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Review

Kallikrein-related peptidases in prostate, breast, and ovarian cancers: from pathobiology to clinical relevance

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Abstract

Tissue kallikrein (KLK1) and kallikrein-related peptidases (KLK2–15) comprise a family of 15 highly conserved secreted serine proteases with similar structural characteristics and a wide spectrum of functional properties. Both gene expression and protein activity of KLKs are rigorously controlled at various levels *via* diverse mechanisms, including extensive steroid hormone regulation, to exert their broad physiological role. Nevertheless, deregulated expression, secretion, and function of *KLK* family members has been observed in several pathological conditions and, particularly, in endocrine-related human malignancies, including those of the prostate, breast, and ovary. The cancer-related abnormal activity of KLKs upon substrates such as growth factors, cell adhesion molecules, cell surface receptors, and extracellular matrix proteins facilitate both tumorigenesis and disease progression to the advanced stages. The well-documented relationship between KLK status and the clinical outcome of cancer patients has led to their identification as promising diagnostic, prognostic, and treatment response monitoring biomarkers for these complex disease entities. The main objective of this review is to summarize the existing knowledge concerning the role of KLKs in prostate, breast, and ovarian cancers and to highlight their continually evolving biomarker capabilities that can provide significant benefits for the management of cancer patients.

Keywords: biomarkers; epithelial-mesenchymal transition; extracellular matrix proteases; KLK; PSA; serine proteases.

Introduction

The study of kallikreins initiated in the 1930s, when Kraut, Frey, and Werle identified a factor of pancreatic (*kallikreas* in Greek) extracts able to release the vasoactive decapeptide kallidin (lys-bradikinin) from low-molecular-weight kininogen (Kraut et al., 1930; Werle et al., 1937). This peptidase was latter named tissue kallikrein (KLK1) to distinguish it from plasma kallikrein (KLKB1), which is secreted by the liver into the circulation and also possesses kininogenase activity. Almost 70 years was needed thereafter to complete the discovery of the human kallikrein family of proteases. Until the early 1990s, only three members of the kallikrein gene family were characterized: *KLK1*, *KLK2* (glandular kallikrein), as well as the most renowned member *KLK3* (prostate-specific antigen, or PSA). Subsequently, a large number of novel kallikrein genes were identified. Today, the human kallikrein gene family consists of 15 serine proteases encoding genes (*KLK1–15*) colocalized in the 19q13.3–13.4 chromosomal region without any interference from non-kallikrein-related genes (Gan et al., 2000; Harvey et al., 2000; Yousef et al., 2000).

The human kallikrein gene family encodes for 15 homologous secreted serine proteases (KLK1–15) possessing trypsin- (KLK1, 2, 4–6, 8, 10–15) or chymotrypsin- (KLK3, 7, and 9) like activities. Despite the significant homology between the family members, only KLK1 exhibits substantial kininogenase activity, leading to the designation of the KLK2–15 family members as kallikrein-related peptidases (Lundwall et al., 2006). The proteolytic function of KLKs is robustly regulated. Specifically, at the transcriptional level, the expression of *KLK* genes relies on the control of steroids hormones (Lawrence et al., 2010), as well as the epigenetic modifications of *KLK* promoters. More recently, microRNA (miRNA)-mediated control of *KLKs* expression was identified as a novel posttranscriptional regulatory mechanism (Yousef, 2008). As far as the posttranslational level is concerned, KLKs are synthesized and secreted as longer inactive precursors (pro-KLKs), the activation of which depends on their tissue-specific proteolytic cleavage, to release the enzymatic active polypeptide (Borgono et al., 2004).

The regulated tissue-specific expression and activation of KLKs enable them to participate in a great number of diverse physiological procedures, such as the regulation of blood pressure (KLK1-mediated cleavage of kininogenase) (Bhoola et al., 1992), semen liquefaction (PSA/KLK3- and KLK2-mediated cleavage of semenogelins) (Pampalakis and Sotiropoulou, 2007), skin desquamation (KLK5-, KLK7-, and KLK14-mediated digestion of desmoglein, desmocollin, corneodesmosin) (Borgono et al., 2007a), tooth maturation (KLK4-mediated processing of enamelin) (Lu et al., 2008), and innate immunity (KLK5- and KLK7-mediated activation of cathelicidin antimicrobial peptide) (Yamasaki et al., 2006).

Bearing in mind the irreversible impact of proteases upon their substrates, as well as the launch of downstream enzyme cascades that lead to the amplification of the initial stimuli, the regulation of KLKs activity becomes crucial for their beneficial physiological function. An enormous amount of evidence highlights the abnormal regulation of KLKs in cancer, in terms of *KLKs* gene expression, as well as KLKs synthesis, secretion, and activation (Borgono and Diamandis, 2004; Emami and Diamandis, 2007; Mavridis and Scorilas, 2010), and reveals also their diagnostic and prognostic clinical exploitation (Obiezu and Diamandis, 2005; Paliouras et al., 2007; Avgeris et al., 2010; Mavridis and Scorilas, 2010). This deregulation is more evident and further studied in endocrine-related tumors – a fact which can be partially explained by the transcriptional control of *KLKs* by steroid hormones. The abnormal activity of KLKs upon substrates, such as proteases [pro-KLKs, matrix metalloproteinases (MMPs), urokinase-type plasminogen activator (uPA)], cell surface receptors [protease-activated receptors (PARs), uPA receptor (uPAR)], growth factors [insulin-like growth factors binding proteins (IGFBPs), latent transforming growth factor β (TGFβ)], and hormones [parathyroid hormone-related protein (PTHrP)] triggers the accumulation of tumorigenic stimuli in the tissue microenvironment. Moreover, the KLK-mediated degradation of extracellular matrix (ECM) proteins facilitates the disruption of physical barriers against tumor cell invasion, as well as other cancer hallmarks such as angiogenesis and metastasis.

In the present review, we describe the involvement of KLKs during the development of the endocrine-related malignancies of the prostate, breast, and ovary to unravel their potential role in tumorigenesis and cancer progression (Table 1). Moreover, we summarize the scientific benefits from the extensive study of KLKs cancer biomarker capability, for the clinical management of cancer patients (Table 2).

Role of kallikrein-related peptidases in cancer pathobiology

Kallikrein-related peptidases and tumor growth

The KLK-dependent degradation of ECM proteins was first considered to be the most essential part of the family's involvement into the pathophysiology of cancer, facilitating tumor cells' invasion and metastasis. However, more recent studies regarding KLK activity allowed the identification of novel candidate substrates and pointed out their crucial role at the initial stages of tumorigenesis and tumor cells growth (Table 1).

The insulin-like growth factor (IGF) axis induces mitogenic and antiapoptotic effects upon normal and tumor cells,

Table 1 KLKs' implication in prostate, breast, and ovarian cancer pathobiology.

Family member	Role in pathobiology	References
Prostate cancer		
KLK1	Promotion of cell invasiveness; angiogenesis	Emanueli et al., 2001; Giusti et al., 2005; Gao et al., 2010
KLK2	Tumor growth promotion; ECM degradation	Deperthes et al., 1996; Takayama et al., 1997; Mikolajczyk et al., 1999; Rehault et al., 2001; Mize et al., 2008
PSA/KLK3	Tumor growth promotion; EMT-like changes; ECM degradation; angiogenesis; metastasis	Webber et al., 1995; Cramer et al., 1996; Fortier et al., 1999; Sun et al., 2001; Yonou et al., 2001; Koistinen et al., 2002; Ishii et al., 2004; Pezzato et al., 2004; Romanov et al., 2004; Dallas et al., 2005; Veveris-Lowe et al., 2005; Goya et al., 2006; Nadiminty et al., 2006
KLK4	Tumor growth promotion; EMT-like changes; ECM degradation; metastasis	Takayama et al., 2001; Matsumura et al., 2005; Veveris-Lowe et al., 2005; Beaufort et al., 2006; Gao et al., 2007; Klokk et al., 2007; Mize et al., 2008; Ramsay et al., 2008a; Wang et al., 2010
KLK7	EMT-like changes; promotion of cell invasiveness	Mo et al., 2010
KLK14	Tumor growth promotion; ECM degradation	Borgono et al., 2007b
Breast cancer		
KLK1	Promotion of cell invasiveness; angiogenesis	Emanueli et al., 2001; Wolf et al., 2001; Giusti et al., 2005
PSA/KLK3	Tumor suppressor	Lai et al., 1996
KLK6	Tumor suppressor; inhibition of cells invasiveness; antiangiogenesis	Bernett et al., 2002; Pampalakis et al., 2009
KLK10	Tumor suppressor	Goyal et al., 1998; Papageorgis et al., 2010; Mian et al., 2011
Ovarian cancer		
KLK4–6	ECM degradation; promotion of cell invasiveness	Takayama et al., 2001; Bernett et al., 2002; Michael et al., 2005; Prezas et al., 2006
KLK7	Tumor growth promotion; ECM degradation; promotion of cell invasiveness; chemoresistance	Prezas et al., 2006; Dong et al., 2010
KLK10	Tumor growth promotion	White et al., 2010
KLK14	Tumor growth promotion; ECM degradation	Borgono et al., 2007b; Zhang et al., 2012

Table 2 Clinical relevance of KLKs in prostate, breast, and ovarian tumors.

Family member	Clinical relevance as biomarker	References
Prostate cancer		
KLK2	Diagnosis; unfavorable prognosis	Kwiatkowski et al., 1998; Magklara et al., 1999; Nam et al., 2000; Steuber et al., 2007
PSA/KLK3	Screening; diagnosis; treatment monitoring; unfavorable prognosis	Stephan et al., 2007; Lilja et al., 2008; Ulmert et al., 2009; Avgeris et al., 2010
KLK4	Unfavorable prognosis	Avgeris et al., 2011b
KLK5	Favorable prognosis	Yousef et al., 2002b; Korbakis et al., 2009
KLK11	Diagnosis; favorable prognosis	Diamandis et al., 2002; Nakamura et al., 2003a,b; Bi et al., 2010
KLK14	Unfavorable prognosis	Yousef et al., 2003e; Rabien et al., 2008
KLK15	Unfavorable prognosis	Mavridis et al., 2010a; Rabien et al., 2010
Breast cancer		
PSA/KLK3	Diagnosis; treatment response; favorable prognosis	Yu et al., 1998; Foekens et al., 1999; Black et al., 2000; Sauter et al., 2004a,b
KLK4	Unfavorable prognosis	Papachristopoulou et al., 2009
KLK5	Diagnosis; unfavorable prognosis	Yousef et al., 2002c, 2003b; Li et al., 2009; Avgeris et al., 2011a; Talieri et al., 2011
KLK7	Unfavorable prognosis	Talieri et al., 2004; Li et al., 2009
KLK9	Favorable prognosis	Yousef et al., 2003d
KLK10	Diagnosis; treatment response; favorable prognosis	Luo et al., 2002; Ewan King et al., 2007; Kioulafa et al., 2009
KLK13	Favorable prognosis	Chang et al., 2002
KLK14	Diagnosis; unfavorable prognosis	Yousef et al., 2002a; Borgono et al., 2003b; Fritzsche et al., 2006; Papachristopoulou et al., 2011
KLK15	Favorable prognosis	Yousef et al., 2002d
Ovarian cancer		
KLK4	Treatment response; unfavorable prognosis	Obiezu et al., 2001; Xi et al., 2004
KLK5	Diagnosis; unfavorable prognosis	Kim et al., 2001; Diamandis et al., 2003a; Dong et al., 2003; Yousef et al., 2003b; Oikonomopoulou et al., 2008b; Dorn et al., 2011a,b
KLK6	Diagnosis; unfavorable prognosis	Diamandis et al., 2000, 2003b; Hoffman et al., 2002; Shih Ie et al., 2007; Kountourakis et al., 2008; Kuzmanov et al., 2009; White et al., 2009; Dorn et al., 2011a; Koh et al., 2011
KLK7	Unfavorable prognosis; treatment response	Dong et al., 2003, 2010; Kyriakopoulou et al., 2003; Shih Ie et al., 2007; Dorn et al., 2011a
KLK8	Diagnosis; prognosis	Magklara et al., 2001; Kishi et al., 2003; Shigemasa et al., 2004b; Borgono et al., 2006; Shih Ie et al., 2007; Kountourakis et al., 2009; Dorn et al., 2011a
KLK9	Favorable prognosis	Yousef et al., 2001
KLK10	Diagnosis; unfavorable prognosis	Luo et al., 2001b, 2003; Shih Ie et al., 2007; Oikonomopoulou et al., 2008b; Batra et al., 2010; Dorn et al., 2011a; Koh et al., 2011
KLK11	Diagnosis; prognosis; treatment response	Borgono et al., 2003a; Diamandis et al., 2004; Shigemasa et al., 2004a; McIntosh et al., 2007; Zheng et al., 2007; Dorn et al., 2011a
KLK13	Prognosis; treatment response	Scorilas et al., 2004; Zheng et al., 2007; White et al., 2009
KLK14	Diagnosis; favorable prognosis; treatment response	Borgono et al., 2003b; Yousef et al., 2003a
KLK15	Unfavorable prognosis	Yousef et al., 2003c

mediated through the attachment of IGFs (IGF1 and IGF2) to the transmembrane IGF receptors (IGFR1 and IGFR2) (Samani et al., 2007). Nonetheless, the availability of IGFs and their interaction with IGFRs is regulated by the IGFBPs (IGFBP1–6), a family of six high-affinity IGF-binding proteins. PARs, a G-protein-coupled cell surface receptor subfamily (PAR1–4), are activated through a cleavage, mediated exclusively by serine proteases with trypsin-like activity, within their N-terminus extracellular domain (Adams et al., 2011). Apart from their physiological roles, PARs can intervene in cancer-associated molecular cascades affecting cell proliferation and migration (Hollenberg et al., 2008; Oikonomopoulou et al., 2010b). Given that IGFBPs and PARs are well-documented substrates of KLKs, an abnormal

KLK-mediated cleavage of IGFBPs and/or PARs deregulates the abovementioned signaling cascades.

In prostate cancer, enhanced IGF axis activation is accompanied by an elevated risk for developing prostate tumors (Monti et al., 2007). Increased circulating IGF1 levels are significantly associated with prostate cancer patients, as well as with advanced disease stages and aggressive phenotypes (Renehan et al., 2004; Rowlands et al., 2009). A great number of prostate-related family members, namely KLK2 (Rehault et al., 2001), PSA/KLK3 (Koistinen et al., 2002), KLK4 (Matsumura et al., 2005), and KLK11 (Sano et al., 2007), have been detected to cleave IGFBP3, which represents the most abundant member among the six IGFBPs. KLK2, PSA/KLK3, and KLK4 were also revealed to cleave efficiently IGFBP2–5, IGFBP4, and IGFBP4–6, respectively (Emami and Diamandis, 2007; Lawrence et al., 2010). Additionally, IGFBP3 induces the apoptosis of prostate cancer cell lines, independently from the IGF-IGFR cascade (Rajah et al., 1997). In summary, a deregulated degradation of IGFBPs at the prostate tissue microenvironment results in increased IGFs availability, promoting in this way the mitogenic and antiapoptotic stimuli of the IGF axis in prostate cells.

As far as the role of PARs in prostate malignancy is concerned, significantly elevated expression of PAR1, PAR2, and PAR4, at both the mRNA and the protein levels, has been revealed in prostate cancer tissues compared with normal or benign ones as well as at advanced disease stages (Ramsay et al., 2008b). Proteolytic activation of PAR1 and PAR2, which have been found to regulate prostate cancer cells' growth, has been confirmed for KLK2 and KLK4. More precisely, KLK2- and KLK4-mediated activation of PAR1 (KLK4) and PAR2 (KLK2 and KLK4) promotes the proliferation of the DU145 prostate cancer cell line *via* the extracellular signal-regulated kinase (ERK) signaling (Mize et al., 2008). KLK4-mediated PAR1 and PAR2 signaling has also been detected in PC3 cells (Ramsay et al., 2008a). Interestingly, PAR1 activation by KLK4 seems to drive the tumor-stroma cells' interactions in prostate carcinoma (Wang et al., 2010). Specifically, overexpressed KLK4 from prostate tumor cells was found to activate the PAR1 of the surrounding stroma cells, triggering the release of interleukin 6 (IL6). IL6 possesses the ability to enhance androgen receptor transcriptional activity, through ERK and signal transducer and activator of transcription 3 (STAT3) signaling pathways (Ueda et al., 2002), promoting the growth and proliferation of prostate cancer cells, as well as the expression of the androgen receptor-regulated genes (Lin et al., 2001).

KLK4 has a unique role in the promotion of prostate cancer cells proliferation and, generally, in malignant phenotype alterations through the regulation of several cell cycle-related genes. The transformation of PC-3 and DU145 KLK4-negative prostate cancer cells to stably express KLK4 resulted in augmented cell proliferation rate and colony formation potential (Klokk et al., 2007). Furthermore, knockdown of *KLK4* in the LNCaP KLK4-positive prostate cancer cell line significantly inhibited cell growth.

Additionally, PSA/KLK3 has been revealed to induce the production of reactive oxygen species (ROS) in prostate cancer cells, a phenomenon that seems to be independent from the proteolytic activity of PSA/KLK3 (Sun et al., 2001). The accumulation of ROS promotes oxidative stress in multiple proteins as well as DNA damage, affecting in this way the activation status of proto-ongogenes or tumor-suppressor genes and their downstream impact upon prostate cells growth and tumorigenesis.

In breast cancer, PSA/KLK3 and KLK10 seem to posses tumor suppressor activities. More precisely, PSA/KLK3, whose expression is decreased in breast malignancy (Yu et al., 1996), has been found to inhibit the growth of the estrogen receptor-positive breast cancer cell line MCF7, by the conversion of estradiol to the less active derivative estrone (Lai et al., 1996). The absence of this regulation upon the estrogen receptor-negative MDA-MB-231 breast cancer cell line indicates that the PSA/KLK3 tumor suppressor function affects the growth of hormone-dependent breast cancers, possibly by its implication in estrogen metabolism.

DNA methylation has been found to be a common modulator of the tumor suppressor genes' repression during breast cancer development and progression (Jovanovic et al., 2010). The epigenetic methylation of the *KLK10* exon 3 results in the loss of gene expression in the majority of advanced breast carcinomas, indicating a correlation between *KLK10* expression and disease progression (Zhang et al., 2006). This is further supported by the observation that stable transfection and expression of *KLK10* in MDA-MB-231 breast cancer cells restrains their anchorage-independent growth, as well as the tumor formation in nude mice after their inoculation with the *KLK10*-transfected cells (Goyal et al., 1998). Recent studies have provided evidence for the central role of the TGFβ receptor (TGFβR) SMAD family member 2 (SMAD2) axis overactivation, which is observed in advanced breast cancer, in the epigenetic silencing of *KLK10*, among other tumor suppressor genes (Papageorgis et al., 2010). Moreover, the disruption of Smad signaling resulted in the demethylation and the subsequent expression of the genes. A strong mediator of *KLK10* epigenetic silencing was revealed recently to be the methyl-CpG binding domain protein 2 (MBD2), which facilitates the repression of methylated genes in breast cancer. Knockdown of MBD2 triggers the restoration of *KLK10* expression, independently from its methylation profile, as well as the inhibition of MDA-MB-231 and MDA-MB-435 breast cancer cells growth (Mian et al., 2011).

The IGF axis is extensively studied in breast malignancy and is considered to be a crucial modulator of the breast cancer cells' phenotype. As has already been mentioned, these cellular effects are mediated by the IGFs mitogenic and antiapoptotic nature. Thus, the bioavailability of IGFs directly controls their downstream impact. In several studies and meta-analyses, IGF1 circulating levels are elevated in premenopausal and postmenopausal breast cancer patients (Renehan et al., 2004, 2006; Werner and Bruchim, 2009). A possible implication of the *KLK* family in the IGF1 elevated levels of breast cancer patients has been proposed recently, after the identification of the IGFBP3 degradation by KLK11 (Sano et al., 2007). Moreover, recent studies dealing with *KLK4* expression in breast carcinomas indicate significantly higher gene expression levels in cancerous tissues compared

with benign or normal ones (Papachristopoulou et al., 2009). This increased *KLK4* expression was attributed mainly to the higher *KLK4* transcription of breast stromal cells, whereas elevated KLK4 levels were detected in the stromal microenvironment (Mangé et al., 2008). Given that KLK4 is an IGFBP3–6-associated protease, it could possibly represent the mediator of a stromal cell-induced elevation of IGFs in the breast tissue microenvironment.

Ovarian cancer represents a unique type of malignancy for the study of KLKs, as the majority of family members, namely *KLK2–8*, *KLK10*, *KLK11*, *KLK13–15*, are up-regulated at both the mRNA and the protein levels. The KLK locus (19q13.3–13.4) was revealed to be a hotspot for genomic instability and copy number changes in ovarian carcinoma (Bayani et al., 2011). This heterogeneity, which is mediated by whole gains of chromosome 19 or *via* unbalanced translocations, has been associated with the increased expression of KLKs in ovarian tumors (Bayani et al., 2008). KLK6 is one of the most extensively studied family members in ovarian malignancies. Although *KLK6* expression is significantly up-regulated in ovarian cancer and its clinical value has been thoroughly demonstrated, only a small proportion of the cancer-derived KLK6 was revealed to be enzymatically active in biological fluids due to neutralization by proteinase inhibitors (Oikonomopoulou et al., 2008a, 2010a).

A functional involvement of KLK10 and KLK14 in ovarian cancer cells growth has also been documented. As already mentioned, miRNAs represent a recently identified posttranscriptional mechanism of *KLKs* expression regulation (Chow et al., 2008). A number of miRNAs have been verified experimentally to target *KLK10* expression (White et al., 2010). Moreover, the transfection of OVCAR-5 ovarian cancer cells to stably express these miRNAs resulted in the down-regulation of KLK10 and the subsequent inhibition of cell proliferation rate *in vitro*, indicating a tumor-promoting role of KLK10 in ovarian cancer. Regarding KLK14, a recent study has highlighted a possible functional role of *KLK14* in ovarian cancer. *KLK14* gene silencing in SKOV-3 and OVCAR-3 ovarian cancer cells suppress cell growth and induce apoptosis through a mechanism that includes survivin down-regulation and caspase up-regulation, indicating a KLK14-mediated tumor growth promoting function (Zhang et al., 2012).

Through a more general approach, the fact that the majority of the *KLK* family members are up-regulated in ovarian carcinomas underlines the deregulated activity upon their substrates and thus their tumorigenic role. In particular, the increased expression and activity of KLK2–5, KLK11, and KLK14 could trigger the massive cleavage of IGFBPs and the downstream overactivation of the tumorigenic and antiapoptotic function of IGFs. In the same view, the overexpression of KLKs could also disrupt the balance of PAR signaling and affect cell growth. Nonetheless, further studies are needed to delineate this possibility.

Kallikrein-related peptidases and tumor progression

Tumorigenesis represents, undoubtedly, the first step that malignant cells have to complete; however, the progression of cancer to the advanced disease stages requires cell invasion into the surrounding tissues, their escape into circulation, and finally the formation of distant metastasis. The progression of tumor cell's malignant phenotype and the remodeling of ECM control these stages.

Extracellular proteolysis is considered to be the corner stone of tissue microenvironment remodeling. The ECM proteins represent a physical barrier that the tumor cells have to surpass to migrate into the surrounding tissues and reach the circulation. MMPs, plasmin, and cathepsins are mainly responsible for the cancer-related ECM degradation (Mason and Joyce, 2011). However, recent studies have also implied KLKs in extracellular proteolysis (Table 1). KLKs have been found to cleave ECM compounds directly, as well as to support, indirectly, their degradation though the activation of other proteases (Borgono and Diamandis, 2004). Many family members can degrade extracellular fimbronectin, laminin, and collagen, while they are also able to activate MMPs or uPA-uPAR cascades. Additionally, the ability of cross- and/or auto-activation of KLKs, known as KLK activome, is well documented (Yoon et al., 2007, 2009). Considering that enzymatic cascades promote the amplification of the starting stimuli, the activation of the abovementioned proteolytic cascades can trigger massive ECM remodeling.

Prostate-related KLK2 (Deperthes et al., 1996), PSA/KLK3 (Webber et al., 1995), and KLK14 (Borgono et al., 2007b) have been found to degrade *in vitro* fimbronectin and laminin, whereas KLK14 extends its activity also upon collagen I–IV. More efficient in ECM degradation is believed to be the activation of the uPA-uPAR-MMPs cascade in prostate cancer (Kessenbrock et al., 2010). KLK2 (Takayama et al., 1997) and KLK4 (Takayama et al., 2001) can cleave pro-uPA to release uPA, which thereafter binds to its transmembrane receptor, uPAR, resulting in this way in the activation of plasmin and MMPs from their precursors, plasminogen, and pro-MMPs, respectively. Alternatively, KLK2 has been found to degrade the plasminogen activator inhibitor 1 (Mikolajczyk et al., 1999), an inhibitor of uPA, promoting in this indirect way the uPA-uPAR axis. Moreover, apart from uPA, KLK4 can also activate the uPA-uPAR cascade by the direct proteolytic cleavage of the uPAR receptor (Beaufort et al., 2006), while PSA/KLK3 is able to cleave pro-MMP2 to activate MMP2 (Pezzato et al., 2004). Additionally, the invasion capacity of LNCaP prostate cancer cells is decreased *in vitro* by the use of PSA/KLK3-neutralizing antibodies (Webber et al., 1995) or zinc (Ishii et al., 2004), which blocks KLKs proteolytic activity.

Epithelial-mesenchymal transition (EMT) represents a cellular process that allows the epithelial cells to undergo transformation to a mesenchymal-like phenotype and thus to enhance their migration, invasiveness, and growth (Thiery et al., 2009). EMT is considered to be a control point for the progression of the majority of solid tumors because of their epithelial origin. EMT promotes the change of the epithelial cells' morphology to obtain a spindle-shaped mesenchymal-like one, as well as the loss of cell-cell and cell-ECM adhesions. More precisely, EMT induces the loss of E-cadherin, thus diminishing cell-cell attachment, and increases the

expression of mesenchymal markers, such as N-cadherin and vimentin, thus improving the mobility and invasiveness of tumor cells.

Several studies have already revealed decreased E-cadherin expression in prostate cancer tissues, compared with normal ones, as well as in the advanced stages and the metastatic disease (Hugo et al., 2007). Additionally, the switch to the mesenchymal-associated N-cadherin, observed in high-Gleason-score patients, has already been documented. A possible association between KLKs and the promotion of EMT-like changes in prostate malignancy has been revealed (Whitbread et al., 2006). Transfected PC-3 prostate cancer cells, stably expressing PSA/KLK3 and KLK4, presented an enhanced migration capacity, spindle-shaped morphology, repressed E-cadherin expression, and up-regulation of vimentin expression (Veveris-Lowe et al., 2005). Although the underlying mechanism of the PSA/KLK3- and KLK4-induced EMT has not been studied yet, TGFβ2 may represent their downstream mediator. Latent TGFβ2, which facilitates EMT-like changes, is a substrate of PSA/KLK3 (Dallas et al., 2005), for which KLK4 represents a potent activator (Takayama et al., 2001). Moreover, KLK7 has been demonstrated to induce EMT-like changes in prostate carcinoma 22RV1 and DU145 cells, increasing in this way their migration and invasion capacity (Mo et al., 2010). KLK1 can also promote DU145 prostate cancer cells' malignant phenotype, in terms of migration and invasion, which is proposed to be mediated by a PAR1-related pathway (Gao et al., 2010).

Although the involvement of KLK10 in the pathobiology of breast cancer is well documented (Zhang et al., 2006), the downstream pathway by which this is mediated is not clear yet. However, studies regarding the significance of KLK10 in breast cancer highlighted its possible implication in both the developmental, as already discussed, and the progression stages of the disease. The correlation of the epigenetic-mediated loss of *KLK10* expression with the advanced infiltrating stages of breast cancer raises the question of the role of KLK10 during the progression of the disease. As has already been mentioned, the TGFβ-TGFβR-Smad2 axis overactivation, which is associated with the EMT-like changes of breast cancer cells, regulates the methylation status of *KLK10* along with other genes, such as *CDH1*, which encodes for E-cadherin (Papageorgis et al., 2010). The inhibition of Smad signaling resulted in the re-expression of the silenced genes, the adoption of epithelial morphology, and the decline of the migration and invasion properties of breast cancer cells. These results are definitely encouraging for further study of the role of KLK10 in breast cancer cells' EMT changes.

Apart from KLK10, another family member, KLK6, has been found to be significantly down-regulated in breast cancer due to epigenetic modifications. More precisely, the hypermethylation of *KLK6* proximal promoter results in the epigenetic silencing of *KLK6* expression in metastatic breast cancer patients (Pampalakis et al., 2009). This indicated a tumor suppressor function of KLK6 in breast malignancy, which was also supported by the inhibition of the MDA-MB-231 breast cancer cells' malignant phenotype, in terms of lower proliferation, inhibition of anchorage-independent growth, and reduced migration, after their transfection with KLK6. Additionally, the expression of KLK6 significantly reduces the breast cancer cells' ability to form tumors in nude mice. A possible mechanism through which KLK6 facilitates its tumor suppressor function is the switching of the EMT changes in breast cancer cells. This is supported by the fact that the stable expression of KLK6 resulted to the reduction of vimentin, as well as the restoration of calreticulin and the epithelial markers cytokeratin 8 and 19.

Furthermore, KLK1 has been reported to facilitate breast cancer cells invasiveness. More precisely, the inhibition of KLK1 by a synthetic peptide-based inhibitor resulted in a dose-dependent decreased invasion of MDA-MB-231 breast cancer cells (Wolf et al., 2001). Additionally, the use of the KLK1 inhibitor weakens the invasion of the breast cancer cells into lung interstitium of an *ex vivo* model.

In ovarian cancer, a number of family members have been reported to stimulate the invasive phenotype of cancer cells. More precisely, the transfection of OV-MZ-6 ovarian cancer cells to stably express KLK4, KLK5, KLK6, and KLK7 resulted in their increased invasiveness *in vitro*. Additionally, infusion of the transfected ovarian cancer cells increases tumor burden *in vivo*, highlighting their significance in ovarian cancer progression (Prezas et al., 2006). This effect could partially be explained by the ability of KLK5 (Michael et al., 2005), KLK6 (Bernett et al., 2002; Magklara et al., 2003), and KLK7 (Borgono and Diamandis, 2004) to degrade ECM proteins, whereas KLK4 promotes this degradation indirectly by the activation of the uPA-uPAR-MMPs axis (Takayama et al., 2001).

The tumor-promoting role of KLK7 in ovarian carcinomas has been further studied, and a possible association with the cell surface adhesion molecules functional status has been demonstrated. More precisely, the stable overexpression of KLK7 in serous epithelial ovarian carcinoma SKOV-3 cells was found to promote the formation of multicellular aggregates, facilitating, in this way, the cancer cells' survival and resistance to paclitaxel *in vitro*. A positive association between the expression levels of the α_5/β_1 integrins and KLK7, in SKOV-3 transformed cells and epithelial ovarian carcinoma tissues, was observed (Dong et al., 2010). Moreover, the formation of multicellular aggregates was inhibited by the antibody-mediated blockage of KLK7 and α_5/β_1 integrins. The above-mentioned data support the hypothesis that KLK7 may have a positive regulatory effect on the integrin-adhesion receptors' functional status, facilitating in this way ovarian cancer cell dissemination and chemoprevention.

Angiogenesis is defined as the growth of new blood vessels from preexisting ones. Although angiogenesis represents a physiological process during growth and development, it is consider as a hallmark of cancer progression. The development of new blood vessels serves the survival and growth of tumor cells, as well as their invasion into surrounding tissues and their subsequent spread to form distant metastasis.

The role of KLKs in angiogenesis is yet to be unraveled (Table 1). The undeniable contribution of KLKs in the remodeling of ECM architecture is definitely an angiogenesis-promoting process. The degradation of ECM proteins, either

directly by KLKs or *via* the activation of uPA- (KLK2, KLK4) and MMPs- (KLK1, PSA/KLK3, KLK7) related cascades, provides the necessary extracellular space for the growth of new vessels. Additionally, the PSA/KLK3-associated (Dallas et al., 2005) activation of the proangiogenic factor TGFβ2, from latent TGFβ2, possibly promotes angiogenesis directly. On the other hand, PSA/KLK3 (Heidtmann et al., 1999), KLK5 (Michael et al., 2005), KLK6 (Bayes et al., 2004), and KLK13 (Sotiropoulou et al., 2003) proteolytic activity upon plasminogen releases angiostatin-like fragments *in vitro*, which block endothelial cells' proliferation and angiogenesis. Moreover, PSA/KLK3 treatment of endothelial cells reduces their proliferation and migration capacities, which is mainly attributed to a PSA/KLK3-mediated inhibition of the treated cells' responses to fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) (Fortier et al., 1999), whereas the angiogenesis-inhibition potential was also demonstrated *in vivo* (Fortier et al., 2003). The antiangiogenic role of PSA/KLK3 is also supported by the increased angiogenesis observed in prostate cancer specimens with decreased PSA/KLK3 expression (Papadopoulos et al., 2001).

Recent studies have highlighted KLK12 as a novel central regulatory molecule in the angiogenesis field. Antibody-mediated blockage of KLK12 reduces *in vitro* the microvascular endothelial cells proliferation, migration, and formation of branching cords. The observation that KLK12 blockage was accompanied by a significant reduction in cAMP and cGMP concentration and in ERK phosphorylation indicates that the angiogenic role of KLK12 relies upon the stimulation of kinin-dependent signaling. KLK1-mediated activation of kinin-downstream cascades has been revealed in prostate cancer angiogenesis (Giusti et al., 2005). A more recent study proposed an alternative molecular pathway for the explanation of KLK12-stimulated angiogenesis. KLK12 was found to cleave the CCN1 and CCN5 matricellular proteins and thus to regulate the bioavailability of VEGF, bone morphogenetic protein 2 (BMP2), TGFβ1 and FGF2 angiogenesis-promoting growth factors (Guillon-Munos et al., 2011). The fact that KLK12 is up-regulated in prostate cancer patients allows us to hypothesize the *in vivo* angiogenic role of KLK12 in prostate malignancy. Additionally, endothelial cells express KLK1 (Plendl et al., 2000), which is believed to support angiogenesis through the production of kinins (Emanueli et al., 2001). KLK1-mediated activation of kinin-downstream cascades has been revealed in prostate cancer-related angiogenesis (Giusti et al., 2005).

Metastasis is a complex procedure that requires tumor cells detachment, invasion into the surrounding tissues, and their spread into circulation. There is an undeniable association of KLKs with these stages, which has already been presented. However, more recent studies pointed out another crucial role of KLKs at the metastasis site (Table 1).

Prostate cancer is characterized by the development of osteoblastic metastasis. PSA/KLK3 has been found to promote the proliferation of osteoblasts and the apoptosis of osteoclasts (Killian et al., 1993; Goya et al., 2006). The proliferation of osteoblasts seems to be induced by a TGFβ-mediated mechanism. This is indicated by the PSA/KLK3-dependent up-regulation of TGFβ mRNA levels, as well as the inhibition of osteoblasts proliferation rate by anti-TGFβ antibodies and serine proteases inhibitors (Yonou et al., 2001). Interestingly, enhanced expression of osteoblast differentiation-promoting genes and osteoblastic-like morphological alterations are induced in PSA/KLK3-transfected human osteosarcoma SaOs2 cells (Nadiminty et al., 2006). Additionally, *in vitro* studies have shown reduced adhesion between prostate cancer and bone marrow endothelial cells after down-regulation of PSA/KLK3 expression by RNA interference. The same result was obtained using anti-PSA/KLK3 antibodies, highlighting the importance of PSA/KLK3 for cell-cell interactions between prostate cancer and bone endothelium cells (Romanov et al., 2004). Finally, the cleavage of PTHrP and latent TGFβ2 by PSA/KLK3 has been proposed to further regulate bone remodeling and the formation of bone metastasis (Cramer et al., 1996; Dallas et al., 2005). Focusing upon KLK4, *in vivo* studies revealed its expression in both the cancer cells and osteoblasts in prostate cancer bone metastasis. The significant up-regulation of *KLK4* expression, at both the mRNA and the protein levels, detected after co-culture of prostate cancer cell lines with the osteoblastic-like SaOs2 cells, promotes the increased migration of prostate cancer cells and their attachment to bone-matrix proteins (Gao et al., 2007).

Clinical relevance of kallikrein-related peptidases for cancer patients

Prostate cancer

Diagnostic significance Prostate cancer diagnosis, nowadays, is characterized by and depends upon the measurement of PSA/KLK3 serum concentration during the screening of the male population (Lilja et al., 2008). Despite the down-regulated PSA/KLK3 expression in prostate malignancy (Hakalahti et al., 1993; Magklara et al., 2000), the tumor-related disruption of prostate tissue architecture triggers the aberrant secretion of PSA/KLK3 (Webber et al., 1995; Pinzani et al., 2008) into circulation. Although the debate about the impact of PSA/KLK3 screening upon the reduction of prostate cancer-related mortality is still ongoing (Andriole et al., 2009; Schroder et al., 2009), the introduction of PSA/KLK3 measurement has been effective for the patients' diagnosis at the early disease stages.

PSA/KLK3 was at first thought to be expressed exclusively in the prostate, which led to its characterization as 'prostate-specific antigen.' Contradictory to what its name denotes, PSA/KLK3 is expressed, in lower levels compared with prostate, in a wide range of human tissues, including breast, colon, testis, uterus, stomach, kidney, and others. Unfortunately, PSA/KLK3 is not a prostate cancer-specific marker because its serum concentration is elevated in benign lesions of the gland, such as benign prostate hyperplasia, giving rise to false-positive results and a low positive predictive value for prostate malignancies. This diagnostic specificity failure of PSA/KLK3 causes a big number of unnecessary biopsies and

patient suffering. Improvements in PSA-dependent diagnosis of prostate cancer have been attempted either using several calculated parameters, such as PSA velocity, PSA doubling time, age-related PSA, and PSA density, or through the measurement of free PSA (fPSA) (Stephan et al., 2007; Ulmert et al., 2009). More precisely, 65%–95% of the serum total PSA (tPSA) form complexes with protease inhibitors, whereas the remaining amount occurs in an uncomplexed fPSA. Patients with prostate cancer present a lower percentage ratio of fPSA to tPSA (%fPSA) compared with those with benign lesions (Catalona et al., 1995). The introduction of %fPSA measurement, together with the determination of tPSA serum levels, significantly improves the prostate cancer diagnostic specificity, especially within the tPSA 'gray zone' and reduces unnecessary biopsies (Catalona et al., 1998).

Apart from PSA/KLK3, diagnostic clinical significance for prostate cancer patients has been demonstrated for KLK2 and KLK11. The ratio of KLK2 to fPSA serum levels has been reported to successfully discriminate the prostate cancer patients from those with benign hyperplasia (Kwiatkowski et al., 1998). Additionally, enhanced diagnostic specificity within the PSA ranges of 2–4 and 4–10 ng/ml was achieved by the use of KLK2/fPSA ratio (Magklara et al., 1999; Nam et al., 2000). Focusing upon KLK11, elevated serum levels were found in prostate cancer patients compared with healthy ones (Diamandis et al., 2002). Moreover, the evaluation of KLK11/tPSA ratio was found to be promising for the discrimination of malignant from benign hyperplasia patients encouraging its clinical use for the avoidance of unnecessary biopsies (Nakamura et al., 2003a).

Prognosis and treatment response PSA/KLK3, before its wide use for the male population screening and the diagnosis of prostate cancer, was first approved and applied for the monitoring of treated patients. In fact, PSA/KLK3 serum concentration is an excellent marker for the supervision of the treatment success (Avgeris et al., 2010). Serum PSA/KLK3 levels are significantly decreased after treatment (radical prostatectomy, androgen exclusion, radiotherapy, or chemotherapy) below the analytical assays' detection limit, due mainly to the restriction of the prostate gland, indicating in this way the treatment course success. The elevation of PSA/KLK3 serum levels above a defined threshold, thereafter, is clinically used for the early detection of patient recurrence. More precisely, this elevation of PSA/KLK3 serum levels, known as biochemical recurrence or 'PSA failure,' indicates the presence of residual tumors and the future clinical recurrence of the disease, providing clinicians with the opportunity for better surveillance of the patients and the earlier initiation of adjuvant treatment (Lilja et al., 2008). More precisely, radical prostatectomy promotes the fall of PSA/KLK3 levels below the assay's detection limit. The biochemical recurrence of a patient after prostatectomy is a strong predictor of treatment failure and metastatic progression. Additionally, for radiation-treated patients, a poor outcome is indicated, in terms of shorter disease-free and distant metastasis-free survival, by the limited PSA drop and the short time interval to reach it. Finally, after androgen deprivation therapy, which is the treatment of choice for advanced prostate cancer, the magnitude of PSA/KLK3 decrease, and the time to PSA nadir represent predictors of favorite patients' disease-free survival (DFS) and overall survival (OS), whereas the rise of PSA/KLK3 serum concentration after its posttreatment nadir level signifies the androgen-independent progression of the disease. Moreover, %fPSA decline represents an unfavorable prognostic marker, as it is inversely correlated to a higher Gleason score, advanced stages of the disease, and positive surgical margins (Stephan et al., 2007).

Apart from PSA/KLK3, *KLK2*, *KLK4*, *KLK5*, *KLK11*, *KLK14*, and *KLK15* gene family members' expression has been demonstrated to serve in the prediction of prostate cancer patients' outcome. High KLK2 serum levels, together with low %fPSA, are strong predictors of a poor outcome for treated patients, revealing rapid disease progression and relapse (Steuber et al., 2007). Additionally, elevated *KLK14* expression is associated with late-stage and high-Gleason-score tumors (Yousef et al., 2003e), whereas patients with higher KLK14 levels exhibit increased risk for relapse after radical prostatectomy (Rabien et al., 2008). Moreover, increased *KLK4* (Avgeris et al., 2011b) and *KLK15* (Mavridis et al., 2010a) mRNA levels have been associated with high-Gleason-score and advanced-stage tumors, supporting, in this way, their unfavorable prognostic nature for the patients. Moreover, KLK15 tissue protein levels are associated with patients' biochemical relapse (Rabien et al., 2010). In contrast to the abovementioned family members, *KLK5* (Yousef et al., 2002b; Korbakis et al., 2009) and *KLK11* (Nakamura et al., 2003b; Bi et al., 2010) expression levels are correlated with early disease stages and low Gleason scores, highlighting the favorable prognostic nature for patients. Finally, treatment of the androgen-independent prostate cancer cell lines, PC3 and DU145, with broadly used chemotherapeutic agents alters *KLK5* expression, indicating its promising value for monitoring of patients' response to chemotherapy (Thomadaki et al., 2009; Mavridis et al., 2010b).

Breast cancer

Diagnostic significance The expression levels of several family members are abnormally altered in breast cancer. Of particular clinical interest are KLK5, KLK10, and KLK14 because they have been proposed as potential diagnostic biomarkers for breast cancer. More precisely, elevated KLK5 (Yousef et al., 2003b), KLK10 (Ewan King et al., 2007), and KLK14 (Borgono et al., 2003b) serum levels have been found in a proportion of women with breast cancer compared with normal individuals. It has also been documented that the detection of fPSA, as the principal molecular form of PSA/KLK3 in the circulation, shows high specificity for breast carcinoma and can successfully differentiate breast cancer patients from individuals with benign breast disease or without malignancy; nonetheless, it lacks sensitivity for breast cancer patients' diagnosis (Black et al., 2000). Finally, it has been proposed that PSA/KLK3 measurements in nipple

aspirate fluid or in serum could aid in the early detection of breast cancer (Sauter et al., 2004b).

Prognosis and treatment response The mRNA levels of *KLK4*, *KLK5*, *KLK7*, and *KLK14*, as well as KLK14 protein levels, measured in tissue samples, are correlated with adverse clinical outcome for breast cancer patients. On the contrary, *KLK9*, *KLK13*, and *KLK15* mRNA expression has been correlated with a more favorable outcome for breast cancer patients (Avgeris et al., 2010; Mavridis and Scorilas, 2010). KLK10 occupies a distinctive place in clinical breast cancer research because it has been described as an important tumor-suppressor gene. Exon 3 methylation of *KLK10* leads to its loss of expression in breast cancer patients, which is also correlated with tumor progression. Breast cancer patients displaying *KLK10* methylation are associated with a high risk of relapse, as well as shorter DFS and OS periods (Kioulafa et al., 2009). Moreover, high KLK10 tissue levels, which are associated with estrogen receptor-negative patients, are predictive of resistance to tamoxifen therapy (Luo et al., 2002).

PSA/KLK3, apart from its dominating role as a biomarker for prostate malignancy, seems to be a promising indicator of breast cancer prognosis and treatment monitoring as well. Elevated PSA/KLK3 levels measured in tissue cytosolic extracts have been related to enhanced DFS and OS intervals of breast cancer patients, highlighting its favorable prognostic significance for the patients' survival (Yu et al., 1998). The analysis of PSA/KLK3 levels in breast cancer patients' biological fluids showed that PSA/KLK3 concentration in nipple aspirate fluid is inversely related to disease progression (Sauter et al., 2004a), unlike the tPSA and fPSA serum levels' association with larger tumor size and higher histological grade, respectively (Black et al., 2000). Focusing upon treatment handling, patients with recurrent breast cancer with high levels of cytosolic PSA/KLK3 are more likely to respond inadequately to tamoxifen therapy (Foekens et al., 1999). Consequently, PSA/KLK3 measurements might represent a biomarker useful for the stratification of breast cancer patients that can actually benefit from tamoxifen administration.

Moreover, *KLK5* and *KLK7* mRNA expression levels, which are down-regulated in breast carcinomas (Li et al., 2009; Avgeris et al., 2011a), were found to correlate with limited DFS and OS of breast cancer patients, unmasking in this way their unfavorable prognostic value (Yousef et al., 2002c; Talieri et al., 2004, 2011). Finally, the increased expression of *KLK4* (Papachristopoulou et al., 2009) and KLK14 (Papachristopoulou et al., 2011) in breast malignancy, along with the *KLK4* expression association with advanced grade and progesterone receptor-negative tumors and the association of *KLK14* expression with advanced grade, large tumor size, and estrogen receptor-negative tumors, highlights their possible prognostic significance for breast cancer patient's outcome.

Ovarian cancer

Diagnostic significance KLK6 (Diamandis et al., 2000, 2003b) and KLK10 (Luo et al., 2003) demonstrate promising capabilities as ovarian diagnostic markers because they have been found to be elevated in the serum of ovarian cancer patients compared with corresponding samples of women without any malignancy and/or those with benign alterations. In ovarian cancer patients of the advanced disease stages, there is a significant correlation of KLK6 and KLK10 with CA125 serum levels (Diamandis et al., 2000, 2003b; Luo et al., 2001a; El Sherbini et al., 2011). These family members not only provide significant sensitivity and specificity diagnostic rates but also enhance the capability of CA125 for early diagnosis (Luo et al., 2003; Yousef and Diamandis, 2009). Interestingly, KLK10 is found elevated in the serum of a proportion of CA125-negative ovarian cancer patients; thus, KLK10 has also been proposed as a component of a multianalyte blood test for ovarian cancer diagnosis. Similarly, increased sensitivity for the early-stage diagnosis of ovarian malignancy is achieved through the combined *KLK6*, *KLK13*, and *MUC16* (CA125 gene) expression analysis in ovarian tissue specimens (White et al., 2009). Moreover, KLK11 has also been suggested to be a putative serological diagnostic biomarker for ovarian cancer, as its concentration is significantly elevated in the serum of patients compared with healthy individuals (McIntosh et al., 2007). Additionally, KLK8 (Kishi et al., 2003) and KLK14 (Borgono et al., 2003b) serum levels are elevated in a proportion of ovarian cancer patients. Recent studies show that KLK5 could also represent a promising biomarker for early and differential diagnosis because it was observed to be augmented in the serum of women with ovarian cancer in relation to patients harboring benign or borderline ovarian tumors (Dorn et al., 2011b).

Ascites fluid from ovarian cancer patients has been shown to contain higher KLK5–8, KLK10, KLK11, KLK13, and KLK14 protein levels compared with benign conditions and other cancer types. KLK6, KLK7, KLK8, and KLK10 were described as the most promising in this study in terms of supporting differential diagnosis (Shih Ie et al., 2007). In addition, taking into account the unique KLK6 N-glycosylation pattern in ascites fluid of women with ovarian cancer, KLK6 could contribute to the early diagnosis of ovarian malignancies (Kuzmanov et al., 2009).

Prognosis and treatment response The expression analyses of *KLK4–KLK7*, *KLK10*, *KLK11*, and *KLK15* genes in ovarian cancer tissue specimens have revealed a positive correlation between their mRNA levels and aggressive phenotypes of ovarian cancer, advanced-stage tumors, and/or limited survival intervals for these patients (Avgeris et al., 2010; Mavridis and Scorilas, 2010). Moreover, the latest data indicate that *KLK6* and *KLK13* mRNA levels are correlated with poor prognosis and can effectively predict tumor recurrence in epithelial ovarian carcinoma (White et al., 2009). On the other hand, the mRNA expressional profiles of *KLK8*, *KLK9*, and *KLK14* genes can be viewed as biomarkers of favorable prognosis for ovarian cancer patients, as their elevated mRNA expression levels are correlated with early disease stages, lower tumor grade, and superior DFS and OS intervals (Avgeris et al., 2010; Mavridis and Scorilas, 2010). Interestingly, high *KLK14* mRNA levels are also associated

with patients responding effectively to chemotherapy (Yousef et al., 2003a). Furthermore, a recent study implies that *KLK10* single-nucleotide polymorphisms may be related to ovarian cancer patients' survival intervals (Batra et al., 2010).

The extended analyses of the majority of KLK proteins in ovarian cancer patients' cytosols, ascitic fluid, and/or serum throughout the years have offered some extremely useful clinical information about this disease. Apart from their significant diagnostic potential, KLK6 and KLK10 represent two of the most promising members of the family in the field of ovarian cancer prognosis as well. Elevated KLK6 and KLK10 concentrations, determined in tissue samples and sera from women with ovarian cancer, have been correlated with poor prognosis, as indicated by disease progression, lower survival rates, and poor response to chemotherapy (Avgeris et al., 2010; Mavridis and Scorilas, 2010; Koh et al., 2011). Moreover, elevated CA125 and KLK6 serum levels are correlated with short OS period of the patients (Koh et al., 2011). The latest data propose that KLK5 could also constitute a very helpful serological biomarker of unfavorable prognosis in ovarian malignancies after the observation of the correlation between the KLK5 serum concentration and the progression-free survival period of the patients (Dorn et al., 2011b). Additionally, a drop in KLK5 serum concentration after the first chemotherapy cycle has been associated with subsequent efficient chemotherapy response (Oikonomopoulou et al., 2008b). Furthermore, increased KLK5 and KLK7 protein concentration in cytosolic extracts have been correlated with unfavorable prognosis, whereas elevated KLK8, KLK11, and KLK13 protein concentrations in cytosolic extracts have been correlated with favorable outcome (Yousef and Diamandis, 2009). The same conclusions are drawn for KLK5 and KLK8 in studies performed in effusion specimens (Kishi et al., 2003; Dorn et al., 2011b). Nevertheless, a recent study conducted in tissue samples of advanced ovarian cancer patients using automated *in situ* quantitative protein analysis showed that KLK8 expression is an adverse predictor of progression-free survival time, highlighting that the prognostic capability of this KLK member requires further investigation (Kountourakis et al., 2009). Additionally, enhanced KLK4 ovarian cancer tissue staining has been correlated with paclitaxel resistance (Xi et al., 2004), whereas elevated KLK11 and KLK13 tissue protein levels are correlated with improved clinical response to chemotherapy (Zheng et al., 2007). Moreover, the latest data show that KLK7 levels in ovarian tissues are indicative of unfavorable outcome for ovarian cancer patients and are associated with paclitaxel chemoresistance (Dong et al., 2010). Finally, recently published data propose that measurements of level differentials (between metastasis and primary tumor) of KLK5–8, KLK10, KLK11, and uPA are correlated significantly with the disease outcome of ovarian cancer patients (Dorn et al., 2011a).

The use of multiparametric models can promote the prognostic capabilities of KLKs even further. Many biomarker panels containing several members of the KLK family, such as KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, and KLK13 along with non-KLK markers, namely, CA125, V-set domain containing T cell activation inhibitor 1 (VTCN1), regenerating islet-derived family, member 4 protein (REG4), and spondin 2, whose expression is determined in sera or tissues of ovarian cancer patients, have been proposed as useful predictors of response to chemotherapy, as well as the DFS, OS, and time-to-progression intervals of the treated patients (Zheng et al., 2007; Oikonomopoulou et al., 2008b).

Overview

In summarizing the existing knowledge about the impact of KLKs on human malignancies, it is obvious that certain members hold a clearer and more crucial role in the cancer-related scene. In prostate cancer, PSA/KLK3, KLK2, and KLK4 hold a central regulatory role. These family members have been found to promote tumor growth through the stimulation of the IGF axis and PARs mitogenic role. Additionally, their direct and indirect impact upon ECM degradation, through the activation of uPA- and MMP-related cascades, as well as the induction of EMT-like changes in prostate cancer cells, becomes essential for the promotion of the tumor cells invasiveness and the formation of distant metastasis. Focusing on the clinical management of prostate cancer patients, this is fully dependent on PSA/KLK3. Determination of its serum levels remains the method of choice for the male population screening and early disease diagnosis. Moreover, PSA/KLK3 serves in the prognosis and treatment monitoring of the patients. The posttreatment elevation of serum PSA/KLK3, known as biochemical recurrence, which indicates treatment failure, can predict efficiently the future clinical recurrence of the patients. Additionally, the determination of KLK2 serum levels has been revealed to significantly support the PSA/KLK3 biomarker role as well as to indicate a poor patients' outcome.

In breast cancer, the loss of KLK10 by the methylation of *KLK10* exon 3 has been established as a hallmark of the advanced stages of the disease and supports its tumor suppressor role. Similarly, the hypermethylation of the *KLK6* promoter region in metastatic breast cancer patients, which is associated with EMT-like changes, underlines KLK6 protecting role from the promotion of breast cancer cells' aggressiveness. Additionally, KLK10 methylation is correlated with a high risk of relapse and shorter DFS and OS periods, whereas a significant diagnostic capability has already been documented for KLK10 serum levels. Finally, the resistance of the patients to tamoxifen is also predicted by the elevated KLK10 tissue levels.

Ovarian malignancy is characterized by the elevated expression of 12 members of the KLK family, whereas KLK chromosomal locus genomic instability occurs very often in ovarian cancer patients. This up-regulation of several KLKs in ovarian cancer is believed to be responsible for a deregulated ECM degradation and thus the increased invasiveness of tumor cells. Specifically, KLK10 and KLK14 have been shown to hold tumor suppression roles, whereas KLK7 has been proposed recently to regulate the functional status of cell surface adhesion molecules. Regarding clinical relevance, KLK6 remains today the most promising family member for ovarian cancer diagnosis, followed by KLK10. The serum levels of the aforementioned KLKs are up-regulated in ovarian cancer patients. Moreover, their combination with CA125

significantly enhances the diagnostic value of the latter. Moreover, KLK6 and KLK10 levels are unfavorable prognostic biomarkers for patient survival and the chemotherapy success.

Despite the fact that endocrine-related malignancies bear strong resemblance to their biological basis and that KLKs share several structural and functional similarities, a big number of opposing impacts have been documented for KLKs in different types of malignancies. In particular, PSA/KLK3 facilitates the growth, invasiveness, and metastasis of prostate cancer cells, and at the same time, it possesses a clear tumor suppressor role in breast malignancy. This is reflected in the unfavorable and favorable prognostic role of PSA/KLK3 in prostate and breast cancer patients, respectively. In the same way, KLK6 and KLK10 are related with tumor aggressiveness in ovarian malignancy, whereas these family members present antitumorigenic properties in the case of breast cancer. The KLK10-related translational research provided similar results because the KLK10 expression profile is related with poor and propitious outcomes in ovarian and breast cancer patients, respectively. The conflicting effects of KLKs on cell growth, tumor invasion, angiogenesis, metastasis, and clinical information can possibly be attributed to the extensive interplay between these proteases. More precisely, given the concurrent tissue expression of KLKs, the endpoint result on tumor behavior depends largely on the specific expression/activation status of each family member. Moreover, one should always keep in mind that the exact function of a protease is largely dependent on the bioavailiability of related substrates in the tumor microenvironment.

Conclusions

Enormous research efforts by a great number of independent laboratories have already established the role of kallikrein-related peptidases as hallmarks of the ECM degradation, EMT-like changes, tumor growth, and cancer progression (Table 1). This is crucial for the endocrine-related cancers, as KLKs expression levels are strongly regulated by steroid hormones. Naturally, this key role of KLKs in the multiparametric scene of cancer establishment and progression displays a strong translation capability. Indeed, KLKs are very well known for the association between their cancer-related deregulated expression/activation and patients' diagnosis/outcome (Table 2). Apart from KLK3/PSA, which is the most recognizable member of the family, KLKs have a great potential as cancer biomarkers. This is even more apparent now for prostate, breast, and ovarian cancers due to their high prevalence and patient heterogeneity. Certainly, KLK associated research will continue to provide novel knowledge regarding the molecular alterations that govern human malignancies.

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