0163-769X/01/$03.00/0
Endocrine Reviews 22(2): 184–204

Printed in U.S.A.

The New Human Tissue Kallikrein Gene Family: Structure, Function, and Association to Disease*

GEORGE M. YOUSEF AND ELEFTHERIOS P. DIAMANDIS

Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5; and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada M5G 1L5

ABSTRACT

The human tissue kallikrein gene family was, until recently, thought to consist of only three genes. Two of these human kallikreins, prostate-specific antigen and human glandular kallikrein 2, are currently used as valuable biomarkers of prostatic carcinoma. More recently, new kallikrein-like genes have been discovered. It is now clear that the human tissue kallikrein gene family contains at least 15 genes. All genes share important similarities, including mapping at the same chromosomal locus (19q13.4), significant homology at both the nucleotide and protein level, and similar genomic organization. All genes encode for putative serine proteases and most of them are regulated by steroid hormones. Recent data suggest that at least a few of these kallikrein genes are connected to malignancy. In this review, we summarize the recently accumulated knowledge on the human tissue kallikrein gene family, including gene and protein structure, predicted enzymatic activities, tissue expression, hormonal regulation, and alternative splicing. We further describe the reported associations of the human kallikreins with various human diseases and identify future avenues for research. (*Endocrine Reviews* **22:** 184–204, 2001)

I. Introduction
II. The Human and Rodent Families of Kallikrein Genes
III. Nomenclature
IV. The Human Kallikrein Gene Locus
  A. Locus organization
  B. Gene organization
V. Protein Homologies and Predicted Enzymatic Activity
VI. Hormonal Regulation of Kallikrein Genes
VII. Tissue Expression of Kallikreins
VIII. Variants of Kallikrein Transcripts
IX. Association of Kallikreins with Human Diseases
X. Physiological Functions
XI. Future Directions
XII. Conclusions

I. Introduction

KALLIKREINS are a group of serine proteases that are found in diverse tissues and biological fluids. The term "Kallikrein" was introduced in the 1930s by Werle and colleagues (1, 2) who found high levels of their original isolates in the pancreas (in Greek, the "Kallikreas"). The kallikrein enzymes are now divided into two major categories: plasma kallikrein and tissue kallikrein (3, 4). These two categories differ significantly in their molecular weight, substrate specificity, immunological characteristics, gene structure, and type of kinin released. Plasma kallikrein or Fletcher factor (official symbol KLKB1)[1] is encoded by a single gene, which is located on human chromosome 4q35 (5, 6). The gene is composed of 15 exons and encodes for an enzyme that releases the bioactive peptide bradykinin from a high molecular weight precursor molecule (high mol wt kininogen) produced by the liver. Plasma kallikrein is exclusively expressed by liver cells. The function of plasma kallikrein includes its participation in the process of blood clotting and fibrinolysis and, through the release of bradykinin, in the regulation of vascular tone and inflammatory reactions (7). Plasma kallikrein will not be discussed further in this review since the gene encoding for this enzyme has no similarities with the tissue kallikrein genes and clearly, is not a member of this multigene family. A historical perspective on the discovery of the kallikrein-kinin system and bradykinin has recently been published (8).

Tissue kallikreins are members of a large multigene family and demonstrate considerable similarities at the gene and protein level as well as in tertiary structure. In this review, we will describe recent developments, exclusively pertinent to the human family of enzymes.

The term "kallikrein" is usually used to describe an enzyme that acts upon a precursor molecule (kininogen) for release of a bioactive peptide (kinin) (7–10). Another term that is also frequently used to describe these enzymes is

Address reprint requests to: Dr. E.P. Diamandis, Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, Canada M5G 1X5. E-mail: ediamandis@mtsinai.on.ca; Web site: http://www.kallikreins.com

* The work done in the authors' laboratory was supported by grants from the National Cancer Institute of Canada (NCIC), the Natural Sciences and Engineering Research Council (NSERC), the Medical Research Council of Canada (MRC), MDS Nordion and Diagnostics Systems Laboratories, Inc., The US Army, The Canadian Breast Cancer Research Initiative, The National Institutes of Health (NCI branch), and MDS Proteomics.

[1] KLK, kallikrein; KLK-L, kallikrein-like; EMSP1, enamel matrix serine proteinase 1; hGK-1, human glandular kallikrein-1; HSCTE, human stratum corneum tryptic enzyme; HSCCE, human stratum corneum chymotryptic enzyme; TADG-14, tumor-associated differentially expressed gene-14; TLSP, trypsin-like serine protease; NES1, normal epithelial cell-specific 1 gene; PRSS, protease serine; PRSSL, protease serine-like; HRE, hormone response element; ARE, androgen response element; CNS, central nervous system; HUGO, human genome organization; uPA, urokinase type plasminogen activator; TGF-β, transforming growth factor β; PSA, prostate specific antigen.

"kininogenases." The term "kininase" is used to describe other enzymes that can inactivate kinins. Among the known human and animal tissue kallikreins, only one enzyme has the ability to release efficiently a bioactive kinin from a kininogen. In humans, this enzyme is known as pancreatic/renal kallikrein or, with the new nomenclature, as the KLK1 gene, encoding for human kallikrein 1 (hK1 protein) (9–12). This enzyme acts upon a liver-derived kininogen (low mol wt kininogen) to release lysyl-bradykinin (also known as kallidin), which is involved in the control of blood pressure, electrolyte balance, inflammation, and other diverse physiological processes. Tissue kallikrein (hK1) may further enzymatically digest other substrates, including growth factors, hormones, and cytokines, to mediate pleiotropic effects (7).

It should be emphasized that the generic term "tissue kallikrein" *is not* restricted to the description of enzymes that release bioactive peptides from precursor molecules. The term is used to describe a group of enzymes with highly conserved gene and protein structure, which also share considerable sequence homology and colocalize in the same chromosomal locus as the KLK1 gene. In this review, the term "kallikrein" will be used to describe a family of 15 genes that have a number of striking similarities, as outlined in point format in Table 1 (13). The use of the term "kallikrein" *does not* necessarily imply that any of these family members (with the exception of KLK1) have kininogenase activity. In fact, for human family members that have been functionally tested, it was found that they possess very low (hK2) (14, 15) or no kininogenase activity [prostate-specific antigen (PSA)] (14). These enzymes are grouped together with KLK1, based on the similarities outlined in Table 1.

II. The Human and Rodent Families of Kallikrein Genes

The tissue kallikrein literature can be roughly separated into various periods as follows. Early in the 1920s and 30s, researchers discovered the basic components of the kallikrein-kinin system and identified the molecular structure of bradykinin and kallidin (lysyl-bradykinin) in the 1960s (8). The molecular biology of the tissue kallikrein gene family was worked out in detail in both the human and rodents in the 1980s (16–19). It was then concluded that the mouse and rat gene families were composed of many genes, clustered in the same chromosomal locus. In particular, the mouse tissue kallikrein gene family is localized on chromosome 7 and consists of 24 genes, of which at least 14 encode for active proteins (the remaining being pseudogenes) (16, 20–22). The area on chromosome 7 encompassing the mouse kallikreins is homologous to an area on human chromosome 19q13.4 that harbors the human kallikrein gene family. The rat tissue kallikrein gene family is composed of approximately 20 homologous genes of which at least 10 are expressed (18, 23–30).

Most of the rodent tissue kallikreins are expressed in the salivary glands, but a few, including the prostate, pituitary gland, and endometrium, have more diverse tissue expression (7, 9, 31–33). It is not the purpose of this review to describe in detail the rodent or other animal tissue kallikrein gene families. Excellent reviews on this subject already exist (9, 16, 17, 21, 22).

The human tissue kallikrein gene family was also discovered in the 1980s and it was then concluded that the entire family is composed of only three genes, namely KLK1, encoding for pancreatic/renal kallikrein (hK1 protein), the KLK2 gene, encoding for human glandular kallikrein 2 (hK2), and the KLK3 gene, encoding for PSA (hK3) (34–38). The major interest in human kallikreins lies in the very restricted tissue expression of hK2 and hK3 in the prostate, which qualifies them as candidate biomarkers for prostatic diseases (39–43). hK3 (PSA), in particular, has gained prominence in recent years as the most valuable tumor marker ever discovered and is currently used widely for the diagnosis, monitoring, and population screening for prostate cancer (44–51). The introduction of this test has had a major impact on prostate cancer diagnosis and monitoring and this field is still evolving (52, 53). More recently, PSA applications have extended beyond the prostate, including breast and other cancers (54–57). Over the last few years, human glandular kallikrein 2 is emerging as an additional prostatic and breast cancer biomarker, and it is now clear that it can supplement PSA testing for improved identification and differential diagnosis of prostate cancer (43, 58–66). It is thus logical to exploit the possible applications of other members of this gene family for cancer and other disease diagnosis and monitoring.

In the last 3 yr, we have witnessed the emergence of new knowledge related to the human kallikrein gene family (13). Independent researchers have cloned a number of new serine protease genes that show significant homologies with the classical human kallikreins; in addition, when these new protease genes were mapped, they were found to colocalize in the known human kallikrein gene locus on chromosome 19q13.3-q13.4 (67–90). The recent detailed molecular description of the human kallikrein gene locus (67, 68) enabled us to construct a physical map containing 15 genes that share

TABLE 1. Similarities between members of the new human kallikrein gene family

1. All genes localize to the same chromosomal region (19q13.4).
2. All genes encode for putative serine proteases with a conserved catalytic triad (histidine, aspartic acid, and serine in the appropriate positions).
3. All genes have five coding exons (some members contain one or more 5′-untranslated exons).
4. Coding exon sizes are similar or identical.
5. Intron phases are fully conserved among all 15 human members and among members of the rodent kallikrein gene families.[a]
6. All genes have significant sequence homologies at the DNA and amino acid levels (40–80%).
7. Many of these genes are regulated by steroid hormones.

[a] Intron phase refers to the location of the intron within the codon: intron phase I, the intron occurs after the first nucleotide of the codon; II, the intron occurs after the second nucleotide; 0, the intron occurs between codons.

significant structural similarities (Table 1). Some of these genes appear to be related to breast, ovarian, and other human cancers, and a few of them appear to encode for functional tumor suppressor genes. In view of these very recent developments, we will describe, in this review, the knowledge that has accumulated on these genes, with special emphasis on the structure of the genes and proteins, their tissue expression and hormonal regulation, and their connection to various human diseases. Where possible, functional aspects of these enzymes will also be described. We hope that the summary of these new findings on the human kallikrein gene family will facilitate further research toward better understanding their physiological function, their pathophysiology and connection to human diseases, and their possible applications in the diagnosis and monitoring of various malignancies and their future suitability as therapeutic targets.

III. Nomenclature

Until 2–3 yr ago, only three human kallikrein genes were recognized: the pancreatic/renal kallikrein (KLK1), the human glandular kallikrein 2 (KLK2), and PSA (KLK3). Rittenhouse and co-workers (43, 49) have recently published the revised nomenclature for these three genes. New developments led to the identification of 15 different genes exhibiting significant homologies and other similarities, as described in Table 1 (13). Since many of these genes were cloned independently by different investigators, various empirical names were initially used for their description.

The Human Genome Organization (HUGO) has recently proposed guidelines for human gene nomenclature. Initially, some members of the new kallikrein gene family were classified by HUGO along with other serine proteases under the prefix "PRSS", standing for "protease serine." It is now clear that this designation does not serve well the needs of the future since members of this multigene family are classified together with other serine proteases that map in different locations of the genome.

The construction of the first detailed map of the human kallikrein gene locus (13, 67, 68) allows for a more rational assignment of official gene symbols. Since the rodent and other animal species kallikrein multigene families were known before 1992, an international working party had reached agreement in 1992 on uniform nomenclature of the animal kallikreins and the three human kallikreins known at that time (91). Based on this paradigm and the guidelines of HUGO (for details please visit the Website: http://www.gene.ucl.ac.uk/nomenclature/), an international group of scientists working in the field agreed to adopt a nomenclature for the newer human kallikreins, consistent with that already defined for KLK1–3, as shown in Table 2 (92). In the same table, we also include previous symbols based on the PRSS system as well as names originally proposed by the discoverers of these genes (93–100). Gene numbering starts from centromere to telomere on chromosome 19q13.4 with the exception of the three classical kallikreins for which the existing nomenclature was retained and one newly discovered gene, which maps between KLK1 and KLK2 genes (69). It is possible that, in the future, new members of this gene family may be identified, either centromeric to KLK1 or telomeric to KLK14 (see below). If new kallikrein genes are identified in this locus, they will be sequentially numbered, starting with KLK16.

IV. The Human Kallikrein Gene Locus

A. Locus organization

The availability of linear genomic sequences around chromosome 19q13.3–q13.4 from the human genome project (the sequences were generated by the Lawrence Livermore National Laboratory) allowed the precise localization of the 15 members of the new human kallikrein gene family with high accuracy (±1 nucleotide) (68) (Fig. 1). The three classical kallikreins, KLK1, KLK3, and KLK2, cluster together within a 60-kb region, as previously described by Riegman *et al.* (36,

TABLE 2. Proposed new nomenclature for human kallikreins

New gene symbol[a,b]	Previous gene symbol(s)	New protein symbol	Other protein names/symbols	GenBank accession no.	Reference
KLK1	KLK1	hK1	Pancreatic/renal kallikrein, hPRK	M25629, M33105	34, 93
KLK2	KLK2	hK2	Human glandular kallikrein 1, hGK-1	M18157	94
KLK3	KLK3	hK3	Prostate-specific antigen, PSA	X14810, M24543, M27274	95–97
KLK4	PRSS17, KLK-L1, KLK4	hK4	Prostase, KLK-L1 protein, EMSP1	AF113141, AF135023, AF148532	70–72, 79
KLK5	KLK-L2	hK5	KLK-L2 protein; HSCTE	AF135028, AF168768	80, 81
KLK6	PRSS9	hK6	Zyme, protease M, neurosin	AF013988, AF149289, U62801, D78203	73, 74, 82, 83
KLK7	PRSS6	hK7	HSCCE	L33404, AF166330	84, 85
KLK8	PRSS19	hK8	Neuropsin; ovasin; TADG-14	AB009849, AF095743, AB010780, AF055982	86, 98
KLK9	KLK-L3	hK9	KLK-L3 protein	AF135026	67
KLK10	PRSSL1, NES1	hK10	NES1 protein	AF055481, NM_002776	76, 99, 87
KLK11	PRSS20	hK11	TLSP/hippostasin	AB012917, AF164623	88, 89, 100
KLK12	KLK-L5	hK12	KLK-L5 protein	AF135025	77
KLK13	KLK-L4	hK13	KLK-L4 protein	AF135024	78
KLK14	KLK-L6	hK14	KLK-L6 protein	AF161221	90
KLK15		hK15		AF242195	69

[a] The order of the genes on chromosome 19q13.4 is shown in Fig. 1.
[b] For full gene names, see abbreviation footnote.

37). and Richards *et al.* (35). Another newly discovered gene, KLK15, maps between KLK1 and KLK2 (69). The remaining kallikrein genes are aligned within this locus, as shown in Fig. 1, without intervention by other genes. The direction of transcription is from telomere to centromere with the exception of KLK3 and KLK2. The genomic lengths of all these genes are relatively small, ranging from 4–10 kb. It is unlikely that this locus harbors more kallikrein-like genes either centromeric from KLK1 or telomeric from KLK14. The next neighboring gene to KLK1 is testicular acid phosphatase (ACPT; GenBank Accession no. AF321918), which is not related to kallikreins. The next neighboring gene from KLK14 is Sigelec 9 (101). Siglecs belong to the immunoglobulin superfamily and encode for transmembrane receptors that have the ability to bind sialic acid (102, 103). These genes have no structural or functional relationship to the human kallikreins.

B. Gene organization

All members of the new human kallikrein multigene family encode for serine proteases. All genes consist of five coding exons, as shown in Fig. 2. The organization of all genes is very similar, with the first coding exon having a short 5′-untranslated region, the second exon harboring the amino acid histidine of the catalytic triad toward the end of the exon, the third exon harboring the aspartic acid of the catalytic triad around the middle, and the fifth exon harboring the serine of the catalytic triad, at the beginning of the exon. Beyond the stop codon, there is a 3′-untranslated region of variable length.

While it is certain that the classical kallikreins do not have 5′-untranslated exons, most other members of this multigene family have one or two 5′-untranslated exons, as shown in Fig. 2. It is possible that some other members of this gene family also harbor 5′-untranslated exons, which have not as yet been identified. In addition, the 3′-untranslated region of many of these genes is sometimes variable, giving rise to variants with different mRNA lengths, but encoding for the same protein (variant kallikrein transcripts are described under a separate heading). It is thus possible that the actual lengths of these genes, as shown in Figs. 1 and 2, may change slightly in the future.

Although the intron lengths of these genes vary considerably, the exon lengths are quite comparable or identical. Additionally, the intron phases between coding exons of all these genes (and those of the rodent kallikreins) are completely conserved among all members, with phases I–II–I–O. The intron phases are defined in Fig. 2.

Although TATA boxes have been identified within the proximal promoter of the classical kallikrein genes (Table 3), no such elements were found for most of the other kallikreins. This may be due to the absence of these elements or to the fact that the proximal promoter of some of these genes has not been accurately defined due to the presence of as yet unidentified 5′-untranslated exons. This issue merits further investigation. Classical (AATAAA) or variant polyadenylation signals have been identified 10–20 bases away from the poly A tail of all kallikrein mRNAs (Table 3). With only one exception, all splice-junction sites are fully conserved among the human kallikrein genes (Table 3).

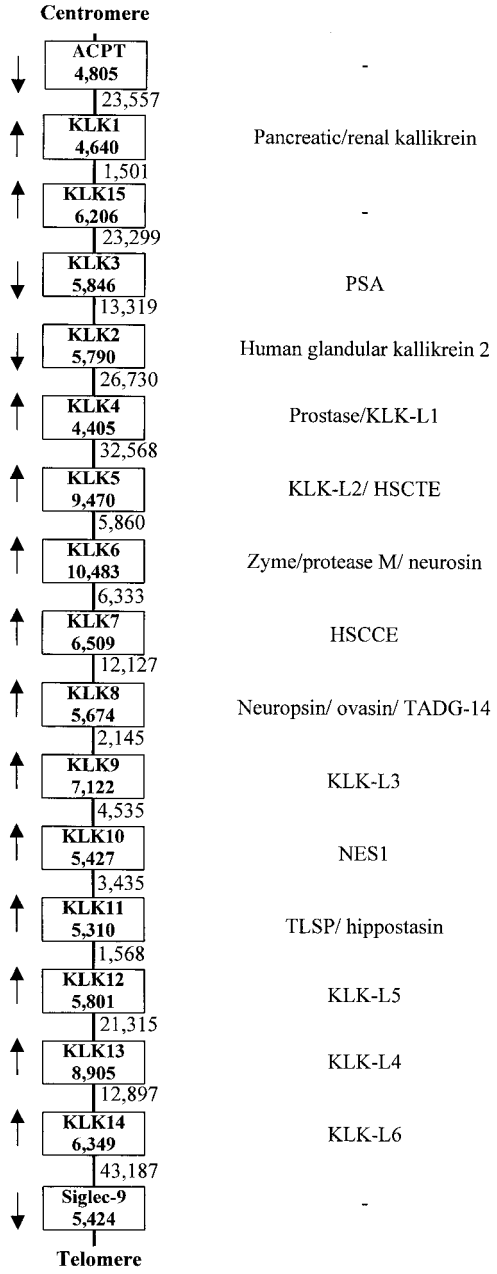

FIG. 1. An approximate 300-kb region of contiguous genomic sequence around chromosome 19q13.4. The direction of transcription of each gene is illustrated by *arrows*. *Boxes* represent genes and contain the gene names and their genomic length, in base pairs. Other commonly used names for these genes are also mentioned. Distances between genes in base pairs are shown between *boxes*. The Siglec and ACPT (testicular acid phosphatase) genes do not belong to the tissue kallikrein gene family. Figure is not drawn to scale. For full gene names, see Table 2 and abbreviation footnote.

V. Protein Homologies and Predicted Enzymatic Activity

The 15 members of the new human kallikrein gene family have been aligned to identify similarities (Fig. 3). Maximum homology between all these proteins is found around the catalytic amino acids histidine (with the conserved region

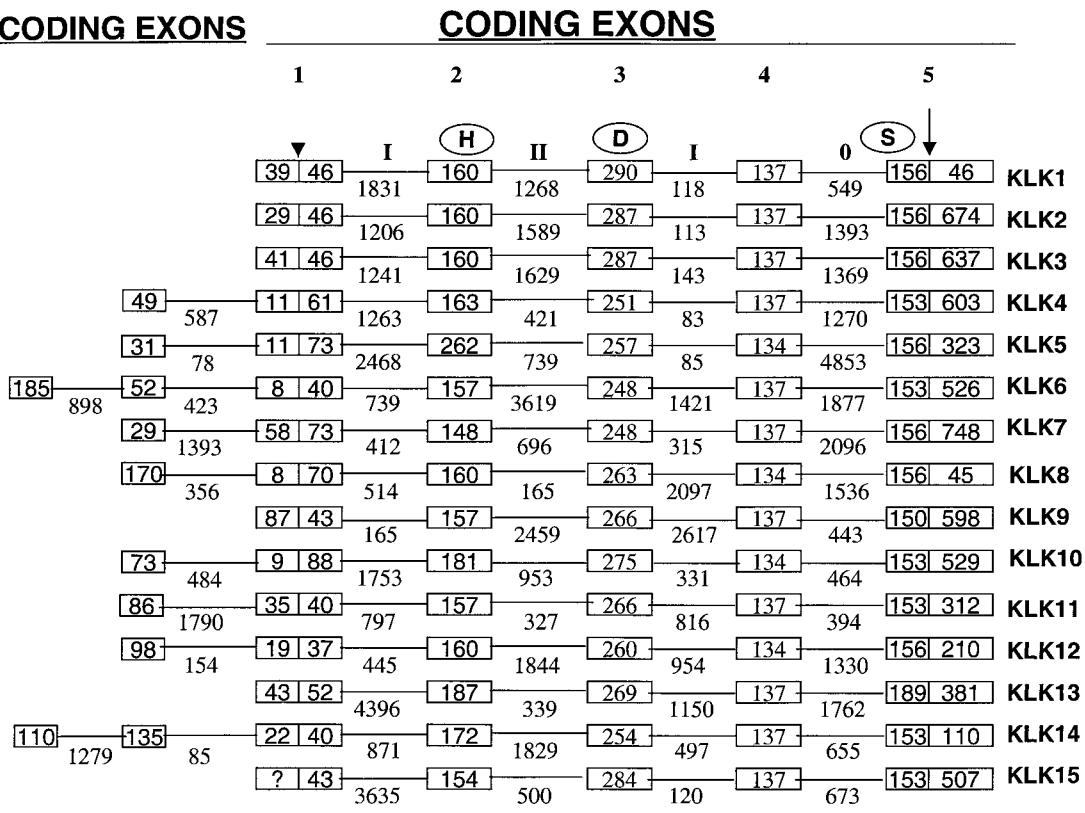

FIG. 2. Schematic diagram showing the comparison of the coding regions of the 15 kallikrein genes. Exons are shown by *solid bars* and introns by the *connecting lines*. *Letters above boxes* indicate relative positions of the catalytic triad that was found to be conserved in all genes; H, histidine; D, aspartic acid; and S, serine. *Roman numbers* indicate intron phases. The intron phase refers to the location of the intron within the codon; I denotes that the intron occurs after the first nucleotide of the codon; II, the intron occurs after the second nucleotide; 0, the intron occurs between codons. The intron phases are conserved in all genes. *Numbers inside boxes* indicate exon lengths and *numbers outside boxes* indicate intron lengths (in base pairs). The *arrowhead* represents the position of the start codon and the *arrow* indicates the position of the stop codon. *Question mark* denotes that region length is unknown. Figure is not drawn to scale.

TABLE 3. TATA, polyadenylation signals, and splice/junctions for the KLK genes[a]

Gene	TATA box	Polyadenylation signal[b]	Splice junctions
KLK1	TTTAAA (−21 bp)[c]	AGTAAA (−15 bp)	Fully conserved
KLK2	TTTATA (−35 bp)	AATAAA (−19 bp)	Fully conserved
KLK3	TTTATA (−22 bp)	AATAAA (−16 bp)	Fully conserved
KLK4	TTATAA (−30 bp)	AATAAA (−15 bp)	Fully conserved
KLK5	Not found	AATAAA (−8 to −11 bp)	Fully conserved
KLK6	Not found	AATAAA (−14 bp)	Fully conserved
KLK7	Not found	AATAAA (−16 bp)	Fully conserved
KLK8	Not found	AATAAA (−15 to −18 bp)	Fully conserved
KLK9	Not found	AGTAAA (−14 bp)	Fully conserved
KLK10	TTAAAA (−35 bp)	ACTAAA (−17 bp)	gc for gt at beginning of intron 4[d]
KLK11	Not found	AATAAA (−17 bp)	Fully conserved
KLK12	Not found	AATAAA (−16 bp)	Fully conserved
KLK13	Not found	TATAAA (−16 bp)	Fully conserved
KLK14	Not found	Putative AATAAA	Fully conserved
KLK15	Not found[e]	ATTAAA (−17 bp)	Fully conserved

[a] The information was derived from the GenBank entries shown in Table 2.
[b] Position of polyadenylation signal in base pairs before the poly-A tail.
[c] Denotes position of TATA box considering first nucleotide of start codon as 1.
[d] See Ref. 87.
[e] TATA box may be present in some genes for which the 5′-proximal promoter sequences have not as yet been accurately defined.

WVLTAAHC), aspartic acid (DLMLL), and serine (GDSGGPL). In general, the amino acid identity between the various members of this family ranges from about 40–80%. The number and position of cysteine residues are highly conserved among the 15 human kallikreins and among other serine proteases. All members of this family possess between 10–12 cysteine residues, which are expected to form disulfide bridges. A number of other invariant amino acids (~25–30),

especially those around the active site of serine proteases, have been described (104). In the case of the human family of genes, there are 39 amino acids that are completely conserved among all 15 kallikreins (Fig. 3). Numerous other conservative amino acid substitutions are shown in Fig. 3. A phylogenetic tree of all human kallikreins and a few other serine proteases is shown in Fig. 4.

All proteins encoded by these genes are initially synthesized as preproenzymes that are then proteolytically processed to yield proenzymes by removal of the signal peptide, followed by activation (also by proteolysis) to the mature, enzymatically active forms. In Table 4, we present the reported signal and activation peptides as well as the length of the mature proteins that are encoded by these genes. It is important to mention that most of these cleavage sites have been predicted by computer programs and have been verified experimentally for only a few members.

The data of Table 4 suggest that most of the pro-forms of these enzymes are activated by cleavage at the carboxy-terminal end of either arginine (R) or lysine (K) residues (the preferred trypsin cleavage site). Since most of the human kallikrein enzymes have trypsin-like activity, they may potentially act as activating enzymes for either themselves (autoactivation) or other pro-forms of kallikreins. Kallikreins may participate in cascade pathways similar to those demonstrated for the digestive enzymes, coagulation, and apoptosis. These possibilities merit further investigation.

Protein sequence examination (Fig. 3) reveals that the three classical kallikreins possess an amino acid sequence of approximately 9–11 amino acids (the kallikrein loop) preceding the aspartic acid residue of serine proteases, which is not present in its entirety in any of the other 12 enzymes. This short sequence is thought to confer specificity for kininogenase activity but, as already mentioned, only hK1 is a potent kininogenase. KLK15 has a unique 8-amino acid sequence at positions 148–155, not found in any other kallikrein protein. Similarly, KLK13 possesses a unique amino-terminal and a unique carboxy-terminal end.

Serine proteases can be divided into two main evolutionary families, the trypsin-like serine proteases and the subtilisin-like pro-protein convertases, which presumably evolved through convergent evolution (105). The trypsin-like serine proteases are believed to have evolved from a single ancestral gene that duplicated in the course of evolution to give rise to other genes that have gradually mutated and evolved to related proteases and protease subfamilies with new functions. The various serine proteases can be markedly different in relation to their substrate specificity (106, 107). The differences are due to very subtle variations in the substrate binding pocket. Trypsin-like serine proteases have an aspartic acid in their binding pocket, which can form strong electrostatic bonds with arginine or lysine residues, which are usually present at the carboxyl-terminal part of the cleavage site. The important amino acid of the binding pocket, responsible for substrate specificity, is usually found six amino acids before the catalytic serine residue. From the 15 proteins aligned in Fig. 3, 11 have aspartic acid in this position and are expected to have trypsin-like activity. The four remaining enzymes, namely hK3 (has serine), hK7 (has asparagine), hK9 (has glycine), and hK15 (has glutamic acid), are expected to have chymotrypsin-like or other specific enzymatic activity (see also Table 4). The cleavage specificity of these enzymes needs to be established experimentally, with the exception of hK3, which has already been characterized (50).

VI. Hormonal Regulation of Kallikrein Genes

KLK1 expression has been studied in animals, and it was concluded, by using gene-specific probes, that this enzyme is not directly regulated by androgens either in the salivary glands or the kidney (31, 108–111). Similarly, no regulation of the KLK1 gene by thyroid hormones has been demonstrated (109–111). Results of KLK1 regulation by mineralocorticoids are inconclusive (112, 113). Other data support the transcriptional up-regulation of KLK1 by estrogens (114, 115) and by dopamine in rat pituitary (116). The demonstration that KLK1 expression in human endometrium is higher during the middle of the menstrual cycle is also suggestive of KLK1 up-regulation by estrogens in this tissue (117).

Murray *et al.* (19) have reported the presence of various motifs that are reminiscent of consensus estrogen-, progestin-, glucocorticoid-, or cAMP-response elements in the 5′-flanking sequence of the human KLK1 gene (19). However, these putative elements have not been functionally tested. Consequently, no conclusion can be drawn regarding direct regulation of KLK1 transcription by steroid or other hormones.

The regulation of the PSA (KLK3) gene by steroid hormones has been extensively studied. Initially, two androgen-response elements were identified in the proximal PSA promoter, at positions −170 [ARE1] and −394 (ARE2), respectively (118–120). These AREs have been functionally tested and found to be active in LNCaP prostate cancer cells. More recently, Schuur *et al.* have identified various regions of 5′-sequences of the PSA gene around −6 to −4 kb and demonstrated presence of a putative androgen-response element at position −4,136 (ARE3), which markedly affects PSA transcription upon induction by androgens (121). It was also demonstrated that this area harbors an enhancer that is contained within a 440-bp fragment (121, 122). The upstream enhancer, containing the putative ARE3, has a dramatic effect on PSA transcription, in comparison to the two AREs in the proximal promoter (122). The hormonal regulation of the PSA gene is not tissue specific since PSA has also been found to be regulated by steroid hormones *in vitro* and *in vivo* in breast tissues and breast carcinoma cell lines (123–125). Despite this, a number of investigators have used the PSA promoter and enhancer region to deliver and express therapeutic vectors to prostate tissue, in experimental gene therapy protocols (126–132).

A number of investigations have clearly demonstrated hormonal regulation of the PSA gene primarily by androgens in the prostatic carcinoma cell line LNCaP (133) and by androgens and progestins in the breast carcinoma cell lines BT-474, T-47D, and MFM223 (123, 125, 134).

The 5′-promoter sequences of the KLK2 gene have been studied by Murtha *et al.* (135) who have identified functional androgen response elements in the promoter of this gene.

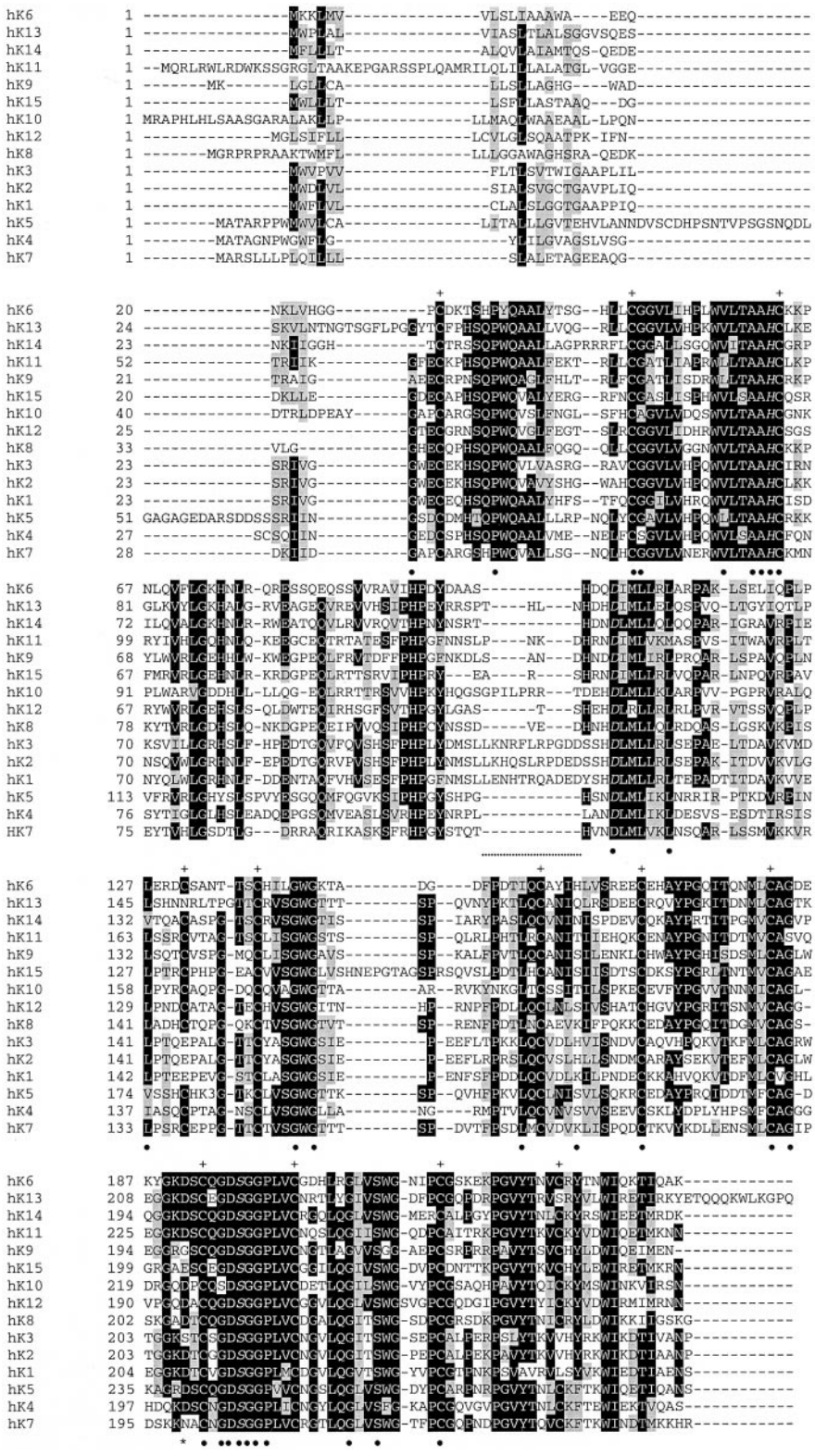

FIG. 3. Alignment of the deduced amino acid sequence of the 15 kallikrein proteins. *Dashes* represent gaps to bring the sequences to better alignment. The amino acids of the catalytic triad (H, D, S) are shown in *italics*. Identical amino acids are highlighted in *black* and similar residues in *gray*. The 29 invariant serine protease residues are marked by (●) on the *bottom*, and the cysteine residues by (+) on *top of each block*. The *dotted area* represents the kallikrein loop sequence. The *asterisk* denotes the position of the amino acid of the binding pocket that is crucial for substrate specificity (for trypsin-like enzymes the amino acid is D). For more details, see text.

The same group has subsequently shown that KLK2 is up-regulated by androgens and progestins in the breast carcinoma cell line T47-D (136) while Riegman *et al.* (137) showed up-regulation by androgens. More recently, it has been demonstrated that, similarly to PSA, a 5′-enhancer region exists about 3–5 kb upstream from the transcription site of the KLK2 gene (138). The enhancer region contains an androgen response element that was shown to be functionally active. Consistent with these data are the findings of hK2 protein secretion and up-regulation by androgens and progestins in

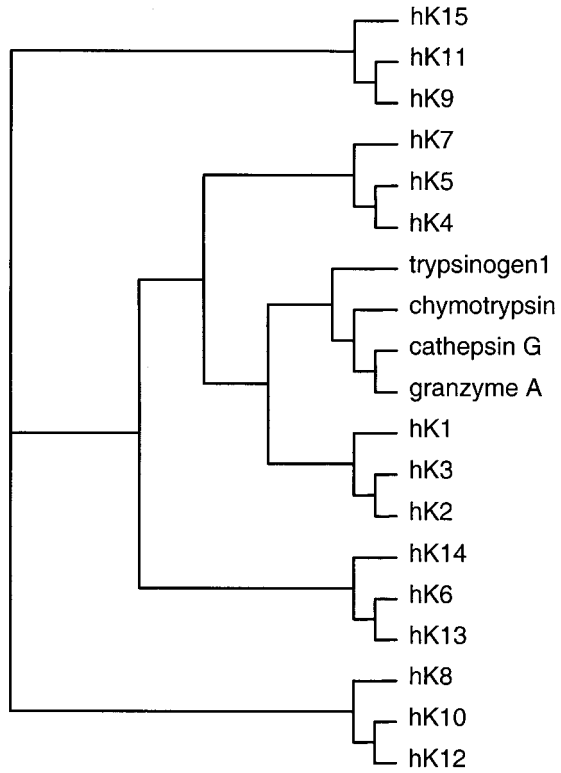

FIG. 4. Dendrogram of the predicted phylogenetic tree for the 15 kallikrein proteins and a few other related serine proteases. The neighbor-joining method was used to align these proteins. The classical kallikreins (hK1, hK2, and PSA) were grouped together; other kallikreins and serine proteases were separated in different groups, as shown. For full protein names, please see Table 2 and abbreviation footnote.

the breast cancer cell lines BT-474, T47-D, and MFM223 (134). Although the KLK2 gene promoter is not exclusively functional in the prostate, gene therapy protocols have used it for prostate cancer therapy (139).

The KLK4 gene was found to be up-regulated by androgens in the prostatic carcinoma cell line LNCaP (70) and by androgens and progestins in the breast carcinoma cell line BT-474 (71). The mode of regulation of KLK2 and KLK4 genes appears to be very similar to the mode of regulation of PSA (KLK3). Stephenson *et al.* (72) have identified putative androgen response elements in the proximal promoter region of the KLK4 gene (up to 553 bp from the transcription initiation site). However, such putative AREs have not been functionally tested, and no data have been published as yet on the characterization of possible enhancer regions further upstream from the proximal KLK4 promoter.

For the remaining 11 human kallikrein genes that have been recently identified, in none of them was the promoter functionally tested for the presence for hormone response elements (HREs). Most studies regarding hormonal regulation of these new genes have been performed with the breast carcinoma cell line BT-474 and, in some cases, with the prostatic carcinoma cell line LNCaP and other breast carcinoma cell lines. It is clear that for 10 of 11 genes under discussion (KLK5-KLK15), transcription is affected by steroid hormones, with the selectivities and potencies shown in Table 5. Most genes appear to be up-regulated by estrogens, androgens, and progestins but with different potencies. It is possible that some of these genes are hormonally regulated through indirect mechanisms, involving *trans*-acting elements (140).

Clearly, there is a need to functionally characterize the promoter and enhancer regions of these genes to understand better the mechanism of transcriptional and posttranscriptional regulation by steroid hormones.

VII. Tissue Expression of Kallikreins

KLK1 gene expression is highest in the pancreas, kidney, and salivary glands (9). The other two classical kallikrein genes, KLK3 and KLK2, were thought, for many years, to be expressed exclusively in the prostate (39–42, 46, 141, 142). By using highly sensitive immunological techniques (143), RT-PCR technology (144) as well as immunohistochemistry (145), it has now been demonstrated unequivocally that both KLK3 and KLK2 genes are expressed in diverse tissues but at relatively much lower concentrations than prostatic tissues (55–57, 146–148). Especially, hK3 (PSA) and hK2 proteins and mRNA have been found in significant amounts in the female breast and at lower levels in many other tissues (Table 6). KLK4 also appears to have prostatic-restricted expression (70) but by RT-PCR, it was demonstrated that it is also expressed in breast and other tissues (71, 72). None of the remaining kallikreins is tissue-specific, although certain genes are preferentially expressed in breast (*e.g.*, KLK5, KLK6, KLK10, KLK13), skin (KLK5, KLK7, KLK8), central nervous system (KLK6, KLK7, KLK8, KLK9,KLK14), salivary glands (almost all kallikreins), etc. A diagrammatic representation of expression of all these kallikreins in human tissues is shown in Fig. 5. Most data have been generated by RT-PCR.

It is clear that there is frequent coexpression of many kallikreins in the same tissues, and this may point to a functional relationship. For example, it has been shown that hK3 and hK2 are regulated by similar mechanisms (134) (see also previous section) and that they are frequently coexpressed in tissues and body fluids (146–148). *In vitro* data have demonstrated that hK2, which has trypsin-like activity, can activate the proform of PSA (149–151). Other functional relationships between members of the kallikrein gene family have not been demonstrated as yet.

VIII. Variants of Kallikrein Transcripts

A relatively large number of variant transcripts have already been identified for the classic and the new human kallikrein genes (Table 7). The functional and diagnostic importance of these transcripts has not as yet been studied in detail. It will be interesting to examine whether any of these transcripts are specific for certain disease states or tissues. Although other forms of some kallikreins in serum have already been described (*e.g.*, kallikreins bound to proteinase inhibitors, internally clipped kallikreins, circulating proforms, etc.), these will not be described in this review. Excellent accounts of these forms and their clinical signifi-

TABLE 4. Proteins encoded by kallikrein genes

Kallikrein	Length of pre-proenzyme	Length of signal peptide (cleavage)[a]	Length of activation peptide/cleavage[a]	Length of mature protein	Amino acid of substrate binding pocket	Reference
hK1	262	17 (A ↓ A)	7 (R ↓ I)	238	D	34, 35, 43, 49
hK2	261	17 (A ↓ V)	7 (R ↓ I)	237	D	43, 49, 94
hK3	261	17 (A ↓ A)	7 (R ↓ I)	237	S	43, 49, 95–97
hK4	254	26 (G ↓ S)	4 (Q ↓ I)	224	D	70–72
hK5	293	29 (A ↓ N)	37 (R ↓ I)	227	D	80, 81
hK6	244	16 (A ↓ E)	5 (K ↓ L)	223	D	73, 74, 82, 83
hK7	253	22 (G ↓ E)	7 (K ↓ I)	224	N	84, 85, 107
hK8	260	28 (A ↓ Q)	4 (K ↓ V)	228	D	86, 98
hK9	251	19 (A ↓ D)	3 (R ↓ A)	229	G	67
hK10	276	33 (A ↓ A)	9 (R ↓ L)	234	D	76
hK11	250	18 (G ↓ E)	3 (R ↓ I)	229	D	88, 89, 100
hK12	248	17 (A ↓ A)	4 (K ↓ I)	227	D	77
hK13	277	20 (S ↓ Q)	5 (K ↓ V)	252	D	78
hK14	251	18 (S ↓ Q)	6 (K ↓ I)	227	D	90
hK15	256	16 (A ↓ Q)	5 (K ↓ L)	235	E	69

[a] Most are predicted; need verification by experiment.

TABLE 5. Hormonal regulation of human kallikreins

Gene	Systems tested	HREs[a]	Functionally tested?	Up-regulating hormone(s)	Reference
KLK1	*In vivo* humans, rodents	Putative	No	Uncertain	19
KLK2	LNCaP; BT-474, T-47D	Yes (2 AREs)	Yes	Androgen, progestin	134–137
KLK3	LNCaP, BT-474, T-47D	Yes (3 AREs)	Yes	Androgen, progestin	118–125 133–134
KLK4	LNCaP; BT-474	Putative (AREs)	No	Androgen, progestin	70–72
KLK5	BT-474	NS (not studied)		Estrogen, progestin > androgen	80
KLK6	BT-474	NS		Estrogen, progestin > androgen	83
KLK7	BT-474	NS		Estrogen > glucocorticoid	85
KLK8	Not studied			–	
KLK9	BT-474	NS		Estrogen, progestin > androgen	67
KLK10	BT-474	Not found		Estrogen > androgen > progestin	140
KLK11	BT-474	NS		Estrogen, glucocorticoid	89
KLK12	LNCaP, BT-474, T-47D	NS		Androgen, progestin > estrogen but in BT-474, estrogen > androgen > progestin	77
KLK13	BT-474	NS		Androgen, progestin > estrogen	78
KLK14	Not studied				
KLK15	LNCaP		No	Androgen > progestin, estrogen	69

LNCaP, Prostate carcinoma cell line; BT-474, T-47D, breast carcinoma cell lines.
[a] Hormone response elements.

cance already exist (43, 47–49, 152–158). It should be emphasized that, in general, the putative proteins encoded by these variant transcripts have not been isolated. By open reading frame analysis, it has been predicted that most transcripts will produce truncated proteins due to frameshifts originating from deleted exons. More details on these variant transcripts and the predicted encoded proteins can be found in the literature cited in Table 7 (34, 69, 77, 78, 86, 94, 96–98, 137, 159–165).

IX. Association of Kallikreins with Human Diseases

As already mentioned, the only enzyme with efficient kininogenase activity, among the human kallikrein family members, is hK1. The biological effects of this enzyme, and of plasma kallikrein, are mediated mainly by kinin release. Kinin binds to specific G protein-coupled cell surface receptors to mediate diverse biological functions. The kallikrein-kinin system is involved in many disease processes, including inflammation (9), hypertension (166), renal disease (167, 168), pancreatitis (169), and cancer (170–174). A recent book summarizes elegantly the physiology, molecular biology, and pathophysiology of the kallikrein-kinin system and its association to various disease processes (175).

Among all other kallikreins, the best studied, by far, is PSA (hK3) and especially, its application to prostate cancer diagnostics. A comprehensive volume on PSA as a tumor marker has been recently published (176). The extensive literature on PSA and prostate cancer does not warrant further discussion in this review.

Although PSA concentration is generally elevated in the serum of prostate cancer patients, one less known and usually not well understood finding is PSA down-regulation in prostate cancer tissue, in comparison to normal or hyperplastic prostatic tissues (177–182). Furthermore, it has been demonstrated that lower tissue PSA concentration is associated with more aggressive forms of prostate cancer (182, 183). These data agree with those published for breast cancer, where it was found that PSA is down-regulated in cancerous breast tissues, in comparison to normal or hyperplastic breast tissues, and in more aggressive forms of breast cancer. Patients with PSA-positive tumors usually have earlier disease stage, live longer, and relapse less frequently (184–186). Furthermore, it was found that lower PSA levels in nipple as-

TABLE 6. Tissue expression of human kallikreins

Gene	Tissue expression[a]		Reference
	Highest	Other tissues	
KLK1	Pancreas, kidney, salivary glands	Sweat glands, intestine, CNS,[b] neutrophils, uterus, prostate, testis, breast, placenta	7, 9
KLK2	Prostate	Breast, thyroid, salivary glands	46, 147, 148
KLK3	Prostate	Breast, thyroid, salivary glands, lung, trachea	39–45, 54–57
KLK4	Prostate	Breast, thyroid, testis, uterus, adrenal, colon, spinal cord	70–72
KLK5	Breast, brain, testis, skin	Salivary glands, thymus, CNS, prostate, thyroid, trachea	80, 81
KLK6	CNS, breast, kidney, uterus	Salivary gland, spleen, testis	73, 74, 82, 83
KLK7	Skin, CNS, kidney, breast	Salivary glands, thymus, uterus, thyroid, placenta, trachea, testis, ovary	84, 85, 107
KLK8	CNS, skin, ovary		86, 98
KLK9	Thymus, testis, CNS, trachea	Breast, prostate, salivary glands, ovary, skin	67
KLK10	Breast, ovary, testis, prostate	Small intestine, lung, colon, pancreas, uterus, CNS, salivary glands, trachea	76
KLK11	Brain, skin, salivary gland, stomach, uterus, lung, thymus, prostate, spleen, liver, small intestine, trachea	Heart, fetal liver, breast, thyroid, skeletal muscle	88, 89
KLK12	Salivary glands, stomach, uterus, trachea, prostate, thymus, lung, colon, brain, breast, thyroid	Testis, pancreas, small intestine, spinal cord	77
KLK13	Breast, prostate, salivary glands, testis	Lung, heart, thymus, adrenal, colon, thyroid, trachea	78
KLK14	CNS	Breast, thyroid, uterus, thymus, colon, spleen, placenta, small intestine, kidney, bone marrow	90
KLK15	Thyroid, salivary glands, prostate	Adrenal, colon, testis, kidney	69

[a] Most data have been produced by RT-PCR technology.
[b] CNS, central nervous system.

pirate fluid of women are associated with higher risk for developing breast cancer (187). Other published data suggest that PSA may be a tumor suppressor (188), an inducer of apoptosis (188), a negative regulator of cell growth (189), and an inhibitor of angiogenesis (190, 191) and bone resorption (192, 193). These data have recently been reviewed (194).

Another set of investigations suggests that PSA may be associated with unfavorable prognosis/outcomes in breast, prostate, and other cancers. More specifically, it was found that breast tumors with higher PSA content do not respond well to tamoxifen therapy (195). Further, patients with breast tumors, which produce PSA after stimulation by medroxyprogesterone acetate (a synthetic progestin/androgen), have a worse prognosis than patients with tumors that do not produce PSA (196). A number of reports have indicated that PSA may cleave insulin-like growth factor binding protein-3, thus liberating insulin-like growth factor I (IGF-I), which is a mitogen for prostatic stromal and epithelial cells (197–199). PSA may activate latent transforming growth factor-β (TGFβ), stimulate cell detachment and facilitate tumor spread (200). Like other serine proteases, PSA may mediate proteolysis of basement membrane, leading to invasion and metastasis (201).

These confusing clinical data are due to differences in methodology, purity, and source of PSA preparations used, selection of patients, etc. Furthermore, the lack of knowledge of the biological pathways in which PSA is participating poses significant difficulties in interpreting these clinical observations, as further exemplified in a recent commentary (194).

Human glandular kallikrein 2 (hK2) appears to be a new, promising biomarker for prostatic carcinoma (43). It is clear that the diagnostic value of hK2 measurement in serum is not superior to PSA; hK2 may aid in the differential diagnosis between prostate cancer and benign prostatic hyperplasia (57–66) as well as in the identification of organ-confined *vs.* non-organ-confined disease (202). Immunohistochemical studies have shown that prostate cancer tissue produces more hK2 than normal or hyperplastic tissue (203, 204). However, recent quantitative data demonstrate that hK2 concentration, although to a lesser extent than PSA, is also decreased in cancerous tissue, in comparison to adjacent normal tissue (181). Although hK2 has been detected in breast and other tissues (146–148), no studies have as yet been performed to examine its biological action or its value as a breast disease biomarker.

Although it has been shown that KLK4 expression is relatively high in prostate (70, 71), there are no reports describing association or usefulness of this kallikrein in prostatic disease. It will be worthwhile to examine the possible clinical value of this kallikrein as a biomarker in prostatic and other diseases. Recently, KLK4 was found to be overexpressed in a subset of ovarian tumors (205).

A single report describes overexpression of KLK5 in ovarian carcinomas and association with less favorable clinical outcomes (206). Further, KLK6 appears to be dramatically down-regulated at metastatic breast cancer sites and up-regulated in a subset of primary breast and ovarian tumors (73). These data should be interpreted with caution since the number of patients was small and the techniques used were qualitative. Additionally, Little *et al*. (74) suggested that KLK6 may be amyloidogenic and may play a role in the development of Alzheimer's disease by cleaving amyloid precursor proteins. Recently, a number of newly cloned aspartyl proteinases were also shown to be amyloidogenic (207). The connection between various types of proteases and

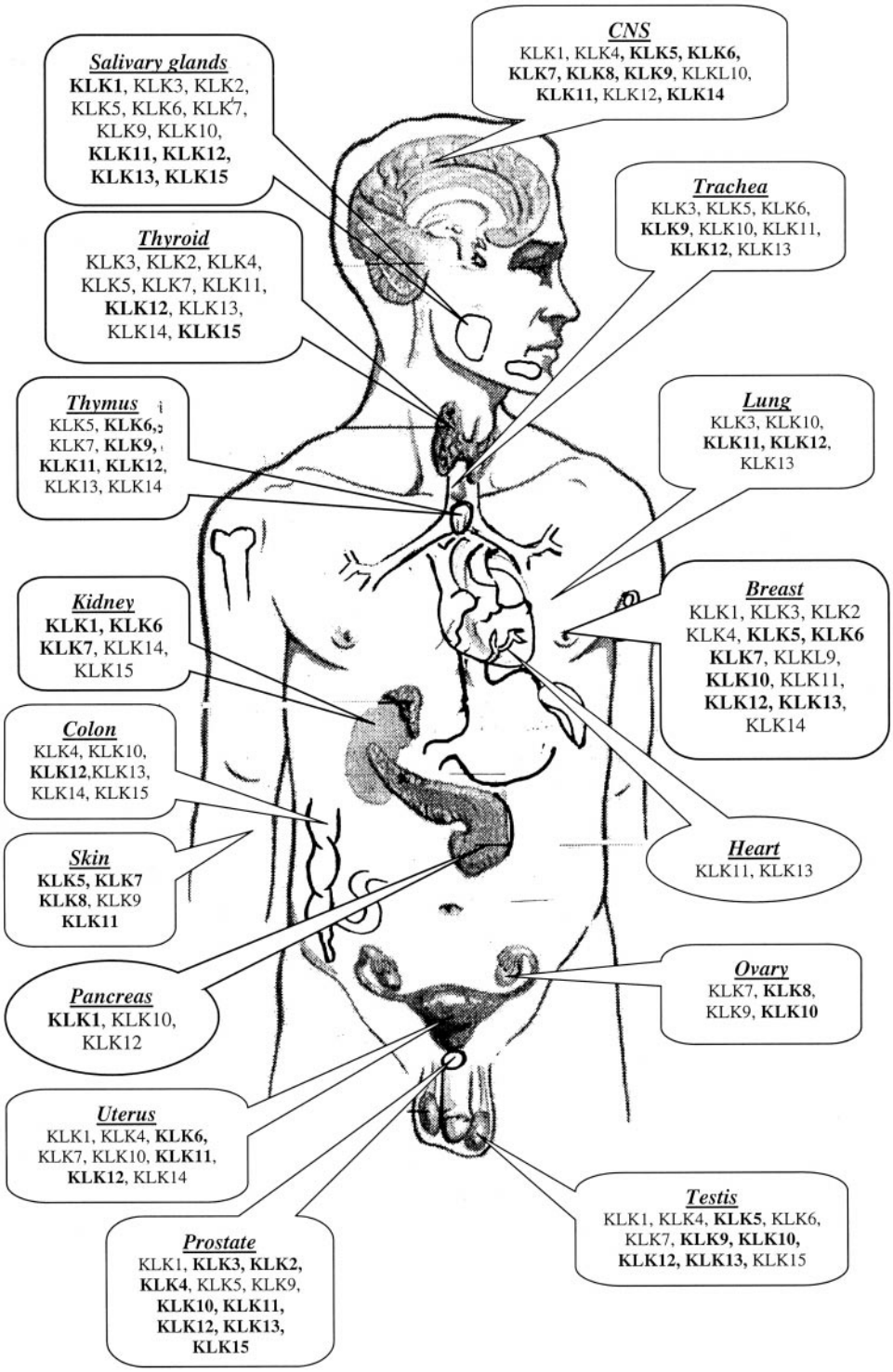

FIG. 5. Schematic representation of tissue kallikrein expression in various tissues. Higher level of expression is shown in *bold*. For more information and discussion, see text and Table 6.

this disease is still ill-defined. The connections of KLK7 with skin diseases, including pathological keratinization and psoriasis, have already been reported (75, 208). KLK7 was also found to be overexpressed in a subset of ovarian carcinomas (107). There are reports describing connection of KLK8 expression with diseases of the central nervous system, including epilepsy (209–212), injury (213, 214), and learning disturbances (215). Another report describes KLK8 overexpression in a subset of ovarian carcinomas (98). Although KLK10 has been shown to be a breast cancer tumor suppressor in animal models (76, 99), there is no report as yet describing prognostic or diagnostic value of KLK10 in breast carcinomas. Recently, KLK10 was found to be down-regulated in more aggressive forms of prostate cancer (216). Preliminary data suggest that KLK12, KLK13, and KLK14 may be down-regulated in a subset of breast carcinomas (77, 78,

90) while KLK15 may be overexpressed in more aggressive forms of prostate cancer (69).

The associations of kallikreins to human diseases are summarized in Table 8. Clearly, except for hK1, hK2, and hK3, the literature is quite limited and the value of the new kallikreins as disease biomarkers is just starting to be examined. Since most studies thus far used small numbers of clinical samples and qualitative methodologies, the data should be interpreted with caution. The knowledge that these kallikreins are secreted proteins supports the idea that they likely circulate in blood and that their concentration may be altered in certain human diseases, including cancer. The experience with hK3 (PSA) and hK2 in prostate cancer may be used to exploit other cancers, including those of breast, ovarian, lung, etc. These possibilities deserve further investigation.

TABLE 7. Variant transcripts of human kallikreins

Gene	No. of known variant transcripts	GenBank accession no.	References
KLK1	3	Not available (N/A)	34, 159, 160
KLK2	6	AF188746	94, 137, 161
KLK3	5	M21896 M21897	96, 97, 162, 163
KLK4	2	N/A	Our unpublished data[a]
KLK7	4	AF166330	85
KLK8	5	AB010780 AF095743 AF251125	86, 98, 164
KLK11	2	AF164623 AB041036	89, 100
KLK12	3	AF135025	77
KLK13	7	AF135024	78, 165
KLK15	3	AF242195	69

[a] Also, J. Clements, personal communication.

X. Physiological Functions

Among the 15 new human kallikrein genes, only 3 have been assigned to a specific biological function (Fig. 6). hK1 exerts its biological activity mainly through the release of lysyl-bradykinin (kallidin) from low molecular weight kininogen. However, the diverse expression pattern of hK1 has led to the suggestion that the functional role of this enzyme may be specific to different cell types (7, 22). Apart from its kininogenase activity, tissue kallikrein has been implicated in the processing of growth factors and peptide hormones (217–220) in light of its presence in pituitary, pancreas, and other tissues. As summarized by Bhoola *et al*. (7), hK1 has been shown to cleave pro-insulin, low density lipoprotein, the precursor of atrial natriuretic factor, prorenin, vasoactive intestinal peptide, procollagenase, and angiotensinogen. Kallikreins, in each cell type, may possess single or multiple functions, common or unique, but Bhoola *et al*. (7) suggest that the release of kinin should still be considered the primary effect of hK1 (7).

The physiological function of hK2 protein has been examined only recently, with the availability of preparations of recombinant origin, which are essentially free of hK3 (PSA)

TABLE 8. Association of kallikreins to human disease

	Disease	Effect/use	Reference
KLK1	Inflammation; sepsis; pancreatitis; bone metabolism; heart disease; renal disease; cancer	Mediation by bradykinin or lysyl-bradykinin	9, 166–178
KLK2	Prostate cancer	Biomarker for diagnosis, monitoring, prognosis	43, 57–66, 202
	Breast cancer	Expressed in breast cancer but prognostic/diagnostic value not as yet examined	146–148
KLK3	Prostate cancer	Biomarker for diagnosis and monitoring	42–53
		Inducer of apoptosis; decreases cell proliferation; inhibitor of angiogenesis	188–191
	Breast cancer	Favorable prognostic indicator; down-regulated in more aggressive disease	184–187
KLK4	Ovarian cancer	Overexpression in a subset of more aggressive ovarian tumors	205
KLK5	Ovarian cancer	Overexpression in a subset of more aggressive ovarian tumors	206
KLK6	Breast cancer	Down-regulation at metastatic sites and up-regulation in a subset of primary tumors	73
	Ovarian cancer	Up-regulation in a subset of ovarian tumors	73
	Alzheimer's disease	Has amyloidogenic potential	74
KLK7	Pathological keratinization	Overexpression in lichen planus and benign oval keratosis	208
	Psoriasis	Overexpression	75
	Ovarian cancer	Overexpression; may be involved in tumor growth and metastasis	107
KLK8	CNS injury	Increased KLK8 expression	213, 214
	Kindling epilepsy	Increased KLK8 expression	209–212
	Ovarian cancer	Overexpression in a subset of tumors	98
KLK10	Breast cancer	Down-regulation; KLK10 may be a tumor suppressor	76, 99
	Prostate cancer	Down-regulated in more aggressive prostate cancer	216
KLK12	Breast cancer	Down-regulated in a subset of breast tumors	77
KLK13	Breast cancer	Down-regulated in a subset of breast and testicular tumors	78
KLK14	Breast cancer	Down-regulation	90
KLK15	Prostate cancer	Overexpressed in more aggressive forms	69

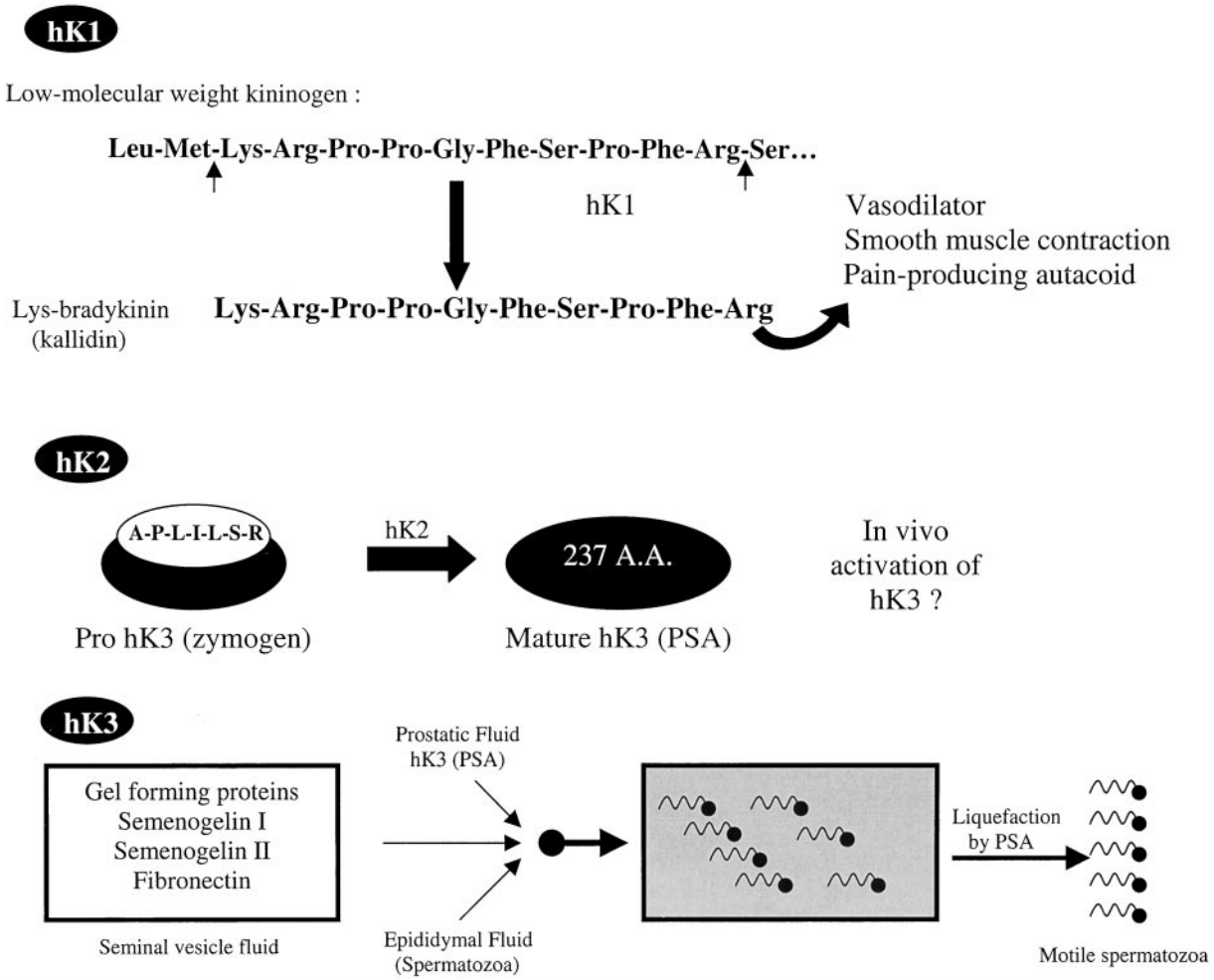

FIG. 6. Biological functions of the three classical kallikreins. hK1 cleaves low molecular weight kininogen and releases lysyl-bradykinin which mediates pleiotropic effects. Human glandular kallikrein 2 activates the pro-form of PSA. Other possible biological functions and substrates of hK2 are described in the text. hK3 (PSA) cleaves semenogelins and fibronectin and mediates seminal clot liquefaction, which increases the motility of spermatozoa. [Adapted with permission from H. G. Rittenhouse *et al.*: *Crit Rev Clin Lab Sci* 35:275–368, 1998 (43). © CRC Press.]

or other kallikrein contaminations (221–224). Three independent groups have reported activation of the pro-form of PSA by hK2 (Fig. 6) (149–151) with a process that is very similar to the autoactivation of hK2 (removal of 7 amino acids) (225). The study of substrate specificities between hK1 and hK2 reveals important differences (106, 226) suggesting that the two proteins have different natural substrates, a notion that is supported by the finding of very low kininogenase activity of hK2 in comparison to hK1 (14, 15). Seminal plasma hK2 was found to be able to cleave seminogelin I and seminogelin II but at different cleavage sites and at a lower efficiency than PSA (227). Since the amount of hK2 in seminal plasma is much lower than PSA (1–5%), the contribution of hK2 in the process of seminal clot liquefaction is expected to be relatively small (43).

In any biological fluid thus far studied, hK3 (PSA) and hK2 were found to coexist (146–148), suggesting a possible functional relationship along the lines described above. Furthermore, a role of hK2 in regulating growth factors, through IGFBP-3 proteolysis, has been suggested (228).

Recently, hK2 was found to activate the zymogen or single-chain form of urokinase-type plasminogen activator (uPA) *in vitro* (229). Since uPA has been implicated in the promotion of cancer metastasis, hK2 may be part of this pathway in prostate cancer.

While both hK1 and hK2 have trypsin-like enzymatic activities, hK3 has chymotrypsin-like substrate specificity (230–233). Since PSA is present at very high levels in seminal plasma, most studies focused on its biological activity within this fluid. Lilja (234) has shown that PSA hydrolyzes rapidly both seminogelin I and seminogelin II, as well as fibronectin, resulting in liquefaction of the seminal plasma clot after ejaculation (234) (Fig. 6). Several other potential substrates for PSA have been identified, including IGFBP-3 (197, 199), TGFβ (200), basement membrane (201), PTH-related peptide (192, 193), and plasminogen (191). The physiological relevance of these findings is still not clear.

hK3 is now known to be found at relatively high levels in nipple aspirate fluid (187, 235), breast cyst fluid (236–240), milk of lactating women (241), amniotic fluid (242), and tumor extracts (184–186). It is thus very likely that hK3 has biological extraprostatic functions in breast and other tissues

TABLE 9. Future directions in kallikrein research

Physiology	Identification of physiological substrates and metabolic pathways in various tissues Delineate mode of regulation—functional characterization of gene promoters Mode of activation and participation in cascade pathways Secretion and deactivation; binding to proteinase inhibitors/serpins Characterization and physiology of enzyme isoforms in different tissues
Pathobiology	Differential expression (overexpression/underexpression) between normal and diseased tissue Tumor promotion/metastasis or tumor suppressor activity Aberrant/ectopic expression, mutation. Activation or inactivation of other effector molecules (growth factors; peptide hormones; cytokines)
Diagnostics	Development of analytical tools (highly sensitive and specific immunoassays) Measurement in biological fluids (especially serum or plasma) Diagnosis; monitoring; prognosis; prediction of therapeutic response; population screening Tumor localization
Therapeutics	Overexpression; underexpression by using external modulators (*e.g.*, steroid hormone agonists/antagonists Serine protease inhibitors; activators Immunotherapy; vaccination Tissue-specific delivery of therapeutic agents by using gene promoters

and may also play a role during fetal development (243). These possibilities merit further investigation.

Among all other human kallikreins, some have been connected to physiological processes and pathological conditions (as described in *Section IX*) but none has been assigned to cleave a specific substrate. Human kallikrein enzymes, with the exception of hK1, hK2, and hK3, are not commercially available and the study of their biological function has not as yet been published. Below, we will attempt to formulate some functional hypotheses for the human kallikreins.

First, all kallikreins are predicted to be secreted proteases, and it is very likely that their biological function is related to their ability to digest one or more substrates. The diversity of expression in human tissues further suggests that they may act on different substrates in different tissues. Their enzymatic activity may initiate, by activation, or terminate, by inactivation, events mediated by other molecules, including hormones, growth factors, receptors, and cytokines. The parallel expression of many kallikreins in the same tissues further suggests that they may participate in cascade reactions similar to those established for the processes of digestion, fibrinolysis, coagulation, and apoptosis. The role of these enzymes in tumor metastasis, as suggested for other proteases (244, 245), should be further investigated.

XI. Future Directions

In Table 9, we summarize some areas that may be fruitful for future kallikrein research. We have already indicated that it will be important to identify the physiological substrates of these enzymes in different tissues and the metabolic pathways in which they participate. The mode of hormonal regulation has been extensively studied only for KLK3 and KLK2. It will be important to functionally characterize gene promoters in view of the preliminary knowledge that the expression of most of these proteases in breast and prostate cancer cell lines is affected by steroid hormones. In addition, the details of activation and deactivation of these enzymes are still obscure. For some of these genes, we already have some information regarding differential expression between normal and diseased tissues. More data are needed. The possible mutational spectrum of these genes in cancer has not been examined.

The most successful clinical application of hK3 (PSA) is currently in the diagnosis and monitoring of prostate cancer. It is anticipated that all these serine proteases circulate in the peripheral blood since they are secreted proteins. It will be important to develop the tools necessary to allow specific and highly sensitive detection of these proteins in biological fluids. Once these tools are available, we should examine whether any of these enzymes have value for diagnosis, monitoring, prediction of therapeutic response, and population screening for diseases such as prostate, breast, ovarian, and other cancers. Applicability to nonmalignant diseases, *e.g.*, Alzheimer's disease, skin pathologies, and inflammatory, autoimmune, and other chronic diseases of many organs in which kallikreins are expressed, should also be examined. Some of these enzymes may be useful targets for tumor localization with specific binding reagents or for therapeutic interventions. If any of these enzymes are shown to participate in cancer metastasis, it may be useful to examine proteinase inhibitors for therapeutic applications. Other possibilities include the use of some of these genes and their promoters for tissue-specific delivery of gene therapy or for over- or underexpression, using exogenously administered modulators (*e.g.,* hormones or hormone blockers) that are known to affect their expression.

XII. Conclusions

In this review, we attempted to summarize the very latest progress in research related to the human kallikrein gene family. For many years, this family was thought to consist of only three genes. We have provided strong evidence suggesting that the human kallikrein gene family now includes at least 15 genes, which are tandemly localized on chromosome 19q13.4 and have significant similarities at both the gene and protein level. Genomic analysis of a large region around the human kallikrein gene locus allowed not only the

precise mapping of these genes but also the delineation of the genomic organization, the prediction of protein sequence and structure, the construction of phylogenetic trees, and the comparison of homologies between all human kallikreins. The diverse tissue expression patterns and the parallel expression of many kallikreins in the same tissues suggest multiple physiological roles as well as possible interactions between the kallikrein enzymes. Many fruitful avenues of investigation are now possible. Most kallikrein genes are regulated by steroid hormones. Protein sequence variation among the kallikreins suggests that each one of them interacts with a specific substrate or a very restricted number of substrates to mediate specific biological events. Much needs to be learned about the substrate specificity of these kallikreins in diverse tissues and the mediation of biological effects from their enzymatic action.

The human kallikrein gene family has contributed the best tumor marker ever developed (PSA). It is possible that other kallikrein members may have applicability as biomarkers in cancer and other chronic and acute diseases. Unfortunately, no methods currently exist to monitor the newly discovered kallikreins with high sensitivity and specificity. The emergence of these new technologies may eventually lead to novel clinical applications of kallikreins other than PSA. We hope that this update will facilitate new developments in this field and lead to practical applications in diverse human diseases.

Acknowledgments

We would like to thank the current and previous members of the Diamandis laboratory, as well as our numerous national and international collaborators who have contributed to the work described in this review.

Note Added in Proof

Since preparation of this review, a few important developments have occurred as follows: The publication of a draft form of the sequence of the human genome will facilitate further genomic analysis within and around the human kallikrein gene locus. A recent paper further summarizes tissue expression data of kallikreins by array analysis (246). Highly sensitive and specific immunoassays for hK6 (247) and hK10 (248) have been published. With these methods, it was found that hK6 may be a biomarker for Alzheimer's disease (249) and a circulating tumor marker for ovarian cancer (250) and that hK10 is a promising new serum tumor marker for ovarian cancer (251).

References

1. **Kraut H, Frey EK, Werle E** 1930 Der Nachweis eines Kreislaufhormon in de Pankreasdruse. Hoppe-Seylers Z Physiol Chem 189: 97–106
2. **Werle E** 1934 Zur Kenntnis des haushalts des Kallikreins. Biochem Z 269:415–434
3. **Fiedler F** 1979 Enzymology of glandular kallikreins. In: Erdos EG (ed) Bradykinin, Kallidin and Kallikrein. Springer-Verlag, Berlin, pp 103–161
4. **Movat HZ** 1979 The plasma kallikrein-kinin system ad its interrelationship with other components of blood. In: Erdos EG (ed) Bradykinin, Kallidin and Kallikrein, Springer-Verlag, Berlin, pp 1–89
5. **Asakai R, Davie EW, Chung DW** 1987 Organization of the gene for human Factor XI. Biochemistry 26:7221–7228
6. **Yu H, Bowden DW, Spray BJ, Roch SS, Freedman BI** 1998 Identification of human plasma kallikrein gene polymorphisms and evaluation of their role in end-stage renal disease. Hypertension 31:906–911
7. **Bhoola KD, Figueroa CD, Worthy K** 1992 Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol Rev 44:1–80
8. **Beraldo WT, Andrade SP** 1997 Discovery of bradykinin and the kallikrein-kinin system. In: Farmer SG (ed) The Kinin System. Academic Press, San Diego, CA, vol 1:1–8
9. **Clements JA** 1997 The molecular biology of the kallikreins and their roles in inflammation. In: Farmer SG (ed) The Kinin System. Academic Press, San Diego, CA, vol 5:71–97
10. **Clements JA** 1989 The glandular kallikrein family of enzymes: tissue-specific expression and hormonal regulation. Endocr Rev 10:393–419
11. **Margolius HS** 1998 Kallikreins, kinins and cardiovascular diseases: a short review. Biol Res 31:135–141
12. **Margolius HS** 1998 Tissue kallikreins structure, regulation, and participation in mammalian physiology and disease. Clin Rev Allergy Immunol 16:337–349
13. **Diamandis EP, Yousef GM, Luo LY, Magklara A, Obiezu CV** 2000 The new human kallikrein gene family: implications in carcinogenesis. Trends Endocrinol Metab 11:54–60
14. **Deperthes D, Marceau F, Frenette G, Lazure C, Tremblay RR, Dube JY** 1997 Human kallikrein hK2 has low kininogenase activity while prostate specific antigen (hK2) has none. Biochim Biophys Acta 1343:102–106
15. **Charlesworth MC, Young CYE, Miller VM, Tindall DJ** 1999 Kininogenase activity of prostate-derived human glandular kallikrein (hK2) purified from seminal fluid. J Androl 20:220–229
16. **Evans BA, Drinkwater CC, Richards RI** 1987 Mouse glandular kallikrein genes. Structure and partial sequence analysis of the kallikrein gene locus. J Biol Chem 262:8027–8034
17. **Bell RA, Fahnestock M** 1988 Sequence and comparative analysis of three cDNAs from *Mastomys natalensis*, an African rat. J Cell Biol 107:615a
18. **Wines DR, Brady JM, Pritchett DB, Roberts JL, MacDonald RJ** 1989 Organization and expression of the rat kallikrein gene family. J Biol Chem 264:7653–7662
19. **Murray SR, Chao J, Lin F, Chao L** 1990 Kallikrein multigene families and the regulation of their expression. J Cardiovasc Pharmacol 15[Suppl]:S7–S15
20. **Howies PN, Dickinson DP, DiCaprio LL, Woodworth-Gutai M, Gross KW** 1984 Use of a cDNA recombinant for the γ-subunit of mouse nerve growth factor to localize members of the multigene family near the TAM-1 locus on chromosome 7. Nucleic Acids Res 12:2791–2805
21. **Mason AJ, Evans BA, Cox DR, Shine J, Richards RI** 1983 Structure of mouse kallikrein gene family suggests a role in specific processing of biologically active peptides. Nature 303:300–307
22. **Drinkwater CC, Evans BA, Richards RI** 1987 Mouse glandular kallikrein genes: identification and characterization of the genes encoding the epidermal growth factor binding proteins. Biochemistry 26:6750–6756
23. **Ashley PL, MacDonald RJ** 1985 Kallikrein-related mRNAs of the rat submaxilliary gland: nucleotide sequences of four distinct types including tonin. Biochemistry 24:4512–4519
24. **Chen Y, Chao J, Chen L** 1988 Molecular cloning and characterization of two rat renal kallikrein genes. Biochemistry 27:7189–7196
25. **Brady JM, Wines DR, MacDonald RJ** 1989 Expression of two kallikrein gene family members in the rat prostate. Biochemistry 28:5203–5210
26. **Inoue H, Fukui K, Miyake Y** 1989 Identification and structure of the rat true tissue kallikrein gene expressed in the kidney. J Biochem (Tokyo) 108:834–840
27. **Shai S, Woodley-Miller C, Chao J, Chao L** 1989 Characterization of genes encoding rat tonin and a kallikrein-like serine protease. Biochemistry 28:5334–5343

28. **Wines DR, Brady JM, Southard EM, MacDonald RJ** 1991 Evolution of the rat kallikrein gene family: gene conversion leads to functional diversity. J Mol Evol 32:476–492
29. **Brady JM, MacDonald RJ** 1990 The expression of two kallikrein gene family members in the rat kidney. Arch Biochem Biophys 278:342–349
30. **Ma JX, Chao J, Chao L** 1992 Molecular cloning and characterization of rKLK10, a cDNA encoding T-kininogenase from rat submandibular gland and kidney. Biochemistry 31:10922–10928
31. **Clements JA, Matheson BA, Funder JW** 1990 Tissue-specific developmental expression of the kallikrein gene family in the rat. J Biol Chem 265:1077–1081
32. **Clements JA, Mukhtar A, Verity K, Pullar M, McNeill P, Cummins J, Fuller PJ** 1996 Kallikrein gene expression in human pituitary tissues. Clin Endocrinol (Oxf) 44:223–231
33. **Clements JA, Mukhtar A, Ehrlich A, Fuller P** 1992 A re-evaluation of the tissue-specific pattern of expression of the rat kallikrein gene family. In: Fritz H, Muller-Esterl W, Jochum M, Roscher A, Lyuppertz K (eds) Recent Progress on Kinins. Agents and Actions Supplement. Birkhauser Verlag, Basel, vol 38/I:34–41
34. **Evans BA, Yun ZX, Close JA, Tregear GW, Kitamura N, Nakanishi S, Callen DF, Baker E, Hyland VJ, Sutherland GR, Richards RI** 1988 Structure and chromosomal localization of the human renal kallikrein gene. Biochemistry 27:3124–3129
35. **Richards RI, Holman K, Shen Y, Kozman H, Harley H, Brook D, Shaw D** 1991 Human glandular kallikrein genes: genetic and physical mapping of the KLK1 locus using a highly polymorphic microsatellite PCR marker. Genomics 11:77–82
36. **Riegman PHJ, Vlietstra RJ, Klaassen P, Van der Korput JAGM, Guerts van Kessel A, Romijn JC, Trapman J** 1989 The prostate-specific antigen gene and the human glandular kallikrein-1 gene are tandemly located on chromosome 19. FEBS Lett 247:123–126
37. **Riegman PHJ, Vlietstra RJ, Suurmeijer L, Cleutjens CBJM, Trapman J** 1992 Characterization of the human kallikrein locus. Genomics 14:6–11
38. **Qin H, Kemp J, Yip M, Lam-Po-Tang PRL, Morris BJ** 1991 Localization of human glandular kallikrein-1 gene to chromosome 19q13.3–13.4 by *in-situ* hybridization. Hum Hered 41:222–226
39. **Wang MC, Valenzuela LA, Murphy GP, Chu TM** 1979 Purification of a human prostate-specific antigen. Invest Urol 17:159–163
40. **Wang MC, Papsidero LD, Kuriyama M, Valenzuela LA, Murphy GP, Chu TM** 1981 Prostate antigen: a new potential marker for prostatic cancer. Prostate 21:89–96
41. **Wang MC, Valenzuela LA, Murphy GP, Chu TM** 1977 Tissue specific and tumor specific antigens in human prostate. Fed Proc 36:1254
42. **Papsidero LD, Wang MC, Valenzuela LA, Murphy GP, Chu TM** 1980 A prostate antigen in sera of prostatic cancer patients. Cancer Res 40:2428–2432
43. **Rittenhouse HG, Finlay JA, Mikolajczyk SD, Partin AW** 1998 Human kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. Crit Rev Clin Lab Sci 35:275–368
44. **Diamandis EP** 1999 Prostate specific antigen—its usefulness in clinical medicine. Trends Endocrinol Metab 25:14–16
45. **Oesterling JE** 1991 Prostate-specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol 145:907–923
46. **Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJ, Petros JA, Andriole GL** 1991 Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med 324:1156–1161
47. **Catalona WJ** 1996 Clinical utility of measurements of free and total prostate-specific antigen (PSA): a review. Prostate Suppl 7:64–69
48. **Pannek J, Partin AW** 1997 Prostate-specific antigen: what's new in 1997. Oncology 9:1273–1278
49. **McCormack RT, Rittenhouse HG, Finlay JA, Sokoloff RL, Wang TJ, Wolfert RL, Lilja H, Oesterling JE** 1995 Molecular forms of prostate-specific antigen and the human kallikrein gene family: a new era. Urology 45:729–744
50. **Lilja H** 1993 Structure, function and regulation of the enzyme activity of prostate-specific antigen. World J Urol 11:188–191
51. **Malm J, Lilja H** 1995 Biochemistry of prostate-specific antigen, PSA. Scand J Clin Lab Invest Suppl 221:15–22
52. **Chu TM** 1997 Prostate-specific antigen and early detection of prostate cancer. Tumour Biol 18:123–134
53. **Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E** 1987 Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 317:909–916
54. **Diamandis EP, Yu H** 1995 New biological function of prostate-specific antigen? J Clin Endocrinol Metab 80:1515–1517
55. **Diamandis EP** 1998 Prostate-specific antigen or human kallikrein 3? Recent developments. Tumor Biol 19:65–68
56. **Diamandis EP, Yu H** 1997 Nonprostatic sources of prostate-specific antigen. In: Oesterling JE (ed) The Urologic Clinics of North America: Prostate-Specific Antigen: The Best Prostatic Tumor Marker. WB Saunders, Philadelphia, PA, vol 14:275–282
57. **Black MH, Diamandis EP** 2000 The diagnostic and prognostic utility of prostate specific antigen for diseases of the breast. Breast Cancer Res Treat 59:1–14
58. **Stenman U-H** 1999 New ultrasensitive assays facilitate studies on the role of human glandular kallikrein (hK2) as a marker for prostatic disease. Clin Chem 45:753–754
59. **Klee G, Goodmanson M, Jacobsen S, Young C, Finlay J, Rittenhouse HG, Wolfert RL, Tindall DJ** 1999 Highly sensitive automated chemiluminometric assay for measuring free human glandular kallikrein-2. Clin Chem 45:800–806
60. **Black M, Magklara A, Obiezu C, Melegos D, Diamandis EP** 1999 Development of an ultrasensitive immunoassay for human glandular kallikrein with no cross-reactivity from prostate-specific antigen. Clin Chem 45:790–799
61. **Kwiatkowski MK, Recker F, Piironen T, Pettersson K, Otto T, Wernli M, Tscholl R** 1998 In prostatism patients the ratio of human glandular kallikrein to free PSA improves the discrimination between prostate cancer and benign hyperplasia within the diagnostic "gray zone" of total PSA 4 to 10 ng/mL. Urology 52:360–365
62. **Magklara A, Scorilas A, Catalona WJ, Diamandis EP** 1999 The combination of human glandular kallikrein and free prostate-specific antigen (PSA) enhances discrimination between prostate cancer and benign prostatic hyperplasia in patients with moderately increased total PSA. Clin Chem 45:1960–1966
63. **Nam RK, Diamandis EP, Toi A, Trachtenberg J, Magklara A, Scorilas A, Papanastasiou P, Jewett MAS, Narod SA** 2000 Serum human glandular kallikrein-2 protease levels predict the presence of prostate cancer among men with elevated prostate-specific antigen. J Clin Oncol 18:1036–1042
64. **Partin AW, Catalona WJ, Finlay JA, Darte C, Tindall DJ, Young CY, Klee GG, Chan DW, Rittenhouse HG, Wolfert RL, Woodrum DL** 1999 Use of human glandular kallikrein 2 for the detection of prostate cancer: preliminary analysis. Urology 54:839–845
65. **Becker C, Piironen T, Kiviniemi J, Lilja H, Pettersson K** 2000 Sensitive and specific immunodetection of human glandular kallikrein 2 in serum. Clin Chem 46:198–206
66. **Becker C, Piironen T, Pettersson K, Bjork T, Wojno KJ, Oesterling JE, Lilja H** 2000 Discrimination of men with prostate cancer from those with benign disease by measurements of human glandular kallikrein 2 (hK2) in serum. J Urol 163:311–316
67. **Yousef GM, Diamandis EP** 2000 The expanded human kallikrein gene family: locus characterization and molecular cloning of a new member KLK-L3. Genomics 65:184–194
68. **Yousef GM, Chang A, Scorilas A, Diamandis EP** 2000 Genomic organization of the human kallikrein gene family on chromosome 19q13.3-q13.4. Biochem Biophys Res Commun 16:125–133
69. **Yousef GM, Scorilas A, June K, Ahsworth LK, Diamandis EP** 2001 Molecular cloning of the human kallikrein 15 gene (KLK15): upregulaton in prostate cancer. J Biol Chem 276:53–61
70. **Nelson PS, Gan L, Ferguson C, Moss P, Gelinas R, Hood L, Wang K** 1999 Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression. Proc Natl Acad Sci USA 96:3114–3119
71. **Yousef GM, Obiezu CV, Luo LY, Black MH, Diamandis EP** 1999 Prostase/KLK-L1 is a new member of the human kallikrein gene family, is expressed in prostate and breast tissues, and is hormonally regulated. Cancer Res 59:4252–4256
72. **Stephenson SA, Verity K, Ashworth LK, Clements JA** 1999 Local-

ization of a new prostate-specific antigen-related serine protease gene, KLK4, is evidence for an expended human kallikrein gene family cluster on chromosome 19q13.3–13.4. J Biol Chem 274:23210–23214
73. **Anisowicz A, Sotiropoulou G, Stenman G, Mok SC, Sager R** 1996 A novel protease homolog differentially expressed in breast and ovarian cancer. Mol Med 2:624–636
74. **Little SP, Dixon EP, Norris F, Buckley W, Becker GW, Johnson M, Dobbins JR, Wyrick T, Miller JR, MacKellar W, Hepburn D, Corvalan J, McClure D, Liu X, Stephenson D, Clements J, Johnstone EM** 1997 Zyme, a novel and potentially amyloidogenic enzyme cDNA isolated from Alzheimer's disease brain. J Biol Chem 272:25135–25142
75. **Ekholm E, Egelrud T** 1999 Stratum corneum chymotryptic enzyme in psoriasis. Arch Dermatol Res 291:195–200
76. **Liu XL, Wazer DE, Watanabe K, Band V** 1996 Identification of a novel serine protease-like gene, the expression of which is down-regulated during breast cancer progression. Cancer Res 56:3371–3379
77. **Yousef GM, Magklara A, Diamandis EP** 2000 KLK12 is a novel serine protease and a new member of the human kallikrein gene family–differential expression in breast cancer. Genomics 69:331–341
78. **Yousef GM, Chang A, Diamandis EP** 2000 Identification and characterization of KLK-L4, a new kallikrein-like gene which appears to be down-regulated in breast cancer tissues. J Biol Chem 275: 11891–11898
79. **Hu JC-C, Zhang C, Sun X, Yang Y, Cao X, Ryu O, Simmer JP** 2000 Characterization of the mouse and human PRSS17 genes their relationship to other serine proteases and the expression of PRSS17 in developing mouse incisors. Gene 251:1–8
80. **Yousef GM, Diamandis EP** 1999 The new kallikrein-like gene KLK-L2: molecular characterization, mapping, tissue expression and hormonal regulation. J Biol Chem 274:37511–37516
81. **Brattsand M, Egelrud T** 1999 Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. J Biol Chem 274: 30033–30040
82. **Yamashiro K, Tsuruoko N, Kodama S, Tsujimoto M, Yamamura T, Tanaka T, Nakazato H, Yamaguchi N** 1997 Molecular cloning of a novel trypsin-like serine protease (neurosin) preferentially expressed in brain. Biochim Biophys Acta 1350:11–14
83. **Yousef GM, Luo LY, Scherer SW, Sotiropoulou G, Diamandis EP** 1999 Molecular characterization of zyme/protease M/neurosin, a hormonally-regulated kallikrein-like serine protease. Genomics 62: 251–259
84. **Hansson L, Stromqvist M, Backman A, Wallbrandt P, Carlstein A Egelrud T** 1994 Cloning, expression and characterization of stratum corneum chymotryptic enzyme. A skin-specific human serine proteinase. J Biol Chem 269:19420–19426
85. **Yousef GM, Scorilas A, Magklara A, Soosaipillai A, Diamandis EP** 2000 The KLK7 (PRSS6) gene encoding for the stratum corneum chymotryptic enzyme is a new member of the human kallikrein gene family–genomic characterization mapping tissue expression and hormonal regulation. Gene 254:119–128
86. **Yoshida S, Taniguchi M, Hirata A Shiosaka S** 1998 Sequence analysis and expression of human neuropsin cDNA and gene. Gene 213:9–16
87. **Luo L, Herbrick J-A, Scherer SW, Beatty B, Squire J, Diamandis EP** 1998 Structural characterization and mapping of the normal epithelial cell-specific 1 gene. Biochem Biophys Res Commun 247: 580–586
88. **Yoshida S, Taniguchi M, Suemoto T, Oka T, He X, Shiosaka S** 1998 cDNA cloning and expression of a novel serine protease, TLSP. Biochim Biophys Acta 1399:225–228
89. **Yousef GM, Scorilas A, Diamandis EP** 2000 Genomic organization mapping tissue expression and hormonal regulation of trypsin-like serine protease (TLSP PRSS20), a new member of the human kallikrein gene family. Genomics 63:88–96
90. **Yousef GM, Magklara A, Chang A, Jung K, Katsaros D, Diamandis EP,** Cloning of a new member of the human kallikrein gene family, KLK14, which is down-regulated in different malignancies. Cancer Res, in press
91. **Berg T, Bradshaw RA, Carretero OA, Chao J, Chao L, Clements JA, Fahnestock M, Fritz H, Gauthier F, MacDonald RJ, Margolius HS, Morris BJ, Richards RI, Scicli AG** 1992 A common nomenclature for members of the tissue (glandular) kallikrein gene families. In: Fritz H, Muller-Esterl W, Jochum M, Roscher A, Luppertz K(eds) Recent Progress on Kinins. Agents and Actions Supplement. Birkhauser Verlag, Basel, vol 38/1:19–25
92. **Diamandis EP, Yousef GM, Clements J, Ashworth LK, Yoshida S, Egelrud T, Nelson PS, Shiosaka S, Little S, Lilja H, Stenman U-H, Rittenhouse HG, Wain** 2000 New nomenclature for the human tissue kallikrein gene family. Clin Chem 46:1855–1858
93. **Fukushima D, Kitamura N, Nakanishi S** 1985 Nucleotide sequence of cloned cDNA for human pancreatic kallikrein. Biochemistry 24:8037–8043
94. **Schedlich LJ, Bennetts BH, Morris BJ** 1987 Primary structure of a human glandular kallikrein gene. DNA 6:429–437
95. **Lundwall A** 1989 Characterization of the gene for prostate-specific antigen, a human glandular kallikrein. Biochem Biophys Res Commun 161:1151–1159
96. **Riegman PHJ, Klassen P, Van der Korput JAGM, Romijn JC, Trapman J** 1988 Molecular cloning and characterization of novel prostate antigen cDNAs. Biochem Biophys Res Commun 155: 181–188
97. **Riegman PHJ, Vlietstra RJ, Van der Korput JAGM, Romijn JC, Trapman J** 1989 Characterization of the prostate-specific antigen gene: a novel human kallikrein-like gene. Biochem Biophys Res Commun 159:95–102
98. **Underwood LJ, Tanimoto H, Wang Y, Shigemasa K, Parmley TH, O'Brien TJ** 1999 Cloning of tumor-associated differentially expressed gene-14, a novel serine protease overexpressed by ovarian carcinoma. Cancer Res 59:4435–4439
99. **Goyal J, Cowan JM, Wazer DE, Lee SW, Band V** 1998 The role for NES1 serine. Protease as a novel tumor suppressor. Cancer Res 58:4782–4786
100. **Mitsui S, Yamada T, Okui A, Kominami K, Uemura H, Yamaguchi N** 2000 A novel isoform of a kallikrein-like protease TLSP/Hippostasin (PRSS20), is expressed in the human brain and prostate. Biochem Biophys Res Commun 272:205–211
101. **Foussias G, Yousef GM, Diamandis EP** 2000 Identification and molecular characterization of a novel member of the siglec family (Siglec 9). Genomics 67:171–178
102. **Crocker PR, Clark EA, Filbin M, Gordon S, Jones Y, Kehrl JH, Kelm S, Le Douarin N, Powell L, Roder J, Schnaar RL, Sgroi DC, Stamenkovic K, Schauer R, Schachner M, van den Berg TK, van der Merwe PA, Watt SM, Varki A** 1998 Siglecs: a family of sialic-acid binding lectins. Glycobiology 8:5–6
103. **Kelm S, Schauer R, Crocker PR** 1996 The sialoadhesins–a family of sialic acid-dependent cellular recognition molecules within the immunoglobulin superfamily. Glycoconj J 13:913–926
104. **Dayhoff MO** 1978 Atlas of protein sequence and structure. Nat Biomed Res Found [Suppl 3]:79–81
105. **Creighton TE** 1993 Proteins: Structures and Molecular Properties, ed 2. WH Freeman and Company, New York
106. **Lovgren J, Airas K, Lilja H** 1999 Enzymatic action of human glandular kallikrein 2 (hK2) substrate specificity and regulation by Zn^{2+} and extracellular protease inhibitors. Eur J Biochem 262:781–789
107. **Tanimoto H, Underwood LJ, Shigemasa K, Yan Y, Clarke J, O'Brien TJ** 1999 The stratum corneum chymotryptic enzyme that mediates shedding and desquamation of skin cells is highly overexpressed in ovarian tumor cells. Cancer 86:2074–2082
108. **Van Leeuwen BH, Evans BA, Tregear GW, Richards R** 1986 Mouse glandular kallikrein genes. Identification, structure and expression of the renal kallikrein gene. J Biol Chem 261:5529–5535
109. **Van Leeuwen BH, Penschow JD, Coghlan JP, Richards RI** 1987 Cellular basis for the differential response of mouse kallikrein genes to hormonal induction. EMBO J 6:1705–1713
110. **Clements JA, Matheson BA, Wines DR, Brady JM, MacDonald RJ, Funder JW** 1988 Androgen dependence of specific kallikrein gene family members expressed in the rat prostate. J Biol Chem 31:16132–16137
111. **Penschow JD, Drinkwater CC, Haralambidis J, Coghlan JP** 1991 Sites of expression and induction of glandular kallikrein gene expression in mice. Mol Cell Endocrinol 81:135–146
112. **Miller DH, Chao J, Margolius HS** 1984 Tissue kallikrein synthesis

and its modification by testosterone or low dietary sodium. Biochem J 218:237–243
113. **Miller DH, Lindley JG, Margolius HS** 1985 Tissue kallikrein levels and synthesis rates are not changed by an acute physiological dose of aldosterone. Proc Soc Exp Biol Med 180:121–125
114. **Clements JA, Fuller PJ, McNally M, Nikolaidis I, Funder JW** 1986 Estrogen regulation of kallikrein gene expression in the rat anterior pituitary. Endocrinology 119:268–273
115. **Clements JA, Matheson BA, MacDonald RJ, Funder JW** 1989 The expression of the kallikrein gene family in the rat pituitary oestrogen effects and the expression of an additional family member in the neurointermediate lobe. J Neuroendocrinol 1:199–203
116. **Pritchett DB, Roberts JL** 1987 Dopamine regulates expression of the glandular-type kallikrein gene at the transcriptional level in the pituitary. Proc Natl Acad Sci USA 84:5545–5549
117. **Clements JA, Mukhtar A, Ehrlich A, Yap B** 1994 Kallikrein gene expression in the human uterus. Braz J Med Biol Res 27:1855–1863
118. **Riegman PHJ, Vlietstra RJ, van der Korput JAGM, Brinkmann AO, Trapman J** 1991 The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol 5:1921–1930
119. **Cleutjens KBJM, van Eekelen CCEM, van der Korput HAGM, Brinkman AO, Trapman J** 1996 Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J Biol Chem 271:6379–6388
120. **Luke MC, Coffey DS** 1994 Human androgen receptor binding to the androgen response element of prostate specific antigen. J Androl 15:41–51
121. **Schuur ER, Henderson GA, Kmetec LS, Miller JD, Lamparski HG, Henderson DR** 1996 Prostate-specific antigen expression is regulated by an upstream enhancer. J Biol Chem 271:7043–7051
122. **Cleutjens KB, van der Korput HA, van Eekelen CC, van Rooij HCJ, Faber PW, Trapman J** 1997 An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate-specific antigen promoter. Mol Endcrinol 11:148–161
123. **Yu H, Diamandis EP, Zarghami N, Grass L** 1994 Induction of prostate specific antigen production by steroids and tamoxifen in breast cancer cell lines. Breast Cancer Res Treat 32:291–300
124. **Yu H, Diamandis EP, Monne M, Croce CM** 1995 Oral contraceptive-induced expression of prostate specific antigen in the female breast. J Biol Chem 270:6615–6618
125. **Zarghami N, Grass L, Diamandis EP** 1997 Steroid hormone regulation of prostate specific antigen gene expression in breast cancer. Br J Cancer 75:579–588
126. **Rodriguez R, Schuur ER, Lim HY, Henderson GA, Simons JW, Henderson DR** 1997 Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. Cancer Res 57: 2559–25563
127. **Cleutjens KB, van der Korput HA, Ehren-van Eekelen CC, Sikes RA, Fasciana C, Chung LW, Trapman J** 1997 A 6-kb promoter fragment mimics in transgenic mice the prostate-specific and androgen-regulated expression of the endogenous prostate-specific antigen gene in humans. Mol Endocrinol 11:1256–1265
128. **Latham JP, Searle PF, Mautner V, James ND** 2000 Prostate-specific antigen promoter/enhancer driven gene therapy for prostate cancer: construction and testing of a tissue-specific adenovirus vector. Cancer Res 60:334–341
129. **Martiniello-Wilks R, Garcia-Aragon J, Daja MM, Russell P, Both GW, Molloy PL, Lockett LJ, Russell PJ** 1998 *In vivo* gene therapy for prostate cancer: preclinical evaluation of two different enzyme-directed prodrug therapy systems delivered by identical adenovirus vectors. Hum Gene Ther 9:1617–1626
130. **Gotoh A, Ko SC, Shirakawa T, Cheon J, Kao C, Miyamoto T, Gardner TA, Ho LJ, Cleutjens CB, Trapman J, Graham FL, Chung LW** 1998 Development of prostate-specific antigen promoter-based gene therapy for androgen-independent human prostate cancer. J Urol 160:220–229
131. **Dannul J, Belldegrun AS** 1997 Development of gene therapy for prostate cancer using a novel promoter of prostate-specific antigen. Br J Urol 79[Suppl 1]:97–103
132. **Lee CG, Liu M, Sie KL, Lee MS** 1996 Prostate-specific antigen promoter driven gene therapy targeting DNA polymerase-α and topoisomerase II α in prostate cancer. Anticancer Res 16:1805–1811
133. **Young CYF, Andrews PE, Tindall DJ** 1995 Expression and androgenic regulation of human prostate-specific kallikreins. J Androl 16:97–99
134. **Magklara A, Grass L, Diamandis EP** 2000 Differential steroid hormone regulation of human glandular kallikrein (hK2) and prostate-specific antigen (PSA) in breast cancer cell lines. Breast Cancer Res Treat 59:263–270
135. **Murtha P, Tindall DJ, Young CYF** 1993 Androgen induction of a human prostate-specific kallikrein, *hKLK2*: characterization of an androgen response element in the 5′ promoter region of the gene. Biochemistry 32:6459–6464
136. **Hsieh M-L, Charlesworth C, Goodmanson M, Zhang S, Seay T, Klee GG, Tindall DJ, Young CY** 1997 Expression of human prostate-specific glandular kallikrein protein (hK2) in the breast cancer cell line T47-D. Cancer Res 57:2651–2656
137. **Riegman PHJ, Vlietstra RJ, van der Korput HA, Romijn JC, Trapman J** 1991 Identification and androgen-regulated expression of two major human glandular kallikrein-1 (hGK-1) mRNA species. Mol Cell Endocrinol 76:181–190
138. **Yu D-C, Sakamoto GT, Henderson DR** 1999 Identification of the transcriptional regulatory sequences of human kallikrein 2 and their use in the construction of Calydon virus 764, an attenuated replication competent adenovirus for prostate cancer therapy. Cancer Res 59:1498–1504
139. **Gotoh A, Kamidono S, Chung LW** 1997 Clinical application for gene therapy in prostate cancer. Hinyokika Kiyo 43:829–833
140. **Luo LY, Grass L, Diamandis EP** 2000 The normal epithelial cell-specific 1 (NES1) gene is up-regulated by steroid hormones in the breast carcinoma cell line BT-474. Anticancer Res 20:981–986
141. **Young CY, Andrews PE, Montgomery BT, Tindall DJ** 1992 Tissue-specific and hormonal regulation of human prostate-specific glandular kallikrein. Biochemistry 31:818–824
142. **Morris BJ** 1989 hGK-1: a kallikrein gene expressed in human prostate. Clin Exp Pharmacol Physiol 16:345–351
143. **Ferguson RA, Yu H, Kalyvas M, Zammit S, Diamandis EP** 1996 Ultrasensitive detection of prostate specific antigen by a time-resolved immunofluorometric assay and the Immulite® Immunochemiluminescent third generation assay: potential applications in prostate and breast cancers. Clin Chem 42:675–684
144. **Zarghami N, Diamandis EP** 1996 Detection of prostate specific antigen mRNA and protein in breast tumors. Clin Chem 42:361–366
145. **Howarth DJC, Aronson IB, Diamandis EP** 1997 Immunohistochemical localization of prostate specific antigen in benign and malignant breast tissues. Br J Cancer 75:1646–1651
146. **Magklara A, Scorilas A, López-Otin C, Vizoso F, Ruibal A, Diamandis EP** 1999 Human glandular kallikrein in breast milk, amniotic fluid, and breast cyst fluid. Clin Chem 45:1774–1780
147. **Black MA, Magklara A, Obiezu C, Levesque MA, Sutherland DJA, Tindall DJ, Young CYF, Sauter ER, Diamandis EP** 2000 Expression of a prostate-associated protein human glandular kallikrein (hK2), in breast tumors and in normal breast secretions. Br J Cancer 82:361–367
148. **Lovgren J, Valtonen-Andre C, Marsal K, Lilja H, Lundwall A** 1999 Measurement of prostate-specific antigen and human glandular kallikrein 2 in different body fluids. J Androl 20:348–355
149. **Lovgren J, Rajakoski K, Karp M, Lundwall A, Lilja H** 1997 Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2. Biochem Biophys Res Commun 238:549–555
150. **Kumar A, Mikolajczyk SD, Goel AS, Millar LS, Saedi MS** 1997 Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2. Cancer Res 57:3111–3114
151. **Takayama TK, Fujikawa K, Davie EW** 1997 Characterization of the precursor of prostate-specific antigen–activation by trypsin and by human glandular kallikrein. J Biol Chem 272:21582–21588
152. **Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A Richie JP, deKernion JB, Walsh PC, Scardino PT, Lange PH, Subong EN, Parson RE, Gasior GH, Loveland KG, Southwick PC** 1998 Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic

disease: a prospective multicenter clinical trial. JAMA 279:1542–1547
153. **Lilja H, Haese A, Bjork T, Frederich MG, Piironen T, Pettersson K, Huland E, Huland H** 1999 Significance and metabolism of complexed and noncomplexed prostate specific antigen forms, and human glandular kallikrein 2 in clinically localized prostate cancer before and after radical prostatectomy. J Urol 162:2029–2034
154. **Lilja H** 1997 Prostate-specific antigen: molecular forms and the human kallikrein gene family. Br J Urol 79[Suppl 1]:44–48
155. **Zhang WM, Finne P, Leinonen J, Vesalainen S, Nordling S, Stenman UH** 1999 Measurement of the complex between prostate-specific antigen and α1-protease inhibitor in serum. Clin Chem 45:814–821
156. **Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Marks LS, Song W, Wheeler TM, Slawin KM** 2000 A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared to benign transition zone prostate tissue. Cancer Res 60:756–759
157. **Christensson A, Laurell CB, Lilja H** 1990 Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. Eur J Biochem 194:755–763
158. **Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O** 1991 A complex between prostate-specific antigen and α1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. Cancer Res 51:222–226
159. **Chen LM, Murray SR, Chai KX, Chao L, Chao J** 1994 Molecular cloning and characterization of a novel kallikrein transcript in colon and its distribution in human tissues. Braz J Med Biol Res 27:1829–1838
160. **Rae F, Bulmer B, Nicol D, Clements J** 1999 The human tissue kallikreins (KLKs 1–3) and a novel KLK1 mRNA transcript are expressed in renal cell carcinoma cDNA library. Immunopharmacology 45:83–88
161. **Liu XF, Essand M, Vasmatzis G, Lee B, Pastan I** 1999 Identification of three new alternate human kallikrein 2 transcripts: evidence of long transcript and alternative splicing. Biochem Biophys Res Commun 264:833–839
162. **Heuze N, Olayat S, Gutman N, Zani ML, Courty Y** 1999 Molecular cloning and expression of an alternative hKLK3 transcript coding for a variant protein of prostate- specific antigen. Cancer Res 59: 2820–2824
163. **Tanaka T, Isono T, Yoshiki T, Yuasa T, Okada Y** 2000 A novel form of prostate-specific antigen transcript produced by alternative splicing. Cancer Res 60:56–59
164. **Magklara A, Scorilas A, Katsaros D, Fracchioli S, Yousef GM, Diamandis EP,** The human KLK8 (neuropsin/ovasin) gene: identification of two novel splice variants and its prognostic value in ovarian cancer. Cancer Res, in press
165. **Chang A, Yousef GM, Jung K, Rajpert-De Meyts E, Diamandis EP,** Identification and molecular characterization of five novel kallikrein gene 13 (KLK13; KLK-L4) splice variants: differential expression in testicular cancer. Gene, in press
166. **Margolius HS, Horwitz D, Pisano JJ, Keiser HR** 1974 Urinary kallikrein in hypertension: relationship to sodium intake and sodium-retaining steroids. Circ Res 35:820–825
167. **Horwitz D, Margolius HS, Keiser HR** 1978 Effects of dietary potassium and race on urinary excretion of kallikrein and aldosterone in man. J Clin Endocrinol Metab 47:296–299
168. **Holland OB, Chud JM, Braunstein H** 1980 Urinary kallikrein excretion in essential and mineralocorticoid hypertension. J Clin Invest 65:347–356
169. **Griesbacher T, Lembeck F** 1997 Putative roles of bradykinin and the kinin system in pancreatitis: In: Farmer SG (ed) The Kinin System. Academic Press, London, vol 12:197–217
170. **Matsumara Y, Kimura M, Yamamoto T, Maeda H** 1988 Involvement of the kinin generating cascade in enhanced vascular permeability in tumour tissue. Jpn J Cancer Res 79:1327–1334
171. **Roberts RA, Gullick WJ** 1989 Bradykinin receptor number and sensitivity to ligand stimulation of mitogenesis by expression of mutant *ras* oncogene. J Cell Sci 94:527–535
172. **Berg T, Johansen L, Bergundhaugen H, Hansen LJ, Reddy JK, Poulsen K** 1985 Demonstration of kallikrein in a rat pancreatic acinar cell carcinoma. Cancer Res 45:226–234
173. **Jones TH, Brown BL, Dobson PRM** 1989 Bradykinin stimulates phosphoinositide metabolism and prolactin secretion in rat anterior pituitary cells. J Mol Endocrinol 2:47–53
174. **Jones TH, Figueroa CD, Smith C, Cullen DR, Bhoola KD** 1990 Characterization of a tissue kallikrein in human prolactin-secreting adenomas. J Endocrinol 124:327–331
175. **Farmer SG (ed)** 1997 The Kinin System. Academic Press, London
176. **Oesterling JE (ed)** 1997 Prostate-specific antigen. The best prostatic tumor marker. Urol Clin North Am 24:247–458
177. **Qiu SD, Young CY, Billhartz DL, Prescott JL, Farrow GM, He WW, Tindall DJ** 1990 In situ hybridization of prostate-specific antigen RNA in human prostate. J Urol 144:1550–1556
178. **Hakalahti L, Vihko P, Henttu P, Autio-Harmainen H, Soini Y, Vihko R** 1993 Evaluation of PAP and PSA gene expression in prostatic hyperplasia and prostatic carcinoma using Northern-blot analyses, *in situ* hybridization and immunohistochemical stainings with monoclonal and bispecific antibodies. Int J Cancer 55:590–597
179. **Gallee MP, Visser-de Jong E, van der Korput JA, van der Kwast TH, Kate FJ, Schroeder FH, Trapman J** 1990 Variation of prostate-specific antigen expression in different tumour growth patterns present in prostatectomy specimens. Urol Res 18:181–187
180. **Pretlow TG, Pretlow TP, Yang P, Kaetzel CS, Delmoro CM, Kamis S, Bodner DR, Kursh E, Resnick MI, Bradley Jr EL** 1991 Tissue concentrations of prostate specific antigen in prostatic carcinoma and benign prostatic hyperplasia. Int J Cancer 49:645–649
181. **Magklara A, Scorilas A, Stephan C, Kristiansen GO, Hauptmann S, Jung K, Diamandis EP** 2000 Decreased concentrations of prostate-specific antigen and human glandular kallikrein 2 in malignant *vs.* nonmalignant prostatic tissue. Urology 56:527–532
182. **Abrahamsson PA, Lilja H, Falkmer S, Wadstrom LB** 1988 Immunohistochemical distribution of the three predominant secretory proteins in the parenchyma of hyperplastic and neoplastic prostate glands. Prostate 12:39–46
183. **Stege R, Grande M, Carlstrom K, Tribukait B, Pousette A** 2000 Prognostic significance of tissue prostate-specific antigen in endocrine-treated prostate carcinomas. Clin Cancer Res 6:160–165
184. **Yu H, Giai M, Diamandis EP, Katsaros D, Sutherland DJA, Levesque MA, Roagna R, Ponzone R, Sismondi P** 1995 Prostate specific antigen is a new favourable prognostic indicator for women with breast cancer. Cancer Res 55:2104–2110
185. **Yu H, Levesque MS, Clark GM, Diamandis EP** 1998 Prognostic value of prostate-specific antigen for women with breast cancer. A large U.S. cohort study. Clin Cancer Res 4:1489–1497
186. **Yu H, Diamandis EP, Levesque M, Giai M, Roagna R, Ponzone R, Sismondi P, Monne M, Croce C** 1996 Prostate specific antigen in breast cancer, benign breast disease and normal breast tissue. Breast Cancer Res Treat 40:171–178
187. **Sauter ER, Daly M, Linahan K, Ehya H, Engstrom PF, Bonney G, Ross EA, Yu H, Diamandis E** 1996 Prostate specific antigen levels in nipple aspirate fluid correlate with breast cancer risk. Cancer Epidemiol Biomarkers Prevent 5:967–970
188. **Balbay MD, Juang P, Ilansa N, Williams S, McConkey D, Fidler IJ, Pettaway CA** 1999 Stable transfection of human prostate cancer cell line PC-3 with prostate specific antigen induces apoptosis both *in-vivo* and *in-vitro*. Proc Am Assoc Cancer Res 40:225–226 (Abstract)
189. **Lai LC, Erbas H, Lennard TWJ, Peaston RT** 1996 Prostate-specific antigen in breast cyst fluid: possible role of prostate-specific antigen in hormone-dependent breast cancer. Int J Cancer 66:743–746
190. **Fortier AH, Nelson BJ, Grella DK, Holaday JW** 1999 Antiangiogenic activity of prostate-specific antigen. J Natl Cancer Inst 91: 1635–1640
191. **Heidtmann HH, Nettelbeck DM, Mingels A, Jager R, Welker HG, Kontermann RE** 1999 Generation of angiostatin-like fragments from plasminogen by prostate-specific antigen. Br J Cancer 81: 1269–1273
192. **Iwamura M, Hellman J, Crockett ATK, Lilha H, Gershagen S** 1996 Alteration of the hormonal bioactivity of parathyroid hormone-related protein (PTHrP) as a result of limited proteolysis by prostate-specific antigen. Urology 48:317–325
193. **Cramer SD, Chen Z, Peehl DM** 1996 Prostate specific antigen

cleaves parathyroid hormone-related protein in the PTH-like domain: inactivation of PTHrP-stimulated cAMP accumulation in mouse osteoblasts. J Urol 156:526–531
194. **Diamandis EP** 2000 Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem 46:896–900
195. **Foekens JA, Diamandis EP, Yu H, Look MP, Meijer-van Gelder ME, van Putten WLJ, Klijn JGM** 1999 Expression of prostate-specific antigen (PSA) correlates with poor response to tamoxifen therapy in recurrent breast cancer. Br J Cancer 79:888–894
196. **Diamandis EP, Helle SJ, Yu H, Melegos DN, Lundgren S, Lonning PE** 1999 Prognostic value of plasma prostate specific antigen after megestrol acetate treatment in patients with metastatic breast carcinoma. Cancer 85:891–898
197. **Cohen P, Graves HCB, Peehl DM, Kamarei M, Guidice LC, Rosenfeld RD** 1991 Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. J Clin Endocrinol Metab 73:401–407
198. **Pollak M, Beamer W, Zhang JC** 1998 Insulin-like growth factors and prostate cancer. Cancer Metastasis Rev 17:383–390
199. **Sutkowski DM, Goode RL, Baniel J, Teater C, Cohen P, McNulty AM, Hsiung HM, Becker GW, Neubauer BL** 1999 Growth regulation of prostatic stromal cells by prostate-specific antigen. J Natl Cancer Inst 91:1663–1669
200. **Killian CS, Corral DA, Kawinski E, Constantine RI** 1993 Mitogenic response of osteoblast cells to prostate-specific antigen suggests an activation of latent TGF-β and a proteolytic modulation of cell adhesion receptor. Biochem Biophys Res Commun 192:940–947
201. **Webber MM, Waghray A, Bello D** 1995 Prostate-specific antigen, a serine protease, facilitates human prostate cancer cells invasion. Clin Cancer Res 1:1089–1094
202. **Heese A, Becker C, Noldus J, Graefen M, Huland E, Huland H, Lilja H** 2000 Human glandular kallikreins 2: a potential serum marker for predicting the organ confined *vs.* non-organ confined growth of prostate cancer. J Urol 163:1491–1497
203. **Darson MF, Pacelli A, Roche Rittenhouse HG, Wolfert RL, Young CY, Klee GG, Tindall DJ, Bostwick DG** 1997 Human glandular kallikrein 2 (hK2) expression in prostatic intraepithelial neoplasia and adenocarcinoma: a novel prostate cancer marker. Urology 49:857–862
204. **Darson MF, Pacelli A, Roche Rittenhouse HG, Wolfert RL, Saeid MS, Young CY, Klee GG, Tindall DJ, Bostwick DG** 1999 Human glandular kallikrein 2 expression in prostate adenocarcinoma and lymph node metastases. Urology 53:939–944
205. **Obiezu CV, Scorilas A, Kim H, Diamandis EP** 2000 Prostase/KLK-L1 is differentially expressed in normal/benign *vs.* malignant ovarian tissues. Clin Biochem 33:234 (Abstract)
206. **Kim H, Scorilas A, Katsaros D, Yousef GM, Fracchioli S, Diamandis EP** 2001 Human kallikrein gene 5 (KLK5) expression is an indicator of poor prognosis in ovarian cancer. Br J Cancer 84:643–650
207. **Haas C, De Strooper B** 1999 The presenilins in Alzheimer's disease –proteolysis holds the key. Science 286:916–919
208. **Sondell B, Dyberg P, Anneroth GK, Ostman PO, Egelrud T** 1996 Association between expression of stratum corneum chymotryptic enzyme and pathological keratinization in human oral mucosa. Acta Derm Venereol 76:177–181
209. **Okabe A, Momota Y, Yoshida S, Hirata A, Ito J, Nishino H, Shiosaka S** 1996 Kindling induces neuropsin mRNA in the mouse brain. Brain Res 728:116–120
210. **Momota Y, Yoshida S, Ito J, Shibata M, Kato K, Sakurai K, Matsumoto K, Shiosaka S** 1998 Blockage of neuropsin, a serine protease, ameliorates kindling epilepsy. Eur J Neurosci 10:760–764
211. **Kishi T, Kato M, Shimizu T, Kato K, Matsumoto K, Yoshida S, Shiosaka S, Hakoshima T** 1999 Crystal structure of neuropsin, a hippocampal protease involved in kindling epileptogenesis. J Biol Chem 274:4220–4224
212. **Yoshida S, Shiosaka S** 1999 Plasticity-related serine proteases in the brain. Int J Mol Med 3:405–409
213. **Mitsui S, Tsuruoka N, Yamashiro K, Nakazato H, Yamaguchi N** 1999 A novel form of human neuropsin, a brain-related serine protease, is generated by alternative splicing a is expressed preferentially in human adult brain. Eur J Biochem 260:627–634
214. **Scarisbrick IA, Towner MD, Isackson PJ** 1997 Nervous system-specific expression of a novel serine protease: regulation in the adult rat spinal cord by excitotoxic injury. J Neurosci 17:8156–8168
215. **Akita H, Matsuyama T, Iso H, Sugita M, Yoshida S** 1997 Effects of exidative stress on the expression of limbic-specific protease neuropsin and avoidance learning in mice. Brain Res 769:86–96
216. **Luo LY, Diamandis EP** 2000 Down-regulation of the normal epithelial cell-specific 1 (NES1) gene is associated with unfavourable outcome of prostate cancer. Clin Biochem 33:237 (Abstract)
217. **Schachter M** 1980 Kallikreins (kininogenases): a group of serine proteases with bioregulatory actions. Pharmacol Rev 31:1–17
218. **Bhoola KD** 1971 Comment on the conversion of pro-insulin to insulin. In: Heller G, Lederis K (eds) Subcellular Organisation and Function in Endocrine Tissues. Cambridge University Press, Cambridge, UK, pp 493–494
219. **Bothwell MA, Wilson WH, Shooter EM** 1979 The relationship between glandular kallikrein and growth factor-processing proteases of mouse submaxillary gland. J Biol Chem 254:7287–7294
220. **Mason AJ, Evans BA, Cox DR, Shine J, Richards RL** 1983 Structure of mouse kallikrein gene family suggests a role in specific processing of biological active peptides. Nature 303:300–307
221. **Kumar A, Goel AS, Hill TM Mikolajczyk SD, Millar LS, Kuus-Reichel K, Saedi MS** 1996 Expression of human glandular kallikrein, hK2, in mammalian cells. Cancer Res 56:5397–5402
222. **Saedi MS, Cass MM, Goel AS, Granger L, Hogen KL, Okaneya T, Griffin BY, Klee GG, Young CY, Tindall DJ** 1995 Overexpression of a human prostate-specific glandular kallikrein, hK2, in *E. coli* and generation of antibodies. Mol Cell Endocrinol 109:237–241
223. **Lovgren J, Piironen T, Overmo C, Dowell B, Karp M, Pettersson K, Lilja H, Lundwall A** 1995 Production of recombinant PSA and hK2 and analysis of their immunologic cross-reactivity. Biochem Biophys Res Commun 213:888–895
224. **Herrala A, Kurkela R, Porvari K, Isomaki R, Henttu P, Vihko P** 1997 Human prostate-specific glandular kallikrein is expressed as an active and an inactive protein. Clin Chem 43:279–284
225. **Mikolajczyk SD, Millar LS, Marker KM, Grauer LS, Goel A, Cass MM, Kumar A, Saedi MS** 1997 Ala217 is important for the catalytic function and autoactivation of prostate-specific human kallikrein 2. Eur J Biochem 246:440–446
226. **Bourgeois L, Brillard-Bourdet M, Deperthes D, Juliano MA, Juliano L, Tremblay RR, Dube JY, Gauthier F** 1997 Serpin-derived peptide substrates for investigating the substrate specificity of human tissue kallikreins hK1 and hK2. J Biol Chem 272:29590–29595
227. **Deperthes D, Frenette G, Brillard-Bourdet M, Bourgeois L, Gauthier F, Tremblay RR, Dube JY** 1996 Potential involvement of kallikrein hK2 in the hydrolysis of the human seminal vesicle proteins after ejaculation. J Androl 17:659–665
228. **Dube JY, Tremblay RR** 1997 Biochemistry and potential roles of prostatic kallikrein hK2. Mol Urol 1:279–285
229. **Frenette G, Tremblay RR, Lazure C, Dube JY** 1997 Prostatic kallikrein hK2, but not prostate-specific antigen (hK3), activates single-chain urokinase-type plasminogen activator. Int J Cancer 71:897–899
230. **Watt KW, Lee PJ, M'Timkulu T, Chan WP, Loor R** 1986 Human prostate-specific antigen: structural and functional similarity with serine proteases. Proc Natl Acad Sci USA 83:3166–3170
231. **Ban Y, Wang MC, Watt KW, Loor R, Chu TM** 1984 The proteolytic activity of human prostate-specific antigen. Biochem Biophys Res Commun 123:482–488
232. **Christensson A, Laurell CB, Lilja H** 1990 Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. Eur J Biochem 194:755–763
233. **Akiyama K, Nakamura T, Iwanaga S, Hara M** 1987 The chymotrypsin-like activity of human prostate-specific antigen, γ-seminoprotein. FEBS Lett 225:168–172
234. **Lilja H** 1985 A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. J Clin Invest 76: 1899–1903
235. **Foretova L, Garber JE, Sadowski NL, Verselis SJ, Li FP** 1996 Prostate-specific antigen in nipple aspirate. Lancet 347:1631
236. **Diamandis EP, Yu H, Lopez-Otin C** 1996 Prostate specific antigen—a new constituent of breast cyst fluid. Breast Cancer Res Treat 38:259–264
237. **Mannello F, Bocchiotti G, Bianchi G, Marcheggiani F, Gazzanelli**

G 1996 Quantification of prostate-specific antigen immunoreactivity in human breast cyst fluids. Breast Cancer Res Treat 38:247–252
238. **Filella X, Molina R, Alcover J, Carretero P, Ballesta AM** 1996 Detection of nonprostatic PSA in serum and non-serum samples from women. Int J Cancer 68:424–427
239. **Filella X, Molina R, Alcover J, Menendez V, Gimenez N, Jo J, Carretero P, Ballesta AM** 1996 Prostate-specific antigen detection by ultrasensitive assay in samples from women. Prostate 29: 311–316
240. **Borchert G, Yu H, Tomlinson G, Giai M, Roagna R, Ponzone R, Sgro L, Diamandis EP** 1999 Prostate specific antigen molecular forms in breast cyst fluid and serum of women with fibrocystic breast disease. J Clin Lab Anal 13:75–81
241. **Yu H, Diamandis EP** 1995 Prostate specific antigen in milk of lactating women. Clin Chem 41:54–58
242. **Yu H, Diamandis EP** 1995 Prostate specific antigen immunoreactivity in amniotic fluid. Clin Chem 41:204–210
243. **Melegos DN, Yu H, Allen LC, Diamandis EP** 1996 Prostate specific antigen in amniotic fluid of normal and abnormal pregnancies. Clin Biochem 29:555–562
244. **Woodhouse EC, Chuaqui RF, Liotta LA** 1997 General mechanisms of metastasis. Cancer [Suppl] 80:1529–1537
245. **Chambers AF, Matrisian LM** 1997 Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 80: 1260–1270
246. **Harvey TJ, Hooper JD, Myers SA, Stephenson SA, Ashworth LK, Clements JA** 2000 Tissue-specific expression patterns and fine mapping of the human kallikrein (KLK) locus on proximal 19q13.4. J Biol Chem 275:37397–37406
247. **Diamandis EP, Yousef GM, Soosaipillai AR, Grass L, Porter A, Little S, Sotiropoulou G** 2000 Immunofluorometric assay of the human kallikrein 6 and preliminary clinical applications. Clin Biochem 33:369–375
248. **Luo LY, Grass L, Howarth DJ, Thibault P, Ong H, Diamandis EP** 2001 Immunofluorometric assay of human kallikrein 10 and its identification in biological fluids and tissues. Clin Chem 47:237–246
249. **Diamandis EP, Yousef GM, Petraki C, Soosaipillai A** 2000 Human kallikrein 6 as a biomarker of Alzheimer's disease. Clin Biochem 33:663–667
250. **Diamandis EP, Yousef GM, Soosaipillai A, Bunting P** 2000 Human kallikrein 6 (zyme/protease M neurosin): a new biomarker of ovarian carcinoma. Clin Biochem 33:369–375
251. **Ying LY, Bunting P, Scorilas A, Diamandis EP,** Human kallikrein 10: a novel tumor marker for ovarian carcinoma? Clin Chim Acta, in press