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Mini-review

Human tissue kallikrein gene family: applications in cancer

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Abstract

Human tissue kallikrein genes, located on the long arm of chromosome 19, are a subgroup of the serine protease family of proteolytic enzymes. Initially thought to consist of three members, the human kallikrein locus has now been extended and includes 15 tandemly located genes. These genes, and their protein products, share a high degree of homology and are expressed in a wide array of tissues, mainly those that are under steroid hormone control. PSA (hK3) is one of the human kallikreins, and is the most useful tumor marker for prostate cancer screening, diagnosis, prognosis and monitoring. hK2, another prostate-specific kallikrein, has also been proposed as a complementary prostate cancer biomarker. In the past 5 years, the newly discovered kallikreins (KLK4–KLK15) have been associated with several types of cancer. For example, hK4, hK5, hK6, hK7, hK8, hK10, hK11, hK13 and hK14 are emerging biomarkers for ovarian, breast, prostate and testicular cancer. New evidence raises the possibility that some kallikreins are directly involved with cancer progression. We here review the evidence linking kallikreins and cancer and their applicability as novel biomarkers for cancer diagnosis and management.

Keywords: Kallikreins; Serine proteases; Cancer; Biomarkers; Prognosis; Diagnosis; Tumor invasion and metastasis

1. Introduction

Serine proteases are the most intensely studied group of proteolytic enzymes; in fact, they are the most intensely studied proteins in biology [1]. They play key roles in a diverse array of physiological processes such as digestion, blood coagulation and fibrionalysis, cellular and humoral immunity, fertilization and embryonic development [2]. To-date, there are 178 human serine proteases, accounting for 32% of the total proteases encoded by the human genome [3,4]. Fuelled by renewed interest in the connection of the human degradome and cancer [5], there have been important advances in the identification of novel serine proteases. The discovery of all 15 members of the human tissue kallikrein family of genes, belonging to the S1A subfamily of serine proteases, added an interesting piece to the puzzle. Originally thought to consist of only three genes, human kallikrein 1 (*KLK1*), human kallikrein 2 (*KLK2*) and prostate-specific antigen (human kallikrein 3; *KLK3*) [6,7], homology studies with mice and

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rat loci led to the hypothesis that other members of this family do exist in humans [8,9]. Sequencing information from the Human Genome Project [10] facilitated data mining strategies and led to the realization that some already discovered genes, namely NES1 [11], zyme/Protease M/neurosin [12–14], neuropsin/TADG-14 [15,16], trypsin-like serine protease (TLSP)/hippostasin [17,18], human stratum corneum tryptic [19] and chymotryptic [20] enzymes (HSCTE and HSCCE, respectively), mapped to the same loci as the three classical kallikreins [21, 22]. Interestingly, most of the former studies that brought about the discovery of these kallikreins were directed towards finding cancer-related genes. Study of the locus eventually led to the cloning of seven additional, formerly unknown serine proteases, increasing the human tissue kallikrein genes to 15 [23] plus an additional pseudogene ΨKLK1 [24].

The genes *KLK1*, *KLK15*, *KLK3*, *KLK2*, *ΨKLK1* and *KLK4–KLK14* are tandemly arranged from centromere to telomere, respectively, without any intervening non-kallikrein genes. The locus consists of approximately 300 kb of genomic sequence at cytogenic region q13.4 of chromosome 19, now known to be the richest gene-containing chromosome [25]. In fact, the kallikrein genes represent the largest contiguous cluster of proteases in the human genome [4,26]. The kallikrein genes range from 4.4 to 10.5 kb in length, and with the exception of *KLK2* and *KLK3*, all genes are transcribed from telomere to centromere [27]. The locus is flanked by the testicular acid phosphatase (*ACPT*) [28] on the centromeric end, and by the cancer-associated gene (*CAG*) [29] and a member of the sialic acid-binding Ig-like lectin family, *Siglec-9* [30] on the telomeric end. An extended kallikrein gene family has recently been characterized in mouse and rat; in these species, human *KLK4–KLK14* and *KLK1* orthologs exist, with conserved orientation and relative localization but *KLK2* and *KLK3* are unique to higher order primates [31–33]. Evolutionary relationships of the human, chimpanzee, rat and mouse kallikreins have recently been reviewed [31–36]. Besides co-localization to chromosome 19q13.4, the human kallikrein genes share many common characteristics, including exon/intron organization (coding sequence spanning 5 exons, conservation in the respective coding exon lengths, conserved intronic phases, presence of 5′ and 3′ untranslated regions and in most one or more 5′ untranslated exons), conserved amino acid residues, and as in all serine proteases, the location of the three catalytic residues His, Asp, Ser [37].

As reviewed recently, the 15 human KLK genes give rise to 15 transcripts, as well as to numerous mRNA forms, that are alternatively spliced and/or have alternate transcription start sites, providing at least another 70 mRNA variants that are predicted to encode truncated proteins [34].

KLK transcripts code for single chain serine protease pre-proenzymes of 248–293 amino acids [38]. At the protein level, these hK proteins have fully conserved amino acids around the catalytic residues, as well as overall amino acid sequence identity of 40–80% with by far the highest degree of homology occurring between hK2 and hK3 [38]. The presence of a signal peptide motif of 16–57 amino acids is also a shared characteristic so that all hK pro-forms (zymogens) are expected to be secreted. With the exception of hK4, all kallikreins have a pro-peptide ending in Lys or Arg, suggesting that these zymogens are activated by enzymes with trypsin-like activity. The presence of an Asp residue at the bottom of their binding pocket (hK1–hK2, hK4–hK6, hK8, hK10–hK14) or Glu (hK15) indicates that most kallikreins have trypsin-like substrate specificity while the presence of Ser for hK3, Asn for hK7 and Gly for hK9 at the same location predict chymotrypsin-like activity [38]. All members have 10–12 cysteine residues, predicted to form 5–6 disulfide bonds [38] that are thought to play a significant role of keeping the protein in its correctly folded conformation [39]. However, only the classical kallikreins (*KLK1*, *KLK2*, *KLK3*) have a loop formed by 9–11 amino acids preceding the Asp catalytic residue; this loop has traditionally been thought to confer kininogenase activity [40]. However, hK1 is the only kallikrein with such activity and the role of this loop is now less defined. In fact, apart from plasma kallikrein, none of the tissue kallikreins save one (hK1) have appreciable kininogenase activity. Indeed, discovery of these novel kallikrein members has led to the modification of the term ‘kallikrein’ [41].

Unlike plasma kallikrein, whose gene is located on chromosome 4 and its expression is restricted to the liver, each tissue kallikrein is expressed in several different organs. KLK1 has its highest expression in

the pancreas, kidney and salivary glands [42,43]. Northern blotting studies have also demonstrated expression of KLK2 [44], KLK3 [45,46] and to a lesser extent KLK4 [47,48] exclusively in the human prostate. For KLK2 and KLK3, prostate tissue specificity has also been confirmed at the protein level [46,49–51] as well as the relative specificity of KLK1 for kidney [52]. In contrast, most of the more recently discovered tissue kallikreins do not exhibit tissue specificity; rather, they are co-expressed in various organs such as CNS (KLK5–KLK9, KLK14), skin (KLK5–KLK7, KLK8, KLK11), breast (KLK5–KLK7, KLK10, KLK11, KLK13) and almost all in the salivary gland, as demonstrated by RT-PCR studies. The presence of hK proteins has been confirmed in various biological fluids (reviewed in Ref. [53]) lending proof to the secreted nature of these proteins.

Hormonal regulation of the expression of many of the human tissue kallikreins is well documented. An exception to this is KLK1; though there is some association of its expression in human endometrial tissue with estrogen levels of the menstrual cycle [54], its regulation by steroid hormones in uterus, kidney, or salivary gland has not been proven [55,56]. In contrast, hormonal control of the expression of the other two classical kallikreins, KLK2 and KLK3, has been extensively studied and demonstrated in prostate cancer cell lines [57–60] as well as in breast cancer cell lines, where their expression is up-regulated in response to androgens and progestins [61], directly attributable to the hormone response elements present in their promoters [62–67]. For these two kallikreins, regulation at the protein level by steroids has also been shown [68]. Their androgen receptor-mediated hormonal control is modulated by other post-transcriptional events such as mRNA stabilization [69] as well as by other, cell-line-specific factors [70]. Though preliminary data suggest responsiveness of many of the newly discovered tissue kallikreins to one or multiple steroid hormones, much less is known about the underlying mechanism, as detailed promoter characterization of these novel kallikreins has not been done.

In light of the fact that hK3 (PSA) and hK2 have already found clinical utility in screening, diagnosis and prognosis of prostate cancer [40], as well as in other cancer types [71], the applicability of newly discovered kallikreins KLK4–KLK15 and their respective proteins in this field have only recently been explored. The next section will focus on summarizing the data on clinical applications of the newly discovered kallikreins in various cancers. We will also present a brief section on the possible roles these kallikreins in cancer pathology, as well as on emerging evidence for their association with some non-cancerous pathological conditions.

2. Kallikreins as cancer biomarkers

2.1. Ovarian cancer

Ovarian cancer remains the second most common malignancy among gynecological cancers in North America but it is the most lethal [72]. Currently, there is no screening strategy for early detection and due to the insidious nature of this disease, most cases are diagnosed at a late stage where treatment options are limited and less effective. Since the last 50 years, no novel strategies were found to diagnose early and combat this malignancy [72], continued research in this area is of primary importance. Among the newly discovered human kallikreins, many have shown utility as ovarian cancer biomarkers at the mRNA and protein levels (Table 1).

At the mRNA level, KLK4 has been shown to be expressed in serous ovarian carcinomas but not in normal ovarian tissues [73]. Moreover, in an RT-PCR study of KLK4 expression in 147 malignant ovarian cancer tissues, KLK4 was shown to be an independent indicator of poor prognostic outcome in grade 1 and grade 2 tumors, suggesting that KLK4 expression is associated with more aggressive forms of ovarian cancer [74]. A similar expression pattern was observed for KLK5 in patents with grade 1 and grade 2 ovarian cancer [75]. This finding was further supported by the demonstration of higher expression in late-stage serous carcinomas in comparison to benign and normal ovarian tissues [76]. This study has also uncovered the presence of a novel KLK5 alternate transcript, characterized by a short 5′ untranslated region, which was highly expressed in OVCAR-3 and PEO1 ovarian carcinoma cell lines, in contrast to low expression in normal ovarian epithelial cells [76]. In this study, a novel transcript of KLK7 with a long 3′ untranslated region was also found to be

Table 1
Applications of human tissue kallikreins in ovarian cancer

Kallikrein (KLK mRNA/hK protein)	Tissue/biological fluid	Biomarker status	References
KLK4	mRNA from normal and cancerous ovarian tissues	Unfavorable prognostic marker	[73,74]
hK4	Cancerous ovarian tissues	Marker of resistance to paclitaxel therapy	[97]
KLK5/hK5	mRNA and cytosols from normal and cancerous ovarian tissues	Unfavorable prognostic marker	[75]
hK5	Cytosols of ovarian cancer tissues	Unfavorable prognostic marker	[76,99]
	Serum and tissue from ovarian cancer patients	Marker of diagnosis	[98]
KLK6/hK6	mRNA and protein extracts from normal, benign and cancerous ovarian tissues	Unfavorable prognostic marker	[78]
hK6	Cytosols of ovarian cancer tissues	Unfavorable prognostic marker	[102]
	Serum of ovarian cancer patients	Marker of diagnosis, prognosis and monitoring	[100,101]
KLK7	mRNA from cancerous ovarian tissues	Unfavorable prognostic marker	[77]
KLK7/hK7	mRNA and protein extracts from normal and cancerous ovarian tissue	Unfavorable prognostic marker	[76] [204]
KLK8	mRNA from ovarian cancer tissues	Favourable prognostic marker	[81,82]
hK8	Serum and tissue from ovarian cancer patients	Marker of diagnosis, prognosis and monitoring	[103]
KLK9	mRNA from ovarian cancer tissues	Favorable prognostic marker	[83]
hK10	Serum and tissue from ovarian cancer patients	Marker of diagnosis, prognosis and monitoring	[107,109]
	Cytosols from normal, benign and cancerous tissues	Unfavorable prognostic marker	[107]
KLK11	mRNA from normal and cancerous ovarian tissues	Unfavorable prognostic marker	[85]
hK11	Cytosols of ovarian cancer tissues	Favorable prognostic marker	[111]
	Serum from ovarian cancer patients	Marker for diagnosis	[140]
hK13	Cytosols of ovarian cancer tissues	Favorable prognostic marker	[110]
KLK14	mRNA from normal, benign and cancerous ovarian tissues	Favorable prognostic marker	[87]
hK14	Serum and tissue from ovarian cancer patients	Marker of diagnosis	[123]
KLK15	mRNA from benign and cancerous ovarian tissues	Unfavorable prognostic marker	[88]

highly expressed in the above ovarian cancer cell lines and displayed a similar pattern of expression in cancer versus normal and benign tissues as KLK5. Furthermore, this work showed a similar expression pattern at the protein level for hK5 and hK7 in the cancerous tissues using Western blotting and immunohistochemistry [76]. In a separate study, using quantitative RT-PCR for the analysis of 125 ovarian tumors for their KLK7 mRNA content, KLK7 negativity in the tumor tissue was associated with longer disease-free survival rates in patients than in those with KLK7-positive tumors [77]. KLK7 expression levels were significantly higher in grade 3 than in lower grade tumors and KLK7 expression was found to be a prognostic marker for disease-free and overall survival in patients with low-grade tumors.

KLK6 (Protease M), one of the most promising ovarian cancer biomarkers among the kallikreins, was discovered by differential display as a novel serine protease with a strong expression pattern in ovarian cancer cell lines and primary ovarian carcinomas [12]. Later studies examined KLK6 expression in

44 ovarian tumors; upon comparison to 10 normal ovarian tissues, it was observed that KLK6 was over-expressed more frequently in the tumors suggesting a connection between KLK6 and invasiveness and promotion of ovarian cancer growth [78]. In a separate study, KLK6 over-expression by real-time quantitative RT-PCR was also demonstrated; Southern blotting results suggested that gene amplification may be a possible explanation for these results [79]. In a recent microarray study comparing the expression of 12,533 genes of 10 primary and two established cell lines of ovarian serous papillary carcinoma to those of five cell lines of normal ovarian epithelial origin, KLK6 (along with KLK10) was among the most highly over-expressed genes in the ovarian cancer cell lines [80]. In contrast, using RT-PCR, KLK8 expression was shown to be a favorable, independent prognostic indicator in ovarian cancer. Ovarian cancer patients expressing higher levels of KLK8 in their tumors had disease of a lower grade, had longer overall survival and relapsed less frequently [81]. The same study also found the presence of two KLK8 transcript variants that were expressed very frequently in ovarian tumor tissues in comparison to normal tissues. In another study, semi-quantitative RT-PCR also found a similar differential expression of KLK8 in ovarian tumors versus normal ovaries and mRNA expression was shown to correlate well with hK8 protein expression [82]. KLK9 is another favorable prognostic indicator in ovarian cancer patients; a semi-quantitative RT-PCR study on a sample population of 168 consecutive ovarian tumors, KLK9 expression proved to be an independent predictor of longer progression-free survival in a subset of patients with early stage and low-grade ovarian tumors [83]. Here, the protective nature of KLK9 expression was also shown by the fact that its mRNA levels tended to be significantly higher in patients with optimal debulking and with early (stage I or II) disease.

Another highly promising kallikrein, KLK10, has recently proven its value in ovarian cancer at the mRNA level, as microarray data comparing KLK10 expression in primary serous ovarian tumors, normal ovarian epithelia, and ovarian cancer cell lines revealed much higher expression in 91.4% of the primary serous ovarian tumors than in normal ovarian epithelium [84]. Findings from this transcriptional profiling were complemented with Northern blotting and in situ hybridization and these findings support previous studies on hK10 protein in ovarian cancer (see below). Another microarray study also confirmed over-expression of KLK10 in serous ovarian cancer-derived cell lines over normal ovarian epithelium-derived cell lines [80]. KLK11 has only recently been shown to be implicated in ovarian cancer: in a study using 64 cancerous and 10 normal ovarian tissues of epithelial origin, quantitative RT-PCR analysis showed mean KLK11 mRNA levels to be significantly higher in cancer than in normal ovarian tissues [85]. While no significant associations were found between KLK11 and clinical stage, histological type and grade, high KLK11 expression and advanced clinical stage did prove to be independent indicators of shorter overall patient survival. For KLK14, preliminary results indicated that it might be down-regulated in various malignancies, among them ovarian cancer [86]. The independent favorable nature of KLK14 as a prognostic marker in ovarian cancer was demonstrated in a later study that focused on KLK14 mRNA expression profiling using semi-quantitative RT-PCR on 155 consecutive ovarian cancer tissue samples [87]. This study demonstrated that KLK14 mRNA tended to be significantly higher in patients with optimal debulking, early stage disease and good response to chemotherapy; patients with KLK14-positive tumors had longer progression-free and overall survival than those with KLK14-negative tumors. In fact, a stepwise decrease was noted in KLK14 mRNA levels in normal, benign, and cancerous tissues, suggesting that KLK14 expression correlates with disease progression of the disease. In contrast, high KLK15 expression in patients with epithelial ovarian cancer proved to be an independent, unfavorable marker of prognosis [88]. Univariate and multivariate analyses showed that KLK15 expression predicted reduced progression-free and overall survival, with KLK15 levels being significantly higher in cancerous tissues than in benign tumors. Lastly, recent in silico studies utilizing serial analysis of gene expression libraries and gene-specific tags for each KLK indicated that KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, and KLK14 are up-regulated in 10 ovarian cancer libraries when compared to two normal ovarian tissue libraries [89]. Immunoassays, available for all except KLK11, confirmed over-expression for

the six kallikreins with a stepwise increase from normal to benign to cancerous tissues; the strong correlation of these six kallikreins at the protein level points to the possibility of existence of a common regulatory mechanism controlling transcription [89]. Furthermore, in several independent, large-scale gene expression microarray studies aiming to find markers with screening potential, many of the kallikreins showed significant differential expression in ovarian cancer over normal ovarian tissue [90–92].

While mRNA studies necessitate availability of tumor tissue, protein measurements in bodily fluids such as serum, urine or ascitic fluid are much preferred. CA125 is one such serum tumor marker. Although its levels may indicate the presence of ovarian cancer, not all ovarian cancer patients have elevated CA125, limiting its use for monitoring treatment response and detecting relapse after surgery [93]. A search for novel diagnostic tools, especially serum biomarkers aiming to detect the disease at earlier stages, should be at the forefront of clinical research. Due to the connection between serine proteases with potential to degrade connective tissue and thus favor tumor cell invasion [94,95], some kallikreins have been evaluated not only as potential diagnostic markers at the protein level but also as extracellular matrix-degrading enzymes [96].

Immunohistochemical analysis of tumor samples from paclitaxel-treated ovarian cancer patients showed that the intensity of staining was significantly associated with unresponsiveness to this agent as well as with higher histological grade [97]. Initial studies for hK5, using highly sensitive immunofluorometric methods, have shown that higher hK5 serum levels were found in 69% of patients, compared to undetectable levels in controls [98]. This very same study noted higher hK5 levels in ascites of patients with metastatic ovarian cancer. In a similar study that focused on hK5 protein levels in ovarian cancer cytosolic extracts, for patients with grade 3 tumors and with optimal debulking, this kallikrein was an independent factor of poor prognostic outcome [99]. This study confirmed expression patterns found in earlier studies, and pointed to the fact that hK5 was associated with more aggressive forms of epithelial ovarian carcinoma.

A promising finding was that hK6, already a prognostic marker at the mRNA level, was significantly increased in serum of ovarian cancer patients but not in serum of patients with other malignancies, suggesting that hK6 is an ovarian cancer-specific serum biomarker [100]. As hK6 levels had a positive correlation with serum CA125 in patients monitored after surgery, this marker may find applications in monitoring recurrence of cancer. Subsequently, this study was expanded to include 97 normal, healthy females, 141 women with benign abdominal diseases and 146 women with histologically confirmed primary ovarian carcinoma to assess diagnostic parameters by serum of hK6 immunofluorometric analysis [101]. With no difference in mean serum hK6 between healthy and benign disease patients, mean pre-surgical serum hK6 in ovarian cancer patients was significantly elevated. Moreover, in 68% of the patients mean serum hK6 decreased significantly post-surgery. Pre-operative serum hK6 levels were a powerful independent predictor of both relapse-free and overall survival. Serum hK6, in combination with serum CA125, may be of value in ovarian cancer diagnosis and prognosis, since the combination of these two markers was superior to each one alone [101]. In another study, using the above hK6-specific immunoassay as well as immunohistochemistry to investigate hK6 levels in ovarian cancer tissue, hK6 positivity in the tumors was an independent indicator of poor prognosis for patients with low-grade (grade 1 and 2) tumors and those with optimal debulking [102]. An immunohistochemical study assessing hK6 expression patterns in ovarian tumor samples, was recently published [79]. Here, hK6 was shown to be elevated in 67 of 80 ovarian tumor samples, with a statistically significant difference in hK6 between normal/benign versus borderline/invasive tumors. As KLK6 expression was found in many of the low-grade and early stage samples, with elevated hK6 in benign epithelia coexisting with borderline and invasive tissues, it is likely that hK6 over-expression is an early phenomenon in ovarian carcinoma development [79] and as such, supports its possible utility for early detection of this disease.

For another kallikrein, hK8, elevated serum levels were observed in 24 of 40 ovarian cancer patients. High levels (up to 1 mg/l) of this protein were found in ascites fluid of ovarian cancer patients [103,104]. Higher hK8 in ascites was associated with better progression-free survival, and there was a positive

correlation between serum CA125 and hK8 levels in both ascites and serum. An immunohistochemical study assessed hK8 in 74 ovarian adenocarcinomas versus six normal ovarian tissues. hK8 was detected in 51.4% of the adenocarcinomas [82]. In these samples, a significantly higher frequency of hK8 positivity was observed in tumors of early stage compared to those of advanced stage. hK8 expression was associated with favorable patient survival outcome in univariate but not in multivariate analysis. This study also demonstrated that hK8 levels changed during the course of ovarian cancer progression, with increases in hK8 as the cancer develops, followed by down-regulation of hK8 during the metastatic phase of the disease.

hK10 was initially discovered as a potential tumor suppressor in breast cancer with absence of mRNA expression [11,105,106]. In ovarian cancer, it has the opposite pattern of expression, as discussed above. Over-expression was also seen at the protein level of primary ovarian tissue lysates, as indicated by western blotting; these data are complemented by in situ hybridization showing KLK10 mRNA over-expression in tumor tissue versus normal epithelial or stromal samples [84]. These results confirm previous findings, where hK10 was quantified by an hK10-specific immunoassay, to show prognostic value of hK10 levels in 182 ovarian cancer tissue extracts, using eight benign and eight normal ovarian tissue extracts as controls [107]. This study indicated that high concentration of hK10 was significantly associated with serous histotype, with advanced stage, large residual tumor size, and suboptimal debulking. Multivariate analyses demonstrated that for stage III and IV patients, hK10 was an independent indicator of reduced overall and progression-free survival. hK10 levels were also shown to be elevated in the serum of 56% of ovarian cancer patients but not in healthy controls, except for an elevation in 15% of patients with gastrointestinal cancer. It seems that hK10 is specific for ovarian cancer [108]. Thus, this study showed possible use of hK10 as a new serological marker for diagnosis and monitoring in ovarian cancer. This was further explored in a larger study [109]. High levels of serum hK10 were strongly associated with serous epithelial cancer type, late stage, large residual tumor size, and suboptimal debulking as well as with advanced grade, and unresponsiveness to chemotherapy. For early cancer detection, in stage I or II patients, the use of serum hK10 levels, in combination with CA125, yielded a 21% increase in sensitivity (at 90% specificity) over the sensitivity obtained when CA125 was used alone [109]. In two separate studies, levels of hK11 and hK13 have also been investigated in tumor extracts and found to be independent indicators of favorable outcome for overall survival (hK11 and hK13) and for progression-free survival (hK13) [110,111]. In these studies, hK11/hK13 positivity in the tumors was more frequently associated with early stage (I and II) disease, complete/partial response to chemotherapy (hK11) and optimal debulking (hK13). While these results are promising, these two kallikreins are yet to be better characterized as serum markers of ovarian cancer. It would also be of interest to evaluate whether the already established kallikrein serum biomarkers, when used together and/or in combination with CA125, can improve diagnosis, prognosis and monitoring, especially for early stage ovarian cancer.

2.2. Breast cancer

After lung cancer, breast cancer is the second leading cause of cancer-related deaths in North America. Although death rates have declined somewhat over the last decade due to earlier detection through mammography, many cancers are missed. Diagnosing this disease in its early stage is important, as 5-year survival rates drop from 97% for localized breast cancer to 79% for regionally spread breast cancer and to 23% for breast cancer with distant metastasis [72]. Many new members of the kallikrein family have been evaluated for their expression in breast cancer. KLK5 expression, assessed by quantitative RT-PCR, was shown to be an indicator of poor prognosis in all patients as well as in a subgroup of patients with early (stage I and II) disease [112]. KLK6 itself has been discovered as a serine protease expressed in primary mammary carcinoma cell lines but was absent in the corresponding cell lines of metastatic origin [12]. By profiling 96 breast cancer samples for KLK7 expression by RT-PCR, Talieri and co-workers have shown that KLK7 had significantly lower expression in stage I and II beast cancers and in patients with progesterone receptor-positive tumors [113]. As this study indicated shorter disease-free and

Table 2
Applications of human tissue kallikreins in breast cancer

Kallikrein (KLK mRNA/hK protein)	Tissue/biological fluid	Application	References
hK3	Serum and tissue from breast cancer patients	Marker of diagnosis and prognosis	Reviewed in Ref. [205]
KLK5	mRNA from cancerous breast tissues	Unfavorable prognostic marker	[112]
hK5	Serum from breast cancer patients	Marker of diagnosis	[98]
KLK7	mRNA from cancerous breast tissues	Unfavorable prognostic marker	[113]
KLK9	mRNA from cancerous breast tissues	Favorable prognostic marker	[114]
KLK10	mRNA in breast ductal carcinoma in situ	Predictive of invasiveness	[116]
hK10	Cytosols from cancerous breast tissues	Predictive value	[126]
KLK13	mRNA from cancerous breast tissues	Favorable prognostic marker	[118]
KLK14	mRNA from cancerous breast tissues	Unfavorable prognostic mark	[120]
hK14	Serum and tissue from breast cancer patients	Marker of diagnosis	[123]
KLK15	mRNA from cancerous breast tissues	Favorable prognostic marker	[121]

overall survival in breast cancer patients with KLK7-positive tumors, KLK7 expression may be useful as a marker of unfavorable prognosis. Another kallikrein, KLK9, was studied by quantitative RT-PCR in 169 breast cancer patients at various disease stages and in tumors of different histological types and grades [114]. Multivariate analysis revealed that patients with KLK9-positive tumors had longer disease-free and overall survival than those with KLK9-negative tumors, suggesting that KLK9 is an independent favorable prognostic indictor in breast cancer (Table 2).

KLK10 (NES1) has also been evaluated at the mRNA level in breast cancer. This gene was originally cloned as a putative tumor suppressor, with loss of expression in breast cancer cell lines without apparent changes in its genetic structure [11]. Using in situ hybridization with antisense KLK10 as a probe, a subsequent study [115] focused on tissue sections from normal breast, of typical and atypical ductal hyperplasia, from ductal carcinoma in situ (DCIS), as well as from infiltrating ductal carcinoma, and has shown that while all 30 of the normal breast tissue samples and 18 of 24 hyperplasia samples showed KLK10 expression, 13 of 28 DCIS completely lacked KLK10 expressing with the remaining 15 having only weak-to-moderate KLK10 levels. More strikingly, 29 of 30 of the infiltrating ductal carcinomas (grades 1–3) completely lacked KLK10, and only weak expression was seen in the remaining one sample. These data strongly suggest that loss of KLK10 expression may be a requirement for tumor progression. Another study focused on biopsy samples of pure DCIS; here, it was demonstrated that all 17 of the high grade DCIS lacked KLK10 expression while only 3 of 7 intermediate grade and 3 of 5 low-grade DCIS samples were KLK10 negative [116]. Of the six KLK10-positive DCIS, none of them were invasive at surgery. In contrast, 40% of KLK10-negative biopsies turned out to be invasive. Thus, lack of KLK10 expression in biopsies of pure DCIS predicts a high risk of invasiveness [116]. Using methylation-specific PCR and sequence analysis of sodium bisulfite-treated genomic DNA, to further investigate the possible cause of loss of KLK10 expression in breast cancer cell lines and primary tumors, a strong correlation between loss of KLK10 mRNA expression and hypermethylation in exon 3 was demonstrated [117].

In a study of 173 patients with breast carcinoma, KLK13 expression was shown to be an independent, favorable indicator of disease-free and overall survival in grade I/II patients, as well as in patients with node, estrogen receptor and progesterone receptor positivity [118]. Pilot studies with KLK12 and KLK14 also indicated down-regulation of its mRNA expression in breast cancer [86,119]. The latter kallikrein was further studied in detail using quantitative RT-PCR in 178 breast carcinoma samples; higher KLK14 expression was more frequently present in patients with advanced (stage III) disease [120]. Thus, KLK14 was shown to be an independent marker of poor prognosis, including patients with large (≥ 2 cm) tumor size and estrogen receptor, progesterone receptor, and node positive status. In a similar study [121] assessing KLK15 levels in 202 breast carcinoma tissues, KLK15 expression was found to be a significant, independent predictor of good prognosis in all patients, as well as in a subgroup of patients with low grade and estrogen and progesterone receptor negative tumors. Lastly, using in silico analysis of the expression of all 15 kallikrein genes in 24 breast cancer and 8 normal breast tissue libraries, parallel down-regulation of KLK5, KLK6, KLK8, KLK10 and KLK12 in breast cancer was detected; these results were verified experimentally using RT-PCR analysis [122].

As many kallikreins have shown promise in breast cancer prognosis at the mRNA level, they were also assessed at the protein level. Higher concentrations of hK5 were detected in serum of 49% of breast cancer patients compared to healthy controls [98]. In another pilot study [123], serum hK14 levels were elevated in 40% of patients suffering with breast cancer. More detailed studies are needed to substantiate these findings. As hK3 (PSA) has been previously shown to be down-regulated in nipple aspirate fluid of women with breast cancer compared to normals [124], a subsequent study aimed at evaluating hK6 and hK10 (along with hK2 and hK3) whether these kallikreins have the ability to detect breast cancer [125]. While hK6 and hK10 levels were the highest among the examined kallikreins in nipple aspirate fluid, only hK2 and hK3 were associated with both pre- and post-menapausal breast cancer. Another study evaluated hK6 and hK10 levels by ELISA in breast cancer cytosolic extracts [126]. While the levels of these two proteins correlated with each other, neither of them was associated with the clinicopathological variables (tumor size, grade, and nodal status) or relapse-free and overall survival. However, higher hK10 levels seemed to have a significant correlation with shorter progression-free and overall survival upon start of tamoxifen treatment; while hK6 was not associated with this regimen, higher hK10 levels significantly correlated with poor response to tamoxifen therapy [126].

2.3. Prostate cancer

Prostate cancer remains the second leading cause of cancer-related mortality in North American men with the highest number of new cases identified per year among all cancers. The fact that prostate cancer accounts for a third of newly diagnosed cancers [72] is partly due to the availability of serum PSA (hK3) screening, the best tumor marker available to-date [40,127]. Results of large-scale clinical trials on whether PSA screening improves prostate cancer survival are still to come [128] and shortcomings of PSA as a diagnostic tool in distinguishing between benign prostatic hyperplasia (BPH) and prostate cancer prompts continued search for novel prostate cancer markers (Table 3).

As discussed below, among the kallikreins, several (KLK4, KLK5, KLK10, KLK11, KLK14 and KLK15) have been characterized at the mRNA level while only hK4 and hK11 have been investigated so far at the protein level. In 29 histologically confirmed prostate cancer tissues with matching samples from the non-cancerous part of each sample, the differential expression of KLK5 was investigated using quantitative RT-PCR [129]. Significantly lower KLK5 expression was noted in cancer with lowest levels found in T3 stage tumors. A negative correlation between KLK5 expression and Gleason score was also observed. In an effort to investigate possible somatic mutations in the KLK10 gene that would explain its observed down-regulation in several cancer types, a germline single nucleotide G–T variation (at codon 50 of exon 3) was discovered that gives rise to an amino acid change (Ala–Ser) [130]. The genotype coding for Ala at this position was significantly less prevalent in prostate cancer patients than in healthy controls while such changes

Table 3
Applications of human tissue kallikreins in prostate cancer

Kallikrein (KLK mRNA/ hK protein)	Tissue/biological fluid	Application	References
hK2	Serum and tissue from prostate cancer patients	Marker of diagnosis, prognosis and monitoring	[40] [206, 207]
hK3	Serum and tissue from prostate cancer patients	Marker of diagnosis, prognosis and monitoring	Reviewed in Ref. [40]
KLK5	mRNA from matched normal and cancerous prostate tissues	Favorable prognostic marker	[129]
KLK11	mRNA from matched normal and cancerous prostate tissues	Favorable prognostic marker	[132]
hK11	Serum from prostate cancer patients	Diagnostic marker	[141]

were not observed in other (testicular, breast and ovarian) cancers. Yet another kallikrein, KLK11 (hippostasin/TLSP) was investigated in prostate cancer chiefly due to initial findings of two murine KLK11 isoforms, brain-type and prostate-type, the latter expressed preferentially in prostate tissue [18]. In a latter study, this prostate-type variant, which has extra 32 amino acids at its N terminus in comparison to the brain-type form, was shown to be preferentially expressed in the secretory epithelium of the normal prostate, versus benign prostate tissue or prostate cancer cell lines using Northern blotting, RT-PCR, in situ hybridization and immunohistochemical analyses [131]. This study also indicated that in prostate cancer cell lines, only the brain-type KLK11 isoform was expressed, suggesting that KLK11 isoforms may be used to distinguish between BPH and prostate cancer [131]. In another study comparing the expression pattern of these KLK11 isoforms in 76 matched (cancerous and normal) human prostate tissue pairs by quantitative RT-PCR, 25–45% higher expression of both isoforms was noted in cancerous over normal tissues [132]. Furthermore, in this study, lower expression was noted only for the prostate-type isoform which had a significant association with higher tumor grade and more advanced disease stage as well as with tumors of higher Gleason score; this suggests the possibility of down-regulation of KLK11 in more advanced forms of prostate cancer. Subsequently, a third type of KLK11 mRNA was found; this variant codes for a predicted enzymatically active form similar to the brain-type, and was also found in epithelial cells of the prostate [133].

As mentioned before, KLK14 has been shown to be down-regulated in several cancer types, and prostate cancer is no exception [86]. These initial findings were followed up by examining KLK14 expression in 100 matched (normal-cancer) samples of prostate tissues from radical prostatectomy by using quantitative, real-time RT-PCR [134]. This study indicated over-expression of this kallikrein in cancer among 74% of the tissue pairs; the ratio of cancerous to non-cancerous KLK14 levels was significantly higher in late stage (stage III) than in earlier stage (stage II) disease; the same relationship held true for KLK14 expression in grade 3 versus lower grade tumors [134]. KLK15 also appears to have prognostic value in prostate cancer, as preliminary studies indicated that KLK15 levels were higher in cancerous than in normal prostate tissues [135]. A subsequent study of 90 pairs of non-cancerous and cancerous prostate tissue samples has a shown clear up-regulation of KLK15 mRNA by quantitative RT-PCR in cancerous tissues in 84% of the pairs [136]. Here, the ratio of cancerous to non-cancerous KLK15 levels was significantly higher in patients with pT3/pT4 than in pT2 patients. Thus, KLK15 levels may have utility in assessing aggressiveness of prostate cancer [136].

As it was identified initially as a prostate-specific kallikrein [47], intense efforts focused on the evaluation of hK4 as a prostate cancer biomarker. hK4 has been shown to be expressed both in normal and cancerous tissues at very low levels as assessed by immunohistochemistry and quantitatively, by an hK4-specific immunoassay [137]. Its over-expression

in prostate cancer over normal prostate tissue has recently been demonstrated by Western blotting [138]. Using quantitative-RT-PCR and immunohistochemistry, the presence of this kallikrein at the mRNA and protein level has been shown to exist both in normal and cancerous (primary and metastatic) prostate tissues, with lower levels seen at the mRNA level in the adrenal, salivary, and thyroid glands [139]. Interestingly, anti-hK4 autoantibodies have been shown to exist in the serum of prostate cancer patients but not in healthy controls, suggesting that this kallikrein may not only be a prostatic biomarker but also a possible target in immunotherapy of prostate cancer [139]. hK11 seems to be another promising novel diagnostic marker of prostate cancer as immunoassay-based quantitative studies demonstrated that this protein is found at the highest levels in prostate tissue, followed by stomach, trachea, skin and colon, and in seminal plasma over various biological fluids [140]. Unlike levels of hK4, the levels of hK11 in seminal plasma are comparable to those of hK2 (300-fold less than that of hK3) and can be easily measured by immunoassay. This study demonstrated that in 60% of cancer patients, serum hK11 seems to be elevated, in comparison to healthy controls, suggesting the possibility of using hK11 as diagnostic/prognostic serum biomarker [140]. Another study by the same group investigated whether serum hK11 levels could be used to distinguish BPH from prostate cancer using 64 and 86 serum samples, respectively [141]. Both hK11 and the ratio of hK11 to total PSA (hK11:tPSA) were significantly lower in serum of prostate cancer patients than in serum of patients with BPH. In fact, in patients with % free PSA less than 20, 54% of biopsies could have been avoided using the hK11:tPSA ratio [141]. Receiver operating characteristic curve analysis pinpointed to the fact that the area under the curve for hK11:tPSA ratio and for % free PSA ($\sim$0.83) was much higher than the area under the curve of total PSA alone (0.69). These results indicate that the combination of serum hK11 and total PSA could be used to reduce the number of prostatic biopsies [141].

2.4. Testicular cancer

The relationships and implications of kallikreins in testicular cancer have recently been reviewed [142] While testicular cancer is a potentially curable disease with incidence much lower than for the cancer types discussed above, a worldwide increase in its incidence has been observed over the past 30 years [143]. Testicular cancer has high morbidity as it is the most common type of malignancy in young males (age 20–34) with treatment often leading to permanent infertility. Coupled with the fact that the risk factors involved are poorly understood, finding possible markers of early detection is imperative. Among the kallikreins, so far only mRNA studies have been performed, showing down-regulation of KLK5, KLK10, KLK11, KLK13 and KLK14 in testicular cancer. Preliminary data on KLK14 expression showed down-regulation of this gene in testicular cancer, prostate, breast and ovarian cancer [86]. As KLK5 is highly expressed in normal testis [144], a pilot study was designed to assess KLK5 expression in tumor tissue obtained from testicular cancer patients. Using quantitative RT-PCR, 13 of 14 patients (93%) had lower KLK5 levels in the cancerous area than in adjacent non-cancerous tissue [145]. This study also demonstrated statistically significant lower levels of KLK5 expression in late stage (II/III) versus early stage (I) carcinomas as well as lower expression in seminomas over non-seminomas. In addition, KLK5 levels were significantly lower in invasive tumors (T2/T3) than in those confined to the testis and epididymis (T1) suggesting association of higher KLK5 levels with less aggressive testicular cancer. In the same set of primary germ cell tumors, KLK10, which is another kallikrein with high expression in testicular tissue (Table 1), was also noted to have significantly lower expression in cancerous areas when compared to corresponding non-cancerous areas by quantitative RT-PCR [146]. Immunohistochemical staining confirmed these findings at the protein level. This study also showed down-regulation of KLK10 in 6 randomly selected germ-cell tumors (GCT) and pre-malignant carcinoma in situ (CIS) in comparison to six random normal testicular tissues. The results testify to the possible tumor suppressive role of KLK10 in testicular cancer progression [146]. Another study identified five novel splice variants of KLK13 that are exclusively expressed in testicular tissue [147]. These splice variants were detected only in non-cancerous parts of germ cell tumor samples, with marked absence in the adjacent cancerous parts;

this is in contrast to full length KLK13 message which was found both in normal as well as cancerous tissue regions [147]. It will be interesting to see in future studies whether the apparently concerted down-regulation of the above five kallikreins also holds true at the protein level.

2.5. Other cancers

Cervical cancer. Two of the kallikreins, KLK7 and KLK8, have been evaluated as possible markers of cervical cancer at the mRNA and protein level. hK7 plays a role in skin shedding via degradation of intercellular cohesive structures [148–150]. Santin and co-workers evaluated KLK8 expression in 18 cervical cancer cell lines, in comparison to eight normal cervical keratinocyte cell lines by RT-PCR and hK7 expression in paraffin-embedded cervical tumors by immunohistochemistry [151]. None of the normal cervical cell lines had KLK7 mRNA expression while half of the cancer cell lines were noted to do so. In addition, all five patients with KLK7-positive tumors had metastasis to regional lymph nodes. Immunohistochemical analyses confirmed presence of hK7 in tumor tissue but not in normal cervical epithelial cells, suggesting that hK7 may have a possible role in invasion and metastasis of squamous cervical carcinoma [151]. A separate study by the same group evaluated KLK8 in the above cell lines and found high KLK8 expression in 9 of 11 (82%) primary cervical cancer cell lines and in 7 of 8 (87%) established cervical cancer cell lines, with no expression in four normal cervical keratinocyte cell lines and in 4 normal cervical biopsies [152]. The same pattern of expression was demonstrated at the protein level by immunohistochemistry, concluding that hK8 may be a good tool for the early detection of cervical carcinoma, and could be a possible target for cervical cancer therapy [152].

Pancreatic cancer. Using serial analysis of gene expression and EST databases, in silico analyses were performed in normal and cancerous pancreatic tissues and cancer cell lines [153]. Gene-specific tags against KLK6 and KLK10 revealed that KLK6 was expressed in 5/6 cancer libraries with a 5-fold increase in average expression in cancerous, compared to normal pancreas; indeed screening of EST databases revealed that KLK6 clones were found only in cancer libraries but not in libraries of normal pancreatic tissues/cell lines. Digital differential display indicated a 13-fold increase in expression of KLK10 in pancreatic cancer, suggesting that perhaps these two kallikreins may be differentially expressed at the protein level as well, especially as 19q14 chromosomal band is non-randomly rearranged in various solid tumors [153]. Another study, using oligonucleotide arrays and serial analysis of gene expression, also identified KLK10 expression to be 12-fold higher in pancreatic ductal carcinoma [154].

Colon cancer. As KLK6 was originally cloned from a human colon adenocarcinoma cell line [14], the above in silico study also looked at differential expression of kallikreins in colon cancer. Over-expression of KLK6, KLK8 and KLK10 as well as down-regulation of KLK1 was found by SAGE and over-expression of KLK6 and KLK10 was also confirmed by EST analysis [153]. These results raise the possibility that these two kallikreins have value in colon cancer diagnostics. In a separate study, KLK6 expression in Caco-2 cells proved to be dependent on the colorectal proto-oncogene K-ras [155].

Lymphoblastic leukemia. As microsatellite markers have indicated that the long arm of chromosome 19 is a common site for loss of heterozygosity in childhood acute lymphoblastic leukemia (ALL), this site is suspected to harbor one or more tumor suppressors [156]. Hypermethylation of exon 3 of KLK10 has been suggested as an explanation for its down-regulation in breast cancer (see above), Roman-Gomez and coworkers investigated whether such *cis*-acting epigenetic regulation of the KLK10 gene may also operate in ALL [157]. Using methylation-specific PCR to investigate methylated alleles of KLK10 gene at exon 3 in four pre-B ALL cell lines and samples from 222 consecutive ALL patients, the results indicated that exon 3 of the KLK10 gene was highly methylated in all tested cell lines and in 60% of patient samples which coincided with strong down-regulation of KLK10 mRNA in ALL cell lines and in 69% of the patients tested. No such methylation was noted in normal cells, with normal bone marrow mononuclear cells clearly expressing KLK10. Lastly, restriction-mediated PCR demonstrated a correlation between hypermethylation of CpGs of exon 3 with those occurring in the promoter and in

the 5′ untranslated region of this gene, strongly suggesting that hypermethylation causes silencing of KLK10 expression, which can be used in the prognosis of ALL [157].

Lung, head and neck cancer. Preliminary evidence has emerged for the utility of kallikreins in these cancers. In one study aimed at classifying lung carcinomas by mRNA expression profiling with microarrays, KLK11 was identified as a marker of neuroendocrine C2 adenocarcinomas which have less favorable outcome [158]. A similar microarray-based study found both KLK5 and KLK10 to be over-expressed in squamous lung carcinomas [159]. Another microarray of squamous cell carcinoma of head and neck also found KLK10 to be highly expressed [160].

3. Kallikreins in non-malignant diseases

While ample studies demonstrate the connection between kallikreins and cancer, many of these genes have also been implicated in non-malignant diseases.

Dental abnormalities. Mutations in the KLK4 gene were shown to be causative of an autosomal dominant dental enamel disorder, recessive hypomaturation amelogenesis imperfecta [161]. These findings suggest that one of the biological functions of KLK4 is the maturation of dental enamel [162,163].

Disorders of the central nervous system (*CNS*). hK6, found predominantly in brain oligodendrocytes and in its zymogen form in cerebrospinal fluid (CSF) [164–166], was proposed to be a homolog of rat myelencephalon-specific protease with implications in demyelinating diseases such as multiple sclerosis (MS) [167,168]. Indeed, this protease was subsequently found in CNS inflammation, particularly in demyelination occurring in MS lesions [169,170]; hK6 was recently shown to be a participant in enzymatic cascades that mediate CNS inflammation, as blocking of hK6 enzymatic activity also blocked the breakdown of myelin basic protein, with mice exhibiting significantly delayed onset of symptoms [171]. Initial isolation of KLK6 cDNA from Alzheimer's disease brain and demonstration of its amyloidogenic activity has also implicated hK6 in Alzheimer's disease [13]. Furthermore, a study comparing hK6 levels in brain tissue from normal and Alzheimer's subjects, found significantly less hK6 in brain of Alzheimer's disease patients [172]. This study was later extended to CSF and whole blood samples [173]; here, 2-fold down-regulation in AD brains reflected previous findings, while 3-fold and 10-fold up-regulation was seen in CSF and blood, respectively, in Alzheimer's patients in comparison to normal controls. In contrast, in another study, correlation of age and hK6 in CSF could only be observed in male and female patients with peripheral neuropathy but not in CSF of Alzheimer's patients who showed a decrease in CSF of hK6 levels compared to the other group [174]. The authors suggested that the decreased levels of hK6 in CSF of Alzheimer's patients may be a risk factor for this disease. Clearly more studies are needed to evaluate the status and diagnostic/prognostic value of hK6 in Alzheimer's patients.

hK6 is also implicated in Parkinson disease as its expression was lower in neurons and microglial cells in brains of both Alzheimer's and Parkinson patients than in those of the normal controls [175]. Indeed, hK6 was later to shown to localize to Lewis bodies and glial cytoplasmic inclusions and could degrade alpha-synuclein [176]. Accumulation of these structures and proteins is a pathology in Parkinson's disease, hK6 is strongly suspected to have direct mechanistic connection to this disease. Recent evidence connects KLK6 and major depressive disorders as microarray data examining differences in serine protease expression between the temporal cortex of 12 such patients and 14 matched controls showed KLK6 to be one of several serine proteases of myelin-regulating function with decreased expression in diseased brain matter [177]. In another study looking at hK6, hK7 and hK10 levels in the CSF of patients suffering from frontotemporal dementia or Alzheimer's disease, significantly lower levels were noted for all three examined kallikreins in frontotemporal dementia as well as higher levels in Alzheimer's for hK10 in comparison to healthy controls [178]. In addition, preliminary studies indicate the involvement of another kallikrein, KLK8, in Alzheimer's, as an 11.5-fold increase of KLK8 expression was noted in hippocampus of Alzheimer's disease patients compared to controls [179]. KLK8 has previously been shown two have two types with type 2 KLK8 preferentially expressed in human adult brain and hippocampus with possible

involvement in adult brain plasticity and development of the nervous system. Its expression was shown to be induced in oligodendrocytes by injury to the CNS of mice [180].

Skin abnormalities. Several kallikreins were shown to be expressed in the skin [181]. KLK1 expression has been shown to be associated with several skin diseases [182,183]. The same applies to some of the newly discovered kallikreins. Overexpression of KLK7, initially cloned from a keratinocyte library [19], has been implicated in mice in chronic itchy dermatitis [184] and psoriasis [185], directly connecting its function to that of yet another epidermal kallikrein KLK5 [20] working in concert in epithelial desquamation [150,186,187]. In addition, insertional mutations in the 3′UTR of KLK7 gene has been linked to atopic dermatitis in a case-control study of 103 children suffering from atopic dermatitis and 261 matched controls [188]. Lastly, Northern blotting and in situ hybridization experiments have shown high over-expression of KLK8 mRNA in skin samples of severe hyperkeratosis obtained from patients with seborrheic keratosis, psoriasis vulgaris, lichen planus, and even squamous cell carcinoma in comparison to normal skin samples where only weak expression of KLK8 was detected [189].

4. Conclusions

As most members of the kallikrein family of serine proteases display differential expression in many forms of cancer, it is very tempting to speculate that they may play a role in cancer initiation and progression. These genes localize at 19q13.3–q13.4, an area of the genome known to undergo rearrangements in various solid tumors and in leukemias/lymphomas [190–192]. Gene rearrangements, mutations or epigenetic changes could lead to up- or down-regulation of expression in various cancers. The involvement of these serine proteases and other classes of enzymes in tumor progression and metastasis, through degradation of the extracellular matrix is likely. Preliminary evidence suggests that kallikreins hK2, hK3, hK5, hK6 and hK14 have the ability to cleave extracellular matrix components [193–195]. These proteases may act alone, or in cascade enzymatic pathways with other proteases [150,196–199]. Involvement in capillary morphogenesis and angiogenesis are another two venues of invasion-modulating roles as shown for hK3 [200–202] and more recently, for hK6 [203]. Studies on the physiological function of kallikreins will shed more light into the roles of these enzymes in cancer and other diseases. These enzymes may represent not only novel biomarkers but also promising future therapeutic targets.

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