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Mini-Review

Utility of Kallikrein-Related Peptidases (KLKs) as Cancer Biomarkers

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BACKGROUND: The human kallikrein-related peptidase (KLK) family consists of 15 highly conserved serine proteases, which are encoded by the largest uninterrupted cluster of protease genes in the human genome. To date, several members of the family have been reported as potential cancer biomarkers. Although primarily known for their biomarker value in prostate, ovarian, and breast cancers, more recent data suggest analogous roles of KLKs in several other cancers, including gastrointestinal, head and neck, lung, and brain malignancies. Among the proposed KLK cancer biomarkers, prostate-specific antigen (also known as KLK3) is the most widely recognized member in urologic oncology.

CONTENT: Despite substantial progress in the understanding of the biomarker utility of individual KLKs, the current challenge lies in devising biomarker panels to increase the accuracy of prognosis, prediction of therapy, and diagnosis. To date, multiparametric KLK panels have been proposed for prostate, ovarian, and lung cancers. In addition to their biomarker utility, emerging evidence has revealed a number of critical functional roles for KLKs in the pathogenesis of cancer and their potential use as therapeutic targets.

SUMMARY: KLKs have biomarker utility in many cancer types but individually lack sufficient specificity or sensitivity to be used in clinical practice; however, groups of KLKs and other candidate biomarkers may offer improved performance.

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Molecular biomarkers of cancer may be used for screening, diagnosis, prognosis, tumor staging, monitoring of pharmacologic response to a therapeutic intervention, and establishing tumor recurrence or remission *(1)*. The interest in kallikrein-related peptidases (KLKs)[4] as cancer biomarkers dates back only 28 years to Papsidero's attempt to measure prostate-specific antigen (PSA) (also known as KLK3) quantitatively in serum and subsequent clinical work on the potential use of the protein as a marker of prostate cancer *(2)*. Since then, PSA has gained tremendous popularity as a prostate cancer biomarker. Given the structural similarity between PSA and other KLKs, the potential role of the remaining members of the family as cancer biomarkers has been widely investigated in recent years.

KLKs belong to the chymotrypisn (S1) family of serine proteases *(3)*. The first member of the KLK family was identified in the 1930s as the most abundant protease in pancreas and hence was named tissue "kallikrein," for pancreas (kallikreas) in Greek *(3)*. Subsequent independent work by Flocks, Ablin, Hara, Li and Beling, Sensabaugh, and Wang between 1960 and the late 1970s led to the discovery of the most well-characterized KLK, i.e., KLK3 (PSA) *(3)*. The KLK family of serine proteases was subsequently defined with the identification of another novel KLK, KLK2 (also known as human glandular kallikrein-1 or hGK-1) *(3)*. Further work from our laboratory and by others at the end of the pregenomic era during the mid 1990s eventually led to the characterization of an additional 12 novel serine protease genes, which are colocalized with the previously identified KLK-encoding genes at chromosomal region 19q13.4 of the human genome *(3)*. Because of their structural and sequence similarities to *KLK1*[5] (kallikrein 1), *KLK2* (kallikrein-related peptidase 2), and *KLK3* (kallikrein-related peptidase 3), these novel genes were also assigned to the *KLK* gene family *(3)*.

KLK genes share many common features, including exon/intron organization, number and length of exonic regions, intron phase, conserved translational start and stop sites, and the catalytic-triad codons *(4)*.

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[4] Nonstandard abbreviations: KLK, kallikrein-related peptidase; PSA, prostate-specific antigen; SNP, single-nucleotide polymorphism; fPSA, free PSA; tPSA, total PSA; MIC-1, macrophage inhibitor cytokine 1; MIF, migration inhibitor factor; CA125, cancer antigen 125; UPSC, uterine papillary serous carcinoma.
[5] Human genes: *KLK1*, kallikrein 1; *KLK2–KLK15*, kallikrein-related peptidases 2–15; *ADAMTS-1*, ADAM metallopeptidase with thrombospondin type 1 motif, 1; *ADAMTS-5*, ADAM metallopeptidase with thrombospondin type 1 motif, 5 (aggrecanase-2); *APAF1*, apoptotic peptidase activating factor 1; *CDKN1C*, cyclin-dependent kinase inhibitor 1C (p57, Kip2).

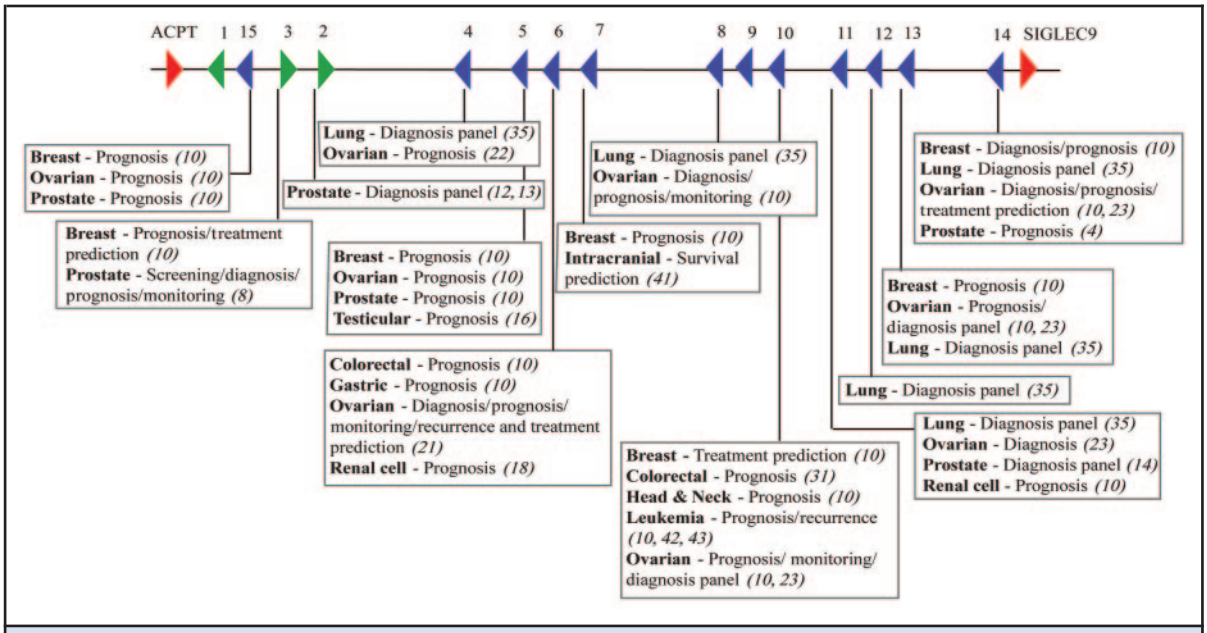

Fig. 1. Schematic presentation of the *KLK* locus and the potential utility of the various *KLK* genes as cancer biomarkers.

Listed biomarker applications have been based on reported differential expression of the respective genes. Note concurrent dysregulation of adjacent *KLK* genes in several cancer types, suggesting transcriptional regulatory mechanisms of groups of genes through common promoter regions. Numbers in parentheses indicate references.

These genes are expressed as single-chain preproenzymes of approximately 30–40 kDa *(4)*. Thus far, the crystal structures of the mature KLK1, KLK3, KLK4, KLK5–7, and pro-KLK6 proteins have been determined and shown to be highly similar *(4–6)*. These KLKs are folded into 2 hydrophobically interacting domains of 6-stranded β-barrels and an α-helix, with the catalytic triad located at the interface between the 2 domains.

According to the most comprehensive expression profiling completed recently, KLKs co-occur in a wide range of biological fluids and tissues, with varying levels of specificity *(7)*. Interestingly, KLKs are coordinately up- or down-regulated at both the transcriptional and protein levels in several neoplastic and nonneoplastic diseases, suggesting common regulatory pathways *(4)*. Furthermore, accumulating evidence is revealing a synergistic hormonal regulation of *KLK* gene transcription, indicating a mechanism passing through a single or a few locus-control regions *(4)*.

Dysregulated *KLK* gene expression has been implicated in cancer and other diseases. In particular, numerous clinical studies have linked the differential expression signatures of *KLK* genes to their potential roles as cancer biomarkers. In recent years, there has been substantial interest in devising multiparametric biomarker panels based on the coordinated dysregulation of groups of KLKs in various cancer types.

Our major objective in this mini-review is to summarize recent applications of KLKs as cancer biomarkers, with special emphasis on work published in the last 2 to 3 years and on multiparametric panels. For more comprehensive reviews of the KLK field, including physiology, pathobiology, genomics, and regulatory aspects, please see Table 1 in the Data Supplement that accompanies the online version of this Mini-Review at http://www.clinchem.org/content/vol54/issue10. Additional references pertaining to the role of KLKs as cancer biomarkers can be found in Fig. 1 and in Table 2 in the online Data Supplement.

Urinary Tract and Male Reproductive Cancers

PROSTATE CANCER

Prostate cancer is the most commonly diagnosed cancer among men in North America *(8)*. According to the latest report of the American Cancer Society, there has been a steady decline in mortality from this cancer since 1990. Although improved treatment options have undoubtedly contributed to this decline, early detection and better monitoring of the disease may also have contributed *(8)*. For instance, along with digital

rectal examination, the PSA test has become part of the routine medical checkup in many countries in recent years *(8)*; however, despite its rising popularity as a screening biomarker, the usefulness of the PSA test is still under scrutiny. The major limitation of this test is its relatively low specificity, especially when high sensitivity is desired (e.g., in screening programs) *(8)*. Because there is already a wealth of information on PSA as a cancer biomarker, we will not cover this subject any further in this mini-review.

To improve the clinical accuracy of PSA and other available biomarkers of prostate cancer, investigators have focused recent efforts on discovering a panel of markers. Several members of the KLK family show promise as clinical indicators of prostate cancer. For instance, on the basis of their differential patterns of transcript and/or protein production, *KLK* genes *2*, *4–6*, *10*, *11*, and *13–15* have been proposed as potential diagnostic and/or prognostic biomarkers of the disease *(9, 10)*. Considering the correlative expression of *KLK* genes *5*, *11*, *14*, and *15* with the Gleason grade of biopsied tumors *(10)*, these KLKs may function as prognostic markers. In the case of *KLK2*, 2 single-nucleotide polymorphisms (SNPs) have recently been identified as risk alleles in prostate cancer *(11)*. These SNPs are located at intron 1 and exon 5 of the *KLK2* gene and may have functional influence on *KLK2* expression levels *(11)*. Given their considerable association with high serum KLK2 concentrations and prostate cancer risk *(11)*, these SNPs may serve as complementary biomarkers of prostate cancer.

Given the individual utility of KLKs as biomarkers, there has been significant progress in designing multiparametric models for identifying potential panels of KLK biomarkers with greater sensitivity/specificity. For instance, logistic regression models that use the ratio of KLK2 to free PSA (fPSA) and the ratio of fPSA to total PSA (tPSA) have been shown to increase the specificity for distinguishing prostate cancer cases from benign prostatic hypertrophy in individuals with "gray zone" PSA (Table 1) *(12, 13)*. Similarly, artificial neural networks with tPSA, fPSA/tPSA, KLK2, KLK2/fPSA, KLK2/(fPSA/tPSA) as input factors have been suggested (Table 1) *(13)*. In addition, at the PSA concentration range of 2–10 μg/L, a combination of percent fPSA, KLK11, macrophage inhibitor cytokine 1 (MIC-1), and migration inhibitor factor (MIF) was found to significantly enhance the ability to distinguish between prostate cancer and benign prostatic hypertrophy (Table 1) *(14)*.

TESTICULAR CANCER

Testicular cancer is one of the most common cancers of young men (15–40 years) *(15)*. According to the National Cancer Institute, the testicular cancer rate has almost doubled among white men since the 1960s *(15)*. Despite the rising incidence of testicular cancer, not much effort has been made to further explore for new biomarkers of the disease. This situation is mainly due to the high survival rate (>95%) of symptomatic patients *(15)*. Regardless of the low mortality rate, however, the adverse effects of treatments on fertility make the identification of complementary biomarkers for early detection highly desirable, because less vigorous treatment is required at asymptomatic stages of the cancer.

Expression profiles of *KLK* genes in testicular cancer remain to be fully characterized. Preliminary data show that KLKs 5, 10, 11, 13, and 14 may be down-regulated in malignant tumors *(16)*. *KLK5* gene expression is clinically correlated with lower-stage tumors and thus may forecast a favorable prognosis *(10)*. Recent studies suggest that other KLKs, including KLK2 and KLK4, are produced in testes *(7)* and therefore may have potential as biomarkers for testicular cancer.

RENAL CELL CARCINOMA

Clinically, renal cell carcinoma presents many challenges in diagnosis and prognosis, because the majority of early-stage tumors are asymptomatic and may be detected only by imaging *(17)*. At presentation, approximately 30% of patients have disease in the advanced-metastatic stage that is refractory to conventional chemotherapy and radiation treatments *(17)*.

Emerging evidence underlines the importance of DNA-methylation patterns as a prognostic indicator of the disease *(17)*. Interestingly, several members of the KLK family were found to be dysregulated via methylation in various cancer types. For instance, hypermethylation of CpG islands of *KLK10* and *KLK6* have been reported in breast cancer and lymphoblastic leukemia *(9)*. Both KLK10 and KLK6 are consistently down-regulated in renal cell carcinoma *(10)*, suggesting a common regulatory mechanism. Similarly, KLK5 was found to be down-regulated in malignant tumors *(10)*. The immunohistochemical expression of these *KLK* genes has been shown to be correlated with tumor size and the histologic type of renal cell carcinoma. In addition, *KLK6* expression may predict a poor disease outcome, because such expression was found to be negatively correlated with disease-free survival *(18)*.

Gynecologic Cancers

OVARIAN CANCER

Ovarian tumors, particularly their most common epithelial subgroup, are difficult to detect. Therefore, such tumors are usually diagnosed in their advanced stages and have poor prognoses *(19)*. The majority of KLKs

Table 1. Multiparametric models of KLK and other biomarkers in human cancers.

Cancer	Tissue/fluid	Model	KLK and other biomarker panels	Clinical relevance	AUC[a]	Reference
Ovarian	Solid tumor	LR	CA125, B7-H4, KLKs 7, 10, 11, 13	Distinguishing primary tumors from healthy tissue	0.97	Zheng et al. *(23)*
	Ascites and pleural effusion	LR	KLKs 5–8, 10, 11, 13, 14	Distinguishing primary tumors from healthy tissue	0.99	Shih et al. *(24)*
	Ascites and pleural effusion	LR	KLKs 5–8, 10, 11, 13, 14	Distinguishing primary tumors from other cancers	0.96	Shih et al. *(24)*
	Solid tumor	LR	CA125, B7-H4, KLKs 4, 5, 7, 8, 11	Distinguishing primary tumors from benign growths	0.92	Zheng et al. *(23)*
	Solid tumor	LR	CA125, KLKs 8, 10, 13	Distinguishing primary tumors from nonovarian metastatic tumors	0.84	Zheng et al. *(23)*
	Solid tumor	LR	KLKs 6, 8, 11, 13	Predictor of 1 year free of disease progression	0.76	Zheng et al. *(23)*
	Solid tumor	LR	B7-H4, KLKs 6, 7, 11, 14	Predictor of 5 years free of disease progression	0.76	Zheng et al. *(23)*
	Solid tumor	LR	KLKs 6, 8, 13	Response to chemotherapy	0.75	Zheng et al. *(23)*
Lung	Serum	LR	KLKs 4, 8, 10–14	Distinguishing cancer cases from healthy tissue	0.90	Planque et al. *(35)*
Prostate	Serum, with PSA of 2–10 μg/L	LR	KLK2/fPSA, fPSA/tPSA	Distinguishing cancer cases from BPH	0.72	Magklara et al. *(12)*
	Serum, with PSA of 1–20 μg/L	ANN	tPSA, fPSA/tPSA, KLK2, KLK2/fPSA, KLK2/(fPSA/tPSA)	Distinguishing cancer cases from BPH	0.72 (tPSA, 1–4 μg/L) 0.74 (tPSA, 2–4 μg/L) 0.78 (tPSA, 4–10 μg/L) 0.83 (tPSA, 2–20 μg/L)	Stephan et al. *(13)*
	Serum, with PSA of 0.5–20 μg/L	LR	tPSA, %fPSA, MIF, MIC-1, KLK11, age, prostate volume (if available)	Distinguishing cancer cases from BPH	0.85 (tPSA, 0.5–20 μg/L) 0.83 (tPSA, 2–10 μg/L) 0.87 (tPSA, 0.5–20 μg/L)[b] 0.83 (tPSA, 2–10 μg/L)[b]	Stephan et al. *(14)*
	Serum, with PSA of 0.5–20 μg/L	ANN	tPSA, %fPSA, MIF, MIC-1, KLK11, age, prostate volume (if available)	Distinguishing cancer cases from BPH	0.86 (tPSA, 0.5–20 μg/L) 0.84 (tPSA, 2–10 μg/L) 0.91 (tPSA, 0.5–20 μg/L)[b] 0.88 (tPSA, 2–10 μg/L)[b]	Stephan et al. *(14)*

[a] AUC, (uncorrected) area under the ROC curve; LR, logistic regression; BPH, benign prostatic hypertrophy; ANN, artificial neural network.
[b] Groups with prostate volume available.

(i.e., KLKs 2–11 and 13–15), are reportedly dysregulated in ovarian cancer cells. Consistent with the cell-specific expression of the genes, the abundances of KLK proteins 5, 6, 8, 10, 11, and 14 and KLKs 5, 6, 7, 8, 10, 13 are dysregulated in serum and in effusion fluids, respectively *(10, 20)*. KLK6 seems to be the most promising candidate biomarker, because it was clinically associated with late stage, high grade, a serous histotype, residual tumor, disease-free survival, and response to chemotherapy *(21)*. Similarly to KLK6, KLKs 4, 5, 10, and 15 may function as indicators of an unfavorable prognosis for ovarian cancer *(10)*. In contrast, KLKs 8 and 14 may have some utility as favorable prognostic markers of the disease *(10)*. In addition to their possible prognostic values, these 2 KLKs, along with KLK11, may have diagnostic potential *(10)*. Finally, *KLK4* expression may serve as a marker for predicting resistance to paclitaxel-based therapy *(22)*.

According to a recent multiparametric analysis of the above-mentioned KLKs, B7-H4, and cancer antigen 125 (CA125) in ovarian tumor biopsies, the combination of KLK7, KLK10, KLK11, KLK13, B7-H4, and CA125 has been found to be superior to CA125 alone in distinguishing between nonpathologic tissues and metastatic tumors (Table 1) *(23)*. Furthermore, inclusion of KLKs 8, 10, and 13 enhanced the ability of CA125 to distinguish primary ovarian tumors from nonovarian metastatic tumors *(23)*. Similar findings were reported for ascites and pleural effusions in ovarian cancer (Table 1) *(24)*. In addition, disease progression at 1 year and 5 years was shown to be predicted more accurately with a panel of KLKs 6, 8, 11, and 13

and a panel of KLK6, KLK7, KLK11, KLK14, and B7-H4, respectively *(23)*. Follow-up experiments are required to verify the validity of the proposed biomarker panels in serum.

UTERINE PAPILLARY SEROUS AND CERVICAL CARCINOMA

Uterine papillary serous carcinoma (UPSC) is a small subtype of endometrial cancer with the highest chance of recurrence and therefore often represents a therapeutic challenge *(25)*. Both KLK6 and KLK10 have been shown to be up-regulated in the sera of patients with UPSC *(10)*. Similarly, *KLK8* expression is reportedly higher in endometrial carcinoma tissues, both at the transcript level and at the protein level *(20)*. *KLK6* in particular is differentially expressed in UPSC vs healthy tissue, as well as vs other subtypes of endometrial carcinomas *(10)*. The potential use of these KLKs in the diagnosis and monitoring of UPSC is currently under investigation.

Cervical cancer is the second most common cancer in women worldwide, with a high prevalence in underdeveloped countries *(26)*. Fortunately, there has been a significant decrease in the incidence and mortality of cervical cancer since the advent of the Papanicolaou (Pap smear) test in the 1940s *(26)*; however, alternative approaches to overcoming the financial and cultural limitations of Pap screening are still required, particularly in developing countries.

Expression profiling of the *KLK* gene family suggests possible involvement of several KLKs as biomarkers of cervical cancer. *KLK7* and *KLK8* show concurrent overexpression in tumor cells and primary tumor cultures, whereas *KLK10* seems to be down-regulated *(10, 27)*. Interestingly, parallel with the augmentation in KLK7 concentration in cervical adenocarcinoma tissues is an observed decline in the production of a KLK7 inhibitor known as antileukoprotease or secretory leukocyte proteinase inhibitor (ALP/SLPI) *(28)*. This finding may indicate an additional regulatory mechanism in neoplastic progression affecting the proteolytic activity of KLK7; however, the potential clinical importance and pathophysiological function of these KLKs remain to be fully elucidated.

Gastrointestinal Cancers

COLORECTAL CANCER

In 2003, the European Group on Tumor Markers established a new guideline on the clinical use of serum markers in colorectal cancer, mainly focusing on carcinoembryonic antigen *(29)*. Several additional markers, including thymidylate synthase, p53, K-*ras*, and microsatellite instability, have been proposed but their use is limited because of the lack of specificity and sensitivity *(29)*.

Bioinformatics analysis with the Serial Analysis of Gene Expression (SAGE) and expressed sequence tag (EST) database of the Cancer Genome Anatomy project has revealed differential expression of *KLK* genes *1*, *6*, *8*, and *10* in colorectal cancer tissues and cell lines *(30)*. Follow-up clinical studies on cancer tissues suggest a potential value of KLKs 6 and 10 as prognostic indicators of the disease, because their dysregulation pattern was correlated with advanced disease stages *(10, 31)*. Recently, caveolin-1, the primary structural protein of caveolae, has been implicated in the aberrant expression of *KLK6* in colon cancer *(32)*. Whether any of the other KLKs have clinical potential as biomarkers requires further elucidation.

ESOPHAGEAL, GASTRIC, AND PANCREATIC CANCERS

The *KLK* gene expression signature has been investigated for the other most common gastrointestinal cancers (i.e., esophageal, gastric, and pancreatic cancers). The up-regulation of KLK6 occurs in all 3 of these gastrointestinal carcinomas and confers an unfavorable prognostic value in gastric cancer *(10)*. Similarly, KLK1 is up-regulated in both esophageal and gastric cancer tissues *(10)*. Reports regarding *KLK10* expression are controversial, however. According to a recent study of gastric cancer tissues with in situ hybridization, the *KLK10* gene is hypermethylated and consequently expresses a reduced transcript concentration *(33)*. This finding contrasts with findings of previous reports of up-regulation of the protein in gastric cancer tissues and its suggested role as an indicator of an unfavorable prognosis. This apparent discrepancy might be attributed in part to the population heterogeneity of the disease; this issue requires further clarification.

Other Cancers

LUNG CANCER

There are currently several available lung cancer biomarkers that have potential application in risk assessment, early detection, treatment selection, prognosis, and monitoring of recurrence *(34)*. Because of their low sensitivity and specificity, however, these markers are not currently recommended for routine clinical use *(34)*.

There is growing evidence that KLKs may be useful as diagnostic/prognostic biomarkers of lung cancer. The up-regulation of the *KLK11* transcript in the C2 subgroup of neuroendocrine tumor tissues has been described to indicate a decreased likelihood of a favorable outcome *(35)*. Similarly, *KLK5* and *KLK10* have been shown to be overexpressed at the transcription level in the squamous cell carcinoma subtype of lung cancer *(35)*. Subsequent studies have identified additional KLKs, namely KLKs 7, 8, and 12–14, as potential

serum protein markers of lung cancer *(35)*. In particular, KLKs 11 and 12 appear to be positively correlated with disease stage *(35)*. Finally, according to a recently proposed multiparametric model, the combination of KLKs 4, 8, and 10–14, may increase the accuracy of detecting the non–small cell subtype of lung cancer (Table 1) *(35)*.

BREAST CANCER

Even though still in the early stages of translational study, several members of the KLK family have been proposed to function as biomarkers of breast cancer, although the investigation is still in the early stages of translational study. KLKs 1, 3, and 5–15 show aberrant expression in breast cancer cells and/or the sera of breast cancer patients *(9, 10)*. KLKs 5, 7, and 14 reportedly exhibit an enhanced and correlated production that is correlated with a poor prognosis *(10)*. In contrast, KLKs 3, 9, 13, and 15 may function as indicators of a favorable disease prognosis *(10)*. In addition, assays of cytosolic PSA in primary breast tumors may have some utility in predicting which patients will have a positive response to systemic tamoxifen therapy. Similarly, KLK10 was shown to function as an independent marker predicting the response to tamoxifen therapy *(10)*.

Although not fully understood, hormonal imbalance and epigenetic factors are believed to play a role in the observed transcriptional dysregulations *(9)*. As mentioned above, hypermethylation of the *KLK10* gene correlates with reduced production of the *KLK10* transcript. In situ hybridization analysis of breast tissues revealed a marked decrease of KLK10 in ductal carcinoma, with complete loss of the gene's expression in approximately 95% of infiltrating ductal carcinoma tissues *(10)*.

Lastly, the functional significance of KLKs in the pathobiology of breast cancer has been substantiated in recent years. For example, KLK11 has been shown to induce tumor progression in estrogen receptor–positive breast cancer cell lines through proteolysis of insulin-like growth factor–binding protein 3 (IGFBP-3) and the subsequent increase in the bioavailability of its ligand, IGF-I *(36)*. In contrast, PSA and KLK10 have been suggested to function as tumor suppressors of breast cancer *(4)*. The tumor-inhibitory effect of PSA is believed to be mediated mainly through activation of the latent form of transforming growth factor β *(4)*.

SALIVARY GLAND AND SQUAMOUS CELL CARCINOMAS

Neoplasms of the major and minor salivary glands pose major histopathologic limitations because of the overlapping histologic patterns of different tumor types and discrepancies between histologic classifications and their prognostic determinants *(37)*. According to a recent expression profiling of *KLK* genes in salivary gland tumors, higher expression of *KLK13* is observed in a number of the most common major and minor salivary gland tumors *(38)*. Interestingly, ductal cells and duct linings appear to express the *KLK13* gene at a higher level, compared with nonductal cells *(38)*. Conversely, KLK6 seems to be down-regulated in salivary gland tumor tissues *(39)*. No appreciable tumor-type specificity has been observed, however, limiting the use of these KLKs as prognostic markers. Given that other KLKs, including KLKs 1, 5, and 8 are produced in the salivary gland *(7)*, their potential clinical applications need to be examined further.

Similarly, the highly heterogeneous nature of the squamous subtype of head and neck cancers represents an inherent limitation in the treatment options for patients of the same risk group *(40)*. To overcome this obstacle, researchers have mainly focused on developing clinical indicators of prognosis and treatment response *(40)*. Although the research is at an early clinical phase, KLK10 shows promise in the prognosis of a subgroup of the disease with the worst clinical outcome *(10)*.

INTRACRANIAL CANCERS

Accumulating evidence implicates KLKs in various processes of neural and/or brain development. For instance, KLK8 is believed to be involved in long-term potentiation, which is required for hippocampus-associated memory formation *(9)*. Similarly, KLK6 may play a critical role in central nervous system development through the maintenance of myelination in oligodendrocytes *(9)*.

Given their function in the normal physiology of the central nervous system, it is tempting to speculate that KLK expression might be dysregulated in intracranial tumors and that KLKs may therefore be used as biomarkers of brain neoplasms. Increased expression of the *KLK7* transcript in tumor tissues was recently shown to be clinically associated with shorter overall survival times *(41)*. Whether KLK7 has clinical application in the prognosis of brain malignancies needs to be determined. Despite KLK8's well-established physiological role, cancer and healthy tissues showed no difference in *KLK8* expression in intracranial biopsy samples *(41)*.

ACUTE LYMPHOBLASTIC LEUKEMIA

Although translational research on the function of KLKs in leukemia is still in its infancy, the preliminary findings for the association of *KLK10* expression with the acute lymphoblastic subtype is encouraging. The expression of the *KLK10* gene is reportedly reduced in acute lymphoblastic leukemia *(42)*. This observed

lower expression was attributed to epigenetic gene silencing through hypermethylation of the CpG islands within the promoter, the 5′ untranslated region, and the coding regions of *KLK10 (42)*. Methylation of the *KLK10* gene was further associated with a poor prognosis and a higher chance of relapse *(42)*. A multiparametric analysis of methylation patterns of *KLK10* and 23 other genes, including *ADAMTS-1* (ADAM metallopeptidase with thrombospondin type 1 motif, 1), *ADAMTS-5* [ADAM metallopeptidase with thrombospondin type 1 motif, 5 (aggrecanase-2)], *APAF1* (apoptotic peptidase activating factor 1), and *CDKN1C* [cyclin-dependent kinase inhibitor 1C (p57, Kip2); also known as *P57*], demonstrated that approximately 76% of patients with T-cell acute lymphoblastic leukemia had more than 2 methylated genes *(43)*. Moreover, lack of methylation is reportedly correlated with a good prognosis, suggesting a potential utility of methylation profiling in assessing the risk of T-cell acute lymphoblastic leukemia *(43)*.

Conclusions

The KLK family of secreted serine proteases includes promising biomarker candidates for a wide range of malignancies. Although the investigations are mostly at early stages of clinical trials, numerous correlative studies have indicated aberrant *KLK* gene expression in various cancer types. Given their often coordinated dysregulation patterns, the current effort is directed at developing multiparametric KLK panels with superior biomarker applicability.

Despite substantial advances in the understanding of the clinical applications of the KLK family, their involvement in the pathophysiology of cancer is just beginning to be understood. As extracellular proteases, KLKs are believed to be involved in neoplastic initiation and/or progression through the remodeling of the tumor microenvironment or by influencing tumor growth, angiogenesis, invasion, and metastasis [for a comprehensive review, see *(9)* and *(4)*].

Advances in genomics and, more recently, proteomics and bioinformatics have provided an array of approaches to further identify the functional role and clinical use of this important family of proteases. Following future validation and blind (randomized) controlled trials, KLKs may provide novel clinical tools for early diagnosis and improving the prospects for cancer survival.

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