Minireview

Kallikrein-related peptidase genes as promising biomarkers for prognosis and monitoring of human malignancies

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Abstract
Tissue kallikrein (KLK1) and the kallikrein-related peptidase (KLK2–15) genes encode for a subgroup of 15 homologous secreted serine proteases possessing numerous physiological roles, such as the regulation of blood pressure, hormone processing and tissue remodeling. The expression of KLKs is detected in a broad spectrum of human tissues where it has been found to be regulated mainly by steroids hormones. The aberrant expression of KLKs, presented in many human malignancies, highlights the significance of this gene family for early diagnosis, prognosis and monitoring of cancer patients, as it is strongly emphasized by the routine use of PSA (KLK3) for prostate cancer management. Here, we review the presently known data regarding the role of KLKs as cancer biomarkers, giving emphasis on novel information about the subject.

Keywords: cancer biomarkers; cancer prognosis; clinical outcome; KLKs; patient monitoring; PSA; treatment response.

Introduction
Cancer is definitely one of the major health problems that the scientific community has to face nowadays. Significant progress has been made in the fields of diagnostic approaches, the surgical management and the therapeutic protocols used to confront this disease. However, only a small percentage of the patients earn the benefit of full recovery. The diagnosis of the disease at early stages is certainly one of the key points for a successful curative treatment, as well as for improving life quality of the patients; therefore, the screening for such specific biomarkers is of clinical priority. Nevertheless, the complex and multiparametric character of cancer that complicates its early diagnosis strengthens the clinical need for cancer prognostic and monitoring biomarkers. These tumor markers should be able to demonstrate the disease progression dynamic, the aggressive or non-aggressive disease phenotype and the patients’ outcome, as well as to indicate the most appropriate therapeutic scheme and patients’ response to it.

Tissue kallikrein gene (KLK1) and kallikrein-related peptidase genes (KLK2–KLK15) encode for a subgroup of 15 homologous secreted serine proteases (KLK1–KLK15) with trypsin- or chymotrypsin-like activities. They are colocalized in tandem to 19q13.3–4 chromosomal region, without interference from other non-kallikrein-related genes, representing, in this way, the largest contiguous family of protease genes of any catalytic class (Yousef et al., 2000; Lundwall et al., 2006). The discovery of the aggregates of KLKs spans almost two decades. The identification of the ‘classical’ members of the family (KLK1–KLK3) took place during late 1980s and the subsequent classification of the ‘novel’ members arose between mid- to late-1990s. KLKs share similar length and amount of exons, intron phases and positioning of the methionine start codon, the catalytic triad residues and the terminal codons, and have 30–80% sequence homology. Furthermore, another common feature of the KLK gene family is the presence of a large number of alternatively spliced transcript variants, which could be responsible for the formation of several structurally and functionally modified protein isoforms (Yousef and Diamandis, 2001; Lundwall et al., 2006).

KLK expression, which is under the transcriptional regulation of steroid hormones, generates a single-strand intracellular and inactive polypeptide (preproenzyme), containing a NH2-terminus signal-peptide (16–30 aa), a pro-peptide (4–9 aa) and the catalytic domain (227–252 aa). Sequential cleavage of the signal- and the pro-peptides results in the secretion and the activation of the mature serine protease (Borgono et al., 2004). The expression of KLKs has been detected in a large number of human tissues and cell lines, implicating the family in a broad spectrum of physiological roles, such as blood pressure regulation, skin desquamation, seminal clot liquefaction, tissue remodeling, peptide hormone processing and inflammatory cascades (Borgono et al., 2004; Yousef et al., 2005).

The value of KLKs as cancer biomarkers is undoubtedly due to the previously established clinical utility of prostate-specific antigen (PSA) for prostate cancer (PCa) diagnosis. Kallikrein-related peptidase 3 (KLK3) or PSA, as it is largely referred to, today remains the serum biomarker of choice for the screening, diagnosis and monitoring of the most common type of male malignancy in the Western world (Schroder et al., 2008). The isolation and the characterization of PSA along with the estimation of its biomarker potential for PCa that took place during the 1970s to late 1980s resulted in the
The main objective of this minireview is to present the clinical benefits that this gene family has to offer in the miscellaneous field of cancer management.  

KLKs as cancer prognostic biomarkers

Prostate cancer

PCa today remains the most frequently diagnosed disease, as well as, the second leading cause of cancer-related deaths among men of the Western world. Undeniably, an enormous part of this high rate of PCa diagnosis belongs to the PSA serum concentration screening of the male population, which leads mainly to the detection of the asymptomatic disease at its initial stages. Therefore, the current clinical aim is focused on the early diagnosis and the determination of PCa patients’ outcome. Preoperative PSA serum measurements are also an independent unfavorable prognostic biomarker, positively correlated with advanced disease stage and poor clinical outcome (Ullah and Aatif, 2009; Ulmert et al., 2009). In parallel to tPSA, recent clinical trials demonstrate the ability of %fPSA to predict the aggressiveness of prostate tumors and the outcome of PCa patients. The %fPSA has been found to be irreversibly associated with increased Gleason score, advanced disease stages, capsular penetration and positive surgical margins of PCa patients (Stephan et al., 2007). Apart from PSA (KLK3), the KLK2, KLK4, KLK5, KLK11, KLK14 and KLK15 members of the family have been found to be promising prognostic biomarkers for PCa.

Kallikrein-related peptidase 2 (KLK2), which is encoded by the KLK2 gene, is expressed mainly in the prostate gland and shares approximately 80% sequence homology with PSA. The combination of KLK2 with tPSA and fPSA measurements in serum increases the ability of discrimination of PCa from benign prostate hyperplasia, particularly in the PSA ‘gray zone’ (Raaijmakers et al., 2007). Additionally, increased KLK2 serum levels in parallel with low tPSA rate are associated with rapid PCa progression (Ulmert et al., 2009). Moreover, an unfavorable prognostic significance for PCa patients has also been revealed for KLK4 expression. It has been observed that higher KLK4 mRNA levels of the prostate tissue biopsy are correlated with higher Gleason score and PCa stage (our unpublished data). Furthermore, KLK14 expression profiles indicate an adverse clinical outcome of PCa patients. Elevated KLK14 mRNA and protein levels have been associated with late stage, aggressive tumors. The overexpression of KLK15 transcript variants, including alternatively spliced ones, which has been detected in prostate malignancies, has been shown to correlate with late stage, more aggressive prostate tumors, and thus, poor prognosis (Paliouras et al., 2007; Mavridis et al., 2010a). The expression analysis of KLK5 and KLK11, differing from this of the aforementioned family members, revealed to have a favorable prognostic significance for PCa patients. In particular, augmented KLK5 transcription levels, as well as elevated KLK11 classical and spliced variants mRNA expression, have been found to be associated with a less advanced stage and lower Gleason score prostate tumors, and with an optimistic disease course for these PCa patients (Obiezu and Diamandis, 2005).

Testicular and renal cancers

Along with prostate cancer, KLKs have previously proved their prognostic significance in two other common malignancies of the urinary track and reproductive system: testicular and renal cancers. Although testicular cancer presents a rising incidence among younger men, the low mortality rates of the disease have not yet encouraged the study of KLK expression. Therefore, a significant prognostic impact has
been revealed only for KLK5 expression, as its correlation with lower stages of the disease indicates a more propitious outcome for these patients (Emami and Diamandis, 2008). In the case of renal cell carcinoma, the fact that a large number of patients suffer from advanced stages of the disease, at the time of diagnosis, indicates that prognostic biomarkers capable of clarifying patients’ outcome should be the most essential clinical aim. At this time, increased KLK6 formation has been correlated with shorter disease-free survival (DFS), whereas patients suffering from advanced pathological stages of the disease present elevated KLK1, KLK7 and KLK11 levels, unmasking the unfavorable features of these KLKs for renal cell carcinoma (Paliouras et al., 2007; Gabri et al., 2009).

Breast cancer

A significant prognostic value for breast cancer (BC), which today constitutes the most frequently diagnosed female malignancy, has been verified for a broad spectrum of KLKs. Among them, KLK4, KLK5, KLK7, KLK10, KLK12 and KLK14 predict a poor clinical outcome for BC patients, opposite to the favorable prognostic value of KLK3, KLK9, KLK13 and KLK15. In particular, the epigenetic methylation of the third exon of KLK10 results in the complete loss of its expression in the majority of breast carcinomas. The hypermethylation of KLK10 indicates an unfavorable prognostic outcome for the early staged diagnosed patients and has been associated with shorter DFS and overall survival (OS) periods (Kioulafa et al., 2009). An important correlation with limited DFS and OS of BC patients has also been observed for the KLK5 and KLK7 mRNA, as well as for the KLK12 transcript variants levels, uncovering a poor prognostic strength of these KLKs for BC (Obiezu and Diamandis, 2005; Pappa et al., 2009). Recently, KLK4 has also been observed to be overexpressed in breast malignancies, as well as correlated with advanced grade and progesterone receptor-negative tumors, a fact that highlights the unfavorable prognostic value of this gene (Papachristopoulou et al., 2009). Finally, elevated KLK14 mRNA expression, observed more frequently in BC patients related to those suffering from benign lesions of the gland, is also associated with higher tumor grade and size, as well as with slight estrogen receptor staining, demonstrating, in this way, the unfavorable nature of KLK14 expression status for BC patients. The same prognostic properties are also revealed by the stronger KLK14 staining of the malignant breast tissues, which has been found to be associated with more aggressive phenotypes of mammary gland tumors (Borgono and Diamandis, 2004). In contradiction to KLK3 (PSA) poor prognostic impact for PCa patients, stronger KLK3 staining of the mammary gland tissue extracts has been associated with longer DFS and OS periods among women suffering from BC, introducing KLK3 as a favorable BC prognostic biomarker (Black and Diamandis, 2000). Additionally, the association of KLK9 mRNA expression with increased DFS and OS, early-stage disease and smaller breast tumor sizes, indicates its significant favorable prognostic value. Finally, the fact that KLK13 and KLK15 expression levels were correlated with a statistically significant elevation of DFS and OS periods of BC patients sets the basis for their acknowledgement as additional promising favorable prognostic biomarkers (Borgono and Diamandis, 2004).

Ovarian cancer

The fact that many ovarian cancer patients are currently diagnosed during late stages of the disease has already triggered enormous research efforts for the establishment of biomarkers that will be able to provide early diagnosis, as well as to clarify the prognostic outcome of these patients. The KLK family represents a large pool of potential ovarian cancer biomarkers, as deregulated gene expression, protein formation and secretion have been reported for the majority of KLKs. To date, KLK6 remains the most studied member of the family, which has also been correlated significantly with a strong unfavorable outcome for these patients. Higher KLK6 tissue concentrations are associated with aggressive phenotypes of the disease and significantly shorter DFS and OS. The same prognostic significance is also supported by KLK6 serum concentrations (Borgono and Diamandis, 2004). Additionally, recent research identified a unique N-glycosylation pattern of ovarian-derived KLK6 in ascites fluid that was found to be correlated significantly with ovarian cancer patient status, providing a promising tool for early diagnosis and prognosis of ovarian cancer (Kuzmanov et al., 2009). Apart from KLK6, poor clinical outcome can also be predicted by the analysis of KLK4, KLK5, KLK7, KLK10 and KLK15 mRNA levels. The expression profiles of the aforementioned genes are positively correlated with aggressive phenotypes of the disease and late-stage tumors, underlining a worse survival probability for these patients. Analogous clinical utility was revealed from the analysis of KLK5 and KLK10 in tissue extracts and patient serum, as well as from KLK7 tissue staining. Elevated levels of the abovementioned KLKs were found to be positively related with rapid progression of the disease and poor survival probabilities for ovarian cancer patients (Obiezu and Diamandis, 2005). Moreover, two alternatively spliced KLK5 and KLK7 transcript variants, presenting a shorter 5′-untranslated region (UTR) and a longer 3′-UTR, respectively, have also been connected with aggressive ovarian cancer phenotypes (Dong et al., 2003), supporting the need for further characterization of the prognostic significance of several transcript variants of KLKs.

A favorable prognostic nature has been reported for the KLK8, KLK9 and KLK14 members of the family. In particular, elevated transcription levels of the aforementioned genes have been associated with less advanced disease stages, improved DFS and OS, lower tumor grade and optimized residual tumor volume, whereas KLK14 expression has also been inversely correlated with CA125 serum concentrations of ovarian cancer patients. Additionally, an interesting and also perplexing finding has been observed for KLK11 and KLK13 prognostic role. An unfavorable clinical outcome is indicated by the increased transcription of these genes, as
their mRNA levels are associated with an aggressive tumor behavior and a worse clinical outcome. However, KLK11 and KLK13 protein accumulation in ovarian cancer tissues is clearly correlated with the early stages of the malignancy and better DFS and OS probabilities (Obiezu and Diamandis, 2005).

**Uterine papillary serous and cervical cancers**

Following gynecological malignancies of the breast and the ovary, KLK6 and KLK7 present their potential prognostic significance in the cases of uterine papillary serous and cervical carcinomas, respectively. In particular, women suffering from uterine papillary serous carcinoma showed elevated KLK6 expression levels and KLK6 serum concentrations. These aberrant concentrations are capable of distinguishing these individuals from normal endometrial controls and endometrioid carcinoma patients. However, the prognostic impact of this KLK needs further clarification. Likewise, cervical cancer patients with lymph node metastasis displayed elevated KLK7 transcription levels, introducing in this way the characterization of the unfavorable prognostic value of this gene for cervical cancer (Paliouras et al., 2007).

**Lung cancer**

Lung cancer currently continues to be the most common fatal malignancy for both genders in Western populations. Therefore, the establishment of a panel of prognostic biomarkers, capable of predicting clinical outcome of lung cancer patients, should be a primary goal. Expression analysis of several KLK members has already set the basis for this requirement. In non-small cell lung cancer, KLK6 and KLK8 expression presents a controversial prognostic impact. In particular, KLK6 expresional status serves as an unfavorable biomarker, opposite to the favorable clinical strength of KLK8 overexpression, which was found to be correlated with longer postoperative recurrence periods (Sher et al., 2006; Nathalie et al., 2009). Moreover, decreased KLK11 mRNA levels were found to correlate significantly with low differentiated adenocarcinomas and poor prognostic outcome (Paliouras et al., 2007). Opposite to the KLK11 favorable nature, overexpression of the KLK13 at both mRNA and protein levels is related to adenocarcinomas of positive nodal status and lower OS probabilities, whereas KLK14 mRNA and protein expression were also related to positive nodal status and increased tumor size, respectively, unmasking their importance in predicting a poor clinical outcome (Planque et al., 2008).

**Gastrointestinal cancers**

A promising prognostic capability of the KLK family has also been presented for gastrointestinal malignancies. In colorectal cancer, deregulated expression has been observed for many family members; however, a strong unfavorable prognostic significance has been revealed only for KLK5, KLK6, KLK7, KLK10, KLK11 and KLK14. More precisely, a shorter OS period for colon cancer patients is indicated by elevated KLK5, KLK7 and KLK14 tissue protein accumulation (Talieri et al., 2009). A worse outcome for low rectal carcinoma is indicated by KLK11 protein levels, which have been found to be positively associated with shorter patient survival and late-stage aggressive disease (Yu et al., 2010). Additionally, higher KLK6 transcription was found to be associated with serosal invasion, liver metastasis, advanced Duke stage and a reduced OS, highlighting in this way the poor clinical outcome of colon cancer patients with augmented KLK6 expression levels (Ogawa et al., 2005). Similarly to the clinical impact of KLK6, KLK10 transcription correlates with lymphatic invasion and advanced clinical stages of the disease (Feng et al., 2006).

Analysis of KLK gene expression has also been applied for other commonly diagnosed gastrointestinal cancers. Recent findings support the favorable prognostic outcome of KLK13 mRNA levels in the case of gastric cancer patients, which were found to significantly correlate with their improved DFS and OS probabilities (Konstantoudakis et al., 2009). An opposite, unfavorable this time, outcome is designated by high KLK6 and KLK10 transcription levels. More precisely, elevated KLK6 expression has been associated with lymphatic invasion and a poorer survival rate, similar to the significant relationship of KLK10 mRNA levels with tumor invasion and more advanced clinical stages of the disease. Analogous prognostic potential of KLK6 and KLK10 expression was further evaluated for pancreatic ductal adenocarcinoma patients (Paliouras et al., 2007). Coexpression of these two family members was found to be correlated with shorter OS periods, underlining in this way the unfavorable prognostic nature of KLK6 and KLK10 for the majority of gastrointestinal malignancies. The strong KLK7 staining of the pancreatic tissue sections during the progression of the pre-malignant ductal lesions to invasive adenocarcinomas clearly supports the prognostic value of KLK7 for pancreatic cancer, as well as the need for further clinical analysis (Ramani and Haun, 2009).

**Other types of cancer**

Finally, a promising prognostic value for intracranial tumors and acute lymphoblastic leukemia has been revealed for KLK7 and KLK10, respectively. Expression analysis of KLK7 in intracranial tumors negatively correlates the mRNA expression levels of this gene with OS probability of patients, whereas KLK7 synthesis has been associated with an enhanced invasive potential of cancer cells, clarifying the unfavorable impact of KLK7 for these tumors (Emami and Diamandis, 2008).

Similar to BC, a significant downregulation of KLK10 expression, attributed to epigenetical hypermethylation within the gene promoter, 5'-UTR and coding sequence, has been observed in acute lymphoblastic leukemia patients. Methylation of the KLK10 gene has been proposed as an indicator of poor prognosis in both childhood and adult patients, based on its direct association with shorter DFS, compared with non-methylated cases (Emami and Diamandis, 2008).
KLKs as cancer monitoring biomarkers

Cancer diagnosis at early stages is considered to be critical for the successful management of the disease. However, the multiparametric basis of cancer, at both the molecular and cellular levels, does not allow the application of a general therapeutic approach that could be utilized for all patients. Therefore, clinicians are aiming towards designing personalized treatment protocols according to the disease features of every individual. In light of this requirement, the establishment of monitoring biomarkers capable of designating appropriate cancer treatment, as well as the patients’ response to it, becomes absolutely necessary for the improvement of cancer management. Until today, a clinical monitoring value has been suggested for some KLKs in the cases of prostate, breast and ovarian cancers. Nevertheless, their diagnostic and prognostic importance in a broad spectrum of human malignancies evidently promotes further characterization of their monitoring significance in many other types of cancer.

It has been previously mentioned that PSA/KLK3 serum concentration measurement was first approved by the FDA for monitoring of the therapeutic course of PCa patients. The treated patients, either with radical prostatectomy, androgen exclusion, radiotherapy, or chemotherapy for more advanced cases, present a significant downregulation of serum PSA concentrations, which drop under the detection limit of the assay, owing to the size restriction of the prostatic tissue. Unfortunately, a significant proportion of these treated patients are certain to display disease recurrences in their lifetime. However, PSA serum concentration measurement today remains the cornerstone of monitoring tools used for the early detection of PCa recurrence. In particular, elevation of serum PSA levels after surgical or radiation treatment, known as biochemical recurrence or PSA failure, is a strong indicator of any residual tumors and putative disease recurrences (Ullah and Aatif, 2009; Ulmert et al., 2009). This biochemical recurrence, which emerges significantly prior to clinical recurrence, provides an opportunity for a better active surveillance of therapy course for these patients, as well for the earlier initiation of an adjuvant treatment, resulting in improved survival probabilities. Moreover, biochemical recurrence can also be used for discrimination between local recurrence and metastatic disease. The levels and the rate of this post-treatment augmentation of serum PSA are correlated with the development of metastatic disease, thus allowing the clinicians to design appropriate management of PCa patients.

Apart from PSA, three other members of the family KLK2, KLK5 and KLK15 have previously demonstrated their monitoring significance for PCa patients. Increasing KLK2 serum levels have been associated with residual tumors and biochemical recurrence after the local treatment of patients, designating in this way the presence of a clinical relapse (Ulmert et al., 2009). Additionally, recent promising studies reported severe overexpression of KLK5 gene transcription levels, after treatment of the androgen-independent prostate cancer cell lines PC3 and DU145 with various and broadly used, in clinical practice, chemotherapeutic agents (Thomadaki et al., 2009; Mavridis et al., 2010b). The fact that modulation of the expression levels of these genes were triggered by the presence of anticancer compounds demonstrates the promising clinical value of these family members in the field of monitoring patients’ response to chemotherapy in androgen-independent PCa.

In the case of BC, analysis of KLK10 expression has exposed the monitoring importance of this family member, in parallel with its strong prognostic significance. More precisely, KLK10 protein tissue accumulation was revealed to be an independent biomarker, predicting patients’ resistance to tamoxifen treatment. Higher KLK10 tissue levels have been correlated with stronger resistance to tamoxifen, indicating the need for a redesign of the initial therapeutic scheme (Luo et al., 2002). Moreover, similar conclusions, regarding tamoxifen resistance, can also be drawn by the assessment of higher cytosolic PSA/KLK3 concentration in primary BC (Foekens et al., 1999).

By contrast, a larger number of KLKs have displayed their monitoring biomarker capability, in the case of ovarian carcinomas. Stronger KLK4 ovarian cancer tissue staining has been observed in patients more resistant to paclitaxel administration, revealing the significance of this peptidase in stratifying patients for paclitaxel-based chemotherapy (Xi et al., 2004). Moreover, presurgically higher KLK10 serum concentrations have been found to be significantly correlated with ovarian cancer patients’ poor response to chemotherapy (Luo et al., 2002). Contrary to KLK10, higher KLK11 and KLK13 tissue protein levels are more frequently associated with women showing an enhanced clinical response to chemotherapy, suggesting that this treatment option is suitable and effective for these patients, whereas the combination of the assessment of KLK6 and KLK8 tissue levels with those of KLK13 yields an enhanced monitoring potential (Borgono et al., 2003; Zheng et al., 2007).

Conclusion and future directions

The promising value of the kallikrein-related serine protease family as diagnostic, prognostic and treatment monitoring biomarkers is well documented and has been unraveled in a wide range of human malignancies. Their deregulated expression, at the mRNA and protein levels in malignant tissues, as well as their abnormal presence in blood circulation, has been revealed for almost all of the family members. The involvement of KLKs in the pathophysiology of tumor cell growth and the establishment of malignancy, as well as the promotion of disease progression, by the stimulation of angiogenesis, tumor invasion and/or metastasis, highlights the importance of KLKs as biomarkers for the estimation of cancer prognosis and patients’ response to various therapeutic schemes. Emerging and ongoing research efforts dealing with KLK-mediated cascade pathways and substrate specificity analysis of the family members are bound to provide further information to support and enrich clinical evaluation of more KLKs. This will result in the improvement of cancer
management efficacy, which is the gamble that researchers and physicians have to win. Additionally, taking into account the intense presence of KLK spliced variants in numerous human malignancies and their putative regulatory role, the expression analysis of these isoforms can provide novel and significant information for the involvement of KLKs in carcinogenesis and broaden the horizons in the field of cancer diagnosis, prognosis and monitoring. Furthermore, biomarker panels that include KLK members could be the answer to the problem of successful confrontation of human neoplasms that comprise complex disease entities, involving deregulations in many critical biochemical pathways. Consequently, members of the kallikrein-related peptidase gene family could be encountered, in future clinical practice, as effective cancer biomarkers, resulting in enhanced patient survival intervals and comprising valuable tools for making critical decisions regarding treatment strategies.

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References


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