Kallikreins on Steroids: Structure, Function, and Hormonal Regulation of Prostate-Specific Antigen and the Extended Kallikrein Locus

Mitchell G. Lawrence, John Lai, and Judith A. Clements

Australian Prostate Cancer Research Centre-Queensland and the Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane 4059, Australia; and the Australian Prostate Cancer BioResource, Adelaide 5000, Australia

The 15 members of the kallikrein-related serine peptidase (KLK) family have diverse tissue-specific expression profiles and putative proteolytic functions. The kallikrein family is also emerging as a rich source of disease biomarkers with KLK3, commonly known as prostate-specific antigen, being the current serum biomarker for prostate cancer. The kallikrein locus is also notable because it is extraordinarily responsive to steroids and other hormones. Indeed, at least 14 functional hormone response elements have been identified in the kallikrein locus. A more comprehensive understanding of the transcriptional regulation of kallikreins may help the field make more informed hypotheses about the physiological functions of kallikreins and their effectiveness as biomarkers. In this review, we describe the organization of the kallikrein locus and the structure of kallikrein genes and proteins. We also focus on the transcriptional regulation of kallikreins by androgens, progestins, glucocorticoids, mineralocorticoids, estrogens, and other hormones in animal models and human prostate, breast, and reproductive tract tissues. The interaction of the androgen receptor with androgen response elements in the promoter and enhancer of *KLK2* and *KLK3* is also summarized in detail. There is evidence that all kallikreins are regulated by multiple nuclear receptors. Yet, apart from *KLK2* and *KLK3*, it is not clear whether all kallikreins are direct transcriptional targets. Therefore, we argue that gaining more detailed information about the mechanisms that regulate kallikrein expression should be a priority of future studies and that the kallikrein locus will continue to be an important model in the era of genome-wide analyses. **(*Endocrine Reviews* 31: 407–446, 2010)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/er.2009-0034 Received August 28, 2009. Accepted December 24, 2009.
First Published Online January 26, 2010

Abbreviations: AP-1, Activator protein-1; AR, androgen receptor; ARE, androgen response element; BLTX, blarina toxin; CBP, cAMP-responsive element-binding protein binding protein; ChIP, chromatin immunoprecipitation; ER, estrogen receptor; ERE, estrogen response element; ERG, ETS-related gene; ERRα, estrogen receptor-related receptor α; ETV1, ETS variant 1; GR, glucocorticoid receptor; GRE, glucocorticoid response element; HDAC, histone deacetylase; HRE, hormone response element; IGFBP, IGF binding protein; KLK, kallikrein-related serine peptidase; KLKB1, plasma kallikrein; KLKP1, kallikrein pseudogene 1; MSR1, minisatellite repeat 1; NCoR, nuclear receptor corepressor; PAR, protease-activated receptor; PPARγ, proliferator-activated receptor γ; Pol II, RNA polymerase II; PR, progesterone receptor; PSA, prostate-specific antigen; RXR, retinoid X receptor; SMG, submandibular gland; TSS, transcription start site; UTR, untranslated region; WFDC, whey acidic protein four disulfide core.

I. Introduction

The 100th anniversary of kallikrein research was celebrated in 2009 (1). From humble beginnings, the kallikrein family is now studied across a remarkable range of human diseases because it has a broad expression profile (2–5). At least one of the 15 kallikreins has been detected in all tissue samples except nerves (6). The expression patterns of kallikreins have always been of immense interest and importance to the field. Tissues that express high levels of kallikreins, like the salivary gland and prostate, were used to clone new members of the kallikrein family (7, 8). Knowledge of the tissue-specific expression profiles of kallikreins has helped identify potential substrates and also underpins their use as disease biomarkers. In many tissues, kallikrein expression is regulated by steroids and other hormones. Indeed, the kallikrein locus is exceptionally hormone responsive because every kallikrein is up-regulated by multiple hormones. For this reason, many researchers use kallikreins as markers of hormone receptor activity. In particular, the actions of the androgen receptor (AR) are often measured through changes in *KLK2* or *KLK3* levels (9). Our laboratory reviewed the tissue-specific expression and hormonal regulation of kallikreins 20 yr ago when just three human genes had been described (10). We now return to this topic to re-examine early reports, analyze the progress that has since been made, and discuss the challenges that still remain.

II. Historical Background

The first observation of kallikrein activity was made when Abelous and Bardier (1, 11, 12) discovered that an alcohol-insoluble fraction of human urine caused peripheral vasodilation and hypotension in dogs. The depressor substance was named "urohypotensine," and the experiments were later confirmed by other laboratories (13–15). In seminal studies during the 1930s, Frey, Kraut, and Werle (16) showed that this factor was abundant in pancreatic extracts, so it was renamed "kallikrein" stemming from the Greek synonym for pancreas, *kallikreas* (16, 17). The hypotensive effect of the kallikrein enzyme, now known as KLK1, was subsequently attributed to its ability to liberate vasoactive kinin peptides from kininogens (17–19). Therefore, kininogenase activity was initially used as the defining characteristic of kallikreins (20). This definition encompassed two distinct enzymes, tissue or glandular kallikrein (KLK1) and plasma kallikrein (KLKB1), which have different expression profiles, protein structure, and substrate specificity (21–23). KLK1 is a 25- to 40-kDa glycoprotein related to trypsin and the founding member of the kallikrein-related serine peptidase (KLK) family. It is highly expressed in a range of visceral organs and able to process kininogens, growth factors, and extracellular matrix molecules (18, 21, 24). KLKB1 is an 85- to 88-kDa multidomain serine peptidase that is structurally related to factor XI. It is secreted by the liver into the blood system, where it activates clotting, fibrinolysis, and inflammation (18, 25). The human *KLKB1* gene spans 15 exons on 4q35 and does not belong to a multigene family (26, 27). *KLKB1* falls outside the scope of this article but has been comprehensively reviewed elsewhere (25, 28).

The use of the term kallikrein evolved with the understanding of the structure and function of the kallikrein-related peptidase locus. Once the amino acid sequence for porcine KLK1 and nucleotide sequence for rat *KLK1* were described, their considerable homology to other serine peptidases suggested that *KLK1* might be part of a multigene family (29, 30). Before long, 24 mouse kallikrein genes were cloned, including 10 pseudogenes (31, 32). At least 12 genes were identified in rats, 10 of which were shown to be transcriptionally active (33, 34). At the time, only three kallikreins were thought to exist in humans: *KLK1* and the recently described prostatic serine peptidases *KLK2* and *KLK3* (35–39). These genes were shown to be clustered together at syntenic loci in humans and rodents, but their evolutionary relationship was enigmatic because *KLK1* was the only gene present in all species (31, 34, 40, 41).

In the 1990s, it became apparent that the human kallikrein family was larger than first described when several other genes encoding serine peptidases were found close to the kallikrein locus. With the help of the draft human genome sequence, three laboratories showed that the extended human kallikrein locus contains 15 genes (42–44). Because they were described earlier, human *KLK1-3* and the rat and mouse genes became known as classical kallikreins, but *KLK1* remained the prototypical kallikrein gene. Whereas KLK2 and KLK3 have 62–67% amino acid identity with KLK1, the more recently identified kallikreins, KLK4–15, have only 27–39% identity with KLK1 (43). This explains why the extended kallikrein family eluded researchers for many years. Orthologs of *KLK4-15* have subsequently been identified in several mammalian species, and it is now clear that most clas-

sical kallikreins are closely related, species-specific genes within the expanded kallikrein locus (45–48). Importantly, unlike KLK1, many kallikreins do not have kininogenase activity (49–53). To acknowledge this discrepancy, KLK2–15 are now formally named kallikrein-related serine peptidases (54). Yet for simplicity, we will continue to refer to them as kallikreins in this review. Previous names of human, rat, and mouse kallikreins are listed in Table 1.

TABLE 1. Previous designations of kallikrein genes and proteins

Symbol	Other designations
KLK	
KLK1	Tissue/renal/pancreatic kallikrein, hK1, mGK-6 (mouse), pMAK3 (mouse), rGK-1 (rat), PS (rat), RSK1105 (rat)
KLK2	Glandular kallikrein, hGK1, hK2
KLK3	Prostate-specific antigen (PSA), APS, KLK2A1, hK3
KLK4	PRSS17, KLK-like 1, enamel matrix serine protease 1 (EMSP1), androgen-regulated message 1 (ARM1), PSTS, prostase, pemB, enamel serine proteinase (pEMS), hK4
KLK5	KLK-like 2, stratum corneum tryptic-like enzyme (SCTE), hK5
KLK6	PRSS9, PRSS18, brain and skin serine protease (BSSP), protease M, zyme, neurosin, myelencephalon specific protease (MSP), hK6
KLK7	PRSS6, stratum corneum chymotrypsin-like enzyme (SCCE), hK7
KLK8	PRSS19, neuropsin, HNP, ovasin, tumor-associated differentially expressed gene-14 (TADG-14), brain serine protease 1 (BSP1), hK8
KLK9	KLK-like 3, hK9
KLK10	PRSSL1, normal epithelial-Specific 1 (NES1), hK10
KLK11	PRSS20, trypsin-like serine protease (TLSP), hippostasin, hK11
KLK12	KLK-like 5, hK12
KLK13	KLK-like 4, hK13
KLK14	KLK-like 6, hK14
KLK15	Prostinogen, ACO protease, HSRNASPH, hK15
Klk1b[a]	
Klk1b3	γ Nerve growth factor subunit, pSM676, mGK-3
Klk1b4	α Nerve growth factor subunit, 2A4, mGK-4
Klk1b8	pMF-2, mGK-8
Klk1b9	Epidermal growth factor-binding protein C (EGF-BP C), MBI-73, mGK-9
Klk1b16	γ-renin, mGK-16
Klk1b22	β Nerve growth factor endopeptidase, epidermal growth factor-binding protein type A (EGF-BP A), enzyme A, mGK-22
Klk1b26	Epidermal growth factor-binding protein type B (EGF-BP B), Egfbp2, pSGP-2, prorenin converting enzyme, pPRECE, pPRECE-2, mGK-26, mGK13
Klk1c	
Klk1c2	rGK-2, RSKG-5, S2, rKLK2, tonin
Klk1c3	rGK-3, RSKG-50, S1, rKLK3
Klk1c4	rGK-4, rKLK4
Klk1c6	rGK-6, rKLK6
Klk1c7	RSKG-7, rKLK7, K1, rK7, esterase B, proteinase A
Klk1c8	rGK-8, rKLK8, P1, rK8
Klk1c9	rKLK9, S3, KLKP-S3, rK9, SEV
Klk1c10	rKLK10, rK10, endopeptidase k, T-kininogenase, proteinase B, antigen D3b region
Klk1c12	RSKG-3, rKLK12

[a] All classical mouse genes previously had the prefix mGK (mGK-1 is KLK1b1, mGK-2 is Klk1b2-ps, etc.).

III. Organization of the Human Kallikrein Locus

The human kallikrein locus spans approximately 265 kb on chromosome 19q13.3–13.4 and is the largest contiguous cluster of peptidases in humans (42–44). The centromeric end of the kallikrein locus is bordered by several hypothetical small nucleolar RNAs and *C19orf48*, a minor histocompatibility antigen of unknown function (55, 56). The *CD33rSiglec* gene family of IgG-like lectin receptors borders the telomeric end of the kallikrein locus (57). Intergenic spacing between kallikrein genes is quite variable, ranging from approximately 1.5 kb between *KLK1* and *KLK15* to 32.5 kb between *KLK4* and *KLK5*. Nevertheless, all but *KLK2* and *KLK3* are transcribed from telomere to centromere. This organization is disrupted in some tumor cells. For example, fusion between *KLK2* and *ETV4*, an ETS family transcription factor on 17q21, was identified in a specimen of prostate cancer (58). The fused gene contains exon 1 of *KLK2* and generates a novel chimeric transcript of unknown function. In addition, copy number gains of the kallikrein locus have been noted in breast, bladder, and ovarian cancer cell lines and ovarian cancer tissues (59–61). Unlike the *KLK2* fusion, the copy number gains are due to large unbalanced translocations of 19q rather than rearrangements within the kallikrein locus.

Repetitive elements comprise 34–52% of the kallikrein locus, whereas protein coding regions make up only 4.3% (42, 62). This is typical for chromosome 19, which is exceptionally rich in repetitive elements (63). The most common repeats in the kallikrein locus are short interspersed nuclear elements (SINEs), such as ALUs and mammalian-wide interspersed repeats (MIRs), which account for about 22% of the total sequence (42, 62). A simple minisatellite repeat, MSR1, is particularly interesting because it is predominantly, although not exclusively, located within the q13.2–13.4 region of chromosome 19 (62, 64). There are MSR1 elements in the 3′ untranslated regions (UTRs) of *KLK4* and *KLK14*, introns of *KLK6*, *7*, and *14*, and several intergenic regions (62, 65–67). Of note, the number of MSR1 repeats within the *KLK4* and *KLK14* 3′ UTRs varies between matched normal and malignant specimens of breast and prostate (62). The functional consequences of these polymorphisms have not been investigated, but could include changes in the stability of kallikrein mRNA transcripts.

Further interest in repetitive elements within the human kallikrein locus was sparked by the identification of a new

kallikrein-like gene, *kallikrein pseudogene 1* (*KLKP1*) (68–70). *KLKP1* lies between *KLK2* and *KLK4* and contains three "exonized" repetitive elements: an AluY repeat for exon 1, a MLT2A2 long-terminal repeat for exon 2, and an ERVL endogenous retrovirus-related repeat for most of exon 5 (69). Exons 3 and 4 are homologous to a segment spanning intron 1 to intron 2 of *KLK1-3*, suggesting that *KLKP1* was created by genomic duplications within the kallikrein locus. At least four different transcripts arise from *KLKP1*, including *ψKLK1*, *KLK31P-short*, *KLK31P-long*, and *KRIP1* (68–70). None of these transcripts encode a serine peptidase; however, a 143-amino acid intracellular protein is translated from *KRIP1* and possibly *KLK31P-long* (68). The *KLKP1* transcripts may not be the last novel transcripts to be identified in the kallikrein locus. Based on expressed sequence tag alignments, it seems that some other regions outside kallikrein genes are also transcribed (our unpublished observations). This suggests that current understanding of the kallikrein locus may still not be complete.

IV. Structure of Kallikrein Genes

In addition to their colocalization in the genome, similarities in form and function unify the kallikrein-related serine peptidase family. As shown in Fig. 1A, all kallikrein genes have five coding exons, which are conserved in size and arrangement (42, 71). Exon 1 contains the 5′UTR and the start codon, whereas exon 5 contains the termination codon and 3′UTR. The histidine, aspartate, and serine residues of the catalytic triad are encoded by exons 2, 3, and 5. The intron phases (1, 2, 1, 0) and splice site sequences between coding exons are also consistent between kallikrein genes (72). The only exception is a variant splice site for intron 4 of *KLK10* (73).

In contrast to the coding regions, there are substantial differences between the untranslated regions of kallikrein genes. For example, the size and sequence of introns varies considerably between kallikreins (42). Furthermore, most human kallikrein genes have one to three additional upstream exons arising from alternative transcription start

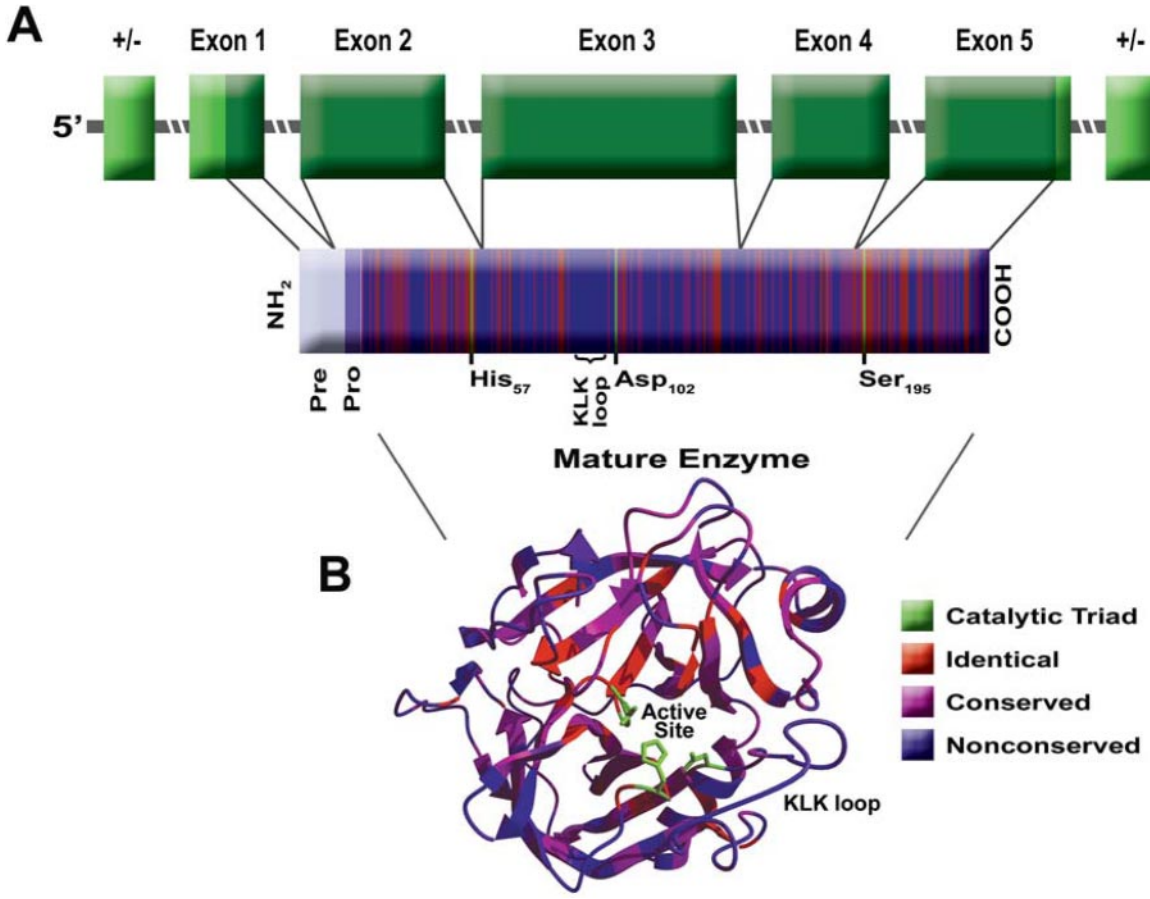

FIG. 1. The structure of kallikrein genes and proteins. A, Kallikrein genes contain five coding exons (*dark green*) and a variable number of noncoding exons (*light green*). Kallikrein proteins have a predomain, required for intracellular trafficking and a prodomain that must be cleaved for proteolytic activity. B, The structure of a mature kallikrein protease is based on the crystal structure of KLK1 reported by Laxmikanthan *et al.* (125). The histidine, aspartate, and serine residues of the catalytic triad are shown in *green*. The position of the kallikrein loop is also noted. The amino acids that are identical among all kallikreins are *red*, whereas those that are conserved in at least eight kallikreins are *purple*. Nonconserved amino acids are *blue*. The residues surrounding the active site are more highly conserved than the surface exposed loops.

sites (TSS) (3). Some of these noncoding exons need to be experimentally validated using rapid amplification of cDNA ends because they have only been predicted *in silico*. *KLK1*, *2*, and *3* do not have additional 5′ exons but instead have TSSs near putative TATA boxes 20 to 30 bp upstream of exon 1 (72). Unlike the 5′UTR, the 3′UTR is contained within a single exon for all kallikrein genes except *KLK14*, which has an additional downstream exon (65). Yet the length of the 3′UTR is quite variable, ranging from 45 bp for *KLK8* to 748 bp for *KLK7* (3). Differences in the 5′ and 3′UTRs may affect the relative stability and translational efficiency of kallikrein transcripts as well as their susceptibility to microRNA (74). Further research into noncoding regions and posttranscriptional regulation of kallikreins will help clarify the relationship between transcriptional regulation and protein abundance.

V. Evolution of the Kallikrein Locus

The evolution of the kallikrein family may underlie similarities in transcriptional regulation and proteolytic function. Kallikreins belong to the S1A family of the PA clan of serine peptidases, which is the largest group of enzymes in the human degradome (75). PA peptidases have a characteristic trypsin-like structure with most S1A subset being secreted proteins (76). The origin of S1A peptidases is contentious but likely occurred in lower eukaryotes before the family underwent substantial duplications and divergence during the evolution of higher metazoates (77). Based on both phylogenetic trees and active site evolutionary markers, kallikreins are most closely related to trypsins and granzymes within the S1A family (78, 79). Kallikrein genes have the same structure as trypsin, but vary from other serine peptidases that have different intron phasing or additional coding exons (80). Indeed, it has been suggested that kallikreins could have easily been classed as trypsins had they not shared a locus with *KLK1* (5). The clustering of kallikreins at a single locus differs from the dynamic evolution of most peptidase families where paralogous genes have translocated to different chromosomes after their duplication (81).

It is possible that kallikreins first arose some time during chordate evolution because they are present in mammals and some reptiles (45, 82, 83). Using bioinformatics, no kallikrein genes were detected in the chicken or frog genomes (45). In placental mammals, the kallikrein family has been described at syntenic regions to the human locus in the mouse (chromosome 7 B2), rat (chromosome 1q21), pig (chromosome 6q12–21), dog (chromosome 1), chimpanzee (chromosome 20), and other species (45–48, 84). Only *KLK5-15* are present in the opossum, which is a marsupial (45). It is not clear whether the lack of *KLK1* and *KLK4* in the opossum means that these genes arose later in mammalian evolution or were deleted in this species.

Given that all placental mammals have *KLK1* and *KLK4-15*, it is likely that these kallikreins have important physiological functions. As shown in Fig. 2, the position and orientation of these genes are highly conserved. Phylogenetic analyses from most studies agree that KLK4 and KLK5 segregate from other kallikreins, as do KLK9 and KLK11, and KLK10 and KLK12 (43, 45–47, 81, 83–86). This implies, for example, that a duplication of *KLK9* and *KLK10* generated *KLK11* and *KLK12*, or vice versa. Currently, there is no consensus on other members of the extended kallikrein locus, but the resolution of phylogenetic trees may improve as kallikreins are identified in other species.

Further comparisons of the kallikrein locus between species suggest that *KLK2* and *KLK3* have a distinct evolutionary history from other human kallikrein genes. *KLK2* and *KLK3* are closely related to *KLK1* because they segregate with *KLK1* in phylogenetic analyses and are the only other genes that encode the kallikrein loop (Fig. 1B) (87). It is likely that a duplication of *KLK1* created the progenitor of *KLK2* in the early evolution of eutherian mammals. It seems this gene was silenced in rats and mice to become *Klk2-ps* and deleted in other species like the cow and pig (83). Humans, dogs, and many primates still have *KLK2*, but it is a pseudogene in some species throughout the primate order (37, 45, 88–91). It is likely that *KLK3* arose through duplication of *KLK2* during primate evolution. In keeping with their close evolutionary relationship, *KLK2* and *KLK3* have highly homologous coding, noncoding, and promoter regions. *KLK3* is present in humans and various species of apes and Old World monkeys, but not New World monkeys or the dog (36, 92–95). Given that high levels of KLK2 and KLK3 are secreted by the prostate, the loss of *KLK2* and gain of *KLK3* in particular mammals may reflect differences in reproductive biology. The major substrates of KLK2 and KLK3 in seminal plasma, semenogelin I and II are coagulum proteins that are unique to primates (96). The semenogelin genes are rapidly evolving with many differences between species (92). *KLK2* may also be under positive selection in primates at certain residues that could alter its substrate specificity, whereas *KLK3* appears to be under weak or variable selection (90, 92).

Further duplications within the centromeric end of the kallikrein locus have led to divergent evolution of this gene family in some species. To avoid confusion, current nomenclature classifies kallikreins into subfamilies when they are only present within a single species or in highly

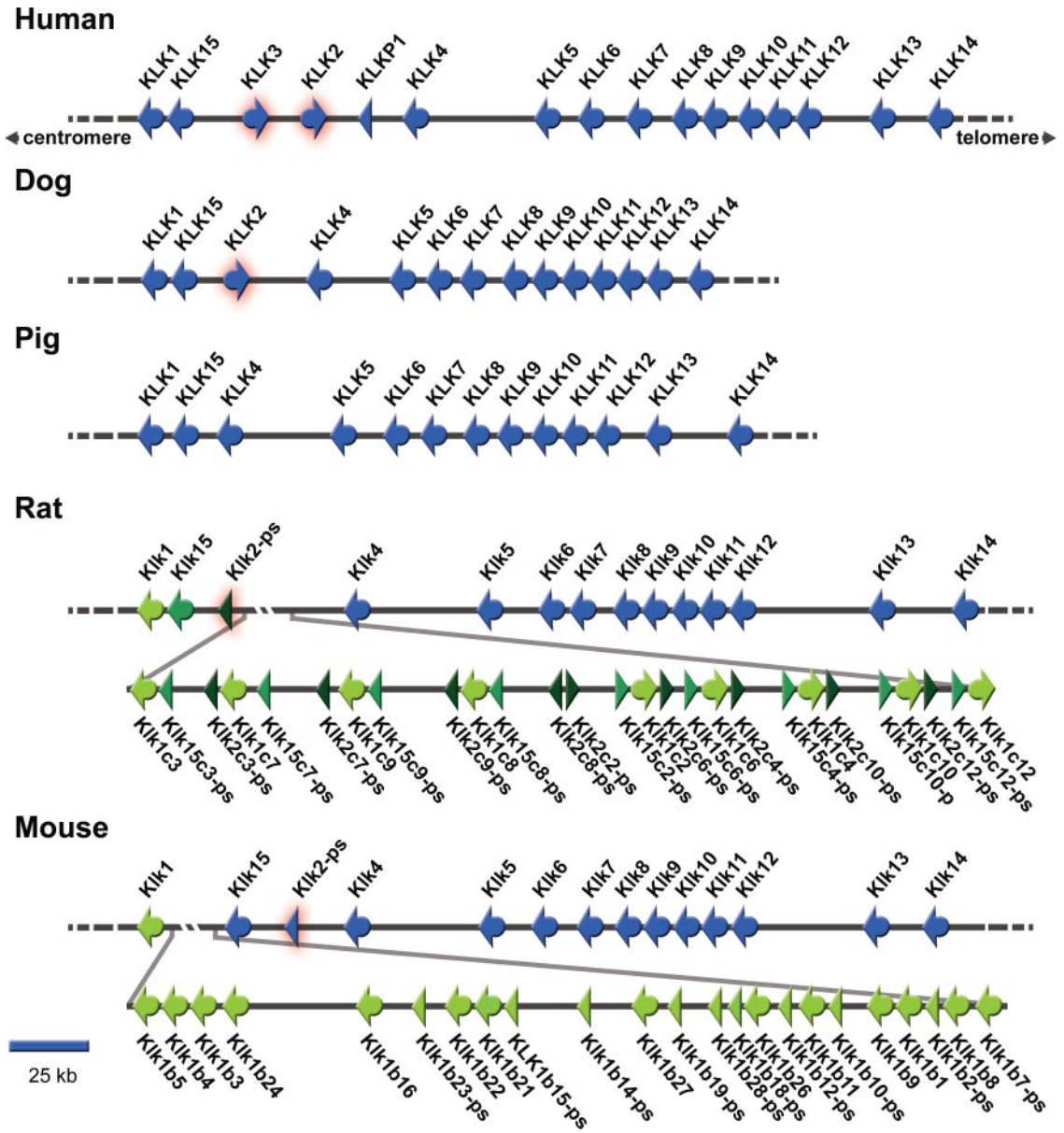

FIG. 2. The organization of the kallikrein locus. The orientation and approximate position of kallikrein genes are shown for mammalian species (human, dog, pig, rat, mouse) where the hormonal regulation of kallikreins has been studied. Kallikrein genes are shown as *arrows*, whereas pseudogenes are shown as *arrowheads*. *KLKP1* is also shown as an *arrowhead* because it does not encode a serine peptidase. The classical rat and mouse kallikrein gene subfamilies are shaded *green* to show whether they are paralogs of *Klk1*, *Klk2-ps*, or *Klk15*. *KLK2*, *KLK3*, and *Klk2-ps* genes are highlighted *red* to emphasize their close evolutionary relationship. This figure is adapted from Refs. 45, 46, 54, and 83.

related species (54). So far these subfamilies include the b-family in the mouse, c-family in the rat, and d-family in the horse. Based on Southern blots with a human *KLK1* probe, at least six classical kallikrein genes have been detected in the horse (47). One of these genes was initially classified as an ortholog of *KLK3*, but recent phylogenetic analyses suggest that *KLK1D2* is more likely to be a paralog of *KLK1* (47, 97). The functions of these horse kallikreins are unknown; however, the presence of KLK1D1 in stallion seminal plasma further implies that there is a link between reproduction and the evolution of the kallikrein locus.

The selective pressures on the kallikrein locus seem to have been different in the shrew, which has three paralogs of *KLK1*: *blarina toxin (BLTX)*, *blarinasin-1*, and *blarinasin-2* (98). These proteins have a higher level of amino acid identity with human KLK1 than it has with human KLK4–15. Like KLK1, BLTX and blasrinasin-1 are secreted from the salivary gland and have kininogenase activity (99, 100). BLTX is a constituent of shrew venom and has acquired toxic properties through small insertions and mutations that are predicted to increase its efficiency as an enzyme (98). Strikingly, venomous snakes and lizards also have KLK1-like enzymes that act as toxins, some with similar insertions to BLTX,

suggesting that particular kallikreins have undergone convergent evolution in reptiles and mammals (82, 98, 101).

The most dramatic differences in the kallikrein locus are in murine rodents. In the mouse, a 290-kb region between *Klk1* and *Klk15* contains 24 *Klk1* paralogs, although 10 are pseudogenes (31, 48). A *KLK2*-like pseudogene, *Klk2-ps*, occupies a similar position to human *KLK2*, between *KLK15* and *KLK4*, but does not have the reverse orientation of human *KLK2*. In rats, a 30-kb segment spanning *Klk1*, *Klk2-ps*, and *Klk15* has been duplicated nine times between *Klk2-ps* and *Klk4* (47). The *Klk1* paralogs encode potentially functional proteins, whereas the *Klk2-ps* and *Klk15* paralogs are all pseudogenes. In contrast to rats and mice, the guinea pig, a cavian rodent, is only estimated to have two or three classical kallikreins including *KLK1* and a prostatic kallikrein (47, 102). The functional relevance of the expanded kallikrein locus in rats and mice is unclear, and research into these classical kallikreins has waned now that it is known that they lack human orthologs. One theory is that murine kallikreins are involved in wound healing through licking because they are highly expressed in the salivary gland and can activate some growth factors. For example, Klk1b9, Klk1b22, and Klk1b26 can activate proepidermal growth factor, and Klk1b3, Klk1b4, and Klk1b22 are part of the 7S nerve growth factor (NGF) complex that cleaves pro-NGF (103). Yet murine kallikreins are also expressed in a range of other tissues where their functions are unknown. It has also been suggested that the expansion of the kallikrein locus is linked to the evolution of the whey acidic protein four disulfide core (WFDC) family of serine protease inhibitors, which have homology to semenogelins and a role in immunity (104). Like the kallikrein locus, the WFDC cluster has undergone separate expansion in mice and rats compared with humans.

VI. Structure of Kallikrein Proteins

Like most members of the S1A peptidase family, kallikreins are produced as preproenzymes (Fig. 1A) and must be correctly processed to become proteolytically active. Newly synthesized kallikreins are first directed to the endoplasmic reticulum and secretory pathway by the 16- to 33-amino acid signal peptide or predomain. After secretion, kallikreins remain as inactive proenzymes or "zymogens" until the 3- to 37-amino acid prodomain is removed. This induces a conformational change in the substrate binding pocket that allows kallikreins to capture and cleave their substrates (105). The prodomain is cleaved at an arginine$_{16}$ or lysine$_{16}$ residue in most kallikreins, indicating that they are activated by peptidases with trypsin-like specificity, including other kallikreins (106–108). KLK4 is the only exception. It has a glutamine$_{16}$ residue and may instead be activated by enzymes such as matrix metallopeptidase-20 and dipeptidyl peptidase I, a cysteine peptidase (109, 110). In general, KLK1, 2, 3, 5, 9, and 11, which have an arginine$_{16}$ residue, are more efficiently activated by other kallikreins than those with a lysine$_{16}$ residue (106, 108). KLK2, 5, 11, 12, and 14 also have the ability to autoactivate (53, 108, 111–114). Several peptidases in the thrombostasis axis including plasmin, tissue plasminogen activator, urokinase-type plasminogen activator, factor Xa, thrombin, and KLKB1 can also cleave the prodomain of particular kallikreins (107).

Once activated, kallikreins function as endopeptidases to cleave bonds within polypeptide chains. Like all members of the PA clan, the proteolytic activity of kallikreins depends on the catalytic triad of histidine$_{57}$, aspartate$_{102}$, and serine$_{195}$ residues (standard bovine chymotrypsin numbering) that span the active site (Fig. 1B) (76). Crystal structures have been solved for human KLK1 and KLK3–7, pig KLK1, horse KLK1D2, rat Klk1c2, and mouse Klk8, Klk13, Klk1b3, and Klk1b4 (85, 115–128). They show that the catalytic triad is brought together from separate parts of the protein by two asymmetric six-stranded β-barrels with "Greek key" topology that is typical of PA clan peptidases (Fig. 1B). The catalytic serine$_{195}$ initiates proteolysis by attacking a carbonyl moiety within a peptide bond of the substrate (105). The substrate specificity of kallikreins depends on residue 189, which lies at the base of the substrate binding pocket. KLK1, 2, 4, 5, 6, and 10–14 all have an aspartate$_{189}$ residue that confers them with trypsin-like specificity to cleave after arginine or lysine residues (83, 129). KLK15 also has trypsin-like specificity, but has a glutamate at position 189 (106, 130). KLK3, with a serine$_{189}$, and KLK7, with an asparagine$_{189}$, both have chymotrypsin-like specificity for tyrosine, leucine, and phenylalanine residues (39, 131). It is difficult to predict the specificity of KLK9 because its glycine$_{189}$ residue is rare among serine peptidases. It is possible that KLK9 has similar specificity to human neutrophil elastase, which also has a glycine$_{189}$ residue and tends to cleave after valine and alanine residues (132, 133). The substrate specificity of kallikreins is further refined by residues in eight loops surrounding the mouth of the active site and charged exosites on the surface of the proteins (Fig. 1B) (129).

Based on multiple sequence alignments, almost half the amino acids (122 residues) are conserved in at least half of the human kallikrein proteins (3). Of the 39 amino acids that are identical between human kallikrein proteins, 37 are also present in human trypsin and 33 in bovine chymotrypsin. The amino acids involved in proteolytic ac-

tivity and protein folding are highly conserved between kallikreins, whereas residues associated with substrate specificity are more divergent. The catalytic triad and several adjacent residues are identical among kallikreins, as are 10 disulfide bridge-forming cysteine residues. KLK4–12 and 15 have an additional pair of cysteine residues (137 and 232) that are unique to kallikreins compared with other S1A peptidases (79). $Glycine_{193}$ is present in all kallikreins except for KLK10, which has a glutamate instead. This residue is highly conserved among serine peptidases because it helps form the oxyanion hole that stabilizes negatively charged intermediates during proteolysis (105). The substitution in KLK10 may account for its apparent lack of proteolytic activity against traditional kallikrein substrates (134). The sequence of kallikrein proteins is most divergent in the eight surface-exposed loops surrounding the active site. The 99 loop is the most extreme example where KLK1–3 have an 11-amino acid insertion known as the "kallikrein loop." KLK8–11 and 13 also have small insertions in this region. The 148 loop also varies between kallikreins, most notably for KLK15, which has a 10-amino acid insertion. Notwithstanding the variation between surface-exposed loops, the core structure of kallikrein proteins is conserved. This suggests that kallikreins can cleave different substrates, but through the same mechanism.

VII. Proteolytic Functions of Kallikreins

Some serine peptidases like trypsin, thrombin, and plasma kallikrein are centrally produced and systemically active. Kallikreins, in contrast, may be locally produced and locally active or secreted as bioactive components of bodily fluids. Because the kallikrein family has a diverse expression profile, it is associated with a broad range of physiological functions. The many potential kallikrein substrates fall into several categories including growth factors and signaling molecules, extracellular matrix proteins, cell adhesion proteins, and cell surface receptors. A complete list of putative substrates is shown in Table 2. It is important to note that many candidate substrates have only been tested in binary biochemical assays where they are combined with purified or recombinant kallikreins. Consequently, the predicted physiological and pathophysiological roles of most kallikreins in most tissues are quite speculative. It is expected that more definitive and biologically relevant substrates will be identified in the next few years due to the increased use of proteomics and *in vivo* models.

Low molecular weight kininogen was the first kallikrein substrate to be identified and is still one of the most extensively studied. KLK1 cleaves low molecular weight kininogen at two sites to release kallidin, a decapeptide also known as lys-bradykinin, which can be further processed by other peptidases into a nonapeptide, des-Arg^{10}-kallidin (135). Kallidin and des-Arg^{10}-kallidin are kinins that mediate the cellular effects of KLK1 by binding to bradykinin receptors 1 and 2. These G protein-coupled receptors subsequently stimulate the production of nitric oxide, prostaglandins, and other secondary mediators that trigger vasodilation, smooth muscle contraction or relaxation, inflammation, and pain (18, 135). This means that the kallikrein-kinin system has a protective role in cardiovascular disease, stroke, and renal dysfunction but exacerbates inflammatory conditions such as asthma (136). KLK2, 5, 8, and 14 cleave either high or low molecular weight kininogen *in vitro*; however, only KLK2 has been shown to generate the kinin peptide, and with a 1000-fold less efficiency compared with KLK1 (6, 52, 53, 137, 138).

A particularly important group of kallikrein substrates are other peptidases. KLK2, 4, 5, 12, and 14 are the most efficient at activating other kallikreins (106, 107). Yet kallikreins do not always only activate one another. KLK5 and KLK14 initially activate KLK3, before inactivating it by cleaving sites within the mature protein (139, 140). Some kallikreins have also been shown to activate other classes of peptidases. For example, KLK1 may activate matrix metallopeptidases 2 and 9, whereas KLK2, 4, and 8 may activate urokinase-type plasminogen activator (141–145). Collectively, these observations suggest that kallikreins participate in enzyme cascades. This means that the proteolytic response to a stimulus may be amplified or modified depending on the particular kallikreins that are present. Indeed, multiple kallikreins are thought to be involved in enzyme cascades in the skin and seminal plasma (111, 140, 146). Therefore, a greater understanding of the substrate specificity and gene expression profiles of kallikreins is needed to predict their biological functions in each tissue.

Kallikreins also have a well-characterized role in skin homeostasis (5). KLK1 and 4–14 are all expressed in skin, but the actions of KLK5, 7, and 14 have been studied in the most detail (147–151). Kallikreins facilitate skin desquamation by degrading desmosomal adhesion proteins such as corneodesmin, desmoglein, and desmocolin in the outermost layer of the skin (146, 152). In addition, KLK5 and KLK7 may bolster innate immunity within the skin by activating antimicrobial cathelicidins (153). The activity of kallikreins within the skin is regulated by the pH gradient, enzyme cascades with other kallikreins, and the levels of protease inhibitors such as lymphoepithelial kazal type inhibitor, which arises from the *Spink5* gene (146, 154). A range of pathologies develop when the balance of kallikrein activity is disrupted in mouse models. *KLK7*

TABLE 2. Putative kallikreins substrates (version 1)

Substrate	Kallikrein/s
Blood pressure regulation	
Angiotensinogen	KLK1
Atrial natriuretic peptide	KLK1
Bradykinin B2 receptor	KLK1
Fibrinogen	KLK2–6, 8, 14
Low molecular weight kininogen	KLK1, 2, 5, 14
High molecular weight kininogen	KLK1, 5, 8, 14
Plasminogen	KLK3, 5, 6, 13, 14
Tissue plasminogen activator	KLK1
Prorenin	KLK1
Cell-cell adhesion molecules	
Corneodesmin	KLK5, 7
Desmocollin	KLK5, 12
Desmoglein 1	KLK1, 3, 5, 6, 12, 14
Desmoglein 2	KLK5
E-cadherin	KLK6, 7
Cell surface receptors	
PAR1	KLK1, 2, 4, 14
PAR2	KLK2, 4, 5, 6, 14
PAR4	KLK14
Urokinase receptor	KLK4
Cytokines, growth factors, and hormones	
GH	KLK4–6, 8, 13, 14
GHRH	KLK1
IGFBP1	KLK5
IGFBP2	KLK5, 14
IGFBP3	KLK1–5, 11
IGFBP4	KLK4, 5
IGFBP5	KLK4, 5
IGFBP6	KLK4, 5
Insulin A and B chains	KLK1, 3
pro-IL-1β	KLK7
IL-2	KLK3
PTHrP	KLK3
Somatostatin	KLK1
Latent TGFβ1	KLK1, 2, 5, 14
Latent TGFβ2	KLK3
Vasoactive intestinal peptide	KLK1
Enzymes and proteases	
β-glucocerebrosidase	KLK7
proHGF activator	KLK4, 5
proKLK1	KLK2–8, 11–15
proKLK2	KLK2–8, 12–14
proKLK3	KLK2–8, 11–15
proKLK5	KLK2, 4–6, 8, 11, 12, 14, 15
proKLK6	KLK4, 5, 11, 14
proKLK7	KLK5, 12, 15
proKLK8	KLK5, 12, 15
proKLK9	KLK1–6, 12–15
proKLK10	KLK14
proKLK11	KLK2–6, 8, 11–15
proKLK12	KLK4, 5, 8, 11, 12, 14
proKLK13	KLK4, 5, 12, 14
proKLK14	KLK4, 5, 11, 12, 14
proKLK15	KLK4, 5, 12, 14, 15
proMeprin β	KLK4
proMMP2	KLK1, 3
proMMP9	KLK1
Sphingomyelinase	KLK7
proUrokinase	KLK2, 4, 6, 8
Extracellular matrix	
Aggrecan	KLK6
Amelogenin	KLK4
Collagen I	KLK5, 6, 13, 14
Collagen II	KLK5, 13, 14
Collagen III	KLK5, 6, 13, 14
Collagen IV	KLK1, 5, 6, 8, 14
Enamelin	KLK4
Fibronectin	KLK1–3, 5–8, 13, 14
Laminin	KLK3, 5, 6, 13, 14
Vitronectin	KLK5, 6, 14
Immune defense	
Cathelicidin hCAP18	KLK5, 7
Defensin α	KLK5
Lysozyme	KLK3
Mucin 4 and 5B	KLK5, 12
Neuronal biology	
Amyloid precursor	KLK6
L1 adhesion molecule	KLK8
Mylein basic protein	KLK6
$\alpha-$Synuclein	KLK6
Seminal plasma	
Prostatic acid phosphatase	KLK4
Seminogelin I and II	KLK2, 3, 5, 14
Miscellaneous	
Apolipoprotein B-100	KLK1
Casein	KLK1, 3, 5, 6, 8, 14
Gelatin	KLK1, 3, 4–8, 14
Myoglobin	KLK3
Ovalbumin	KLK3

(*Continued*)

TABLE 2. Continued

References for these substrates are provided in Version 2 of Table 2 in Supplemental Data. Please note that some experiments with purified kallikreins may contain contaminating peptidases.

overexpression causes inflammation and itching, *KLK8* knockout delays the recovery of the epidermis from UVB-induced inflammation, and *Spink5* knockout leads to a loss of skin barrier function (155–157). Moreover, *Spink5* mutations in humans cause Netherton syndrome, which is a severe autosomal recessive skin disorder (158). These observations suggest that kallikreins are potential therapeutic targets for skin diseases.

As previously mentioned, kallikreins have a functional role in reproductive biology through the postejaculatory cleavage of seminogelin I and II. Seminogelins are produced by the seminal vesicles and are the major structural gel-forming proteins in human semen (96). All kallikreins are present in seminal plasma; however, KLK2, 3, and 11 are by far the most abundant (159, 160). Interestingly, seminogelins regulate their own degradation by chelating the high concentration of zinc ions in seminal fluid that would otherwise inhibit kallikrein enzyme activity (140, 161–163). Degradation of seminogelins by kallikreins causes liquefaction of seminal fluid which facilitates sperm motility (164). So far, KLK2, 3, 5, and 14 have all been shown to cleave seminogelins (140, 161, 165, 166). Other kallikreins might also cleave seminogelins, other constit-

uents of seminal plasma such as fibronectin and prostatic acid phosphatase, or simply participate in enzyme cascades (144, 167).

In addition to their normal physiological functions, there has been great interest in the potential roles of kallikreins in cancer progression (2). For example, kallikreins are thought to enhance the invasion of tumor cells by cleaving cell-cell adhesion proteins like E-cadherin and degrading extracellular matrix molecules including fibronectin, laminin, vitronectin, and collagen I-IV. Indeed, KLK1, 3–10, and 13 all increase the *in vitro* invasiveness of tumor cell lines through reconstituted extracellular matrix solutions like Matrigel (61, 168–175). KLK6 also promotes the invasion of MCA3D keratinocytes through chicken chorioallantoic membranes (176). KLK1, 2, 4, 5, 6, and 14 may also facilitate the dissemination of tumor cells through protease activated receptors (PARs), a family of G protein-coupled receptors that initiate downstream signaling and cellular migration upon protease cleavage (177–186).

The functions of some putative substrates imply that kallikreins might also stimulate the proliferation of tumor cells. For example, IGF binding proteins (IGFBPs) are cleaved by KLK2–5, 11, and 14 (6, 138, 140, 187–192). Because cleaved IGFBPs have reduced affinity for IGF-I, it is possible that kallikreins increase the bioavailability of this mitogenic, antiapoptotic growth factor in the tumor microenvironment (193). Degradation of IGFBP3 by KLK3 increases the proliferation of prostate stromal fibroblasts (194), but there is no clear correlation between kallikrein levels and the proliferation of tumor cells. KLK3, 4, and 6 increase the *in vitro* proliferation of some cell lines, but not others (176, 195–199). Therefore, the biological significance of IGFBP degradation by kallikreins is unclear. This emphasizes that the relevance of kallikrein substrates identified in biochemical assays needs to be confirmed in biological contexts.

In addition to invasion and proliferation, kallikreins have been suggested to have several other functions in cancer progression, although many of the substrates involved have not been identified. KLK3 is the most extensively studied kallikrein in tumor progression, and its putative functions are quite varied. For instance, KLK3 may regulate oxygen balance in tumors. It inhibits the migration and tube formation of endothelial cells *in vitro* (200, 201) and stimulates the production of reactive oxygen species in prostate cancer cells (202). It is also possible that KLK3 modulates the immune response. For example, KLK3 has immunosuppressive effects, by inhibiting T cell proliferation and dendritic cell maturation (203, 204), as well as proinflammatory effects, by stimulating interferon γ secretion by natural killer cells (205). KLK3 has also been implicated in the metastasis of prostate cancer cells to bone. Overexpression of KLK3 increases the migration of prostate cancer cells through epithelial to mesenchymal transition (199). KLK3 also stimulates the proliferation and osteoblastic differentiation of bone cells (206–210), perhaps in part by activating latent TGFβ2 and degrading PTHrP (208, 211, 212). In turn, conditioned medium from bone cells up-regulates KLK3 expression in prostate cancer cells (213). Finally, it has been proposed that KLK3 enhances androgen-regulated gene expression in castrate-resistant prostate cancer cells by binding to a cofactor of the AR, ARA70 (197). These data show that even within one type of tumor, the functions of kallikreins depend on the context. Similarly, KLK6 and KLK10 either increase or decrease the aggressiveness of cancer cells depending on the tumor models that are tested (134, 173, 176, 198). This means that kallikreins cannot easily be classified as tumor promoters or suppressors without detailed functional studies.

VIII. Tissue-Specific Expression Profiles

Kallikreins are expressed throughout the body with distinct expression profiles that often overlap. Figure 3 shows the expression of human kallikrein genes in a broad range of tissues based on microarray data from Gene Atlas (214). The results are consistent with previous Northern blot, RT-PCR, and ELISA data showing that some kallikreins are produced at very high levels in specific tissues (42, 43, 160). For example, *KLK2* and *KLK3* are two of the most highly expressed genes in the prostate (215). *KLK1* is abundant in the kidney, salivary gland, and pancreas, whereas *KLK6* is highly expressed in the central nervous system. Yet none of these kallikreins is completely tissue-specific. KLK3 has been detected in salivary gland, brain, breast, and other tissues, albeit at more than 100-fold lower concentrations than the prostate (160, 216–218). Several kallikreins are usually expressed in the same tissue, although at quite different levels. In keeping with previous findings, Fig. 3 also shows that *KLK14* and *KLK15* are more lowly expressed than other kallikreins (160). Almost all kallikreins are expressed in the salivary gland, including the species-specific rat and mouse kallikrein genes (160, 219, 220). Many kallikreins are also produced in the skin, prostate, female reproductive tract, and other tissues (150, 199, 221). Intriguingly, adjacent genes in the kallikrein locus sometimes have similar expression profiles. For example, *KLK2*, *3*, and *4* are all highly expressed in the prostate compared with other tissues. Similarly, *KLK5–8* have parallel expression profiles in ovarian cancer (222). This has led some researchers to suggest that kallikreins

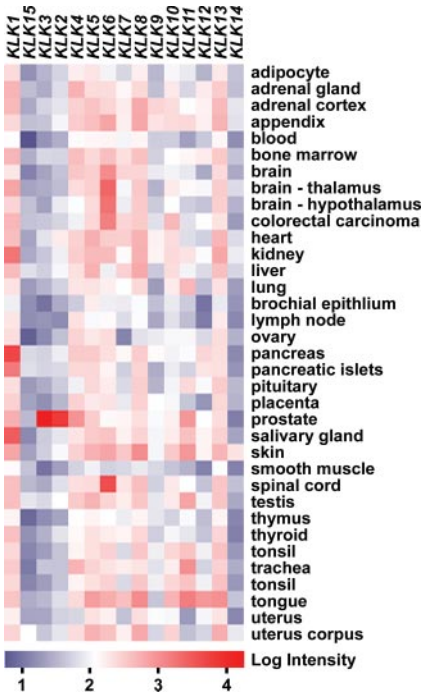

FIG. 3. Tissue-specific expression of the kallikrein family. A heatmap showing the Log_2 intensity of kallikrein gene expression in a selection of tissues from Gene Atlas (214). Data were downloaded from the Genome Expression Omnibus (GDS594 and GDS596). Genes are displayed in their order, from centromere to telomere, within the kallikrein locus. As shown in the key, *darker shades of red* represent highest gene expression. For genes with multiple probes (*KLK2*, *3*, *10*, *13*), the average probe intensity is shown. Please refer to Supplemental Methods for more information about data analysis.

may be coordinately regulated through "cassette-type" expression (223).

Kallikreins are expressed in specific cell types within each tissue. This means that data from whole tissue extracts provide a valuable overview of kallikrein expression but should be complemented by immunohistochemistry experiments. Figure 4A shows that KLK2, 3, 4, 14, and 15 are all expressed in luminal epithelial cells within the prostate, which is in keeping with their secretion into seminal plasma (167). A detailed study by Petraki *et al.* (224) and data on KLK2, 3, 5, 7, 8, 10, 13, 14, and 15 staining from the Human Protein Atlas (225) both show that in visceral organs kallikreins are primarily expressed by glandular epithelial cells. This is consistent with the secretion of kallikreins into a range of bodily fluids.

It is notable that the tissue-specific expression profiles of kallikreins are conserved among different mammalian species. For example, KLK3 is produced in the prostate of humans and various species of Old World monkeys (93, 95, 226). *KLK1* has been detected in the human, pig, dog, cat, rat, and mouse salivary gland and pancreas (24). *KLK4* is expressed during tooth development in humans, mice, and pigs (227). *KLK8* has been studied in the skin and brain of both humans and mice (228–231). Finally, different splice variants of *KLK11* are expressed in the brain and prostate of humans and mice (232, 233). Two conclusions can be drawn from these observations. First, the consistent expression patterns imply that there are evolutionarily conserved regulatory elements within kallikrein promoters. Second, the conserved expression profiles suggest that kallikreins have important and nonredundant functions that might involve different substrates in each tissue. Indeed, *KLK1*, *KLK4*, and *KLK8* knockout mice have confirmed the functional importance of these kallikreins in some tissues (234–237). KLK1 controls the production of kinins, KLK4 regulates the mineralization of tooth enamel, and KLK8 is involved in synaptogenesis and skin desquamation. Although there are many examples of conserved tissue-specific kallikrein expression between species, the complete expression profile of the extended kallikrein locus has only been reported for humans and the pig (42, 43, 46, 160). More extensive studies with other animal models are needed to determine whether the tissue-specific expression profile of each kallikrein is completely conserved or exhibits some species-specific differences.

IX. Kallikreins as Biomarkers of Disease

As a result of their tissue-specific expression profiles, the kallikrein family is a rich source of potential biomarkers (238). Indeed, KLK3 [prostate-specific antigen (PSA)] has been used as the serum biomarker for prostate cancer for more than 20 yr (239, 240). Normally, PSA is only secreted into seminal plasma, and very little enters the bloodstream, but in prostate cancer, it leaks into the circulation due to loss of glandular architecture and breakdown of basement membrane within the tumor (241, 242). Yet PSA is a prostate-specific marker, not a cancer-specific marker. Benign prostatic hyperplasia, a common condition in older men, also disrupts prostatic architecture and increases serum PSA levels (240, 241). Whether PSA screening reduces the rate of deaths from prostate cancer is controversial (243, 244). Furthermore, widespread use of the PSA test has led to stage migration where fewer cases of advanced and metastatic prostate cancer are being diagnosed compared with low grade and clinically insignificant disease (245, 246). The PSA test may be more valuable in younger men where benign prostatic hyperplasia is less common. For men in their forties, small increases in PSA concentration are highly predictive of the development of advanced prostate cancer in future decades (247).

In the search for ways to improve the PSA test, other members of the kallikrein family have been examined as potential adjunct biomarkers (248). There are promising

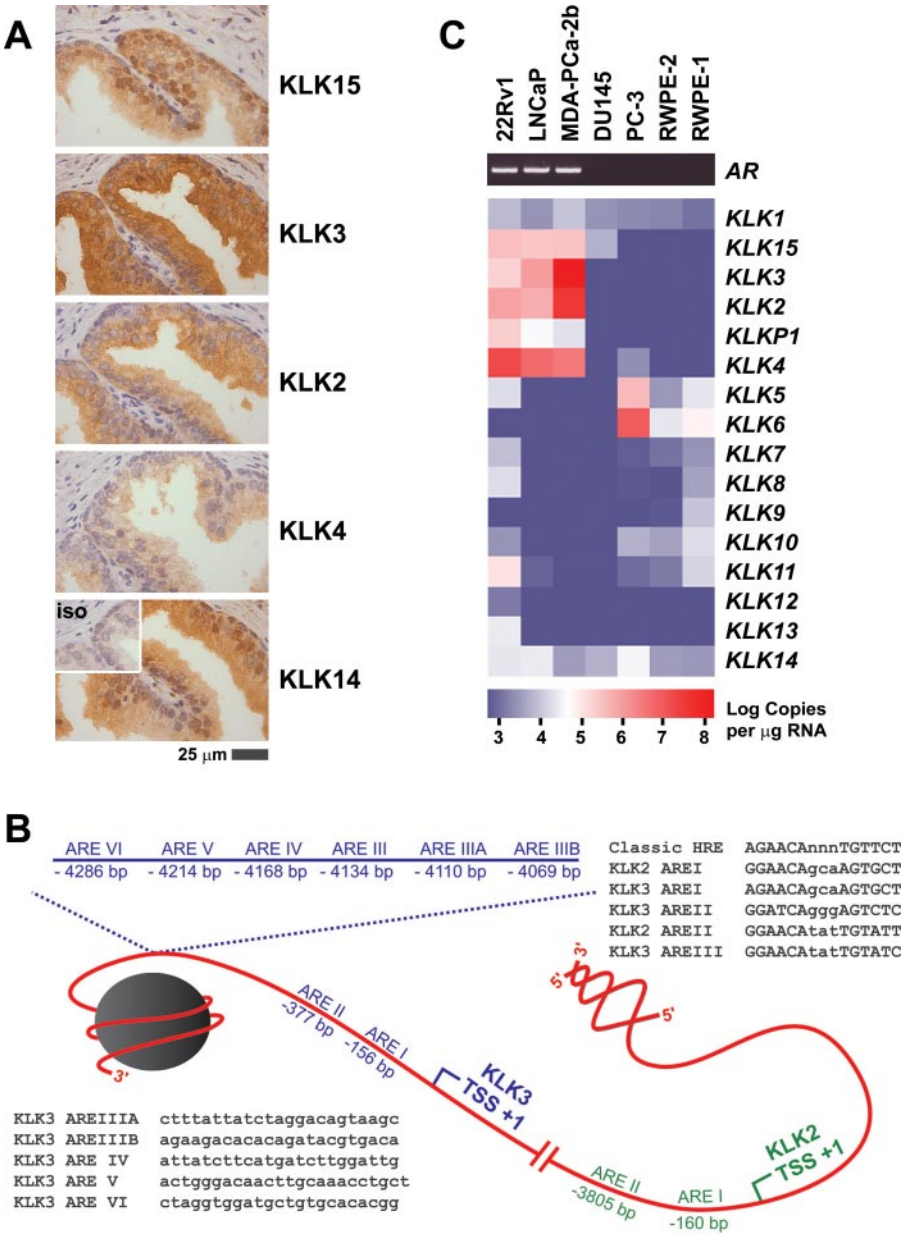

FIG. 4. Expression and androgen regulation of kallikreins in prostate. A, Immunohistochemistry for KLK2, 3, 4, 14, and 15 on serial sections of a sample of benign prostate. All kallikreins are expressed in luminal epithelial cells, but not the stroma. The isotype negative control (iso) is shown as an *inset*. Tissue specimens were obtained from the Australian Prostate Cancer BioResource. Scale bar, 25 μm. B, A diagram of functional AREs in the promoter and enhancers of the *KLK2* (*green*) and *KLK3* (*purple*) genes. ARE positions are relative to the TSSs where the first transcribed nucleotide is +1. Typically, the DNA is double-stranded when bound by the AR and histones (*circle*). The sequences of *KLK2* and *KLK3* AREI-III are shown, as are the DNase protected regions containing *KLK3* AREIIIA, B, IV, V and VI. C, A heatmap of Log copies of kallikrein transcripts per microgram of RNA in a panel of prostate cell lines. Genes are listed in their order in the kallikrein locus from centromere (*top*) to telomere (*bottom*). As shown in the color scale, *red cells* represent higher gene expression. The AR status of each cell line was confirmed using RT-PCR. *KLK2*, *3*, *4*, *15*, and *KLKP1* are highly expressed in the androgen-responsive 22Rv1, LNCaP, and MDA-PCa-2b cell lines. Intriguingly, genes that are adjacent in the kallikrein locus have similar expression profiles.

results for KLK2 that may help distinguish between prostate cancer and benign prostatic hyperplasia (249). KLK2 may also be a useful prognostic marker because its levels have been correlated with increased grade and stage of prostate cancer in several, although not all, studies (248). Serum levels of KLK5, 6, 8, 10, 11, and 14 have also been examined, albeit in comparatively small numbers of patients (250–253). There is a trend of reduced KLK5, 6, 8, and 10 levels in men with prostate cancer compared with normal controls, but increased KLK11 and KLK14 levels.

Kallikreins have also been studied as biomarkers for a range of other diseases from breast, lung, and ovarian cancer to dermatological diseases and even neurodegeneration. Readers are referred to a recent review by Paliouras *et al.* (238) for a detailed summary of this topic.

It is a significant challenge to determine which kallikreins may be successful biomarkers for particular diseases. The levels of some kallikreins change in several diseases. The serum levels of KLK6, for instance, are increased in ovarian cancer, uterine serous papillary carcinoma, multiple sclerosis, and psoriasis (254–257). Similarly, in some diseases, the levels of multiple kallikreins change. For example, *KLK2-11* and *13–15* are all differentially expressed in ovarian cancer compared to normal or benign tissue (238). Several recent studies suggest that the key to using kallikreins as biomarkers is to test them in combination. Indeed, for colorectal, ovarian, and non-small cell lung cancer, kallikreins are more effective as multiparametric panels of biomarkers than as individual antigens (258–261). This emphasizes the need for a thorough understanding of which kallikreins are coexpressed in different tissues, cell types, and diseases. More detailed information about the transcriptional regulation of kallikreins would also explain why they are dysregulated in particular diseases and, therefore, the biological changes that they may represent as biomarkers.

X. Steroid Hormone Regulation

Kallikreins are expressed at different levels in a diverse range of tissues. Although many of the factors that coordinate this complex expression profile are unknown, in a subset of tissues it is clear that kallikrein expression is strictly regulated by steroid hormones including androgens, estrogens, progestins, mineralocorticoids, and glucocorticoids. Genomic signaling by steroid hormones is mediated by cognate nuclear receptors. Upon ligand binding, nuclear receptors translocate to the nucleus and stimulate transcription by binding to hormone response elements (HREs) in the promoters of target genes (262). Since the identification of the first glucocorticoid response element (GRE) (263), it has been widely accepted that HREs are composed of palindromic repeats of the 5′–TGTTCT–3′ motif separated by a three nucleotide spacer. Classically, the 5′–AGAACAnnnTGTTCT–3′ motif is recognized by the glucocorticoid receptor (GR), mineralocorticoid receptor, progesterone receptor (PR), and AR, whereas the estrogen receptor (ER) recognizes the 5′–AGGTCAnnnTGACCT–3′ motif (264).

The traditional model of hormone receptor action has recently been overhauled through the results of genome-wide studies. Technological advances have helped develop new chromatin immunoprecipitation (ChIP)-based techniques such as ChIP display, ChIP-on-chip, ChIP with paired-end diTag analysis (ChIP-PET), ChIP-sequencing, and chromatin interaction analyses by paired end tag sequencing (ChIA-PET) that allow the unbiased identification of functional hormone receptor binding sites (265–269). Bioinformatic analyses of these sites have revealed some important findings. First, many hormone receptor binding sites do not contain canonical HRE motifs, but rather 6-bp half-sites. For example, only 7.1–26.8% of AR binding sites have a near consensus androgen response element (ARE), whereas 78–79.2% at least contain an ARE half-site (270–272). ERα binding sites are more likely to contain a consensus-like estrogen response element (ERE) (49–71%), but those that don't usually have an ERE half-site (268, 269, 273). Many PR and GR binding sites are also reported to only have half-site motifs (274, 275). Another significant observation from genome-wide ChIP studies is that hormone receptor binding sites seldom lie in the 5′ promoter of hormone-regulated genes. Instead, many binding sites are intragenic, particularly intronic, or at distal sites upstream or downstream from currently annotated genes (266–269, 272, 273, 276, 277). Indeed, only 28% of AR binding sites and 5–7% of ERα sites are located within 5 kb of the TSS (268, 269, 276). This means that many hormone receptor binding sites may be distal enhancers that bridge large distances through extensive chromatin looping to interact with the proximal promoters of target genes (265, 266, 272, 278). Alternatively, future unbiased transcriptome studies using next-generation RNA sequencing platforms may reveal other transcripts that are located proximal to these HREs.

By analyzing large numbers of binding sites, genome-wide studies have been able to explore the mechanisms that regulate the recruitment and activity of hormone receptors. Several non-HRE motifs were found to be overrepresented in steroid hormone binding sites including those for activator protein-1 (AP-1), octomer-binding transcription factor (Oct-1/POU2F1), GATA binding proteins (GATA-2), CCAAT/enhancer binding protein (C/EBP) and Forkhead (FoxA1) (265, 268, 270, 272, 273, 279, 280). In addition, v-ets erythroblastosis virus E26 oncogene homolog 1 (Ets1) was found to share a subset of AR binding sites in prostate cancer cells (271). Many of these molecules act as pioneer factors to prime the promoters of tissue-specific target genes in readiness for ligand-bound hormone receptors. ChIP-on-chip studies have also shown that epigenetic marks like histone 3 methylation and acetylation colocalize with hormone receptor binding sites (279, 281, 282). Indeed, mono- and dimethylation of histone 3 lysine 4 residues is critical for the recruitment of coactivators, ERα, and the AR to enhancers (281, 282). Collectively, the results from genome-wide ChIP studies show that the specificity of hormone receptor binding to distal sites relies on epigenetic marks and coactivators rather than the similarity of the HRE to the consensus motif.

XI. Androgen Regulation of Kallikrein Expression

A. Classical kallikreins in animal models

The most extensively studied and well-characterized aspect of kallikrein gene expression is their androgen responsiveness. Initial studies focused on the mouse and rat submandibular gland (SMG), a rich source of classical kallikreins that is well known to have a sexually dimorphic pattern of cellular differentiation and gene expression (283). Most classical kallikreins in the mouse (*Klk1b1*, *1b3*, *1b4*, *1b5*, *1b8*, *1b9*, *1b11*, *1b16*, *1b21*, *1b22*, *1b26*) and rat (*Klk1c2*, *1c3*, *1c7*, *1c8*, and *1c9*) are more abundant in the SMG of male animals, decrease after castration, and increase after testosterone treatment (284–288). *KLK1* is a notable exception; it does not display sexually dimorphic expression in the rat SMG and may actually be down-regulated by androgens in the mouse (220, 285, 288–292). Importantly, the *KLK1* paralogs are unlikely to be direct AR target genes either because it takes up to 1 wk of testosterone treatment to increase the levels of these kallikreins in the rat SMG (288). Instead, the increased expression of *KLK1* paralogs is a secondary effect of androgen-dependent differentiation of the granular convoluted tubule cells in which these kallikreins are expressed (220, 288, 293). Indeed, *KLK1* is expressed in a different subset of cells that are unresponsive to androgens. These early experiments highlight the need to distinguish between direct and indirect hormonal regulation of kallikrein genes.

A subset of classical kallikreins may be direct AR target genes in rodent testes. Rat *Klk1c8* and mouse *Klk1b9*, *Klk1b21*, *Klk1b24*, and *Klk1b27* are all produced by Leydig cells, which secrete testosterone (220, 294–297). *Klk1b21*, *Klk1b24*, and *Klk1b27* are all down-regulated in the testes of mice with attenuated androgen or AR levels (295, 298). In contrast, *Klk1b21* is up-regulated by testosterone treatment of primary and immortalized mouse Leydig cells (297). Notably, a *KLK1b27* promoter construct is activated by androgens in the MA-10 mouse Leydig tumor cell line (295). Based on the activity of deletion constructs, the androgen-responsiveness of the *KLK1b27* promoter relies on the −175 to −440 region of the promoter, which contains three putative AREs (295). BLAST searches show that this whole region is highly conserved in the promoters of *Klk1b21* and *Klk1b24*, but not human kallikrein genes. Although several human kallikreins are also expressed in the testes, their androgen responsiveness has not been examined (299, 300).

Kallikreins are also highly expressed in the prostate where they are under direct transcriptional regulation by the AR. Canine *KLK2*, originally known as canine arginine esterase, was one of the first prostatic kallikreins shown to be androgen responsive (301, 302). Canine *KLK2* levels decreased more than 100-fold in dogs that were castrated or injected with AR antagonists such as flutamide (303, 304). Nuclear run-on experiments demonstrated that canine *KLK2* was rapidly down-regulated at the mRNA level in castrated animals, implying direct transcriptional regulation by the AR (305). A subset of rat classical kallikrein genes (*Klk1c2*, *Klk1c8*, *Klk1c9*) was also shown to be expressed in the luminal epithelial cells of the prostate in response to androgens (8, 285, 294, 306, 307).

B. Human kallikreins in the prostate

Androgens regulate the prostatic expression of several human kallikreins, in particular *KLK2* and *KLK3*. The earliest evidence for androgen-regulated KLK3 expression came from immunohistochemistry experiments showing that prostatic KLK3 levels mirror serum testosterone concentrations: low in prenatal development and childhood, greater in puberty, and highest in adulthood (308–310). Soon after the *KLK2* and *KLK3* genes were cloned, their androgen responsiveness was confirmed at the mRNA level using Northern blots of androgen-treated LNCaP prostate cancer cells (38, 311–315). These observations were verified with a range of *in vitro* and *in vivo* experiments (316–319). Numerous studies have since used *KLK2* and *KLK3* as prototypical AR target genes to investigate different aspects of androgen signaling in prostate cells. KLK3 levels are also monitored in patients undergoing androgen ablation therapy for prostate cancer because KLK3 is re-expressed when AR signaling is reactivated in castrate-resistant tumors. KLK3 levels, however, are highly heterogeneous in castrate-resistant prostate cancer and do not directly correlate with tumor growth (320, 321). This variability may be due to the different ways that tumors adapt to castrate androgen levels including overexpression and mutation of the AR, up-regulation of transcriptional coactivators, and intratumoral steroidogenesis (322).

C. The *KLK3* promoter and enhancer

AREs were identified within the promoter of *KLK3* soon after its androgen-dependent expression was established. Using DNase I footprinting assays, the Trapman group showed that the *KLK3* promoter is bound by nuclear proteins in LNCaP cells (323). They then identified the first *KLK3* ARE, AREI (AGAACAgcaAGTGCT), at −170 to −156 bp from the TSS using a series of promoter deletion and mutation constructs (323). Other groups confirmed this finding using similar reporter experiments (324) and EMSAs (325). The results from reporter assays suggested that another ARE might be present between −320 and −539 bp from the *KLK3* TSS (323). Subsequently, AREII (GGATCAgggAGTCTC) was identified at −400 bp

from the TSS and found to be a low-affinity AR binding site that cooperates with AREI (326). This was confirmed by other studies that also suggested that Fos-related complexes, distinct from AP-1, might be important in mediating AR transactivation of the *KLK3* promoter (327–329).

KLK3 expression is also regulated by a highly androgen-responsive enhancer located between −5824 and −3738 bp from the TSS (330). Importantly, the *KLK3* enhancer is prostate specific, unlike the proximal promoter AREs, which are active in nonprostatic cell lines such as Panc-1 and Ovcar-3 cells (330). The *KLK3* enhancer contains AREIII (GGAACAtatTGTATC) at −4148 to −4134 bp as well as GAGATA motifs that bind a putative coactivator (331–334). The observation that AREIII has lower activity in isolation than AREI suggested that other AR binding sites are located within the enhancer (330, 331). Accordingly, AREs IIIA, IIIB, IV, V, and VI were later identified within the enhancer between −3870 bp and −4366 bp from the *KLK3* TSS (335). These sites do not contain consensus ARE motifs, but DNase I footprinting assays show they directly bind recombinant AR. The location of all *KLK3* AREs is shown in Fig. 4B. Chromatin looping may bring the promoter and enhancer AREs together to form a coordinated transcription complex (336) as supported by ChIP chromosome conformation capture assays (278). It is also possible that there is a separate transcriptional complex at the *KLK3* enhancer because reporter constructs spanning the −4685 to −3862 bp region have basal promoter activity in LNCaP cells (337).

ChIP assays have confirmed *in vivo* binding of the AR to the *KLK3* promoter and enhancer in LNCaP cells (336, 338). ChIP experiments also show that androgen-induced recruitment of the AR is cyclic and coincides with histone H3 acetylation as well as binding of RNA polymerase II (Pol II) and AR coactivators including cAMP-responsive element-binding protein binding protein (CBP), p300, steroid receptor coactivator-1 (SRC-1/NCOA1), SRC-2 (GRIP1/TIF2/NCOA2), SRC-3 (AIB1/NCOA3), mediator complex subunit 1 (MED1/TRAP220), coactivator-associated arginine methyltransferase 1 (CARM1) and others (278, 336, 338, 339). This may in part be regulated by upstream protein kinase A signaling (340). The AR still occupies the *KLK3* promoter in cells that are treated with AR antagonists such as bicalutamide, although this results in the corecruitment of AR corepressors like nuclear receptor corepressor (NCoR), silencing mediator of retinoic acid and thyroid hormone receptors (SMRT), histone deacetylase (HDAC) 1 and HDAC2 instead of Pol II and AR coactivators (278, 336, 338, 339). Notably, IL-6 might have a role as an endogenous AR antagonist because it inhibits androgen-mediated recruitment of AR, CBP, and p300 to the *KLK3* enhancer and promoter (341). Generally, Pol II and AR coactivators are recruited to both the promoter and enhancer of *KLK3*. In contrast, AR corepressors only bind the *KLK3* promoter (278, 336), although one study has shown bicalutamide-mediated recruitment of NCoR to the enhancer (339). Table 3 lists all proteins that have been shown to bind to the *KLK3* gene using ChIP assays, EMSAs, and promoter deletion constructs. Readers are referred to an excellent review (342) for details of all factors that interact with the AR, some of which modulate *KLK3* expression, but have not yet been shown to be directly recruited to the *KLK3* gene.

Two particularly interesting coregulators are ETV1 (ETS variant 1) and ERG (ETS-related gene). Both factors are commonly overexpressed in prostate cancer due to chromosomal rearrangements that fuse their coding regions to the androgen-regulated promoter of *TMPRSS2*. ETV1 and ERG both bind the *KLK3* enhancer, but they have opposing effects; ETV1 synergistically increases AR-dependent *KLK3* expression, whereas ERG represses *KLK3* expression (343, 344). Perhaps different gene fusions account for some of the heterogeneity in *KLK3* expression between prostate tumors.

D. KLK3 as a marker of prostatic differentiation

Kallikreins can be used as markers of particular cell types, especially when their patterns of tissue-specific expression and hormonal regulation converge. *KLK3* is a good example because it is one of the most highly expressed genes in the prostate (215). This means that *KLK3* may have several clinical applications in prostate cancer. In addition to its use as the serum biomarker, KLK3 has been tested as a marker of circulating tumor cells, as an antigen to prime dendritic cells for targeted immunotherapy, and as an enzyme to activate cytotoxic prodrugs (345–347). Furthermore, the *KLK3* promoter and enhancer have been used to design prostate-specific expression vectors for gene therapy (348). KLK3 is more precisely a marker of terminally differentiated luminal epithelial cells of the prostate. It is not produced by stem, transit amplifying, or intermediate cells, which make up the basal layer of the epithelium and express little or no *AR* (349). Although the prostate stroma is androgen-responsive, it does not express *KLK3* (350). This suggests that *KLK3* expression in luminal epithelial cells depends on more than just androgens and AR. Recent genome-wide ChIP studies have shown that epigenetic marks, such as histone 3 lysine 4 methylation and pioneer coactivators guide hormone receptors to enhancers of tissue-specific target genes (282). This holds true for AR-mediated expression of *KLK3*. Prostate cancer cell lines that express endogenous *KLK3* have high levels of di- and trimethylated histone 3 lysine 4 at the promoter and enhancer of *KLK3* (351). Furthermore, pioneer factors like GATA2 bind to the *KLK3* enhancer in prostate cells and are re-

TABLE 3. Factors that bind to the *KLK3* gene and their effect on KLK3 expression (version 1)

Transcription factors			
ATF2	U	GATA3	↑
c/EBPα	↓	HIF1α	↑
c/EBPβ	↓	NF1	↑
CREB	↑	p50/p65 complex	↑
Fos	↓	p53	↓
c-Jun	↑/↓	PDEF/SPDEF	↑
c-Rel	↓	Oct1/POUF1	↑
EGR1	↑	RREB-1	↓
ERG	↓	Runx1/CBFα1	↑
ERRα	↑	Sp1	↑
ETV1	↑	Sp3	↑
FOXA1/HNF3α	↑/↓	SREBP-1c	↓
FOXP1	↓	Thyroid hormone receptor	↑
FOXO1	↓	USF2	↓
GATA2	↑	Wilms tumor 1	U
Chaperone and co-chaperones			
Bag-1L	U/↑	Hsp70	U/↑
Hsp27	↑		
Chromatin remodeling complex			
BAF57/SMARC1	↑	SNF5/SMARCB1	U
BRG1/SMARCA4	↓	SRG3/BAF155	↑
BRM/SMARCA2	↑		
DNA repair			
Ku70/XRCC6	↑	Ku80/XRCC5	↑
Histone acetyltransferases and deacetylases			
CBP	↑	SIRT1	↓
DBC1/KIAA1967	↑	SRC-1/NCOA1	↑
HDAC1 and 2	↑/↓	SRC-2/GRIP1/ TIF2/ NCOA2	↑
p300	↑	SRC-3/AIB1/ NCOA3	↑
P/CAF	↑	Tip60/KAT5	↑
Sin3a	↓		
Histone methytransferases and demethylases			
CARM1/PRMT4	↑	JMJD2C/KDM4C	↑
G9a/EHMT2	↑	LSD1/KDM1A	↑
JHDM2A/KDM3a	↑	NSD2/WHSC1	↑
Kinases and phosphatases			
LATS2/KPM	↓	PDK1	U/↓
MAK	↑	SCP1-3/CTDSP	↓
Nuclear receptor co-regulators			
Alien/COPS2	↓	SMRT/NCOR2	↓
NCoR	↓	TRAP220/MED1	↑
RIP140/NRIP	↓		
Splicing and RNA metabolism			
Ddx5/p68	↑	p54nrb/NONO	↓
PSF/SFPQ	↓	Sam68/KHDRBS1	U/↑
p44/WDR77	↑		
Signal integrators and transducers, scaffolds, and adaptors			
Ack1/TNK2	↑	PRK1/PKN1	↑
β-catenin	↑	Smad1	↓
IRS-1	↑	EBP1/PA2G4	↓
Nucleophosmin	↑		
Ubiquitination/proteosome pathway			
E6-AP/UBE3A	↑	PIRH2/ARNIP RCHY1	↑
Mdm2	↓	RNF6	↑
Diverse functions			
CDK6 (cell cycle)	↑	Pdx1 (Antioxidant enzyme)	↑
CRIF1/GADD45GIP1 (cell cycle)	↓	Pontin/RUVBL1 (AAA+ ATPase)	↑
DACH1 (tumor suppressor)	↓	Prohibitin (Nuclear and Mitochondrial Protein)	↓
HIP1 (endocytosis)	↑	Ubc9/UBE E2I (Sumoylation)	↑

Full gene names and references for these transcription factors are provided in Version 2 of Table 3 in Supplemental Data. ↑, Increased KLK3 expression; ↓, decreased expression; U, unknown effect on KLK3 expression.

quired for maximum androgen-regulated gene expression (272, 279, 352). Within the prostate, GATA2 and KLK3 are both produced by luminal epithelial cells, but not the stroma (242, 353). As previously noted, *KLK3* is expressed in some other tissues, but at much lower levels. Presumably, these tissues lack the precise combination of methylation, coactivator expression, and AR activity that stimulates such high levels of KLK3 in the prostate.

E. *KLK3* promoter polymorphisms

The *KLK3* promoter and enhancer harbor numerous polymorphisms, some of which have been reported to alter androgen responsiveness (354). The G−158A polymorphism (rs266882) in AREI is of particular interest because it may alter AR binding and consequently androgen-induced *KLK3* expression (355). Contrary to two previous reports (355, 356), our laboratory found that the A-allele increases both the binding affinity of AREI for the AR and the transcriptional response to androgens (357). We also contend that the data presented by Shibahara *et al.* (356) actually support our findings because they show higher binding affinity of the A allele to the AR in EMSAs and a qualitative, but not statistically significant, increase in transcriptional activity for the A allele in reporter assays. The G−158A polymorphism has been associated with higher serum KLK3 (PSA) levels, tumor volume, stage and grade of disease, lymph node invasion, and circulating tumor cells in some but not all studies (358–365). An initial association study found that men with the GG genotype have a 3-fold increased risk of developing prostate cancer that is compounded 5-fold when associated with short AR alleles (364). Subsequent studies have reported increased risk of prostate cancer for either the A (361, 366, 367) or G (358, 364, 368, 369) allele, whereas others have found no significant association for either allele (370–373). Furthermore, a meta-analysis of 12 previous studies found that neither allele is associated with prostate cancer risk, nor any of the clinical parameters previously investigated (374). Other *KLK3* promoter polymorphisms have also been examined, but preliminary studies suggest that the G−4643A polymorphism (rs925013) may be the only one to confer increased risk of prostate cancer (366, 370, 373, 375–377). The inconsistencies between studies likely stem from many factors including differences in the size and ethnicity of sample populations, confounding cultural and environmental effects, and the presence or absence of other protective alleles.

F. The *KLK2* promoter and enhancer

The *KLK2* and *KLK3* promoters share over 80% sequence identity in the regions between −1 to −196 bp and −1673 to −4165 bp from the *KLK2* TSS (Supple-

mental Fig. 1A, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Not surprisingly, similar motifs to the *KLK3* AREs have been identified within the *KLK2* promoter and enhancer. In fact, the sequence (GGAACAgcaAGTGCT) and position (−160 bp) of *KLK2* AREI is almost identical to *KLK3* AREI (Fig. 4B) (319). Promoter constructs spanning *KLK2* AREI have also confirmed that it is androgen responsive (324, 327). Like *KLK3*, much of the androgen responsiveness of *KLK2* is mediated by the distal enhancer (378, 379). The data from several studies using a series of deletion constructs collectively show that the *KLK2* enhancer spans the region from −4.4 to −3.8 kb from the TSS (378, 379). This *KLK2* minimal enhancer region was also identified by Southern blotting with a *KLK3* enhancer probe (380). The *KLK2* enhancer contains the functionally validated *KLK2* ARE (GGAACAtatTGTATT) located at −3819 to −3805 bp from the TSS, which is similar to *KLK3* AREIII (Fig. 4B) (378, 379). *KLK3* AREs IIIA, IIIB, and IV also seemed to be conserved within the *KLK2* enhancer (Supplemental Fig. 1B). ChIP studies have shown that the AR is recruited to the *KLK2* promoter and enhancer within 1 h of androgen stimulation in LNCaP cells (338, 339). Furthermore, Pol II is synergistically recruited with the AR in a cyclic event (339). Methylation of histone 3 Arg-17 at the *KLK2* enhancer and androgen-induced recruitment of CARM1, a histone methyltransferase, are also necessary for activation of *KLK2* gene expression (381). The AREs within the human *KLK2* promoter and enhancer are shown in Fig. 4B.

It is notable that the promoter of canine *KLK2* is similar to its human ortholog. Canine *KLK2* has an ARE in the same position as the human AREI with a similar, although not identical, sequence (AGGACAacaGGTGTT) that binds the AR with 100-fold lower affinity compared with *KLK3* AREI (382). The enhancer ARE is also conserved for canine *KLK2* (GGGAACtatTAATACT) (47). Intriguingly, this motif has been deleted in the cotton-top tamarin where *KLK2* is a pseudogene (84). This implies that the function of KLK2 is intertwined with its transcriptional regulation.

G. Other prostatic kallikreins

In addition to *KLK2* and *KLK3*, other kallikreins are also up-regulated by androgens in the prostate. There has been particular interest in *KLK4* because it is highly expressed in the prostate. *KLK4* expression is up-regulated by androgens at the mRNA and protein level in LNCaP cells, but down-regulated in the CWR22 prostate cancer xenograft in castrated mice (66, 383–386). Our laboratory recently confirmed previous findings that the most abundant *KLK4* transcript in prostate cancer cells arises from an alternative downstream TSS in exon 2 (384, 385). This truncated transcript and the classical form of *KLK4* are both androgen regulated. Several putative AREs have been identified upstream of *KLK4 in silico* (184). One element located 1005 bp upstream of the exon 2 TSS (GGTGCAggaGATTGT) indirectly binds the AR in EMSAs, but does not mediate androgen-regulated expression in reporter assays (385).

The *KLK31P* and *KRIP1* transcripts of the *KLKP1* gene, which lies adjacent to *KLK4*, are also androgen regulated in LNCaP cells (68, 69). Notably, recent AR ChIP-on-chip studies have shown that there is an AR-occupied region close to the *KLKP1* gene (279, 282). Of the remaining kallikreins, there are preliminary data that androgens stimulate *KLK12* and *KLK15* gene expression in LNCaP cells as well as KLK5 and KLK8 protein levels in AR-transfected PC-3 cells (387–390). To distinguish between androgen-dependent and androgen-responsive kallikreins, we have examined the expression of all 16 genes in a range of prostate cell lines using quantitative RT-PCR (Fig. 4C). *KLK1-4*, *KLKP1*, and *KLK15* levels correlate with AR status, implying that the expression of these kallikreins in prostate epithelial cells is AR-dependent. Other kallikreins have AR-independent expression profiles, including *KLK14*, which is ubiquitously expressed.

H. Kallikrein expression in breast

The breast is also a prominent site of androgen-regulated kallikrein expression. Most studies have used BT-474 and T47D breast cancer cells, which express the AR, ER, and PR (391). *KLK2*, *3*, *8*, *10*, *11*, *13*, *14*, and *15* are all up-regulated by androgens at the mRNA and protein level in breast cancer cell lines (223, 392–396). Based on qualitative RT-PCR, *KLK4*, *5*, *6*, *9*, and *12* expression may also be stimulated by androgens in breast cancer cell lines, but these observations require further validation (71, 223, 389, 397, 398). Notably, KLK3 has been detected in 98% of breast cancer specimens that express AR (399). Moreover, ChIP experiments have shown that AR is recruited to the proximal promoter of *KLK3* in dihydrotestosterone-treated T47D cells (400). These observations suggest that *KLK3* expression in breast is predominantly AR-dependent, just as it is in prostate. In contrast, other kallikreins may be regulated by both direct and indirect mechanisms. In BT-474 cells that are co-stimulated with androgens and estrogens, membrane-bound AR indirectly enhances estrogen-dependent KLK10, 11, and 14 expression by increasing Akt phosphorylation and downstream ER activity (401). An AR antagonist blocks the synergistic effect of androgens, whereas an ER antagonist completely inhibits kallikrein expression. In T47D cells treated with androgens alone, the AR may directly mediate a more modest increase in KLK10 and KLK11 levels (400). ChIP experiments suggest that the AR binds within

two regions of the *KLK10* (−2000 to −2500 bp and +1000 to +1500 bp) and *KLK11* (−1000 to −1500 bp and +1 to +500 bp) promoters, which contain predicted AREs. Yet a reporter construct spanning the −2000 to −2500 bp region of *KLK10* is not responsive to androgens in T47D cells (394). This is similar to our observations for *KLK4* in prostate cells (385). Perhaps AR-mediated induction of *KLK4*, *10*, and *11* involves indirect interactions of more distal AR complexes.

XII. Progestin Regulation of Kallikrein Expression

Like androgens, progestins coordinately stimulate the expression of multiple kallikreins. KLK2 and KLK3 are both up-regulated by progestins in breast cancer cell lines (392, 395, 402). *KLK2* (−493 to +27) and *KLK3* (−620 to +40) reporter constructs are also activated by progesterone in PC-3 prostate cancer cells cotransfected with PR (327). Therefore, it is tempting to speculate that the PR is recruited to AREI and AREII. A reporter construct spanning the *KLK3* promoter and enhancer was also stimulated by progestins in T47D cells (331). Yet, a construct only containing the *KLK3* enhancer was not responsive to progesterone in PR-transfected BHK cells (335). This is consistent with the *KLK3* enhancer being more AR-dependent and prostate-specific than the promoter. Progesterone also stimulates *KLK4* expression in KLE endometrial cancer and T47D breast cancer cell lines, possibly through a functional progesterone response element (AGAACAtgagagAGAACA) located 2419 bp upstream of the primary TSS in breast cells (385, 403). The *KLK4* progesterone response element has two strong half-site motifs rather than the classical 15-bp HRE sequence. Although this element binds the PR in EMSA and ChIP experiments, it is only moderately responsive to progesterone in luciferase reporter assays. In addition to *KLK2*, *3*, and *4*, preliminary data suggest that *KLK6* and *8-15* expression are also up-regulated by progestins in breast cancer cells (71, 388, 389, 393, 396, 404–407). Progestins may also stimulate kallikrein expression in the female reproductive tract because KLK5, 6, 7, 11, and 12 levels in human cervico-vaginal fluid all peak in the secretory phase of the menstrual cycle (221). Furthermore, *KLK5* and *KLK6* may be direct PR target genes because they are up-regulated in the uteri of wild-type mice, but not PR knockout mice, within 4 h of progesterone treatment (408). Collectively, these observations show that progestins regulate kallikrein expression in different tissues, in some instances through direct binding of the PR to kallikrein promoters.

XIII. Corticosteroid Regulation of Kallikrein Expression

Unlike androgens and progestins, glucocorticoids either activate or repress kallikrein expression, particularly in breast and cervical cancer cell lines. For example, KLK10 levels are increased by dexamethasone treatment of MCF7, T47D, and MDA-MB-468 breast cancer cells, but decreased in MCF-10A cells (388, 409). The effect of dexamethasone on *KLK5*, *6*, *8*, and *11* expression also varies between cell lines. Generally, these kallikreins are coordinately regulated and exhibit the same response to glucocorticoids in each cell line (388). The GR can activate or repress gene expression in *cis* by directly binding to different types of GREs, or in *trans* by interacting with other transcription factors (410). The mechanisms underlying kallikrein expression and their differences between cell lines remain to be determined. Numerous GRE half-sites have been identified in the promoters of *KLK5*, *6*, *7*, *8*, *10*, and *13 in silico*, but none have been experimentally verified (388). Notably, *KLK2* and *KLK3* promoter constructs are just as responsive to dexamethasone as androgens, so it is possible that the GR binds AREI and AREII (326, 327). Yet androgens are much more potent than glucocorticoids at increasing endogenous KLK2 and KLK3 levels, probably because the *KLK2* and *KLK3* enhancers are only activated by androgens (392, 395). Overall, there is mounting evidence that glucocorticoids modulate kallikrein expression, but more research is required to define the molecular mechanisms and *in vivo* significance of these findings.

The effects of adrenal hormones on *KLK1* expression have been more extensively studied. Initial reports suggested that mineralocorticoids directly regulate renal *KLK1* expression. Urinary KLK1 levels are sometimes, although not always, increased in patients with primary hyperaldosteronism, a condition where the adrenal glands overproduce the potent mineralocorticoid aldosterone (411, 412). Normal patients on low-salt diets that stimulate endogenous mineralocorticoid production also have increased urinary KLK1 levels, but not if they are treated with mineralocorticoid receptor antagonists (413, 414). Yet experiments with animal models yielded conflicting results. Studies that measured the amount of KLK1 protein or activity after extended mineralocorticoid treatments reported an increase in renal or urinary KLK1 levels (415, 416). In contrast, studies that used acute mineralocorticoid treatments or measured *KLK1* mRNA levels found no change in KLK1 expression (417, 418). Therefore, the increase in KLK1 protein may be due to post-transcriptional regulation or simply a secondary effect of changes in renal physiology.

In contrast to mineralocorticoids, glucocorticoids are generally associated with decreased renal KLK1 expres-

sion. Yet differences in the dose and duration of hormone treatments and methods of measuring *KLK1* levels have led to conflicting data between studies. Nevertheless, experiments with short time points, which are arguably the most informative, suggest that glucocorticoids have a direct effect on KLK1 expression. For example, a low physiological dose of the glucocorticoid methylprednisolone reduces *in vivo* protein synthesis of rat renal KLK1 within 2 h (419). Renal KLK1 levels in the rat are also inversely proportional to diurnal changes in corticosterone concentrations (419). In addition, dexamethasone decreases *KLK1* mRNA and protein levels in AR42J rat pancreatic cancer cells within 12 h of treatment (420). Although these observations suggest that corticosteroids modulate KLK1 levels, they do not regulate basal *KLK1* expression. *KLK1* is still expressed in the rat kidney, pancreas, and SMG when adrenalectomy is used to abolish endogenous mineralocorticoid and glucocorticoid production (418, 421).

XIV. Estrogen Regulation of Kallikrein Expression

The first studies to examine the effect of estrogens on kallikrein expression focused on the rat anterior pituitary, where there is greater kininogenase activity in female animals (422). KLK1 is produced by lactotrophs within the rat anterior pituitary, which also secrete prolactin in response to estrogens (423). *KLK1* and prolactin levels follow similar trends; both are produced after the onset of puberty, increase in animals treated with 17β-estradiol, but decrease in ovariectomized animals (294, 424–426). Yet changes in *KLK1* and prolactin expression have different kinetics. High doses of the nonsteroidal estrogen diethylstilbestrol cause lactotroph proliferation and anterior pituitary adenomas in rats. There is a rapid 250-fold increase in KLK1 levels in these tumors, but KLK1 levels subsequently plateau, even as prolactin levels and tumor size continue to increase (427, 428). There is also discordance between *KLK1* and prolactin levels in human pituitary specimens and immortalized rat pituitary cell lines (429, 430). These discrepancies might be due to the mixture of direct and indirect mechanisms that seem to contribute to estrogen-regulated *KLK1* expression. Estrogens increase the number of lactotrophs in the anterior pituitary, so changes in KLK1 levels are partly a secondary effect of variations in cellular differentiation (431). Based on immunohistochemistry experiments, estrogens also increase the intensity of KLK1 staining in each lactotroph, suggesting that KLK1 may indeed be a direct ER target gene (431). Potential EREs have been noted in the proximal promoter of rat *KLK1*, but have not been experimentally analyzed (431, 432).

The effect of estrogens on kallikrein expression has also been investigated in the female reproductive tract. *KLK1* expression in the human endometrium fluctuates with the menstrual cycle, peaking with maximum estrogen concentrations in the mid to late proliferative phase (433). There is a similar trend in rats and mice where endometrial *KLK1* levels are highest in the estrogen-regulated proestrous phase (434–436). *KLK1* may be a direct ER target gene because it is up-regulated in the endometrium of ovariectomized mice within 3 to 6 h of estradiol treatment (435, 437). There are some species-specific differences because *KLK1* levels do not change across the estrous cycle in the pig (438). In all species, however, endometrial *KLK1* expression increases at sites of embryo implantation, possibly due to estrogen secreted by blastocysts (436, 439, 440). Estrogen also stimulates *KLK1* expression in the vagina of ovariectomized mice (437).

KLK8 has a similar expression pattern to KLK1. It is highly expressed in the human endometrium during the proliferative phase of the menstrual cycle and up-regulated by estrogen in the mouse vagina (441, 442). Yet *KLK8* levels in the mouse do not change until 48 h of estrogen treatment, suggesting that this response is a secondary effect of changes in cellular differentiation (442). Little is known about the effect of estrogens on the other kallikreins in the female reproductive tract. Preliminary data suggest that estrogens up-regulate KLK4 expression in KLE human endometrial cancer cells and OVCAR-3 human ovarian cancer cells (403, 443). Estrogen stimulates KLK11 secretion from Me-180 cervical cancer cells, but it slightly decreases KLK6, 10, and 11 secretion from VK2 vaginal epithelial cells (221).

Estrogen has different effects on kallikrein expression in the breast compared with the reproductive tract. Unlike the endometrium and vagina, *KLK1* expression is not up-regulated by estrogen in the mouse mammary gland (437). Interestingly, estrogen actually inhibits the androgen-dependent expression of KLK2 and KLK3 in human breast cancer cell lines (392, 402). In contrast, KLK5, 6, 8, 10, 11, 13, and 14 mRNA and protein levels are all stimulated by estrogen (223, 250, 388, 393, 401, 404, 405). Qualitative RT-PCR experiments suggest that estrogen may also increase *KLK7*, *9*, *12*, and *15* expression in breast cancer cells (71, 389, 444, 445). Notably, the estrogen-responsiveness of each kallikrein differs between breast cancer cell lines that are all ERα- and ERβ-positive (223, 446). For example, KLK5 is up-regulated by estrogen in BT-474 and MCF7 cells, but is not expressed in T47D cells. In contrast, KLK8 is estrogen-regulated in T47D and MCF7 cells, but not in BT-474 cells. KLK10, 11, and 14 are produced by all three cell lines, but are only up-regulated by estrogen in BT-474 and MCF7 cells. It has been proposed that the discrepancies between cell lines are due to ge-

netic or epigenetic changes at the kallikrein locus or differences in the cross-talk between the ER and other signaling pathways (223). Indeed, it is not clear whether kallikreins are direct or indirect targets of ER in breast cancer cell lines. Using bioinformatics, one study failed to identify any EREs within 6 kb of *KLK5*, *6*, or *8*, although another noted that there are several ERE half-sites upstream of *KLK5* and *7* (223, 447). The data are more promising for *KLK10*. An ERα ChIP-on-chip study noted that *KLK10* is up-regulated in MCF7 cells within 1 h of estrogen treatment and has a nearby ERα binding site (448).

Although many kallikreins are estrogen-responsive in breast cancer cell lines, few are likely to be estrogen-dependent. KLK5–11 and 15 are all expressed in ER-negative breast cancer cell lines such as BT-20, MDA-MB-231, and MDA-MB-468 (223, 388). Furthermore, there is no correlation between *KLK9*, *11*, *14*, and *15* levels and ER status in breast cancer tissue specimens (192, 407, 449, 450). Only KLK3 and *KLK13* are more highly expressed in ER-positive tumors (451, 452). For KLK3, this trend may be due to coexpression of other hormone receptors with ER given that estrogen down-regulates KLK3 *in vitro* (451). Although *KLK5*, *6*, and *10* are estrogen-responsive in breast cancer cell lines, these kallikreins are more highly expressed in ER-negative tumors (453, 454). In addition, KLK10 is an independent predictive marker of tumors that fail to respond to the ER antagonist tamoxifen and may therefore lack the ER (453).

Our analysis of published microarray data from 586 breast cancer specimens confirms the trend where estrogen-regulated kallikreins are more highly expressed in ER-negative tumors (Supplemental Fig. 2). We examined other microarray datasets to further investigate this paradox. Data from the Neve *et al.* study (455) of 51 breast cancer cell lines shows that *KLK5*, *6*, *8*, and *10* are more highly expressed in the "Basal A" subset of ER-negative cell lines than the "Luminal" subset of ER-positive cells (Fig. 5A). Indeed, *KLK6* and *8* were included in the list of 305 classifier genes that could be used to discriminate between different subtypes of breast cancer (455). *KLK5*, *6*, *8*, and *10* are also more highly expressed in patient specimens of ER-negative basal breast cancer compared with ER-positive luminal breast cancer (Fig. 5B). There is little variation in the expression of other kallikreins between cell lines or tumor specimens. These observations are surprising because *KLK6* and *10* have long been classed as tumor suppressor genes in breast cancer (134, 198, 456, 457). The association of *KLK6* and *10* with breast cancer may be more complex where they are down-regulated in the luminal, but not basal, subset of tumors. Although *in vitro* experiments with luminal cell lines show that kallikreins are estrogen-responsive in some contexts, the microarray data also strongly suggest that kallikrein expression in breast is not estrogen-dependent.

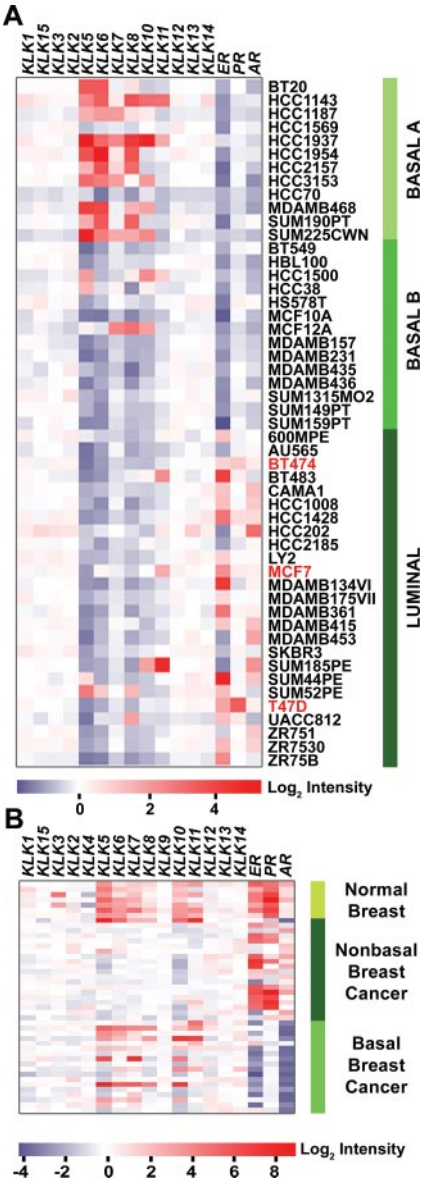

FIG. 5. Median-centered kallikrein and sex hormone receptor expression in breast cancer cell lines and tissues. A, Data from the Neve *et al.* study (455) of 51 breast cancer cell lines shows that *KLK5-8* and *10* tend to be more highly expressed in the Basal subset, whereas the ER, PR, and AR are more highly expressed in the Luminal subset of cells. There is little variation in *KLK1-3* and *12–15* between cell lines. BT-474, MCF7, and T47D cells are labeled in *red* because they are commonly used to study the hormonal regulation of kallikreins in breast. B, Gene expression in breast cancer specimens from the Richardson *et al.* dataset (498). *KLK5-8*, *10*, and *11* are more highly expressed in normal breast and basal breast cancer than luminal breast cancer. In contrast, ER, PR, and AR are lowly expressed in basal breast cancer. There is little variation in *KLK1-4*, *9*, and *12-15* expression between samples.

XV. Nonsteroid Hormone Regulation of Kallikrein Expression

A. Thyroid hormone

Compared with steroid hormone receptors, relatively little is known about the role of other members of the nuclear receptor superfamily in regulating kallikrein expression. Nevertheless, several early studies examined whether

kallikrein genes are responsive to thyroid hormone in animal models. For example, in the rat SMG, thyroid hormone increases the levels of *Klk1c2*, *Klk1c3*, *Klk1c7*, *Klk1c8*, and *Klk1c9*, but not *Klk1* (458). Little change in kallikrein expression was detected in other organs (458, 459). There is a similar trend in the mouse where thyroid hormone induces *Klk1b3*, but either does not change or decreases *Klk1* expression depending on the experimental model (288, 460). Yet it takes 1 wk for these changes to occur, suggesting that increased expression of classical rat and mouse kallikrein is probably a secondary effect of changes in cellular differentiation (288). Thyroid hormone seems to have a more direct effect on the human *KLK3* gene that has a functional thyroid HRE (GGTGCAtccaGGGTGA) at −183 to −198 bp from the TSS (461). Through this element, thyroid hormone synergistically increases the androgen-dependent expression of *KLK3* in LNCaP prostate cancer cells (461, 462).

B. Vitamin D

Just as *KLK3* is widely used as a prototypical androgen-regulated gene, *KLK6* has emerged as a reliable marker of vitamin D receptor activity. Vitamin D3 and related analogs rapidly up-regulate *KLK6* expression in head and neck, breast, colon, and prostate cancer cell lines as well as normal and transformed human skin keratinocytes (463–466). In addition, a vitamin D response element (AGTTCAacgAGTTCT) has been identified 489 bp upstream of the *KLK6* TSS using ChIP assays (467). Significantly, vitamin D also up-regulates *KLK5*, *7*, *8*, *10*, and *13* in keratinocytes, suggesting that the vitamin D receptor may regulate the expression of the kallikrein cascade in skin (468). In prostate cancer cells, vitamin D indirectly stimulates the androgen-dependent expression of *KLK2* and *KLK3* by increasing the expression and nuclear translocation of the AR (378, 469–471). In the absence of androgens, vitamin D has no effect.

C. Retinoic acid

Kallikreins are differentially regulated by retinoic acid: *KLK10* is up-regulated in breast cancer cells, but *KLK3* is down-regulated in prostate cancer cells. *KLK10* has a retinoic acid response element (TGACCTcgTGATCC) 1014 bp upstream of the TSS (472). Reporter assays show that this element is necessary and sufficient for retinoic acid-regulated gene expression. In 76R-30 breast cells, retinoic acid receptor and retinoid X receptor (RXR) both bind the *KLK10* retinoic acid response element in a ligand-independent manner, whereas their coactivator, ADA3, is recruited in response to retinoic acid treatment. RXR also binds the promoter of *KLK3*, but its association with gene expression is more complex (473). In the absence of retinoic acid, RXR acts as a weak coactivator of androgen-regulated *KLK3* expression in prostate cancer cells. Under these conditions, the RXR and AR bind to each other as well as to *KLK3* AREI and AREIII. In cells treated with both 9-*cis* retinoic acid and androgens, the RXR and AR still interact; however, their recruitment to AREI and AREIII is reduced. As a result, *KLK3* expression decreases. Because retinoic acid also down-regulates *KLK2* expression in prostate cancer cells (474), it is likely that RXR will repress all androgen-regulated kallikreins through its interaction with the AR.

D. Other nuclear receptors

KLK3 expression in prostate cancer cells is modulated by several other members of the nuclear receptor superfamily, mostly through indirect mechanisms. For example, the liver X receptor reduces AR-dependent *KLK3* expression by up-regulating SULT2A1, an enzyme that metabolizes androgens (475). In addition, the synthetic liver X receptor agonist T0901317 has been shown to decrease *KLK3* expression by acting as an AR antagonist (476). Peroxisome proliferator-activated receptor γ agonists (PPARγ) also down-regulate *KLK3* expression in prostate cancer cells (477). Intriguingly, this effect is independent of the PPARγ (478). Low concentrations of PPARγ agonists reduce AR recruitment to the *KLK3* promoter, whereas higher concentrations cause down-regulation of the AR (479). Estrogen receptor-related receptor α (ERRα) has also been shown to alter *KLK3* expression (480). ERRα binds to the *KLK3* enhancer in the presence of the AR and stimulates *KLK3* expression. A specific ERRα inverse agonist, XCT790, increases the occupancy of ERRα at the *KLK3* enhancer, but transforms the receptor into a repressor, which down-regulates androgen-dependent *KLK3* expression. Collectively, these studies show that although the AR is the most important regulator of *KLK3* expression, other members of the nuclear receptor superfamily may influence *KLK3* levels under some conditions.

XVI. Future Challenges

A. Are kallikreins direct targets of hormone receptors?

Uncertainty about kallikreins being direct or indirect targets of hormone receptors is a recurring theme among studies. This is an important distinction if kallikrein genes are to be used as models of hormone responsiveness. The problem is exemplified by studies with animal models from the 1970s and 1980s. These experiments often involved chronic hormone treatments or complete hormone ablation though castration, ovariectomy, or adrenalectomy. In some cases, these studies generated valuable preliminary data that have since been confirmed and expanded upon. Other results are now considered to be secondary effects of hormone-related changes in cellular dif-

ferentiation and proliferation or feedback from other endocrine pathways. More recent studies have relied on cell lines, which are less complex and more convenient. Yet many of these experiments still fail to distinguish between the direct and indirect effects of hormones. For instance, when cells are treated with hormones for days, not hours, changes in kallikrein levels may reflect differences in proliferation or differentiation rather than hormone receptor activity. Rapid changes in kallikrein expression can also be ambiguous. Most studies use tumor cell lines, some of which have mutated hormone receptors with more promiscuous affinity for ligands. For example, the AR is activated by nonandrogenic steroid hormones in LNCaP prostate cancer cells due to the T877A mutation in the ligand-binding domain (481, 482). Therefore, the increase in *KLK2*, *3*, *4*, and *15* expression with progestins in LNCaP cells may be mediated by the AR rather than the PR (384, 388, 390, 481). Another source of ambiguity may be the metabolism of the ligand of interest into other steroid hormones. For example, breast cancer cells can metabolize dihydrotestosterone, an androgen, into 5α androstane-3β,17b-diol, an estrogenic hormone that activates ERα and ERβ (483). Conversely, prostate cancer cells use steroidogenic enzymes to generate dihydrotestosterone from progesterone (484). Future studies can overcome these problems by using shorter time courses, specific hormone receptor antagonists, and promoter-based assays.

B. Where are the hormone response elements?

Hormone receptors may trigger rapid and specific changes in kallikrein expression through genomic or nongenomic actions. The classical model of a ligand-bound hormone receptor activating gene expression by binding to a HRE is well established for AR-mediated expression of *KLK2* and *KLK3*. Other kallikreins may also have functional HREs, but they could be difficult to find given that recent genome-wide ChIP studies have shown that few HREs are located within proximal promoters. The prevalence of hormone receptor binding sites that differ from the classical HRE motif adds further complexity. If kallikrein HREs are nonconsensus and far removed from the locus, they will be challenging to identify using binding site prediction software and promoter deletion constructs. Alternative approaches would be to mine genome-wide ChIP data for putative hormone receptor binding sites, or use chromosome conformation capture assays to investigate long-range genomic interactions. Because many kallikreins have conserved tissue-specific expression profiles between species, novel HREs might also be identified by focusing on the conserved regions of kallikrein promoters.

In addition to binding to HREs, hormone receptors can also modulate gene expression by interacting with other transcription factors, including AP-1 and nuclear factor κB, and activating extranuclear signaling cascades, such as the phosphatidylinositol-3-kinase and Src pathways (485, 486). Therefore, some kallikreins might not have HREs. Instead, they may be specific, but indirect, targets of hormone receptor signaling. One factor that could mediate the nongenomic actions of hormone receptors is cFos because it is associated with hormone-dependent as well as hormone-independent kallikrein expression (400, 487). A more detailed understanding of the structure of kallikrein promoters would help resolve whether they are direct targets of hormone receptors.

C. Do kallikreins have shared enhancers?

Given that kallikreins are colocalized in the genome and coexpressed in some tissues, there has been much speculation that they are jointly regulated by shared enhancer elements. The kallikrein field has referred to shared enhancers as "locus control regions," but this misconstrues the use of this term by other researchers. The term locus control region arose from studies with transgenic mice to describe a potent enhancer that stimulates correct tissue-specific expression of a transgene (488). Rather than coordinately up-regulating several genes, many locus control regions near multigene families only activate one gene at a time. For example, the β-globin locus control region stimulates sequential, not simultaneous, expression of β-globin genes throughout development (489). The human *KLK3* enhancer actually fits the criteria of a locus control region because it drives high levels of KLK3 or LacZ expression in the prostate of transgenic mice (490, 491). Interestingly, one study found that maximal KLK3 expression in transgenic mice requires both the *KLK2* and *KLK3* enhancers (380). This suggests that *KLK2* AREII may act as a shared enhancer for *KLK2* and *KLK3*. Further experiments, such as chromatin conformation capture assays, are needed to confirm this observation. It would also be interesting to investigate whether the *KLK2* and *KLK3* enhancers influence the expression of other kallikreins, especially *KLK4*, *KLK15*, and *KLKP1*, which are clustered around *KLK2* and *KLK3* and are also androgen-regulated in the prostate. Given the location and orientation of these genes in the kallikrein locus, interactions between the promoters of *KLK4*, *KLK15*, and *KLKP1* and the enhancers of *KLK2* and *KLK3* would require complex patterns of chromatin looping. Because the kallikrein locus probably evolved through a series of gene duplications, it is also possible that the expression of each kallikrein is independently controlled by conserved regulatory elements. Indeed, Kroon *et al.* (492) showed that the coexpression of rat classical kallikreins in the SMG is due to autonomous rather than shared enhancers. When separate fragments of the kallikrein locus were in-

troduced into mice, all rat kallikreins were still expressed in the SMG at physiological levels. Overall, the evidence for shared enhancers in the kallikrein locus is fairly circumstantial. In future studies, the use of novel techniques like chromatin conformation capture assays may help identify distal regulatory elements, some of which may be shared enhancers.

D. Is the kallikrein locus relevant in the era of genome-wide analyses?

The kallikrein locus, in particular *KLK3*, has served as a model of hormonal regulation for more than 15 yr. Studies with the *KLK3* promoter have provided important insights into AR-mediated gene expression and steroid hormone receptor biology in general (278, 336). Some of these findings, such as the importance of coactivators and chromatin looping, presaged similar observations from genome-wide ChIP studies that have since confirmed their broader significance. Yet, with the increasing use of genome-wide ChIP techniques, it is pertinent to consider whether *KLK3* and the kallikrein locus are still relevant. The main advantage of ChIP-on-chip, ChIP-sequencing, and related methods is their breadth, which is the main disadvantage of gene- and locus-centric experiments. Unbiased global ChIP techniques can be used to study many transcription factor binding sites under different contexts, whereas kallikreins represent a subset of target genes in specific contexts. *KLK2* and *KLK3* for example, are useful androgen-responsive genes for studies of luminal epithelial prostate cells, but not prostatic stroma or other androgen-regulated cell types such as skeletal muscle. Conversely, the major downside of genome-wide ChIP techniques is their cost and complexity compared with experiments with candidate genes that are relatively simple and inexpensive. This suggests that model genes like *KLK3* will continue to be widely used, especially for studies on generic rather than gene-specific mechanisms of transcriptional regulation. Overall, it seems that genome-wide and gene-centric experiments are complementary approaches. The advantages of each technique compensate for the disadvantages of the other, so they can be used separately or in conjunction depending on the hypotheses being tested. The most compelling reason for the ongoing use of *KLK3* as a model of hormonal regulation is that it is among the most well-characterized genes in the human genome. Indeed, genome-wide AR ChIP studies have often used *KLK3* to test their new hypotheses (272, 279). Therefore, as a model gene, *KLK3* can be used to compare results between laboratories and will continue to provide a link between past and future studies on AR-mediated gene expression.

XVII. Conclusion

The kallikrein family holds great promise, not just as a panel of biomarkers and potential therapeutic targets, but also as an important model of hormonal regulation. Indeed, *KLK3* has been extensively used as a prototypical androgen-regulated gene to investigate the mechanisms of AR-mediated gene expression. Yet, it is unlikely that *KLK3* will always represent the spectrum of androgen-regulated genes in the normal or diseased prostate. For example, *KLK2*, *KLK3*, prostatic acid phosphatase, and *TMPRSS2* are all well-characterized androgen-dependent genes, but KLK3 and prostatic acid phosphatase are down-regulated during prostate cancer progression, whereas KLK2 and TMPRSS2 are up-regulated (493–497). By analyzing a range of prostatic kallikreins, including *KLK2*, *3*, *4*, *15*, and *KLKP1*, and other androgen-regulated genes, studies would be able to distinguish between generic changes in AR signaling and gene- or locus-specific effects. Other kallikreins may also be useful models to study the actions of other nuclear receptors. For instance, KLK6 has emerged as a reliable marker of vitamin D receptor activity, and KLK5 and KLK6 are promising markers of PR signaling in the uterus. Although all kallikrein genes have been shown to be hormone-responsive, it is not yet clear which genes are specific and direct targets of each hormone receptor. Some changes in kallikrein expression may be secondary effects of differences in proliferation or cellular differentiation. Future studies on the hormonal regulation of kallikreins should clarify this point and distinguish between hormone-related and hormone-regulated kallikrein expression. Thus, the kallikrein-related peptidase gene family locus will continue to be a powerful tool for testing hypotheses of hormonal regulation.

Acknowledgments

We gratefully acknowledge the Australian Prostate Cancer Bio-Resource for providing tissue specimens and Dr. Preston Alexander, Triple Point Biologics, for providing primary antibodies. We also thank Ms. Pak Ling Leung, Ms. Satomi Okano, and Dr. Scott Stansfield for their assistance in preparing the figures in this manuscript.

Address all correspondence and requests for reprints to: Judith A. Clements, Institute of Health and Biomedical Innovation, 60 Musk Avenue, Kelvin Grove, Brisbane, Queensland, 4059, Australia. E-mail: j.clements@qut.edu.au.

This work was supported by grants and fellowships from the Queensland Government Growing the Smart State Fund (to M.G.L.), the Cancer Australia and Prostate Cancer Foundation of Australia (to J.L. and J.A.C.), The Cancer Council Queensland (to J.A.C.), the National Breast Cancer Foundation (to J.A.C.), and the National Health and Medical Research Council (to J.A.C. and the Australian Prostate Cancer BioResource).

Disclosure Summary: The authors have nothing to disclose.

References

1. **Abelous JE, Bardier E** 1909 Les substances hypotensive de l'urine humaine normale. Compt Rend Soc Biol 66:511–512
2. **Borgoño CA, Diamandis EP** 2004 The emerging roles of human tissue kallikreins in cancer. Nat Rev Cancer 4:876–890
3. **Clements JA, Willemsen NM, Myers SA, Dong Y** 2004 The tissue kallikrein family of serine proteases: functional roles in human disease and potential as clinical biomarkers. Crit Rev Clin Lab Sci 41:265–312
4. **Clements JA** 2008 Reflections on the tissue kallikrein and kallikrein-related peptidase family—from mice to men—what have we learnt in the last two decades? Biol Chem 389:1447–1454
5. **Lundwall A, Brattsand M** 2008 Kallikrein-related peptidases. Cell Mol Life Sci 65:2019–2038
6. **Borgoño CA, Michael IP, Shaw JL, Luo LY, Ghosh MC, Soosaipillai A, Grass L, Katsaros D, Diamandis EP** 2007 Expression and functional characterization of the cancer-related serine protease, human tissue kallikrein 14. J Biol Chem 282:2405–2422
7. **Ashley PL, MacDonald RJ** 1985 Kallikrein-related mRNAs of the rat submaxillary gland: nucleotide sequences of four distinct types including tonin. Biochemistry 24:4512–4520
8. **Brady JM, Wines DR, MacDonald RJ** 1989 Expression of two kallikrein gene family members in the rat prostate. Biochemistry 28:5203–5210
9. **Kim J, Coetzee GA** 2004 Prostate specific antigen gene regulation by androgen receptor. J Cell Biochem 93: 233–241
10. **Clements JA** 1989 The glandular kallikrein family of enzymes: tissue-specific expression and hormonal regulation. Endocr Rev 10:393–419
11. **Abelous JE, Bardier E** 1909 De l'action hypotensive et myotique de l'urine humaine normales. Compt Rend Soc Biol 66:876
12. **Abelous JE, Bardier E** 1909 L'urohypotensine. J Physiol Pathol Gener 11:777–786
13. **Frey E** 1926 Zusammenhänge zwischen Herzarbeit und Nierentätigkeit. Arch Clin Kir 142:663
14. **Frey EK, Kraut H** 1928 Ein neues Kreislaufhormon und seine Wirkung. Arch Exp Pathol Pharmkol 133:1
15. **Pribram H, Hernheiser G** 1920 Zur Kenntnis der dialysierbaren Bestandteile des Menschenharnes. Biochem Z 111:30
16. **Kraut H, Frey EK, Werle E** 1930 Der Nachweis eines Kreislaufhormons in der Pankreasdrüse. Hoppeseylers Z Physiol Chem 189:97–106
17. **Werle E, Gotze W, Keppler A** 1937 Über die Wirkung des Kallikreins aus dem isolierten Darm und über eine neue darmkontrahierende Substanz. Biochem J 289:217–233
18. **Bhoola KD, Figueroa CD, Worthy K** 1992 Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol Rev 44:1–80
19. **Rocha E, Silva M, Beraldo WT, Rosenfeld G** 1949 Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. Am J Physiol 156:261–273
20. **Werle E, Fiedler F** 1969 Kallikreins. Biochem J 115:4P–6P
21. **Fiedler F** 1979 Enzymology of glandular kallikreins. In: Erdos EG, ed. Bradykinin, kallidin, and kallikrein. Berlin, Heidelberg, New York: Spinger-Verlag; 103–161
22. **Movat HZ** 1979 The plasma kallikrein-kinin and its interrelationship with other components of blood. In: Erdos EG, ed. Bradykinin, kallikin, and kallikrein. Berlin, Heidelberg, New York: Springer-Verlag; 1–89
23. **Webster ME, Pierce JV** 1960 Studies on plasma kallikrein and its relationship to plasmin. J Pharmacol Exp Ther 130:484–491
24. **Schachter M** 1979 Kallikreins (kininogenases)–a group of serine proteases with bioregulatory actions. Pharmacol Rev 31:1–17
25. **Sainz IM, Pixley RA, Colman RW** 2007 Fifty years of research on the plasma kallikrein-kinin system: from protein structure and function to cell biology and in-vivo pathophysiology. Thromb Haemost 98:77–83
26. **Beaubien G, Rosinski-Chupin I, Mattei MG, Mbikay M, Chrétien M, Seidah NG** 1991 Gene structure and chromosomal localization of plasma kallikrein. Biochemistry 30: 1628–1635
27. **Yu H, Bowden DW, Spray BJ, Rich SS, Freedman BI** 1998 Identification of human plasma kallikrein gene polymorphisms and evaluation of their role in end-stage renal disease. Hypertension 31:906–911
28. **Schmaier AH** 2008 Assembly, activation, and physiologic influence of the plasma kallikrein/kinin system. Int Immunopharmacol 8:161–165
29. **Swift GH, Dagorn JC, Ashley PL, Cummings SW, MacDonald RJ** 1982 Rat pancreatic kallikrein mRNA: nucleotide sequence and amino acid sequence of the encoded preproenzyme. Proc Natl Acad Sci USA 79:7263–7267
30. **Tschesche H, Mair G, Godec G, Fiedler F, Ehret W, Hirschauer C, Lemon M, Fritz H, Schmidt-Kastner G, Kutzbach C** 1979 The primary structure of porcine glandular kallikreins. In: Fuji S, Moriya H, Suzuki T, eds. Kinins II: biochemistry, pathophysiology, and clinical aspects. New York: Plenum Press; 245
31. **Evans BA, Drinkwater CC, Richards RI** 1987 Mouse glandular kallikrein genes. Structure and partial sequence analysis of the kallikrein gene locus. J Biol Chem 262:8027–8034
32. **Mason AJ, Evans BA, Cox DR, Shine J, Richards RI** 1983 Structure of mouse kallikrein gene family suggests a role in specific processing of biologically active peptides. Nature 303:300–307
33. **Southard-Smith M, Pierce JC, MacDonald RJ** 1994 Physical mapping of the rat tissue kallikrein family in two gene clusters by analysis of P1 bacteriophage clones. Genomics 22:404–417
34. **Wines DR, Brady JM, Pritchett DB, Roberts JL, MacDonald RJ** 1989 Organization and expression of the rat kallikrein gene family. J Biol Chem 264:7653–7662
35. **Digby M, Zhang XY, Richards RI** 1989 Human prostate specific antigen (PSA) gene: structure and linkage to the kallikrein-like gene, hGK-1. Nucleic Acids Res 17:2137
36. **Lundwall A** 1989 Characterization of the gene for prostate-specific antigen, a human glandular kallikrein. Biochem Biophys Res Commun 161:1151–1159
37. **Morris BJ** 1989 hGK-1: a kallikrein gene expressed in human prostate. Clin Exp Pharmacol Physiol 16:345–351
38. **Riegman PH, Klaassen P, van der Korput JA, Romijn JC, Trapman J** 1988 Molecular cloning and characterization of novel prostate antigen cDNA's. Biochem Biophys Res Commun 155:181–188
39. **Watt KW, Lee PJ, M'Timkulu T, Chan WP, Loor R** 1986 Human prostate-specific antigen: structural and functional

similarity with serine proteases. Proc Natl Acad Sci USA 83:3166–3170
40. **Howles PN, Dickinson DP, DiCaprio LL, Woodworth-Gutai M, Gross KW** 1984 Use of a cDNA recombinant for the γ-subunit of mouse nerve growth factor to localize members of this multigene family near the TAM-1 locus on chromosome 7. Nucleic Acids Res 12:2791–2805
41. **Riegman PH, Vlietstra RJ, Suurmeijer L, Cleutjens CB, Trapman J** 1992 Characterization of the human kallikrein locus. Genomics 14:6–11
42. **Gan L, Lee I, Smith R, Argonza-Barrett R, Lei H, McCuaig J, Moss P, Paeper B, Wang K** 2000 Sequencing and expression analysis of the serine protease gene cluster located in chromosome 19q13 region. Gene 257:119–130
43. **Harvey TJ, Hooper JD, Myers SA, Stephenson SA, Ashworth LK, Clements JA** 2000 Tissue-specific expression patterns and fine mapping of the human kallikrein (KLK) locus on proximal 19q13.4. J Biol Chem 275:37397–37406
44. **Yousef GM, Chang A, Scorilas A, Diamandis EP** 2000 Genomic organization of the human kallikrein gene family on chromosome 19q13.3-q13.4. Biochem Biophys Res Commun 276:125–133
45. **Elliott MB, Irwin DM, Diamandis EP** 2006 In silico identification and Bayesian phylogenetic analysis of multiple new mammalian kallikrein gene families. Genomics 88:591–599
46. **Fernando SC, Najar FZ, Guo X, Zhou L, Fu Y, Geisert RD, Roe BA, DeSilva U** 2007 Porcine kallikrein gene family: genomic structure, mapping, and differential expression analysis. Genomics 89:429–438
47. **Olsson AY, Lilja H, Lundwall A** 2004 Taxon-specific evolution of glandular kallikrein genes and identification of a progenitor of prostate-specific antigen. Genomics 84:147–156
48. **Olsson AY, Lundwall A** 2002 Organization and evolution of the glandular kallikrein locus in *Mus musculus*. Biochem Biophys Res Commun 299:305–311
49. **Borgoño CA, Gavigan JA, Alves J, Bowles B, Harris JL, Sotiropoulou G, Diamandis EP** 2007 Defining the extended substrate specificity of kallikrein 1-related peptidases. Biol Chem 388:1215–1225
50. **Bothwell MA, Wilson WH, Shooter EM** 1979 The relationship between glandular kallikrein and growth factor-processing proteases of mouse submaxillary gland. J Biol Chem 254:7287–7294
51. **Charlesworth MC, Young CY, Miller VM, Tindall DJ** 1999 Kininogenase activity of prostate-derived human glandular kallikrein (hK2) purified from seminal fluid. J Androl 20:220–229
52. **Deperthes D, Marceau F, Frenette G, Lazure C, Tremblay RR, Dubé JY** 1997 Human kallikrein hK2 has low kininogenase activity while prostate-specific antigen (hK3) has none. Biochim Biophys Acta 1343:102–106
53. **Michael IP, Sotiropoulou G, Pampalakis G, Magklara A, Ghosh M, Wasney G, Diamandis EP** 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
54. **Lundwall A, Band V, Blaber M, Clements JA, Courty Y, Diamandis EP, Fritz H, Lilja H, Malm J, Maltais LJ, Olsson AY, Petraki C, Scorilas A, Sotiropoulou G, Stenman UH, Stephan C, Talieri M, Yousef GM** 2006 A comprehensive nomenclature for serine proteases with homology to tissue kallikreins. Biol Chem 387:637–641
55. **Hüttenhofer A, Kiefmann M, Meier-Ewert S, O'Brien J, Lehrach H, Bachellerie JP, Brosius J** 2001 RNomics: an experimental approach that identifies 201 candidates for novel, small, non-messenger RNAs in mouse. EMBO J 20:2943–2953
56. **Tykodi SS, Fujii N, Vigneron N, Lu SM, Mito JK, Miranda MX, Chou J, Voong LN, Thompson JA, Sandmaier BM, Cresswell P, Van den Eynde B, Riddell SR, Warren EH** 2008 C19orf48 encodes a minor histocompatibility antigen recognized by CD8+ cytotoxic T cells from renal cell carcinoma patients. Clin Cancer Res 14:5260–5269
57. **Cao H, de Bono B, Belov K, Wong ES, Trowsdale J, Barrow AD** 2009 Comparative genomics indicates the mammalian CD33rSiglec locus evolved by an ancient large-scale inverse duplication and suggests all Siglecs share a common ancestral region. Immunogenetics 61:401–417
58. **Hermans KG, Bressers AA, van der Korput HA, Dits NF, Jenster G, Trapman J** 2008 Two unique novel prostate-specific and androgen-regulated fusion partners of ETV4 in prostate cancer. Cancer Res 68:3094–3098
59. **Bayani J, Paliouras M, Planque C, Shan SJ, Graham C, Squire JA, Diamandis EP** 2008 Impact of cytogenetic and genomic aberrations of the kallikrein locus in ovarian cancer. Mol Oncol 2:250–260
60. **Ni X, Zhang W, Huang KC, Wang Y, Ng SK, Mok SC, Berkowitz RS, Ng SW** 2004 Characterisation of human kallikrein 6/protease M expression in ovarian cancer. Br J Cancer 91:725–731
61. **Shinoda Y, Kozaki K, Imoto I, Obara W, Tsuda H, Mizutani Y, Shuin T, Fujioka T, Miki T, Inazawa J** 2007 Association of KLK5 overexpression with invasiveness of urinary bladder carcinoma cells. Cancer Sci 98:1078–1086
62. **Yousef GM, Bharaj BS, Yu H, Poulopoulos J, Diamandis EP** 2001 Sequence analysis of the human kallikrein gene locus identifies a unique polymorphic minisatellite element. Biochem Biophys Res Commun 285:1321–1329
63. **Grimwood J, Gordon LA, Olsen A, Terry A, Schmutz J, Lamerdin J, Hellsten U, Goodstein D, Couronne O, Tran-Gyamfi M, Aerts A, Altherr M, Ashworth L, Bajorek E, Black S, Branscomb E, Caenepeel S, Carrano A, Caoile C, Chan YM, Christensen M, Cleland CA, Copeland A, Dalin E, Dehal P, Denys M, Detter JC, Escobar J, Flowers D, Fotopulos D, Garcia C, Georgescu AM, Glavina T, Gomez M, Gonzales E, Groza M, et al.** 2004 The DNA sequence and biology of human chromosome 19. Nature 428:529–535
64. **Das HK, Jackson CL, Miller DA, Leff T, Breslow JL** 1987 The human apolipoprotein C-II gene sequence contains a novel chromosome 19-specific minisatellite in its third intron. J Biol Chem 262:4787–4793
65. **Hooper JD, Bui LT, Rae FK, Harvey TJ, Myers SA, Ashworth LK, Clements JA** 2001 Identification and characterization of KLK14, a novel kallikrein serine protease gene located on human chromosome 19q13.4 and expressed in prostate and skeletal muscle. Genomics 73:117–122
66. **Nelson PS, Gan L, Ferguson C, Moss P, Gelinas R, Hood L, Wang K** 1999 Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression. Proc Natl Acad Sci USA 96:3114–3119
67. **Yoshida S, Taniguchi M, Hirata A, Shiosaka S** 1998 Sequence analysis and expression of human neuropsin cDNA and gene. Gene 213:9–16
68. **Kaushal A, Myers SA, Dong Y, Lai J, Tan OL, Bui LT,**

Hunt ML, Digby MR, Samaratunga H, Gardiner RA, Clements JA, Hooper JD 2008 A novel transcript from the KLKP1 gene is androgen regulated, down-regulated during prostate cancer progression and encodes the first non-serine protease identified from the human kallikrein gene locus. Prostate 68:381–399

69. **Lu W, Zhou D, Glusman G, Utleg AG, White JT, Nelson PS, Vasicek TJ, Hood L, 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
70. **Yousef GM, Borgono CA, Michael IP, Diamandis EP** 2004 Cloning of a kallikrein pseudogene. Clin Biochem 37:961–967
71. **Yousef GM, Diamandis EP** 2000 The expanded human kallikrein gene family: locus characterization and molecular cloning of a new member, KLK-L3 (KLK9). Genomics 65:184–194
72. **Yousef GM, Diamandis EP** 2001 The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr Rev 22:184–204
73. **Luo L, Herbrick JA, Scherer SW, Beatty B, Squire J, Diamandis EP** 1998 Structural characterization and mapping of the normal epithelial cell-specific 1 gene. Biochem Biophys Res Commun 247:580–586
74. **Yousef GM** 2008 microRNAs: a new frontier in kallikrein research. Biol Chem 389:689–694
75. **Rawlings ND, Morton FR, Kok CY, Kong J, Barrett AJ** 2008 MEROPS: the peptidase database. Nucleic Acids Res 36:D320–D325
76. **Di Cera E** 2009 Serine proteases. IUBMB Life 61:510–515
77. **Page MJ, Di Cera E** 2008 Evolution of peptidase diversity. J Biol Chem 283:30010–30014
78. **Krem MM, Di Cera E** 2001 Molecular markers of serine protease evolution. EMBO J 20:3036–3045
79. **Yousef GM, Elliott MB, Kopolovic AD, Serry E, Diamandis EP** 2004 Sequence and evolutionary analysis of the human trypsin subfamily of serine peptidases. Biochim Biophys Acta 1698:77–86
80. **Patthy L** 1999 Protein evolution. 1st ed. Oxford, UK: Blackwell Science
81. **Puente XS, López-Otín C** 2004 A genomic analysis of rat proteases and protease inhibitors. Genome Res 14:609–622
82. **Fry BG, Vidal N, Norman JA, Vonk FJ, Scheib H, Ramjan SF, Kuruppu S, Fung K, Hedges SB, Richardson MK, Hodgson WC, Ignjatovic V, Summerhayes R, Kochva E** 2006 Early evolution of the venom system in lizards and snakes. Nature 439:584–588
83. **Lundwall A, Clauss A, Olsson AY** 2006 Evolution of kallikrein-related peptidases in mammals and identification of a genetic locus encoding potential regulatory inhibitors. Biol Chem 387:243–249
84. **Olsson AY, Valtonen-André C, Lilja H, Lundwall A** 2004 The evolution of the glandular kallikrein locus: identification of orthologs and pseudogenes in the cotton-top tamarin. Gene 343:347–355
85. **Bernett MJ, Blaber SI, Scarisbrick IA, Dhanarajan P, Thompson SM, 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
86. **Yousef GM, Diamandis EP** 2003 Human kallikreins: common structural features, sequence analysis and evolution. Curr Genomics 4:147–165
87. **Yousef GM, Kishi T, Diamandis EP** 2003 Role of kallikrein enzymes in the central nervous system. Clin Chim Acta 329: 1–8
88. **Chapdelaine P, Dubé JY, Frenette G, Tremblay RR** 1984 Identification of arginine esterase as the major androgen-dependent protein secreted by dog prostate and preliminary molecular characterization in seminal plasma. J Androl 5:206–210
89. **Chapdelaine P, Paradis G, Tremblay RR, Dubé JY** 1988 High level of expression in the prostate of a human glandular kallikrein mRNA related to prostate-specific antigen. FEBS Lett 236:205–208
90. **Clark NL, Swanson WJ** 2005 Pervasive adaptive evolution in primate seminal proteins. PLoS Genet 1:e35
91. **Lazure C, Leduc R, Seidah NG, Chrétien M, Dubé JY, Chapdelaine P, Frenette G, Paquin R, Tremblay RR** 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
92. **Dorus S, Evans PD, Wyckoff GJ, Choi SS, Lahn BT** 2004 Rate of molecular evolution of the seminal protein gene SEMG2 correlates with levels of female promiscuity. Nat Genet 36:1326–1329
93. **Mubiru JN, Hubbard GB, Dick Jr EJ, Furman J, Troyer DA, Rogers J** 2008 Nonhuman primates as models for studies of prostate specific antigen and prostatic diseases. Prostate 68:1546–1554
94. **Sato I, Yoshikawa A, Ishiwari A, Shimizu K** 2007 Seasonal changes in urinary prostate-specific antigenic activity in male Japanese macaques (*Macaca fuscaa fuscata*). J Androl 28:821–826
95. **Valtonen-André C, Olsson AY, Nayudu PL, Lundwall A** 2005 Ejaculates from the common marmoset (*Callithrix jacchus*) contain semenogelin and β-microseminoprotein but not prostate-specific antigen. Mol Reprod Dev 71:247–255
96. **Jonsson M, Lundwall A, Malm J** 2006 The semenogelins: proteins with functions beyond reproduction? Cell Mol Life Sci 63:2886–2888
97. **Carvalho AL, Sanz L, Barettino D, Romero A, Calvete JJ, Romão MJ** 2002 Crystal structure of a prostate kallikrein isolated from stallion seminal plasma: a homologue of human PSA. J Mol Biol 322:325–337
98. **Aminetzach YT, Srouji JR, Kong CY, Hoekstra HE** 2009 Convergent evolution of novel protein function in shrew and lizard venom. Curr Biol 19:1925–1931
99. **Kita M, Nakamura Y, Okumura Y, Ohdachi SD, Oba Y, Yoshikuni M, Kido H, 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
100. **Kita M, Okumura Y, Ohdachi SD, Oba Y, Yoshikuni M, Nakamura Y, Kido H, Uemura D** 2005 Purification and characterisation of blarinasin, a new tissue kallikrein-like protease from the short-tailed shrew *Blarina brevicauda*: comparative studies with blarina toxin. Biol Chem 386:177–182
101. **Utaisincharoen P, Mackessy SP, Miller RA, Tu AT** 1993 Complete primary structure and biochemical properties of gilatoxin, a serine protease with kallikrein-like and angiotensin-degrading activities. J Biol Chem 268:21975–21983

102. **Fiedler F, Betz G, Hinz H, Lottspeich F, Raidoo DM, Bhoola KD** 1999 Not more than three tissue kallikreins identified from organs of the guinea pig. Biol Chem 380:63–73
103. **Isackson PJ, Dunbar JC, Bradshaw RA** 1987 Role of glandular kallikreins as growth factor processing enzymes: structural and evolutionary considerations. J Cell Biochem 33: 65–75
104. **Clauss A, Lilja H, Lundwall A** 2005 The evolution of a genetic locus encoding small serine proteinase inhibitors. Biochem Biophys Res Commun 333:383–389
105. **Hedstrom L** 2002 Serine protease mechanism and specificity. Chem Rev 102:4501–4524
106. **Yoon H, Blaber SI, Debela M, Goettig P, Scarisbrick IA, Blaber M** 2009 A completed KLK activome profile: investigation of activation profiles of KLK9, 10, and 15. Biol Chem 390:373–377
107. **Yoon H, Blaber SI, Evans DM, Trim J, Juliano MA, Scarisbrick IA, Blaber M** 2008 Activation profiles of human kallikrein-related peptidases by proteases of the thrombostasis axis. Protein Sci 17:1998–2007
108. **Yoon H, Laxmikanthan G, Lee J, Blaber SI, Rodriguez A, Kogot JM, Scarisbrick IA, Blaber M** 2007 Activation profiles and regulatory cascades of the human kallikrein-related peptidases. J Biol Chem 282:31852–31864
109. **Simmer JP, Hu JC** 2002 Expression, structure, and function of enamel proteinases. Connect Tissue Res 43:441–449
110. **Tye CE, Pham CT, Simmer JP, Bartlett JD** 2009 DPPI may activate KLK4 during enamel formation. J Dent Res 88:323–327
111. **Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T** 2005 A proteolytic cascade of kallikreins in the stratum corneum. J Invest Dermatol 124:198–203
112. **Memari N, Jiang W, Diamandis EP, Luo LY** 2007 Enzymatic properties of human kallikrein-related peptidase 12 (KLK12). Biol Chem 388:427–435
113. **Mikolajczyk SD, Millar LS, Marker KM, Grauer LS, Goel A, Cass MM, Kumar A, Saedi MS** 1997 Ala217 is important for the catalytic function and autoactivation of prostate-specific human kallikrein 2. Eur J Biochem 246: 440–446
114. **Väisänen V, Lövgren J, Hellman J, Piironen T, Lilja H, Pettersson K** 1999 Characterization and processing of prostate specific antigen (hK3) and human glandular kallikrein (hK2) secreted by LNCaP cells. Prostate Cancer Prostatic Dis 2:91–97
115. **Bax B, Blaber M, Ferguson G, Sternberg MJ, Walls PH** 1993 Prediction of the three-dimensional structures of the nerve growth factor and epidermal growth factor binding proteins (kallikreins) and an hypothetical structure of the high molecular weight complex of epidermal growth factor with its binding protein. Protein Sci 2:1229–1241
116. **Bode W, Chen Z, Bartels K, Kutzbach C, Schmidt-Kastner G, Bartunik H** 1983 Refined 2A 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
117. **Cavallaro U, Schaffhauser B, Christofori G** 2002 Cadherins and the tumour progression: is it all in a switch? Cancer Lett 176:123–128
118. **Chen Z, Bode W** 1983 Refined 2.5A x-ray crystal structure of the complex formed by porcine kallikrein A and the bovine pancreatic trypsin inhibitor. Crystallization, Patterson search, structure determination, refinement, structure and comparison with its components and with the bovine trypsin-pancreatic trypsin inhibitor complex. J Mol Biol 164: 283–311
119. **Debela M, Goettig P, Magdolen V, Huber R, Schechter NM, Bode W** 2007 Structural basis of the zinc inhibition of human tissue kallikrein 5. J Mol Biol 373:1017–1031
120. **Debela M, Hess P, Magdolen V, Schechter NM, Steiner T, Huber R, Bode W, 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
121. **Debela M, Magdolen V, Grimminger V, Sommerhoff C, Messerschmidt A, Huber R, Friedrich R, Bode W, Goettig P** 2006 Crystal structures of human tissue kallikrein 4: activity modulation by a specific zinc binding site. J Mol Biol 362:1094–1107
122. **Fujinaga M, James MN** 1987 Rat submaxillary gland serine protease, tonin. Structure solution and refinement at 1.8 A resolution. J Mol Biol 195:373–396
123. **Gomis-Rüth FX, Bayés A, Sotiropoulou G, Pampalakis G, Tsetsenis T, Villegas V, Avilés FX, Coll M** 2002 The structure of human prokallikrein 6 reveals a novel activation mechanism for the kallikrein family. J Biol Chem 277: 27273–27281
124. **Kishi T, Kato M, Shimizu T, Kato K, Matsumoto K, Yoshida S, Shiosaka S, Hakoshima T** 1997 Crystallization and preliminary x-ray analysis of neuropsin, a serine protease expressed in the limbic system of mouse brain. J Struct Biol 118:248–251
125. **Laxmikanthan G, Blaber SI, Bernett MJ, Scarisbrick IA, Juliano MA, 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
126. **Ménez R, Michel S, Muller BH, Bossus M, Ducancel F, Jolivet-Reynaud C, Stura EA** 2008 Crystal structure of a ternary complex between human prostate-specific antigen, its substrate acyl intermediate and an activating antibody. J Mol Biol 376:1021–1033
127. **Mittl PR, Di Marco S, Fendrich G, Pohlig G, Heim J, Sommerhoff C, Fritz H, Priestle JP, Grütter MG** 1997 A new structural class of serine protease inhibitors revealed by the structure of the hirustasin-kallikrein complex. Structure 5:253–264
128. **Timm DE** 1997 The crystal structure of the mouse glandular kallikrein-13 (prorenin converting enzyme). Protein Sci 6:1418–1425
129. **Debela M, Beaufort N, Magdolen V, Schechter NM, Craik CS, Schmitt M, Bode W, Goettig P** 2008 Structures and specificity of the human kallikrein-related peptidases KLK 4, 5, 6, and 7. Biol Chem 389:623–632
130. **Takayama TK, Carter CA, 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
131. **Skytt A, Strömqvist M, Egelrud T** 1995 Primary substrate specificity of recombinant human stratum corneum chymotryptic enzyme. Biochem Biophys Res Commun 211:586–589
132. **Blow AM** 1977 Action of human lysosomal elastase on the oxidized B chain of insulin. Biochem J 161:13–16

133. **Sinha S, Watorek W, Karr S, Giles J, Bode W, Travis J** 1987 Primary structure of human neutrophil elastase. Proc Natl Acad Sci USA 84:2228–2232
134. **Zhang Y, Bhat I, Zeng M, Jayal G, Wazer DE, Band H, Band V** 2006 Human kallikrein 10, a predictive marker for breast cancer. Biol Chem 387:715–721
135. **Moreau ME, Garbacki N, Molinaro G, Brown NJ, Marceau F, Adam A** 2005 The kallikrein-kinin system: current and future pharmacological targets. J Pharmacol Sci 99:6–38
136. **Chao J, Bledsoe G, Yin H, Chao L** 2006 The tissue kallikrein-kinin system protects against cardiovascular and renal diseases and ischemic stroke independently of blood pressure reduction. Biol Chem 387:665–675
137. **Rajapakse S, Ogiwara K, Yamano N, Kimura A, Hirata K, Takahashi S, Takahashi T** 2006 Characterization of mouse tissue kallikrein 5. Zoolog Sci 23:963–968
138. **Rajapakse S, Takahashi T** 2007 Expression and enzymatic characterization of recombinant human kallikrein 14. Zoolog Sci 24:774–780
139. **Emami N, Diamandis EP** 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
140. **Michael IP, Pampalakis G, Mikolajczyk SD, Malm J, Sotiropoulou G, Diamandis EP** 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
141. **Beaufort N, Debela M, Creutzburg S, Kellermann J, Bode W, Schmitt M, Pidard D, Magdolen V** 2006 Interplay of human tissue kallikrein 4 (hK4) with the plasminogen activation system: hK4 regulates the structure and functions of the urokinase-type plasminogen activator receptor (uPAR). Biol Chem 387:217–222
142. **Rajapakse S, Ogiwara K, Takano N, Moriyama A, Takahashi T** 2005 Biochemical characterization of human kallikrein 8 and its possible involvement in the degradation of extracellular matrix proteins. FEBS Lett 579:6879–6884
143. **Takayama TK, Fujikawa K, Davie EW** 1997 Characterization of the precursor of prostate-specific antigen. Activation by trypsin and by human glandular kallikrein. J Biol Chem 272:21582–21588
144. **Takayama TK, McMullen BA, Nelson PS, Matsumura M, 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
145. **Tschesche H, Michaelis J, Kohnert U, Fedrowitz J, Oberhoff R** 1989 Tissue kallikrein effectively activates latent degrading metalloenzymes. Adv Exp Med Biol 247A:545–548
146. **Borgoño CA, Michael IP, Komatsu N, Jayakumar A, Kapadia R, Clayman GL, Sotiropoulou G, Diamandis EP** 2007 A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. J Biol Chem 282:3640–3652
147. **Brattsand M, Egelrud T** 1999 Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. J Biol Chem 274:30033–30040
148. **Hansson L, Strömqvist M, Bäckman A, Wallbrandt P, Carlstein A, Egelrud T** 1994 Cloning, expression, and characterization of stratum corneum chymotryptic enzyme. A skin-specific human serine proteinase. J Biol Chem 269:19420–19426
149. **Komatsu N, Saijoh K, Toyama T, Ohka R, Otsuki N, Hussack G, Takehara K, Diamandis EP** 2005 Multiple tissue kallikrein mRNA and protein expression in normal skin and skin diseases. Br J Dermatol 153:274–281
150. **Komatsu N, Takata M, Otsuki N, Toyama T, Ohka R, Takehara K, Saijoh K** 2003 Expression and localization of tissue kallikrein mRNAs in human epidermis and appendages. J Invest Dermatol 121:542–549
151. **Stefansson K, Brattsand M, Ny A, Glas B, 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
152. **Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, Egelrud T, Simon M, 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
153. **Yamasaki K, Schauber J, Coda A, Lin H, Dorschner RA, Schechter NM, Bonnart C, Descargues P, Hovnanian A, Gallo RL** 2006 Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. FASEB J 20:2068–2080
154. **Deraison C, Bonnart C, Lopez F, Besson C, Robinson R, Jayakumar A, Wagberg F, Brattsand M, Hachem JP, Leonardsson G, 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
155. **Descargues P, Deraison C, Bonnart C, Kreft M, Kishibe M, Ishida-Yamamoto A, Elias P, Barrandon Y, Zambruno G, Sonnenberg A, Hovnanian A** 2005 Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat Genet 37:56–65
156. **Hansson L, Bäckman A, Ny A, Edlund M, Ekholm E, Ekstrand Hammarström B, Törnell J, Wallbrandt P, Wennbo H, Egelrud T** 2002 Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. J Invest Dermatol 118:444–449
157. **Kirihara T, Matsumoto-Miyai K, Nakamura Y, Sadayama T, Yoshida S, Shiosaka S** 2003 Prolonged recovery of ultraviolet B-irradiated skin in neuropsin (KLK8)-deficient mice. Br J Dermatol 149:700–706
158. **Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, Bonafé JL, Wilkinson J, Taïeb A, Barrandon Y, Harper JI, de Prost Y, Hovnanian A** 2000 Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat Genet 25:141–142
159. **Emami N, Scorilas A, Soosaipillai A, Earle T, Mullen B, Diamandis EP** 2009 Association between kallikrein-related peptidases (KLKs) and macroscopic indicators of semen analysis: their relation to sperm motility. Biol Chem 390:921–929
160. **Shaw JL, Diamandis EP** 2007 Distribution of 15 human kallikreins in tissues and biological fluids. Clin Chem 53:1423–1432
161. **Emami N, Deperthes D, Malm J, Diamandis EP** 2008 Ma-

jor role of human KLK14 in seminal clot liquefaction. J Biol Chem 283:19561–19569
162. **Jonsson M, Linse S, Frohm B, Lundwall A, Malm J** 2005 Semenogelins I and II bind zinc and regulate the activity of prostate-specific antigen. Biochem J 387:447–453
163. **Lövgren J, Airas K, Lilja H** 1999 Enzymatic action of human glandular kallikrein 2 (hK2). Substrate specificity and regulation by Zn2+ and extracellular protease inhibitors. Eur J Biochem 262:781–789
164. **de Lamirande E** 2007 Semenogelin, the main protein of the human semen coagulum, regulates sperm function. Semin Thromb Hemost 33:60–68
165. **Deperthes D, Frenette G, Brillard-Bourdet M, Bourgeois L, Gauthier F, Tremblay RR, Dubé JY** 1996 Potential involvement of kallikrein hK2 in the hydrolysis of the human seminal vesicle proteins after ejaculation. J Androl 17:659–665
166. **Lilja H** 1985 A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. J Clin Invest 76:1899–1903
167. **Veveris-Lowe TL, Kruger SJ, Walsh T, Gardiner RA, Clements JA** 2007 Seminal fluid characterization for male fertility and prostate cancer: kallikrein-related serine proteases and whole proteome approaches. Semin Thromb Hemost 33:87–99
168. **Ghosh MC, Grass L, Soosaipillai A, Sotiropoulou G, Diamandis EP** 2004 Human kallikrein 6 degrades extracellular matrix proteins and may enhance the metastatic potential of tumour cells. Tumour Biol 25:193–199
169. **Ishii K, Otsuka T, Iguchi K, Usui S, Yamamoto H, Sugimura Y, Yoshikawa K, Hayward SW, Hirano K** 2004 Evidence that the prostate-specific antigen (PSA)/Zn(2+) axis may play a role in human prostate cancer cell invasion. Cancer Lett 207:79–87
170. **Johnson SK, Ramani VC, Hennings L, Haun RS** 2007 Kallikrein 7 enhances pancreatic cancer cell invasion by shedding E-cadherin. Cancer 109:1811–1820
171. **Kapadia C, Ghosh MC, Grass L, Diamandis EP** 2004 Human kallikrein 13 involvement in extracellular matrix degradation. Biochem Biophys Res Commun 323:1084–1090
172. **Prezas P, Arlt MJ, Viktorov P, Soosaipillai A, Holzscheiter L, Schmitt M, Talieri M, Diamandis EP, Krüger A, Magdolen V** 2006 Overexpression of the human tissue kallikrein genes KLK4, 5, 6, and 7 increases the malignant phenotype of ovarian cancer cells. Biol Chem 387:807–811
173. **Rückert F, Hennig M, Petraki CD, Wehrum D, Distler M, Denz A, Schröder M, Dawelbait G, Kalthoff H, Saeger HD, Diamandis EP, Pilarsky C, Grützmann R** 2008 Co-expression of KLK6 and KLK10 as prognostic factors for survival in pancreatic ductal adenocarcinoma. Br J Cancer 99:1484–1492
174. **Webber MM, Waghray A, Bello D** 1995 Prostate-specific antigen, a serine protease, facilitates human prostate cancer cell invasion. Clin Cancer Res 1:1089–1094
175. **Wolf WC, Evans DM, Chao L, Chao J** 2001 A synthetic tissue kallikrein inhibitor suppresses cancer cell invasiveness. Am J Pathol 159:1797–1805
176. **Klucky B, Mueller R, Vogt I, Teurich S, Hartenstein B, Breuhahn K, Flechtenmacher C, Angel P, Hess J** 2007 Kallikrein 6 induces E-cadherin shedding and promotes cell proliferation, migration, and invasion. Cancer Res 67:8198–8206
177. **Briot A, Deraison C, Lacroix M, Bonnart C, Robin A, Besson C, Dubus P, Hovnanian A** 2009 Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. J Exp Med 206:1135–1147
178. **Gao L, Chao L, Chao J** 2010 A novel signaling pathway of tissue kallikrein in promoting keratinocyte migration: activation of proteinase-activated receptor 1 and epidermal growth factor receptor. Exp Cell Res 316:376–389
179. **Mize GJ, Wang W, Takayama TK** 2008 Prostate-specific kallikreins-2 and -4 enhance the proliferation of DU-145 prostate cancer cells through protease-activated receptors-1 and -2. Mol Cancer Res 6:1043–1051
180. **Oikonomopoulou K, Hansen KK, Saifeddine M, Tea I, Blaber M, Blaber SI, Scarisbrick I, Andrade-Gordon P, Cottrell GS, Bunnett NW, Diamandis EP, Hollenberg MD** 2006 Proteinase-activated receptors, targets for kallikrein signaling. J Biol Chem 281:32095–32112
181. **Oikonomopoulou K, Hansen KK, Saifeddine M, Vergnolle N, Tea I, Blaber M, Blaber SI, Scarisbrick I, Diamandis EP, Hollenberg MD** 2006 Kallikrein-mediated cell signalling: targeting proteinase-activated receptors (PARs). Biol Chem 387:817–824
182. **Ramsay AJ, Dong Y, Hunt ML, Linn M, Samaratunga H, Clements JA, Hooper JD** 2008 Kallikrein-related peptidase 4 (KLK4) initiates intracellular signaling via protease-activated receptors (PARs). KLK4 and PAR-2 are co-expressed during prostate cancer progression. J Biol Chem 283:12293–12304
183. **Ramsay AJ, Reid JC, Adams MN, Samaratunga H, Dong Y, Clements JA, Hooper JD** 2008 Prostatic trypsin-like kallikrein-related peptidases (KLKs) and other prostate-expressed tryptic proteinases as regulators of signalling via proteinase-activated receptors (PARs). Biol Chem 389:653–668
184. **Stephenson SA, Verity K, Ashworth LK, Clements JA** 1999 Localization of a new prostate-specific antigen-related serine protease gene, KLK4, is evidence for an expanded human kallikrein gene family cluster on chromosome 19q13.3–13.4. J Biol Chem 274:23210–23214
185. **Swedberg JE, Nigon LV, Reid JC, de Veer SJ, Walpole CM, Stephens CR, Walsh TP, Takayama TK, Hooper JD, Clements JA, Buckle AM, Harris JM** 2009 Substrate-guided design of a potent and selective kallikrein-related peptidase inhibitor for kallikrein 4. Chem Biol 16:633–643
186. **Vandell AG, Larson N, Laxmikanthan G, Panos M, Blaber SI, Blaber M, Scarisbrick IA** 2008 Protease-activated receptor dependent and independent signaling by kallikreins 1 and 6 in CNS neuron and astroglial cell lines. J Neurochem 107:855–870
187. **Cohen P, Graves HC, Peehl DM, Kamarei M, Giudice LC, Rosenfeld RG** 1992 Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. J Clin Endocrinol Metab 75:1046–1053
188. **Koistinen H, Paju A, Koistinen R, Finne P, Lövgren J, Wu P, Seppälä M, Stenman UH** 2002 Prostate-specific antigen and other prostate-derived proteases cleave IGFBP-3, but prostate cancer is not associated with proteolytically cleaved circulating IGFBP-3. Prostate 50:112–118
189. **Matsumura M, Bhatt AS, Andress D, Clegg N, Takayama TK, Craik CS, Nelson PS** 2005 Substrates of the prostate-specific serine protease prostase/KLK4 defined by positional-scanning peptide libraries. Prostate 62:1–13

190. **Plymate SR, Rosen CJ, Paulsen CA, Ware JL, Chen J, Vessella RE, Birnbaum RS** 1996 Proteolysis of insulin-like growth factor-binding protein-3 in the male reproductive tract. J Clin Endocrinol Metab 81:618–624
191. **Réhault S, Monget P, Mazerbourg S, Tremblay R, Gutman N, Gauthier F, Moreau T** 2001 Insulin-like growth factor binding proteins (IGFBPs) as potential physiological substrates for human kallikreins hK2 and hK3. Eur J Biochem 268:2960–2968
192. **Sano A, Sangai T, Maeda H, Nakamura M, Hasebe T, Ochiai A** 2007 Kallikrein 11 expressed in human breast cancer cells releases insulin-like growth factor through degradation of IGFBP-3. Int J Oncol 30:1493–1498
193. **Fielder PJ, Rosenfeld RG, Graves HC, Grandbois K, Maack CA, Sawamura S, Ogawa Y, Sommer A, Cohen P** 1994 Biochemical analysis of prostate specific antigen-proteolyzed insulin-like growth factor binding protein-3. Growth Regul 4:164–172
194. **Sutkowski DM, Goode RL, Baniel J, Teater C, Cohen P, McNulty AM, Hsiung HM, Becker GW, Neubauer BL** 1999 Growth regulation of prostatic stromal cells by prostate-specific antigen. J Natl Cancer Inst 91:1663–1669
195. **Denmeade SR, Litvinov I, Sokoll LJ, Lilja H, Isaacs JT** 2003 Prostate-specific antigen (PSA) protein does not affect growth of prostate cancer cells in vitro or prostate cancer xenografts in vivo. Prostate 56:45–53
196. **Klokk TI, Kilander A, Xi Z, Waehre H, Risberg B, Danielsen HE, Saatcioglu F** 2007 Kallikrein 4 is a proliferative factor that is overexpressed in prostate cancer. Cancer Res 67:5221–5230
197. **Niu Y, Yeh S, Miyamoto H, Li G, Altuwaijri S, Yuan J, Han R, Ma T, Kuo HC, Chang C** 2008 Tissue prostate-specific antigen facilitates refractory prostate tumor progression via enhancing ARA70-regulated androgen receptor transactivation. Cancer Res 68:7110–7119
198. **Pampalakis G, Prosnikli E, Agalioti T, Vlahou A, Zoumpourlis V, Sotiropoulou G** 2009 A tumor-protective role for human kallikrein-related peptidase 6 in breast cancer mediated by inhibition of epithelial-to-mesenchymal transition. Cancer Res 69:3779–3787
199. **Veveris-Lowe TL, Lawrence MG, Collard RL, Bui L, Herington AC, Nicol DL, Clements JA** 2005 Kallikrein 4 (hK4) and prostate-specific antigen (PSA) are associated with the loss of E-cadherin and an epithelial-mesenchymal transition (EMT)-like effect in prostate cancer cells. Endocr Relat Cancer 12:631–643
200. **Fortier AH, Holaday JW, Liang H, Dey C, Grella DK, Holland-Linn J, Vu H, Plum SM, Nelson BJ** 2003 Recombinant prostate specific antigen inhibits angiogenesis in vitro and in vivo. Prostate 56:212–219
201. **Koistinen H, Wohlfahrt G, Mattsson JM, Wu P, Lahdenperä J, Stenman UH** 2008 Novel small molecule inhibitors for prostate-specific antigen. Prostate 68:1143–1151
202. **Sun XY, Donald SP, Phang JM** 2001 Testosterone and prostate-specific antigen stimulate generation of reactive oxygen species in prostate cancer cells. Carcinogenesis 22: 1775–1780
203. **Aalamian M, Tourkova IL, Chatta GS, Lilja H, Huland E, Huland H, Shurin GV, Shurin MR** 2003 Inhibition of dendropoiesis by tumor derived and purified prostate specific antigen. J Urol 170:2026–2030
204. **Kennedy-Smith AG, McKenzie JL, Owen MC, Davidson PJ, Vuckovic S, Hart DN** 2002 Prostate specific antigen inhibits immune responses in vitro: a potential role in prostate cancer. J Urol 168:741–747
205. **Kodak JA, Mann DL, Klyushnenkova EN, Alexander RB** 2006 Activation of innate immunity by prostate specific antigen (PSA). Prostate 66:1592–1599
206. **Goya M, Ishii G, Miyamoto S, Hasebe T, Nagai K, Yonou H, Hatano T, Ogawa Y, Ochiai A** 2006 Prostate-specific antigen induces apoptosis of osteoclast precursors: potential role in osteoblastic bone metastases of prostate cancer. Prostate 66:1573–1584
207. **Gygi CM, Leibovitch IY, Adlington R, Baldwin JE, Chen B, Mccoull W, Pritchard GJ, Becker GW, Dixon EP, Little SP, Sutkowski DM, Teater C, Neubauer BL** 2002 Prostate-specific antigen (PSA)-mediated proliferation, androgenic regulation and inhibitory effects of LY312340 in HOS-TE85 (TE85) human osteosarcoma cells. Anticancer Res 22:2725–2732
208. **Killian CS, Corral DA, Kawinski E, Constantine RI** 1993 Mitogenic response of osteoblast cells to prostate-specific antigen suggests an activation of latent TGF-β and a proteolytic modulation of cell adhesion receptors. Biochem Biophys Res Commun 192:940–947
209. **Nadiminty N, Lou W, Lee SO, Mehraein-Ghomi F, Kirk JS, Conroy JM, Zhang H, Gao AC** 2006 Prostate-specific antigen modulates genes involved in bone remodeling and induces osteoblast differentiation of human osteosarcoma cell line SaOS-2. Clin Cancer Res 12:1420–1430
210. **Yonou H, Aoyagi Y, Kanomata N, Kamijo T, Oda T, Yokose T, Hasebe T, Nagai K, Hatano T, Ogawa Y, Ochiai A** 2001 Prostate-specific antigen induces osteoplastic changes by an autonomous mechanism. Biochem Biophys Res Commun 289:1082–1087
211. **Cramer SD, Chen Z, Peehl DM** 1996 Prostate specific antigen cleaves parathyroid hormone-related protein in the PTH-like domain: inactivation of PTHrP-stimulated cAMP accumulation in mouse osteoblasts. J Urol 156:526–531
212. **Dallas SL, Zhao S, Cramer SD, Chen Z, Peehl DM, Bonewald LF** 2005 Preferential production of latent transforming growth factor β-2 by primary prostatic epithelial cells and its activation by prostate-specific antigen. J Cell Physiol 202: 361–370
213. **Gao J, Collard RL, Bui L, Herington AC, Nicol DL, Clements JA** 2007 Kallikrein 4 is a potential mediator of cellular interactions between cancer cells and osteoblasts in metastatic prostate cancer. Prostate 67:348–360
214. **Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, Cooke MP, Walker JR, Hogenesch JB** 2004 A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci USA 101:6062–6067
215. **Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG, Balk SP** 2006 Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 66: 2815–2825
216. **Narita D, Cimpean AM, Anghel A, Raica M** 2006 Prostate-specific antigen value as a marker in breast cancer. Neoplasma 53:161–167
217. **Olsson AY, Bjartell A, Lilja H, Lundwall A** 2005 Expression of prostate-specific antigen (PSA) and human glan-

dular kallikrein 2 (hK2) in ileum and other extraprostatic tissues. Int J Cancer 113:290–297
218. **Stone JG, Rolston RK, Ueda M, Lee HG, Richardson SL, Castellani RJ, Perry G, Smith MA** 2009 Evidence for the novel expression of human kallikrein-related peptidase 3, prostate-specific antigen, in the brain. Int J Clin Exp Pathol 2:267–274
219. **MacDonald RJ, Southard-Smith EM, Kroon E** 1996 Disparate tissue-specific expression of members of the tissue kallikrein multigene family of the rat. J Biol Chem 271: 13684–13690
220. **Penschow JD, Drinkwater CC, Haralambidis J, Coghlan JP** 1991 Sites of expression and induction of glandular kallikrein gene expression in mice. Mol Cell Endocrinol 81:135–146
221. **Shaw JL, Petraki C, Watson C, Bocking A, Diamandis EP** 2008 Role of tissue kallikrein-related peptidases in cervical mucus remodeling and host defense. Biol Chem 389:1513–1522
222. **Yousef GM, Polymeris ME, Yacoub GM, Scorilas A, Soosaipillai A, Popalis C, Fracchioli S, Katsaros D, Diamandis EP** 2003 Parallel overexpression of seven kallikrein genes in ovarian cancer. Cancer Res 63:2223–2227
223. **Paliouras M, Diamandis EP** 2007 Coordinated steroid hormone-dependent and independent expression of multiple kallikreins in breast cancer cell lines. Breast Cancer Res Treat 102:7–18
224. **Petraki CD, Papanastasiou PA, Karavana VN, Diamandis EP** 2006 Cellular distribution of human tissue kallikreins: immunohistochemical localization. Biol Chem 387:653–663
225. **Berglund L, Björling E, Oksvold P, Fagerberg L, Asplund A, Szigyarto CA, Persson A, Ottosson J, Wernérus H, Nilsson P, Lundberg E, Sivertsson A, Navani S, Wester K, Kampf C, Hober S, Pontén F, Uhlén M** 2008 A genecentric human protein atlas for expression profiles based on antibodies. Mol Cell Proteomics 7:2019–2027
226. **Williams SA, Singh P, Isaacs JT, Denmeade SR** 2007 Does PSA play a role as a promoting agent during the initiation and/or progression of prostate cancer? Prostate 67:312–329
227. **Lu Y, Papagerakis P, Yamakoshi Y, Hu JC, Bartlett JD, Simmer JP** 2008 Functions of KLK4 and MMP-20 in dental enamel formation. Biol Chem 389:695–700
228. **Chen ZL, Yoshida S, Kato K, Momota Y, Suzuki J, Tanaka T, Ito J, Nishino H, Aimoto S, Kiyama H** 1995 Expression and activity-dependent changes of a novel limbic-serine protease gene in the hippocampus. J Neurosci 15: 5088–5097
229. **Inoue N, Kuwae K, Ishida-Yamamoto A, Iizuka H, Shibata M, Yoshida S, Kato K, Shiosaka S** 1998 Expression of neuropsin in the keratinizing epithelial tissue-immunohistochemical analysis of wild-type and nude mice. J Invest Dermatol 110:923–931
230. **Kuwae K, Matsumoto-Miyai K, Yoshida S, Sadayama T, Yoshikawa K, Hosokawa K, Shiosaka S** 2002 Epidermal expression of serine protease, neuropsin (KLK8) in normal and pathological skin samples. Mol Pathol 55:235–241
231. **Mitsui S, Tsuruoka N, Yamashiro K, Nakazato H, Yamaguchi N** 1999 A novel form of human neuropsin, a brain-related serine protease, is generated by alternative splicing and is expressed preferentially in human adult brain. Eur J Biochem 260:627–634
232. **Mitsui S, Okui A, Kominami K, Uemura H, Yamaguchi N, Yoshida S, Taniguchi M, Suemoto T, Oka T, He X, Shiosaka S** 2000 cDNA cloning and tissue-specific splicing variants of mouse hippostasin/TLSP (PRSS20). Biochim Biophys Acta 1494:206–210
233. **Mitsui S, Yamada T, Okui A, Kominami K, Uemura H, Yamaguchi N** 2000 A novel isoform of a kallikrein-like protease, TLSP/hippostasin, (PRSS20), is expressed in the human brain and prostate. Biochem Biophys Res Comm 272:205–211
234. **Hirata A, Yoshida S, Inoue N, Matsumoto-Miyai K, Ninomiya A, Taniguchi M, Matsuyama T, Kato K, Iizasa H, Kataoka Y, Yoshida N, Shiosaka S** 2001 Abnormalities of synapses and neurons in the hippocampus of neuropsin-deficient mice. Mol Cell Neurosci 17:600–610
235. **Kishibe M, Bando Y, Terayama R, Namikawa K, Takahashi H, Hashimoto Y, Ishida-Yamamoto A, Jiang YP, Mitrovic B, Perez D, Iizuka H, Yoshida S** 2007 Kallikrein 8 is involved in skin desquamation in cooperation with other kallikreins. J Biol Chem 282:5834–5841
236. **Meneton P, Bloch-Faure M, Hagege AA, Ruetten H, Huang W, Bergaya S, Ceiler D, Gehring D, Martins I, Salmon G, Boulanger CM, Nussberger J, Crozatier B, Gasc JM, Heudes D, Bruneval P, Doetschman T, Ménard J, Alhenc-Gelas F** 2001 Cardiovascular abnormalities with normal blood pressure in tissue kallikrein-deficient mice. Proc Natl Acad Sci USA 98:2634–2639
237. **Simmer JP, Hu Y, Lertlam R, Yamakoshi Y, Hu JC** 2009 Hypomaturation enamel defects in Klk4 knockout/LacZ knockin mice. J Biol Chem 284:19110–19121
238. **Paliouras M, Borgono C, Diamandis EP** 2007 Human tissue kallikreins: the cancer biomarker family. Cancer Lett 249:61–79
239. **Lilja H, Ulmert D, Vickers AJ** 2008 Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. Nat Rev Cancer 8:268–278
240. **Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E** 1987 Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 317:909–916
241. **Kuriyama M, Wang MC, Papsidero LD, Killian CS, Shimano T, Valenzuela L, Nishiura T, Murphy GP, Chu TM** 1980 Quantitation of prostate-specific antigen in serum by a sensitive enzyme immunoassay. Cancer Res 40:4658–4662
242. **Pinzani P, Lind K, Malentacchi F, Nesi G, Salvianti F, Villari D, Kubista M, Pazzagli M, Orlando C** 2008 Prostate-specific antigen mRNA and protein levels in laser microdissected cells of human prostate measured by real-time reverse transcriptase-quantitative polymerase chain reaction and immuno-quantitative polymerase chain reaction. Hum Pathol 39:1474–1482
243. **Andriole GL, Crawford ED, Grubb 3rd RL, Buys SS, Chia D, Church TR, Fouad MN, Gelmann EP, Kvale PA, Reding DJ, Weissfeld JL, Yokochi LA, O'Brien B, Clapp JD, Rathmell JM, Riley TL, Hayes RB, Kramer BS, Izmirlian G, Miller AB, Pinsky PF, Prorok PC, Gohagan JK, Berg CD** 2009 Mortality results from a randomized prostate-cancer screening trial. N Engl J Med 360:1310–1319
244. **Schröder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, Kwiatkowski M, Lujan M, Lilja H, Zappa M, Denis LJ, Recker F, Berenguer A, Määttänen L, Bangma CH, Aus G, Villers A, Rebillard X, van der Kwast**

T, Blijenberg BG, Moss SM, de Koning HJ, Auvinen A 2009 Screening and prostate-cancer mortality in a randomized European study. N Engl J Med 360:1320–1328

245. **Stamey TA, Caldwell M, McNeal JE, Nolley R, Hemenez M, Downs J** 2004 The prostate specific antigen era in the United States is over for prostate cancer: what happened in the last 20 years? J Urol 172:1297–1301
246. **Ung JO, Richie JP, Chen MH, Renshaw AA, D'Amico AV** 2002 Evolution of the presentation and pathologic and biochemical outcomes after radical prostatectomy for patients with clinically localized prostate cancer diagnosed during the PSA era. Urology 60:458–463
247. **Ulmert D, Cronin AM, Björk T, O'Brien MF, Scardino PT, Eastham JA, Becker C, Berglund G, Vickers AJ, Lilja H** 2008 Prostate-specific antigen at or before age 50 as a predictor of advanced prostate cancer diagnosed up to 25 years later: a case-control study. BMC Med 6:6
248. **Stephan C, Jung K, Lein M, Diamandis EP** 2007 PSA and other tissue kallikreins for prostate cancer detection. Eur J Cancer 43:1918–1926
249. **Kwiatkowski MK, Recker F, Piironen T, Pettersson K, Otto T, Wernli M, Tscholl R** 1998 In prostatism patients the ratio of human glandular kallikrein to free PSA improves the discrimination between prostate cancer and benign hyperplasia within the diagnostic "gray zone" of total PSA 4 to 10 ng/ml. Urology 52:360–365
250. **Borgoño CA, Grass L, Soosaipillai A, Yousef GM, Petraki CD, Howarth DH, Fracchioli S, Katsaros D, Diamandis EP** 2003 Human kallikrein 14: a new potential biomarker for ovarian and breast cancer. Cancer Res 63:9032–9041
251. **Luo LY, Bunting P, Scorilas A, Diamandis EP** 2001 Human kallikrein 10: a novel tumor marker for ovarian carcinoma? Clin Chim Acta 306:111–118
252. **Parekh DJ, Ankerst DP, Baillargeon J, Higgins B, Platz EA, Troyer D, Hernandez J, Leach RJ, Lokshin A, Thompson IM** 2007 Assessment of 54 biomarkers for biopsy-detectable prostate cancer. Cancer Epidemiol Biomarkers Prev 16:1966–1972
253. **Sardana G, Marshall J, Diamandis EP** 2007 Discovery of candidate tumor markers for prostate cancer via proteomic analysis of cell culture-conditioned medium. Clin Chem 53:429–437
254. **Diamandis EP, Yousef GM, Soosaipillai AR, Bunting P** 2000 Human kallikrein 6 (zyme/protease M/neurosin): a new serum biomarker of ovarian carcinoma. Clin Biochem 33:579–583
255. **Komatsu N, Saijoh K, Kuk C, Shirasaki F, Takehara K, Diamandis EP** 2007 Aberrant human tissue kallikrein levels in the stratum corneum and serum of patients with psoriasis: dependence on phenotype, severity and therapy. Br J Dermatol 156:875–883
256. **Santin AD, Diamandis EP, Bellone S, Soosaipillai A, Cane S, Palmieri M, Burnett A, Roman JJ, Pecorelli S** 2005 Human kallikrein 6: a new potential serum biomarker for uterine serous papillary cancer. Clin Cancer Res 11: 3320–3325
257. **Scarisbrick IA, Linbo R, Vandell AG, Keegan M, Blaber SI, Blaber M, Sneve D, Lucchinetti CF, Rodriguez M, Diamandis EP** 2008 Kallikreins are associated with secondary progressive multiple sclerosis and promote neurodegeneration. Biol Chem 389:739–745
258. **Oikonomopoulou K, Li L, Zheng Y, Simon I, Wolfert RL, Valik D, Nekulova M, Simickova M, Frgala T, Diamandis EP** 2008 Prediction of ovarian cancer prognosis and response to chemotherapy by a serum-based multiparametric biomarker panel. Br J Cancer 99:1103–1113
259. **Planque C, Li L, Zheng Y, Soosaipillai A, Reckamp K, Chia D, Diamandis EP, Goodglick L** 2008 A multiparametric serum kallikrein panel for diagnosis of non-small cell lung carcinoma. Clin Cancer Res 14:1355–1362
260. **Talieri M, Li L, Zheng Y, Alexopoulou DK, Soosaipillai A, Scorilas A, Xynopoulos D, Diamandis EP** 2009 The use of kallikrein-related peptidases as adjuvant prognostic markers in colorectal cancer. Br J Cancer 100:1659–1665
261. **Zheng Y, Katsaros D, Shan SJ, de la Longrais IR, Porpiglia M, Scorilas A, Kim NW, Wolfert RL, Simon I, Li L, Feng Z, Diamandis EP** 2007 A multiparametric panel for ovarian cancer diagnosis, prognosis, and response to chemotherapy. Clin Cancer Res 13:6984–6992
262. **Walters MR** 1985 Steroid hormone receptors and the nucleus. Endocr Rev 6:512–543
263. **Scheidereit C, Geisse S, Westphal HM, Beato M** 1983 The glucocorticoid receptor binds to defined nucleotide sequences near the promoter of mouse mammary tumour virus. Nature 304:749–752
264. **Klock G, Strähle U, Schütz G** 1987 Oestrogen and glucocorticoid responsive elements are closely related but distinct. Nature 329:734–736
265. **Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoute J, Shao W, Hestermann EV, Geistlinger TR, Fox EA, Silver PA, Brown M** 2005 Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell 122:33–43
266. **Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, Chew EG, Huang PY, Welboren WJ, Han Y, Ooi HS, Ariyaratne PN, Vega VB, Luo Y, Tan PY, Choy PY, Wansa KD, Zhao B, Lim KS, Leow SC, Yow JS, Joseph R, Li H, Desai KV, Thomsen JS, Lee YK, Karuturi RK, Herve T, Bourque G, Stunnenberg HG, Ruan X, Cacheux-Rataboul V, Sung WK, Liu ET, Wei CL, Cheung E, Ruan Y** 2009 An oestrogen-receptor-α-bound human chromatin interactome. Nature 462:58–64
267. **Jariwala U, Prescott J, Jia L, Barski A, Pregizer S, Cogan JP, Arasheben A, Tilley WD, Scher HI, Gerald WL, Buchanan G, Coetzee GA, Frenkel B** 2007 Identification of novel androgen receptor target genes in prostate cancer. Mol Cancer 6:39
268. **Lin CY, Vega VB, Thomsen JS, Zhang T, Kong SL, Xie M, Chiu KP, Lipovich L, Barnett DH, Stossi F, Yeo A, George J, Kuznetsov VA, Lee YK, Charn TH, Palanisamy N, Miller LD, Cheung E, Katzenellenbogen BS, Ruan Y, Bourque G, Wei CL, Liu ET** 2007 Whole-genome cartography of estrogen receptor α binding sites. PLoS Genet 3:e87
269. **Welboren WJ, van Driel MA, Janssen-Megens EM, van Heeringen SJ, Sweep FC, Span PN, Stunnenberg HG** 2009 ChIP-Seq of ERα and RNA polymerase II defines genes differentially responding to ligands. EMBO J 28: 1418–1428
270. **Lin B, Wang J, Hong X, Yan X, Hwang D, Cho JH, Yi D, Utleg AG, Fang X, Schones DE, Zhao K, Omenn GS, Hood L** 2009 Integrated expression profiling and ChIP-seq

analyses of the growth inhibition response program of the androgen receptor. PLoS One 4:e6589
271. **Massie CE, Adryan B, Barbosa-Morais NL, Lynch AG, Tran MG, Neal DE, Mills IG** 2007 New androgen receptor genomic targets show an interaction with the ETS1 transcription factor. EMBO Rep 8:871–878
272. **Wang Q, Li W, Liu XS, Carroll JS, Jänne OA, Keeton EK, Chinnaiyan AM, Pienta KJ, Brown M** 2007 A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. Mol Cell 27:380–392
273. **Carroll JS, Meyer CA, Song J, Li W, Geistlinger TR, Eeckhoute J, Brodsky AS, Keeton EK, Fertuck KC, Hall GF, Wang Q, Bekiranov S, Sementchenko V, Fox EA, Silver PA, Gingeras TR, Liu XS, Brown M** 2006 Genome-wide analysis of estrogen receptor binding sites. Nat Genet 38:1289–1297
274. **Jacobsen BM, Jambal P, Schittone SA, Horwitz KB** 2009 ALU repeats in promoters are position-dependent co-response elements (coRE) that enhance or repress transcription by dimeric and monomeric progesterone receptors. Mol Endocrinol 23:989–1000
275. **Schoneveld OJ, Gaemers IC, Lamers WH** 2004 Mechanisms of glucocorticoid signalling. Biochim Biophys Acta 1680:114–128
276. **Bolton EC, So AY, Chaivorapol C, Haqq CM, Li H, Yamamoto KR** 2007 Cell- and gene-specific regulation of primary target genes by the androgen receptor. Genes Dev 21:2005–2017
277. **Takayama K, Kaneshiro K, Tsutsumi S, Horie-Inoue K, Ikeda K, Urano T, Ijichi N, Ouchi Y, Shirahige K, Aburatani H, Inoue S** 2007 Identification of novel androgen response genes in prostate cancer cells by coupling chromatin immunoprecipitation and genomic microarray analysis. Oncogene 26:4453–4463
278. **Wang Q, Carroll JS, Brown M** 2005 Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. Mol Cell 19:631–642
279. **Jia L, Berman BP, Jariwala U, Yan X, Cogan JP, Walters A, Chen T, Buchanan G, Frenkel B, Coetzee GA** 2008 Genomic androgen receptor-occupied regions with different functions, defined by histone acetylation, coregulators and transcriptional capacity. PLoS ONE 3:e3645
280. **Phuc Le P, Friedman JR, Schug J, Brestelli JE, Parker JB, Bochkis IM, Kaestner KH** 2005 Glucocorticoid receptor-dependent gene regulatory networks. PLoS Genet 1:e16
281. **Lupien M, Eeckhoute J, Meyer CA, Wang Q, Zhang Y, Li W, Carroll JS, Liu XS, Brown M** 2008 FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription. Cell 132:958–970
282. **Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, Chen Z, Beroukhim R, Wang H, Lupien M, Wu T, Regan MM, Meyer CA, Carroll JS, Manrai AK, Jänne OA, Balk SP, Mehra R, Han B, Chinnaiyan AM, Rubin MA, True L, Fiorentino M, Fiore C, Loda M, Kantoff PW, Liu XS, Brown M** 2009 Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell 138:245–256
283. **Barka T** 1980 Biologically active polypeptides in submandibular glands. J Histochem Cytochem 28:836–859
284. **Chen YP, Chao J, Chao L** 1988 Molecular cloning and characterization of two rat renal kallikrein genes. Biochemistry 27:7189–7196
285. **Clements JA, Matheson BA, Wines DR, Brady JM, MacDonald RJ, Funder JW** 1988 Androgen dependence of specific kallikrein gene family members expressed in rat prostate. J Biol Chem 263:16132–16137
286. **Shai SY, Woodley-Miller C, Chao J, Chao L** 1989 Characterization of genes encoding rat tonin and a kallikrein-like serine protease. Biochemistry 28:5334–5343
287. **Shih HC, Chao L, Chao J** 1986 Age and hormonal dependence of tonin levels in rat submandibular gland as determined by a new direct radioimmunoassay. Biochem J 238:145–149
288. **van Leeuwen BH, Penschow JD, Coghlan JP, Richards RI** 1987 Cellular basis for the differential response of mouse kallikrein genes to hormonal induction. EMBO J 6:1705–1713
289. **Bhoola KD, Dorey G, Jones CW** 1973 The influence of androgens on enzymes (chymotrypsin-and trypsin-like proteases, renin, kallikrein and amylase) and on cellular structure of the mouse submaxillary gland. J Physiol 235:503–522
290. **Clements JA, Matheson BA, MacDonald RJ, Funder JW** 1990 Oestrogen administration and the expression of the kallikrein gene family in the rat submandibular gland. J Steroid Biochem 35:55–60
291. **Kurabuchi S, Hosoi K, Gresik EW** 2002 Developmental and androgenic regulation of the immunocytochemical distribution of mK1, a true tissue kallikrein, in the granular convoluted tubule of the mouse submandibular gland. J Histochem Cytochem 50:135–145
292. **van Leeuwen BH, Evans BA, Tregear GW, Richards RI** 1986 Mouse glandular kallikrein genes. Identification, structure, and expression of the renal kallikrein gene. J Biol Chem 261:5529–5535
293. **Berg T, Wassdal I, Sletten K** 1992 Immunohistochemical localization of rat submandibular gland esterase B (homologous to the RSKG-7 kallikrein gene) in relation to other serine proteases of the kallikrein family. J Histochem Cytochem 40:83–92
294. **Clements JA, Matheson BA, Funder JW** 1990 Tissue-specific developmental expression of the kallikrein gene family in the rat. J Biol Chem 265:1077–1081
295. **Eacker SM, Shima JE, Connolly CM, Sharma M, Holdcraft RW, Griswold MD, Braun RE** 2007 Transcriptional profiling of androgen receptor (AR) mutants suggests instructive and permissive roles of AR signaling in germ cell development. Mol Endocrinol 21:895–907
296. **Matsui H, Moriyama A, Takahashi T** 2000 Cloning and characterization of mouse klk27, a novel tissue kallikrein expressed in testicular Leydig cells and exhibiting chymotrypsin-like specificity. Eur J Biochem 267:6858–6865
297. **Matsui H, Takahashi T** 2001 Mouse testicular Leydig cells express Klk21, a tissue kallikrein that cleaves fibronectin and IGF-binding protein-3. Endocrinology 142:4918–4929
298. **Strauss L, Kallio J, Desai N, Pakarinen P, Miettinen T, Gylling H, Albrecht M, Mäkelä S, Mayerhofer A, Poutanen M** 2009 Increased exposure to estrogens disturbs maturation, steroidogenesis, and cholesterol homeostasis via estrogen receptor α in adult mouse Leydig cells. Endocrinology 150:2865–2872
299. **Petraki CD, Karavana VN, Diamandis EP** 2003 Human

kallikrein 13 expression in normal tissues: an immunohistochemical study. J Histochem Cytochem 51:493–501
300. **Yousef GM, Obiezu CV, Jung K, Stephan C, Scorilas A, Diamandis EP** 2002 Differential expression of Kallikrein gene 5 in cancerous and normal testicular tissues. Urology 60:714–718
301. **Frenette G, Dubé JY, Tremblay RR** 1983 Effect of castration and steroid treatments on the activity of some hydrolytic enzymes in dog prostate. Prostate 4:383–390
302. **Isaacs WB, Shaper JH** 1983 Isolation and characterization of the major androgen-dependent glycoprotein of canine prostatic fluid. J Biol Chem 258:6610–6615
303. **Chapdelaine P, Potvin C, Ho-Kim MA, Larouche L, Bellemare G, Tremblay RT, Dubé JY** 1988 Androgen regulation of canine prostatic arginine esterase mRNA using cloned cDNA. Mol Cell Endocrinol 56:63–70
304. **Juniewicz PE, Hoekstra SJ, Lemp BM, Barbolt TA, Devin JA, Gauthier E, Frenette G, Dube JY, Tremblay RR** 1993 Effect of combination treatment with zanoterone (WIN 49596), a steroidal androgen receptor antagonist, and finasteride (MK-906), a steroidal 5α-reductase inhibitor, on the prostate and testes of beagle dogs. Endocrinology 133: 904–913
305. **Gauthier ER, Chapdelaine P, Tremblay RR, Dubé JY** 1993 Transcriptional regulation of dog prostate arginine esterase gene by androgens. Mol Cell Endocrinol 94:155–163
306. **Berg T, Schøyen H** 1995 Immunohistochemical localization of rK9, an enzyme of the kallikrein gene family, in the rat ventral prostate. J Histochem Cytochem 43:61–65
307. **Winderickx J, Swinnen K, Van Dijck P, Verhoeven G, Heyns W** 1989 Kallikrein-related protease in the rat ventral prostate: cDNA cloning and androgen regulation. Mol Cell Endocrinol 62:217–226
308. **Goldfarb DA, Stein BS, Shamszadeh M, Petersen RO** 1986 Age-related changes in tissue levels of prostatic acid phosphatase and prostate specific antigen. J Urol 136:1266–1269
309. **Popek EJ, Tyson RW, Miller GJ, Caldwell SA** 1991 Prostate development in prune belly syndrome (PBS) and posterior urethral valves (PUV): etiology of PBS—lower urinary tract obstruction or primary mesenchymal defect? Pediatr Pathol 11:1–29
310. **Xia T, Blackburn WR, Gardner Jr WA** 1990 Fetal prostate growth and development. Pediatr Pathol 10:527–537
311. **Henttu P, Vihko P** 1989 cDNA coding for the entire human prostate specific antigen shows high homologies to the human tissue kallikrein genes. Biochem Biophys Res Commun 160:903–910
312. **Riegman PH, Vlietstra RJ, van der Korput HA, Romijn JC, Trapman J** 1991 Identification and androgen-regulated expression of two major human glandular kallikrein-1 (hGK-1) mRNA species. Mol Cell Endocrinol 76:181–190
313. **Riegman PH, Vlietstra RJ, van der Korput JA, Romijn JC, Trapman J** 1989 Characterization of the prostate-specific antigen gene: a novel human kallikrein-like gene. Biochem Biophys Res Commun 159:95–102
314. **Schedlich LJ, Bennetts BH, Morris BJ** 1987 Primary structure of a human glandular kallikrein gene. DNA 6:429–437
315. **Young CY, Montgomery BT, Andrews PE, Qui SD, Bilhartz DL, Tindall DJ** 1991 Hormonal regulation of prostate-specific antigen messenger RNA in human prostatic adenocarcinoma cell line LNCaP. Cancer Res 51:3748–3752
316. **Gleave ME, Hsieh JT, Wu HC, von Eschenbach AC, Chung LW** 1992 Serum prostate specific antigen levels in mice bearing human prostate LNCaP tumors are determined by tumor volume and endocrine and growth factors. Cancer Res 52:1598–1605
317. **Henttu P, Liao SS, Vihko P** 1992 Androgens up-regulate the human prostate-specific antigen messenger ribonucleic acid (mRNA), but down-regulate the prostatic acid phosphatase mRNA in the LNCaP cell line. Endocrinology 130: 766–772
318. **Wolf DA, Schulz P, Fittler F** 1992 Transcriptional regulation of prostate kallikrein-like genes by androgen. Mol Endocrinol 6:753–762
319. **Young CY, Andrews PE, Montgomery BT, Tindall DJ** 1992 Tissue-specific and hormonal regulation of human prostate-specific glandular kallikrein. Biochemistry 31:818–824
320. **Denmeade SR, Sokoll LJ, Dalrymple S, Rosen DM, Gady AM, Bruzek D, Ricklis RM, Isaacs JT** 2003 Dissociation between androgen responsiveness for malignant growth vs. expression of prostate specific differentiation markers PSA, hK2, and PSMA in human prostate cancer models. Prostate 54:249–257
321. **Mostaghel EA, Page ST, Lin DW, Fazli L, Coleman IM, True LD, Knudsen B, Hess DL, Nelson CC, Matsumoto AM, Bremner WJ, Gleave ME, Nelson PS** 2007 Intraprostatic androgens and androgen-regulated gene expression persist after testosterone suppression: therapeutic implications for castration-resistant prostate cancer. Cancer Res 67:5033–5041
322. **Schröder FH** 2008 Progress in understanding androgen-independent prostate cancer (AIPC): a review of potential endocrine-mediated mechanisms. Eur Urol 53:1129–1137
323. **Riegman PH, Vlietstra RJ, van der Korput JA, Brinkmann AO, Trapman J** 1991 The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol 5:1921–1930
324. **Murtha P, Tindall DJ, Young CY** 1993 Androgen induction of a human prostate-specific kallikrein, hKLK2: characterization of an androgen response element in the 5′ promoter region of the gene. Biochemistry 32:6459–6464
325. **Luke MC, Coffey DS** 1994 Human androgen receptor binding to the androgen response element of prostate specific antigen. J Androl 15:41–51
326. **Cleutjens KB, van Eekelen CC, van der Korput HA, Brinkmann AO, Trapman J** 1996 Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J Biol Chem 271:6379–6388
327. **Shan JD, Porvari K, Ruokonen M, Karhu A, Launonen V, Hedberg P, Oikarinen J, Vihko P** 1997 Steroid-involved transcriptional regulation of human genes encoding prostatic acid phosphatase, prostate-specific antigen, and prostate-specific glandular kallikrein. Endocrinology 138:3764–3770
328. **Sun Z, Pan J, Balk SP** 1997 Androgen receptor-associated protein complex binds upstream of the androgen-responsive elements in the promoters of human prostate-specific antigen and kallikrein 2 genes. Nucleic Acids Res 25: 3318–3325
329. **Zhang J, Zhang S, Murtha PE, Zhu W, Hou SS, Young CY** 1997 Identification of two novel cis-elements in the pro-

moter of the prostate-specific antigen gene that are required to enhance androgen receptor-mediated transactivation. Nucleic Acids Res 25:3143–3150

330. **Schuur ER, Henderson GA, Kmetec LA, Miller JD, Lamparski HG, Henderson DR** 1996 Prostate-specific antigen expression is regulated by an upstream enhancer. J Biol Chem 271:7043–7051
331. **Cleutjens KB, van der Korput HA, van Eekelen CC, van Rooij HC, Faber PW, Trapman J** 1997 An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate-specific antigen promoter. Mol Endocrinol 11:148–161
332. **Tsui KH, Wu L, Chang PL, Hsieh ML, Juang HH** 2004 Identifying the combination of the transcriptional regulatory sequences on prostate specific antigen and human glandular kallikrein genes. J Urol 172:2029–2034
333. **Wang C, Yeung F, Liu PC, Attar RM, Geng J, Chung LW, Gottardis M, Kao C** 2003 Identification of a novel transcription factor, GAGATA-binding protein, involved in androgen-mediated expression of prostate-specific antigen. J Biol Chem 278:32423–32430
334. **Zhang S, Murtha PE, Young CY** 1997 Defining a functional androgen responsive element in the 5′ far upstream flanking region of the prostate-specific antigen gene. Biochem Biophys Res Commun 231:784–788
335. **Huang W, Shostak Y, Tarr P, Sawyers C, Carey M** 1999 Cooperative assembly of androgen receptor into a nucleoprotein complex that regulates the prostate-specific antigen enhancer. J Biol Chem 274:25756–25768
336. **Shang Y, Myers M, Brown M** 2002 Formation of the androgen receptor transcription complex. Mol Cell 9:601–610
337. **Pang S, Dannull J, Kaboo R, Xie Y, Tso CL, Michel K, deKernion JB, Belldegrun AS** 1997 Identification of a positive regulatory element responsible for tissue-specific expression of prostate-specific antigen. Cancer Res 57:495–499
338. **Kang Z, Pirskanen A, Jänne OA, Palvimo JJ** 2002 Involvement of proteasome in the dynamic assembly of the androgen receptor transcription complex. J Biol Chem 277: 48366–48371
339. **Kang Z, Jänne OA, Palvimo JJ** 2004 Coregulator recruitment and histone modifications in transcriptional regulation by the androgen receptor. Mol Endocrinol 18:2633–2648
340. **Kim J, Jia L, Stallcup MR, Coetzee GA** 2005 The role of protein kinase A pathway and cAMP responsive element-binding protein in androgen receptor-mediated transcription at the prostate-specific antigen locus. J Mol Endocrinol 34:107–118
341. **Jia L, Kim J, Shen H, Clark PE, Tilley WD, Coetzee GA** 2003 Androgen receptor activity at the prostate specific antigen locus: steroidal and non-steroidal mechanisms. Mol Cancer Res 1:385–392
342. **Heemers HV, Tindall DJ** 2007 Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. Endocr Rev 28:778–808
343. **Shin S, Kim TD, Jin F, van Deursen JM, Dehm SM, Tindall DJ, Grande JP, Munz JM, Vasmatzis G, Janknecht R** 2009 Induction of prostatic intraepithelial neoplasia and modulation of androgen receptor by ETS variant 1/ETS-related protein 81. Cancer Res 69:8102–8110
344. **Sun C, Dobi A, Mohamed A, Li H, Thangapazham RL, Furusato B, Shaheduzzaman S, Tan SH, Vaidyanathan G, Whitman E, Hawksworth DJ, Chen Y, Nau M, Patel V, Vahey M, Gutkind JS, Sreenath T, Petrovics G, Sesterhenn IA, McLeod DG, Srivastava S** 2008 TMPRSS2-ERG fusion, a common genomic alteration in prostate cancer activates C-MYC and abrogates prostate epithelial differentiation. Oncogene 27:5348–5353
345. **Christensen SB, Skytte DM, Denmeade SR, Dionne C, Møller JV, Nissen P, Isaacs JT** 2009 A Trojan horse in drug development: targeting of thapsigargins towards prostate cancer cells. Anticancer Agents Med Chem 9:276–294
346. **Helo P, Cronin AM, Danila DC, Wenske S, Gonzalez-Espinoza R, Anand A, Koscuiszka M, Väänänen RM, Pettersson K, Chun FK, Steuber T, Huland H, Guillonneau BD, Eastham JA, Scardino PT, Fleisher M, Scher HI, Lilja H** 2009 Circulating prostate tumor cells detected by reverse transcription-PCR in men with localized or castration-refractory prostate cancer: concordance with CellSearch assay and association with bone metastases and with survival. Clin Chem 55:765–773
347. **Madan RA, Gulley JL, Arlen PM** 2006 PSA-based vaccines for the treatment of prostate cancer. Expert Rev Vaccines 5:199–209
348. **Latham JP, Searle PF, Mautner V, James ND** 2000 Prostate-specific antigen promoter/enhancer driven gene therapy for prostate cancer: construction and testing of a tissue-specific adenovirus vector. Cancer Res 60:334–341
349. **Taylor RA, Risbridger GP** 2008 The path toward identifying prostatic stem cells. Differentiation 76:671–681
350. **Cano P, Godoy A, Escamilla R, Dhir R, Onate SA** 2007 Stromal-epithelial cell interactions and androgen receptor-coregulator recruitment is altered in the tissue microenvironment of prostate cancer. Cancer Res 67:511–519
351. **Jia L, Shen HC, Wantroba M, Khalid O, Liang G, Wang Q, Gentzschein E, Pinski JK, Stanczyk FZ, Jones PA, Coetzee GA** 2006 Locus-wide chromatin remodeling and enhanced androgen receptor-mediated transcription in recurrent prostate tumor cells. Mol Cell Biol 26:7331–7341
352. **Perez-Stable CM, Pozas A, Roos BA** 2000 A role for GATA transcription factors in the androgen regulation of the prostate-specific antigen gene enhancer. Mol Cell Endocrinol 167:43–53
353. **Böhm M, Locke WJ, Sutherland RL, Kench JG, Henshall SM** 2009 A role for GATA-2 in transition to an aggressive phenotype in prostate cancer through modulation of key androgen-regulated genes. Oncogene 28:3847–3856
354. **Cramer SD, Chang BL, Rao A, Hawkins GA, Zheng SL, Wade WN, Cooke RT, Thomas LN, Bleecker ER, Catalona WJ, Sterling DA, Meyers DA, Ohar J, Xu J** 2003 Association between genetic polymorphisms in the prostate-specific antigen gene promoter and serum prostate-specific antigen levels. J Natl Cancer Inst 95:1044–1053
355. **Rao A, Chang BL, Hawkins G, Hu JJ, Rosser CJ, Hall MC, Meyers DA, Xu J, Cramer SD** 2003 Analysis of G/A polymorphism in the androgen response element I of the PSA gene and its interactions with the androgen receptor polymorphisms. Urology 61:864–869
356. **Shibahara T, Onishi T, Franco OE, Arima K, Nishikawa K, Yanagawa M, Hioki T, Watanabe M, Hirokawa Y,**

Shiraishi T, Sugimura Y 2006 A G/A polymorphism in the androgen response element 1 of prostate-specific antigen gene correlates with the response to androgen deprivation therapy in Japanese population. Anticancer Res 26:3365–3371

357. **Lai J, Kedda MA, Hinze K, Smith RL, Yaxley J, Spurdle AB, Morris CP, Harris J, Clements JA** 2007 PSA/KLK3 AREI promoter polymorphism alters androgen receptor binding and is associated with prostate cancer susceptibility. Carcinogenesis 28:1032–1039
358. **Chiang CH, Chen KK, Chang LS, Hong CJ** 2004 The impact of polymorphism on prostate specific antigen gene on the risk, tumor volume and pathological stage of prostate cancer. J Urol 171:1529–1532
359. **Cramer SD, Sun J, Zheng SL, Xu J, Peehl DM** 2008 Association of prostate-specific antigen promoter genotype with clinical and histopathologic features of prostate cancer. Cancer Epidemiol Biomarkers Prev 17:2451–2457
360. **dos Santos RM, de Jesus CM, Trindade Filho JC, Trindade JC, de Camargo JL, Rainho CA, Rogatto SR** 2008 PSA and androgen-related gene (AR, CYP17, and CYP19) polymorphisms and the risk of adenocarcinoma at prostate biopsy. DNA Cell Biol 27:497–503
361. **Medeiros R, Morais A, Vasconcelos A, Costa S, Pinto D, Oliveira J, Carvalho R, Lopes C** 2002 Linkage between polymorphisms in the prostate specific antigen ARE1 gene region, prostate cancer risk, and circulating tumor cells. Prostate 53:88–94
362. **Schatzl G, Marberger M, Remzi M, Grösser P, Unterlechner J, Haidinger G, Zidek T, Preyer M, Micksche M, Gsur A** 2005 Polymorphism in ARE-I region of prostate-specific antigen gene associated with low serum testosterone level and high-grade prostate cancer. Urology 65:1141–1145
363. **Xu J, Meyers DA, Sterling DA, Zheng SL, Catalona WJ, Cramer SD, Bleecker ER, Ohar J** 2002 Association studies of serum prostate-specific antigen levels and the genetic polymorphisms at the androgen receptor and prostate-specific antigen genes. Cancer Epidemiol Biomarkers Prev 11: 664–669
364. **Xue W, Irvine RA, Yu MC, Ross RK, Coetzee GA, Ingles SA** 2000 Susceptibility to prostate cancer: interaction between genotypes at the androgen receptor and prostate-specific antigen loci. Cancer Res 60:839–841
365. **Xue WM, Coetzee GA, Ross RK, Irvine R, Kolonel L, Henderson BE, Ingles SA** 2001 Genetic determinants of serum prostate-specific antigen levels in healthy men from a multiethnic cohort. Cancer Epidemiol Biomarkers Prev 10: 575–579
366. **Cicek MS, Liu X, Casey G, Witte JS** 2005 Role of androgen metabolism genes CYP1B1, PSA/KLK3, and CYP11α in prostate cancer risk and aggressiveness. Cancer Epidemiol Biomarkers Prev 14:2173–2177
367. **Gsur A, Preyer M, Haidinger G, Zidek T, Madersbacher S, Schatzl G, Marberger M, Vutuc C, Micksche M** 2002 Polymorphic CAG repeats in the androgen receptor gene, prostate-specific antigen polymorphism and prostate cancer risk. Carcinogenesis 23:1647–1651
368. **Binnie MC, Alexander FE, Heald C, Habib FK** 2005 Polymorphic forms of prostate specific antigen and their interaction with androgen receptor trinucleotide repeats in prostate cancer. Prostate 63:309–315
369. **Das K, Cheah PY, Lim PL, Zain YB, Stephanie FC, Zhao Y, Cheng C, Lau W** 2008 Shorter CAG repeats in androgen receptor and non-GG genotypes in prostate-specific antigen loci are associated with decreased risk of benign prostatic hyperplasia and prostate cancer. Cancer Lett 268: 340–347
370. **Mononen N, Seppälä EH, Duggal P, Autio V, Ikonen T, Ellonen P, Saharinen J, Saarela J, Vihinen M, Tammela TL, Kallioniemi O, Bailey-Wilson JE, Schleutker J** 2006 Profiling genetic variation along the androgen biosynthesis and metabolism pathways implicates several single nucleotide polymorphisms and their combinations as prostate cancer risk factors. Cancer Res 66:743–747
371. **Okugi H, Nakazato H, Matsui H, Ohtake N, Nakata S, Suzuki K** 2006 Association of the polymorphisms of genes involved in androgen metabolism and signaling pathways with familial prostate cancer risk in a Japanese population. Cancer Detect Prev 30:262–268
372. **Salinas CA, Austin MA, Ostrander EO, Stanford JL** 2005 Polymorphisms in the androgen receptor and the prostate-specific antigen genes and prostate cancer risk. Prostate 65:58–65
373. **Wang LZ, Sato K, Tsuchiya N, Yu JG, Ohyama C, Satoh S, Habuchi T, Ogawa O, Kato T** 2003 Polymorphisms in prostate-specific antigen (PSA) gene, risk of prostate cancer, and serum PSA levels in Japanese population. Cancer Lett 202:53–59
374. **Jesser C, Mucci L, Farmer D, Moon C, Li H, Gaziano JM, Stampfer M, Ma J, Kantoff P** 2008 Effects of G/A polymorphism, rs266882, in the androgen response element 1 of the PSA gene on prostate cancer risk, survival and circulating PSA levels. Br J Cancer 99:1743–1747
375. **Ahn J, Berndt SI, Wacholder S, Kraft P, Kibel AS, Yeager M, Albanes D, Giovannucci E, Stampfer MJ, Virtamo J, Thun MJ, Feigelson HS, Cancel-Tassin G, Cussenot O, Thomas G, Hunter DJ, Fraumeni Jr JF, Hoover RN, Chanock SJ, Hayes RB** 2008 Variation in KLK genes, prostate-specific antigen and risk of prostate cancer. Nat Genet 40:1032–1034; author reply 1035–1036
376. **Severi G, Hayes VM, Neufing P, Padilla EJ, Tilley WD, Eggleton SA, Morris HA, English DR, Southey MC, Hopper JL, Sutherland RL, Boyle P, Giles GG** 2006 Variants in the prostate-specific antigen (PSA) gene and prostate cancer risk, survival, and circulating PSA. Cancer Epidemiol Biomarkers Prev 15:1142–1147
377. **Sieh W, Edwards KL, Fitzpatrick AL, Srinouanprachanh SL, Farin FM, Monks SA, Kronmal RA, Eaton DL** 2006 Genetic susceptibility to prostate cancer: prostate-specific antigen and its interaction with the androgen receptor (United States). Cancer Causes Control 17:187–197
378. **Mitchell SH, Murtha PE, Zhang S, Zhu W, Young CY** 2000 An androgen response element mediates LNCaP cell dependent androgen induction of the hK2 gene. Mol Cell Endocrinol 168:89–99
379. **Yu DC, Sakamoto GT, Henderson DR** 1999 Identification of the transcriptional regulatory sequences of human kallikrein 2 and their use in the construction of calydon virus 764, an attenuated replication competent adenovirus for prostate cancer therapy. Cancer Res 59:1498–1504
380. **Wei C, Callahan BP, Turner MJ, Willis RA, Lord EM, Barth RK, Frelinger JG** 1998 Regulation of human prostate-specific antigen gene expression in transgenic mice:

evidence for an enhancer between the PSA and human glandular kallikrein-1 genes. Int J Mol Med 2:487–496
381. **Majumder S, Liu Y, Ford 3rd OH, Mohler JL, Whang YE** 2006 Involvement of arginine methyltransferase CARM1 in androgen receptor function and prostate cancer cell viability. Prostate 66:1292–1301
382. **Dubé JY, Chapdelaine P, Guérin S, Leclerc S, Rennie PS, Matusik RJ, Tremblay RR** 1995 Search for androgen response elements in the proximal promoter of the canine prostate arginine esterase gene. J Androl 16:304–311
383. **Dong Y, Bui LT, Odorico DM, Tan OL, Myers SA, Samaratunga H, Gardiner RA, Clements JA** 2005 Compartmentalized expression of kallikrein 4 (KLK4/hK4) isoforms in prostate cancer: nuclear, cytoplasmic and secreted forms. Endocr Relat Cancer 12:875–889
384. **Korkmaz KS, Korkmaz CG, Pretlow TG, Saatcioglu F** 2001 Distinctly different gene structure of KLK4/KLK-L1/prostase/ARM1 compared with other members of the kallikrein family: intracellular localization, alternative cDNA forms, and regulation by multiple hormones. DNA Cell Biol 20:435–445
385. **Lai J, Myers SA, Lawrence MG, Odorico DM, Clements JA** 2009 Direct progesterone receptor and indirect androgen receptor interactions with the kallikrein-related peptidase 4 gene promoter in breast and prostate cancer. Mol Cancer Res 7:129–141
386. **Xi Z, Klokk TI, Korkmaz K, Kurys P, Elbi C, Risberg B, Danielsen H, Loda M, Saatcioglu F** 2004 Kallikrein 4 is a predominantly nuclear protein and is overexpressed in prostate cancer. Cancer Res 64:2365–2370
387. **Kishi T, Grass L, Soosaipillai A, Shimizu-Okabe C, Diamandis EP** 2003 Human kallikrein 8: immunoassay development and identification in tissue extracts and biological fluids. Clin Chem 49:87–96
388. **Shaw JL, Diamandis EP** 2008 Regulation of human tissue kallikrein-related peptidase expression by steroid hormones in 32 cell lines. Biol Chem 389:1409–1419
389. **Yousef GM, Magklara A, Diamandis EP** 2000 KLK12 is a novel serine protease and a new member of the human kallikrein gene family-differential expression in breast cancer. Genomics 69:331–341
390. **Yousef GM, Scorilas A, Jung K, Ashworth LK, Diamandis EP** 2001 Molecular cloning of the human kallikrein 15 gene (KLK15). Up-regulation in prostate cancer. J Biol Chem 276:53–61
391. **Hall RE, Lee CS, Alexander IE, Shine J, Clarke CL, Sutherland RL** 1990 Steroid hormone receptor gene expression in human breast cancer cells: inverse relationship between oestrogen and glucocorticoid receptor messenger RNA levels. Int J Cancer 46:1081–1087
392. **Hsieh ML, Charlesworth MC, Goodmanson M, Zhang S, Seay T, Klee GG, Tindall DJ, Young CY** 1997 Expression of human prostate-specific glandular kallikrein protein (hK2) in the breast cancer cell line T47-D. Cancer Res 57:2651–2656
393. **Luo LY, Grass L, Diamandis EP** 2000 The normal epithelial cell-specific 1 (NES1) gene is up-regulated by steroid hormones in the breast carcinoma cell line BT-474. Anticancer Res 20:981–986
394. **Luo LY, Grass L, Diamandis EP** 2003 Steroid hormone regulation of the human kallikrein 10 (KLK10) gene in cancer cell lines and functional characterization of the KLK10 gene promoter. Clin Chim Acta 337:115–126
395. **Magklara A, Grass L, Diamandis EP** 2000 Differential steroid hormone regulation of human glandular kallikrein (hK2) and prostate-specific antigen (PSA) in breast cancer cell lines. Breast Cancer Res Treat 59:263–270
396. **Yousef GM, Fracchioli S, Scorilas A, Borgoño CA, Iskander L, Puopolo M, Massobrio M, Diamandis EP, Katsaros D** 2003 Steroid hormone regulation and prognostic value of the human kallikrein gene 14 in ovarian cancer. Am J Clin Pathol 119:346–355
397. **Yousef GM, Obiezu CV, Luo LY, Black MH, Diamandis EP** 1999 Prostase/KLK-L1 is a new member of the human kallikrein gene family, is expressed in prostate and breast tissues, and is hormonally regulated. Cancer Res 59:4252–4256
398. **Yousef GM, White NM, Kurlender L, Michael I, Memari N, Robb JD, Katsaros D, Stephan C, Jung K, Diamandis EP** 2004 The kallikrein gene 5 splice variant 2 is a new biomarker for breast and ovarian cancer. Tumour Biol 25:221–227
399. **Hall RE, Clements JA, Birrell SN, Tilley WD** 1998 Prostate-specific antigen and gross cystic disease fluid protein-15 are co-expressed in androgen receptor-positive breast tumours. Br J Cancer 78:360–365
400. **Paliouras M, Diamandis EP** 2008 Intracellular signaling pathways regulate hormone-dependent kallikrein gene expression. Tumour Biol 29:63–75
401. **Paliouras M, Diamandis EP** 2008 Androgens act synergistically to enhance estrogen-induced upregulation of human tissue kallikreins 10, 11, and 14 in breast cancer cells via a membrane bound androgen receptor. Mol Oncol 1:413–424
402. **Yu H, Diamandis EP, Zarghami N, Grass L** 1994 Induction of prostate specific antigen production by steroids and tamoxifen in breast cancer cell lines. Breast Cancer Res Treat 32:291–300
403. **Myers SA, Clements JA** 2001 Kallikrein 4 (KLK4), a new member of the human kallikrein gene family is up-regulated by estrogen and progesterone in the human endometrial cancer cell line, KLE. J Clin Endocrinol Metab 86:2323–2326
404. **Yousef GM, Chang A, Diamandis EP** 2000 Identification and characterization of KLK-L4, a new kallikrein-like gene that appears to be down-regulated in breast cancer tissues. J Biol Chem 275:11891–11898
405. **Yousef GM, Luo LY, Scherer SW, Sotiropoulou G, Diamandis EP** 1999 Molecular characterization of zyme/protease M/neurosin (PRSS9), a hormonally regulated kallikrein-like serine protease. Genomics 62:251–259
406. **Yousef GM, Scorilas A, Diamandis EP** 2000 Genomic organization, mapping, tissue expression, and hormonal regulation of trypsin-like serine protease (TLSP PRSS20), a new member of the human kallikrein gene family. Genomics 63:88–96
407. **Yousef GM, Scorilas A, Magklara A, Memari N, Ponzone R, Sismondi P, Biglia N, Abd Ellatif M, Diamandis EP** 2002 The androgen-regulated gene human kallikrein 15 (KLK15) is an independent and favourable prognostic marker for breast cancer. Br J Cancer 87:1294–1300
408. **Jeong JW, Lee KY, Kwak I, White LD, Hilsenbeck SG, Lydon JP, DeMayo FJ** 2005 Identification of murine uter-

ine genes regulated in a ligand-dependent manner by the progesterone receptor. Endocrinology 146:3490–3505
409. **Kulasingam V, Diamandis EP** 2007 Glucocorticoid receptor-mediated expression of kallikrein 10 in human breast cancer cell lines. Biol Chem 388:1113–1119
410. **Gross KL, Cidlowski JA** 2008 Tissue-specific glucocorticoid action: a family affair. Trends Endocrinol Metab 19: 331–339
411. **Lechi A, Covi G, Lechi C, Corgnati A, Arosio E, Zatti M, Scuro LA** 1978 Urinary kallikrein excretion and plasma renin activity in patients with essential hypertension and primary aldosteronism. Clin Sci Mol Med 55:51–55
412. **Margolius HS, Pisano JJ, Geller RG, Sjoerdsma A** 1971 Altered urinary kallikrein excretion in human hypertension. Lancet 298:1063–1065
413. **Lieberthal W, Oza NB, Arbeit L, Bernard DB, Levinsky NG** 1983 Effects of alterations in sodium and water metabolism on urinary excretion of active and inactive kallikrein in man. J Clin Endocrinol Metab 56:513–519
414. **Margolius HS, Horwitz D, Geller RG, Alexander RW, Gill Jr JR, Pisano JJ, Keiser HR** 1974 Urinary kallikrein excretion in normal man. Relationships to sodium intake and sodium-retaining steroids. Circ Res 35:812–819
415. **Bönner G, Autenrieth R, Marin-Grez M, Rascher W, Gross F** 1981 Effects of sodium loading, desoxycorticosterone acetate, and corticosterone on urinary kallikrein excretion. Horm Res 14:87–94
416. **Marin-Grez M** 1985 The influence of tetracosactide and adrenal steroids on renal kallikrein activity and urinary kallikrein excretion in rats. Biochem Pharmacol 34:4013–4017
417. **Fuller PJ, Clements JA, Nikolaidis I, Hiwatari M, Funder JW** 1986 Expression of the renal kallikrein gene in mineralocorticoid-treated and genetically hypertensive rats. J Hypertens 4:427–433
418. **Miller DH, Lindley JG, Margolius HS** 1985 Tissue kallikrein levels and synthesis rates are not changed by an acute physiological dose of aldosterone. Proc Soc Exp Biol Med 180:121–125
419. **Jaffa AA, Miller DH, Silva RH, Margolius HS, Mayfield RK** 1990 Regulation of renal kallikrein synthesis and activation by glucocorticoids. Kidney Int 38:212–218
420. **Rosewicz S, Detjen K, Logsdon CD, Chen LM, Chao J, Riecken EO** 1991 Glandular kallikrein gene expression is selectively down-regulated by glucocorticoids in pancreatic AR42J cells. Endocrinology 128:2216–2222
421. **Rosewicz S, Logsdon CD** 1989 Pancreatic kallikrein gene expression: effects of glucocorticoids in vivo and in vitro. Gastroenterology 97:1005–1010
422. **Powers CA, Nasjletti A** 1984 A major sex difference in kallikrein-like activity in the rat anterior pituitary. Endocrinology 114:1841–1844
423. **Vio CP, Roa JP, Silva R, Powers CA** 1990 Localization of immunoreactive glandular kallikrein in lactotrophs of the rat anterior pituitary. Neuroendocrinology 51:10–14
424. **Chao J, Chao L, Swain CC, Tsai J, Margolius HS** 1987 Tissue kallikrein in rat brain and pituitary: regional distribution and estrogen induction in the anterior pituitary. Endocrinology 120:475–482
425. **Clements JA, Fuller PJ, McNally M, Nikolaidis I, Funder JW** 1986 Estrogen regulation of kallikrein gene expression in the rat anterior pituitary. Endocrinology 119:268–273
426. **Fuller PJ, Clements JA, Whitfeld PL, Funder JW** 1985 Kallikrein gene expression in the rat anterior pituitary. Mol Cell Endocrinol 39:99–105
427. **Fuller PJ, Matheson BA, MacDonald RJ, Verity K, Clements JA** 1988 Kallikrein gene expression in estrogen-induced pituitary tumors. Mol Cell Endocrinol 60:225–232
428. **Hatala MA, Powers CA** 1988 Glandular kallikrein in estrogen-induced pituitary tumors: time course of induction and correlation with prolactin. Cancer Res 48:4158–4162
429. **Clements JA, Mukhtar A, Verity K, Pullar M, McNeill P, Cummins J, Fuller PJ** 1996 Kallikrein gene expression in human pituitary tissues. Clin Endocrinol (Oxf) 44:223–231
430. **Faurobert E, Albaladéjo V, Joly-Pharaboz MO, Girolami JP, André J** 1992 The control by estradiol of pituitary tumor and cell growth is not correlated with that of kallikrein gene expression. Cancer Lett 64:211–218
431. **Roa JP, Powers CA, Silva R, Vio CP** 1993 Cellular mechanisms of estrogen- and dopamine-induced control of glandular kallikrein in the anterior pituitary of the rat. Cell Tissue Res 274:421–427
432. **Murray SR, Chao J, Lin FK, Chao L** 1990 Kallikrein multigene families and the regulation of their expression. J Cardiovasc Pharmacol 15:S7–S16
433. **Clements J, Mukhtar A, Ehrlich A, Yap B** 1994 Glandular kallikrein gene expression in the human uterus. Braz J Med Biol Res 27:1855–1863
434. **Corthorn J, Valdés G** 1994 Variations in uterine kallikrein during cycle and early pregnancy in the rat. Biol Reprod 50:1261–1264
435. **Rajapakse S, Yamano N, Ogiwara K, Hirata K, Takahashi S, Takahashi T** 2007 Estrogen-dependent expression of the tissue kallikrein gene (Klk1) in the mouse uterus and its implications for endometrial tissue growth. Mol Reprod Dev 74:1053–1063
436. **Valdés G, Figueroa CD, Corthorn J** 1996 Temporospatial changes of kallikrein-like enzymes during the estrous cycle and pregnancy in the rat uterus. Biol Reprod 55:236–245
437. **Suzuki A, Urushitani H, Watanabe H, Sato T, Iguchi T, Kobayashi T, Ohta Y** 2007 Comparison of estrogen responsive genes in the mouse uterus, vagina and mammary gland. J Vet Med Sci 69:725–731
438. **Fernando SC, Buck JS, Ashworth MD, Ross JW, Geisert RD, DeSilva U** 2006 Porcine endometrial and conceptus tissue kallikrein 1, 4, 11, and 14 gene expression. Reproduction 132:939–947
439. **Corthorn J, Figueroa C, Valdés G** 1997 Estrogen and luminal stimulation of rat uterine kallikrein. Biol Reprod 56:1432–1438
440. **Vonnahme KA, Malayer JR, Spivey HO, Ford SP, Clutter A, Geisert RD** 1999 Detection of kallikrein gene expression and enzymatic activity in porcine endometrium during the estrous cycle and early pregnancy. Biol Reprod 61:1235–1241
441. **Jin H, Nagai N, Shigemasa K, Gu L, Tanimoto H, Yunokawa M, Ohama K, Kudo Y, O'Brien TJ** 2006 Expression of tumor-associated differentially expressed Gene-14 (TADG-14/KLK8) and its protein hK8 in uterine endometria and endometrial carcinomas. Tumour Biol 27:274–282
442. **Katsu Y, Takasu E, Iguchi T** 2002 Estrogen-independent expression of neuropsin, a serine protease in the vagina of mice exposed neonatally to diethylstilbestrol. Mol Cell Endocrinol 195:99–107

443. **Dong Y, Kaushal A, Bui L, Chu S, Fuller PJ, Nicklin J, Samaratunga H, Clements JA** 2001 Human kallikrein 4 (KLK4) is highly expressed in serous ovarian carcinomas. Clin Cancer Res 7:2363–2371
444. **Yousef GM, Borgoño CA, Michael IP, Davidian C, Stephan C, Jung K, Diamandis EP** 2004 Molecular cloning of a new gene which is differentially expressed in breast and prostate cancers. Tumour Biol 25:122–133
445. **Yousef GM, Scorilas A, Magklara A, Soosaipillai A, Diamandis EP** 2000 The KLK7 (PRSS6) gene, encoding for the stratum corneum chymotryptic enzyme is a new member of the human kallikrein gene family—genomic characterization, mapping, tissue expression and hormonal regulation. Gene 254:119–128
446. **Tong D, Schuster E, Seifert M, Czerwenka K, Leodolte S, Zeillinger R** 2002 Expression of estrogen receptor β isoforms in human breast cancer tissues and cell lines. Breast Cancer Res Treat 71:249–255
447. **Li X, Liu J, Wang Y, Zhang L, Ning L, Feng Y** 2009 Parallel underexpression of kallikrein 5 and kallikrein 7 mRNA in breast malignancies. Cancer Sci 100:601–607
448. **Bhat-Nakshatri P, Wang G, Appaiah H, Luktuke N, Carroll JS, Geistlinger TR, Brown M, Badve S, Liu Y, Nakshatri H** 2008 AKT alters genome-wide estrogen receptor α binding and impacts estrogen signaling in breast cancer. Mol Cell Biol 28:7487–7503
449. **Yousef GM, Borgoño CA, Scorilas A, Ponzone R, Biglia N, Iskander L, Polymeris ME, Roagna R, Sismondi P, Diamandis EP** 2002 Quantitative analysis of human kallikrein gene 14 expression in breast tumours indicates association with poor prognosis. Br J Cancer 87:1287–1293
450. **Yousef GM, Scorilas A, Nakamura T, Ellatif MA, Ponzone R, Biglia N, Maggiorotto F, Roagna R, Sismondi P, Diamandis EP** 2003 The prognostic value of the human kallikrein gene 9 (KLK9) in breast cancer. Breast Cancer Res Treat 78:149–158
451. **Black MH, Diamandis EP** 2000 The diagnostic and prognostic utility of prostate-specific antigen for diseases of the breast. Breast Cancer Res Treat 59:1–14
452. **Chang A, Yousef GM, Scorilas A, Grass L, Sismondi P, Ponzone R, Diamandis EP** 2002 Human kallikrein gene 13 (KLK13) expression by quantitative RT-PCR: an independent indicator of favourable prognosis in breast cancer. Br J Cancer 86:1457–1464
453. **Luo LY, Diamandis EP, Look MP, Soosaipillai AP, Foekens JA** 2002 Higher expression of human kallikrein 10 in breast cancer tissue predicts tamoxifen resistance. Br J Cancer 86: 1790–1796
454. **Yousef GM, Scorilas A, Kyriakopoulou LG, Rendl L, Diamandis M, Ponzone R, Biglia N, Giai M, Roagna R, Sismondi P, Diamandis EP** 2002 Human kallikrein gene 5 (KLK5) expression by quantitative PCR: an independent indicator of poor prognosis in breast cancer. Clin Chem 48:1241–1250
455. **Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, Clark L, Bayani N, Coppe JP, Tong F, Speed T, Spellman PT, DeVries S, Lapuk A, Wang NJ, Kuo WL, Stilwell JL, Pinkel D, Albertson DG, Waldman FM, McCormick F, Dickson RB, Johnson MD, Lippman M, Ethier S, Gazdar A, Gray JW** 2006 A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell 10:515–527
456. **Anisowicz A, Sotiropoulou G, Stenman G, Mok SC, Sager R** 1996 A novel protease homolog differentially expressed in breast and ovarian cancer. Mol Med 2:624–636
457. **Liu XL, Wazer DE, Watanabe K, Band V** 1996 Identification of a novel serine protease-like gene, the expression of which is down-regulated during breast cancer progression. Cancer Res 56:3371–3379
458. **Clements JA, Matheson BA, Funder JE** 1990 Tissue-specific regulation of the expression of rat kallikrein gene family members by thyroid hormone. Biochem J 267:745–750
459. **Chao J, Margolius HS** 1983 Differential effects of testosterone, thyroxine, and cortisol on rat submandibular gland versus renal kallikrein. Endocrinology 113:2221–2225
460. **Kurabuchi S, Gresik EW, Hosoi K** 2004 Additive and/or synergistic action (downregulation) of androgens and thyroid hormones on the cellular distribution and localization of a true tissue kallikrein, mK1, in the mouse submandibular gland. J Histochem Cytochem 52:1437–1446
461. **Zhu W, Young CY** 2001 Androgen-dependent transcriptional regulation of the prostate-specific antigen gene by thyroid hormone 3,5,3′-L-triiodothyronine. J Androl 22: 136–141
462. **Zhang S, Hsieh ML, Zhu W, Klee GG, Tindall DJ, Young CY** 1999 Interactive effects of triiodothyronine and androgens on prostate cell growth and gene expression. Endocrinology 140:1665–1671
463. **Lin R, Nagai Y, Sladek R, Bastien Y, Ho J, Petrecca K, Sotiropoulou G, Diamandis EP, Hudson TJ, White JH** 2002 Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D3 analog EB1089 signaling on cell proliferation, differentiation, and immune system regulation. Mol Endocrinol 16:1243–1256
464. **Pálmer HG, Sánchez-Carbayo M, Ordóñez-Morán P, Larriba MJ, Cordón-Cardó C, Muñoz A** 2003 Genetic signatures of differentiation induced by 1α,25-dihydroxyvitamin D3 in human colon cancer cells. Cancer Res 63:7799–7806
465. **Pampalakis G, Sotiropoulou G** 2006 Multiple mechanisms underlie the aberrant expression of the human kallikrein 6 gene in breast cancer. Biol Chem 387:773–782
466. **Ting HJ, Bao BY, Reeder JE, Messing EM, Lee YF** 2007 Increased expression of corepressors in aggressive androgen-independent prostate cancer cells results in loss of 1α,25-dihydroxyvitamin D3 responsiveness. Mol Cancer Res 5:967–980
467. **Wang TT, Tavera-Mendoza LE, Laperriere D, Libby E, MacLeod NB, Nagai Y, Bourdeau V, Konstorum A, Lallemant B, Zhang R, Mader S, White JH** 2005 Large-scale in silico and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes. Mol Endocrinol 19:2685–2695
468. **Lu J, Goldstein KM, Chen P, Huang S, Gelbert LM, Nagpal S** 2005 Transcriptional profiling of keratinocytes reveals a vitamin D-regulated epidermal differentiation network. J Invest Dermatol 124:778–785
469. **Hsieh T, Wu JM** 1997 Induction of apoptosis and altered nuclear/cytoplasmic distribution of the androgen receptor and prostate-specific antigen by 1α,25-dihydroxyvitamin D3 in androgen-responsive LNCaP cells. Biochem Biophys Res Commun 235:539–544
470. **Hsieh TY, Ng CY, Mallouh C, Tazaki H, Wu JM** 1996 Regulation of growth, PSA/PAP and androgen receptor

expression by 1 α,25-dihydroxyvitamin D3 in the androgen-dependent LNCaP cells. Biochem Biophys Res Commun 223:141–146
471. **Zhao XY, Ly LH, Peehl DM, Feldman D** 1997 1α,25-dihydroxyvitamin D3 actions in LNCaP human prostate cancer cells are androgen-dependent. Endocrinology 138: 3290–3298
472. **Zeng M, Zhang Y, Bhat I, Wazer DE, Band H, Band V** 2006 The human kallikrein 10 promoter contains a functional retinoid response element. Biol Chem 387:741–747
473. **Chuang KH, Lee YF, Lin WJ, Chu CY, Altuwaijri S, Wan YJ, Chang C** 2005 9-Cis-retinoic acid inhibits androgen receptor activity through activation of retinoid X receptor. Mol Endocrinol 19:1200–1212
474. **Young CY, Murtha PE, Andrews PE, Lindzey JK, Tindall DJ** 1994 Antagonism of androgen action in prostate tumor cells by retinoic acid. Prostate 25:39–45
475. **Lee JH, Gong H, Khadem S, Lu Y, Gao X, Li S, Zhang J, Xie W** 2008 Androgen deprivation by activating the liver X receptor. Endocrinology 149:3778–3788
476. **Chuu CP, Hiipakka RA, Kokontis JM, Fukuchi J, Chen RY, Liao S** 2006 Inhibition of tumor growth and progression of LNCaP prostate cancer cells in athymic mice by androgen and liver X receptor agonist. Cancer Res 66: 6482–6486
477. **Hisatake JI, Ikezoe T, Carey M, Holden S, Tomoyasu S, Koeffler HP** 2000 Down-regulation of prostate-specific antigen expression by ligands for peroxisome proliferator-activated receptor γ in human prostate cancer. Cancer Res 60:5494–5498
478. **Chintharlapalli S, Papineni S, Safe S** 2007 1,1-Bis(3′-indolyl)-1-(p-substitutedphenyl)methanes inhibit growth, induce apoptosis, and decrease the androgen receptor in LNCaP prostate cancer cells through peroxisome proliferator-activated receptor γ-independent pathways. Mol Pharmacol 71:558–569
479. **Yang CC, Wang YC, Wei S, Lin LF, Chen CS, Lee CC, Lin CC, Chen CS** 2007 Peroxisome proliferator-activated receptor γ-independent suppression of androgen receptor expression by troglitazone mechanism and pharmacologic exploitation. Cancer Res 67:3229–3238
480. **Teyssier C, Bianco S, Lanvin O, Vanacker JM** 2008 The orphan receptor ERRα interferes with steroid signaling. Nucleic Acids Res 36:5350–5361
481. **Montgomery BT, Young CY, Bilhartz DL, Andrews PE, Prescott JL, Thompson NF, Tindall DJ** 1992 Hormonal regulation of prostate-specific antigen (PSA) glycoprotein in the human prostatic adenocarcinoma cell line, LNCaP. Prostate 21:63–73
482. **Veldscholte J, Voorhorst-Ogink MM, Bolt-de Vries J, van Rooij HC, Trapman J, Mulder E** 1990 Unusual specificity of the androgen receptor in the human prostate tumor cell line LNCaP: high affinity for progestagenic and estrogenic steroids. Biochim Biophys Acta 1052:187–194
483. **Sikora MJ, Cordero KE, Larios JM, Johnson MD, Lippman ME, Rae JM** 2009 The androgen metabolite 5α-androstane-3β,17β-diol (3βAdiol) induces breast cancer growth via estrogen receptor: implications for aromatase inhibitor resistance. Breast Cancer Res Treat 115:289–296
484. **Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, Ettinger SL, Gleave ME, Nelson CC** 2008 Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res 68:6407–6415
485. **Björnström L, Sjöberg M** 2005 Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol 19: 833–842
486. **Heinlein CA, Chang C** 2004 Androgen receptor in prostate cancer. Endocr Rev 25:276–308
487. **Prassas I, Paliouras M, Datti A, Diamandis EP** 2008 High-throughput screening identifies cardiac glycosides as potent inhibitors of human tissue kallikrein expression: implications for cancer therapies. Clin Cancer Res 14:5778–5784
488. **Grosveld F, van Assendelft GB, Greaves DR, Kollias G** 1987 Position-independent, high-level expression of the human β-globin gene in transgenic mice. Cell 51:975–985
489. **Liang S, Moghimi B, Yang TP, Strouboulis J, Bungert J** 2008 Locus control region mediated regulation of adult β-globin gene expression. J Cell Biochem 105:9–16
490. **Cleutjens KB, van der Korput HA, Ehren-van Eekelen CC, Sikes RA, Fasciana C, Chung LW, Trapman J** 1997 A 6-kb promoter fragment mimics in transgenic mice the prostate-specific and androgen-regulated expression of the endogenous prostate-specific antigen gene in humans. Mol Endocrinol 11:1256–1265
491. **Wei C, Willis RA, Tilton BR, Looney RJ, Lord EM, Barth RK, Frelinger JG** 1997 Tissue-specific expression of the human prostate-specific antigen gene in transgenic mice: implications for tolerance and immunotherapy. Proc Natl Acad Sci USA 94:6369–6374
492. **Kroon E, MacDonald RJ, Hammer RE** 1997 The transcriptional regulatory strategy of the rat tissue kallikrein gene family. Genes Funct 1:309–319
493. **Abrahamsson PA, Lilja H, Falkmer S, Wadström LB** 1988 Immunohistochemical distribution of the three predominant secretory proteins in the parenchyma of hyperplastic and neoplastic prostate glands. Prostate 12:39–46
494. **Darson MF, Pacelli A, Roche P, Rittenhouse HG, Wolfert RL, Young CY, Klee GG, Tindall DJ, Bostwick DG** 1997 Human glandular kallikrein 2 (hK2) expression in prostatic intraepithelial neoplasia and adenocarcinoma: a novel prostate cancer marker. Urology 49:857–862
495. **Lucas JM, True L, Hawley S, Matsumura M, Morrissey C, Vessella R, Nelson PS** 2008 The androgen-regulated type II serine protease TMPRSS2 is differentially expressed and mislocalized in prostate adenocarcinoma. J Pathol 215: 118–125
496. **Qiu SD, Young CY, Bilhartz DL, Prescott JL, Farrow GM, He WW, Tindall DJ** 1990 In situ hybridization of prostate-specific antigen mRNA in human prostate. J Urol 144: 1550–1556
497. **Sinha AA, Gleason DF, Wilson MJ, Wick MR, Reddy PK, Blackard CE** 1988 Relationship of prostatic acid phosphatase localization in human prostate by a monoclonal antibody with the Gleason grading system. Prostate 13:1–15
498. **Richardson AL, Wang ZC, De Nicolo A, Lu X, Brown M, Miron A, Liao X, Iglehart JD, Livingston DM, Ganesan S** 2006 X Chromosomal abnormalities in basal-like human breast cancer. Cancer Cell 9:121–132