The New Human Kallikrein Gene Family: Implications in Carcinogenesis

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The traditional human kallikrein gene family consists of three genes, namely KLK1 *[encoding human kallikrein 1 (hK1) or pancreatic/renal kallikrein],* KLK2 *(encoding hK2, previously known as human glandular kallikrein 1) and* KLK3 *[encoding hK3 or prostate-specific antigen (PSA)].* KLK2 *and* KLK3 *have important applications in prostate cancer diagnostics and, more recently, in breast cancer diagnostics. During the past two to three years, new putative members of the human kallikrein gene family have been identified, including the* PRSSL1 *gene [encoding normal epithelial cell-specific 1 gene (NES1)], the gene encoding zyme/protease M/neurosin, the gene encoding prostase/KLK-L1, and the genes encoding neuropsin, stratum corneum chymotryptic enzyme and trypsin-like serine protease. Another five putative kallikrein genes, provisionally named* KLK-L2, KLK-L3, KLK-L4, KLK-L5 *and* KLK-L6, *have also been identified. Many of the newly identified kallikrein-like genes are regulated by steroid hormones, and a few kallikreins (NES1, protease M, PSA) are known to be downregulated in breast and possibly other cancers. NES1 appears to be a novel breast cancer tumor suppressor protein and PSA a potent inhibitor of angiogenesis. This brief review summarizes recent developments and possible applications of the newly defined and expanded human kallikrein gene locus.*

The kallikrein gene family is a subfamily of serine proteases. Kallikreins were originally defined as enzymes that cleave vasoactive peptides (kinins) from kininogen[1,2]. In rodents, the kallikreins comprise a large family of genes (13 and 26 genes in rat and mouse, respectively)[1–3]. In humans, the kallikrein gene family was, until recently, known to include only three members: the gene encoding pancreatic/renal kallikrein (*KLK1*), the gene encoding kallikrein 2 (*KLK2*) and the gene encoding prostate-specific antigen (PSA; *KLK3*)[4]. The mouse kallikrein genes are all clustered on chromosome 7 in a gene-dense area that is similar in organization to the human kallikrein gene locus. All three established human kallikrein genes have been assigned to chromosome 19q13.3–13.4, and they are in close proximity to one another[4]. In fact, among the three human kallikreins, only human kallikrein 1 (hK1) fulfills the functional definition, based on enzymatic activity. The *KLK2* and *KLK3* genes are assigned to the same family based on the striking structural similarities of the genes and the encoded proteins, and the close localization of all three genes to the same chromosomal region[5,6].

Recently, our research group and others have contributed to the expansion of the human kallikrein gene family. In humans, this gene family now appears to include at least 14 genes[7]. Here, we will describe the new human kallikrein gene locus, outline the similarities between the genes of this family and review the recent evidence suggesting that at least some members of this gene family are implicated in breast, prostate and other human cancers.

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• The New Human Kallikrein Gene Locus

We have recently studied a genomic region of approximately 300 kb around human chromosome 19q13.3–13.4 and established the position of each of the known human kallikrein and kallikrein-like genes. In addition, we have identified new members of this gene family, using the method of 'positional candidate' cloning[7]. In Fig. 1, we present the relative localization and direction of transcription of 14 genes that appear to be members of the same family. In Table 1, we present the currently used names of these genes and proteins, and in Table 2 we present a summary of tissue expression of these genes and their hormonal regulation. Because many of these genes have been identified only recently, detailed information concerning their actions is lacking. In Box 1 we summarize some important similarities between these genes. These similarities support the view that all genes belong to the same family and that they have probably originated from an ancestral gene via the process of gene duplication. Below, we provide some information for each member of this gene family, with special emphasis on their connection to carcinogenesis.

• The *KLK1* Gene

Pancreatic/renal kallikrein (hK1) is found in various tissues (salivary glands, pancreas, kidney, heart, etc.) and catalyzes the release of lysyl-bradykinin or bradykinin from low- and high-molecular weight kininogen[1,2]. Bradykinin is involved in a number of physiological and pathological processes, including control of blood flow, vascular tone, inflammation and cell proliferation. Because the *KLK1* gene is expressed abundantly in many

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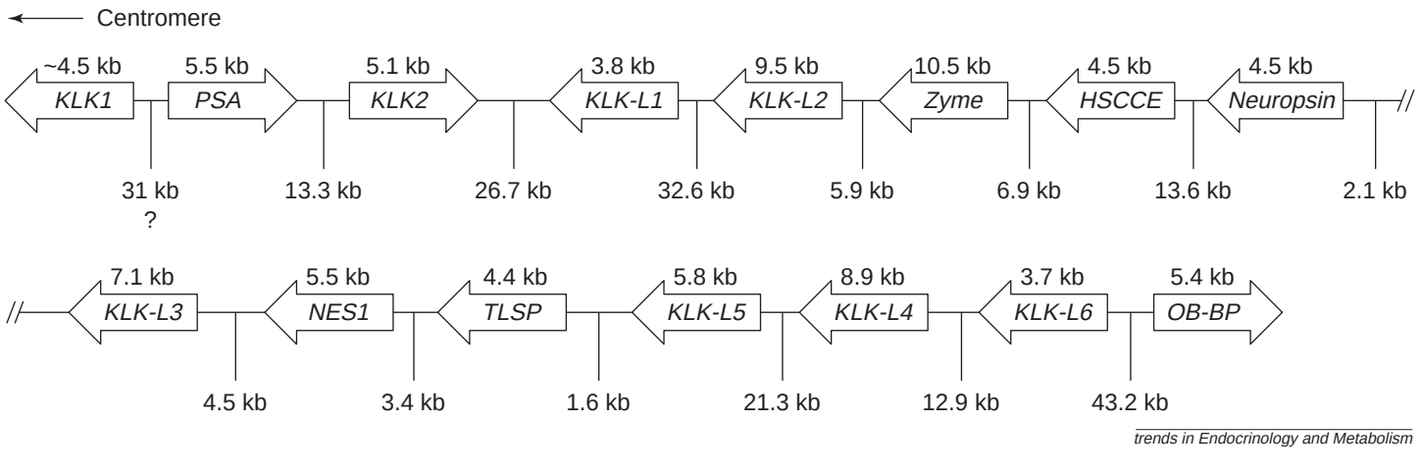

Figure 1. The new human kallikrein gene locus (19q13.3–13.4) showing the physical position of each gene. Arrows denote the direction of transcription. On top of each gene, we show its approximate genomic length in kilobases (kb). On the bottom part, we show the distance between genes. Gene symbols and the proteins they encode are described in Table 1. The full genomic structure of some genes is not yet known. The question mark denotes a sequence gap and uncertainty in the length of DNA sequence. The relative position of the *KLK-L4*, *KLK-L5* and *KLK-L6* genes needs further confirmation. The OB-BP-like gene (encoding a putative leptin-binding protein) does not belong to the kallikrein gene family. It was added to show the end of the genomic area containing the kallikrein genes. It is possible that more kallikreins are present centrometric to *KLK1*. The figure is not drawn to scale.

tissues, it has not found any specific applications in cancer. The *KLK1* gene appears to be upregulated by estrogens, most probably through indirect mechanisms[1]. Detailed reviews summarize current knowledge on this classic kallikrein[1,2].

• The *KLK3* Gene

The *KLK3* gene encodes PSA, which is the best cancer marker currently available, and it is widely used for screening, diagnosis and management of prostate cancer. There are numerous recent reviews of this molecule[5,6,8] and of its clinical applications. More recently, PSA has been identified in normal, hyperplastic and cancerous breast tissues[9,10]. PSA is a favorable prognostic indicator in breast cancer, and its synthesis is generally reduced in breast cancer compared with normal or hyperplastic breast tissue[11,12]. Breast carcinoma cell lines have been utilized to demonstrate the hormonal regulation of this gene by steroids. *KLK3* is upregulated by androgens and progestins[13]. The concentration of PSA in nipple aspirate fluid may serve as a marker of risk for developing breast carcinoma[14]. PSA-positive breast tumors are usually steroid hormone receptor-positive, are of a smaller size, are euploid, have low S-phase fraction and are found in younger women[12]. Women with PSA-positive tumors live longer and relapse less frequently[11,12]. Thus, it appears that PSA is a favorable prognostic marker in breast cancer and that downregulation of *KLK3* is associated with more aggressive, less differentiated tumors and decreased patient survival. Recently, new data have emerged regarding the possible biological function of PSA (Refs 15–17). When

Table 1. The new kallikrein gene family in humans

Kallikrein gene name (old nomenclature)	*Official gene symbol*[a]	*Protein name(s)*
KLK1	*KLK1*	Pancreatic/renal kallikrein (hK1)
KLK3	*KLK3*	Prostate-specific antigen (PSA; hK3)
KLK2	*KLK2*	Human kallikrein 2 (hK2)
PROSTASE/KLK-L1/KLK4	–	Prostase, KLK-L1 protein, human kallikrein 4 (hK4)
KLK-L2/HSCTE	–	KLK-L2 protein, human stratum corneum trypsin-like serine protease
ZYME/PROTEASE M/NEUROSIN	*PRSS9*	Zyme, protease M, neurosin
HSCCE	*PRSS6*	Human stratum corneum chymotryptic enzyme
NEUROPSIN/TADG14	*PRSS19*	Neuropsin, ovasin, tumor-associated differentially expressed gene 14
KLK-L3	–	KLK-L3 protein
NES1	*PRSSL1*	NES1 protein
TLSP	*PRSS20*	Trypsin-like serine protease
KLK-L5	–	KLK-L5 protein
KLK-L4	–	KLK-L4 protein
KLK-L6	–	KLK-L6 protein

Abbreviation: NES1, normal epithelial cell-specific 1.
[a]Assigned by the human gene nomenclature committee (http://www.gene.ucl.ac.uk/nomenclature/). Genes are presented from the most centromeric (*KLK1*) to the most telomeric (*KLK-L6*).

Table 2. Tissue expression and hormonal regulation of human kallikrein genes

Gene	Main tissue expression	Steroid hormone upregulation?	Refs
KLK1	Pancreas, kidney, salivary glands	Yes (estrogen)	1
KLK3	Prostate, breast	Yes (androgen, progestin)	8–10
KLK2	Prostate, breast	Yes (androgen, progestin)	6, 22, 23, 26
KLK-L1/KLK4	Prostate, breast, testis, uterus, thyroid	Yes (androgen, progestin)	28–30
KLK-L2	Breast, brain, testis, skin	Yes (progestin > estrogen > androgen)	46, 47
PRSS9	Brain, breast, ovary, kidney, uterus	Yes (progestin > estrogen > androgen)	31–34
PRSS6	Skin, brain, kidney, breast, salivary glands, thymus	Yes (estrogen, glucocorticoids)	35, 36,[a]
PRSS19/TADG14	Brain, skin	Unknown	38–40
KLK-L3	Skin, thymus, trachea, cerebellum, spinal cord	Yes (progestin, estrogen)	7,[a]
PRSSL1	Breast, ovary, testis, prostate, small intestine, lung, pancreas	Yes (estrogen > androgen > progestin)	41–44
PRSS20	Brain, skin	Unknown	45
KLK-L5	Unknown	Unknown	7
KLK-L4	Prostate, breast, testis, salivary gland	Yes (progestin > androgen > estrogen)	7,[a]
KLK-L6	Unknown	Unknown	7

[a]E.P. Diamandis *et al.*, unpublished.

prostatic carcinoma cell lines are transfected with *KLK3* cDNA, they become apoptotic. Moreover, cancer cell lines transfected with *KLK3* cDNA have decreased proliferation rates and they give rise to tumors with decreased metastatic potential[15]. Furthermore, PSA appears to have potent anti-angiogenic activity, and thereby inhibits tumor formation[17]. These data suggest that PSA might act as a tumor suppressor, an anticarcinogen or an inducer of apoptosis. Interestingly, data for other kallikrein-like genes [for example, genes encoding normal epithelial cell-specific 1 (NES1) and protease M] also suggest that the encoded proteins might have similar activities (see below).

- **The *KLK2* Gene**

The *KLK2* gene (encoding hK2) is located at the same chromosomal region as *KLK3* (only 13 kb further telomeric) and has 80% homology with *KLK3*. An extensive review of the literature regarding hK2 has been published recently[6]. Only within the past two to three years has it been possible to measure hK2 with immunological techniques[18–20]. Human glandular kallikrein is now emerging as an additional prostatic tumor marker that might have clinical applications complementary to those of PSA, as reviewed by Stenman[21] and Rittenhouse and colleagues[6]. Similar to PSA, it was thought initially that hK2 was produced by prostatic epithelial cells only. However, recent studies have unequivocally localized hK2 to the female breast and other tissues[22–24]. It appears that hK2, which has trypsin-like activity, can activate the pro-form of PSA to its enzymatically active, mature form[25]. Until now, PSA and hK2 have been found together in all biological fluids examined, especially the secretions of the prostate (seminal plasma) and breast (nipple aspirate fluid, breast cyst fluid). These data suggest that the two molecules might act in concert, hK2 being the activating enzyme of the pro-form of PSA. The mode of regulation of *KLK2* by steroid hormones in breast cancer cell lines is similar to the one already reported for *KLK3* (upregulation by androgens and progestins)[26]. *KLK2* was shown to be upregulated in prostatic carcinomas[27], in contrast to *KLK3*, which is generally downregulated compared with normal tissue[6]. There are no data regarding the prognostic value of hK2 in breast or other cancers.

- **The Gene Encoding Prostase/KLK-L1**

This gene is known as the *KLK-L1/KLK4* gene. It was cloned independently by Nelson *et al.*, who used subtractive hybridization[28], and by our own group (using the positional candidate approach[7,29]), as well as by others[30]. On the basis of northern blot analysis, it appeared initially that this gene was expressed predominantly, if not exclusively, in prostatic tissue. However, with the use of the more sensitive reverse transcription polymerase chain reaction (RT–PCR) technique, we found that this gene is expressed in various tissues, including prostate (highest expression), testis, adrenals, uterus, thyroid, mammary gland, colon and spinal cord[29]. *KLK-L1/KLK4* is regulated by androgens and progestins in both prostatic carcinoma and breast carcinoma cell lines[28,29]. We do not as yet know whether the gene is upregulated or downregulated in prostate cancer, in comparison to normal tissue. In addition, we do not know much about the expression levels of this gene in breast and other cancers, or its possible prognostic value. It has not yet been shown whether or not the prostase/KLK-L1 protein is circulating in the blood, because no methods are available to measure it. Clearly, this gene product holds promise of becoming a valuable tumor marker for prostate, breast and possibly other cancers, but more research is necessary.

- **The Gene Encoding Zyme/Protease M/Neurosin**

This gene, now known as *PRSS9* (for protease, serine, 9), was cloned

Box 1. Similarities between the 14 kallikrein-like genes

- All genes colocalize to the same chromosomal region (q13.3–13.4) in a linear arrangement (Fig. 1), apparently without intervention of non-kallikrein genes
- All genes encode putative serine proteases with a conserved catalytic triad (histadine, aspartic acid and serine)
- All genes appear to have five coding exons, although some members of the family do contain one or more untranslated exons (examples: *PRSS19*, *PRSS9*, *PRSSL1*)
- Intron phase completely conserved among all 14 members[a]
- All genes have significant sequence homologies at the DNA and amino acid levels (30–80%)
- Many of these genes are regulated by steroid hormones (Table 2)
- Some kallikrein genes (*KLK3*, *PRSSL1* and *PRSS9*) are downregulated in breast cancer and breast cancer cell lines and the proteins appear to act as tumor suppressors

[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.

independently by three different groups and was given three different names[31–33]. One group cloned this gene by the method of differential display, as a gene that is expressed differently in normal human mammary epithelial cells (high expression) versus tumor cell lines (low expression)[31]. *PRSS9* encodes a serine protease and maps very close to the human kallikrein gene locus[31,32]. The gene has about 50% homology with the other kallikrein genes and it exhibits a number of additional important similarities with genes encoding other kallikreins (Table 3). The hallmark of *PRSS9* gene discovery is its dramatic downregulation in metastatic breast carcinoma cells, in comparison to primary carcinoma cells or normal breast epithelial cells[31]. The gene is expressed in various tissues, including brain, spinal cord, cerebellum, mammary gland, kidney, uterus, salivary gland, thymus, spleen and testis[34]. *PRSS9* is upregulated by steroid hormones in the breast cancer cell line BT-474, with potencies estrogen > progestin > androgen[34]. Little *et al.* have provided evidence that zyme has amyloidogenic potential and might play a role in amyloid precursor processing and Alzheimer's disease[32]. The zyme protein is present in cerebrospinal fluid and seminal plasma (E.P. Diamandis *et al.*, unpublished). Although zyme is predicted to be a secreted serine protease, we do not know yet whether it circulates in blood and whether it has any diagnostic, prognostic or predictive value in breast or other cancers, or in other human diseases, because methods to measure the protein with high sensitivity are not yet available.

- **The Gene Encoding Human Stratum Corneum Chymotryptic Enzyme (HSCCE)**

HSCCE is a new serine proteinase produced by keratinocytes in the epidermis[35,36]. The gene encoding this proteinase is known as *PRSS6*. The putative function of the protein is to catalyze the degradation of intercellular cohesive structures in the cornified layer of the skin, thus facilitating the continuous shedding of cells from the skin surface. HSCCE is found mainly in human skin, the central nervous system, kidney, mammary gland, thymus and salivary glands. One of the possible functions of HSCCE is to activate interleukin 1β (IL-1β) in human epidermis. The enzyme has been linked to skin diseases, including psoriasis, acne and hair growth disturbances[36]. Recently, one report described the overproduction of the enzyme in ovarian carcinomas[37].

- **The Gene Encoding Neuropsin**

This gene is now known as *PRSS19*. Neuropsin is a serine protease that is thought to function in a number of different tissues, including the skin and brain. In mice, this protease has been shown to have important roles in neural plasticity[38–40]. The human gene has 72% homology with cDNA encoding murine neuropsin and the highest level of expression is also seen in brain and skin. Neuropsin might be involved in the production of cerebrospinal fluid, the formation of memory, and in some forms of epilepsy. Lowell *et al.* cloned an alternatively spliced form of the same gene and named it *TADG14* (for tumor-associated, differentially expressed gene 14), and the encoded protein was named ovasin. *TADG14* appears to be overexpressed in a subset of ovarian carcinomas[37].

- **The Gene Encoding NES1**

This gene was cloned by Vimla Band and her associates using subtractive hybridization techniques[41] and is now known as *PRSSL1*. The discovery of this gene was based on the finding that it was expressed abundantly in a normal breast cell line but was absent from the same cell line that had been irradiated to become tumorous. Thus, it was suggested that the downregulation of *PRSSL1* might be part of the tumorous phenotype in this cell line. This was confirmed by expression studies, which indicated that *PRSSL1* is expressed in normal breast cells but not in breast carcinoma cell lines or metastatic breast tumor tissue[41]. Some interesting findings related to *PRSSL1* include the following: (1) the gene encodes a novel serine protease that has 50–60% homology with PSA and other members of the human kallikrein gene family; (2) the gene is expressed in various tissues in humans including breast, ovarian and prostatic tissue; (3) *PRSSL1* is dramatically downregulated in breast and prostate cancer cells compared with

Table 3. Kallikreins that might be involved in cancer

Kallikrein gene or protein	*Possible role/findings in cancer*	*Refs*
PSA	Inducer of apoptosis	15
	Reduces cell proliferation	15, 16
	Reduces tumorigenic potential of cancer cell lines	15
	Gene downregulated in breast/prostate cancer[a]	11, 12
	Favorable prognostic indicator in breast cancer	11, 12
	Gene downregulated in high-risk breast cancer patients	14
	Inhibitor of angiogenesis; anticarcinogenic	17
	Might regulate growth factor/cytokine networks	9
Human kallikrein 2 (hK2)	Gene coexpressed with *KLK3* in breast tissues and present in breast secretions	22–24
	Gene overexpressed in prostatic tumors	27
	Might activate zymogen form of PSA	25
	Might regulate growth factor/cytokine networks	6
Prostase/KLK-L1 protein	Gene expressed in prostate and breast tissue and it is hormonally regulated	28–30
	Might play a role in bony metastasis of breast and prostate cancers	28
	Might regulate growth factor/cytokine networks	28
Zyme/protease M/neurosin	Gene expressed in breast tissue but it is dramatically downregulated in breast cancer, and more so at metastatic sites	31
NES1	Gene expressed in breast and prostate tissues and dramatically downregulated in cancer and cancer cell lines	
	NES1 abolishes ability of tumorigenic cells to grow in an anchorage-independent manner	42
	NES1 slows down cell proliferation	42
	NES1 reduces tumorigenic potential of cell lines in a nude mouse model of carcinogenesis	42
	NES1 is considered a novel tumor suppressor	42
Neuropsin/ovasin	Gene overexpressed in a subset of ovarian carcinomas	37
HSCCE	Gene overexpressed in a subset of ovarian carcinomas	37

Abbreviations: HSCCE, human stratum corneum chymotryptic enzyme; NES1, normal epithelial cell-specific 1; PSA, prostate-specific antigen.
[a]*KLK3* is downregulated in prostate cancer cells compared with normal tissues. The observed increase in serum PSA in prostate cancer is the result of increased diffusion of PSA into the circulation.

normal tissues; (4) more recently, it has been shown that *PRSSL1* is a novel tumor suppressor gene[42]. These data were based on experiments indicating that the expression of *PRSSL1* suppressed the anchorage-independent growth of tumor cells in soft agar and inhibited the formation of tumors in nude mice. These findings are reminiscent of the already described findings for protease M (Ref. 31). Our group was able to characterize the genomic structure, mapping and the hormonal regulation of *PRSSL1* (Refs 43,44).

- **The Gene Encoding Trypsin-like Serine Protease (TLSP)**

Recently, Yoshida *et al*. cloned a novel human cDNA that encodes a putative novel serine protease, TLSP (Ref. 45). This gene is now known as *PRSS20* and is expressed primarily in the brain and in keratinocytes. It appears to have a number of important similarities to neuropsin. There are no reports yet describing the connection of this gene to any human disease.

- **Newly Identified Kallikrein-like Genes**

As shown in Fig. 1, another six kallikrein-like genes have been discovered within the human kallikrein gene locus[7]. These genes are dispersed throughout the 300-kb genomic region between the classic kallikrein genes and other genes previously identified and described in this review. All these new genes are predicted to encode serine proteases and have significant homologies, at both the DNA and amino acid level, with the classic kallikreins (GenBank accession numbers AF135023, AF135024, AF135025, AF135026, AF135028 and AF161221). These genes also have other similar features to those of the classic kallikreins, as described in Box 1. There are strong indications that at least the *KLK-L2*, *KLK-L3* and *KLK-L4* genes are expressed in various tissues, including breast and prostate[46]. Recently, a cDNA identical to the cDNA for *KLK-L2* was cloned and found to encode a serine protease, named human stratum corneum trypsin-like serine protease, which might have some function in skin desquamation[47].

Other data on the encoded proteins of these genes are lacking.

- **Connection of the Human Kallikrein Gene Family to Cancer: An Integrated View**

The connection between breast and prostate cancer and steroid hormones has been established in many epidemiological studies, but we do not yet understand which genes are involved in the pathogenesis of the sporadic forms of these two diseases[48]. It is logical to speculate that at least some of the genes involved in the pathogenesis of breast and prostatic cancer are regulated directly or indirectly through the action of steroid hormones. In this respect, the *KLK3*, *KLK2*, *KLK-L1*/*KLK4*, *PRSS9*, *PRSSL1*, *KLK-L2*, *KLK-L3* and *KLK-L4* genes have all been shown to be regulated by steroid hormones (Table 2).

PSA is currently the best tumor marker available and is used widely for prostate cancer diagnostics and, more recently, for breast cancer diagnostics. Although *KLK3* is downregulated in both breast and prostate cancer cells, we have no direct evidence that this downregulation is related to either the pathogenesis or the progression of breast or prostatic cancer. It has been suggested that PSA might be involved in growth factor and cytokine networks through its proteolytic action[9]. Other data indicate that PSA synthesis might be reduced in the breast tissue of patients who are at high risk for breast cancer development[14], and high tumor PSA concentrations are known to be a favorable prognostic indicator in breast cancer[11,12]. Lower or no expression of *KLK3* is found in less differentiated and more aggressive tumors. More recently, it has been shown that *KLK3* cDNA transfected into prostatic cell lines induces apoptosis, negatively regulates cell proliferation and reduces the tumorigenic potential of cancer cell lines[15,16]. Striking new data demonstrate the anti-angiogenic activity of PSA (Ref. 17). These data suggest that PSA might act as a tumor suppressor (anticarcinogenic) protein. Remarkably, similar data have been reported independently for the putative tumor suppressor gene *PRSSL1* (Refs 41,42). *PRSSL1* expression is downregulated in cancerous breast and prostate tissue, compared with normal tissue, and in a number of cancer cell lines[41]. NES1 abolishes the ability of tumorigenic cells to grow in an anchorage-independent manner, reduces their proliferation rates and their ability to form tumors in nude mice[42]. Furthermore, it is now clear that *PRSS9* is also dramatically downregulated in breast cancer, especially in the more aggressive, metastatic forms of the disease[31]. In addition, new data suggest that *KLK2* is expressed not only in prostatic tissue but also in breast tissue[22–24]. Although a functional connection between hK2 and PSA has been suggested[25], no studies have been done to examine whether *KLK2* is up- or downregulated in breast cancer, compared with normal tissue. This possibility merits investigation. We have also shown that the newly discovered gene *KLK-L1*/*KLK4* is expressed in both prostate and breast tissue[29]. However, we do not know yet whether this gene is either up- or downregulated in cancer cells, compared with normal tissue. A number of other kallikrein-like genes, including *KLK-L2*, *KLK-L3* and *KLK-L4*, are now known to be expressed in breast, prostate, testicular and other tissues[46]. It remains to be established whether any of these genes are involved in cancer pathogenesis or progression, and whether they will be useful tumor markers for disease diagnosis or monitoring. In this respect HSCCE and neuropsin/ovasin might have some value because these proteins are found in abundance in ovarian carcinomas[37]. In Table 3, we summarize the evidence that some members of the kallikrein gene family are connected to breast, prostate and other cancers.

Proteolytic enzymes are usually considered unfavorable prognostic indicators in cancer. A number of proteolytic enzymes facilitate metastasis through degradation of the basement membrane. It is possible that some of the serine proteases shown in Fig. 1 are involved in the process of metastasis, angiogenesis or in the osteoblastic and osteolytic manifestations of cancer in bone. These possibilities should be investigated further.

- **Conclusion: Future Directions**

We have provided evidence that the human kallikrein gene family consists of as many as 14 genes. Some of these genes have been identified only recently and not much is known about their expression, hormonal regulation or involvement in human diseases. On the other hand, there is convincing evidence that at least some kallikreins, including PSA, NES1 and zyme/protease M/neurosin, are involved in breast and prostate cancer in a way that is reminiscent of tumor suppressor activity. Some of the well-known kallikreins, including PSA and hK2, are valuable tumor markers for prostate cancer diagnosis and monitoring. At least two other kallikreins, HSCCE and neuropsin/ovasin, are overproduced in ovarian carcinomas[37]. It is conceivable that at least some members of this gene family might prove to be useful tumor markers for prostate, breast, ovarian and other cancers. Clearly, there is a need to develop reliable immunological assays for measuring extremely small amounts of these kallikrein-like proteins in biological fluids. The availability of such assays might open up new ways for diagnosis and monitoring of cancer. Furthermore, there is a need to understand the possible physiological function of all these newly identified proteins. It is possible that some of these kallikreins participate in pathways that are involved in either tumor initiation or tumor progression. Some kallikreins might be good therapeutic targets, but both overexpression and underexpression of these genes should be examined, because the enzymatic activity of these proteins might be beneficial (as reported in Ref. 17) or it could be deleterious, by promoting cancer metastasis. Clearly, more intensive research is necessary to explore this interesting and newly expanded family of proteolytic enzymes.

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