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Review

Kallikrein-related peptidases

Å. Lundwall[a,*] and M. Brattsand[b]

[a]Department of Laboratory Medicine Malmö, Clinical Chemistry, Lund University, University Hospital MAS, 205 02 Malmö (Sweden), Fax: +46 – 40337043, e-mail: Ake.Lundwall@med.lu.se
[b]Department of Public Health and Clinical Medicine, Dermatology and Venereology, Umeå University, 901 85 Umeå (Sweden)

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Abstract. Kallikrein 1 (KLK1), a key component of the kallikrein-kinin system, originates from a locus on the long arm of chromosome 19 that contains several related serine endopeptidases. The biological role of these kallikrein-related peptidases is not clear, but emerging evidence suggests that they might be important in several physiological systems, *e.g.*, in male reproduction, skin homeostasis, tooth enamel formation and neural development and plasticity. The kallikrein locus has undergone some major evolutionary events. Most spectacular are relatively recent duplications of KLK1 that have created 13 and 9 functional genes that are unique to the mouse and the rat, respectively. Human paralogs are KLK2 and KLK3: the latter encoding the cancer biomarker prostate-specific antigen. In this review on kallikrein-related peptidases, the focus is on their evolution, their role in skin homeostasis and semen liquefaction, and their utility as cancer biomarkers.

Keywords. Semen, liquefaction, skin, desquamation, protease, cancer, evolution, prostate.

Introduction

In 1930, it was reported that the pancreas is a rich source of an endogenous hypotensive substance, which, based on the Greek word for pancreas, was given the name kallikrein [1]. For many years, the substance, now known as kallikrein 1 (KLK1) or the tissue kallikrein, was named the glandular kallikrein to distinguish it from the later discovered plasma kallikrein (KLKB1). Both the plasma and the tissue kallikrein are serine endopeptidases that by specific proteolysis of high and low molecular mass kininogen (HMWK and LMWK, respectively), the products of two splice variants of the kininogen gene (KNG1), can generate vasoactive and spasmogenic kinins. KLKB1, which is part of the plasma contact activation system, is a complex multi-domain protein that is structurally related to coagulation factor XI, whereas KLK1 display a more simple structure related to that of trypsin.

In a classic paper from 1983, Mason et al. [2] demonstrated that the mouse and the rat genomes carry several genes that are closely related to KLK1. The homologous genes were assigned to a novel gene family, denoted the glandular kallikrein gene family, and the number of genes was estimated to a total of 25 in the mouse and 10 transcribed genes in the rat [3, 4]. Many of the genes were found to be transcribed in the rodent salivary glands, from where their translation products could also be isolated [5]. Some of the glandular kallikreins were found in high molecular mass complexes with precursors of growth factors, *e.g.*, the epidermal (EGF) and nerve (NGF) growth

* Corresponding author.

factors, from which they could also liberate bioactive peptides by proteolysis [6, 7]. Others, like gammarenin and tonin, were also found to release bioactive peptides from precursor molecules and, as a similar function was attributed to KLK1, it was hypothesized that the glandular kallikreins formed a gene family of protein processing endopeptidases, which in a regulated way could generate bioactive peptides from high molecular mass precursors [2]. However, analysis of DNA from other species indicated that a large number of glandular kallikreins perhaps was unique to murine rodents and that other mammals might have substantially fewer genes. There were only three glandular kallikreins identified in humans: KLK1 [8], KLK2 (initially called human glandular kallikrein 1, hGK1, and later human kallikrein 2, hK2) [9], and KLK3, also known as prostate-specific antigen (PSA) [10]. Since none of the novel, potentially important, glandular kallikreins discovered in rodents was present in humans, much of the scientific interest in the gene family was lost.

At around the turn of the millennium, three research groups independently discovered that the human kallikrein locus on the long arm of chromosome 19 carries a number of genes encoding serine endopeptidases that are related to KLK1 [11–13]. The novel genes were merged with the existing genes at the kallikrein locus to form an extended kallikrein gene family. Unfortunately, there was initially some confusion regarding the nomenclature of the genes, as some of the novel genes accidentally were given names already occupied by unique genes at the rodent kallikrein loci. This has since been corrected and a new comprehensive nomenclature has been worked out [14]. In the new nomenclature, all genes at the locus, except kallikrein 1, are denoted kallikrein-related peptidases. The discovery of the extended kallikrein locus has had a vitalizing effect on the research field, both in terms of new projects on previously unknown proteins, but also by bringing together researchers from different fields, *e.g.*, reproduction, dermatology, neurology, dentistry and oncology.

In this review, we give an overview on the current knowledge on the proteins encoded by genes at the kallikrein locus. Special focus is given to the organization and evolution of the locus and the function of some constituent genes in well-defined biological systems and compartments. We also briefly address the utility of kallikrein-related peptidases as tumor markers.

The human kallikrein-related peptidase gene family

The human kallikrein locus is situated 7.5 Mb from the telomeres of the long arm of chromosome 19, in the cytogenic region q13.3–4. The locus spans 265 kb and carries 15 functional genes and at least 1 pseudogene (Fig. 1A). There is conflicting information regarding the pseudogene, as overlapping DNA sequences have been reported to give rise to either a pseudogene of five exons or a processed pseudogene consisting of one exon [15, 16]. Both are reported to be transcribed, yielding transcripts encompassing interspersed repeat sequences and nucleotides that are homologous with exon 2 of KLK1–3 (Fig. 2). The two nucleotide sequences with homology to exon 2 of KLK1–3 could also be regarded as two short pseudogenes, as depicted in Figure 1A. Except for KLK2 and KLK3, all functional genes are transcribed from the negative DNA strand. They are organized in a similar way as genes of other simple serine peptidases of the chymotrypsin clan, with five exons encompassing the coding nucleotides and with the residues of the catalytic triad located on separate exons. The translation products are synthesized with amino terminal signal peptides, 16–34 amino acid residues in length, which targets them to the endoplasmic reticulum for secretion. The signal peptide is cleaved off to yield the inactive zymogen form of the peptidase, which carries a short N-terminal propeptide. The peptide chains of the zymogens have molecular masses of 24–29 kDa, but due to glycosylation the sizes of the mature proteins are slightly larger with apparent molecular masses in the range of 30–40 kDa (Table 1). The fully active enzyme is generated by removal of the propeptide by specific proteolysis. This generates a conformational change that opens up the catalytic cleft due to an interaction between the amino group of the newly formed N-terminal residue and the carboxylate group of the conserved Asp located next to the catalytic Ser. This highly regulated step is accomplished by trypsin-like enzymes in 14 out of 15 human kallikrein-related peptidases, as revealed by the presence of an Arg or Lys residue in the zymogen immediately before the N terminus of the mature enzyme. It is only KLK4 that differ, by having a Gln residue at this position.

The kallikrein-related peptidases are homologous with trypsins. Amino acid sequence comparison with the prototype trypsin from bovine pancreas shows that 40–47 % of the trypsin sequence is conserved in the kallikrein-related peptidases. This relatively high sequence conservation is of the same order of magnitude as between most of the kallikrein-related peptidases (Table 2). Were it not for the location close to KLK1, most of the kallikrein-related peptidases

Table 1. Molecular properties of human kallikrein-related peptidases.

	Precursor (No. of amino acid residues)			Zymogen		
Gene	Signal peptide	Propeptide	Zymogen	Mol. mass (kDa)	p*I*	*N*-linked glycosyl.
KLK1	17	7	245	27.2	4.45	3
KLK2	17	7	244	27.0	7.10	1
KLK3	17	7	244	26.8	7.73	1
KLK4	26	4	228	24.4	4.56	1
KLK5	29	37	264	28.9	8.38	4
KLK6	16	5	228	25.1	7.09	1
KLK7	22	7	231	25.2	8.56	1
KLK8	23	9	237	25.5	6.76	1
KLK9	19	3	231	25.6	7.17	3
KLK10	33	13	243	26,7	8.42	1
KLK11	18	3	232	25.6	8.38	4
KLK12	17	4	231	25.0	7.68	2
KLK13	20	15	257	28.6	8.55	2
KLK14	34	6	233	25.5	9.10	1
KLK15	16	5	240	26.4	8.03	2

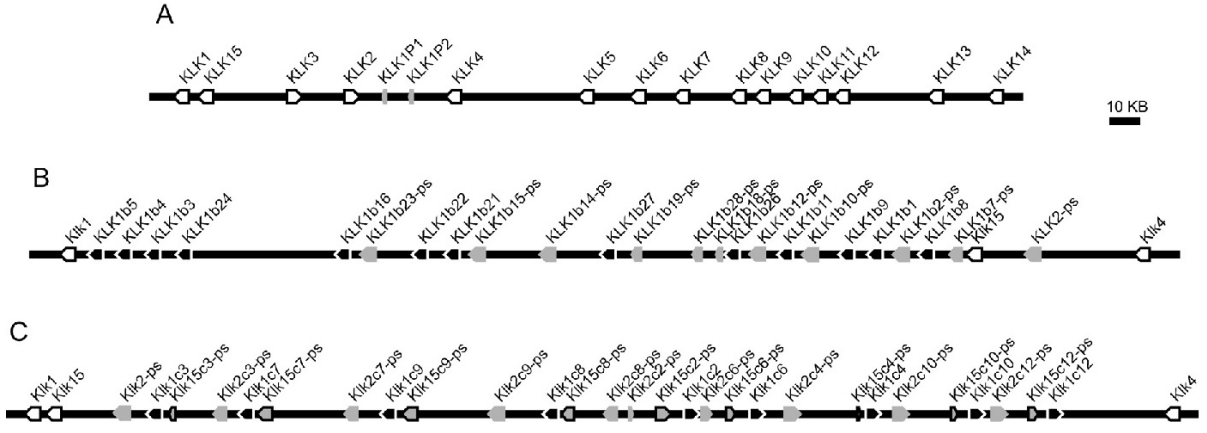

Figure 1. Schematic representation of the kallikrein locus showing the location and relative orientation of genes. The complete human kallikrein locus is illustrated in (*A*) and the regions encompassing unique rodent genes are shown for the mouse (*B*) and the rat (*C*). Functional human genes and their orthologs in mouse and rat are indicated with open arrowheads. The filled arrowheads denote unique functional genes in the mouse and the rat. Pseudogenes are represented by shaded arrowheads. The size of the arrowheads reflects the number of coding exons so that one exon is equivalent to 1 kb: functional genes carry five coding exons, which is equivalent to an arrowhead of 5 kb. The sequences with homology to exon 2 of KLK1–3 were given the tentative gene symbols KLK1P1 and KLK1P2.

could therefore equally well have been named trypsins. It should also be noted that the primary structures of KLK1–3 are more similar to each other than to the remainder of the kallikrein-related peptidases, suggesting that they form a phylogenetically young subgroup of the gene family. KLK1–3 also lack the disulfide that bridges Cys109 and Cys210 in bovine cationic trypsin, which is present in the other kallikrein-related peptidases, except KLK13.

The similarity of kallikrein-related peptidases to trypsins is not only confined to the primary structure, as they also display similar catalytic properties. Extensive studies on the structure and function of pancreatic trypsin have demonstrated that the enzyme consists of two similarly folded domains with the catalytic residues located in a shallow depression between these structural domains [17]. The catalytic specificity of trypsin very much depends on a negatively charged Asp located at the base of the substrate-binding pocket, which can interact with positively charged Lys and Arg residues in trypsin substrates. A similarly located Asp is also present in KLK1 and is vital for the catalytic recognition of a specific Arg residue in the kininogens [18]. The

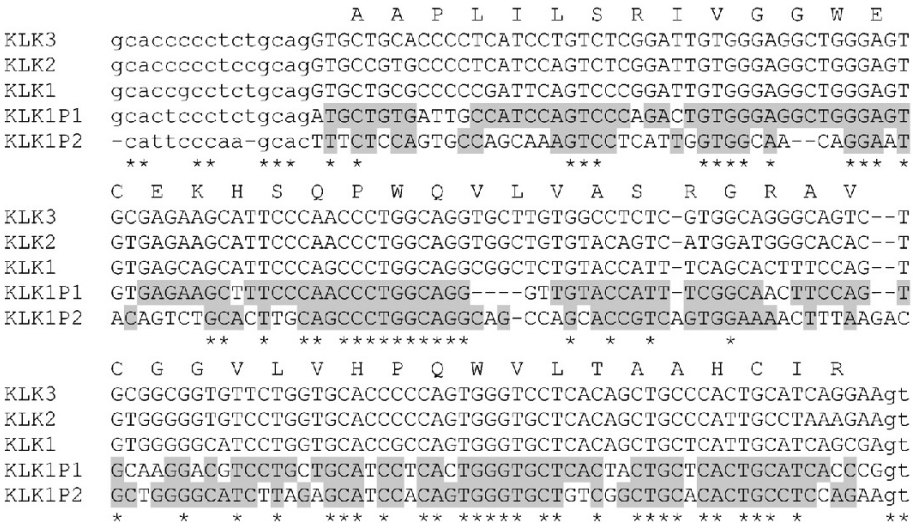

Figure 2. Alignment of KLK1–3 and homologous pseudogenes. Nucleotides of exon 2 and flanking sequences were aligned with CLUSTAL W. The aligned sequences are displayed with the translation of KLK3 on top and conserved nucleotides in the pseudogenes shaded. Exon sequences are written in capital letters and intron sequences in lower case letters. Dashes represent gapped nucleotides and nucleotides conserved in all sequences are indicated by stars.

Table 2. Conservation of human kallikrein-related peptidases. The amino acid sequences were aligned with CLUSTALW and the percent of identical amino acids were calculated. For comparison, the sequence of bovine trypsin was included in the alignment.

	KLK1	KLK2	KLK3	KLK4	KLK5	KLK6	KLK7	KLK8	KLK9	KLK10	KLK11	KLK12	KLK13	KLK14	KLK15
KLK1		65	61	41	40	39	42	45	41	36	44	42	41	44	40
KLK2			79	41	44	47	44	46	42	40	46	43	46	45	42
KLK3				42	43	41	43	43	41	36	42	42	46	44	42
KLK4					52	41	46	42	38	37	43	43	43	45	40
KLK5						46	48	49	49	40	51	46	51	50	44
KLK6							42	48	44	41	46	46	53	47	46
KLK7								46	41	43	44	46	46	45	43
KLK8									50	46	50	50	50	50	48
KLK9										40	57	46	47	48	49
KLK10											39	49	43	42	43
KLK11												50	52	49	52
KLK12													48	48	49
KLK13														51	47
KLK14															48
Trypsin	43	46	42	40	47	44	43	47	43	43	47	47	46	46	47

homologous position in all but four kallikrein-related peptidases is also occupied by Asp, suggesting a trypsin-like specificity (Fig. 3). A tryptic or Arg-restricted specificity has also been confirmed with KLK2 [19, 20], KLK4 [21–23], KLK5 [22–26], KLK6 [22, 27–29], KLK8 [30, 31], KLK10 [22], KLK11 [22, 32], KLK12 [33], KLK13 [23] and KLK14 [23, 34, 35]. The architecture of the catalytic cleft in KLK10, KLK11 and KLK14 also allow the enzymes to accept amino acid residues with large hydrophobic side chains, *e.g.*, Tyr, Met, and Leu, similar to chymotrypsin. This is in accordance with the catalytic properties of KLK1, which beside the Arg specificity also cleaves C-terminal to Met379 in kininogens when liberating kallidin. In KLK3, KLK7, KLK9 and KLK15, the Asp is replaced by Ser, Asn, Gly and Glu residues. By having the same charge, the Glu182 in KLK15 might fulfill a similar function as the Asp171 in trypsin. The specificity of KLK7 is chymotrypsin-like as indicated by its alternative name: stratum corneum chymotryptic enzyme [36, 37]. The Ser183 in KLK3 is equivalent to Ser174 in chymotrypsin and it was early speculated that KLK3 might display chymotrypsin-like activity [10]. Studies with its natural substrates, semenogelin 1 (SEMG1) and semenogelin 2 (SEMG2), confirms a chymotrypsin-like activity with peptide chain cleavage following Tyr, Phe and Leu, but also an extended spectrum with cleavages, *e.g.*, after Gln and His [38, 39]. Using peptide substrates KLK3 was also shown to cleave after Lys and Arg, and it has been suggested

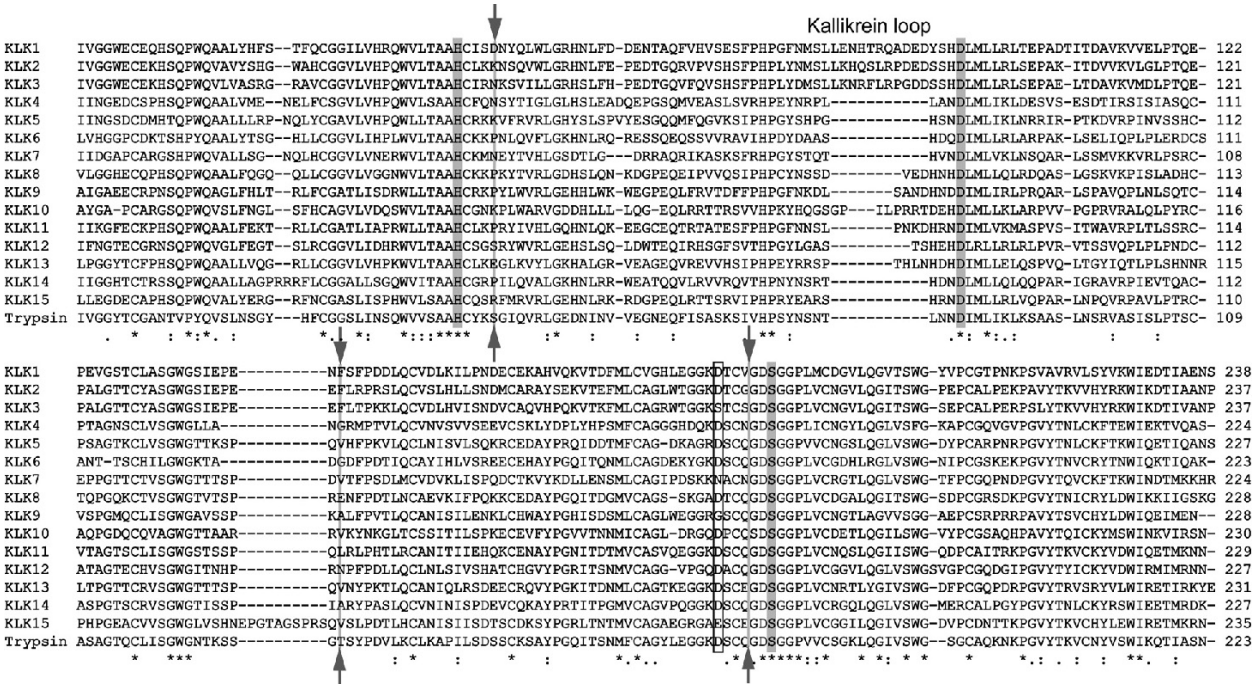

Figure 3. Sequence alignment of human kallikrein-related peptidases. Gapped residues are marked by dashes, residues of the catalytic triad are shaded and the residues at the base of the catalytic pocket are framed. The locations of introns are indicated with arrows. A sign below the aligned sequences denote conserved residues; a star indicates that the same residue is present in all sequences and a colon or a dot indicates that there is at least one conserved or semi-conserved substitution.

that the peptidase preferentially cleaves after residues with medium-sized side chains [22]. KLK9 is similar to elastase in having a Gly at the base of the catalytic cleft, but whether KLK9 display elastase-like activity is not yet known.

Early studies on the structure of porcine KLK1 showed that the protein has a structural element of 11 amino acid residues following Asn78 of the mature peptide chain, which is not present in trypsin and chymotrypsin. The structure, denoted the kallikrein loop, flanks and partly covers the catalytic cleft and probably affects the access of protein substrates (Fig. 4). The kallikrein loop is conserved in KLK2 and KLK3, but is absent or shorter in the remaining kallikrein-related peptidases (Fig. 3). Molecular dynamics simulations have shown that the kallikrein loop can adopt two different conformations: one open with substrate access to the catalytic residues and one closed where the kallikrein loop acts as lid over the catalytic cleft [40]. Crystallographic studies have also confirmed two such structures: an open conformation was observed in the porcine urinary kallikrein, whereas the kallikrein-related peptidase isolated from stallion seminal plasma displayed the closed conformation [18, 41]. Other unique features of members to the kallikrein-related peptidase gene family are an extended C-terminus in KLK13 and a 10-residue insertion between residue 129 and 138 in KLK15 (Fig. 3). In trypsin, and also in chymotrypsin, there is a loop between Trp121 and Asp133. The loop is recognized by trypsin, which can cleave itself at Lys125 and chymotrypsin at a homologous Arg, leading to two-chain molecules that are sensitive to further degradation and inactivation. The 10-residue insertion in KLK15 will presumably enlarge this loop, but whether this is of any importance for the stability of KLK15 is not known.

The tissue tropism of the expression has been studied with various methods, *e.g.*, RT-PCR, hybridization to tissue blots, immunoassays, immunohistochemistry and *in situ* hybridization [12, 42–44]. An early conclusion from these studies was that many of the kallikrein-related peptidases are expressed in hormone-sensitive tissues, *e.g.*, in prostate, breast and ovary [45]. The relative expression levels of genes can also be monitored by counting expressed sequence tags (EST), which basically reflects the mRNA composition of a certain tissue. Compiled human EST data from the UniGene database (http://www.ncbi.nlm.nih.gov/UniGene/) shows that most of the kallikrein-related peptidases are moderately expressed, with expression levels in the range of a few hundred transcripts per million (tpm) or less (Table 3). Exceptions are KLK2 and KLK3, which display very high transcript levels, particularly in the prostate. On the other end of the scale are KLK9, KLK12, KLK14

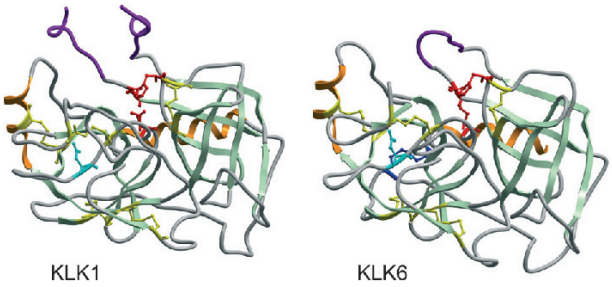

Figure 4. Ribbon structure displaying the kallikrein loop. Protein databank structure files 1SPJ [171] and 1LO6 [27] were displayed using the ICM Browser (Molsoft, La Jolla). Residues of the catalytic triad (painted red) and the Asp at the base of the catalytic pocket (painted light blue) are given as stick and ball representations. An extended kallikrein loop from Phe77 to Tyr93 in KLK1 is colored purple: the gap in the loop structure is due to lack of coordinates for His85 and Thr86. The homologous loop in KLK6 is also colored in red and extends from Tyr77 to His82. The disulfide bridges are colored yellow, except the one in KLK6 that is missing in KLK1, which is colored dark blue.

and KLK15, for which the total number of EST in the database is less then 10. The tissue distribution of EST confirms the previously noted expression in hormone-sensitive tissues, but also show that the genes are transcribed in many hormone-insensitive tissues as well, *e.g.*, in the gastrointestinal tract and a variety of glandular tissues. Another general conclusion that can be drawn from the expression studies is that, although the genes are expressed in many different tissues, they are rarely transcribed in the liver. This suggests that the kallikrein-related peptidases are locally acting in the tissues and at the mucosal sites where they are synthesized, as opposed to liver-secreted systemic proteases, such as thrombin, plasmin and plasma kallikrein.

Evolution of kallikrein-related peptidase genes

As mentioned in the introduction, studies in the 1980s indicated that the family of kallikrein-related peptidases is larger in rodents than in most other mammals, including humans. When the new genes of the extended human kallikrein locus were discovered, many scientists believed that they were the equivalents of the missing rodent genes and that in fact there was no major difference in the number of genes between humans and rodents. These assumptions were soon refuted by the demonstration of mouse orthologs to the human KLK4–15 [46]. Furthermore, a comparison of the kallikrein loci on human chromosome 19 and mouse chromosome 7 showed that most of the excess kallikrein-related peptidases in the mouse are located between Klk1 and Klk15: a region comprising 1.5 kb in the human genome, but that in the mouse genome is 286 kb and carries 13 functional genes and 10 pseudogenes (Fig. 1B). These genes of the Klk1b subfamily are very conserved and have 67–91 % of the primary structure in common (Table 4). Presumably, they are the result of repeated duplications that started with a duplication of Klk1. The location of the genes also indicates that there is no mouse ortholog to KLK2 and KLK3. Instead there is a pseudogene at the mouse kallikrein locus with a similar location as of KLK2 and KLK3 at the human locus. The pseudogene, Klk2-ps, is closely related to a progenitor of KLK2 and KLK3, as it segregates with these genes in phylogenetic analyses, as opposed to the genes of the Klk1b subfamily, which segregate with KLK1.

The kallikrein locus on rat chromosome 1 also carries Klk4–14 in a similar way as at the human and mouse loci. Given the relative closeness in phylogeny between the mouse and the rat, it might have been expected that the excess Klk1-related genes in the two species are orthologous. However, this is not the case, as analysis of the rat kallikrein locus indicates a different evolutionary history than in the mouse. A DNA segment consisting of Klk1, Klk15 and Klk2 has been subjected to repeated duplications, yielding an expansion of the locus with more than 300 kb [47]. The net result is ten copies of each gene (Fig. 1C). All copies of Klk2, and all but one copy of Klk15, have been silenced, whereas all copies of Klk1, the Klk1c subfamily, are functional. They are very similar in structure, with the transcribed genes yielding translation products with 74–89 % conservation of the amino acid sequence (Table 5).

Studies on primates show that the human kallikrein locus is fully conserved in the chimpanzee [48]. The locus is presumably also conserved in Old Word monkeys, as revealed by genes identified in the still not fully completed *Macaca mulatta* Genome Project. A functional macaque KLK3 gene was also demonstrated by cloning of its cDNA from a prostate tissue specimen [49]. Furthermore, phylogenetic studies show that duplication of KLK1 predates the split of the lineages leading to New and Old World monkeys [50]. A closer examination of kallikrein-related peptidase genes in the cotton-top tamarin, a New World monkey, revealed that this primate carries a KLK2 pseudogene but no KLK3 gene [51]. However, based on sequence conservation, it was concluded that the KLK3 gene had been deleted from the genome of an ancestor to the cotton-top tamarin.

Analysis of kallikrein-related peptidase genes with Southern blotting using a probe that recognize KLK1–3 yielded a single hybridizing fragment in DNA from cow and pig [47]. Earlier studies had clearly demonstrated that both the cow and the pig

Table 3. Tissue distribution of kallikrein-related peptidase transcripts. The expression of kallikrein-related peptidase genes is based on the number of identified EST in tissues as compiled in the UniGene database. The relative expression levels are displayed as transcripts per million (tpm). KLK9, KLK12, KLK14 and KLK15 were omitted from the table as they were represented by less than ten transcripts in the database.

Tissue	KLK1	KLK2	KLK3	KLK4	KLK5	KLK6	KLK7	KLK8	KLK10	KLK11	KLK13
Adrenal gland			30					30			
Ascites	25					25	250		150	50	
Bladder	68										
Blood				8							
Bone	28	210	294								
Bone marrow		20	249								
Brain			5	1		42	15	1	4	3	
Cervix					20						
Connective tissue	6									6	
Ear											
Embryonic tissue						4					
Esophagus						198	149	49	49		
Eye									19		
Heart	22				45		22			45	
Intestine	17		4			60	4		21	43	
Kidney	99				19	52	14				
Larynx								87	87		
Liver								4			
Lung		2			2	2	2	2	20	17	
Lymph											
Lymph node					154	628	44	33			
Mammary gland			1515		6	87	20	13	13	6	
Muscle		28	93							9	
Nerve		1341	1725								
Ovary	9				128	228	79	59	79	79	
Pancreas	131					4	9		65	4	
Parathyroid											
Pharynx		24				322	24		24	24	247
Pituitary gland										60	
Placenta								10			
Prostate		3223	5854	130					5	54	
Salivary gland	199		99								
Skin	4				14		71		9	9	
Soft tissue								76		76	
Spleen											
Stomach					41	209	20	20		20	
Testis		12			3				6	30	18
Thymus											
Thyroid	43								21		
Tongue					30	15	15		107	61	46
Tonsil	118										
Trachea										38	
Umbilical cord											
Uterus				17	8	17	13	17	34	17	
Vasculature											
Total EST	71	640	1385	30	50	210	76	31	76	75	19

have a functional KLK1 gene [18]. This was also verified by sequencing of KLK1 transcripts derived from kidneys, and it was concluded that the hybridizing DNA fragment was derived from the KLK1 gene [47]. This lends support to the hypothesis that the kallikrein locus in artiodactyls contains a single KLK1 gene and no KLK2 or KLK3 gene. This was also partly verified by analysis of DNA sequences generated from the Swine Genome Project [48]. Interestingly, the horse, an ungulate like the cow and the pig, but of the perissodactyl family, yielded at least six hybridizing DNA fragments [47]. This might suggest repeated duplications of the equine KLK1 gene in a similar way as in the rat and the mouse, but with less number of duplications. Phylogenetic analysis also revealed that a kallikrein-related peptidase gene expressed in the horse prostate, and which had been considered to be the equine PSA [41], was more closely related to KLK1 than to KLK3 [47]. Thus, it is not the equine KLK3/PSA, but a paralog.

Both Southern blotting and analysis of DNA sequences generated through the Dog Genome Project showed that there are two kallikrein-related peptidase genes in the dog. These are the canine KLK1 and a related arginine esterase [52, 53]. Both the location on the chromosome and the phylogenetic analysis sug-

Table 4. Conservation of mouse kallikrein-related peptidases of the Klk1b subfamily. The amino acid sequences were aligned with CLUSTAL W and the percent of identical amino acids were calculated.

	Klk1 homolog												
	b1	b3	b4	b5	b8	b9	b11	b16	b21	b22	b24	b26	b27
Klk1	74	78	82	91	72	75	75	69	77	75	75	72	73
Klk1b1		80	75	77	72	87	79	72	78	77	77	74	78
Klk1b3			79	80	74	84	79	72	79	79	77	74	78
Klk1b4				82	70	76	75	67	74	73	75	71	73
Klk1b5					73	78	79	72	79	79	78	75	77
Klk1b8						73	77	80	77	77	78	85	75
Klk1b9							78	71	78	77	76	73	79
Klk1b11								73	83	79	85	77	84
Klk1b16									74	76	75	84	73
Klk1b21										80	89	78	89
Klk1b22											79	78	80
Klk1b24												80	88
Klk1b26													75

Table 5. Conservation of rat kallikrein-related peptidases of the Klk1c subfamily. The amino acid sequences were aligned with CLUSTAL W and the percent of identical amino acids were calculated. Mouse Klk1 and bovine trypsin were included in the alignment for comparison.

	Klk1	Klk1c2	Klk1c3	Klk1c4	Klk1c6	Klk1c7	Klk1c8	Klk1c9	Klk1c10	Klk1c12
Klk1		74	79	79	79	80	81	75	80	75
Klk1c2			79	76	79	78	74	84	79	79
Klk1c3				84	85	87	80	78	85	82
Klk1c4					86	83	77	76	89	83
Klk1c6						86	79	77	88	87
Klk1c7							80	78	85	84
Klk1c8								72	79	77
Klk1c9									78	74
Klk1c10										83
Mouse Klk1	71	61	67	67	67	69	69	61	67	63
Bovine trypsin	39	39	42	43	43	42	42	39	41	41

gested that the arginine esterase is more closely related to KLK2 and KLK3 than to KLK1. Thus, in contrast to mouse Klk2-ps, the arginine esterase seems to represent a functional progenitor of KLK2 and KLK3. It was named canine KLK2, as the enzyme's catalytic properties more resemble those of KLK2 than of KLK3 [47].

The DNA sequences available through the Opossum Genome Project allowed analysis of the kallikrein locus also in a Metatherian species [48]. A total of 11 kallikrein-related peptidase genes for KLK5–KLK15 were identified. As expected, the opossum did not have KLK2 and KLK3 genes, but most conspicuously, it was also lacking the KLK1 and KLK4 genes. Whether the lack of KLK1 is unique to the opossum or is a general feature of primitive mammals remains to be seen. In any case, there seems to be a functional kallikrein/kinin system also in the opossum, as its genome contains both the plasma kallikrein and two bradykinin receptors.

It can be concluded that the majority of kallikrein-related peptidase genes are present in most mammals, although a thorough analysis of the kallikrein locus in monotremes is yet to be done. All or at least most eutherian mammals seem to carry one functional gene for each of KLK1 and KLK4-KLK15. Early in the phylogeny of eutherian mammals, there was a duplication of KLK1, yielding a progenitor of dog KLK2 and mouse Klk2-ps. In primates, there was an additional duplication yielding KLK2 and KLK3. Interestingly, KLK2 and KLK3, or their progenitor, has since been silenced in several species. The KLK1 gene was again duplicated, this time at a late phylogenetic stage following the separation of artiodactyls and

perissodactyl and also after the separation of the lineages leading to mouse and rat. These events yielded unique genes with close similarity to KLK1 in the mouse, the rat and the horse. Presumably, these phylogenetically late events were driven by an evolutionary pressure exerted by something in common to these three species. It has been speculated that the common property is a similar setup of NK cell receptors [54].

The function of kallikrein-related peptidases

As mentioned before, KLK1 was discovered due to its effect on blood pressure. The primary substrate of KLK1 is LMWK [55]. Proteolysis generates the decapeptide kallidin, which may then be converted to the nonapeptide bradykinin by ubiquitously present amino peptidases. The biology of the kallikrein-kinin system has been the subject of several excellent recent reviews and is only briefly addressed here [55, 56]. The physiological effects of kallidin and bradykinin are exerted through the bradykinin receptors, of which there are two, denoted B1 and B2 [57, 58]. The blood pressure-lowering effect is primarily effectuated by signaling through the B2 receptors. In addition to vasodilatation, signaling through the G-protein coupled bradykinin receptors also generates edema by increasing vascular permeability, smooth muscle spasm by stimulating contraction of non-vascular smooth muscle cells and pain by stimulating C-fiber nociceptors. Beside of the vascular effects, the signaling through bradykinin receptors is important in innate immunity by also stimulating the secretion of pro-inflammatory cytokines. Another interesting function of KLK1 was identified in the short-tailed shrew [59]. The very potent venom in the saliva of this small mammal was identified as KLK1. It is believed that the toxicity is due to massive generation of bradykinin, with subsequent hypotension and other effects generated through the B1 and B2 receptors.

The excess paralogs of Klk1 in rodents are puzzling and their biological role is not understood. A little is known about the biochemical properties of some individual components, *e.g.*, it has clearly been demonstrated that mouse Klk1b9 can liberate EGF by specific proteolysis of its precursor protein [6]. In a similar way, Klk1b3, the gamma subunits of the mouse 7S NGF complex, generates NGF [60]. These effects suggest that at least some of the murine kallikrein-related peptidases are important in the regulation of cell proliferation and survival. It could be speculated that the presence in saliva of growth factor precursors and processing enzymes might have a role in wound healing. Presumably, these substances would initiate repair following wound licking.

Several kallikrein-related peptidases have been detected in the skin and in semen where they are important components of biological processes like skin desquamation and semen liquefaction, as addressed below. Endopeptidases of the extended kallikrein family have also been identified in other biological systems. KLK4 was originally named enamel matrix serine proteinase 1 (EMSP1), as it was identified as an important factor in the maturation stage of amelogenesis, *i.e.*, the process of teeth enamel formation [61]. KLK8, also known as brain serine peptidase or neuropsin, have been reported to play an important role in synaptic plasticity by degrading and rearranging extracellular matrix proteins [30]. By affecting long-term potentiation KLK8 is believed to be involved in learning and memory [62]. There are also reports on a pathological role of KLK8 in neurodegenerative diseases [63]. Similarly, KLK6, which is alternatively named neurosin or zyme, is also reported to be involved in neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease and multiple sclerosis [64–68].

Kallikrein-related peptidases in skin homeostasis

The skin is a multifunctional organ that protects the body from mechanical stress, UV-light and harmful agents like toxins, bacteria and viruses. It also keeps the homeostasis of the organism intact by preventing free diffusion of water molecules and ions over the skin barrier. The epidermis is the outermost layer of the skin that confers the barrier function. It is mainly built up from keratinocytes that are formed in a basal cell layer. From this layer, the cells migrate up towards the skin surface, at the same time as they undergo a tightly regulated differentiation. When the cells reach the outermost surface, the stratum corneum, they are metabolically dead, flattened and filled with keratin [69]. The cells normally reside at the skin surface for 1–4 weeks before they are shed in a process termed desquamation. To maintain a healthy epidermis, it is very important that there is a balance between proliferation in the basal cell layer and shedding of cells at the surface. Imbalance between the two processes causes problems, like in psoriasis (too high proliferation rate) or ichtyosis (shedding process too slow) [70].

In the late 1980s, it was shown that protease activity is required for the desquamation process to occur. Skin biopsies incubated in buffer showed a gradual shedding of cells. The shedding process was polar, *i.e.*, only cells from the outermost layers of stratum corneum were shed. Experiments showed that proteolytic degradation of desmosomes, the protein structures holding the keratinocytes together, were necessary for the desquamation to take place *in vitro* [71]. Addition

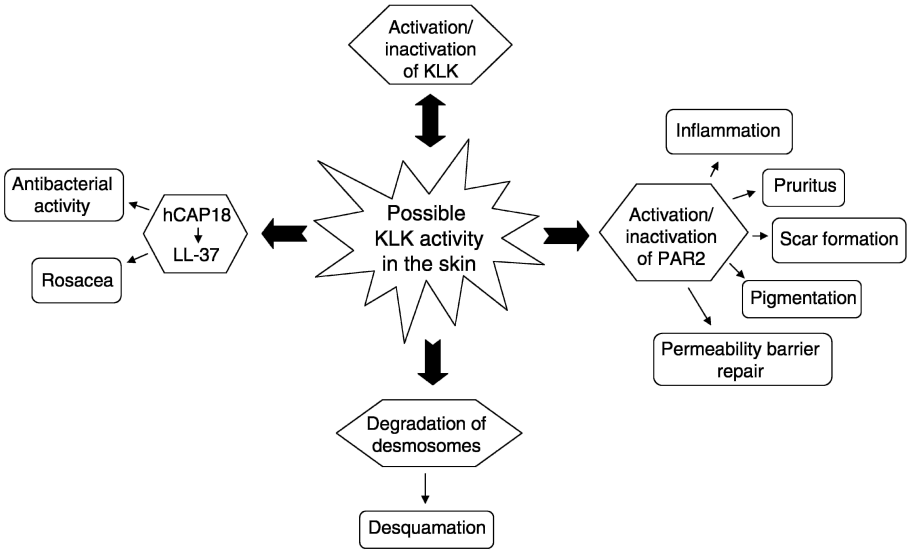

Figure 5. Schematic illustration of possible functions of kallikrein-related peptidases in the skin.

of different kinds of protease inhibitors demonstrated that serine proteases with both trypsin-like and chymotrypsin-like substrate specificity are involved in this process [72–76].

The first *in vivo* data in support of the idea that a well-regulated protease activity is important for the maintenance of a healthy skin was presented in 1992 by Wachter and Lezdey [77]. They treated patients suffering from severe atopic dermatitis with topical application of the serine protease inhibitor alpha-1 proteinase inhibitor. Application of the inhibitor stopped pain, pruritus and promoted tissue healing without scar formation in all tested patients.

Today, three kallikrein-related peptidases, KLK5, KLK7 and KLK14, have been isolated in active form from the outermost layers of the stratum corneum [24, 35, 78]. KLK5 and KLK7 are both expressed in the outermost living layers of the stratum corneum, the stratum granulosum, and outwards through the stratum corneum towards the skin surface. Expression can also be detected in the keratinized layers of the hair shaft and sebaceous glands [79–82]. KLK14 is primarily expressed in the sweat glands, but low levels have also been detected in the outer layers of the epidermis [35, 83]. According to RT-PCR and *in situ* hybridization data KLK1, KLK4, KLK6, KLK8–11 and KLK13 are also expressed in the epidermis [84, 85]. If these proteins also are present as active enzymes remains to be shown. No function of any of the kallikrein-related peptidases present in the epidermis is currently known, but data are accumulating that propose the involvement in skin physiology in various ways (Fig. 5).

Possible functions for kallikrein-related peptidases in the skin

Overexpression of human KLK7 in the mouse epidermis results in an itchy phenotype with hyperproliferation and reduced permeability barrier, measured as an increase in water loss that show many similarities with inflammatory skin conditions in human beings [86]. So far, the only knockout mouse published is the KLK8$^{-/-}$. This mouse has an apparently healthy skin unless inflammation is induced; the KLK8-deficient mouse shows a delayed recovery from UV-induced inflammation [87]. However, the apparent absence of phenotype does not exclude that KLK8 is involved in the normal skin physiology, as redundancy is a quite common feature in knockout animals.

Most work regarding kallikrein-related peptidases in skin physiology has been focused on KLK5 and KLK7 and their putative involvement in desquamation. The presence of active enzyme at the skin surface [24, 35, 78], immunolocalization coinciding spatially with the detachment region of desmosomes [79–82, 88], and ability to degrade desmosomal proteins at physiological pH [89] indicate that KLK5 and KLK7 are involved in desquamation. Lately, KLK1, KLK6, KLK13, and KLK14 have also been suggested to be involved in this process due to immunolocalization and ability to degrade desmoglein-1, one of the adhesive proteins in the corneodesmosome [90]. It has also been suggested that KLK8 is involved in the terminal differentiation of keratinocytes [91]. So far, KLK5 is the only enzyme found that has the ability to activate the pro-form of KLK7. KLK5 has also been shown to activate its own pro-form as well as pro-KLK14 *in vitro* [25, 89].

Kallikrein-related peptidases may also be involved in inflammation in the epidermis through the protease-

activated receptor (PAR) system. This is a G protein-coupled transmembrane group of receptors where cleavage of a tethered ligand on the receptor results in a conformational change that leads to intracellular calcium release and thereby signaling to downstream processes [92]. The PAR2 receptor is found on the surface of keratinocytes in the granular layer, endothelial cells, hair follicles, in myoepithelial cells of sweat glands and dermal dendritic cells [93]. It has been shown that KLK5, KLK6 and KLK14, but neither KLK7 nor KLK8, have the ability to activate the PAR2 receptor and KLK14 can also inactivate the PAR2 receptor at certain concentrations [83, 94]. Activation of PAR2 receptors located at nerve fiber ends may also contribute to itch in the epidermis [95]. Another possible involvement for kallikrein-related peptidases could be in scar tissue formation as PAR1 and PAR2 expression has been observed in all types of scars [96]. PAR2 has also been implicated in pigmentation [97] and epidermal permeability barrier homeostasis [98].

Antimicrobial activity is another important aspect of skin biology. Cathelicidin antimicrobial peptides are an important part of the innate immunity. The activity of the cathelicidins is, like the kallikrein-related peptidases, regulated by an enzymatic processing of the inactive proform (named hCAP18 in humans) to the mature, active peptide LL-37 (released from human neutrophils). Recently, it was shown that both KLK5 and KLK7 can cleave hCAP18 into LL-37 as well as into other smaller peptides with alternate biological activity. It has also been speculated that increased amounts of cathelicidins in combination with increases in KLK5 activity would be responsible for the pathogenesis of the inflammatory skin disorder rosacea [99, 100].

Regulation of kallikrein-related peptidase activity in the skin

The unique microenvironment of the outer epidermis influences the activity of the present enzymes. One important aspect is the pH gradient that exists over the outermost living layers and out towards the skin surface, with a neutral pH in the living layers, and an acidic environment at the skin surface [101]. The kallikrein-related peptidases 5, 7 and 14 show activity *in vitro* at the low pH found at the surface. As mentioned earlier, the propeptide of the kallikrein-related molecules have to be cleaved off to make the enzyme active. KLK5 has been suggested to play a key role, as it can activate 11 out of 15 zymogens of kallikrein-related peptidases [102]. In a proposed cascade reaction taking place in the skin, KLK5 autoactivates, and can then activate both proKLK7 and proKLK14. Activated KLK14 may then form a positive feed-back loop as it has the ability to activate proKLK5. The rate of these activation reactions has been shown to be pH dependent *in vitro* and the pH gradient in epidermis has been suggested to be important also for the amount of activated enzyme present [25, 89]. Besides KLK7 and KLK14, KLK5 can activate the pro-forms of KLK1, KLK6, KLK11 and KLK12 that have also been suggested to be part of the cascade reaction of kallikrein-related peptidases in the skin. KLK14 can furthermore activate proKLK1 and proKLK11, and there are also other examples of "cross-talks" in the suggested cascade reactions [102, 103].

Another important aspect of the skin physiology is the water content at the surface. It has been shown that the rate of desquamation is influenced by the humidity in the environment, and thereby the water content of the skin. When the relative humidity is below 80 % the desquamation rate decreases. The activity of KLK7 is water dependent, but compared to trypsin and chymotrypsin it seems to be adapted to function also in relatively dry environments [104, 105]. The water content also influences the concentration of electrolytes. Even at low concentrations, Zn^{2+} is a very potent inhibitor of different kallikrein-related peptidases [25, 106].

The importance of regulation of the proteolytic activity in epidermis has been emphasized in patients suffering from the severe skin disorder Netherton's syndrome, which is a rare, autosomal recessive disorder of cornification. The patients suffer from ichtyosis, hair shaft defects and atopy. The disease is caused by mutations in the *SPINK5* gene encoding a Kazal type serine protease inhibitor with the alternative name lymphoepithelial kazal type inhibitor (LEKTI) [107]. This protein is synthesized as a proform that consists of 15 different kazal type domains that all theoretically have inhibitory capacity [108]. After synthesis, the large inhibitor is processed into several single or multi-domain fragments with different inhibitory specificity. Several of the domains have been shown to be efficient inhibitors for KLK5, KLK6, KLK7, KLK13 and KLK14 *in vitro* [90, 109–111]. Fragment D8–D11 forms a very stable complex with KLK5. Reduction of pH eventually leads to a dissociation, which is in favor of the hypothesis that the complex keeps the KLK5 inactive until it reaches the outermost layers of the epidermis with lower pH where increased proteolytic activity is required for the desquamation to occur [109].

Two other serine protease inhibitors suggested to be involved in the desquamation process are skin-derived anti-leukoprotease (SKALP), also known as elafin or

peptidase inhibitor 3 (PI3), and secretory leukocyte protease inhibitor (SLPI), with the alternative names anti-leukoprotease (ALP) and human seminal proteinase inhibitor 1 (HUSI-1). Both inhibitors have the ability to potently reduce desquamation *in vitro*. In particular, SLPI is a potent inhibitor of KLK7, but SKALP also shows a weak inhibition of this enzyme [112]. Both inhibitors are naturally present in the epidermis, but SKALP is primarily expressed in association with inflammatory skin [113]. SLPI has also been suggested to have an important antimicrobial function in the skin [114].

Patients suffering from X-linked ichtyosis, which is caused by a deletion in the gene encoding steroid sulfatase [115], show hyperkeratosis with an increased thickness of stratum corneum. This has been associated with an increase in cholesterol sulfate. *In vitro* studies show that cholesterol sulfate acts as a potent inhibitor for both trypsin and chymotrypsin, and it could therefore be speculated that this substance would also regulate the activity of the kallikrein-related peptidases present in the epidermis [116]. Putative regulating factors in the epidermis are summarized in Figure 6.

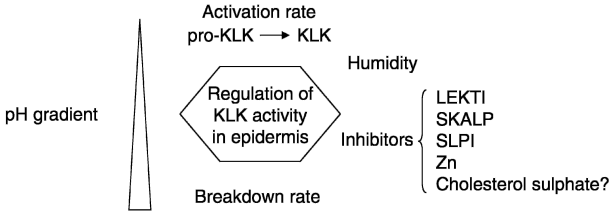

Figure 6. Different factors that may influence the activity of kallikrein-related peptidases present in the epidermis.

To summarize, the only thing that is known without question is that several kallikrein-related peptidases are present in the skin in active form. The true nature of their role in skin physiology is still to be proven.

Kallikrein-related peptidases in semen liquefaction

Human semen is formed at ejaculation, when epididymal fluid rich in spermatozoa is mixed with secretions from the accessory sex glands. The contribution of spermatozoa and epididymal fluid to the ejaculated volume is less than 5 %, whereas around 2/3 is provided by the seminal vesicles and 1/3 by the prostate. The newly ejaculated human semen is a semisolid coagulum that gradually dissolves: a process that within 10–20 min leads to complete liquefaction. The predominating structural components of the semen coagulum are the seminal vesicle-secreted SEMG1 and SEMG2 [117, 118]. It has been estimated that there are three to five times more SEMG1 than SEMG2 in seminal plasma [119, 120]. Monomeric SEMG1 is a non-glycosylated polypeptide of 50 kDa with an unusually high isoelectric point of 9.8. The unmodified SEMG2 monomer has an isoelectric point of 9.6 and a molecular mass of 63 kDa, but around half of the molecules are slightly larger due to glycosylation of a single Asn. SEMG1 and SEMG2 are homologous and carry 78 % fully conserved amino acid residues. The primary structures consist to more than 80 % of tandem repeats of 60 amino acid residues [118]. One Cys in SEMG1 and two in SEMG2 form disulfides that lead to the formation of both homo- and hetero-multimers [121]. There are also strong non-covalent interactions between semenogelin molecules, leading to high molecular mass complexes with poor solubility in ordinary buffers [122].

The semenogelin molecules bind Zn^{2+}. Each semenogelin monomer binds at least ten Zn^{2+} molecules with an average K_d of 5 μM [123]. The semenogelin concentration in liquefied semen has been determined to an average 153 μM [124], but this is probably an underestimation of the concentration of intact semenogelin molecules in unliquified semen, which has been estimated to be around 50 mg/ml or 1 mM [123]. Thus, the semenogelin molecules in semen should be able to chelate most of seminal plasma Zn^{2+}, with an average concentration of 2 mM.

During semen liquefaction, the semenogelin molecules are digested to peptide fragments by seminal plasma endopeptidases and simultaneously the spermatozoa acquire propulsive motility [125, 126]. The major semenogelin-degrading enzyme in semen is KLK3 secreted by the prostate gland [127, 128]. As pointed out earlier, KLK3 cleaves C-terminal to several amino acid residues with large and medium-sized side chains, resulting in extensive degradation of both SEMG1 and SEMG2. The proteolytic activity can be regulated by protease inhibitors. In seminal plasma, the major serine protease inhibitor is the serpin protein C inhibitor (PCI) [129], but the concentration of PCI is only 0.2 mg/ml and hence it can only inhibit a fraction of KLK3, which has an average concentration of 0.8 mg/ml in seminal plasma and a molecular size that is around half that of PCI. Furthermore, PCI is a relatively slow inhibitor of KLK3 and only 40 % of the total concentration in seminal plasma is involved in complex formation with KLK3 [130]. Thus, only around 5 % of KLK3 in seminal plasma is inhibited by PCI, leaving a majority of the molecules in an uncomplexed form that is either proteolytically active or inactive due to internal cleavages [130, 131]. In prostate secretion the proteolytic activity would be extinguished by Zn^{2+}, which

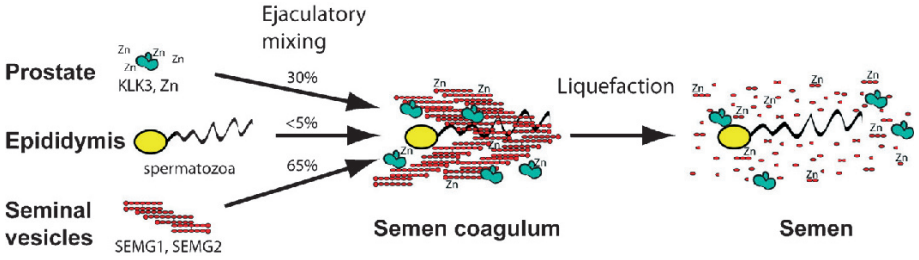

Figure 7. Semen coagulum formation and liquefaction with illustration of important components. Zn^{2+} binding to SEMG1 and SEMG2 terminates the inhibition of KLK3.

binds to and inhibits KLK3 and several other kallikrein-related peptidases at sub-millimolar concentrations. It has been shown that KLK3 inhibited by low concentration of Zn^{2+} can regain activity when semenogelin is added to chelate the Zn^{2+} [123]. Therefore, a consequence of the ejaculatory mixing of secretions from the seminal vesicles and the prostate is activation of KLK3 due to chelation of Zn^{2+} by the semenogelins. In other words, the semenogelins initiate their own destruction by removing the blockade of KLK3 activity by Zn^{2+} (Fig. 7).

As KLK3 is synthesized as a zymogen, it has to undergo proteolytic activation to fulfill its physiological function. An important question is then: which enzyme is the physiological activator of the KLK3 zymogen? Early studies demonstrated that catalytic concentrations of trypsin and KLK2 readily could activate proKLK3 [132–134]. More recent studies indicate that also KLK4, KLK5 and KLK15 are present in seminal plasma and can activate KLK3 [135–137]. There are also additional kallikrein-related peptidases expressed in the gonads or the accessory sex glands, as indicated in Table 3, that potentially could also activate KLK3 [102]. However, studies of KLK3 in prostatic fluid, using a monoclonal antibody directed against the active site or direct titration with radioactive di-isopropylfluorophosphate, suggests that zymogen activation takes place in the prostate prior to ejaculation [127, 138]. In that case it is not very likely that KLK3 is activated by kallikrein-related peptidases, as many of them, *e.g.*, KLK2, KLK4 and KLK5, would be inhibited by the high concentration of Zn^{2+} in prostatic fluid [20, 135, 139, 140]. Instead, the zymogen activation must be effectuated by a not-yet-identified Zn^{2+} insensitive enzyme, perhaps in conjugation with secretion.

Kallikrein-related peptidases as tumor markers

There are good reasons to study proteolytic enzymes as tumor markers, as many characteristic features of cancer cells depend on proteolysis, or could be affected by proteolysis. Invasive growth and metastasis are processes that require degradation of protein components in basal membranes and interstitial matrix. Peptidases could also affect cell proliferation and survival, directly by signaling through protease-activated receptors or indirectly by degrading growth factors or growth factor-binding proteins and decoy receptors.

The focus on kallikrein-related peptidases as tumor markers was initiated in the late 1970 s and early 1980 s, by the discovery that KLK3, as indicated by its alternative name prostate-specific antigen, with certain specificity was expressed in the prostate and also in malignantly transformed prostate and in the serum of prostate cancer (PCa) patients [141–143]. Elevated serum PSA levels were also detected in men with nodular prostate hyperplasia, a condition often referred to as benign prostate hyperplasia (BPH). The overlap in serum concentration between BPH and PCa has, ever since the introduction of KLK3 as a serum marker of PCa, been a matter of concern and various improvements have been introduced to increase the discriminating power of KLK3 measurements. In blood serum, a large proportion of KLK3 is found in complex with the serpin alpha1-antichymotrypsin (ACT) [144, 145]. Measurement of KLK3-ACT complexes in serum shows that the level of complexes is higher in PCa sera than in BPH sera [146, 147]. Thus, measurements of uncomplexed "free" KLK3 and the total concentration of KLK3 provides information that increases the discrimination between BPH and PCa. Other means of improving the specificity has been to follow the KLK3 concentration over time. This concept, known as PSA velocity, shows that the serum KLK3 level increased faster in men with PCa than in healthy men [148]. Another approach is to determine PSA density, in which the serum KLK3 levels are divided by the prostate volume, as measured by trans-rectal ultrasound [149, 150]. Patients with high PSA density are more likely to have PCa, as the release of KLK3 to the blood circulation is higher from cancer tissue than from benign tissue.

Patients with a serum KLK3 value exceeding 4 ng/ml are generally investigated for PCa with fine needle biopsy [151]. However, at this cut-off value more than 1/3 of PCa will not be detected. Studies on the long-

term risk of developing PCa in relation to serum or plasma KLK3 levels (plasma KLK3 is more stable when stored) showed increased KLK3 values in the PCa cohort compared to controls up to 25 years before diagnosis [152]. This suggests that the disease process may be ongoing for many years prior to diagnosis, but whether the proteolytic activity of KLK3 is part of it remains to be seen.

To improve the diagnostic power, studies have been conducted on serum KLK2, as a complement to KLK3 testing. It was found that patients with PCa had significantly higher serum KLK2 than apparently healthy controls or subjects with BPH [153–155]. The discrimination between PCa and BPH was greatest when KLK2 values were combined with the fraction of uncomplexed PSA. Furthermore, there are also results that indicates that KLK2 measurement might be useful in the prediction of organ *versus* non-organ confined PCa, something that might be very useful for the staging of the disease [156, 157].

Both KLK2 and KLK3 are expressed in many tissues, as demonstrated with several techniques [42, 131]. Still, they are relatively specific for the prostate, as their concentration in this organ exceeds those in other organs by several orders of magnitude [42]. In a search of additional prostate-specific proteins, KLK4 was identified, by way of its transcript, as a protein that with certain specificity is expressed in the prostate [158]. Although there are studies that point to its potential as PCa marker, there is insignificant information regarding KLK4 expression in cancer and benign tissue [159, 160]. In addition to these genes, there are also reports that KLK5 [161, 162], KLK11 [163, 164], KLK14 [165, 166] and KLK15 [167] are expressed in the prostate and might be of value as diagnostic or prognostic biomarkers in PCa.

What about the utility of kallikrein-related peptidases as biomarkers in tumors other than PCa? There have been numerous reports on their potential as cancer biomarkers ever since the discovery of the extended kallikrein locus less than a decade ago. A survey of kallikrein-related peptidases in different cancers can be found in a recent review [168]. One of the first reports on kallikrein-related peptidases in non-prostatic cancer was about KLK3 in breast cancer. By measuring the KLK3 levels in breast cancer specimens, it was demonstrated that KLK3 is a favorable prognostic indicator [169]. Unfortunately, measurement of KLK3 in serum showed no correlation with disease and thereby also excluded it as a diagnostic marker [170]. Furthermore, KLK3 has not been established as a prognostic marker in breast cancer and its utility compared to other prognostic indicators, *e.g.*, histological grading and presence of steroid hormone receptors, is not obvious. Hopefully, the fate of the novel kallikrein-related peptidases with potential as biomarkers will be different and that some of them will find their way into clinical practice.

Perspectives

Much has been learned about the kallikrein-related peptidases in recent years: 3D structures, sites of expression and catalytic properties have been mapped. Still, knowledge that pertains to their biological importance is very limited. It is well established that KLK3 is the major effector molecule in semen liquefaction, but the physiological role of semen coagel formation and liquefaction is still not known. What is the function of KLK3 in organs outside of the male genital tract? In the case of KLK4, a function has been established in enamel formation, but what about the expression in other tissues? The same goes for KLK5 and KLK7, which both probably have important roles in skin desquamation, but these proteins also seem to be expressed in more tissues than any of the other kallikrein-related peptidases (Table 3). The obvious answer is that kallikrein-related peptidases must have several and perhaps overlapping functions. In biological systems with overlapping and redundant components, it could be hard to identify the contribution and importance of each individual component for the proper function of the whole system. On the other hand, redundancies could also loosen the grip on individual components and allow deficiency states and gene losses to a greater extent than in non-redundant systems. Overlapping functions of kallikrein-related peptidases may prohibit recognizable phenotypes that are easy to detect, but hopefully they will show up during certain circumstances, *e.g.*, in experimental animals subjected to various challenges. From what we now know, redundancy is probably a rather common phenomenon among kallikrein-related peptidases. Thus, deficiency states of individual components might not be that uncommon and it could be worth screening for them.

Another approach of addressing the function of kallikrein-related peptidases is gene targeting, where the production of kallikrein-related peptidase knockout mice is of primary importance. There are already now a few kallikrein-related peptidase knockout animals available for research and presumably there will be more in the future. It is most likely that there has to be a certain amount of cross breeding between different knockout strains before conclusions can be drawn regarding biological role. Unfortunately, the wider function of KLK2 and KLK3 cannot be addressed in this way as there are no murine orthologs to these genes.

The function of individual kallikrein-related peptidase genes could of course also be addressed in many other ways, *e.g.*, by identifying natural substrates and by studies of certain biological systems, as has already been done with components of importance for skin desquamation and semen liquefaction. In any case, the coming years will be exciting, when gradually the function of these genes, which form the largest contiguous cluster of endopeptidase genes in the human genome, is revealed.

1 Kraut, H., Frey, E. K. and Werle, E. (1930) Der Nachweis eines Kreislaufhormons in der Pankreasdrüse. Hoppe-Seyler's Z. Physiol. Chem. 192, 1–21.
2 Mason, A. J., Evans, B. A., Cox, D. R., Shine, J. and Richards, R. I. (1983) Structure of mouse kallikrein gene family suggests a role in specific processing of biologically active peptides. Nature 303, 300–307.
3 Evans, B. A., Drinkwater, C. C. and Richards, R. I. (1987) Mouse glandular kallikrein genes. Structure and partial sequence analysis of the kallikrein gene locus. J. Biol. Chem. 262, 8027–8034.
4 Wines, D. R., Brady, J. M., Pritchett, D. B., Roberts, J. L. and MacDonald, R. J. (1989) Organization and expression of the rat kallikrein gene family. J. Biol. Chem. 264, 7653–7662.
5 Bothwell, M. A., Wilson, W. H. and Shooter, E. M. (1979) The relationship between glandular kallikrein and growth factor-processing proteases of mouse submaxillary gland. J. Biol. Chem. 254, 7287–7294.
6 Frey, P., Forand, R., Maciag, T. and Shooter, E. M. (1979) The biosynthetic precursor of epidermal growth factor and the mechanism of its processing. Proc. Natl. Acad. Sci. USA 76, 6294–6298.
7 Thomas, K. A., Baglan, N. C. and Bradshaw, R. A. (1981) The amino acid sequence of the gamma-subunit of mouse submaxillary gland 7 S nerve growth factor. J. Biol. Chem. 256, 9156–9166.
8 Fukushima, D., Kitamura, N. and Nakanishi, S. (1985) Nucleotide sequence of cloned cDNA for human pancreatic kallikrein. Biochemistry 24, 8037–8043.
9 Schedlich, L. J., Bennetts, B. H. and Morris, B. J. (1987) Primary structure of a human glandular kallikrein gene. DNA 6, 429–437.
10 Lundwall, A. and Lilja, H. (1987) Molecular cloning of human prostate specific antigen cDNA. FEBS Lett. 214, 317–322.
11 Gan, L., Lee, I., Smith, R., Argonza-Barrett, R., Lei, H., McCuaig, J., Moss, P., Paeper, B. and Wang, K. (2000) Sequencing and expression analysis of the serine protease gene cluster located in chromosome 19q13 region. Gene 257, 119–130.
12 Harvey, T. J., Hooper, J. D., Myers, S. A., Stephenson, S. A., Ashworth, L. K. and Clements, J. A. (2000) Tissue-specific expression patterns and fine mapping of the human kallikrein (KLK) locus on proximal 19q13.4. J. Biol. Chem. 275, 37397–37406.
13 Yousef, G. M., Chang, A., Scorilas, A. and Diamandis, E. P. (2000) Genomic organization of the human kallikrein gene family on chromosome 19q13.3-q13.4. Biochem. Biophys. Res. Commun. 276, 125–133.
14 Lundwall, A., Band, V., Blaber, M., Clements, J. A., Courty, Y., Diamandis, E. P., Fritz, H., Lilja, H., Malm, J., Maltais, L. J., Yvonne Olsson, A., Petraki, C., Scorilas, A., Sotiropoulou, G., Stenman, U. H., Stephan, C., Talieri, M. and Yousef, G. M. (2006) A comprehensive nomenclature for serine proteases with homology to tissue kallikreins. Biol. Chem. 387, 637–641.
15 Lu, W., Zhou, D., Glusman, G., Utleg, A. G., White, J. T., Nelson, P. S., Vasicek, T. J., Hood, L. and Lin, B. (2006) KLK31P is a novel androgen regulated and transcribed pseudogene of kallikreins that is expressed at lower levels in prostate cancer cells than in normal prostate cells. Prostate 66, 936–944.
16 Yousef, G. M., Borgono, C. A., Michael, I. P. and Diamandis, E. P. (2004) Cloning of a kallikrein pseudogene. Clin. Biochem. 37, 961–967.
17 Bode, W. and Schwager, P. (1975) The refined crystal structure of bovine beta-trypsin at 1.8 Å resolution. II. Crystallographic refinement, calcium binding site, benzamidine binding site and active site at pH 7.0. J. Mol. Biol. 98, 693–717.
18 Bode, W., Chen, Z., Bartels, K., Kutzbach, C., Schmidt-Kastner, G. and Bartunik, H. (1983) Refined 2 Å X-ray crystal structure of porcine pancreatic kallikrein A, a specific trypsin-like serine proteinase. Crystallization, structure determination, crystallographic refinement, structure and its comparison with bovine trypsin. J. Mol. Biol. 164, 237–282.
19 Bourgeois, L., Brillard-Bourdet, M., Deperthes, D., Juliano, M. A., Juliano, L., Tremblay, R. R., Dube, J. Y. and Gauthier, F. (1997) Serpin-derived peptide substrates for investigating the substrate specificity of human tissue kallikreins hK1 and hK2. J. Biol. Chem. 272, 29590–29595.
20 Lovgren, J., Airas, K. and Lilja, H. (1999) Enzymatic action of human glandular kallikrein 2 (hK2). Substrate specificity and regulation by Zn^{2+} and extracellular protease inhibitors. Eur. J. Biochem. 262, 781–789.
21 Matsumura, M., Bhatt, A. S., Andress, D., Clegg, N., Takayama, T. K., Craik, C. S. and Nelson, P. S. (2005) Substrates of the prostate-specific serine protease prostase/KLK4 defined by positional-scanning peptide libraries. Prostate 62, 1–13.
22 Debela, M., Magdolen, V., Schechter, N., Valachova, M., Lottspeich, F., Craik, C. S., Choe, Y., Bode, W. and Goettig, P. (2006) Specificity profiling of seven human tissue kallikreins reveals individual subsite preferences. J. Biol. Chem. 281, 25678–25688.
23 Borgono, C. A., Gavigan, J. A., Alves, J., Bowles, B., Harris, J. L., Sotiropoulou, G. and Diamandis, E. P. (2007) Defining the extended substrate specificity of kallikrein 1-related peptidases. Biol. Chem. 388, 1215–1225.
24 Brattsand, M. and Egelrud, T. (1999) Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. J. Biol. Chem. 274, 30033–30040.
25 Brattsand, M., Stefansson, K., Lundh, C., Haasum, Y. and Egelrud, T. (2005) A proteolytic cascade of kallikreins in the stratum corneum. J. Invest. Dermatol. 124, 198–203.
26 Michael, I. P., Sotiropoulou, G., Pampalakis, G., Magklara, A., Ghosh, M., Wasney, G. and Diamandis, E. P. (2005) Biochemical and enzymatic characterization of human kallikrein 5 (hK5), a novel serine protease potentially involved in cancer progression. J. Biol. Chem. 280, 14628–14635.
27 Bernett, M. J., Blaber, S. I., Scarisbrick, I. A., Dhanarajan, P., Thompson, S. M. and Blaber, M. (2002) Crystal structure and biochemical characterization of human kallikrein 6 reveals that a trypsin-like kallikrein is expressed in the central nervous system. J. Biol. Chem. 277, 24562–24570.
28 Blaber, S. I., Scarisbrick, I. A., Bernett, M. J., Dhanarajan, P., Seavy, M. A., Jin, Y., Schwartz, M. A., Rodriguez, M. and Blaber, M. (2002) Enzymatic properties of rat myelencephalon-specific protease. Biochemistry 41, 1165–1173.
29 Angelo, P. F., Lima, A. R., Alves, F. M., Blaber, S. I., Scarisbrick, I. A., Blaber, M., Juliano, L. and Juliano, M. A. (2006) Substrate specificity of human kallikrein 6: Salt and glycosaminoglycan activation effects. J. Biol. Chem. 281, 3116–3126.
30 Shimizu, C., Yoshida, S., Shibata, M., Kato, K., Momota, Y., Matsumoto, K., Shiosaka, T., Midorikawa, R., Kamachi, T., Kawabe, A. and Shiosaka, S. (1998) Characterization of recombinant and brain neuropsin, a plasticity-related serine protease. J. Biol. Chem. 273, 11189–11196.
31 Kishi, T., Cloutier, S. M., Kundig, C., Deperthes, D. and Diamandis, E. P. (2006) Activation and enzymatic character-

ization of recombinant human kallikrein 8. Biol. Chem. 387, 723–731.
32 Luo, L. Y., Shan, S. J., Elliott, M. B., Soosaipillai, A. and Diamandis, E. P. (2006) Purification and characterization of human kallikrein 11, a candidate prostate and ovarian cancer biomarker, from seminal plasma. Clin. Cancer Res. 12, 742–750.
33 Memari, N., Jiang, W., Diamandis, E. P. and Luo, L. Y. (2007) Enzymatic properties of human kallikrein-related peptidase 12 (KLK12). Biol. Chem. 388, 427–435.
34 Felber, L. M., Kundig, C., Borgono, C. A., Chagas, J. R., Tasinato, A., Jichlinski, P., Gygi, C. M., Leisinger, H. J., Diamandis, E. P., Deperthes, D. and Cloutier, S. M. (2006) Mutant recombinant serpins as highly specific inhibitors of human kallikrein 14. FEBS J. 273, 2505–2514.
35 Stefansson, K., Brattsand, M., Ny, A., Glas, B. and Egelrud, T. (2006) Kallikrein-related peptidase 14 may be a major contributor to trypsin-like proteolytic activity in human stratum corneum. Biol. Chem. 387, 761–768.
36 Debela, M., Hess, P., Magdolen, V., Schechter, N. M., Steiner, T., Huber, R., Bode, W. and Goettig, P. (2007) Chymotryptic specificity determinants in the 1.0 A structure of the zinc-inhibited human tissue kallikrein 7. Proc. Natl. Acad. Sci. USA 104, 16086–16091.
37 Egelrud, T. and Lundstrom, A. (1991) A chymotrypsin-like proteinase that may be involved in desquamation in plantar stratum corneum. Arch. Dermatol. Res. 283, 108–112.
38 Malm, J., Hellman, J., Hogg, P. and Lilja, H. (2000) Enzymatic action of prostate-specific antigen (PSA or hK3): Substrate specificity and regulation by Zn(2+), a tight-binding inhibitor. Prostate 45, 132–139.
39 Robert, M., Gibbs, B. F., Jacobson, E. and Gagnon, C. (1997) Characterization of prostate-specific antigen proteolytic activity on its major physiological substrate, the sperm motility inhibitor precursor/semenogelin I. Biochemistry 36, 3811–3819.
40 Villoutreix, B. O., Getzoff, E. D. and Griffin, J. H. (1994) A structural model for the prostate disease marker, human prostate-specific antigen. Protein Sci. 3, 2033–2044.
41 Carvalho, A. L., Sanz, L., Barettino, D., Romero, A., Calvete, J. J. and Romao, M. J. (2002) Crystal structure of a prostate kallikrein isolated from stallion seminal plasma: A homologue of human PSA. J. Mol. Biol. 322, 325–337.
42 Olsson, A. Y., Bjartell, A., Lilja, H. and Lundwall, A. (2005) Expression of prostate-specific antigen (PSA) and human glandular kallikrein 2 (hK2) in ileum and other extraprostatic tissues. Int. J. Cancer 113, 290–297.
43 Petraki, C. D., Papanastasiou, P. A., Karavana, V. N. and Diamandis, E. P. (2006) Cellular distribution of human tissue kallikreins: Immunohistochemical localization. Biol. Chem. 387, 653–663.
44 Shaw, J. L. and Diamandis, E. P. (2007) Distribution of 15 human kallikreins in tissues and biological fluids. Clin. Chem. 53, 1423–1432.
45 Diamandis, E. P. and Yousef, G. M. (2002) Human tissue kallikreins: A family of new cancer biomarkers. Clin. Chem. 48, 1198–1205.
46 Olsson, A. Y. and Lundwall, A. (2002) Organization and evolution of the glandular kallikrein locus in Mus musculus. Biochem. Biophys. Res. Commun. 299, 305–311.
47 Olsson, A. Y., Lilja, H. and Lundwall, A. (2004) Taxon-specific evolution of glandular kallikrein genes and identification of a progenitor of prostate-specific antigen. Genomics 84, 147–156.
48 Elliott, M. B., Irwin, D. M. and Diamandis, E. P. (2006) *In silico* identification and Bayesian phylogenetic analysis of multiple new mammalian kallikrein gene families. Genomics 88, 591–599.
49 Gauthier, E. R., Chapdelaine, P., Tremblay, R. R. and Dube, J. Y. (1993) Characterization of rhesus monkey prostate specific antigen cDNA. Biochim. Biophys. Acta 1174, 207–210.
50 Olsson, A. Y., Persson, A. M., Valtonen-Andre, C. and Lundwall, A. (2000) Glandular kallikreins of the cotton-top tamarin: Molecular cloning of the gene encoding the tissue kallikrein. DNA Cell Biol. 19, 721–727.
51 Olsson, A. Y., Valtonen-Andre, C., Lilja, H. and Lundwall, A. (2004) The evolution of the glandular kallikrein locus: Identification of orthologs and pseudogenes in the cotton-top tamarin. Gene 343, 347–355.
52 Gauthier, E. R., Dumas, C., Chapdelaine, P., Tremblay, R. R. and Dube, J. Y. (1994) Characterization of canine pancreas kallikrein cDNA. Biochim. Biophys. Acta 1218, 102–104.
53 Lazure, C., Leduc, R., Seidah, N. G., Chretien, M., Dube, J. Y., Chapdelaine, P., Frenette, G., Paquin, R. and Tremblay, R. R. (1984) The major androgen-dependent protease in dog prostate belongs to the kallikrein family: Confirmation by partial amino acid sequencing. FEBS Lett. 175, 1–7.
54 Lundwall, A., Clauss, A. and Olsson, A. Y. (2006) Evolution of kallikrein-related peptidases in mammals and identification of a genetic locus encoding potential regulatory inhibitors. Biol. Chem. 387, 243–249.
55 Moreau, M. E., Garbacki, N., Molinaro, G., Brown, N. J., Marceau, F. and Adam, A. (2005) The kallikrein-kinin system: Current and future pharmacological targets. J. Pharmacol. Sci. 99, 6–38.
56 Madeddu, P., Emanueli, C. and El-Dahr, S. (2007) Mechanisms of disease: The tissue kallikrein-kinin system in hypertension and vascular remodeling. Nature Clin. Pract. 3, 208–221.
57 Leeb-Lundberg, L. M., Marceau, F., Muller-Esterl, W., Pettibone, D. J. and Zuraw, B. L. (2005) International union of pharmacology. XLV. Classification of the kinin receptor family: From molecular mechanisms to pathophysiological consequences. Pharmacol. Rev. 57, 27–77.
58 Marceau, F., Hess, J. F. and Bachvarov, D. R. (1998) The B1 receptors for kinins. Pharmacol. Rev. 50, 357–386.
59 Kita, M., Nakamura, Y., Okumura, Y., Ohdachi, S. D., Oba, Y., Yoshikuni, M., Kido, H. and Uemura, D. (2004) Blarina toxin, a mammalian lethal venom from the short-tailed shrew *Blarina brevicauda:* Isolation and characterization. Proc. Natl. Acad. Sci. USA 101, 7542–7547.
60 Berger, E. A. and Shooter, E. M. (1977) Evidence for pro-beta-nerve growth factor, a biosynthetic precursor to beta-nerve growth factor. Proc. Natl. Acad. Sci. USA 74, 3647–3651.
61 Simmer, J. P., Fukae, M., Tanabe, T., Yamakoshi, Y., Uchida, T., Xue, J., Margolis, H. C., Shimizu, M., DeHart, B. C., Hu, C. C. and Bartlett, J. D. (1998) Purification, characterization, and cloning of enamel matrix serine proteinase 1. J. Dent. Res. 77, 377–386.
62 Tamura, H., Ishikawa, Y., Hino, N., Maeda, M., Yoshida, S., Kaku, S. and Shiosaka, S. (2006) Neuropsin is essential for early processes of memory acquisition and Schaffer collateral long-term potentiation in adult mouse hippocampus *in vivo.* J. Physiol. 570, 541–551.
63 Terayama, R., Bando, Y., Murakami, K., Kato, K., Kishibe, M. and Yoshida, S. (2007) Neuropsin promotes oligodendrocyte death, demyelination and axonal degeneration after spinal cord injury. Neuroscience 148, 175–187.
64 Iwata, A., Maruyama, M., Akagi, T., Hashikawa, T., Kanazawa, I., Tsuji, S. and Nukina, N. (2003) Alpha-synuclein degradation by serine protease neurosin: Implication for pathogenesis of synucleinopathies. Hum. Mol. Genet. 12, 2625–2635.
65 Little, S. P., Dixon, E. P., Norris, F., Buckley, W., Becker, G. W., Johnson, M., Dobbins, J. R., Wyrick, T., Miller, J. R., MacKellar, W., Hepburn, D., Corvalan, J., McClure, D., Liu, X., Stephenson, D., Clemens, J. and Johnstone, E. M. (1997) Zyme, a novel and potentially amyloidogenic enzyme cDNA isolated from Alzheimer's disease brain. J. Biol. Chem. 272, 25135–25142.
66 Terayama, R., Bando, Y., Jiang, Y. P., Mitrovic, B. and Yoshida, S. (2005) Differential expression of protease M/

neurosin in oligodendrocytes and their progenitors in an animal model of multiple sclerosis. Neurosci. Lett. 382, 82–87.
67 Blaber, S. I., Ciric, B., Christophi, G. P., Bernett, M. J., Blaber, M., Rodriguez, M. and Scarisbrick, I. A. (2004) Targeting kallikrein 6 proteolysis attenuates CNS inflammatory disease. FASEB J. 18, 920–922.
68 Scarisbrick, I. A., Blaber, S. I., Lucchinetti, C. F., Genain, C. P., Blaber, M. and Rodriguez, M. (2002) Activity of a newly identified serine protease in CNS demyelination. Brain 125, 1283–1296.
69 Candi, E., Schmidt, R. and Melino, G. (2005) The cornified envelope: A model of cell death in the skin. Nat. Rev. Mol. Cell. Biol. 6, 328–340.
70 Egelrud, T. (2000) Desquamation in the stratum corneum. Acta Derm. Venereol. Suppl. (Stockh). 208, 44–45.
71 Egelrud, T., Hofer, P. A. and Lundström, A. (1988) Proteolytic degradation of desmosomes in plantar stratum corneum leads to cell dissociation *in vitro.* Acta Derm. Venereol. 68, 93–97.
72 Lundström, A. and Egelrud, T. (1988) Cell shedding from human plantar skin *in vitro:* Evidence of its dependence on endogenous proteolysis. J. Invest. Dermatol. 91, 340–343.
73 Lundström, A. and Egelrud, T. (1990) Cell shedding from human plantar skin *in vitro:* Evidence that two different types of protein structures are degraded by a chymotrypsin-like enzyme. Arch. Dermatol. Res. 282, 234–237.
74 Lundström, A. and Egelrud, T. (1990) Evidence that cell shedding from plantar stratum corneum *in vitro* involves endogenous proteolysis of the desmosomal protein desmoglein I. J. Invest. Dermatol. 94, 216–220.
75 Suzuki, Y., Koyama, J., Moro, O., Horii, I., Kikuchi, K., Tanida, M. and Tagami, H. (1996) The role of two endogenous proteases of the stratum corneum in degradation of desmoglein-1 and their reduced activity in the skin of ichthyotic patients. Br. J. Dermatol. 134, 460–464.
76 Suzuki, Y., Nomura, J., Koyama, J. and Horii, I. (1994) The role of proteases in stratum corneum: Involvement in stratum corneum desquamation. Arch. Dermatol. Res. 286, 249–253.
77 Wachter, A. M. and Lezdey, J. (1992) Treatment of atopic dermatitis with alpha 1-proteinase inhibitor. Ann. Allergy. 69, 407–414.
78 Hansson, L., Strömqvist, M., Bäckman, A., Wallbrandt, P., Carlstein, A. and Egelrud, T. (1994) Cloning, expression, and characterization of stratum corneum chymotryptic enzyme. A skin-specific human serine proteinase. J. Biol. Chem. 269, 19420–19426.
79 Ekholm, E. and Egelrud, T. (1998) The expression of stratum corneum chymotryptic enzyme in human anagen hair follicles: Further evidence for its involvement in desquamation-like processes. Br. J. Dermatol. 139, 585–590.
80 Ekholm, E., Sondell, B., Strandén, P., Brattsand, M. and Egelrud, T. (1998) Expression of stratum corneum chymotryptic enzyme in human sebaceous follicles. Acta Derm. Venereol. 78, 343–347.
81 Ekholm, I. E., Brattsand, M. and Egelrud, T. (2000) Stratum corneum tryptic enzyme in normal epidermis: A missing link in the desquamation process? J. Invest. Dermatol. 114, 56–63.
82 Sondell, B., Thornell, L. E., Stigbrand, T. and Egelrud, T. (1994) Immunolocalization of stratum corneum chymotryptic enzyme in human skin and oral epithelium with monoclonal antibodies: Evidence of a proteinase specifically expressed in keratinizing squamous epithelia. J. Histochem. Cytochem. 42, 459–465.
83 Stefansson, K., Brattsand, M., Roosterman, D., Kempkes, C., Bocheva, G., Steinhoff, M. and Egelrud, T. (2008) Activation of proteinase-activated receptor-2 by human kallikrein-related peptidases. J. Invest. Dermatol. 128, 18–25.
84 Komatsu, N., Takata, M., Otsuki, N., Toyama, T., Ohka, R., Takehara, K. and Saijoh, K. (2003) Expression and localization of tissue kallikrein mRNAs in human epidermis and appendages. J. Invest. Dermatol. 121, 542–549.
85 Komatsu, N., Saijoh, K., Toyama, T., Ohka, R., Otsuki, N., Hussack, G., Takehara, K. and Diamandis, E. P. (2005) Multiple tissue kallikrein mRNA and protein expression in normal skin and skin diseases. Br. J. Dermatol. 153, 274–281.
86 Hansson, L., Bäckman, A., Ny, A., Edlund, M., Ekholm, E., Ekstrand Hammarström, B., Törnell, J., Wallbrandt, P., Wennbo, H. and Egelrud, T. (2002) Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: A model for chronic itchy dermatitis. J. Invest. Dermatol. 118, 444–449.
87 Kirihara, T., Matsumoto-Miyai, K., Nakamura, Y., Sadayama, T., Yoshida, S. and Shiosaka, S. (2003) Prolonged recovery of ultraviolet B-irradiated skin in neuropsin (KLK8)-deficient mice. Br. J. Dermatol. 149, 700–706.
88 Sondell, B., Thornell, L. E. and Egelrud, T. (1995) Evidence that stratum corneum chymotryptic enzyme is transported to the stratum corneum extracellular space *via* lamellar bodies. J. Invest. Dermatol. 104, 819–823.
89 Caubet, C., Jonca, N., Brattsand, M., Guerrin, M., Bernard, D., Schmidt, R., Egelrud, T., Simon, M. and Serre, G. (2004) Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. J. Invest. Dermatol. 122, 1235–1244.
90 Borgoño, C. A., Michael, I. P., Komatsu, N., Jayakumar, A., Kapadia, R., Clayman, G. L., Sotiropoulou, G. and Diamandis, E. P. (2007) A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. J. Biol. Chem. 282, 3640–3652.
91 Kuwae, K., Matsumoto-Miyai, K., Yoshida, S., Sadayama, T., Yoshikawa, K., Hosokawa, K. and Shiosaka, S. (2002) Epidermal expression of serine protease, neuropsin (KLK8) in normal and pathological skin samples. Mol. Pathol. 55, 235–241.
92 Déry, O., Corvera, C. U., Steinhoff, M. and Bunnett, N. W. (1998) Proteinase-activated receptors: Novel mechanisms of signaling by serine proteases. Am. J. Physiol. 274, C1429–1452.
93 Steinhoff, M., Corvera, C. U., Thoma, M. S., Kong, W., McAlpine, B. E., Caughey, G. H., Ansel, J. C. and Bunnett, N. W. (1999) Proteinase-activated receptor-2 in human skin: Tissue distribution and activation of keratinocytes by mast cell tryptase. Exp. Dermatol. 8, 282–294.
94 Oikonomopoulou, K., Hansen, K. K., Saifeddine, M., Vergnolle, N., Tea, I., Blaber, M., Blaber, S. I., Scarisbrick, I., Diamandis, E. P. and Hollenberg, M. D. (2006) Kallikrein-mediated cell signalling: Targeting proteinase-activated receptors (PARs). Biol. Chem. 387, 817–824.
95 Steinhoff, M., Neisius, U., Ikoma, A., Fartasch, M., Heyer, G., Skov, P. S., Luger, T. A. and Schmelz, M. (2003) Proteinase-activated receptor-2 mediates itch: A novel pathway for pruritus in human skin. J. Neurosci. 23, 6176–6180.
96 Materazzi, S., Pellerito, S., Di Serio, C., Paglierani, M., Naldini, A., Ardinghi, C., Carraro, F., Geppetti, P., Cirino, G., Santucci, M., Tarantini, F. and Massi, D. (2007) Analysis of protease-activated receptor-1 and -2 in human scar formation. J. Pathol. 212, 440–449.
97 Paine, C., Sharlow, E., Liebel, F., Eisinger, M., Shapiro, S. and Seiberg, M. (2001) An alternative approach to depigmentation by soybean extracts *via* inhibition of the PAR-2 pathway. J. Invest. Dermatol. 116, 587–595.
98 Hachem, J. P., Houben, E., Crumrine, D., Man, M. Q., Schurer, N., Roelandt, T., Choi, E. H., Uchida, Y., Brown, B. E., Feingold, K. R. and Elias, P. M. (2006) Serine protease signaling of epidermal permeability barrier homeostasis. J. Invest. Dermatol. 126, 2074–2086.
99 Yamasaki, K., Di Nardo, A., Bardan, A., Murakami, M., Ohtake, T., Coda, A., Dorschner, R. A., Bonnart, C., Descargues, P., Hovnanian, A., Morhenn, V. B. and Gallo, R. L. (2007) Increased serine protease activity and cathelici-

din promotes skin inflammation in rosacea. Nat. Med. 13, 975–980.
100 Yamasaki, K., Schauber, J., Coda, A., Lin, H., Dorschner, R. A., Schechter, N. M., Bonnart, C., Descargues, P., Hovnanian, A. and Gallo, R. L. (2006) Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. FASEB J. 20, 2068–2080.
101 Ohman, H. and Vahlquist, A. (1994) *In vivo* studies concerning a pH gradient in human stratum corneum and upper epidermis. Acta Derm. Venereol. 74, 375–379.
102 Yoon, H., Laxmikanthan, G., Lee, J., Blaber, S. I., Rodriguez, A., Kogot, J. M., Scarisbrick, I. A. and Blaber, M. (2007) Activation profiles and regulatory cascades of the human kallikrein-related peptidases. J. Biol. Chem. 282, 31852–31864.
103 Emami, N. and Diamandis, E. P. (2008) Human kallikrein-related peptidase 14 (KLK14) is a new activator component of the KLK proteolytic cascade: Possible function in seminal plasma and skin. J. Biol. Chem. 283, 3031–3041.
104 Sato, J., Denda, M., Nakanishi, J. and Koyama, J. (1998) Dry condition affects desquamation of stratum corneum *in vivo*. J. Dermatol. Sci. 18, 163–169.
105 Watkinson, A., Harding, C., Moore, A. and Coan, P. (2001) Water modulation of stratum corneum chymotryptic enzyme activity and desquamation. Arch. Dermatol. Res. 293, 470–476.
106 Egelrud, T. (1993) Purification and preliminary characterization of stratum corneum chymotryptic enzyme: A proteinase that may be involved in desquamation. J. Invest. Dermatol. 101, 200–204.
107 Chavanas, S., Bodemer, C., Rochat, A., Hamel-Teillac, D., Ali, M., Irvine, A. D., Bonafé, J. L., Wilkinson, J., Taïeb, A., Barrandon, Y., Harper, J. I., de Prost, Y. and Hovnanian, A. (2000) Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat. Genet. 25, 141–142.
108 Mägert, H. J., Ständker, L., Kreutzmann, P., Zucht, H. D., Reinecke, M., Sommerhoff, C. P., Fritz, H. and Forssmann, W. G. (1999) LEKTI, a novel 15-domain type of human serine proteinase inhibitor. J. Biol. Chem. 274, 21499–21502.
109 Deraison, C., Bonnart, C., Lopez, F., Besson, C., Robinson, R., Jayakumar, A., Wagberg, F., Brattsand, M., Hachem, J. P., Leonardsson, G. and Hovnanian, A. (2007) LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. Mol. Biol. Cell 18, 3607–3619.
110 Egelrud, T., Brattsand, M., Kreutzmann, P., Walden, M., Vitzithum, K., Marx, U. C., Forssmann, W. G. and Mägert, H. J. (2005) hK5 and hK7, two serine proteinases abundant in human skin, are inhibited by LEKTI domain 6. Br. J. Dermatol. 153, 1200–1203.
111 Schechter, N. M., Choi, E. J., Wang, Z. M., Hanakawa, Y., Stanley, J. R., Kang, Y., Clayman, G. L. and Jayakumar, A. (2005) Inhibition of human kallikreins 5 and 7 by the serine protease inhibitor lympho-epithelial Kazal-type inhibitor (LEKTI). Biol. Chem. 386, 1173–1184.
112 Franzke, C. W., Baici, A., Bartels, J., Christophers, E. and Wiedow, O. (1996) Antileukoprotease inhibits stratum corneum chymotryptic enzyme. Evidence for a regulative function in desquamation. J. Biol. Chem. 271, 21886–21890.
113 Nonomura, K., Yamanishi, K., Yasuno, H., Nara, K. and Hirose, S. (1994) Up-regulation of elafin/SKALP gene expression in psoriatic epidermis. J. Invest. Dermatol. 103, 88–91.
114 Wiedow, O., Harder, J., Bartels, J., Streit, V. and Christophers, E. (1998) Antileukoprotease in human skin: An antibiotic peptide constitutively produced by keratinocytes. Biochem. Biophys. Res. Commun. 248, 904–909.
115 Shapiro, L. J., Weiss, R., Buxman, M. M., Vidgoff, J., Dimond, R. L., Roller, J. A. and Wells, R. S. (1978) Enzymatic basis of typical X-linked icthyosis. Lancet 2, 756–757.
116 Sato, J., Denda, M., Nakanishi, J., Nomura, J. and Koyama, J. (1998) Cholesterol sulfate inhibits proteases that are involved in desquamation of stratum corneum. J. Invest. Dermatol. 111, 189–193.
117 Lilja, H., Abrahamsson, P. A. and Lundwall, A. (1989) Semenogelin, the predominant protein in human semen. Primary structure and identification of closely related proteins in the male accessory sex glands and on the spermatozoa. J. Biol. Chem. 264, 1894–1900.
118 Lilja, H. and Lundwall, A. (1992) Molecular cloning of epididymal and seminal vesicular transcripts encoding a semenogelin-related protein. Proc. Natl. Acad. Sci. USA 89, 4559–4563.
119 Lundwall, A., Bjartell, A., Olsson, A. Y. and Malm, J. (2002) Semenogelin I and II, the predominant human seminal plasma proteins, are also expressed in non-genital tissues. Mol. Hum. Reprod. 8, 805–810.
120 Malm, J., Hellman, J., Magnusson, H., Laurell, C. B. and Lilja, H. (1996) Isolation and characterization of the major gel proteins in human semen, semenogelin I and semenogelin II. Eur. J. Biochem. 238, 48–53.
121 Lilja, H. (1988) Structure and function of prostatic- and seminal vesicle-secreted proteins involved in the gelation and liquefaction of human semen. Scand. J. Clin. Lab. Invest. 191, 13–20.
122 Lilja, H. and Laurell, C. B. (1985) The predominant protein in human seminal coagulate. Scand. J. Clin. Lab. Invest. 45, 635–641.
123 Jonsson, M., Linse, S., Frohm, B., Lundwall, A. and Malm, J. (2005) Semenogelins I and II bind zinc and regulate the activity of prostate-specific antigen. Biochem. J. 387, 447–453.
124 Koistinen, H., Soini, T., Leinonen, J., Hyden-Granskog, C., Salo, J., Halttunen, M., Stenman, U. H., Seppala, M. and Koistinen, R. (2002) Monoclonal antibodies, immunofluorometric assay, and detection of human semenogelin in male reproductive tract: No association with *in vitro* fertilizing capacity of sperm. Biol. Reprod. 66, 624–628.
125 Lilja, H. and Laurell, C. B. (1984) Liquefaction of coagulated human semen. Scand. J. Clin. Lab. Invest. 44, 447–452.
126 McGee, R. S. and Herr, J. C. (1987) Human seminal vesicle-specific antigen during semen liquefaction. Biol. Reprod. 37, 431–439.
127 Lilja, H. (1985) A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. J. Clin. Invest. 76, 1899–1903.
128 McGee, R. S. and Herr, J. C. (1988) Human seminal vesicle-specific antigen is a substrate for prostate-specific antigen (or P-30). Biol. Reprod. 39, 499–510.
129 Laurell, M., Christensson, A., Abrahamsson, P. A., Stenflo, J. and Lilja, H. (1992) Protein C inhibitor in human body fluids. Seminal plasma is rich in inhibitor antigen deriving from cells throughout the male reproductive system. J. Clin. Invest. 89, 1094–1101.
130 Christensson, A. and Lilja, H. (1994) Complex formation between protein C inhibitor and prostate-specific antigen *in vitro* and in human semen. Eur. J. Biochem. 220, 45–53.
131 Lovgren, J., Valtonen-Andre, C., Marsal, K., Lilja, H. and Lundwall, A. (1999) Measurement of prostate-specific antigen and human glandular kallikrein 2 in different body fluids. J. Androl. 20, 348–355.
132 Kumar, A., Mikolajczyk, S. D., Goel, A. S., Millar, L. S. and Saedi, M. S. (1997) Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2. Cancer Res. 57, 3111–3114.
133 Lovgren, J., Rajakoski, K., Karp, M., Lundwall, A. and Lilja, H. (1997) Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2. Biochem. Biophys. Res. Commun. 238, 549–555.
134 Takayama, T. K., Fujikawa, K. and Davie, E. W. (1997) Characterization of the precursor of prostate-specific antigen.

Activation by trypsin and by human glandular kallikrein. J. Biol. Chem. 272, 21582–21588.
135 Michael, I. P., Pampalakis, G., Mikolajczyk, S. D., Malm, J., Sotiropoulou, G. and Diamandis, E. P. (2006) Human tissue kallikrein 5 is a member of a proteolytic cascade pathway involved in seminal clot liquefaction and potentially in prostate cancer progression. J. Biol. Chem. 281, 12743–12750.
136 Takayama, T. K., Carter, C. A. and Deng, T. (2001) Activation of prostate-specific antigen precursor (pro-PSA) by prostin, a novel human prostatic serine protease identified by degenerate PCR. Biochemistry 40, 1679–1687.
137 Takayama, T. K., McMullen, B. A., Nelson, P. S., Matsumura, M. and Fujikawa, K. (2001) 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 40, 15341–15348.
138 Denmeade, S. R., Sokoll, L. J., Chan, D. W., Khan, S. R. and Isaacs, J. T. (2001) Concentration of enzymatically active prostate-specific antigen (PSA) in the extracellular fluid of primary human prostate cancers and human prostate cancer xenograft models. Prostate 48, 1–6.
139 Debela, M., Goettig, P., Magdolen, V., Huber, R., Schechter, N. M. and Bode, W. (2007) Structural basis of the zinc inhibition of human tissue kallikrein 5. J. Mol. Biol. 373, 1017–1031.
140 Debela, M., Magdolen, V., Grimminger, V., Sommerhoff, C., Messerschmidt, A., Huber, R., Friedrich, R., Bode, W. and Goettig, P. (2006) Crystal structures of human tissue kallikrein 4: Activity modulation by a specific zinc binding site. J. Mol. Biol. 362, 1094–1107.
141 Kuriyama, M., Wang, M. C., Lee, C. I., Papsidero, L. D., Killian, C. S., Inaji, H., Slack, N. H., Nishiura, T., Murphy, G. P. and Chu, T. M. (1981) Use of human prostate-specific antigen in monitoring prostate cancer. Cancer Res. 41, 3874–3876.
142 Wang, M. C., Papsidero, L. D., Kuriyama, M., Valenzuela, L. A., Murphy, G. P. and Chu, T. M. (1981) Prostate antigen: A new potential marker for prostatic cancer. Prostate 2, 89–96.
143 Wang, M. C., Valenzuela, L. A., Murphy, G. P. and Chu, T. M. (1979) Purification of a human prostate specific antigen. Investig. Urol. 17, 159–163.
144 Christensson, A., Laurell, C. B. and Lilja, H. (1990) Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. Eur. J. Biochem. 194, 755–763.
145 Lilja, H., Christensson, A., Dahlen, U., Matikainen, M. T., Nilsson, O., Pettersson, K. and Lovgren, T. (1991) Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. Clin. Chem. 37, 1618–1625.
146 Christensson, A., Bjork, T., Nilsson, O., Dahlen, U., Matikainen, M. T., Cockett, A. T., Abrahamsson, P. A. and Lilja, H. (1993) Serum prostate specific antigen complexed to alpha 1-antichymotrypsin as an indicator of prostate cancer. J. Urol. 150, 100–105.
147 Stenman, U. H., Leinonen, J., Alfthan, H., Rannikko, S., Tuhkanen, K. and Alfthan, O. (1991) A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: Assay of the complex improves clinical sensitivity for cancer. Cancer Res. 51, 222–226.
148 Carter, H. B., Pearson, J. D., Metter, E. J., Brant, L. J., Chan, D. W., Andres, R., Fozard, J. L. and Walsh, P. C. (1992) Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. JAMA 267, 2215–2220.
149 Babaian, R. J., Fritsche, H. A. and Evans, R. B. (1990) Prostate-specific antigen and prostate gland volume: Correlation and clinical application. J. Clin. Lab. Anal. 4, 135–137.
150 Veneziano, S., Pavlica, P., Querze, R., Nanni, G., Lalanne, M. G. and Vecchi, F. (1990) Correlation between prostate-specific antigen and prostate volume, evaluated by transrectal ultrasonography: Usefulness in diagnosis of prostate cancer. Eur. Urol. 18, 112–116.
151 Catalona, W. J., Smith, D. S., Ratliff, T. L., Dodds, K. M., Coplen, D. E., Yuan, J. J., Petros, J. A. and Andriole, G. L. (1991) Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N. Engl. J. Med. 324, 1156–1161.
152 Lilja, H., Ulmert, D., Bjork, T., Becker, C., Serio, A. M., Nilsson, J. A., Abrahamsson, P. A., Vickers, A. J. and Berglund, G. (2007) Long-term prediction of prostate cancer up to 25 years before diagnosis of prostate cancer using prostate kallikreins measured at age 44 to 50 years. J. Clin. Oncol. 25, 431–436.
153 Becker, C., Piironen, T., Pettersson, K., Bjork, T., Wojno, K. J., Oesterling, J. E. and Lilja, H. (2000) Discrimination of men with prostate cancer from those with benign disease by measurements of human glandular kallikrein 2 (HK2) in serum. J. Urol. 163, 311–316.
154 Partin, A. W., Catalona, W. J., Finlay, J. A., Darte, C., Tindall, D. J., Young, C. Y., Klee, G. G., Chan, D. W., Rittenhouse, H. G., Wolfert, R. L. and Woodrum, D. L. (1999) Use of human glandular kallikrein 2 for the detection of prostate cancer: Preliminary analysis. Urology 54, 839–845.
155 Saedi, M. S., Hill, T. M., Kuus-Reichel, K., Kumar, A., Payne, J., Mikolajczyk, S. D., Wolfert, R. L. and Rittenhouse, H. G. (1998) The precursor form of the human kallikrein 2, a kallikrein homologous to prostate-specific antigen, is present in human sera and is increased in prostate cancer and benign prostatic hyperplasia. Clin. Chem. 44, 2115–2119.
156 Haese, A., Becker, C., Noldus, J., Graefen, M., Huland, E., Huland, H. and Lilja, H. (2000) Human glandular kallikrein 2: A potential serum marker for predicting the organ confined *versus* non-organ confined growth of prostate cancer. J. Urol. 163, 1491–1497.
157 Recker, F., Kwiatkowski, M. K., Piironen, T., Pettersson, K., Huber, A., Lummen, G. and Tscholl, R. (2000) Human glandular kallikrein as a tool to improve discrimination of poorly differentiated and non-organ-confined prostate cancer compared with prostate-specific antigen. Urology 55, 481–485.
158 Nelson, P. S., Gan, L., Ferguson, C., Moss, P., Gelinas, R., Hood, L. and Wang, K. (1999) Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression. Proc. Natl. Acad. Sci. USA 96, 3114–3119.
159 Obiezu, C. V., Soosaipillai, A., Jung, K., Stephan, C., Scorilas, A., Howarth, D. H. and Diamandis, E. P. (2002) Detection of human kallikrein 4 in healthy and cancerous prostatic tissues by immunofluorometry and immunohistochemistry. Clin. Chem. 48, 1232–1240.
160 Xi, Z., Klokk, T. I., Korkmaz, K., Kurys, P., Elbi, C., Risberg, B., Danielsen, H., Loda, M. and Saatcioglu, F. (2004) Kallikrein 4 is a predominantly nuclear protein and is overexpressed in prostate cancer. Cancer Res. 64, 2365–2370.
161 Kurlender, L., Yousef, G. M., Memari, N., Robb, J. D., Michael, I. P., Borgono, C., Katsaros, D., Stephan, C., Jung, K. and Diamandis, E. P. (2004) Differential expression of a human kallikrein 5 (KLK5) splice variant in ovarian and prostate cancer. Tumour Biol. 25, 149–156.
162 Yousef, G. M., Scorilas, A., Chang, A., Rendl, L., Diamandis, M., Jung, K. and Diamandis, E. P. (2002) Down-regulation of the human kallikrein gene 5 (KLK5) in prostate cancer tissues. Prostate 51, 126–132.
163 Scorilas, A. and Gregorakis, A. K. (2006) mRNA expression analysis of human kallikrein 11 (KLK11) may be useful in the discrimination of benign prostatic hyperplasia from prostate cancer after needle prostate biopsy. Biol. Chem. 387, 789–793.

164 Stavropoulou, P., Gregorakis, A. K., Plebani, M. and Scorilas, A. (2005) Expression analysis and prognostic significance of human kallikrein 11 in prostate cancer. Clin. Chim. Acta 357, 190–195.
165 Borgono, C. A., Michael, I. P., Shaw, J. L., Luo, L. Y., Ghosh, M. C., Soosaipillai, A., Grass, L., Katsaros, D. and Diamandis, E. P. (2007) Expression and functional characterization of the cancer-related serine protease, human tissue kallikrein 14. J. Biol. Chem. 282, 2405–2422.
166 Yousef, G. M., Stephan, C., Scorilas, A., Ellatif, M. A., Jung, K., Kristiansen, G., Jung, M., Polymeris, M. E. and Diamandis, E. P. (2003) Differential expression of the human kallikrein gene 14 (KLK14) in normal and cancerous prostatic tissues. Prostate 56, 287–292.
167 Yousef, G. M., Scorilas, A., Jung, K., Ashworth, L. K. and Diamandis, E. P. (2001) Molecular cloning of the human kallikrein 15 gene (KLK15). Up-regulation in prostate cancer. J. Biol. Chem. 276, 53–61.
168 Paliouras, M., Borgono, C. and Diamandis, E. P. (2007) Human tissue kallikreins: The cancer biomarker family. Cancer Lett. 249, 61–79.
169 Yu, H., Giai, M., Diamandis, E. P., Katsaros, D., Sutherland, D. J., Levesque, M. A., Roagna, R., Ponzone, R. and Sismondi, P. (1995) Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. Cancer Res. 55, 2104–2110.
170 Giai, M., Yu, H., Roagna, R., Ponzone, R., Katsaros, D., Levesque, M. A. and Diamandis, E. P. (1995) Prostate-specific antigen in serum of women with breast cancer. Br. J. Cancer 72, 728–731.
171 Laxmikanthan, G., Blaber, S. I., Bernett, M. J., Scarisbrick, I. A., Juliano, M. A. and Blaber, M. (2005) 1.70 A X-ray structure of human apo kallikrein 1: Structural changes upon peptide inhibitor/substrate binding. Proteins 58, 802–814.