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Mechanistic and Diagnostic Roles of Kallikrein Related Peptidases 2 (KLK2) in Prostate Cancer

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Abstract:
Kallikrein related peptidases (KLKs) are a group of serine-like proteases such as chemo trypsin and trypsin, which are regulated by steroid hormones and play a vital role in a variety of natural and physiological functions through their proteolytic activity. However, involvement of these proteases has been reported in many pathological conditions, such as various types of malignancies. Deregulation of the expression of genes encoding kallikrein, including KLK2, is often associated with many types of cancer, in particular prostate cancer. This review provides an overview of the gene and protein structures and function of KLKs particularly, KLK2, at the molecular level, and also summarizes the role of KLK2 in the pathobiology of prostate cancer and the possible mechanisms involved in its progression. Finally, the importance of this protein is studied as a specific diagnostic marker along with PSA marker as well as therapeutic target of KLK2 in treatment of prostate cancer. A comprehensive understanding the structure and activity of this protein in prostate cancer can provide a valuable tool for future clinical practice that can be used to evaluate the clinical outcome and select the most appropriate treatment strategy. The critical role of KLK2 in promoting cell growth, migration, metastasis, angiogenesis and inhibiting apoptosis in prostate cancer cells, suggests KLK2 as the second diagnostic biomarker along with PSA with high specificity.

INTRODUCTION

Tissue kallikrein (KLK1) and kallikrein-related peptidase (KLK2-KLK15) are included of a subset of 15 chemotrypsin-like or trypsin-like serine protease encoded by a 265 kb multigene cluster family in chromosome 19q13.3-4 (Figure 1A) (1). The term of kallikrin refers to pancreas, firstly used in 1930 by Warl et al due to high concentration of the component in the pancreas. Firstly, kallikrin was identified as a proteolytic enzyme and named tissue kallikrin or glandular kallikrin/kallikrin 1 (KLK1) (2). Following this, kallikrein coding gene (KLK1) was discovered in 1985, and then two very structurally similar genes, KLK2 and KLK3 / prostate-specific antigen (PSA), were found in the same chromosomal region. After gene cloning and mapping, it was proposed that 14 of 15 genes of tissue kallikrein should be introduced as kallikrein-related peptidases (KLK2-KLK15) (3).

All KLK genes and proteins are identified by a series of similar structural and functional features, including the presence of: (Figure 1B) and (Figure 2) (4).

- Five coding exons with the same exon length and four intron regions with different length
- Conserved catalytic subunits, histidine (His), aspartate (Asp) and serine (Ser) in exons 1, 3 and 5, respectively.
- A number of spliced alternative copies
- A single peptide
- Amino acid sequence with 80-40% identity

In addition, the expression of KLKs is regulated by steroid hormones, and the proteolytic activity of KLK proteins is mediated by several ways, including zymogenic activation and complex formation with plasma and / or tissue inhibitors, whereas the inactivation of KLKs is mediated by cleavage and fragmentation. KLKs are expressed in a wide range of human tissues, mainly in the cytoplasm of glandular epithelial cells which are involved in a variety of natural and physiological functions, from regulating blood pressure to homeostasis and tissue regeneration as well as hormone processing (5). However, deregulation of KLK gene expression at the level of mRNA and / or protein in many types of cancer is

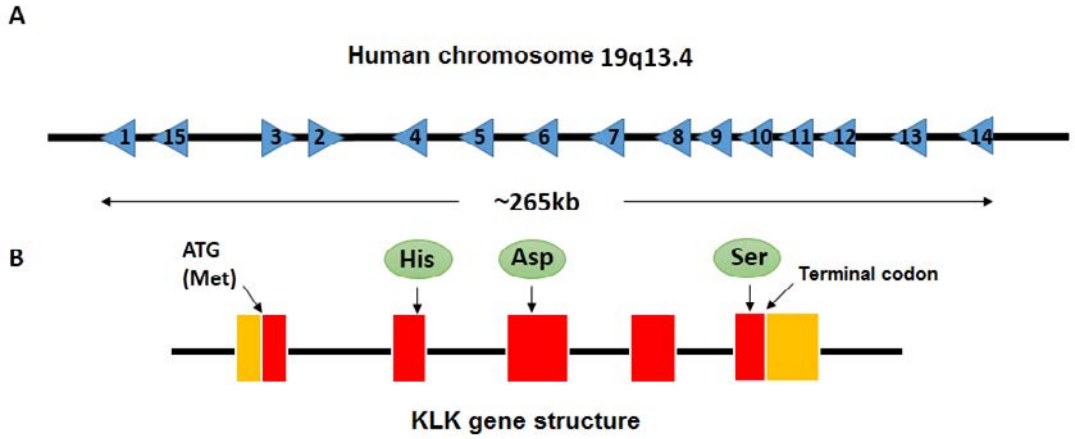

Fig1. A) The largest peptidase cluster in the human genome is related to kallikreins, B) The coding gene contains 5 exons separated by 4 introns.

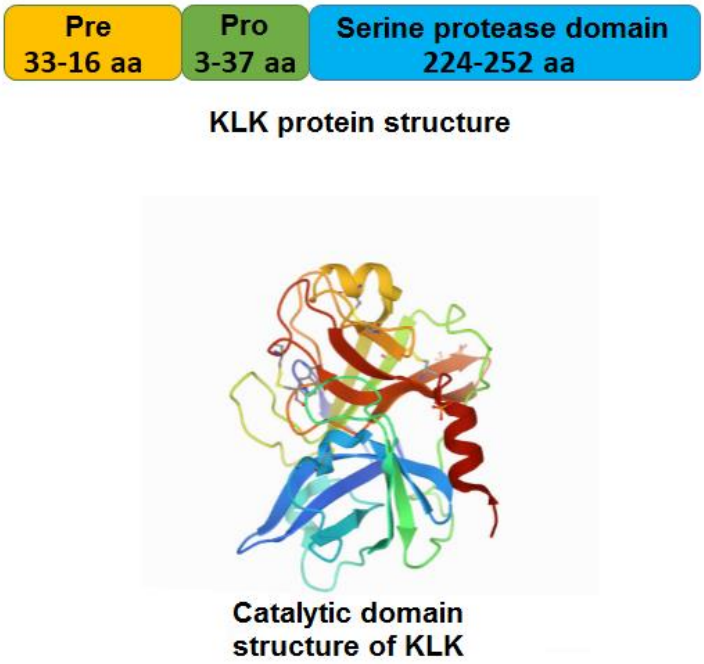

Fig 2. Linear and three-dimensional structure of KLK proteins (https://www.rcsb.org/)

often associated with the pathological profile of patients as well as using as a potential prognostic biomarker (4-7). The mechanisms influencing deregulation of KLK expression in cancers have not yet been fully clarified. However, a recent study suggests that chromosomal aberrations and changes in copy number of genes are responsible for the deregulation of KLK genes in many human malignancies. Numerous studies have provided evidence that KLKs are contributed to the development and progression of several cancer cells by modulating different factors and proteins (4, 7-9). They may stimulate or inhibit cancer cell growth through activating growth factors and interacting with other proteases. Furthermore, KLKs can mediate angiogenesis by activating and releasing angiogenic factors, and may also be involved in invasion and metastasis through proteolytic processing and degradation of extracellular matrix compounds (6, 7, 10).

The multiplicity and diverse role of these enzymes have introduced them as diagnostic markers and therapeutic targets in a large number of diseases such as cancer, nervous system disorders, skin diseases and diabetes (11-15). Interest in KLKs as cancer biomarkers began in the late 1970s to mid-1980s, followed by scientific groups that specifically elucidated KLKs, especially KLK3 or PSA, in malignant prostate gland in patients's serum (16, 17). Besides PSA, many other KLKs were proposed in screening, differential diagnosis and prognosis of prostate cancer that can be used as prostate cancer biomarkers in tissue or serum, providing valuable information about the prognosis of prostate cancer as well as rate of malignancy (1, 8, 17). This study provides a review on KLK2 as a member of the KLK family that plays a key role in the progression and diagnosis of prostate cancer. The role of KLK2 in the pathobiology of prostate cancer and the possible mechanisms involved in the progression of this cancer are summarized. Finally, this review presents

the importance of this protein as a specific diagnostic marker along with PSA marker in prostate cancer. Understanding the structure and activity of KLK2 and the mechanistic pathways implicated by KLK2 in the progression of prostate cancer, could offer the way for further studies to apply new treatment strategies as well as usage as a specific diagnostic marker in the diagnosis of prostate cancer.

Kalikrin-2 peptidase (KLK2)

Kalikrin-2 (KLK2) gene is a member of the KLK gene family and KLK2 protein is a trypsin-like serine protease which is highly expressed in prostate tissue. KLK2 is also called hK2. KLK2 is usually co-expressed with KLK3 or PSA and co-located in the prostate gland. KLK2 is about 1% of the total PSA concentration in seminal plasma and shares 80% homology with PSA or KLK3. Both KLK2 and KLK3 are highly expressed in prostate luminal epithelial cells and as secretary proteins are produced by prostate gland (18). Although expression of KLK2 may be observed in other organs, the level of expression in other tissues is much less than prostate and their biological activity remains unknown. Expression levels of KLK2 and KLK3 which are regulated by androgens, reflect the function and activity of the nuclear androgen receptor (AR) and their response to testosterone or other androgen hormones (19, 20).

KLK2 gene and protein

It has been hypothesized that the KLK2 precursor gene has been originated from the replication of the KLK1 gene, early in mammalian evolution. The KLK2 precursor is a non-functional pseudo gene in many mammals, including rodents, however it is highly expressed in prostate of other mammals whose expression of KLK2 is regulated by androgen. There is an enhancer containing androgen response element (ARE) in 5′UTR of transcription initiation site (21). KLK2 as a proto-oncogene can be regulated and overexpressed by androgens and androgen receptor signaling pathway in prostate cancer (22). KLK2 can also act as an androgen regulator, cooperating with the ARA70 regulatory protein to increase androgen activity and regulate cell growth during cancer development (23).

The identity of amino acid sequence between KLK1 and KLK2 or KLK3 (67-62%) is much greater than amino acid sequence of KLK4-15 (27-29%). Firstly, these proteases are produced as non-catalytic pre-proenzymes which require several post-translational modifications to form a catalytically active form. To obtain the active form of KLK2, a proteolytic cleavage in the signal sequence occurs by the signal peptidase, followed by a secondary breakdown with a trypsin-like peptidase enzyme, releasing a short peptide from the N-terminal region. Finally, the non-catalytic zymogen form is converted to the active single-stranded form of KLK2 with 237 amino acids. Substitution of alanine with valine 217 has shown a reduction in the catalytic efficiency of KLK2 (22, 24, 25). Figure 3 shows the three-dimensional structure of KLK2.

KLK2 activity and its role in the pathobiology of prostate cancer

Regulation of KLK2 activity is crucial for the maintenance of cell and tissue function and homeostasis, whereas KLK2 deregulation can be used as a cancer biomarker. Although there are some similarities between KLK2 and PSA in terms of their function, tissue expression and regulatory properties, KLK2 has different enzymatic activity compared to PSA. One of the most significant differences between KLK2 and PSA is that KLK2 is a trypsin-like serine protease, whereas PSA is a chymotrypsin-like one. KLK2 has a much higher catalytic activity than PSA and is able to activate either itself or PSA. Therefore, it suggests that KLK2 may also regulate biological activity of PSA (24-26).

KLK2 is secreted and activated into the seminal fluid. The gel-forming proteins (SEMG1 and SEMG2) are then proteolytically degraded by KLK2 to liquefy the seminal gel. This is an essential step for the release of sperm to the uterus in order to fertilize the ovum (21).

Moreover, KLK2 contributes to the normal

Fig3. KLK2 protein structure (https://www.rcsb.org/)

physiology of the prostate through hydrolyzing seminal vesicle proteins, hydrolyzing seminogelin I and II and fibronectin. Proteolytic cleavage of seminogelin and fibronectin by KLK2 indicates that this enzyme is also able to degrade cellular matrix proteins. This leads to liquefaction of the seminal clot and increases sperm motility (21, 22). However, KLK2 can also play a critical role in the pathological condition of the prostate gland. Research has also focused on the association between KLK2 and prostate cancer proliferation and invasion. Tumorigenicity activity of KLK2 is mediated by activating several molecules and proteins. KLK2 is able to inhibit the plasminogen activator urokinase inhibitor (uPAI) and activate uPA which is a protease initiating the proteolytic cascade, converting plasminogen to plasmin as well as degrading extracellular matrix proteins such as collagen types I and IV, fibronectin and laminin. It is contributed to tumor development and strongly associated with prostate cancer invasion and metastasis (22, 27, 28). Furthermore, KLK2 also has a vital role in activating matrix metalloproteases (MMPs) through proteolytic cleavage of pro-peptides (27, 28).

It has been reported that KLK2 may directly enhance the growth of cancer cells (10). Studies have shown that KLK2 is able to cleave insulin-like growth factor proteins and activate growth factor, and may also modulate the activity of parathyroid protein (PTHrP). Insulin-like growth factor (IGF) causes mitogenic and anti-apoptotic effects on normal and tumor cells through binding to IGF receptor. IGF availability and binding to IGF receptor can be regulated by IGF-binding proteins (IGFBPs). KLK2 is also able to degrade IGFBP2-5, thus leads to the high availability of IGF and may indirectly contribute to the proliferation and progression of cancer cells. High level of IGF in the blood and therefore high activity of the IGF pathway has been shown in prostate cancer (20-22). IGFBP3 can also induce apoptosis in prostate cancer cells. Therefore, KLK2-mediated degradation of IGFBP3 leads to inhibit apoptosis in these cells as well as increase in IGF availability which is implicated in proliferation and progression of prostate cancer cells. In addition, studies have shown that KLK2 plays a crucial role in promoting the growth and metastasis of prostate cancer cells by activating TGFβ (28-30).

The role of KLK2 in angiogenesis of prostate cancer cells is also mediated by proteolytic degradation of plasminogen and high molecular weight kininogen (HMWK). KLK2 cleaves HMWK proteolytically to activate and release bradykinin, a factor that induces smooth muscle cell contraction, which is implicated in formation of blood vessels in prostate tumors (28-30).

Another target of KLK2 is PARs (PAR1-4), a subtype of G protein-coupled receptors. Proteolytic activation of PAR1 is involved in regulating proliferation of prostate cancer as the increased expression of PARs has also been confirmed in prostate cancer. These proteins are activated by a proteolytic cleavage by KLK2 in the N-terminal domain (31). Besides of the physiological roles, PARs can interfere with the development of cancer-related signaling pathways such as MAP kinase and ERK, which promote cell proliferation and migration (Figure 4) (29, 30).

Table 1 presents the role of KLKs in particular KLK2 in prostate cancer.

Different forms of KLK2 and KLK3

There are various forms of PSA detected in the blood due to the catalytic activity of PSA and the existence of more than 10,000 times proteinase inhibitors in extracellular fluid. Once, enzymatically active PSA releases into the blood, can form irreversibly active complexes with extracellular protease inhibitors such

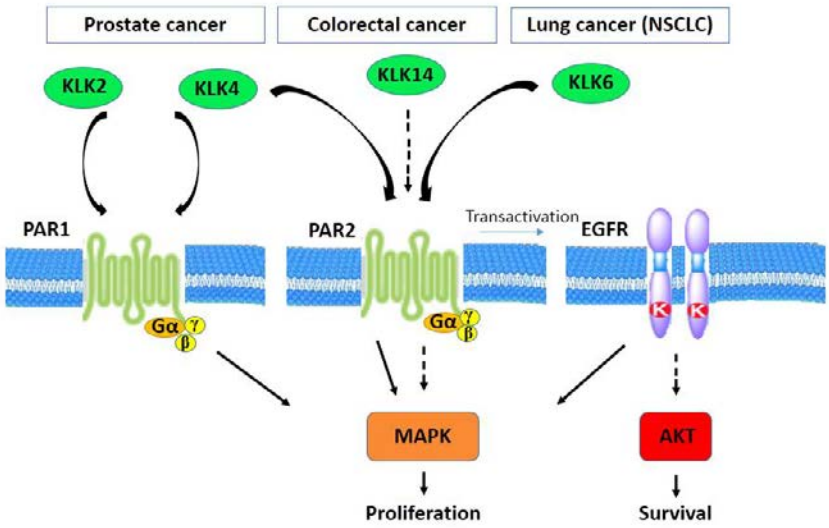

Fig 4. *The role of KLKs in cancer and specifically the role of KLK2 in activating PAR1 and activating the MAPK pathway in prostate cancer*

Table 1. An overview on the activity and role of KLKs, specifically KLK2, in prostate cancer

KLK	Target	Phenotype of cancer cell
KLK2-5, KLK11	IGFBP1-5 TGFβ1, 2 Growth hormones	Proliferation
KLK14 ،KLK2-4	IGFBP3 PAR-1	Fibroblast signaling
KLK2-3	HMWK Plasminogen	Endothelial signaling
KLK2-3	HMWK Plasminogen	Angiogenesis
KLK13-14 ،KLK2-5	Collagen type I, IV Fibronectin Laminin	EMT and migration
KLK13-14 ،KLK2-5	Collagen type I, IV Fibronectin Laminin Plasminogen	Invasion
KLK2-4	TGFβ1, 2 Plasminogen	Metastasis

as α1-antichemotrypsin (ACT or SERPINA3), α2-macroglobulin (A2M), pregnancyzone protein (PZP), α1-antitrypsin (SERPINA1) or protein inhibitor C (SERPINA5). Binding PSA to the inhibitors is the immune active dominant form of PSA in the blood (32, 33). Besides of these irreversible PSA complexes, there are non-catalytic PSA forms which don't bind to the inhibitors and are commonly referred to as "free PSA". Free PSA is proteolytically processed and creates its inactive type which is not able to form complexes with the inhibitors. This type of PSA is not implicated in the cleavage and proteolysis of vascular substrates (34, 35). In the same manner, it is hypothesized that most non-catalytic forms of KLK2 do not also appear to bind to the mentioned inhibitors in the blood.

Recently, a new complex of KLK2 with protease inhibitor 6 (PI-6) has been discovered in prostate cancer tissue. PI-6 is an intracellular serine protease inhibitor with both anti-trypsin and anti-chemotrypsin activity. It is estimated that this 64-kDa KLK2-PI6 complex comprises 10% of total KLK2 in prostate tissue, whereas it increases in prostate tumor tissue. The KLK2-PI6 complex provides evidence which may indicate tissue damage and necrosis associated with neoplasia and therefore play a vital role in the development of neoplasia (36). Moreover, KLK2 may be observed as complexes with protein C (PC), ACT, α2-antiplasmin, and antithrombin III in the blood, seminal plasma, and prostate cancer tissue.

One the other hand, KLK2 without complex (inactive) or free form of KLK2 in serum includes the following (37):

- Decayed KLK2
- Denatured KLK2
- Pre-enzyme form of KLK2 or zymogen

Prostate cancer and KLK2 as biomarker

Prostate cancer is the second cause of death in men with the highest number of new cases among all cancers. This type of cancer is highly dependent on the androgen signaling pathway in order to proliferate and survive. Therefore, cancer dependence on androgen is a benefit to treat the disease though androgen deprivation. The process involved in this disease leads to destruction of the basement membrane, destruction of the basal cell layer and glandular structure (38). Prostate cancer is usually diagnosed by digital rectal examination or PSA test in the blood. Numerous studies indicate that an increase in level of PSA in the blood is a sensitive (not specific) way which is associated with the diagnosis of the risk or occurrence of prostate cancer as well as usage as an effective factor in monitoring relapse after treatment (16).

KLK2 is well-known as the second biomarker among all of the kallikrins in detecting prostate cancer. Various histological studies have confirmed an increase in the ratio of KLK2 to PSA expression during carcinogenesis and progression of prostate cancer. Therefore, it was hypothesized that KLK2 may be a useful biomarker for the diagnosis of advanced prostate cancer (2). The KLK2 expression in patients with prostate cancer is higher than the healthy people, therefore, KLK2 gene could be considered as a useful factor in prostate cancer, whose expression is contributed to the

development of prostate cancer (39). Concentrations of PSA and KLK2 in tissue is 10^6 times higher compared to concentrations in blood. Recent research on prostate volume in prostatectomy specimens have shown that serum KLK2 concentration is significantly associated with extracapsular extension (ECE) of prostate cancer (2, 40).

Statistical analysis has revealed that the ratio of KLK2 to free PSA (fPSA) can specifically diagnose prostate cancer from benign prostatic hypertrophy. Therefore, the usage of KLK2 as a crucial diagnostic marker may increase the specificity of prostate cancer diagnosis. The prognostic information can be obtained by measuring KLK2. High level of KLK2 expression along with a low ratio of free PSA to total PSA (tPSA) are associated with prostate cancer malignancy and may also predict disease recurrence after local treatment (2, 41, 42). Therefore, it suggests that assessment KLK2 in patients with prostate cancer is a valuable biomarker which can be used to evaluate the clinical outcome and select the most appropriate therapy strategy.

KLK2-targeted therapies for prostate cancer

Due to the critical role of KLK2 as a prostate cancer biomarker in the diagnosis of this type of cancer, this biomarker has also been introduced as an important therapeutic target for the treatment of prostate cancer. There are several naturally inhibitors such as serpins, Kazal-type serine protease inhibitors and α2-macroglobulin which inhibit KLKs, however they act non-specifically (43). Recently, a KLK2 inhibitor that is a modified version of ACT (MD-PK67b) has been shown to reduce the development of prostate cancer tumors producing KLK2 (44). In addition, prodrugs activated by KLKs have been also described by some studies (45, 46). These prodrugs are capable to deliver drugs to a specific tissue. The inactive form of a prodrug comprises of a toxic molecule conjugated to a peptide. The activated prodrug is generated in the target tissue through cleavage of the peptide by a specific protease, resulting in release of the active form of drug molecule (43). A KLK2-activated prodrug consisting of a KLK2 peptide substrate conjugated to the thapsigargin analog, L12ADT, has shown anti-tumor activity in human prostate tumors (47).

CONCLUSION

KLK2 is a trypsin-like serine protease which is highly expressed in prostate tissue. Beside of the physiological functions of KLK2 to maintain normal cell and tissue integrity and homeostasis, androgen-regulated KLK2 gene expression can also contribute to prostate malignancies and the progression of prostate cancer. Mechanistic roles of KLK2 in the progression of prostate cancer are included the activation of plasminogen activator urokinase, inhibition of uPA inhibitors, activation of MMPs and proteolytic degradation of extracellular matrix proteins. Moreover, KLK2 induces proliferation of cancer cells as well as inhibition of apoptosis through activating IGF factor and MAP kinase signaling pathway, regulating PTHrP protein activity and degrading IGFBP3 protein. KLK2 also plays an important role in promoting migration, metastasis and angiogenesis of prostate cancer cells. Therefore, KLK2 can be recognized as the second diagnostic biomarker of prostate cancer. It is specifically able to distinguish prostate cancer from benign hypertrophy, thus it increases the specificity of prostate cancer diagnosis. In conclusion, evaluation of KLK2 provides a valuable method for future clinical procedures that can be used to assess the clinical outcome and select the most appropriate treatment for patients with prostate cancer.

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Research involving human participants and/or animals

This article does not contain any studies with animals and human participants performed by author.

Informed consent

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Author Contribution

S.Y.: Conception, Providing the data and design, Manuscript writing.

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