RJ, Hammer RE. The transcriptional regulatory strategy of the rat tissue kallikrein gene family. Genes Funct 1997;1:309–19.
47. Goard CA, Bromberg IL, Elliott MB, Diamandis EP. A consolidated catalogue and graphical annotation of dbsnp polymorphisms in the human tissue kallikrein (klk) locus. Mol Oncol 2007;1:303–12.
48. Cramer SD, Chang BL, Rao A, Hawkins GA, Zheng SL, Wade WN, et al. Association between genetic polymorphisms in the prostate-specific antigen gene promoter and serum prostate-specific antigen levels. J Natl Cancer Inst 2003;95:1044–53.
49. Nam RK, Zhang WW, Klotz LH, Trachtenberg J, Jewett MA, Sweet J, et al. Variants of the hk2 protein gene (klk2) are associated with serum hk2 levels and predict the presence of prostate cancer at biopsy. Clin Cancer Res 2006;12:6452–8.
50. Pal P, Xi H, Sun G, Kaushal R, Meeks JJ, Thaxton CS, et al. Tagging snps in the kallikrein genes 3 and 2 on 19q13 and their associations with prostate cancer in men of European origin. Hum Genet 2007;122:251–9.
51. Weinsheimer S, Goddard KA, Parrado AR, Lu Q, Sinha M, Lebedeva ER, et al. Association of kallikrein gene polymorphisms with intracranial aneurysms. Stroke 2007;38:2670–6.
52. Zhao W, Wang L, Lu X, Yang W, Huang J, Chen S, et al. A coding polymorphism of the kallikrein 1 gene is associated with essential hypertension: a tagging snp-based association study in a chinese han population. J Hypertens 2007;25:1821–7.
53. Zheng SL, Sun J, Wiklund F, Smith S, Stattin P, Li G, et al. Cumulative association of five genetic variants with prostate cancer. N Engl J Med 2008;358:910–9.
54. Batra J, Nagle CM, O'Mara T, Higgins M, Dong Y, Tan OL, et al. A kallikrein 15 (klk15) single nucleotide polymorphism located close to a novel exon shows evidence of association with poor ovarian cancer survival. BMC Cancer 2011;11:119.
55. Shen M, Vermeulen R, Rajaraman P, Menashe I, He X, Chapman RS, et al. Polymorphisms in innate immunity genes and lung cancer risk in Xuanwei, China. Environ Mol Mutagen 2009;50:285–90.
56. Barbarotto E, Schmittgen TD, Calin GA. Micrornas and cancer: Profile, profile, profile. Int J Cancer 2008;122:969–77.
57. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small rnas with antisense complementarity to lin-14. Cell 1993;75:843–54.
58. Chow TF, Crow M, Earle T, El-Said H, Diamandis EP, Yousef GM. Kallikreins as microrna targets: an in silico and experimental-based analysis. Biol Chem 2008;389:731–8.
59. Jung M, Schaefer A, Steiner I, Kempkensteffen C, Stephan C, Erbersdobler A, et al. Robust microrna stability in degraded rna preparations from human tissue and cell samples. Clin Chem 2010;56:998–1006.
60. Kojima K, Fujita Y, Nozawa Y, Deguchi T, Ito M. Mir-34a attenuates paclitaxel-resistance of hormone-refractory prostate cancer pc3 cells through direct and indirect mechanisms. Prostate 2010;70:1501–12.
61. Sehic A, Risnes S, Khan QE, Khuu C, Osmundsen H. Gene expression and dental enamel structure in developing mouse incisor. Eur J Oral Sci 2010;118:118–30.
62. White NM, Bui A, Mejia-Guerrero S, Chao J, Soosaipillai A, Youssef Y, et al. Dysregulation of kallikrein-related peptidases in renal cell carcinoma: Potential targets of mirnas. Biol Chem 2010;391:411–23.
63. White NM, Chow TF, Mejia-Guerrero S, Diamandis M, Rofael Y, Faragalla H, et al. Three dysregulated mirnas control kallikrein 10 expression and cell proliferation in ovarian cancer. Br J Cancer 2010;102:1244–53.
64. Wissmann M, Yin N, Muller JM, Greschik H, Fodor BD, Jenuwein T, et al. Cooperative demethylation by jmjd2c and lsd1 promotes androgen receptor-dependent gene expression. Nat Cell Biol 2007;9:347–53.

65. Yousef GM. Micrornas: a new frontier in kallikrein research. Biol Chem 2008;389:689–94.
66. Anisowicz A, Sotiropoulou G, Stenman G, Mok SC, Sager R. A novel protease homolog differentially expressed in breast and ovarian cancer. Mol Med 1996;2:624–36.
67. Yamashiro K, Tsuruoka N, Kodama S, Tsujimoto M, Yamamura Y, Tanaka T, et al. Molecular cloning of a novel trypsin-like serine protease (neurosin) preferentially expressed in brain. Biochim Biophys Acta 1997;1350:11–4.
68. Yousef GM, Luo LY, Scherer SW, Sotiropoulou G, Diamandis EP. Molecular characterization of zyme/protease m/neurosin (prss9), a hormonally regulated kallikrein-like serine protease. Genomics 1999;62:251–9.
69. Yousef GM, Borgono CA, White NM, Robb JD, Michael IP, Oikonomopoulou K, et al. In silico analysis of the human kallikrein gene 6. Tumour Biol 2004;25:282–9.
70. Shan SJ, Scorilas A, Katsaros D, Diamandis EP. Transcriptional upregulation of human tissue kallikrein 6 in ovarian cancer: Clinical and mechanistic aspects. Br J Cancer 2007;96:362–72.
71. Little SP, Dixon EP, Norris F, Buckley W, Becker GW, Johnson M, et al. Zyme, a novel and potentially amyloidogenic enzyme cdna isolated from Alzheimer's disease brain. J Biol Chem 1997;272:25135–42.
72. Christophi GP, Isackson PJ, Blaber S, Blaber M, Rodriguez M, Scarisbrick IA. Distinct promoters regulate tissue-specific and differential expression of kallikrein 6 in cns demyelinating disease. J Neurochem 2004;91:1439–49.
73. Pampalakis G, Kurlender L, Diamandis EP, Sotiropoulou G. Cloning and characterization of novel isoforms of the human kallikrein 6 gene. Biochem Biophys Res Commun 2004;320:54–61.
74. Klucky B, Mueller R, Vogt I, Teurich S, Hartenstein B, Breuhahn K, et al. Kallikrein 6 induces e-cadherin shedding and promotes cell proliferation, migration, and invasion. Cancer Res 2007;67:8198–206.
75. Kim JT, Song EY, Chung KS, Kang MA, Kim JW, Kim SJ, et al. Up-regulation and clinical significance of serine protease kallikrein 6 in colon cancer. Cancer 2011;117:2608–19.
76. Diamandis EP, Yousef GM, Soosaipillai AR, Grass L, Porter A, Little S, et al. Immunofluorometric assay of human kallikrein 6 (zyme/protease m/neurosin) and preliminary clinical applications. Clin Biochem 2000;33:369–75.
77. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet 2006;7:85–97.
78. Pang AW, MacDonald JR, Pinto D, Wei J, Rafiq MA, Conrad DF, et al. Towards a comprehensive structural variation map of an individual human genome. Genome Biol 2010;11:R52.
79. Collins FS, Brooks LD, Chakravarti A. A DNA polymorphism discovery resource for research on human genetic variation. Genome Res 1998;8:1229–31.
80. Brookes AJ. The essence of snps. Gene 1999;234:177–86.
81. Kruglyak L, Nickerson DA. Variation is the spice of life. Nat Genet 2001;27:234–6.
82. Ladiges W, Kemp C, Packenham J, Velazquez J. Human gene variation: From snps to phenotypes. Mutat Res 2004;545:131–9.
83. Kurlender L, Borgono C, Michael IP, Obiezu C, Elliott MB, Yousef GM, et al. A survey of alternative transcripts of human tissue kallikrein genes. Biochim Biophys Acta 2005;1755:1–14.
84. Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, et al. Generation and initial analysis of more than 15,000 full-length human and mouse cdna sequences. Proc Natl Acad Sci USA 2002;99:16899–903.
85. Cheng J, Kapranov P, Drenkow J, Dike S, Brubaker S, Patel S, et al. Transcriptional maps of 10 human chromosomes at 5-nucleotide resolution. Science 2005;308:1149–54.
86. Yousef GM, Polymeris ME, Yacoub GM, Scorilas A, Soosaipillai A, Popalis C, et al. Parallel overexpression of seven kallikrein genes in ovarian cancer. Cancer Res 2003;63:2223–7.
87. Komatsu N, Saijoh K, Otsuki N, Kishi T, Micheal IP, Obiezu CV, et al. Proteolytic processing of human growth hormone by multiple tissue kallikreins and regulation by the serine protease inhibitor kazal-type5 (spink5) protein. Clin Chim Acta 2007;377:228–36.
88. Landry JR, Mager DL, Wilhelm BT. Complex controls: the role of alternative promoters in mammalian genomes. Trends Genet 2003;19:640–8.
89. Tan OL, Whitbread AK, Clements JA, Dong Y. Kallikrein-related peptidase (klk) family mrna variants and protein isoforms in hormone-related cancers: Do they have a function? Biol Chem 2006;387:697–705.
90. Scarisbrick IA, Isackson PJ, Ciric B, Windebank AJ, Rodriguez M. Msp, a trypsin-like serine protease, is abundantly expressed in the human nervous system. J Comp Neurol 2001;431:347–61.
91. Scarisbrick IA, Linbo R, Vandell AG, Keegan M, Blaber SI, Blaber M, et al. Kallikreins are associated with secondary progressive multiple sclerosis and promote neurodegeneration. Biol Chem 2008;389:739–45.
92. Ozsolak F, Milos PM. Rna sequencing: advances, challenges and opportunities. Nat Rev Genet 2011;12:87–98.
93. Tanimoto H, Underwood LJ, Shigemasa K, Parmley TH, O'Brien TJ. Increased expression of protease m in ovarian tumors. Tumour Biol 2001;22:11–8.
94. Rosen DG, Wang L, Atkinson JN, Yu Y, Lu KH, Diamandis EP, et al. Potential markers that complement expression of ca125 in epithelial ovarian cancer. Gynecol Oncol 2005;99:267–77.
95. White NM, Mathews M, Yousef GM, Prizada A, Fontaine D, Ghatage P, et al. Human kallikrein related peptidases 6 and 13 in combination withca125 is a more sensitive test for ovarian cancer than ca125 alone. Cancer Biomark 2009;5:279–87.
96. White NM, Mathews M, Yousef GM, Prizada A, Popadiuk C, Dore JJ. Klk6 and klk13 predict tumor recurrence in epithelial ovarian carcinoma. Br J Cancer 2009;101:1107–13.
97. Wang SM, Mao J, Li B, Wu W, Tang LL. Expression of klk6 protein and mrna in primary breast cancer and its clinical significance. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2008;24:1087–9.
98. Santin AD, Diamandis EP, Bellone S, Soosaipillai A, Cane S, Palmieri M, et al. Human kallikrein 6: a new potential serum biomarker for uterine serous papillary cancer. Clin Cancer Res 2005;11:3320–5.
99. Ruckert F, Hennig M, Petraki CD, Wehrum D, Distler M, Denz A, et al. Co-expression of klk6 and klk10 as prognostic factors for survival in pancreatic ductal adenocarcinoma. Br J Cancer 2008;99:1484–92.
100. Ogawa K, Utsunomiya T, Mimori K, Tanaka F, Inoue H, Nagahara H, et al. Clinical significance of human kallikrein gene 6 messenger rna expression in colorectal cancer. Clin Cancer Res 2005;11:2889–93.
101. Henkhaus RS, Gerner EW, Ignatenko NA. Kallikrein 6 is a mediator of k-ras-dependent migration of colon carcinoma cells. Biol Chem 2008;389:757–64.
102. Nagahara H, Mimori K, Utsunomiya T, Barnard GF, Ohira M, Hirakawa K, et al. Clinicopathologic and biological significance of kallikrein 6 overexpression in human gastric cancer. Clin Cancer Res 2005;11:6800–6.

103. Shinoda Y, Kozaki K, Imoto I, Obara W, Tsuda H, Mizutani Y, et al. Association of klk5 overexpression with invasiveness of urinary bladder carcinoma cells. Cancer Sci 2007;98:1078–86.
104. Ogawa K, Yamada T, Tsujioka Y, Taguchi J, Takahashi M, Tsuboi Y, et al. Localization of a novel type trypsin-like serine protease, neurosin, in brain tissues of alzheimer's disease and parkinson's disease. Psychiatry Clin Neurosci 2000;54:419–26.
105. Debela M, Beaufort N, Magdolen V, Schechter NM, Craik CS, Schmitt M, et al. Structures and specificity of the human kallikrein-related peptidases klks 4, 5, 6, and 7. Biol Chem 2008;389:623–32.
106. Bernett MJ, Blaber SI, Scarisbrick IA, Dhanarajan P, Thompson SM, Blaber M. Crystal structure and biochemical characterization of human kallikrein 6 reveals that a trypsin-like kallikrein is expressed in the central nervous system. J Biol Chem 2002;277:24562–70.
107. Gomis-Ruth FX, Bayes A, Sotiropoulou G, Pampalakis G, Tsetsenis T, Villegas V, et al. The structure of human prokallikrein 6 reveals a novel activation mechanism for the kallikrein family. J Biol Chem 2002;277:27273–81.
108. Blaber SI, Yoon H, Scarisbrick IA, Juliano MA, Blaber M. The autolytic regulation of human kallikrein-related peptidase 6. Biochemistry 2007;46:5209–17.
109. Yoon H, Laxmikanthan G, Lee J, Blaber SI, Rodriguez A, Kogot JM, et al. Activation profiles and regulatory cascades of the human kallikrein-related peptidases. J Biol Chem 2007;282:31852–64.
110. Yoon H, Blaber SI, Evans DM, Trim J, Juliano MA, Scarisbrick IA, et al. Activation profiles of human kallikrein-related peptidases by proteases of the thrombostasis axis. Protein Sci 2008;17:1998–2007.
111. Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T. A proteolytic cascade of kallikreins in the stratum corneum. J Invest Dermatol 2005;124:198–203.
112. Angelo PF, Lima AR, Alves FM, Blaber SI, Scarisbrick IA, Blaber M, et al. Substrate specificity of human kallikrein 6: salt and glycosaminoglycan activation effects. J Biol Chem 2006;281:3116–26.
113. Bayes A, Tsetsenis T, Ventura S, Vendrell J, Aviles FX, Sotiropoulou G. Human kallikrein 6 activity is regulated via an autoproteolytic mechanism of activation/inactivation. Biol Chem 2004;385:517–24.
114. Magklara A, Mellati AA, Wasney GA, Little SP, Sotiropoulou G, Becker GW, et al. Characterization of the enzymatic activity of human kallikrein 6: autoactivation, substrate specificity, and regulation by inhibitors. Biochem Biophys Res Commun 2003;307:948–55.
115. Kuzmanov U, Jiang N, Smith CR, Soosaipillai A, Diamandis EP. Differential n-glycosylation of kallikrein 6 derived from ovarian cancer cells or the central nervous system. Mol Cell Proteomics 2009;8:791–8.
116. Ghosh MC, Grass L, Soosaipillai A, Sotiropoulou G, Diamandis EP. Human kallikrein 6 degrades extracellular matrix proteins and may enhance the metastatic potential of tumour cells. Tumour Biol 2004;25:193–9.
117. Oikonomopoulou K, Hansen KK, Saifeddine M, Vergnolle N, Tea I, Blaber M, et al. Kallikrein-mediated cell signalling: Targeting proteinase-activated receptors (pars). Biol Chem 2006;387:817–24.
118. Oikonomopoulou K, Scorilas A, Michael IP, Grass L, Soosaipillai A, Rosen B, et al. Kallikreins as markers of disseminated tumour cells in ovarian cancer – a pilot study. Tumour Biol 2006;27:104–14.
119. Iwata A, Maruyama M, Akagi T, Hashikawa T, Kanazawa I, Tsuji S, et al. Alpha-synuclein degradation by serine protease neurosin: implication for pathogenesis of synucleinopathies. Hum Mol Genet 2003;12:2625–35.
120. Borgono CA, Michael IP, Komatsu N, Jayakumar A, Kapadia R, Clayman GL, et al. A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. J Biol Chem 2007;282:3640–52.
121. Heuze-Vourc'h N, Ainciburu M, Planque C, Brillard-Bourdet M, Ott C, Jolivet-Reynaud C, et al. Recombinant kallikrein expression: site-specific integration for hk6 production in human cells. Biol Chem 2006;387:687–95.
122. Petraki CD, Karavana VN, Skoufogiannis PT, Little SP, Howarth DJ, Yousef GM, et al. The spectrum of human kallikrein 6 (zyme/protease m/neurosin) expression in human tissues as assessed by immunohistochemistry. J Histochem Cytochem 2001;49:1431–41.
123. Petraki CD, Karavana VN, Diamandis EP. Human kallikrein 13 expression in normal tissues: an immunohistochemical study. J Histochem Cytochem 2003;51:493–501.
124. Petraki CD, Papanastasiou PA, Karavana VN, Diamandis EP. Cellular distribution of human tissue kallikreins: Immuno histochemical localization. Biol Chem 2006;387:653–63.
125. Nathalie HV, Chris P, Serge G, Catherine C, Benjamin B, Claire B, et al. High kallikrein-related peptidase 6 in non-small cell lung cancer cells: An indicator of tumour proliferation and poor prognosis. J Cell Mol Med 2009;13:4014–22.
126. Singh J, Naran A, Misso NL, Rigby PJ, Thompson PJ, Bhoola KD. Expression of kallikrein-related peptidases (krp/hk5, 7, 6, 8) in subtypes of human lung carcinoma. Int Immunopharmacol 2008;8:300–6.
127. Strojnik T, Kavalar R, Zajc I, Diamandis EP, Oikonomopoulou K, Lah TT. Prognostic impact of cd68 and kallikrein 6 in human glioma. Anticancer Res 2009;29:3269–79.
128. Darling MR, Jackson-Boeters L, Daley TD, Diamandis EP. Human kallikrein 6 expression in salivary gland tumors. J Histochem Cytochem 2006;54:337–42.
129. Petraki CD, Karavana VN, Revelos KI, Luo LY, Diamandis EP. Immunohistochemical localization of human kallikreins 6 and 10 in pancreatic islets. Histochem J 2002;34:313–22.
130. Diamandis EP, Yousef GM, Soosaipillai AR, Bunting P. Human kallikrein 6 (zyme/protease m/neurosin): a new serum biomarker of ovarian carcinoma. Clin Biochem 2000;33:579–83.
131. Hoffman BR, Katsaros D, Scorilas A, Diamandis P, Fracchioli S, Rigault de la Longrais IA, et al. Immunofluorometric quantitation and histochemical localisation of kallikrein 6 protein in ovarian cancer tissue: a new independent unfavourable prognostic biomarker. Br J Cancer 2002;87:763–71.
132. Koh SC, Razvi K, Chan YH, Narasimhan K, Ilancheran A, Low JJ, et al. The association with age, human tissue kallikreins 6 and 10 and hemostatic markers for survival outcome from epithelial ovarian cancer. Arch Gynecol Obstet 2011;284:183–90.
133. Kountourakis P, Psyrri A, Scorilas A, Camp R, Markakis S, Kowalski D, et al. Prognostic value of kallikrein-related peptidase 6 protein expression levels in advanced ovarian cancer evaluated by automated quantitative analysis (aqua). Cancer Sci 2008;99:2224–9.
134. Zheng Y, Katsaros D, Shan SJ, de la Longrais IR, Porpiglia M, Scorilas A, et al. A multiparametric panel for ovarian cancer diagnosis, prognosis, and response to chemotherapy. Clin Cancer Res 2007;13:6984–92.

135. Luo LY, Soosaipillai A, Grass L, Diamandis EP. Characterization of human kallikreins 6 and 10 in ascites fluid from ovarian cancer patients. Tumour Biol 2006;27:227–34.
136. Prezas P, Arlt MJ, Viktorov P, Soosaipillai A, Holzscheiter L, Schmitt M, et al. Overexpression of the human tissue kallikrein genes klk4, 5, 6, and 7 increases the malignant phenotype of ovarian cancer cells. Biol Chem 2006;387:807–11.
137. Gabril M, White NM, Moussa M, Chow TF, Metias SM, Fatoohi E, et al. Immunohistochemical analysis of kallikrein-related peptidases in the normal kidney and renal tumors: potential clinical implications. Biol Chem 2010;391:403–9.
138. Scarisbrick IA. The multiple sclerosis degradome: Enzymatic cascades in development and progression of central nervous system inflammatory disease. Curr Top Microbiol Immunol 2008;318:133–75.
139. Menendez-Gonzalez M, Castro-Santos P, Suarez A, Calatayud MT, Perez-Pinera P, Martinez M, et al. Value of measuring plasmatic levels of neurosin in the diagnosis of alzheimer's disease. J Alzheimers Dis 2008;14:59–67.
140. Zarghooni M, Soosaipillai A, Grass L, Scorilas A, Mirazimi N, Diamandis EP. Decreased concentration of human kallikrein 6 in brain extracts of alzheimer's disease patients. Clin Biochem 2002;35:225–31.
141. Komatsu N, Saijoh K, Kuk C, Liu AC, Khan S, Shirasaki F, et al. Human tissue kallikrein expression in the stratum corneum and serum of atopic dermatitis patients. Exp Dermatol 2007;16:513–9.
142. Komatsu N, Saijoh K, Kuk C, Shirasaki F, Takehara K, Diamandis EP. Aberrant human tissue kallikrein levels in the stratum corneum and serum of patients with psoriasis: dependence on phenotype, severity and therapy. Br J Dermatol 2007;156:875–83.
143. Bayani J, Selvarajah S, Maire G, Vukovic B, Al-Romaih K, Zielenska M, et al. Genomic mechanisms and measurement of structural and numerical instability in cancer cells. Semin Cancer Biol 2007;17:5–18.
144. Holland AJ, Cleveland DW. Boveri revisited: Chromosomal instability, aneuploidy and tumorigenesis. Nat Rev Mol Cell Biol 2009;10:478–87.
145. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature 2010;463: 899–905.
146. Murtha P, Tindall DJ, Young CY. Androgen induction of a human prostate-specific kallikrein, hklk2: Characterization of an androgen response element in the 5′ promoter region of the gene. Biochemistry 1993;32:6459–64.
147. Riegman PH, Vlietstra RJ, van der Korput JA, Brinkmann AO, Trapman J. The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol 1991;5:1921–30.
148. Smith MS, Lechago J, Wines DR, MacDonald RJ, Hammer RE. Tissue-specific expression of kallikrein family transgenes in mice and rats. DNA Cell Biol 1992;11:345–58.
149. Pampalakis G, Arampatzidou M, Amoutzias G, Kossida S, Sotiropoulou G. Identification and analysis of mammalian klk6 orthologue genes for prediction of physiological substrates. Comput Biol Chem 2008;32:111–21.
150. Lin R, Nagai Y, Sladek R, Bastien Y, Ho J, Petrecca K, et al. Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin d3 analog eb1089 signaling on cell proliferation, differentiation, and immune system regulation. Mol Endocrinol 2002;16:1243–56.
151. Palmer HG, Sanchez-Carbayo M, Ordonez-Moran P, Larriba MJ, Cordon-Cardo C, Munoz A. Genetic signatures of differentiation induced by 1alpha,25-dihydroxyvitamin d3 in human colon cancer cells. Cancer Res 2003;63:7799–806.
152. Pampalakis G, Diamandis EP, Sotiropoulou G. The epigenetic basis for the aberrant expression of kallikreins in human cancers. Biol Chem 2006;387:795–9.
153. Croce CM. Causes and consequences of microrna dysregulation in cancer. Nat Rev Genet 2009;10:704–14.
154. Sun D, Lee YS, Malhotra A, Kim HK, Matecic M, Evans C, et al. Mir-99 family of micrornas suppresses the expression of prostate specific antigen and prostate cancer cell proliferation. Cancer Res 2011;71:1313–24.
155. Ignatenko NA, Babbar N, Mehta D, Casero RA Jr, Gerner EW. Suppression of polyamine catabolism by activated ki-ras in human colon cancer cells. Mol Carcinog 2004;39:91–102.
156. Ignatenko NA, Zhang H, Watts GS, Skovan BA, Stringer DE, Gerner EW. The chemopreventive agent alpha-difluoromethylornithine blocks ki-ras-dependent tumor formation and specific gene expression in caco-2 cells. Mol Carcinog 2004;39:221–33.
157. Henkhaus RS, Roy UK, Cavallo-Medved D, Sloane BF, Gerner EW, Ignatenko NA. Caveolin-1-mediated expression and secretion of kallikrein 6 in colon cancer cells. Neoplasia 2008;10:140–8.
158. Chai KX, Chen LM, Chao J, Chao L. Kallistatin: a novel human serine proteinase inhibitor. Molecular cloning, tissue distribution, and expression in escherichia coli. J Biol Chem 1993;268:24498–505.
159. Luo LY, Jiang W. Inhibition profiles of human tissue kallikreins by serine protease inhibitors. Biol Chem 2006;387:813–6.
160. Matsui H, Kimura A, Yamashiki N, Moriyama A, Kaya M, Yoshida I, et al. Molecular and biochemical characterization of a serine proteinase predominantly expressed in the medulla oblongata and cerebellar white matter of mouse brain. J Biol Chem 2000;275:11050–7.
161. Hutchinson S, Luo LY, Yousef GM, Soosaipillai A, Diamandis EP. Purification of human kallikrein 6 from biological fluids and identification of its complex with alpha(1)-antichymotrypsin. Clin Chem 2003;49:746–51.
162. Sotiropoulou G, Pampalakis G. Kallikrein-related peptidases: Bridges between immune functions and extracellular matrix degradation. Biol Chem 2010;391:321–31.
163. Komatsu N, Saijoh K, Toyama T, Ohka R, Otsuki N, Hussack G, et al. Multiple tissue kallikrein mrna and protein expression in normal skin and skin diseases. Br J Dermatol 2005;153:274–81.
164. Naldi L. Scoring and monitoring the severity of psoriasis. What is the preferred method What is the ideal method? Is pasi passe? Facts and controversies. Clin Dermatol 2010;28: 67–72.
165. Komatsu N, Suga Y, Saijoh K, Liu AC, Khan S, Mizuno Y, et al. Elevated human tissue kallikrein levels in the stratum corneum and serum of peeling skin syndrome-type b patients suggests an over-desquamation of corneocytes. J Invest Dermatol 2006;126:2338–42.
166. Johnson SK, Ramani VC, Hennings L, Haun RS. Kallikrein 7 enhances pancreatic cancer cell invasion by shedding e-cadherin. Cancer 2007;109:1811–20.
167. Scarisbrick IA, Blaber SI, Tingling JT, Rodriguez M, Blaber M, Christophi GP. Potential scope of action of tissue kallikreins in cns immune-mediated disease. J Neuroimmunol 2006;178:167–76.

168. Blaber SI, Scarisbrick IA, Bernett MJ, Dhanarajan P, Seavy MA, Jin Y, et al. Enzymatic properties of rat myelencephalon-specific protease. Biochemistry 2002;41:1165–73.
169. Scarisbrick IA, Blaber SI, Lucchinetti CF, Genain CP, Blaber M, Rodriguez M. Activity of a newly identified serine protease in cns demyelination. Brain 2002;125:1283–96.
170. Scarisbrick IA, Epstein B, Cloud BA, Yoon H, Wu J, Renner DN, et al. Functional role of kallikrein 6 in regulating immune cell survival. PLoS One 2011;6:e18376.
171. Rezze GG, Fregnani JH, Duprat J, Landman G. Cell adhesion and communication proteins are differentially expressed in melanoma progression model. Hum Pathol 2011;42:409–18.
172. Frisch SM, Screaton RA. Anoikis mechanisms. Curr Opin Cell Biol 2001;13:555–62.
173. Nagaprashantha LD, Vatsyayan R, Lelsani PC, Awasthi S, Singhal SS. The sensors and regulators of cell-matrix surveillance in anoikis resistance of tumors. Int J Cancer 2011;128:743–52.
174. Kupferman ME, Patel V, Sriuranpong V, Amornphimoltham P, Jasser SA, Mandal M, et al. Molecular analysis of anoikis resistance in oral cavity squamous cell carcinoma. Oral Oncol 2007;43:440–54.
175. Aimes RT, Zijlstra A, Hooper JD, Ogbourne SM, Sit ML, Fuchs S, et al. Endothelial cell serine proteases expressed during vascular morphogenesis and angiogenesis. Thromb Haemost 2003;89:561–72.
176. Hansen KK, Oikonomopoulou K, Li Y, Hollenberg MD. Proteinases, proteinase-activated receptors (pars) and the pathophysiology of cancer and diseases of the cardiovascular, musculoskeletal, nervous and gastrointestinal systems. Naunyn Schmiedebergs Arch Pharmacol 2008;377:377–92.
177. Hollenberg MD, Oikonomopoulou K, Hansen KK, Saifeddine M, Ramachandran R, Diamandis EP. Kallikreins and proteinase-mediated signaling: Proteinase-activated receptors (pars) and the pathophysiology of inflammatory diseases and cancer. Biol Chem 2008;389:643–51.
178. Oikonomopoulou K, Hansen KK, Saifeddine M, Tea I, Blaber M, Blaber SI, et al. Proteinase-activated receptors, targets for kallikrein signaling. J Biol Chem 2006;281:32095–112.
179. Oikonomopoulou K, Hansen KK, Saifeddine M, Vergnolle N, Tea I, Diamandis EP, et al. Proteinase-mediated cell signalling: targeting proteinase-activated receptors (pars) by kallikreins and more. Biol Chem 2006;387:677–85.
180. Hollenberg MD, Compton SJ. International union of pharmacology. XXVIII. Proteinase-activated receptors. Pharmacol Rev 2002;54:203–17.
181. Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. Proteinase-activated receptors. Pharmacol Rev 2001;53:245–82.
182. Oikonomopoulou K, Diamandis EP, Hollenberg MD. Kallikrein-related peptidases: proteolysis and signaling in cancer, the new frontier. Biol Chem 2010;391:299–310.
183. Aggarwal BB, Vijayalekshmi RV, Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. Clin Cancer Res 2009;15:425–30.
184. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–7.
185. Gonda TA, Tu S, Wang TC. Chronic inflammation, the tumor microenvironment and carcinogenesis. Cell Cycle 2009;8:2005–13.
186. Castellano E, Downward J. Role of ras in the regulation of pi 3-kinase. Curr Top Microbiol Immunol 2010;346:143–69.
187. Ramsay AJ, Dong Y, Hunt ML, Linn M, Samaratunga H, Clements JA, et al. Kallikrein-related peptidase 4 (klk4) initiates intracellular signaling via protease-activated receptors (pars). Klk4 and par-2 are co-expressed during prostate cancer progression. J Biol Chem 2008;283:12293–304.
188. Ramsay AJ, Reid JC, Adams MN, Samaratunga H, Dong Y, Clements JA, et al. Prostatic trypsin-like kallikrein-related peptidases (klks) and other prostate-expressed tryptic proteinases as regulators of signalling via proteinase-activated receptors (pars). Biol Chem 2008;389:653–68.
189. Mize GJ, Wang W, Takayama TK. Prostate-specific kallikreins-2 and -4 enhance the proliferation of du-145 prostate cancer cells through protease-activated receptors-1 and -2. Mol Cancer Res 2008;6:1043–51.
190. Petraki CD, Gregorakis AK, Vaslamatzis MM, Papanastasiou PA, Yousef GM, Levesque MA, et al. Prognostic implications of the immunohistochemical expression of human kallikreins 5, 6, 10 and 11 in renal cell carcinoma. Tumour Biol 2006;27:1–7.
191. Mitsui S, Okui A, Uemura H, Mizuno T, Yamada T, Yamamura Y, et al. Decreased cerebrospinal fluid levels of neurosin (klk6), an aging-related protease, as a possible new risk factor for alzheimer's disease. Ann N Y Acad Sci 2002;977:216–23.
192. Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, et al. Global prevalence of dementia: a delphi consensus study. Lancet 2005;366:2112–7.
193. Kalaria RN, Maestre GE, Arizaga R, Friedland RP, Galasko D, Hall K, et al. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. Lancet Neurol 2008;7:812–26.
194. Zhang YW, Thompson R, Zhang H, Xu H. App processing in alzheimer's disease. Mol Brain 2011;4:3.
195. Baig S, Wilcock GK, Love S. Loss of perineuronal net n-acetylgalactosamine in alzheimer's disease. Acta Neuropathol 2005;110:393–401.
196. Diamandis EP, Yousef GM, Petraki C, Soosaipillai AR. Human kallikrein 6 as a biomarker of alzheimer's disease. Clin Biochem 2000;33:663–7.
197. Shulman JM, De Jager PL, Feany MB. Parkinson's disease: Genetics and pathogenesis. Annu Rev Pathol 2011;6:193–222.
198. Kasai T, Tokuda T, Yamaguchi N, Watanabe Y, Kametani F, Nakagawa M, et al. Cleavage of normal and pathological forms of alpha-synuclein by neurosin in vitro. Neurosci Lett 2008;436:52–6.
199. Dev KK, Hofele K, Barbieri S, Buchman VL, van der Putten H. Part ii: alpha-synuclein and its molecular pathophysiological role in neurodegenerative disease. Neuropharmacology 2003;45:14–44.
200. Tatebe H, Watanabe Y, Kasai T, Mizuno T, Nakagawa M, Tanaka M, et al. Extracellular neurosin degrades alpha-synuclein in cultured cells. Neurosci Res 2010;67:341–6.
201. Hebb AL, Bhan V, Wishart AD, Moore CS, Robertson GS. Human kallikrein 6 levels are elevated in multiple sclerosis. Curr Drug Discov Technol 2010;7:137–40.
202. Stadelmann C, Wegner C, Bruck W. Inflammation, demyelination, and degeneration-recent insights from ms pathology. Biochim Biophys Acta 2011;1812:275–82.
203. Vandell AG, Larson N, Laxmikanthan G, Panos M, Blaber SI, Blaber M, et al. Protease-activated receptor dependent and independent signaling by kallikreins 1 and 6 in cns neuron and astroglial cell lines. J Neurochem 2008;107:855–70.
204. Terayama R, Bando Y, Takahashi T, Yoshida S. Differential expression of neuropsin and protease m/neurosin in oligodendrocytes after injury to the spinal cord. Glia 2004;48:91–101.
205. Bando Y, Ito S, Nagai Y, Terayama R, Kishibe M, Jiang YP, et al. Implications of protease m/neurosin in myelination during

experimental demyelination and remyelination. Neurosci Lett 2006;405:175–80.
206. El Sherbini MA, Sallam MM, Shaban EA, El-Shalakany AH. Diagnostic value of serum kallikrein-related peptidases 6 and 10 versus ca125 in ovarian cancer. Int J Gynecol Cancer 2011;21:625–32.
207. Gilks CB, Prat J. Ovarian carcinoma pathology and genetics: recent advances. Hum Pathol 2009;40:1213–23.
208. Helzlsouer KJ, Alberg AJ, Norkus EP, Morris JS, Hoffman SC, Comstock GW. Prospective study of serum micronutrients and ovarian cancer. J Natl Cancer Inst 1996;88:32–7.
209. Husseinzadeh N. Status of tumor markers in epithelial ovarian cancer has there been any progress? A review. Gynecol Oncol 2011;120:152–7.
210. Cramer DW, Bast RC Jr, Berg CD, Diamandis EP, Godwin AK, Hartge P, et al. Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. Cancer Prev Res (Phila) 2011;4:365–74.
211. Zhu CS, Pinsky PF, Cramer DW, Ransohoff DF, Hartge P, Pfeiffer RM, et al. A framework for evaluating biomarkers for early detection: validation of biomarker panels for ovarian cancer. Cancer Prev Res (Phila) 2011;4:375–83.
212. Schuster R, Max N, Mann B, Heufelder K, Thilo F, Grone J, et al. Quantitative real-time rt-pcr for detection of disseminated tumor cells in peripheral blood of patients with colorectal cancer using different mrna markers. Int J Cancer 2004;108:219–27.
213. Oikonomopoulou K, Hansen KK, Baruch A, Hollenberg MD, Diamandis EP. Immunofluorometric activity-based probe analysis of active klk6 in biological fluids. Biol Chem 2008;389:747–56.
214. Pan Z, Jeffery DA, Chehade K, Beltman J, Clark JM, Grothaus P, et al. Development of activity-based probes for trypsin-family serine proteases. Bioorg Med Chem Lett 2006;16:2882–5.
215. Petrocca F, Lieberman J. Promise and challenge of rna interference-based therapy for cancer. J Clin Oncol 2011;29:747–54.
216. Urban JH, Merten CA. Retroviral display in gene therapy, protein engineering, and vaccine development. ACS Chem Biol 2011;6:61–74.

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