Review

TumorBiology

Tumor Biol 2002;23:185–192

Received: April 12, 2002
Accepted after revision: May 1, 2002

Expanded Human Tissue Kallikrein Family – A Novel Panel of Cancer Biomarkers

George M. Yousef Eleftherios P. Diamandis

Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada

Key Words
Kallikrein · Tumor markers · Prostate cancer · Breast cancer · Ovarian cancer · Testicular cancer · Cancer prognosis · Prostate-specific antigen

Abstract
The full characterization of the human kallikrein gene locus has allowed identification of all members of this gene family on chromosome 19q13.4 and the establishment of common structural criteria, at both the mRNA and protein level. The human kallikrein gene family now consists of 15 members; their mRNA and protein structure, tissue expression and hormonal regulation patterns have been delineated. In addition to prostate-specific antigen (PSA, hK3), which is an established tumor marker for prostate cancer diagnosis and follow-up, and human glandular kallikrein (hK2), an emerging prostate cancer biomarker, accumulating evidence indicates that many other members of the human kallikrein gene family are also implicated in endocrine-related malignancies. Many kallikreins are differentially regulated in breast, prostate, ovarian and testicular cancers. In addition, preliminary reports indicate that three newly identified kallikreins (hK6, hK10 and hK11) are serum biomarkers for diagnosis and monitoring of ovarian and prostate cancer. The mechanism by which kallikreins might be involved in the pathogenesis and/or progression of cancer is not as yet fully understood. Preliminary reports indicate a possible role of kallikreins in controlling vital processes, like apoptosis, angiogenesis and tumor metastasis by cleavage of critical substrates such as growth factors, hormones or extracellular matrix. In this review, we present data on the differential expression of kallikreins in cancer at both the mRNA and protein levels, and propose future directions of research towards our understanding of the involvement of kallikreins in cancer and their possible diagnostic, prognostic and therapeutic applications.

Definitions

The term ‘kallikrein’ was introduced in the 1930s to describe proteolytic enzymes which can release small vasoactive peptides from high-molecular-weight precursors. There are two categories of kallikrein enzymes: plasma and tissue kallikreins. Plasma kallikrein is encoded by a single gene on chromosome 4 [1]. This enzyme (a serine protease) releases the vasoactive peptide bradykinin from a high-molecular-weight precursor synthesized in the liv-

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E.P. Diamandis, MD, PhD, FRCPC
Department of Pathology and Laboratory Medicine
Mount Sinai Hospital, 600 University Avenue
Toronto, Ont. M5G 1X5 (Canada)
E-Mail ediamandis@mtsinai.on.ca

er. The tissue kallikreins are a family of genes localized on chromosome 19q13.4 and also encode for serine protease enzymes.

According to the original definition of kallikreins, which is based on their kininogenase activity, only one of the tissue kallikreins, pancreatic/renal kallikrein, fulfills this criterion. Until a few years ago, two other enzymes, the human glandular kallikrein 2 and human kallikrein 3 (prostate-specific antigen, PSA), were also classified as members of the human tissue kallikrein gene family, based on significant sequence homologies and proximity of gene location. More recently, other genes encoding for similar enzymes are also classified as members of the human kallikrein gene family. This classification is not based on the functional definition but rather on other criteria, including structural similarities and tight mapping of all genes on chromosome 19q13.4. Based on the newer definition, the number of genes that are included in the human tissue kallikrein gene family has now been increased to fifteen.

The expansion of the human tissue kallikrein gene family and the identification of new genes has led to the conclusion that the human gene family, originally thought to be much smaller than similar families found in rodents, is now as large as the families found in rat and mouse.

Human Tissue Kallikrein Gene Family

A schematic diagram of the human tissue kallikrein gene locus on chromosome 19q13.4 is shown in figure 1. All known kallikrein genes map within a 300-kb region and the lengths of the genes, the distances between them as well as the direction of transcription have now been accurately defined [2]. Telomerically from the last kallikrein gene identified, KLK14 [3] starts another family of

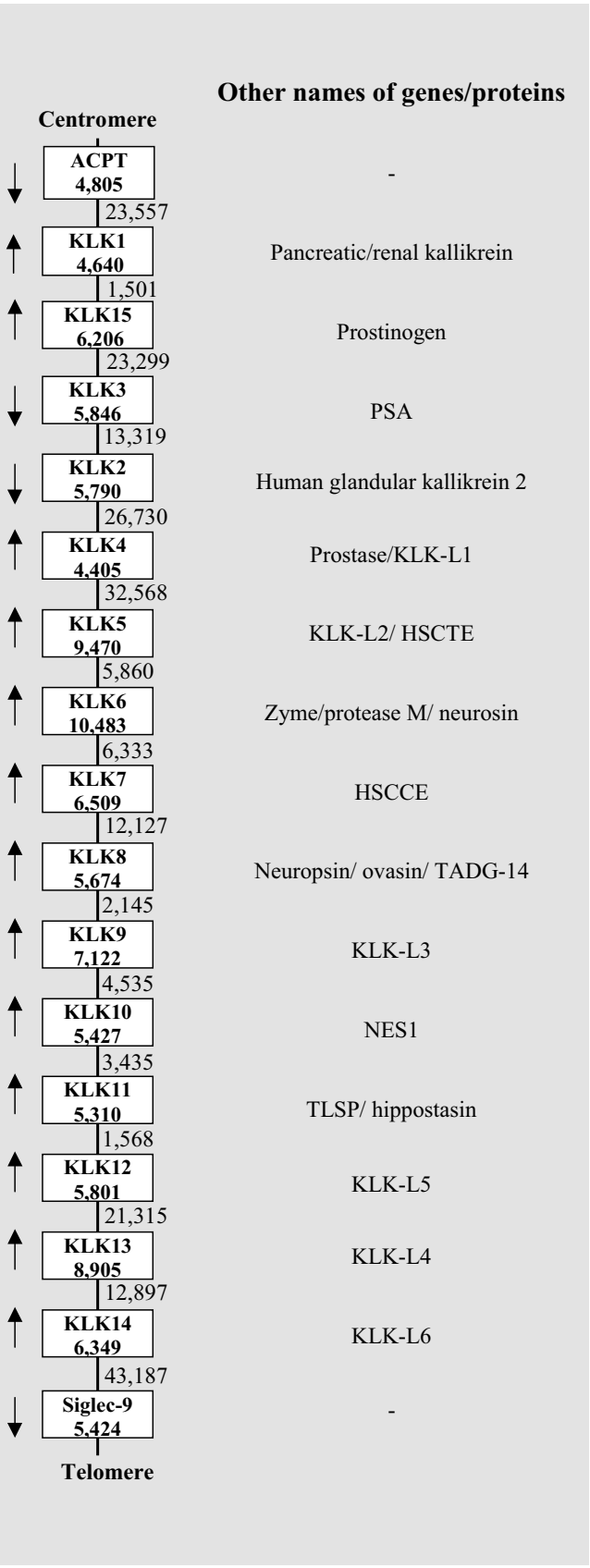

Fig. 1. An approximate 300-kb region of contiguous genomic sequence around chromosome 19q13.4. The direction of transcription of each gene is illustrated by arrows. Boxes represent genes and contain the gene names and their genomic length, in base pairs (bp). Other commonly used names for these genes are also mentioned. Distances between genes in bp are shown between boxes. Figure is not drawn to scale. HSCTE = Human stratum corneum tryptic enzyme; HSCCE = human stratum corneum chymotryptic enzyme; TADG-14 = tumor-associated differentially expressed gene-14; NES1 = normal epithelial cell-specific 1; TLSP = trypsin-like serine protease.

genes, the Siglec gene family [4, 5]. This finding suggests that this area defines the end of the kallikrein gene family and the beginning of the Siglec family of genes. Centromerically from the KLK1 gene, a novel gene was cloned, named 'testicular acid phosphatase' (ACPT) which is not a kallikrein [6] and denotes the end of the kallikrein gene family from this side. Thus, it appears that between these two nonkallikrein genes (ACPT and Siglec9), there are fifteen kallikrein genes which are tandemly aligned, as shown in figure 1. No other genes map within this locus, which represents the largest cluster of serine proteases within the whole human genome.

The genomic organization of each one of these kallikrein genes is very similar. All genes share significant sequence homologies at both the DNA and amino acid level and many of them are regulated by steroid hormones. For more information about similarities between human kallikreins, see recent reviews [7, 8]. In order to simplify communication, an international group of scientists working in the field has established uniform nomenclature for the kallikrein genes and the encoded proteins [9].

Despite their structural similarities, the tissue expression patterns of these genes are different. Some genes are expressed in very few tissues, while others are abundantly expressed. Detailed tissue expression data can be found elsewhere [3, 10–14]. An interesting observation is the coexpression of groups of kallikreins in the same tissue, reviewed in detail elsewhere [7, 8].

Kallikreins and Cancer

Kallikreins are mostly known as cancer biomarkers. PSA (hK3), human glandular kallikrein (hK2) and prostase (hK4) are tandemly localized and they are highly expressed in the prostate. This restricted tissue expression, and their secretion into biological fluids, makes them ideal markers for prostatic diseases. A more detailed discussion on hK2 and hK3 as cancer biomarkers can be found elsewhere [15]. In addition to hK3 being the premier marker for prostate cancer diagnosis and monitoring, recent reports suggest some usefulness of hK3 as a prognostic and diagnostic marker for breast cancer [16, 17].

With the full identification and characterization of all members of the kallikrein gene family, accumulating reports suggest that other kallikreins might also be related to hormonal malignancies (for instance, breast, prostate, testicular and ovarian cancers). KLK6 (zyme/protease M) was isolated by differential display from an ovarian cancer cDNA library [18], and KLK10 (NES1) was cloned by subtractive hybridization from a breast cancer cDNA library [19]. This gene has later shown to act as a tumor suppressor [20]. Underwood et al. [21] and Magklara et al. [22] have shown that KLK8 (also known as neuropsin, ovasin or TADG14) is differentially expressed in ovarian cancer. KLK7 is upregulated in ovarian cancer patients [23], and KLK4 and KLK5 are indicators of poor prognosis of ovarian cancer [24–26]. More recently, KLK9 has been shown to be a marker of favorable ovarian cancer prognosis [10].

At the protein level, recent reports indicate that kallikreins can be useful serum biomarkers for diagnosis, monitoring and prognosis of cancer. In addition to hK3 and hK2, hK6 and hK10 are emerging diagnostic markers for ovarian cancer [27–30]. More recently, hK11 has also been shown to be a potential marker for ovarian and prostate cancer [31]. A synthetic hK1 inhibitor has recently been found to suppress cancer cell invasiveness in human breast cancer cell lines [32].

Added to the above evidence are the findings that most of the known kallikreins are under sex steroid hormone regulation in cancer cell lines [11–13, 33–36]. In tables 1–4, we summarize published data on the expression of kallikrein genes and proteins in tumor tissue extracts and serum of cancer patients for the purpose of disease diagnosis, monitoring, prognosis or subclassification. It is clear from these data that at least a few kallikreins have already found important clinical applications, while some others show promise. The availability of sensitive analytical methods for the remaining kallikreins will allow for their examination as candidate cancer biomarkers.

It is possible that some kallikreins may become valuable therapeutic targets when the biological pathways that are involved are delineated. For example, the enzymatic activity of these serine proteases may initiate biological events (e.g. tumor invasion, activation of hormones, growth factors, other enzymes, receptors or cytokines, amyloid formation) or terminate biological events (e.g. inhibition of angiogenesis, inactivation of growth factors, hormones, enzymes, cytokines or receptors). Once known, these events could be manipulated, for therapeutic purposes, by enzyme inhibitors or activators. These are simply possibilities since the function of most kallikrein enzymes is not known at present.

Table 1. Clinical applications of kallikrein genes and proteins as ovarian cancer biomarkers

Gene/ protein name	Sample used	Method	Application	References
KLK4	mRNA from ovarian cancer tissues	RT-PCR	prognosis; overexpression in more aggressive cancers (late stage; higher grade; shorter disease-free and overall survival)	24
	mRNA from ovarian cancer tissues	SQ RT-PCR	prognosis; overexpression in late stage cancer	26
KLK5	mRNA from ovarian cancer tissues	RT-PCR	prognosis; over-expression in more aggressive cancers (late stage; higher grade; shorter disease-free and overall survival)	25
KLK6	mRNA from ovarian cancer cell lines and tissues	Northern blot	higher level of message in some cell lines and tissues; no application specified	18
	serum	immunoassay	diagnosis and monitoring of ovarian cancer	27
	ovarian cancer cytosols	immunoassay	prognosis; higher levels associated with late stage and decreased disease-free and overall patient survival	our unpublished data
KLK7	mRNA from ovarian cancer tissues	SQ RT-PCR	prognosis; overexpression in ovarian cancer	47
	ovarian cancer mRNA and extracts	RT-PCR; Northern blots; Western blots; immunohistochemistry	overexpression of mRNA and protein in the majority of ovarian tumors	23
KLK8	mRNA from ovarian cancer tissues	RT-PCR	prognosis; higher expression is associated with lower grade and improved patient survival	22
	mRNA from ovarian cancer tissues	Northern blot	prognosis; high expression in ovarian cancer	21
KLK9	mRNA from ovarian cancer tissues	Q RT-PCR	prognosis; higher expression is associated with early stages and optimal debulking	10
KLK10	serum	immunoassay	diagnosis and monitoring of ovarian cancer	30
	ovarian cancer cytosolic extracts	immunoassay; immunohistochemistry	prognosis; high levels are associated with late stage disease and decreased disease-free and overall patient survival	29
KLK11	serum of ovarian cancer patients	immunoassay	diagnosis and prognosis; elevated in 70% of ovarian cancer patients	31
KLK14	mRNA from ovarian cancer tissues	Q RT-PCR	prognostic, higher expression associated with improved survival	our unpublished data
KLK15	mRNA from ovarian cancer tissues	Q RT-PCR	prognosis; higher expression associated with reduced survival	our unpublished data

RT-PCR = Reverse transcriptase-polymerase chain reaction; SQ RT-PCR = semi-quantitative RT-PCR; Q RT-PCR = quantitative RT-PCR.

Table 2. Clinical applications of kallikrein genes and proteins as breast cancer biomarkers

Gene/ protein name	Sample used	Method	Application	References
hK3	tissue extracts; serum	immunoassay	prognostic, overexpression associated with better prognosis	16, 17
KLK5	mRNA from breast cancer tissues	Q RT-PCR	prognosis; overexpression associated with reduced survival	48
KLK6	breast cancer cytosols	immunoassay	prognosis; association with hormone receptors	56
KLK10	mRNA and extracts from breast cancer tissue and cell lines	Northern blots, Western blots	downregulation in cancer cell lines	20, 49
	breast cancer cytosols	immunoassay	prognosis (negatively associated with estrogen and progesterone receptors)	55
KLK12	mRNA from breast cancer tissues	RT-PCR	downregulation in a subset of breast tumors	36
KLK13	mRNA from breast cancer tissues	RT-PCR	downregulation in a subset of breast tumors	12
	Q RT-PCR	prognosis; overexpression associated with better prognosis		50
KLK14	mRNA from breast cancer tissues	RT-PCR	downregulation in a subset of breast tumors	3
KLK15	mRNA from breast cancer tissues	Q RT-PCR	prognosis; overexpression associated with favorable prognosis	our unpublished data

RT-PCR = Reverse transcriptase-polymerase chain reaction; Q RT-PCR = quantitative reverse transcriptase-polymerase chain reaction.

Table 3. Clinical applications of kallikrein genes and proteins as prostate cancer biomarkers

Gene/ protein name	Sample used	Method	Application	References
KLK2	serum of prostate cancer patients	immunoassay	adjuvant tumor marker for prostate cancer diagnosis	15, 51
KLK3	serum of prostate cancer patients	immunoassay	established tumor marker; diagnosis, prognosis and follow-up	15, 52
KLK5	mRNA from matched pairs of normal and cancerous prostate tissues	Q RT-PCR	prognosis; lower expression associated with more aggressive tumors	53
KLK11	serum of prostate cancer patients	immunoassay	diagnosis/prognosis; elevated in 60% of prostate cancer patients	31
KLK15	mRNA from matched pairs of normal and cancerous prostate tissues	Q RT-PCR	prognosis; overexpression associated with unfavorable prognosis	11

Q RT-PCR = Quantitative reverse transcriptase-polymerase chain reaction.

Table 4. Clinical applications of kallikrein genes and proteins as testicular cancer biomarkers

Gene/ protein name	Sample used	Method	Application	References
KLK5	mRNA from matched pairs of normal and cancerous testicular tissues	Q RT-PCR	prognosis; down-regulation in advanced cancer	54
KLK10	testicular tissue extracts	RT-PCR; immunohistochemistry	prognosis; downregulation in cancer in comparison to normal tissues	54
KLK14	mRNA from matched pairs of normal and cancerous testicular tissues	RT-PCR	downregulation in cancerous tissues	3

RT-PCR = Reverse transcriptase-polymerase chain reaction; Q RT-PCR = quantitative RT-PCR.

Pathobiology of Kallikreins in Cancer

Several hypotheses can be proposed regarding the possible mechanism(s) by which kallikreins are associated with the pathogenesis of endocrine-related malignancies. Proteolytic enzymes are thought to be involved in tumor progression because of their role in extracellular matrix degradation. Many studies have shown that a variety of proteolytic enzymes are overproduced either by the cancer cells themselves or by the surrounding stromal cells and that this overexpression is associated with unfavorable clinical prognosis [37].

Breast, prostate, testicular and ovarian cancers are all known steroid hormone-dependent malignancies. Sex hormones affect the initiation, and/or progression of these malignancies. On the other hand, most kallikreins are under sex steroid hormonal regulation. Taken together, these data suggest that kallikreins may represent downstream targets by which hormones affect the initiation or progression of these tumors.

It has been shown that hK2 and hK4 can activate the pro-form of another serine protease, the urokinase-type plasminogen activator (uPA) [38, 39]. Urokinase activates plasmin (another serine protease) from its inactive form (plasminogen) which is ubiquitously located in the extracellular space, leading to degradation of extracellular matrix proteins. This might suggest a role of kallikreins in cancer progression and could explain the differential expression of several kallikreins in tumors. Plasminogen can also activate the precursor forms of collagenases, thus promoting the degeneration of collagen in the basement membrane surrounding the capillaries and lymph nodes. Another kallikrein, hK7, can degrade the alpha chain of native human fibrinogen [40] and it is also hypothesized that it is involved in an apoptotic-like mechanism that leads to desquamation of the skin [40]. The involvement in growth/apoptotic activities is reported for hK3, which can digest insulin-like growth factor-binding protein IGFBP-3 [41], and also inactivate the amino terminal fragment of the parathyroid hormone-related protein (PTHrP) by cleavage [42]. Similar findings were observed for some rodent kallikreins [43]. Also, hK3 was reported to have antiangiogenic activities [44]. Given the parallel expression of kallikreins in the same malignancy (table 1–4), it is possible to hypothesize that kallikreins are involved in a cascade enzymatic pathway which drives tumor progression.

Another possible mechanism by which kallikreins are involved in malignancy is the activation of the proteinase-activated receptors (PAR). PAR is a novel family of G-protein-coupled receptors which is activated by cleavage of their N-termini by a serine protease rather than by ligand-receptor occupancy [45]. Activation of these receptors elicits different responses in several tissues. In addition, they switch on cell-signaling pathways, e.g. the MAP-kinase pathway, leading to cell growth and division.

Bhoola et al. [46] have recently provided strong evidence indicating the presence of hK1 activity in the chemotactically attracted inflammatory cells of esophageal and renal cancers, suggesting a role for kallikreins in the body reaction towards the malignant process.

The elevation of these serum concentration of kallikreins in cancer is likely due to the increased vasculature (angiogenesis) of cancerous tissues, the destruction of the glandular architecture of the tissues involved and the subsequent leakage of these proteins into the general circula-

tion. It is also likely that the serum concentration of kallikreins may be increased due to gene overexpression, a mechanism that was shown to be true for several kallikreins (for details, see table 1).

Future Directions

Very few cancer biomarkers have had a major impact in clinical practice; a notable exception is the PSA (hK3). One of the reasons for this low impact is that most of the current cancer biomarkers are not tissue or cancer specific; they are elevated in benign as well as in malignant diseases of many organs and they lack sensitivity and specificity for early disease diagnosis. For monitoring, the time window from biochemical to clinical relapse is usually short (less than 1 year) and therapeutic approaches for treating relapsing disease are not generally very successful. It is clear that we need more specific and sensitive cancer biomarkers for early detection and more efficient monitoring of cancer.

It is anticipated, with the completion of the human genome project that many new analytes will be examined as disease biomarkers. In our opinion, not a single biomarker will be effective for any disease (with some notable exceptions). It is conceivable that a combination of a carefully selected panel of biomarkers will offer the required sensitivity and specificity. The advent of new, miniaturized, multiparametric testing (e.g. microarray technology) will likely facilitate the introduction of multiple tests for each disease, and bioinformatic approaches (e.g. neural networks, pattern recognition and logistic regression) will bring about the required sensitivity and specificity.

The human kallikrein gene family has already contributed the best-known cancer biomarker (PSA, hK3). Another new biomarker, human glandular kallikrein 2 (hK2), has been examined and it shows promise of being a complementary test. The new information presented here pinpoints to the fact that a few other members of this gene family may have applicability as diagnostic, prognostic and predictive indicators in various cancers. The availability of reliable analytical methodologies for all members of the kallikrein gene family will facilitate further research. It is conceivable, that the kallikrein chip (multiparametric testing of all kallikreins simultaneously) as well as their combination with other biomarkers may bring about a powerful diagnostic and prognostic multiparametric procedure.

The challenge over the next 5–10 years is to identify the biological function of these enzymes, their physiological substrates and the connection of overexpression, underexpression and mutation of these genes with the pathogenesis of various human diseases, including cancer.

References

1 Beaubien G, Rosinski-Chupin I, Mattei MG, Mbikay M, Chretien M, Seidah NG: Gene structure and chromosomal localization of plasma kallikrein. Biochemistry 1991;30: 1628–1635.
2 Yousef GM, Chang A, Scorilas A, Diamandis EP: Genomic organization of the human kallikrein gene family on chromosome 19q13.3–q13.4. Biochem Biophys Res Commun 2000; 276:125–133.
3 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 2001;61:3425–3431.
4 Foussias G, Yousef GM, Diamandis EP: Identification and molecular characterization of a novel member of the siglec family (SIGLEC9). Genomics 2000;67:171–178.
5 Yousef GM, Ordon M, Foussias G, Diamandis EP: Genomic organization of the Siglec gene locus on chromosome 19q13.4 and cloning of two new siglec pseudogenes. Gene 2002;286: 259–270.
6 Yousef GM, Diamandis M, Jung K, Diamandis EP: Molecular cloning of a novel human acid phosphatase gene (ACPT) that is highly expressed in the testis. Genomics 2001;74: 385–395.
7 Yousef GM, Diamandis EP: The new human tissue kallikrein gene family: Structure, function, and association to disease. Endocr Rev 2001;22:184–204.
8 Yousef GM, Diamandis EP: Human kallikreins: Common structural features, sequence analysis and evolution. Curr Genomics, in press.
9 Diamandis EP, Yousef GM, Clements J, et al: New nomenclature for the human tissue kallikrein gene family. Clin Chem 2000;46:1855–1858.
10 Yousef GM, Kyriakopoulou LG, Scorilas A, et al: Quantitative expression of the human kallikrein gene 9 (KLK9) in ovarian cancer: A new independent and favorable prognostic marker. Cancer Res 2001;61:7811–7818.
11 Yousef GM, Scorilas A, Jung K, Ashworth LK, Diamandis EP: Molecular cloning of the human kallikrein 15 gene (KLK15). Up-regulation in prostate cancer. J Biol Chem 2001;276: 53–61.
12 Yousef GM, Chang A, Diamandis EP: Identification and characterization of KLK-L4, a new kallikrein-like gene that appears to be down-regulated in breast cancer tissues. J Biol Chem 2000;275:11891–11898.
13 Yousef GM, Scorilas A, Magklara A, Soosaipillai A, Diamandis EP: 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 2000;254:119–128.
14 Yousef GM, Scorilas A, Diamandis EP: 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 2000;63:88–96.

15 Rittenhouse HG, Finlay JA, Mikolajczyk SD, Partin AW: Human kallikrein 2 (hK2) and prostate-specific antigen (PSA): Two closely related, but distinct, kallikreins in the prostate. Crit Rev Clin Lab Sci 1998;35:275–368.
16 Yu H, Giai M, Diamandis EP, et al: Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. Cancer Res 1995;55:2104–110.
17 Black MH, Diamandis EP: The diagnostic and prognostic utility of prostate-specific antigen for diseases of the breast. Breast Cancer Res Treat 2000;59:1–14.
18 Anisowicz A, Sotiropoulou G, Stenman G, Mok SC, Sager R: A novel protease homolog differentially expressed in breast and ovarian cancer. Mol Med 1996;2:624–636.
19 Liu XL, Wazer DE, Watanabe K, Band V: Identification of a novel serine protease-like gene, the expression of which is down-regulated during breast cancer progression. Cancer Res 1996;56:3371–3379.
20 Goyal J, Smith KM, Cowan JM, Wazer DE, Lee SW, Band V: The role for NES1 serine protease as a novel tumor suppressor. Cancer Res 1998;58:4782–4786.
21 Underwood LJ, Tanimoto H, Wang Y, Shigemasa K, Parmley TH, O'Brien TJ: Cloning of tumor-associated differentially expressed gene-14, a novel serine protease overexpressed by ovarian carcinoma. Cancer Res 1999;59:4435–4439.
22 Magklara A, Scorilas A, Katsaros D, et al: The human KLK8 (neuropsin/ovasin) gene: Identification of two novel splice variants and its prognostic value in ovarian cancer. Clin Cancer Res 2001;7:806–811.
23 Tanimoto H, Underwood LJ, Shigemasa K, et al: The stratum corneum chymotryptic enzyme that mediates shedding and desquamation of skin cells is highly overexpressed in ovarian tumor cells. Cancer 1999;86:2074–2082.
24 Obiezu CV, Scorilas A, Katsaros D, et al: Higher human kallikrein gene 4 (KLK4) expression indicates poor prognosis of ovarian cancer patients. Clin Cancer Res 2001;7:2380–6.
25 Kim H, Scorilas A, Katsaros D, et al: Human kallikrein gene 5 (KLK5) expression is an indicator of poor prognosis in ovarian cancer. Br J Cancer 2001;84:643–650.
26 Dong Y, Kaushal A, Bui L, et al: Human kallikrein 4 (KLK4) is highly expressed in serous ovarian carcinomas. Clin Cancer Res 2001;7:2363–2371.
27 Diamandis EP, Yousef GM, Soosaipillai AR, Bunting P: Human kallikrein 6 (zyme/protease M/neurosin): A new serum biomarker of ovarian carcinoma. Clin Biochem 2000;33:579–583.
28 Diamandis EP, Yousef GM, Soosaipillai AR, et al: Immunofluorometric assay of human kallikrein 6 (zyme/protease M/neurosin) and preliminary clinical applications. Clin Biochem 2000;33:369–375.
29 Luo LY, Katsaros D, Scorilas A, et al: Prognostic value of human kallikrein 10 expression in epithelial ovarian carcinoma. Clin Cancer Res 2001;7:2372–2379.
30 Luo L, Bunting P, Scorilas A, Diamandis EP: Human kallikrein 10: A novel tumor marker for ovarian carcinoma? Clin Chim Acta 2001;306:111–118.
31 Diamandis EP, Okui A, Mitsui S, et al: Human kallikrein 11: A new biomarker of prostate and ovarian carcinoma. Cancer Res 2002;62:295–300.
32 Wolf WC, Evans DM, Chao L, Chao J: A synthetic tissue kallikrein inhibitor suppresses cancer cell invasiveness. Am J Pathol 2001;159:1797–1805.
33 Nelson PS, Gan L, Ferguson C, et al: Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression. Proc Natl Acad Sci USA 1999;96:3114–3119.
34 Hooper JD, Bui LT, Rae FK, et al: Identification and characterization of KLK14, a novel kallikrein serine protease gene located on human chromosome 19q13.4 and expressed in prostate and skeletal muscle. Genomics 2001;73:117–122.
35 Yousef GM, Scorilas A, Diamandis EP: 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 2000;63:88–96.
36 Yousef GM, Magklara A, Diamandis EP: KLK12 is a novel serine protease and a new member of the human kallikrein gene family-differential expression in breast cancer. Genomics 2000;69:331–341.
37 Sappino AP, Busso N, Belin D, Vassalli JD: Increase of urokinase-type plasminogen activator gene expression in human lung and breast carcinomas. Cancer Res 1987;47:4043–4046.
38 Takayama TK, Fujikawa K, Davie EW: Characterization of the precursor of prostate-specific antigen. Activation by trypsin and by human glandular kallikrein. J Biol Chem 1997;272:21582–21588.
39 Takayama TK, McMullen BA, Nelson PS, Matsumura M, Fujikawa K: Characterization of hK4 (prostase), a prostate-specific serine protease: Activation of the precursor of prostate-specific antigen (pro-PSA) and single-chain urokinase-type plasminogen activator and degradation of prostatic acid phosphatase. Biochemistry 2001;40:15341–15348.
40 Smyth MJ: Starum corneum chymotryptic enzyme; in Barrett AJ, Rawlings NP, Woessner JF (eds): Handbook of Proteolytic Enzymes. London, Academic Press, 1998, pp 87–89.
41 Sutkowski DM, Goode RL, Baniel J, et al: Growth regulation of prostatic stromal cells by prostate-specific antigen. J Natl Cancer Inst 1999;91:1663–1669.
42 Iwamura M, Hellman J, Cockett AT, Lilja H, Gershagen S: Alteration of the hormonal bioactivity of parathyroid hormone-related protein (PTHrP) as a result of limited proteolysis by prostate-specific antigen. Urology 1996;48:317–325.
43 Matsui H, Takahashi T: Mouse testicular Leydig cells express KLK21, a tissue kallikrein that cleaves fibronectin and IGF-binding protein-3. Endocrinology 2001;142:4918–4929.
44 Fortier AH, Nelson BJ, Grella DK, Holaday JW: Antiangiogenic activity of prostate-specific antigen. J Natl Cancer 1999;91:1635–1640.
45 Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R: Proteinase-activated receptors. Pharmacol Rev 2001;53:245–282.
46 Bhoola K, Ramsaroop R, Plendl J, Cassim B, Dlamini Z, Naicker S: Kallikrein and kinin receptor expression in inflammation and cancer. Biol Chem 2001;382:77–89.
47 Tanimoto H, Underwood LJ, Shigemasa K, Parmley TH, O'Brien TJ: Increased expression of protease M in ovarian tumors. Tumour Biol 2001;22:11–18.
48 Yousef GM, Scorilas A, Kyriakopoulou LG, et al: Human kallikrein gene 5 (KLK5) expression by quantitative PCR: An independent indicator of poor prognosis in breast cancer. Clin Chem, in press.
49 Dhar S, Bhargava R, Yunes M, et al: Analysis of normal epithelial cell specific-1 (NES1)/kallikrein 10 mRNA expression by in situ hybridization, a novel marker for breast cancer. Clin Cancer Res 2001;7:3393–3398.
50 Chang A, Yousef GM, Scorilas A, et al: Human kallikrein gene 13 (KLK13) expression by quantitative RT-PCR: An independent indicator of favorable prognosis in breast cancer. Br J Cancer 2002;86:1457–1464.
51 Partin AW, Catalona WJ, Finlay JA, et al: Use of human glandular kallikrein 2 for the detection of prostate cancer: Preliminary analysis. Urology 1999;54:839–845.
52 Diamandis EP: Prostate-specific antigen – its usefulness in clinical medicine. Trends Endocrinol Metab 1998;9:310–316.
53 Yousef GM, Scorilas A, Chang A, et al: Down-regulation of the human kallikrein gene 5 (KLK5) in prostate cancer tissues. Prostate 2002;86:1457–1464.
54 Yousef GM, Obiezu C, Jung K, Stephan C, Scorilas A, Diamandis EP: Differential expression of kallikrein gene 5 (KLK5) in cancerous and normal testicular tissues. Urology, in press.
55 Luo LY, Rajpert-De Meyts ER, Jung K, Diamandis EP: Expression of the normal epithelial cell-specific 1 (NES1; KLK10) candidate tumour suppressor gene in normal and malignant testicular tissue. Br J Cancer 2001;85:220–224.
56 Luo L-Y, Diamandis EP, Look MP, Soosaipillai AP, Foekens JA: Higher expression of human kallikrein 10 in breast cancer tissue predicts tamoxifen resistance. Br J Cancer, in press.