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Mini-review

Human tissue kallikreins: The cancer biomarker family ☆

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Abstract

Human tissue kallikreins (KLKs) are attracting increased attention due to their role as biomarkers for the screening, diagnosis, prognosis, and monitoring of various cancers including those of the prostate, ovarian, breast, testicular, and lung. Human tissue kallikrein genes represent the largest contiguous group of proteases within the human genome. Originally thought to consist of three genes, the identification of the human kallikrein locus has expanded this number to fifteen. These genes, and their encoded proteins, share a high degree of homology and are expressed in different tissues. Prostate-specific antigen (PSA), the most commonly known kallikrein, is a useful biomarker for prostate cancer. Several other kallikreins, including kallikreins 2 (KLK2) and 11 (KLK11) are emerging as complementary prostate cancer biomarkers. Along with these kallikreins, several others have been implicated in the other cancers. For example, *KLK5, 6, 7, 10, 11*, and *14* are emerging biomarkers for ovarian cancer. The identification of kallikrein substrates and the development of proteolytic cascade models implicate kallikrein proteins in cancer progression. This review describes the current status of kallikreins as cancer biomarkers.

Keywords: Kallikreins; Serine proteases; Cancer; Biomarkers; Clinical applications

1. Introduction

Human tissue kallikrein research has evolved with these accomplishments having been thoroughly chronicled in recent reviews, as well as in many research articles [2–5]. However, increased attention has been paid to the valuable role of kallikreins as cancer biomarkers.

Of the 178 known human serine proteases, accounting for 32% of all proteases, the human tissue kallikreins represent the largest contiguous cluster of protease genes in the human genome. The *KLK* genes are tightly grouped and arranged tandemly without any intervention by non-*KLK* genes. The three classical kallikreins KLK1, KLK2, and prostate-specific antigen (PSA) and *KLK15* are clustered in a 60 kb region, followed by the pseudogene *ΨKLK1*, and the 11 other *KLK*

☆ *Nomenclature*. In this review, kallikrein genes will be denoted as *KLK1* … *KLK15* and kallikrein proteins as KLK1 … KLK15, in accordance with the recently approved nomenclature [1]. *KLK3* will be referred to as *Prostate-specific antigen (PSA)* throughout the review.

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genes, with the direction of transcription of all genes from telomere to centromere with the exception of *KLK3 (PSA)* and *KLK2*. *KLK* genes share many common characteristics including; five coding exons, similar or identical coding exon lengths and conserved serine protease catalytic triad residues His, Asp, and Ser in exons 1, 3, and 5, respectively [2,6]. Most *KLK* genes also have a number of splice variants and/or alternative transcriptional start sites. With the exception of *KLK14*, all kallikreins have at least one alternative transcript, exclusive of their reference form, with *PSA*, followed by *KLK13*, having the highest number of alternative transcripts [7–10]. Most of these alternative *KLK* transcripts are predicted to code for truncated proteins. The biological and physiological significance, if any, of truncated KLK proteins, or the regulation of alternative *KLK* transcripts is unknown.

Groups of *KLK* genes are often expressed within a specific tissue. For example, *KLK2*, *KLK3*, *KLK4*, *KLK5*, *KLK11*, and *KLK15* mRNA and/or proteins are found in the prostate. As well, almost every kallikrein is expressed in the salivary gland, while other groups are found in the skin (*KLK5*, *KLK7*, *KLK8*, *KLK9*, *KLK11*, *KLK13*, and *KLK14)*, breast (*KLK3*, *KLK4*, *KLK5*, *KLK6*, *KLK8*, *KLK10*, *KLK13*, *KLK14*), pancreas (*KLK1*, *KLK10*, *KLK12*) and the central nervous system *KLK5-KLK9, KLK11, KLK14*. KLK proteins have also been found in biological fluids such as serum, seminal plasma, and milk of lactating women, confirming that these are secreted proteins. Tissue-specific expression patterns have also been identified for a few alternative *KLK* transcripts [3,11–13]. Some splice variants of both *KLK2* and *KLK3* seem to be exclusively expressed in the prostatic epithelium [14].

1.1. Physiological protein function

KLK transcripts code for a single chain serine protease pre-proenzyme, a characteristic of most secreted proteases. Kallikreins share an overall amino acid sequence identity of 40–80%, with the highest degree of similarity between KLK2 and PSA. With the exception of KLK4, all have a pro-peptide ending with Lys or Arg, suggesting that these zymogens are activated by enzymes with trypsin-like activity [15]. The majority of KLK proteins (KLK1–2, KLK4–6, KLK8, and KLK10–14) have an Asp residue in their binding pocket (or Glu for KLK15) suggesting that they possess trypsin-like substrate specificity. Other kallikreins, such as PSA, KLK7, and KLK9, possess chymotrypsin-like activity [16]. It has recently been shown that pro-KLK proteins can serve as substrates for activated KLKs, thereby setting the stage, potentially, for a proteolytic cascade, whereby differentially expressed kallikreins within the tissue microenvironment proteolytically activate other kallikrein proenzymes, with the entire array of activated species subsequently acting on extracellular substrates to either mediate physiological functions or contribute to disease progression [17–20]. Cascade models are currently being applied to skin desquamation and semen liquefaction, and may have relevance in tumor invasion and metastasis.

Kallikreins may have clinical utility in serving as targets for a number of novel therapeutic approaches currently under investigation. There is increasing evidence that a group of serine protease inhibitors (collectively known as serpins) may play a role in blocking KLK activity. The design of specific kallikrein serpins exploits the flexible reactive-site loop (RSL) of the inhibitors which is implicated in the interaction with the putative protease [21–23]. Binding of the enzyme and cleavage of the serpin leads to covalent bond formation between the two proteins, irreversibly trapping the protease in a non-reactive state. The specificity of serpin inhibition depends on both the amino acid sequence and length of the RSL. Several serpin inhibitors of kallikrein activity have been identified; but many of these lack specificity for the kallkrein family or specific members, which adversely affects their therapeutic potential. In particular, phage display technology has been used in conjunction with amino acid substitutions in the RSL of α_1-antichymotrypsin (ACT) to construct novel KLK2 specific inhibitors. Several potential serpin inhibitors were identified and tested against other serine proteases including chymotrypsin, PSA and urokinase Plasminogen Activator, with only one showing KLK2-specific inhibition [24,25]. The same technology is being used to discover additional kallikrein-specific serpin inhibitors.

Other therapeutic strategies have taken advantage of kallikrein activity or tissue specificity (e.g., PSA in prostate) to deliver tissue-specific toxic genes and induce active immunotherapy using KLK-based vaccines. Using an adenoviral or non-viral/liposomal vector delivery system containing a cell suicide gene, under the regulation of prostate-specific *PSA* promoter and enhancer elements, it is possible to selectively stimulate gene expression within PSA-producing prostate cancer cells, resulting in prostate

cancer cell death *in vitro* and inhibition of tumor growth in xenografted mice in preclinical investigations [26–28].

More recently, efforts have focused on understanding the physiological and biological functions of kallikreins in different tissues and diseases. Specific degradomic tools have been developed such as phage display and combinatorial peptide-based specific profiling, in order to identify specific kallikrein substrates [4,29]. Phage display and fluorogenic substrates have also been developed to determine the enzymatic kinetics of kallikrein activity. These methods have led to the characterization of highly selective substrates and potential kallikrein biological targets implicated in cancer progression.

1.2. Regulation

The regulation of kallikrein expression has been widely analyzed. Many kallikreins show regulation by specific steroid hormones in a variety of cancer cell lines [3,30]. The androgenic regulation of *PSA* has been particularly well-characterized in both prostate cancer cells and *in vivo*. Upstream androgen response elements (AREs) have been identified in the proximal promoters of *PSA* and *KLK2* [31–34]. Putative AREs have also been identified for KLK4, but have not as yet been functionally tested [35,36]. Other kallikreins that are sensitive to hormone stimulation in cancer cells are *KLK5, 6, 10, 11, 13*, and *14* [30], although the mechanism by which they are stimulated by estrogen in breast cancer cells is currently uncertain given that putative hormone response elements have not as yet been identified in proximal promoter regions. Some suggest that there may be a central control locus (locus control region) that regulates the coordinate expression of these kallikreins.

Recently, work with *PSA* has implicated major components of the cell signaling pathways including RAS-MEK-ERK, PI3K-AKT, NFkappaB and JNK, and p38MAPK stress signaling pathways [37–41]. It appears that many signal transduction pathways regulate kallikrein gene expression. For example, approximately 30% of all breast cancers either have a deletion or mutation in the gene encoding the tumor suppressor protein *phosphatase and tensin homologue deleted from chromosome 10 (PTEN)*, a negative regulator of AKT function, resulting in increases in cell growth and proliferation. It is worth noting that *PTEN* deficient cells are no longer sensitive to current therapeutic agents such as CCI-779 and Tamoxifen [42,43]. Loss of PTEN protein or activity is also a prognostic marker in prostate cancer, especially in androgen-independent cancers [40,44]. Turning to another cell signaling pathway, overexpression of the signaling receptor HER2/EGFR has been found to carry prognostic significance in the evaluation of breast cancer progression [45]. Because of the unique organization of the kallikrein locus and the coordinated expression of many of these genes, we believe that these pathways may not be solely exclusive to the regulation of *PSA* but may also be implicated in the regulation of other locus family members.

Kallikrein expression has also been shown to be regulated by DNA methylation, an epigenetic mechanism. Hypermethylation of CpG islands within promoter or gene coding sequences correlates with transcriptional silencing of the gene. Such regulation has been most extensively studied in the case of *KLK10* [46,47], but recent work has identified several other kallikreins including *KLK5, 6, 11*, and *12*, in a limited number of cell lines, that appear to be regulated via DNA methylation [48–50]. As discussed below, this epigenetic regulation (of *KLK1)* has the potential to account for the differential expression of the kallikreins in breast and lymphoblast leukemia.

2. Kallikreins as cancer biomarkers

Carcinogenesis is a complex process that includes alterations in gene structure and expression. Table 1 highlights the expression pattern and prognostic significance of all members of the kallikrein family in ovarian, breast and prostate, and other malignancies. Several kallikreins such as *KLK6* in ovarian and breast cancer appear to exclusively indicate poor prognosis, whereas others such as *KLK10* may herald either favorable or unfavorable prognosis depending on the cancer type (in this case, favorable if breast cancer and unfavorable if ovarian cancer).

Currently, two well-established technologies are in use to quantify kallikrein expression, RT-PCR and ELISA assays. Kallikrein expression is assessed by RT-PCR to detect the presence of any individual kallikrein transcript from a tissue source (much of the tissue expression profiles, along with steroid hormone studies of kallikrein gene regulation, took advantage of this simple and highly sensitive technique). More recently, certain kallikrein splice variants have also been found to have prognostic

Table 1
Differential expression and potential clinical applicability of human tissue kallikreins in human disease

Cancer	KLK	Localization in tissues/fluids	Expression	Clinical relevance	Ref.
Acute lymphoblastic leukemia (ALL)	10		↓ Expression (↑ methylation) in ALL cell lines compared to normal fresh bone marrow mononuclear cells	Unfavorable prognosis (↓ survival)	[50]
Brain	6		↓ Expression in malignant tumors compared to normal tissues	ND[a]	[92]
Breast	1	Cancer cells	ND	ND	[93,94]
	PSA	Cancer cells Serum	↓ Expression in malignant tumors compared to benign tissues	Favorable prognosis (↑ survival) Predictive value (predicts response to tamoxifen therapy)	[95–100]
	5	Serum	↓ Expression in malignant tumors compared to normal tissues ↑ Levels in serum of cancer patients compared to normal	Diagnosis Unfavorable prognosis (↓ survival)	[60,61,101]
	6		↓ Expression in metastatic tumors, ↑ expression in primary tumors ↓ Expression in malignant tumors compared to normal tissues	ND	[92,102,101]
	7		ND	Unfavorable prognosis (↑ stage, ↓ survival)	[103]
	8		↓ Expression in malignant tumor compared to normal tissues		[101]
	9		ND	Favorable prognosis (↓ stage, ↑ survival)	[104]
	10		↓ Expression in malignant tumors and cell lines compared to normal and benign tissues ↑ Expression in subset of malignant cell lines	Predictive value (predicts response to tamoxifen therapy)	[7,47,63,105,106]
	12		↓ Expression in malignant tumors compared to normal tissues	ND	[107]
	13		↓ Expression in malignant tumors and cell lines compared to normal tissues	Favorable prognosis (↑ survival)	[81,108]
	14	Cancer cells	↓ Expression in malignant tumors and cell lines compared to normal tissues ↑ Levels in serum of cancer patients compared to normal	Diagnosis Unfavorable prognosis (↑ stage, ↓ survival)	[66,85,109]

	15		ND	Favorable prognosis (↑ survival)	
Cervical	7	Cancer cells	↑ Expression in tumors compared to normal tissues	Expression not correlated with survival	[110,111]
	8	Cancer cells	↑ Expression in cancer cell lines and primary tumor cultures compared to normal tissues	ND	[112]
Colon	1		↓ Expression in malignant tumors compared to normal tissues	ND	[88]
	6		↑ Expression in malignant tumors compared to normal tissues	ND	[88,92]
	8		↑ Expression in malignant tumors compared to normal tissues	ND	[88]
	10		↑ Expression in malignant tumors compared to normal tissues	ND	[7,88]
Colorectal	6	Cancer cells	↑ Expression in malignant tumors compared to normal tissues	Unfavorable prognosis (↑ stage, ↓ survival)	[113]
Esophageal	6		↑ Expression in malignant tumors compared to normal tissues	ND	[92]
Gastric	6	Cancer cells	↑ Expression in malignant tumors and cell lines compared to normal tissues	Unfavorable prognosis (↓ survival)	[88,114]
	10		↑ Expression in malignant tumors and cell lines compared to normal tissues	ND	[7]
Lung	5		↑ Expression in squamous cell tumors compared to normal tissues	ND	[115]
	7		↓ Expression in adenocarcinomas compared to normal tissues	ND	[115]
	10	Cancer cells	↑ Presence of splice variants in non-small-cell lung cancer	ND	[90]
	11	Cancer cells	↑ Expression in a subgroup (cluster "C2) of neuroendocrine tumors [89] ↑ Presence of splice variants non-small-cell lung cancer	Unfavorable prognosis (↓ survival): worst outcome For "C2" tumor subtype compared to others	[89,90]
Ovarian	2		↑ Expression in malignant tumors compared to normal tissues	ND	[116]
	PSA		↑ Expression in malignant tumors compared to normal tissues	ND	[116,117]

(*continued on next page*)

Table 1 (*continued*)

Cancer	KLK	Localization in tissues/fluids	Expression	Clinical relevance	Ref.
			↑ Expression in low malignant potential serous tumors compared to serous carcinomas		
	4	Cancer cells, Stromal cells	↑ Expression in malignant tumors and cell lines compared to benign and normal tissues	Unfavorable prognosis (↑ stage, ↑ tumor grade, ↓ survival)	[67,118–120]
			↑ Expression in tumor cells in effusions compared with primary tumors and solid metastases	Expression not correlated with survival	
			↑ Expression in stromal cells of primary tumors than from solid metastases	Predictive value (predicts resistance to paclitaxel resistance)	
	5	Serum, Ascites	↑ Expression in malignant tumors and cell lines compared to benign and normal tissues	Diagnosis	[60,68,78,79,121,122]
			↑ Levels in serum and ascites fluid of cancer patients compared to normal	Unfavorable prognosis (↑ stage, ↑ tumor grade, ↓ survival)	
	6	Cancer cells Serum	↑ Expression in malignant tumors and tumors of low malignant potential compared to benign and normal tissues	Diagnosis	[69–74,79,92,102,116, 117,121,123–125]
			↑ Expression in low malignant potential serous tumors compared to serous carcinomas	Unfavorable prognosis (↑ stage, ↑ tumor grade, ↓ survival)	
			↑ Levels in serum of cancer patients compared to normal women and those with benign disease	Monitoring	
	7		↑ Expression in malignant tumors and cell lines compared to benign and normal tissues	Unfavorable prognosis (↑ tumor grade, ↓ survival)	[78–80,116,117,121,126–128]
			↑ Expression in low malignant potential serous tumors compared to serous carcinomas	Favorable prognosis	

	8	Cancer cells, Serum Ascites	↑ Expression in malignant tumors compared to benign and normal tissues ↑ Expression in low malignant potential serous tumors compared to serous carcinomas ↑ Levels in serum of cancer patients compared to normal	Diagnosis Favorable prognosis (↓ stage, ↓ tumor grade,↑ survival) Monitoring	[9,79,116,121,129–131]
	9	Cancer cells		Favorable prognosis (↓ stage, ↑ survival)	[132]
	10	Cancer cells Serum	↑ Expression in malignant tumors and cell lines compared to benign and normal tissues ↑ Expression in low malignant potential serous tumors compared to serous carcinomas ↑ Levels in serum of cancer patients compared to normal women and those with benign disease	Diagnosis Unfavorable prognosis (↑ tumor grade, ↑ stage, ↓ survival) Monitoring	[7,47,73,75–77,79,116,117, 123–125,133]
	11	Cancer cells	↑ Expression in malignant tumors compared to benign and normal tissues ↑ Expression in low malignant potential serous tumors compared to serous carcinomas ↑ Levels in serum of cancer patients compared to normal	Diagnosis Favorable prognosis (↓ stage, ↑ survival) Unfavorable prognosis (↑ stage,↓ survival)	[79,82,116,117,134–136]
	13	Ascites	↑ Expression in malignant tumors compared to benign and normal tissues	Favorable prognosis (↓ stage, ↑ survival)	[83,116,137]
	14	Cancer cells	↓ Expression in malignant tumors compared to normal tissues ↑ Expression in malignant tumors compared to benign and normal tissues ↑ Levels in serum of cancer patients compared to normal	Diagnosis Favorable prognosis (↓ stage, ↑ survival)	[66,79,85,138]
	15		↑ Expression in malignant tumors compared to benign tissues	Unfavorable prognosis (↓ survival)	[139]
Pancreatic	1	Cancer cells Fibroblasts Neutrophils Lymphocytes	ND	ND	[140]
	6		↑ Expression in malignant tumors compared to normal tissues	ND	[88,92]

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Table 1 (*continued*)

Cancer	KLK	Localization in tissues/fluids	Expression	Clinical relevance	Ref.
	10		↑ Expression in malignant tumors compared to benign and normal tissues	ND	[7,87,88]
Prostate	2	Cancer cells	↓ Expression in malignant tumors compared to normal tissues	Diagnosis	[52,141,142]
	PSA	Cancer cells	↓ Expression in malignant tumors compared to normal tissues	Population screening, Diagnosis, Prognosis, Monitoring	[52,142,143]
	4	Cancer cells	↑ Expression in malignant tumors compared to benign and normal tissues	ND	[35,36,58,144–147]
	5		↓ Expression in malignant tumors compared to normal tissues	Favorable prognosis (↓ tumor grade, ↓ Gleason score)	[148]
	6		↓ Expression in malignant tumors compared to normal and benign tissues	ND	[12]
	10		↓ Expression in malignant tumors compared to normal and benign tissues ↑ Expression in subset of malignant cell lines	ND	[12,47,149]
	11		↑ Expression in malignant tumors compared to normal ↑ Levels in serum of cancer patients compared to normal	Diagnosis Favorable prognosis (↓ stage, ↓ tumor grade, ↓ Gleason score)	[54,134,150,151]
	13	Cancer cells	↓ Expression in malignant tumors compared to normal and benign tissues	ND	[137,152]
	14	Cancer cells	↑ Expression in malignant tumors compared to normal tissues	Unfavorable prognosis (↑ stage, ↑ tumor grade, ↑ Gleason score)	[57,153]
	15		↑ Expression in malignant tumors compared to normal tissues	Unfavorable prognosis (↑ stage, ↑ tumor grade, [56,154] ↑ Gleason score)	[56,154]
Renal cell carcinoma	1	Cancer cells	ND	ND	[155]
	5	Cancer cells	↓ Expression in malignant tumors compared to normal tissues	ND	[156]
	6	Cancer cells	↓ Expression in malignant tumors compared to normal tissues; ↑ Expression in high malignant compared to low malignant tumors	Unfavorable prognosis (↑ stage, ↓ survival)	[156]
	10	Cancer cells	↓ Expression in malignant tumors compared to normal tissues ↑ Expression in high malignant compared to low malignant tumors	ND	[156]

	11	Cancer cells	↓ Expression in malignant tumors compared to normal tissues	Unfavorable prognosis (↑ stage)	[156]
Salivary gland tumors	6	Cancer cells	↓ Expression in malignant tumors compared to normal tissues	ND	[157]
Squamous cell carcinoma: head and neck	10		↑ Expression in "Group 1" tumor subtype	Unfavorable prognosis (↓ survival): worst outcome for "Group 1" tumor subtype compared to others	[158]
Testicular	5		↓ Expression in malignant tumors compared to normal tissues	Favorable prognosis (↓ stage)	[159]
	10	Cancer cells	↓ Expression in malignant tumors compared to normal tissues	ND	[64]
	14		↓ Expression in malignant tumors compared to normal tissues	ND	[85]
Uterine (serous papillary)	6	Serum	↑ Expression in malignant tumors compared to normal tissues ↑ Expression in uterine serous papillary tumors compared to endometriod carcinoma ↑ Levels in serum of patients with uterine serous papillary tumors compared to those with endometroid carcinoma and normal women	ND	[92,125,160]
	10		↑ Expression in malignant tumors compared to normal tissues	ND	[161]

[a] ND, not determined.

and diagnostic value. However, since KLK proteins are secreted into the extracellular matrix and fluids, ELISA assays were developed to measure protein concentration from a wide variety of biological samples such as serum and seminal plasma. To date, ELISA assays have been developed for all KLK proteins (our unpublished data). KLK ELISA assays have contributed to our understanding of the potential importance of KLKs in cancer biology. Thus, many KLKs have been identified as new biomarkers for several forms of cancer.

2.1. Prostate cancer

Prostate cancer is the most commonly diagnosed malignancy among North American men, accounting for almost 33% of all male cancers. PSA is the best prostate cancer screening marker available to date. However, PSA when used as the sole biomarker for prostatic cancer has shortcomings in that circulating levels are not sensitive to early stage prostate cancer nor specific, having limited ability to differentiate benign prostatic hyperplasia (BPH) from prostate cancer [51,52]. Whether other members of the kallikrein family are also dysregulated in prostate cancer is currently being explored and their combination with PSA to improve clinical performance further assessed. *KLK4, 5, 10, 11*, and *14* mRNA and/or protein expression in prostate cancer have all been investigated. In the case of KLK11, ELISA studies have shown that it is highly expressed in prostate tissue, secreted along with PSA into the seminal plasma and elevated in the circulation of 65 patients with prostate cancer versus controls [53–55]. Of interest, the ratio of KLK11 to total PSA (tPSA) was significantly lower in the patients with prostate cancer than in those with BPH, thereby potentially providing assistance in differentiating these two prostate disorders.

KLK15 may prove to be a marker of late stage prostate cancer. Recent studies using RT-PCR have shown that tissue levels are significantly lower in late stage tumors, with the lowest levels occurring in stage T3. A negative correlation was also observed between the Gleason score and *KLK15* expression from the analysis of 129 histologically confirmed prostate cancer tissues of varying malignancy [56].

KLK14 is overexpressed in prostate cancer. Quantitative RT-PCR of 100 matched (normal-cancer) samples of prostatic tissue showed this in 74% of the examined tissue pairs [57]. In addition, KLK14 levels tended to be higher in late stage (stage III) compared to earlier stage (stage II) disease [57]. ELISA and immunohistochemical techniques has also found KLK4 to be overexpressed in prostate cancer over normal prostate tissue [35,36]. Antibodies to KLK4 have been found in the serum of some prostate cancer patients raising the possibility that this kallikrein might be a target in the immunotherapeutic treatment of prostate cancer [58].

2.2. Breast cancer

Five year survival rates drop dramatically from 97% for localized tumors, to 79% for regionally spread tumors, to 23% for metastatic tumors [59]. Breast and prostate cancers have very similar progression phenotypes. Quantitative RT-PCR analysis has shown that enhanced *KLK5* expression indicates poor prognosis across the spectrum of breast cancer, as well as in a subgroup of early stage (I and II) tumors [60,61]. KLK6 protein is another kallikrein found to be expressed in mammary carcinoma cell lines but it is apparently absent in corresponding cell lines of metastatic origin [10,62]. It is unclear whether hormone influences, epigenetic controls or a combination can explain why these kallikreins are dysregulated in breast cancer.

KLK10 has been extensively studied in breast tumors, since it was originally cloned as a putative tumor suppressor, with loss of expression in breast cancer. Study of *KLK10* mRNA by *in situ* hybridization on tissue sections from normal breast, typical and atypical hyperplasia, as well as in infiltrating ductal carcinoma, has shown that while all normal and a large majority of hyperplasia samples showed *KLK10* expression, more than half of the ductal carcinoma and 29 of 30 of the infiltrating ductal carcinomas completely lacked *KLK10* expression [63–65]. This study suggests that loss of *KLK10* expression is required for tumor progression. The expression of *KLK10* in breast cancer also underlies a unique mechanism of regulation for the gene, with down regulation seen in many cancer types including prostate and testicular cancers and in leukemia.

Correlative clinical data also indicates that KLK14 is associated with parameters of poor prognosis in breast cancer including higher tumor grade, positive nodal status, advanced stage disease and a decreased disease-free and overall survival. Analysis of 178 breast carcinoma samples suggests that higher *KLK14* expression is more frequently present in patients with advanced stage disease, indicating that

KLK14 expression is associated with an increase in tumor stage and reduced/poor prognosis for survival [66]. Thus, KLK14 may have clinical applicability as a diagnostic/prognostic breast cancer biomarker.

2.3. Ovarian cancer

KLK4, *5, 6, 7, 8, 10, 11, 13, 14*, and *15* have been shown to be overexpressed in ovarian carcinoma tissue, serum from women with ovarian cancer, and ovarian cancer cell lines at either the mRNA or protein level or both [3,5]. In particular, *KLK4* and *KLK5* mRNAs have been shown to be overexpressed and are indicators of poor prognostic outcome in grade 1 and grade 2 tumors, suggesting that these genes are associated with more aggressive forms of ovarian cancer [67,68]. *KLK6*/KLK6 appears to be one of the most promising ovarian cancer biomarkers among the kallikreins. KLK6 was initially discovered by differential display, in efforts to identify serine proteases with a strong expression pattern in ovarian cancer cell lines and ovarian carcinomas. Follow-up work examined KLK6 protein expression in 44 ovarian tumors versus 10 normal ovarian tissues, and found KLK6 overexpression more often in tumors. Ovarian cancer patients who show higher levels of KLK6 protein in serum were not responsive to chemotherapies and showed an overall decrease in disease-free and overall survival [69–72]. Combination of KLK6 with CA125 (a well-characterized and widely used ovarian cancer marker), yielded a 21% increase in sensitivity (at 90% specificity) over sensitivity of CA125 alone [73]. At the genetic level, Southern blot analysis of ovarian tumor samples suggests that amplification of the *KLK6* gene may be a possible explanation for the dysregulated expression [74].

Unlike its expression profile in breast cancer, *KLK10*/KLK10 is an unfavorable ovarian cancer prognostic/predictive biomarker. Overexpression of KLK10 protein was seen in primary ovarian tissue lysates; mRNA by *in situ* hybridization was shown to also be overexpressed in tumor tissue versus normal epithelial or stromal cells [75]. A study of ovarian cancer tissue extracts indicated that high concentrations of KLK10 were significantly associated with serous histotype, advanced stage, and large residual tumor size. KLK10 protein levels were also found at higher concentrations in serum of the majority of ovarian cancer patients when compared to healthy controls [75,76]. Other data indicate that for stage III and IV patients, KLK10 was an independent indicator of reduced overall and progression-free survival [77]. All these studies, taken together, suggest that KLK10 is a new serological and tissue marker for diagnosis, monitoring and prognosis of ovarian cancer.

More recently, *KLK7* has been examined as a biomarker for ovarian cancer [78,79]. It was found that KLK7 tissue protein expression was much higher in ovarian cancers versus normal, benign ovarian tumors or non-ovarian cancers that metastasized to the ovary. Expression levels also correlated to cancer stage with highest concentrations forming at stage III/IV. However, KLK7 expression did not show strong correlations with survival, tumor grade or disease stage and bears no prognostic importance for ovarian cancer. Rather, KLK7 may be a surrogate marker of advanced stage disease [80].

Whereas *KLK6* and *KLK10* are unfavorable markers in ovarian cancer, studies of *KLK11* and *KLK13* expression have found them to be independent indicators of favorable outcome for overall survival. In these studies, KLK11 and KLK13-positive tumors were associated with early stage (I and II) cancer and complete or partial response to chemotherapy [81–83].

2.4. Testicular cancer

Several kallikreins have been shown to have some value as biomarkers in testicular cancer, including *KLK5, 10, 11, 13*, and *14*. mRNA data suggest that these kallikreins are downregulated in the disease. *KLK5* is highly expressed in the testis and quantitative RT-PCR showed that 13 of 14 (93%) patients with testicular cancer had lower *KLK5* in the cancerous area than in the surrounding, adjacent non-cancerous tissue. The lower levels in late stage (II/III) versus early stage (I) carcinomas, suggests that *KLK5* is a favorable prognostic marker [60,84]. Furthermore, the differential expression of *KLK10* and *KLK14*, along with *KLK13* splice variants in testicular cancer tissues, have been recently reported to be favorable markers, also showing reduced expression in malignant forms of the disease than in healthy individuals [64,85,86].

2.5. Pancreatic, colon, lung cancers, and leukemias

Although not as extensively studied, kallikreins have been analyzed in the above malignancies and found to undergo distinctive differential expression.

Such studies have been limited primarily to RT-PCR, in silico, and microarray analysis.

2.5.1. Pancreatic cancer

In silico analysis of gene-specific tags against *KLK6* and *KLK10*, of EST databases of normal and cancerous pancreatic tissues and cancer cell lines, revealed *KLK6* to be expressed in 5 of 6 libraries with a 5-fold increase in cancerous versus normal pancreatic tissues. Likewise, *KLK10* expression was 13-fold greater in cancerous pancreatic tissue in the same study, while another study of pancreatic ductal carcinoma indicated a 12-fold increase [87,88].

2.5.2. Colon cancer

The above in silico study also looked at the differential expression of kallikreins in colon cancer. As KLK6 was originally cloned from a human colon adenocarcinoma, *KLK6* was also overexpressed within the database. Together with *KLK6, KLK8* and *KLK10* were also overexpressed, in addition to downregulation of *KLK1*. Therefore, *KLK6* and *KLK10* may provide clinical diagnostic value for both pancreatic and colon cancer [88].

2.5.3. Lung cancer

A microarray study has identified at least one kallikrein that is overexpressed in lung carcinoma *(KLK11)*, particularly in neuroendocrine tumors with less favorable outcome [89]. Current quantitative RT-PCR evidence has also identified several splice variants of *KLK11* that are expressed along with *KLK10* in 47 patients with non-small-cell lung cancer (NSCLC). Although the expression profile of these kallikreins did not correlate with each other or patient survival, it does suggest the possibility that these two genes are co-regulated and are likely involved in normal physiological processes in the bronchus [90].

2.5.4. Lymphoblastic leukemia

Epigenetic regulation of *KLK10* in breast cancers by DNA methylation has been inferred from several studies. Now it appears that this mechanism may also contribute to the expression of *KLK10* in childhood acute lymphoblastic leukemia (ALL). In a study of the methylated status of the *KLK10* gene in 222 ALL patients, exon 3 was found to be highly methylated in 60% of samples. No such methylation was noted in normal cells, with normal bone marrow cells clearly expressing *KLK10*. The methylation of *KLK10* at exon 3, together with methylation within the 5' untranslated region and upstream promoter sequences, suggested that gene silencing via hypermethylation of the *KLK10* gene can be used in the prognosis of ALL [50].

3. Conclusions and future directions

It is clear that many members of the kallikrein gene family are differentially expressed in a wide variety of carcinomas. Although not in clinical use it has been shown that the kallikreins can serve as new biomarkers for diagnosis, prognosis, and monitoring of cancer, the understanding of their regulation and the discovery of their physiological substrates are also of high priority. Kallikrein pathways are starting to emerge which will undoubtedly help in our efforts to understand tumor biology, as it relates to cell invasion and angiogenesis. It has been shown that the overexpression of several kallikreins in ovarian cancer cells increases their malignant potential as studied by *in vitro* assays and animal models [91]. This line of research is still in its infancy. Despite these knowledge gaps, the examination of kallikreins as candidate biomarkers is still expanding. The development of more sensitive ELISAs and multiparametric analysis of kallikreins in tissues and biological fluids, together with other established cancer biomarkers will likely move some of these markers from the investigational arena into routine clinical practice.

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