Ovarian Cancer

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Ovarian Cancer

Second Edition

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As we struggle to understand, treat, and cure this deadly disease, this book is dedicated to the women who suffer with ovarian cancer and those who love them.

Preface

This volume provides a set of comprehensive reviews from experts in the field on key clinical, translational, and basic research issues in ovarian cancer for clinicians and scientists. Chemoprevention, staging, and novel therapeutic targets are addressed in Part I with a series of reviews highlighting prevention strategies, surgical treatments, and translation of novel targets into clinical practice. Part II is focused on tumorigenesis and biomarkers. Reviews highlight genetic and epigenetic changes active in transformation of ovarian surface epithelium and biomarkers currently under investigation as diagnostic/prognostic indicators or therapeutic targets. Part III includes comprehensive overviews of tumor progression, metastasis, and translational research models. These reviews evaluate key signal transduction pathways in ovarian cancer, describe the novel adhesive microenvironment unique to ovarian tumors, and provide a comprehensive description of in vitro organotypic and in vivo murine models used to study ovarian cancer onset, progression, and metastasis.

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Chapter 8
The Human Kallikrein Gene Family: New Biomarkers for Ovarian Cancer

George M. Yousef and Eleftherios P. Diamandis

The Human Kallikrein Gene Family

Structure and Genomic Organization

The term "kallikrein" (derived from the Greek *kallikreas*, for pancreas) was introduced in the 1930s to describe proteolytic enzymes that can release small vasoactive peptides from high-molecular-weight precursors. There are two categories of human kallikreins; the plasma and the tissue kallikreins. The plasma kallikrein is encoded by a single gene on chromosome 4. This enzyme (a serine protease) releases the vasoactive peptide bradykinin from a high-molecular-weight precursor synthesized in the liver.[1] The human tissue kallikrein family is localized on chromosome 19 and also encodes for serine protease enzymes.[2–4]

Recently, a new classification emerged for tissue kallikreins that is not based on the functional definition but rather on structural criteria and map location. Based on the newer definition, the number of genes that are included in this family increased to 15, a number that is comparable with that of homologous families found in rat and mouse.[5,6] Because all kallikreins (except KLK1) do not have classic "kallikrein" activity, they are better defined as "kallikrein-related peptidases." A list of the official names of all kallikrein genes and proteins is included in Table 8.1, and a schematic diagram showing the human tissue kallikrein gene locus on chromosome 19q13.4 is shown in Fig. 8.1. All kallikrein genes map within an approximately 300-kb region, and the lengths of the genes, the distances between them, as well as the direction of transcription have now been accurately defined.[7,8] The kallikrein family is bounded from the telomeric side by the Siglec family of genes[9] and

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Table 8.1 Official and other gene and protein names for members of the human kallikrein gene family

Official Gene Symbol	Other Names/Symbols	GenBank Accession Number	UniGene Cluster	OMIM ID	SwissProt ID
KLK1	Pancreatic/renal kallikrein, hPRK	M25629 M33105	Hs.123107	147910	Q07276
KLK2	Kallikrein-related peptidase 2 Human glandular kallikrein 1, hGK-1	M18157	Hs.181350	147960	P20151
KLK3	Kallikrein-related peptidase 3 Prostate-specific antigen, PSA, APS	X14810 M24543 M27274	Hs.171995	176820	P07288
KLK4	Kallikrein-related peptidase 4 Prostase, KLK-L1, EMSP1, PRSS17, ARM1	AF113141 AF135023 AF148532	Hs.218366	603767	Q9Y5K2
KLK5	Kallikrein-related peptidase 5 KLK-L2, HSCTE	AF135028 AF168768	Hs.50915	605643	Q9Y337
KLK6	Kallikrein-related peptidase 6 Zyme, Protease M, Neurosin, PRSS9	AF013988 AF149289 U62801 D78203	Hs.79361	602652	Q92876
KLK7	Kallikrein-related peptidase 7 HSCCE, PRSS6	L33404 AF166330	Hs.151254	604438	P49862
KLK8	Kallikrein-related peptidase 8 Neuropsin; Ovasin; TADG-14, PRSS19, HNP	AB009849 AF095743 AB010780 AF055982	Hs.104570	605644	O60259
KLK9	Kallikrein-related peptidase 9 KLK-L3	AF135026	Hs.448942	605504	Q9UKQ9
KLK10	Kallikrein-related peptidase 10 NES1, PSSSL1	AF055481 NM_002776	Hs.69423	602673	O43240
KLK11	Kallikrein-related peptidase 11 TLSP/Hippostasin, PRSS20	AB012917	Hs.57771	604434	Q9UBX7
KLK12	Kallikrein-related peptidase 12 KLK-L5	AF135025	Hs.159679	605539	Q9UKR0
KLK13	Kallikrein-related peptidase 13 KLK-L4	AF135024	Hs.165296	605505	Q9UKR3
KLK14	Kallikrein-related peptidase 14 KLK-L6 protein	AF161221	Hs.283925	606135	Q9P0G3
KLK15	Kallikrein-related peptidase 15 Prostinogen, HSRNASPH	AF303046	Hs.250770	610601	Q9H2R5

OMIM, Online Mendelian Inheritance in Man.

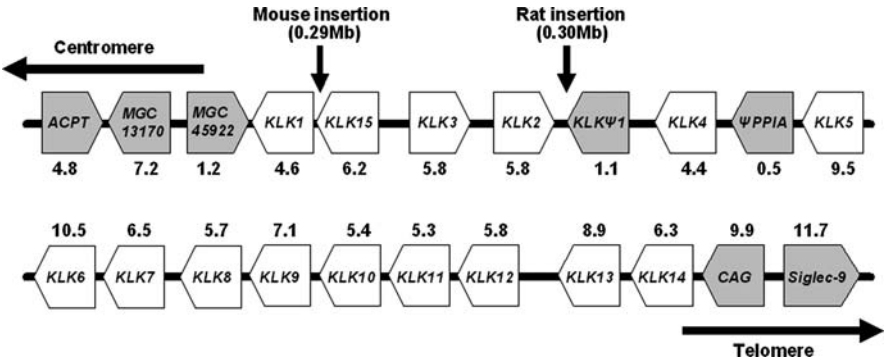

Fig. 8.1 Schematic presentation of the human kallikrein locus on chromosome 19q13.4. Gene locations are indicated by arrowheads that show the direction of transcription. Gene lengths are shown in kilobases. Position of the location of expanded regions in the mouse and rat genome that contain closely related *KLK1* paralogs is indicated. Non-kallikrein genes are shaded in gray

centromerically by the testicular acid phosphatase gene (ACPT).[10] New, uniform nomenclatures are now established for the human tissue kallikreins and their rodent orthologs.[11,12] There are many common structural features of the human kallikrein genes and proteins.[13] All genes are formed of five coding exons, and most of them have one or more extra 5′ untranslated exons. The first coding exon always contains a 5′ untranslated region, followed by the methionine start codon, located ~50 bp away from the end of the exon. The stop codon is always located ~156 bp from the beginning of the last coding exon. Moreover, exon sizes are nearly identical, and the positions of the residues of the catalytic triad of serine proteases are conserved. All kallikrein proteins are synthesized as a pre/pro peptides with a signal peptide of about 17–20 amino acids at the amino terminus, followed by an activation peptide of about 4–9 amino acids (with the exception of KLK5), followed by the mature (enzymatically active) protein. Finally, all proteins contain 10–12 cysteine residues that will form 5–6 disulfide bonds. The position of the cysteine residues is also fully conserved.

Tissue Expression and Hormonal Regulation

Many kallikreins are transcribed predominately in few tissues, as indicated by Northern blotting. By using the more sensitive RT-PCR technique, kallikreins are found to be expressed at lower amounts in several other tissues. The tissue expression of kallikrein mRNAs and proteins is summarized elsewhere.[14] Kallikrein abundance can be categorized as highly restricted (KLK2 and KLK3 in prostate), restricted (KLK5 in skin, salivary gland, breast, and esophagus; KLK6 in brain and central nervous system; KLK7 in esophagus, heart, liver, and skin; KLK8 in breast, esophagus, skin, and tonsil; KLK13 in

esophagus and tonsil), or wide (KLK1, 4, 9–12, 14, and 15). Interestingly, many kallikreins are expressed in endocrine-related organs, including the prostate, testis, ovary, and breast.[15]

In the ovary, there is abundant expression of the mRNA of KLK6–8 and KLK10, followed by lower levels of KLK1, 9, 11, 14, and 15. At the protein level, KLK1, 6, 7, 10–11 show highest expression levels, followed by KLK8 and KLK14.[14] Given the coexpression of many kallikreins in the same tissue, it is possible that these kallikreins may act in concert in cascade pathways, reminiscent of the coagulation and apoptotic processes.

Several reports confirmed that many kallikreins are under steroid hormone regulation.[16–18] An interesting observation is the different patterns of hormonal regulation in different tissues (e.g., KLK4 is upregulated by androgen in prostate and breast cancer cell lines and by estrogen in endometrial cancer cell lines).

Kallikreins in Normal Physiology

From a functional point of view, kallikreins are serine proteases (SPs). SPs are peptidases with a uniquely activated serine residue in the substrate-binding pocket. They are involved in many vital functions such as digestion, blood clotting, fibrinolysis, fertilization, and complement activation and are related to many diseases including cancer, arthritis, and emphysema.[19]

Accumulating evidence indicates that kallikreins might have diverse functions in different tissues and developmental stages. KLK1 has a known role in blood pressure regulation by cleaving low-molecular-weight kininogen to produce vasoactive kinin peptides. Intact kinin binds to bradykinin B_2 receptor in target tissues and exerts a broad spectrum of biological effects including blood pressure reduction via vasodilation, smooth muscle relaxation or contraction, pain induction, and mediation of the inflammatory response.[19] Low renal synthesis and urinary excretion of tissue kallikreins have been linked to hypertension in animals and humans.[20] Apart from its kininogenase activity, KLK1 has been implicated in the processing of growth factors and peptide hormones in light of its presence in pituitary, pancreas, and other tissues. As summarized by Bhoola et al.,[19] KLK1 has been shown to cleave proinsulin, low-density lipoprotein, prorenin, angiotensinogen, vasoactive intestinal peptide, procollagenase, and the precursor of atrial natriuretic factor.

KLK3 (also known as prostate specific antigen; PSA) has been shown to rapidly hydrolyze semenogelin I and semenogelin II, as well as fibronectin, resulting in liquefaction of the seminal clot after ejaculation.[21] Several other potential substrates for KLK3 have been identified, including IGFBP-3, TGF-β, parathyroid hormone-related peptide, and plasminogen.[22] KLK2 is found to be able to cleave semenogelin I and semenogelin II but at different cleavage sites and with lower efficiency than that of KLK3.[23] The mouse and porcine orthologs of KLK4 were originally designated "enamel matrix serine proteases" because of their predicted role in normal teeth development.[24] Recent evidence

shows that a splice variant of kallikrein 4 is a predominately nuclear protein that might have a role in controlling gene expression.[25]

A few kallikreins, especially KLK5 and KLK7, are expressed in the stratum corneum of the skin and are known to be involved in desquamation of corneocytes.[26] Another group of kallikreins, KLK6, KLK8, and KLK11, are highly expressed in the central nervous system where they are thought to play a role in neural plasticity.[27] Another possible mechanism for kallikrein action is the activation of proteinase-activated receptors (PARs). Activation of these receptors elicits different responses in several tissues. In addition, they switch-on cell signaling pathways (e.g., the MAP kinase pathway), leading to cell growth and division.[28]

Regulation of Kallikrein Activity

Kallikrein activity is controlled at both the mRNA and protein levels. Besides KLK3 and KLK2, and more recently KLK10, no other kallikrein gene promoter has been functionally tested. TATA box variants are found in the three classic kallikreins (KLK1–3).[29] Also, androgen response elements have been identified and experimentally verified.[30] No obvious TATA boxes are found in the promoter of other kallikreins. At the protein level, there are different mechanisms for controlling serine protease activity by which unwanted activation is avoided and precise spatial and temporal regulation of the proteolytic activity is achieved. One important mechanism is by producing kallikreins in an inactive "proenzyme" (or zymogen) form, which is activated as necessary. The N-terminal extension of the mature enzyme, or the "prosegment," sterically blocks the active site and thus prevents binding of substrates. The activation of the zymogen can occur intracellularly (i.e., in the trans-Golgi apparatus or in the secretory granules) or extracellularly after secretion, and it can be autolytic or dependent on the activity of another enzyme. Autoactivation is a common phenomenon among kallikreins. KLK2, but not KLK3, is capable of autoactivation.[31] KLK4 is also autoactivated during the refolding process, and there is evidence that KLK6 is also capable of autoactivation.[32]

Proteolytic activation is irreversible. Hence, other means of switching off the activity of these enzymes are needed. Once activated, serine proteases are controlled by ubiquitous endogenous inhibitors.[33] Some molecular complexes of kallikreins with protease inhibitors have clinical applicability because they can improve the diagnostic sensitivity or specificity of cancer biomarkers such as PSA.[34]

The coexpression of many kallikreins in the same tissues and the parallel differential regulation of groups of kallikreins in pathologic conditions raise the possibility of the existence of a common mechanism that controls expression of groups of kallikrein genes in a cluster, as a "locus control region." Added to this are the relatively short distances between adjacent kallikreins (which could be as short as the 1.5 kb between *KLK1* and *KLK15*) and the absence of classic promoter sequences, as shown by prediction analysis, in all kallikreins except *KLK1–3*.

Kallikreins can be also targeted by microRNAs (miRNAs). The first bioinformatic prediction of the potential interaction between miRNAs and kallikreins with experimental verification has been recently published.[35] miRNAs represent an important tool of posttranscriptional regulation of kallikrein activity that can explain aberrancies between the mRNA and protein expression levels.[36]

Kallikrein Expression in Cancer

Kallikreins as Cancer Biomarkers

Accumulating evidence indicates that many kallikreins are differentially expressed in various malignancies. KLK6 (zyme/protease M) was originally isolated by differential display from an ovarian cancer library,[37] and KLK10 was cloned by subtractive hybridization from a breast cancer library[38] and later proved to act as a tumor suppressor gene.[39]

A number of kallikreins were shown to be putative prognostic and/or predictive cancer markers. In breast cancer, the expression of *KLK5* and *KLK14* is indicative of poor patient prognosis,[40] whereas higher levels of *KLK9*, *KLK13,* and *KLK15* mRNA and the KLK3 protein forecast a favorable disease outcome.[41] The apparent relationship between kallikreins and testicular cancer has been published,[42] and the differential expression of *KLK10*, *KLK14,* and *KLK13* splice variants in testicular cancer tissues have also been reported.[43]

A microarray study has identified at least one kallikrein (*KLK11*) is overexpressed in lung carcinoma.[44] Recently, *in silico* analysis provided evidence that some kallikreins are differentially regulated in pancreatic cancer.[45] This was confirmed by microarray analysis.[46] Recent evidence also indicates overexpression of three kallikreins (*KLK7*, *KLK8*, and *KLK10*) in colon cancer.[45] Another report showed downregulation of the KLK10 gene in acute lymphoblastic leukemia.[47]

The potential clinical utility of kallikreins as cancer biomarkers has been proved by many reports. Prostate-specific antigen (KLK3) and, more recently, human glandular kallikrein (KLK2) are useful biomarkers for prostate cancer.[48] KLK11 is also shown to be a potential marker for ovarian and prostate cancer.[49] Recent reports demonstrate that kallikrein mRNA and proteins can be useful serum biomarkers for diagnosis, monitoring, and prognosis of different cancers.[15] In addition to their diagnostic/prognostic utilities, kallikreins have potential for being used for therapeutic applications. A synthetic KLK1 inhibitor is found to suppress cancer cell invasiveness in human breast cancer cell lines.[50]

An interesting observation is that many kallikreins were found to be dysregulated in malignancies of different tissues, for example, KLK5 in ovarian and breast cancer[51] and the downregulation of *KLK14* in multiple malignancies.[52] This lack of "tissue specificity" points to the possibility that kallikreins might be involved in a "common" pathway or biological process that is involved in cancer initiation and/or progression.

Differential Expression of Kallikreins in Ovarian Cancer

The dysregulation of kallikreins in ovarian cancer is well documented. KLK6 was isolated by differential display from an ovarian cancer library.[37] Kallikreins were identified among the top differentially expressed genes in ovarian cancer in a global analysis.[53] Recently, an *in silico* analysis of kallikrein gene expression in ovarian cancer was performed by using the databases of the Human Genome Anatomy Project. This study showed that at least seven kallikreins are upregulated in ovarian cancer compared with that in normal ovarian tissues. This was also confirmed at the protein level.[54] A review showing the prognostic value of many members of the human kallikrein family in ovarian cancer has been also published.[18]

Subcellular Localization of Kallikreins in Ovarian Cancer

Immunohistochemistry (IHC) enables kallikrein protein distribution in different cell types, independently from its quantity in the tissue. In addition, it provides a semiquantitative analysis of expression levels. Because kallikreins are secreted proteins, it was not unexpected that immunostaining of kallikreins was mainly cytoplasmic and in some tissues displayed a characteristic pattern that was membranous, droplet-like, supranuclear, subnuclear, or luminal. KLK4 appears to be a notable exception. Recently, Xi et al. suggested that one variant of KLK4 is a predominately nuclear protein that is overexpressed in prostate cancer.[25] Many kallikreins were analyzed by IHC and showed upregulation in ovarian cancer compared with that in normal ovarian tissues. KLK4 is localized to the cytoplasm of ovarian cancer, but not normal cells, with focal membranous staining.[55] KLK10 and KLK14 are found to have an intracytoplasmic pattern of staining in the epithelial cells (and occasional stromal cells) of serous ovarian cancers.[56,57] Underwood et al., using peptide antibody against the KLK8 protein, showed cytoplasmic granular staining (that might represent a secretion pathway) in tumor cells of different histologic types. In endometrioid carcinoma, the staining was most prominent in the glandular lumens.[58] KLK9 shows moderate cytoplasmic staining, with no nuclear or stromal staining pattern in ovarian cancer cells.[59]

Kallikrein Splice Variants in Ovarian Cancer

The mechanism by which a single gene gives rise to more than one mRNA transcript is referred to as differential splicing. This system is often tightly regulated in a cell type–specific or developmental stage–specific manner and increases genome complexity by generating different proteins from the same mRNA. The presence of more than one mRNA form for the same gene is common among kallikreins. These variant mRNAs may result from alternative splicing, a retained intronic segment, or use of an alternative transcription

initiation site. To date, there are at least 82 documented splice variants of the 15 kallikrein genes.[60] A better understanding of alternative splicing can lead to the use of gene variants as drug targets, therapeutic agents, or diagnostic markers.[60] Slawin et al. reported a prognostic significance of a splice variant–specific RT-PCR assay for *KLK2* in detecting prostate cancer metastasis.[61] Nakamura et al. reported differential expression of the brain and prostate types of *KLK11* between benign, hyperplastic, and malignant prostate cancer cell lines.[62] Some of the alternatively spliced forms were also found to be tissue specific.

Several kallikrein splice variants were identified in ovarian cancer. Dong et al.[55] identified three alternative splice forms of KLK4 expressed in ovarian cancer tumor tissues and cell lines, but not in normal ovaries: one with intronic insertion from intron 3; the second has intronic insertion from intron 2 and exon 4 deletion, and the third has deleted exon 4. A novel *KLK5* mRNA transcript with a short 5′ untranslated region and a *KLK7* splice variant with a long 3′ untranslated region are highly expressed in ovarian cancer cell lines but are expressed in very low levels in normal ovarian epithelial cells.[63] Another splice variant was identified, *KLK5-SV2*, which is overexpressed in ovarian cancer tissues and cell lines.[64] A recent report showed that *KLK6-splice variant 1* is expressed at much higher levels in ovarian cancer compared with the "classic" variant.[65]

Mechanisms of Kallikrein Involvement in Ovarian Cancer

The mechanism by which kallikreins might be involved in the pathogenesis and/or progression of ovarian cancer is not yet fully understood. Preliminary reports indicate a possible role of kallikreins in controlling vital processes, like apoptosis, angiogenesis, and tumor metastasis by cleavage of specific substrates, including growth factors, hormone receptors, or connective tissue. The involvement in growth and apoptotic activities was reported for KLK3 (PSA), which can digest insulin-like growth factor–binding protein (IGFBP-3)[66] and parathyroid hormone–related protein (PTHrP).

As proteolytic enzymes, kallikreins can be involved in tumor progression because of their role in extracellular matrix degradation. Many studies have shown that a variety of proteolytic enzymes are overproduced either by the cancer cells themselves or by the surrounding stromal cells, with an associated unfavorable clinical prognosis. Experimental evidence indicates that KLK2 and KLK4 can activate the proform of another serine protease, the urokinase-type plasminogen activator (uPA).[67] Urokinase activates plasmin from its inactive form (plasminogen), which is ubiquitously located in the extracellular space leading to degradation of the extracellular matrix proteins. Plasmin can also activate precursor forms of collagenases, thus promoting the degeneration of collagen in the basement membrane surrounding the capillaries and lymph nodes. Another kallikrein, KLK7, can degrade the alpha chain of human fibrinogen.[68]

Modulation of angiogenic activity is another possible mechanism for kallikrein involvement in cancer. The kinin family of vasoactive peptides, liberated by

KLK1 action, is believed to regulate the angiogenic process.[69] It was recently reported that immunolabeling of KLK1 is intense in the angiogenic endothelial cells derived from mature corpora lutea.[69] Also, KLK3 is reported to have anti-angiogenic activities.[70]

A recent study has shown that expression of kallikreins increases the malignant behavior of ovarian cancer cells.[71] Transfecting cancer cells with kallikreins led to significantly increased invasive behavior, and when these cells were inoculated into the peritoneum of nude mice, they resulted in a remarkable increase in tumor burden.

Ovarian cancer is a "hormone-related" malignancy. Sex hormones are known to affect its initiation and/or progression.[72] Oral contraceptive pills decrease the risk of ovarian cancer,[73] and the growth of ovarian carcinoma cell lines is sensitive to estrogen.[74] Progesterone promotes cell differentiation and apoptosis, and it has been shown to inhibit DNA synthesis and cell division.[75] Also, studies have shown a prognostic value of the progesterone receptor in ovarian cancer.[76] Moreover, appreciable evidence implicates androgens in the pathogenesis of ovarian cancer[77] and supports the existence of a physiologic interaction between androgens and the ovarian surface epithelium, as well as the possible role of this interaction in ovarian neoplasia.[78] Androgens have also been shown to stimulate growth of rodent ovarian epithelial cells in vivo, leading to benign ovarian neoplasms.[79] Ovarian cancer patients have higher levels of circulating androgens than do women without cancer.[80] Additionally, the majority of ovarian cancers express androgen receptor (AR),[81,82] and ovarian cancer cell growth is inhibited in vitro by antiandrogens.[83] Recent observations show a correlation between AR and susceptibility to ovarian cancer.[82] Given the fact that most kallikreins are regulated by sex hormones,[17] kallikreins could represent downstream targets by which steroids are involved in the malignant process. This, however, could not be verified in a recent study.[65] The elevation of serum concentration of kallikreins in cancer could also be due to the increased vasculature (angiogenesis) of cancerous tissues and the destruction of the glandular architecture of the tissues involved, with subsequent leakage of these proteins into the general circulation.

Clinical Utility of Kallikreins in Ovarian Cancer

Kallikreins as Diagnostic Markers

The clinical utility of kallikreins in ovarian cancer spans both the diagnostic and prognostic applications. For diagnostic purposes, many kallikreins have been shown to be elevated, at both the mRNA and protein levels, in ovarian cancer compared with patients with normal ovaries. Kallikreins can be measured in serum, tissue, or ascites fluid.[84] Reports on the diagnostic value of kallikreins in ovarian cancer are summarized in Table 8.2. Among all kallikreins, KLK6 and KLK10 show the best promise as serum biomarkers for ovarian cancer, specially the serous type.

Table 8.2 Kallikreins as diagnostic markers for ovarian cancer

Type of Analysis	Kallikrein	Clinical Significance	References
Protein in serum	KLK6	• Serum levels elevated in 66% to 68% of cancer patients • The diagnostic sensitivities at 90% and 95% specificity are 52% and 47%, respectively	90, 92
	KLK10	• Serum levels elevated in cancer compared with normal and benign disease (54% sensitivity at 90% specificity) • Elevated in 35% of CA-125–negative cancers (at 90% specificity) • Significantly associated with serous type	89
Protein in serum and ovarian tissue	KLK10	• A member of a multianalyte test for ovarian cancer diagnosis	88
	KLK14	• Elevated serum levels in 65% of ovarian cancer patients versus normal • Higher levels in 40% of ovarian cancer tissues compared with normal	57
Protein in ovarian tissue extract	KLK5	• Elevated in 55% of ovarian cancers compared with normal	97
mRNA from ovarian tissue	KLK4	• Elevated in 100% of serous carcinoma of late stage	55
	KLK6	• Significantly elevated in low-malignant-potential tumors and ovarian cancer	98
	KLK5, KLK7	• Significantly elevated in ovarian cancer, especially serous type	63
	KLK7	• Elevated in 67% of low-malignant-potential tumors and 78% of carcinomas	99
	KLK8	• Overexpressed in 67% of ovarian cancers and 40% of low-malignant-potential tumors compared with normal	58
	KLK10	• Significantly elevated in 91% of serous cancers, 73% of nonserous cancers, and 73% of primary peritoneal carcinoma compared with normal	100
	KLK14	• Downregulated in ovarian cancer • Stepwise decrease in normal > benign > cancer	52, 101
IHC of ovarian cancer	KLK6 and KLK10	• Expressed in 100% of CA-125–negative cancers	87
Protein from ascites fluid	KLK5–8, 10, 11, 13, 14	• Ovarian cancer ascites contained higher levels compared with benign effusions and ascites from other cancer types	86
Microarray	KLK5–8	• Among the top upregulated genes in ovarian cancer compared with normal tissue and other diseases	102

Table 8.2 (continued)

	KLK6–8, 10, 11	• Significantly upregulated in ovarian cancer	103
	KLK6–8, 10, 11	• Overexpressed in ovarian cancer	104
Bioinformatics analysis	KLK5–8, 10,11, 14	• Parallel overexpression in ovarian cancer compared with normal	54
	KLK6	• Elevated 25-fold in ovarian cancer	53, 63

Recent reports indicate the potential diagnostic utility of kallikreins in ascites fluid. Among kallikreins, KLK6–10 showed the highest statistical power in distinguishing ovarian cancer ascites from that of benign causes and other cancer groups. It was shown that kallikreins could identify false-negative cases of cytology.[85,86] Combinations of kallikreins achieved areas under the receiver operating characteristics (ROC) curve of 0.994 and 0.961 in separating ovarian cancer from benign effusion and from other cancer groups, respectively.[86]

The diagnostic utility of kallikreins can also extend to their use as immunohistochemical markers. A recent study has shown that in ovarian cancers that lacked CA125 expression by IHC, all specimens (100%) expressed KLK10 and KLK6.[87] Kallikrein mRNAs are also detected in circulating tumor cells in the blood and ascites fluid of ovarian cancer patients, but this application lacked sensitivity and specificity for detecting disseminated disease.[85]

Although the sensitivity and specificity of individual kallikrein proteins are not superior to that of standard markers, like CA-125, the use of kallikreins as a part of a multianalyte test significantly improves the diagnostic sensitivity and specificity.[88] In patients with early-stage cancer (stage I/II), the combination of CA-125 and KLK10 results in 21% increase in sensitivity compared with that of CA-125 alone.[89] The combination of KLK6 and CA-125 can also lead to improved sensitivity of detection of early-stage disease. When combined with CA-125, at 90% specificity, sensitivity increases to 72% (for all patients) and to 42% in stage I or II disease.[90] A recent review included kallikreins among the most promising new markers that are now being investigated to complement CA-125 for ovarian cancer diagnosis/prognosis.[91]

Another interesting application is distinguishing benign and borderline ovarian tumors. Preliminary reports indicate that KLK10 and KLK6 can have better ability than CA-125 in distinguishing ovarian cancer from benign ovarian tumors.[89,90]

Prognostic Applications

Clinical utility of kallikreins in ovarian cancer extends beyond diagnosis. Table 8.3 summarizes published data about the prognostic utility of different kallikreins in ovarian cancer. These data show that a group of kallikreins,

Table 8.3 Prognostic utility of kallikrein genes/proteins in ovarian cancer

Kallikrein Gene/Protein	Sample Type	Analysis Method	Prognostic Value	References
KLK4	mRNA	RT-PCR	*Unfavorable* prognosis: • overexpressed in late-stage, higher-grade tumors and no response to chemotherapy • associated with shorter DFS and OS • independent indicator of poor prognosis in patients with low-grade tumors	105
	Protein from effusion cells and solid tumor	IHC and immunoblotting	*Favourable* prognosis: • lower in grade IV compared with grade III • associated with longer OS	106
KLK5	mRNA	RT-PCR	*Unfavorable* prognosis: • overexpressed in late-stage and higher-grade tumors • associated with shorter DFS and OS • independent indicator of poor prognosis in patients with low-grade tumors	107
	mRNA and protein	SQ-RT-PCR, Southern, Northern, and Western blots and immunohistochemistry	*Unfavorable* prognosis: • overexpressed in ovarian tumor tissues and cell lines mainly of late stage and serous histotype	63
	Ovarian cancer cytosols	Immunoassay	*Unfavorable* prognosis: • overexpressed in patients with late-stage and higher-grade tumors • associated with shorter DFS and OS	108

Table 8.3 (continued)

Kallikrein Gene/Protein	Sample Type	Analysis Method	Prognostic Value	References
			• independent indicator of poor prognosis in patients with high-grade tumors and optimal debulking success	
KLK6	Ovarian cancer cytosols	Immunoassay	*Unfavorable* prognosis: • overexpressed in late-stage and serous tumors • associated with shorter DFS and OS • independent indicator of poor prognosis in low-grade tumors and optimal debulking success	109
	Serum from normal women, women with benign disease, and women with ovarian cancer	Immunoassay	*Unfavorable* prognosis: • higher serum levels in late-stage, higher-grade, serous tumors, suboptimal debulking, and a poor response to chemotherapy • indicator of decreased DFS and OS	90
KLK7	Ovarian cancer cytosols	Immunoassay	*Unfavorable* prognosis: • overexpressed in advanced stage, higher grade, suboptimal debulking, and serous or undifferentiated histotypes • associated with significantly shorter DFS but not OS • not an independent prognosticator for ovarian cancer	110
	mRNA from normal, benign, and cancerous ovarian tissues and late-stage	SQ-RT-PCR, Southern, Northern, and Western blots and immunohistochemistry	*Unfavorable* prognosis: • overexpressed in ovarian tumor tissues and cell lines mainly of late-stage and serous histotype	63

Table 8.3 (continued)

Kallikrein Gene/Protein	Sample Type	Analysis Method	Prognostic Value	References
	serous ovarian cancer cell lines			
	mRNA from cancerous ovarian tissue	Q-RT-PCR	*Unfavorable* prognosis: • overexpressed in higher-grade tumors • associated with shorter DFS • independent indicator of poor prognosis in patients with low-grade tumors and optimal debulking success	111
KLK8	mRNA from ovarian cancer tissues	RT-PCR	*Favorable* prognosis: • overexpressed in lower-grade tumors • associated with longer DFS and OS • independent indicator of longer DFS	112
	Protein from ovarian cancer cytosols	Immunoassay	*Favorable* prognosis: • overexpressed in lower grade, no residual tumor, and optimal debulking success • associated with significantly longer DFS and OS • an independent marker of favorable prognosis	113
	mRNA from ovarian cancer tissues	RT-PCR	*Favorable* prognosis: • overexpressed in younger age, lower-grade, and early-stage tumors	114
KLK9	mRNA from ovarian cancer tissues	Q-RT-PCR	*Favorable* prognosis: • overexpressed in early stage and optimal debulking success • associated with longer DFS and OS	59

Table 8.3 (continued)

Kallikrein Gene/Protein	Sample Type	Analysis Method	Prognostic Value	References
			• independent indicator of prolonged DFS in patients with low-grade tumors and optimal debulking success	
KLK10	Normal, benign, and cancerous ovarian cytosols	Immunoassay	*Unfavorable* prognosis: • overexpressed in cancer patients with late-stage, serous tumors and suboptimal debulking success • associated with shorter DFS and OS • independent indicator of DFS and OS in patients with late-stage tumors	56
	Serum from normal women, women with benign disease, and women with ovarian cancer	Immunoassay	*Unfavorable* prognosis: • higher serum levels in late-stage, advanced-grade, large residual tumor, suboptimal debulking, and poor response to chemotherapy • indicator of decreased DFS and OS • independent indicator of OS	89
KLK11	Proteins of ovarian tumor extracts	Immunoassay	*Favorable* prognosis: • overexpressed in early-stage and low-grade tumors • associated with longer DFS and OS • independent indicator of DFS	115
	Ovarian cancer cytosols	Immunoassay	*Favorable* prognosis: • overexpressed in patients with early-stage disease, and good response to chemotherapy • associated with longer DFS and OS	116

Table 8.3 (continued)

Kallikrein Gene/Protein	Sample Type	Analysis Method	Prognostic Value	References
			• independent indicator of OS • independent indicator of DFS and OS in patients with low-grade tumors	
KLK13	Ovarian cancer cytosols	Immunoassay	*Favorable* prognosis: • overexpressed in early stage, no residual tumor after surgery, and optimal debulking success • independent indicator of longer DFS and OS	117
KLK14	mRNA from normal, benign, and cancerous ovarian tissues	Q-RT-PCR	*Favorable* prognosis: • overexpressed in early stage, optimal debulking success, and good response to chemotherapy • independent indicator of longer DFS and OS	101
KLK15	mRNA from benign and cancerous ovarian tissues	Q-RT-PCR	*Unfavorable* prognosis: • independent indicator of decreased DFS and OS	118

IHC, immunohistochemistry; RT-PCR, reverse transcriptase–polymerase chain reaction; DFS, disease-free survival; OS, overall survival; SQ-RT-PCR, semiquantitative RT-PCR; Q-RT-PCR, quantitative RT-PCR.

namely *KLK4–7*, *KLK8*, *KLK10,* and *KLK15*, are markers of poor prognosis in ovarian cancer. That is, higher kallikrein mRNA and/or protein levels correlate with more aggressive forms of the disease and a decreased disease-free survival (DFS) and overall survival (OS) of patients. The remaining subset of kallikreins, namely *KLK8, KLK9*, *KLK11*, and *KLK13–14*, seem to be markers of favorable prognosis, with higher levels of their mRNA or proteins associated with earlier-stage disease and increased DFS and OS.

Data from a recent report suggested that KLK6 might have value for patient monitoring in ovarian cancer.[92] A recent pilot study shows that some kallikrein proteins could be included with other clinical variables to develop a multiparametric "score" that can predict the surgical outcome and thus help in preoperative risk stratification and identifying candidates for alternative or adjuvant therapeutic strategies.[93] Another study showed evidence that a group of kallikreins and multiparametric combinations with other biomarkers and clinical variables can significantly assist with ovarian cancer classification, prognosis, and response to platinum-based chemotherapy.[94]

Kallikrein expression in ovarian cancer may also be clinically useful in determining the prognosis in subgroups of patients. Subclassification of large heterogeneous groups into smaller subgroups is becoming an important tool for individualizing treatment options in ovarian cancer patients and thus avoiding unnecessary treatments with high costs and unwanted side effects.

Therapeutic Applications

It is possible that some kallikreins may become valuable therapeutic targets when the biological pathways that are involved are delineated. For example, the enzymatic activity of these serine proteases may initiate or terminate biological events (e.g., tumor invasion, angiogenesis, activation or inhibition of hormones, growth factors, other enzymes, receptors, or cytokines). Once known, these events could be manipulated, for therapeutic purposes, by specific enzyme inhibitors or activators. Another potential therapeutic approach is the cell-specific activation of therapeutic agents.[95] Preliminary reports show potential success by using the KLK3 (PSA) promoter to express molecules in a tissue-specific fashion.[96] A third possible therapeutic approach involves immunotherapy and/or development of cancer vaccines. With our increasing knowledge of the hormonal regulation of kallikreins, hormonal activation (or repression) of kallikrein activity could be investigated in the future.

Conclusion

Accumulating evidence, at both the mRNA and protein levels, indicates that many kallikreins are differentially expressed in ovarian cancer. The mechanism by which kallikreins are involved in the pathogenesis and/or progression of

cancer is not fully understood and is possibly through controlling vital processes, like apoptosis, angiogenesis, and tumor metastasis, by cleavage of specific substrates. Kallikreins can be measured in serum, tissue, and ascites fluid of ovarian cancer patients. Reports show that kallikreins can be useful serum biomarkers for diagnostic, monitoring, and prognostic purposes. They can be also useful immunohistochemical markers. In addition, kallikreins have potential for being used for therapeutic applications. KLK6 and KLK10 show the best promise as serum biomarkers for ovarian cancer. Although kallikreins might not have superior sensitivity and specificity to that of existing markers, the use of kallikreins as a part of a multianalyte test significantly improves the diagnostic and prognostic accuracy. Further large-scale studies are needed to evaluate the applicability of this approach.

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