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Clinical Biochemistry 36 (2003) 443–452

CLINICAL BIOCHEMISTRY

An overview of the kallikrein gene families in humans and other species: Emerging candidate tumour markers☆

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Abstract

Kallikreins are serine proteases with diverse physiologic functions. They are represented by multigene families in many animal species, especially in rat and mouse. Recently, the human kallikrein gene family has been fully characterized and includes 15 members, tandemly localized on chromosome 19q13.4. A new definition has now been proposed for kallikreins, which is not based on function but, rather, on close proximity and structural similarities. In this review, we summarize available information about kallikreins in many animal species with special emphasis on human kallikreins. We discuss the common structural features of kallikreins at the DNA, mRNA and protein levels and overview their evolutionary history. Kallikreins are expressed in a wide range of tissues including the salivary gland, endocrine or endocrine-related tissues such as testis, prostate, breast and endometrium and in the central nervous system. Most, if not all, genes are under steroid hormone regulation. Accumulating evidence indicates that kallikreins are involved in many pathologic conditions. Of special interest is the potential role of kallikreins in the central nervous system. In addition, many kallikreins seem to be candidate tumor markers for many malignancies, especially those of endocrine-related organs.

Keywords: Kallikrein; Tumor markers; Cancer biomarkers; Prostate cancer; Breast cancer; Ovarian cancer; Alzheimer's disease; Serine proteases; Chromosome 19; Kallikrein evolution; Rodent kallikreins; Hormonally regulated genes

1. Introduction

Proteolytic enzymes can be classified based on their catalytic mechanisms. Classes of proteolytic enzymes include proteases which have an activated cysteine residue (cysteine proteases), an aspartate (aspartate proteases), a metal ion (metalloproteases) and proteases with an active serine (serine proteases). Serine proteases are a family of enzymes that utilize a uniquely activated serine residue in the substrate-binding site to catalytically hydrolyze peptide bonds [1]. This large family includes the digestive enzymes (*e.g.*, trypsin, chymotrypsin), the kringle domain-containing growth factors (*e.g.*, tissue plasminogen activator), some of the blood clotting factors and the kallikreins. Serine proteases are involved in many vital functions such as digestion, coagulation and fibrinolysis, tissue remodeling, activation of hormones, growth factors and receptors and extracellular matrix protein degradation.

The term 'Kallikrein' was introduced in the 1930s by Werle and colleagues who found high levels of their original isolates in the pancreas (in Greek, the "Kallikreas") [2]. It was first observed that when human urine is injected into dogs, it leads to hypotension. Later, a pancreatic extract was found to contain the same hypotensive substance. The term 'kallikrein' was originally introduced to describe an enzyme that acts upon a precursor molecule (kininogen) and releases a bioactive peptide (kinin) [3]. Another term which is also frequently used to describe these enzymes is 'kininogenases'. The kallikrein enzymes are now divided into two major categories: plasma kallikrein and tissue kallikreins [4]. These two categories differ significantly in their molecular weight, substrate specificity, immunologic characteristics, gene structure and type of kinin released.

More recently, the concept of the "kallikrein multigene family" was introduced, in which the generic term 'tissue kallikrein' is not restricted to the description of enzymes that release bioactive peptides from precursor molecules, but rather, used to describe a group of enzymes with highly conserved gene and protein structure, which also share con-

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☆ Abbreviations: KLK, human kallikrein (gene); hK, human kallikrein (protein); mGK, mouse glandular kallikrein; rKLK, rat glandular kallikrein; CNS, central nervous system.

0009-9120/03/$ – see front matter
doi:10.1016/S0009-9120(03)00055-9

siderable sequence homology and co-localize in the same chromosomal locus [5]. The use of the term 'kallikrein' does not necessarily imply that any of these family members (with the exception of hK1) has kininogenase activity. In fact, for the human family members that have been functionally tested, it was found that they possess very low (hK2) or no (hK3, PSA) kininogenase activity [6]. These enzymes are grouped together with KLK1, based on structural similarities and map location.

Kallikreins in animal species other than humans have been extensively described in a number of excellent reviews [7–11]. Here, we provide a quick overview on kallikreins in different species, followed by a more detailed description of the human kallikrein gene family.

2. Kallikreins in different animal species

Kallikreins are found in both primates and nonprimates. Kallikrein proteins have been identified in six different mammalian orders; Primates, Rodentia, Carnivora, Proboscidea, Perissodactyla, and Artiodactyla [12]. The number of kallikrein genes varies between species.

2.1. The mouse kallikrein gene family

At least 25 kallikreins have been reported in mouse, and among them, 14 genes are presumed to be encoding serine proteases [7], the rest being pseudogenes. Data obtained from the Mouse Genome Project (http://www.ncbi.nlm.nih.gov/genome/guide/mouse/) indicate that this number could be increased to 37 genes (including annotated genes). Most mouse genes are located in the mouse kallikrein gene locus that spans an area of about 310 kb region at, or near, the Tam-1 locus on mouse chromosome 7. Data from the Human/Mouse homology maps (http://www.ncbi.nlm.nih.gov/Homology/) show that this region is highly syntenic to the human chromosome 19q13.4 locus which harbors the human kallikrein genes (up to 75% sequence similarity). All mouse kallikrein genes are transcribed in the same direction and share a high degree of structural homology at both the mRNA and protein levels (70–90%). They also share the same genomic organization, being formed of five coding exons and four introns, with completely conserved exon-intron splice sites. A TATA box variant "TTTAAA" and consensus polyadenylation signal sequences were found in all mouse kallikreins [13–15]. All mouse kallikreins code for prepro-kallikreins that are 261 amino acids in length, with an 18-amino acid signal peptide followed by a prefragment of 6 amino acids. Similar to the human family, only one mouse kallikrein (mKLK1 or mGK6) has true kininogenase activity [13]. Other mouse kallikrein proteins have different types of activities; mGK3 and mGK4 being nerve growth factor binding and processing enzymes and mGK9, mGK13 and mGK22 being epidermal growth factor-binding proteins; mGK22 is a nerve growth factor-inactivating enzyme; mGK16 is γ-renin and mGK26 is prorenin-converting enzyme-2 [16,17]. With the expansion of the mouse family of kallikreins, it is becoming necessary to develop a revised nomenclature of mouse kallikreins, that takes into consideration the newly cloned genes, their map location and phylogenetic relatedness to their human orthologues.

2.2. The rat kallikrein gene family

Another large family of about 13 kallikreins was identified in the rat, of which at least 10 are transcriptionally active [18]. More recent data from the Rat Genome Database (http://ratmap.gen.gu.SE/) points to the possibility for more kallikreins in the rat genome. Rat kallikreins are clustered together in the same chromosomal region and share a high degree of structural homology. They also have the same conserved structure of five coding exons and four introns, with most of the similarity in the exonic, rather than intronic, regions [19]. Ten out of the 13 rat kallikreins code for potentially active serine proteases which are 261 amino acids in length; the rest are assumed to be pseudogenes. As is the situation in human and mouse, only one rat kallikrein (rKLK1) meets the functional definition of a kallikrein [20]. rKLK2 codes for tonin, which converts angiotensinogen to angiotensin [21] and rKLK10 codes for a kininogenase which cleaves T-kininogen to release T-kinin [21]. Rat kallikrein mRNA levels were found to be responsive to hormonal manipulation and castration of male animals resulted in a decrease of mRNA which could be restored by testosterone [22]. Pituitary rKLK1 has been found to be up-regulated by estrogen [11]. Interestingly, rodent kallikreins are mainly expressed in the salivary gland with very few of them having a wider tissue expression pattern [11].

2.3. The mastomys kallikrein gene family

Mastomys is an African rodent intermediate in size and characteristics between the mouse and rat. It has been studied because of the presence of an androgen-responsive prostate in the female. Fahnestock reported the cloning of kallikrein cDNAs from Mastomys. Two of these were expressed in the kidney as well as the submandibular gland, and one is hypothesized to code for a true tissue kallikrein [23]. A third kallikrein was found only in the submandibular gland. DNA sequence analysis and hybridization studies demonstrate that Mastomys represents an interesting hybrid between mouse and rat [23].

2.4. The monkey kallikrein gene family

A cynomolgus monkey tissue kallikrein has been characterized from a monkey renal cDNA library, and shown to be 90% homologous to its human counterpart at the nucleotide level [24]. The cmKLK1 encodes a tissue kallikrein of 257 amino acids, which is 93% homologous to the human kallikrein protein. The key residues important for kininoge-

nase activity are entirely conserved. A rhesus monkey prostatic KLK3 cDNA encoding the simian counterpart of prostate specific antigen (KLK3) has also been cloned [25]. It consists of 1,515 nucleotides, encoding a preproenzyme of 261 amino acids, with a long 3′ untranslated region. The deduced amino acid sequence is 89% homologous to human hK3 and 71% to human hK2. Tyr^{93}, a residue important for the kininogenase activity of human kallikrein 1, is replaced by a serine, indicating that rmKLK3 will lack kininogenase activity, as does its human counterpart.

2.5. The dog kallikrein gene family

Only two kallikreins have been identified in the dog; dKLK1 encoding for a true tissue kallikrein based on functional definition, and dKLK2 encoding a canine arginine esterase [26]. dKLK1 encodes for a polypeptide of 261 amino acids with a typical 24-residue prepropeptide, a conserved catalytic triad of serine proteases, and a tissue kallikrein substrate-binding pocket. A prostatic cDNA and the gene encoding for canine arginine esterase (dKLK2) have both been cloned. As for all other mammalian kallikrein genes, dKLK2 consists of five exons and four introns with fully conserved exon/intron boundaries, and an "AGTAAA" polyadenylation signal identical to that of human hK1. The dKLK2 gene product shows a wide pattern of tissue expression and has less overall conservation (~50%) with kallikreins of other species.

2.6. Kallikreins in other species

No more than three tissue kallikreins were identified from organs of the guinea pig [27]. Southern blot analysis detected KLK2 and KLK3 positive bands in several nonhuman primate species including macaque, orangutan, chimpanzee and gorilla but not cows and rabbits [28]. Kallikreins were also isolated from the pancreas, colon and submadibular gland of the cat [29]. Nonprimates do not contain any prostate-localized proteins homologous to PSA [30].

3. The human kallikrein gene family

In humans, there are two classes of kallikreins; plasma kallikrein and tissue kallikreins. The plasma kallikrein gene is encoded by a 15-exon single gene on chromosome 4q35. This enzyme (a serine protease) releases the vasoactive peptide bradykinin from a high molecular weight precursor synthesized in the liver [31]. The human tissue kallikreins are a family of genes localized on chromosome 19, and also encode for serine protease enzymes. The following discussion will focus on the human tissue kallikrein gene family.

The human tissue kallikrein gene family was discovered and extensively studied in the 1980s. In 1986, Watt and co-workers generated peptides of purified PSA from seminal plasma [32] and in 1987, Lundwall and Lilja reported a nearly complete cDNA sequence of prepro-PSA [33]. In 1987, Schedlich and co-workers isolated the genomic DNA for hK2 and determined the sequence for prepro-hK2 [34]. The locus was also investigated and it was then concluded that the entire family is composed of only 3 genes, namely KLK1, encoding for pancreatic/renal kallikrein (hK1 protein); KLK2, encoding the human glandular kallikrein 2 (hK2) and KLK3, encoding the prostate-specific antigen (PSA, hK3) [35,36]. The major interest in human kallikreins lies in the highly restricted tissue expression of hK2 and hK3 in the prostate, which qualifies them as candidate biomarkers for prostatic diseases [30].

Extensive recent work by many laboratories has led to the identification of all members of the human kallikrein gene family and their characteristic structural features (as described below) and the adoption of a uniform nomenclature [37–49]. According to the approved revised nomenclature of the human kallikreins, a kallikrein gene is denoted as (KLK) and a kallikrein protein as (hK). The human kallikrein family currently includes 15 genes (KLK1-15). Table 1 summarizes the official names, GenBank accession numbers and synonyms of all genes and proteins.

4. Structure of the human kallikrein genes and proteins

Human kallikrein genes range from 4 to 10 kb of genomic length with most of the differences attributed to intron lengths. The common structural features of this family of genes are as follows [50-53]:

1. All genes are formed of 5 coding exons and some have one or more extra 5′ untranslated exons. The first coding exon always contains a 5′ untranslated region, followed by the methionine start codon, located ~ 50 bp away from the end of the exon. The stop codon is always located ~156 bp from the beginning of the last coding exon.
2. Exon sizes are very similar or identical
3. The intron phases are conserved in all genes, with a pattern of I-II-I-0
4. Positions of the catalytic triad residues of serine proteases are conserved, with the histidine occurring near the end of the second coding exon, the aspartate at the middle of the third exon and the serine residue at the beginning of the fifth exon.
5. All kallikrein proteins are predicted to be synthesized as a pre/pro peptides with a signal peptide of about 17 to 20 amino acids at the amino terminus, followed by an activation peptide of about 4 to 9 amino acids (with the exception of hK5 which has a longer peptide), followed by the mature (enzymatically active) protein.
6. The amino acid of the substrate-binding pocket is either aspartate, indicating trypsin-like specificity (11 enzymes), or another amino acid (probably conferring

Table 1
Official names and synonyms of members of the human kallikrein gene family

Official name	Synonyms	GenBank submission	Reference
KLK1	Pancreatic/renal kallikrein, hPRK	M25629 M33105	[35]
KLK2	Human glandular kallikrein 1, hGK-1	M18157	[34]
KLK3	Prostate-specific antigen, PSA	X14810 M24543 M27274	[105–108]
KLK4	Prostase, KLK-L1, EMSP1, PRSS17, ARM1	AF113141	[44]
KLK5	KLK-L2, HSCTE	AF135028	[84]
KLK6	Zyme, Protease M, Neurosin, PRSS9	D78203 (mRNA) AF149289 (Full gene)	[83] [109]
KLK7	HSCCE, PRSS6	L33404 (mRNA) AF166330 (Full gene)	[110] [111]
KLK8	Neuropsin; Ovasin; TADG-14, PRSS19, HNP	AB009849	[47]
KLK9	KLK-L3 protein	AF135026	[39]
KLK10	NES1, PSSSL1	NM_002776 (mRNA) AF055481 (Full gene)	[89] [112]
KLK11	TLSP/Hippostasin, PRSS20	AB012917 (mRNA) AF164623 (Full gene)	[48] [113]
KLK12	KLK-L5 protein	AF135025	[40]
KLK13	KLK-L4 protein	AF135024	[41]
KLK14	KLK-L6 protein	AF161221	[42]
KLK15	prostinogen, HSRNASPH	AF242195	[43]

chymotryptic or other activity), as is the case with hK3 (PSA)
7. Most, if not all, genes are under steroid hormonal regulation
8. All proteins contain 10 to 12 cysteine residues, that will form 5 (classical kallikreins and hK13) or 6 (in the rest) disulphide bonds. The positions of the cysteine residues are also fully conserved

Classical or variant polyadenylation signals have been predicted 10 to 20 bases away from the poly A tail of all kallikreins [51]. Multiple alignments of all kallikrein proteins have been published before [43]. In addition to the conservation of the catalytic amino acid triad, other protein motifs have been recently reported [52]. The functional significance of these motifs is yet to be elucidated. The crystal structure of two human kallikrein proteins has been recently reported [54,55].

5. Tissue expression and hormonal regulation of the kallikrein genes

Many kallikreins are transcribed predominantly in a few tissues, as indicated by Northern blotting. By using the more sensitive RT-PCR technique, kallikreins were found to be expressed at lower amounts in several other tissues. The tissue expression of all kallikreins, assessed by RT-PCR and or Northern blot, has been summarized elsewhere [51,56]. Many kallikreins are expressed in the salivary gland, the tissue where most of the rodent kallikreins are expressed. Also, several kallikreins were found in the central nervous system and preliminary reports suggest that they are involved in brain physiology and pathobiology, as discussed below. An interesting phenomenon is the co-expression of many kallikreins in the same tissue. It is possible that these kallikreins may act in concert, in cascade enzymatic pathways, reminiscent of the coagulation and apoptotic pathways.

6. Evolution of the kallikrein gene family

Based on the originally presumed small size of the family in humans and because two of the three classical kallikreins (KLK2, KLK3) have no known orthologues among the rodent kallikreins, it was suggested that the mouse and human kallikrein families have diverged from a common ancestor after the separation of the primate and rodent lineages [57]. After characterization of all members of the human kallikrein family, five rodent orthologues were identified; the mouse EMSP (similar to KLK4) [58], mouse SCCE (similar to KLK7) [59], mouse protease M and rat MSP (similar to KLK6) [60,61], mouse and rat neuropsin (KLK8) [62,63] and mouse hippostasin (KLK11) [64]. In addition, data obtained from the Mouse Genome Project predicted the presence of mouse orthologues to human kallikreins 9 and 14 (http://www.ncbi.nlm.nih.gov/genome/guide/mouse/).

More recently, Olsson and Lundwall analyzed the organization of the kallikrein gene family in the mouse through sequence analysis of different databases [65]. This analysis indicated the presence of mouse orthologues for the human kallikreins 4 to 15, but not for the classical kallikreins KLK2 and KLK3. Interestingly, all mouse orthologues are clustered together on the mouse genome and are in the same order as their human counterparts. Comparing the human and mouse kallikrein loci indicated that while the distance between the human KLK1 and KLK15 genes is only 1.5 kb, the same area in the mouse genome is 290 kb in length and is occupied by the classical mouse kallikrein genes [65].

Several phylogenetic analyses were performed for all 15 human kallikrein proteins along with some mouse and rat kallikreins and other serine proteases. Kallikrein proteins were found to be clustered in one branch with reasonable confidence. Consequently, this branch can be rooted by using other protease sequences as outgroup [43,66]. All methods showed that the classical kallikreins (hK1-hK3) constitute a distinct monophyletic group in the kallikrein family, in agreement with other previously published reports [56]. The separation of the classical human kallikreins (hK1-hK3) from the "new" human kallikreins (hK4-hK15) is also supported by other findings. The degree of similarity, at the protein level, between the classical kallikreins is about 80%, which is much higher than between them and the "new" kallikreins (30–50%). Also, the three classical kallikreins have a distinct "kallikrein loop" that is not found in the "new" kallikreins, and the new kallikreins (with the exception of hK13) have six disulphide bonds as opposed to five in the classical kallikreins. Finally, two of the three classical kallikreins (KLK2, KLK3) do not have rodent orthologues, but all of the new kallikreins do [64]. Thus, we conclude that the new kallikreins likely diverged before the rodent-primate split, which has been dated between 65 and 85 million years ago [67] and also that either the classical kallikreins have diverged from the so-called "new" kallikreins, or the two groups are monophyletic and share a common ancestor. These conclusions will have to be reconsidered in case of future cloning of rodent orthologues of the classical kallikreins. The same conclusion was also suggested by Olsson and Lundwall by phylogenetic analysis of the human and mouse kallikreins [65].

Hu *et al.* [58], by comparing the amino acid sequences of the rat and mouse kallikreins and other trypsin-like serine proteases, found that the kallikrein loop, while present in all mouse and rat kallikreins, is not present in the mouse KLK4 (EMSP1). In addition, there is an amino acid insertion (leucine at position 98) that was not found in other serine proteases, suggesting an evolutionary divergence. This report, in addition to a more recent one, raise the possibility that human KLK4 might have a unique evolutionary history [68].

Within the human kallikrein family itself, it is hard to precisely determine the degree of divergence of the different branches due to the low branch numbers. However, most of the trees agreed that kallikreins can be roughly divided into 5 main branches using distance matrix methods; hK1-hK3 are clustered in one branch, hK9, hK11, hK15 in the second, hK6, hK13, hK14 in the third, hK8, hK10, hK12 in the fourth and hLK4, hK5, hK7 in the fifth [43,69].

The most accepted theory is that this gene family arose through gene duplication and exon shuffling [39,44]. The mechanism proposed involves unequal crossing-over of sister chromatids during meiotic recombination. In fact, chromosome 19-specific minisatellites have been identified in the long arm of chromosome 19, which may have facilitated such gene duplication [70–72]. Studies of the γ-globin gene in a wide range of species have led to the development of a gene duplication model similar to that proposed for the kallikreins. Fitch et. al., found that interspersed repetitive elements may act as nucleation sites for unequal crossover events [73]. Further, these exchanges can also introduce nucleotide changes in the coding and untranslated regions of these genes, which have been implicated in the regulatory changes that delayed expression of some globin genes from embryonic to fetal life (reviewed in [73]).

7. Association of kallikreins with human diseases

The KLK1 gene is involved in many disease processes, including inflammation [11], hypertension [74], renal nephritis and diabetic renal disease [75]. The connections of HSCCE (KLK7) to skin diseases, including pathologic keratinization and psoriasis, have already been reported [76,77]. Much focus is now placed on the relation of kallikreins to diseases of the central nervous system and cancer, as discussed below.

7.1. Kallikreins in CNS diseases

Many kallikreins seem to play important physiologic roles in the central nervous system (CNS). In mouse, neuropsin appears to have an important role in neural plasticity, and the amount of neuropsin mRNA is related to memory retention after a chemically induced ischemic insult [78]. The human neuropsin gene (hK8) was first isolated from the hippocampus [47]. A recent report described the association of hK8 expression with diseases of the central nervous system, including epilepsy [79,80]. In addition, an 11.fivefold increase in KLK8 mRNA levels in Alzheimer's disease hippocampus compared to controls was recently reported. The same study has shown that KLK1, 4, 5, 6, 7, 8, 10, 11, 13 and 14 are expressed in both cerebral cortex and hippocampus, while KLK9 is expressed in cortex but not hippocampus [81]. Another kallikrein, the zyme/protease M/neurosin gene (KLK6), was isolated from Alzheimer's disease brain, and was shown to have amyloidogenic activity [82,83]. KLK6 was also found to be localized in perivascular cells and microglial cells in human Alzheimer's disease brain [82]. Little *et al.* suggested that hK6 may play a

Table 2
The role of kallikreins in cancer diagnosis/prognosis[1]

Kallikrein	Sample type	Application	Reference
hk2	Serum and tissue	Diagnosis, prognosis and monitoring of prostate and breast cancer	[114]
hK3 (PSA)	Serum and tissue	Diagnosis, prognosis and monitoring of prostate and breast cancer	[30]
KLK4	Ovarian cancer tissue	Unfavorable prognostic marker	[94]
KLK5	Ovarian cancer tissue	Unfavorable prognostic marker	[95]
	Breast tumor cytosols	Unfavorable prognostic marker	[98]
	Normal and prostate cancer tissues	Lower expression in more aggressive tumors	[115]
	Normal and testicular cancer tissues	Down-regulation in advanced cancer	[116]
hK6	Serum	Diagnosis, prognosis and monitoring of ovarian cancer	[99]
	Breast tumor cytosols	Prognosis: association with hormone receptors	our unpublished data
KLK6	Ovarian cancer mRNA	Overexpression in ovarian cancer	[117]
KLK7	Ovarian cancer mRNA	Overexpression in ovarian cancer	[93]
KLK8	Ovarian cancer mRNA	Marker of favorable prognosis	[92]
	Ovarian cancer mRNA	Higher expression in ovarian cancer	[91]
KLK9	Ovarian cancer mRNA	Marker of favorable prognosis	[97]
KLK9	breast cancer mRNA		[118]
hK10	Serum	Diagnosis and monitoring of ovarian cancer	[101,102]
	Ovarian cancer cytosols	Prognosis; high levels associated decreased survival	Our unpublished data
hK11	Serum	Diagnosis and prognosis of ovarian cancer	[103]
KLK12	Breast cancer mRNA	down-regulation in breast cancer	[40]
KLK13	Breast cancer mRNA	down-regulation in a subset of breast tumors	[41]
KLK14	Ovarian cancer mRNA	Marker of favorable prognosis	[119]
	Breast cancer mRNA	Down-regulated in breast cancer	[42]
	Normal and testicular cancer mRNA pairs	Down-regulation in cancerous tissue	[42]
KLK15	Ovarian cancer mRNA	Marker of poor prognosis	Our unpublished data
	Breast cancer mRNA	Marker of favorable prognosis	[118]
	Matched tissue from normal and cancerous tissues	Marker of favorable prognosis	Our unpublished data

1. hK, kallikrein protein; KLK, kallikrein gene transcript.

role in the development of Alzheimer's disease [82]. Trypsin-like serine protease (KLK11), another newly discovered kallikrein gene, was isolated from brain hippocampus cDNA and is thought to play a role in brain plasticity [48]. Similarly, KLK5 and KLK14 are also expressed at high levels in the brain [42,84], and might have roles in brain physiology and pathobiology. For more detailed discussion about the possible involvement of kallikreins in the CNS, we refer the reader to our recent review [85].

7.2. Kallikreins in cancer

The relation between kallikreins and cancer is well established [50,86,87]. Prostate specific antigen (hK3) and human glandular kallikrein 2 (hK2) are established or evolving markers for prostate cancer, respectively, and more recently, they were also linked to breast cancer [88]. With the identification and characterization of all members of the kallikrein gene family, accumulating evidence has suggested that other kallikreins might be also related to hormonal malignancies (for instance, breast, prostate, testicular and ovarian cancers). KLK6 was originally isolated by differential display from an ovarian cancer library [46] and KLK10 was cloned by subtractive hybridization from a breast cancer library [89], and later suggested to act as a tumor suppressor gene [90]. Underwood *et al.* [91] and Magklara *et al.* [92] have shown that KLK8 is differentially expressed in ovarian cancer. KLK7 is also up-regulated in ovarian cancer patients [93], and KLK4 and KLK5 are indicators of poor prognosis of ovarian cancer [94–96]. Also, KLK9 is a marker of favorable prognosis for ovarian cancer [97], and KLK5 is a prognostic marker for breast cancer [98]. In addition, we have shown that KLK13 is down-regulated in breast cancer tissues, and that KLK15 is up-regulated in prostate cancer tissues compared to their normal counterparts (our data, submitted for publication).

At the protein level, recent reports show that kallikrein proteins could be useful serum biomarkers for diagnosis and prognosis of cancer. In addition to the diagnostic value of hK3 and hK2 in prostate cancer, hK6 and hK10 are emerging diagnostic markers for ovarian cancer [99-102]. More recently, hK11 was also shown to be a potential marker for ovarian and prostate cancer [103]. A synthetic hK1 inhibitor was recently found to suppress cancer cell invasiveness in human breast cancer cell lines [104].

In Table 2, we summarize published data on the assessment of kallikrein gene and protein expression 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 kal-

likreins have already found important clinical applications while some other members 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 biologic pathways involved are delineated. For example, the enzymatic activity of these serine proteases may initiate biologic events (*e.g.*, tumor invasion, activation of hormones, growth factors, other enzymes, receptors or cytokines, amyloid formation) or terminate biologic 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. Literature on these topics does not currently exist since the function of most kallikrein enzymes is not known.

8. Conclusions

Knowledge on human kallikrein genes is evolving rapidly. Now that all members of the human kallikrein gene family are characterized, it will be interesting to study the physiologic function of the proteins and their possible connection to pathologic processes. Also, interesting is the recent association of kallikrein gene expression with cancer, CNS, skin and other systems and the finding that many circulating kallikreins are biomarkers for cancer. The examination of tissue kallikreins as therapeutic targets (through activation or inhibition) may also be interesting. Clearly, over the next 3 to 5 yr, the physiology and pathobiology of this large family of serine proteases will be more precisely defined.

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