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Mini-review

Human tissue kallikreins: The cancer biomarker family ☆

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Abstract

Human tissue kallikreins (KLKs) are attracting increased attention due to their role as biomarkers for the screening, diagnosis, prognosis, and monitoring of various cancers including those of the prostate, ovarian, breast, testicular, and lung. Human tissue kallikrein genes represent the largest contiguous group of proteases within the human genome. Originally thought to consist of three genes, the identification of the human kallikrein locus has expanded this number to fifteen. These genes, and their encoded proteins, share a high degree of homology and are expressed in different tissues. Prostate-specific antigen (PSA), the most commonly known kallikrein, is a useful biomarker for prostate cancer. Several other kallikreins, including kallikreins 2 (KLK2) and 11 (KLK11) are emerging as complementary prostate cancer biomarkers. Along with these kallikreins, several others have been implicated in the other cancers. For example, *KLK5, 6, 7, 10, 11*, and *14* are emerging biomarkers for ovarian cancer. The identification of kallikrein substrates and the development of proteolytic cascade models implicate kallikrein proteins in cancer progression. This review describes the current status of kallikreins as cancer biomarkers.

Keywords: Kallikreins; Serine proteases; Cancer; Biomarkers; Clinical applications

1. Introduction

Human tissue kallikrein research has evolved with these accomplishments having been thoroughly chronicled in recent reviews, as well as in many research articles [2–5]. However, increased attention has been paid to the valuable role of kallikreins as cancer biomarkers.

Of the 178 known human serine proteases, accounting for 32% of all proteases, the human tissue kallikreins represent the largest contiguous cluster of protease genes in the human genome. The *KLK* genes are tightly grouped and arranged tandemly without any intervention by non-*KLK* genes. The three classical kallikreins KLK1, KLK2, and prostate-specific antigen (PSA) and *KLK15* are clustered in a 60 kb region, followed by the pseudogene *ΨKLK1*, and the 11 other *KLK*

☆ *Nomenclature*. In this review, kallikrein genes will be denoted as *KLK1* … *KLK15* and kallikrein proteins as KLK1 … KLK15, in accordance with the recently approved nomenclature [1]. *KLK3* will be referred to as *Prostate-specific antigen (PSA)* throughout the review.

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genes, with the direction of transcription of all genes from telomere to centromere with the exception of *KLK3 (PSA)* and *KLK2*. *KLK* genes share many common characteristics including; five coding exons, similar or identical coding exon lengths and conserved serine protease catalytic triad residues His, Asp, and Ser in exons 1, 3, and 5, respectively [2,6]. Most *KLK* genes also have a number of splice variants and/or alternative transcriptional start sites. With the exception of *KLK14*, all kallikreins have at least one alternative transcript, exclusive of their reference form, with *PSA*, followed by *KLK13*, having the highest number of alternative transcripts [7–10]. Most of these alternative *KLK* transcripts are predicted to code for truncated proteins. The biological and physiological significance, if any, of truncated KLK proteins, or the regulation of alternative *KLK* transcripts is unknown.

Groups of *KLK* genes are often expressed within a specific tissue. For example, *KLK2*, *KLK3*, *KLK4*, *KLK5*, *KLK11*, and *KLK15* mRNA and/or proteins are found in the prostate. As well, almost every kallikrein is expressed in the salivary gland, while other groups are found in the skin (*KLK5*, *KLK7*, *KLK8*, *KLK9*, *KLK11*, *KLK13*, and *KLK14)*, breast (*KLK3*, *KLK4*, *KLK5*, *KLK6*, *KLK8*, *KLK10*, *KLK13*, *KLK14*), pancreas (*KLK1*, *KLK10*, *KLK12*) and the central nervous system *KLK5-KLK9, KLK11, KLK14*. KLK proteins have also been found in biological fluids such as serum, seminal plasma, and milk of lactating women, confirming that these are secreted proteins. Tissue-specific expression patterns have also been identified for a few alternative *KLK* transcripts [3,11–13]. Some splice variants of both *KLK2* and *KLK3* seem to be exclusively expressed in the prostatic epithelium [14].

1.1. Physiological protein function

KLK transcripts code for a single chain serine protease pre-proenzyme, a characteristic of most secreted proteases. Kallikreins share an overall amino acid sequence identity of 40–80%, with the highest degree of similarity between KLK2 and PSA. With the exception of KLK4, all have a pro-peptide ending with Lys or Arg, suggesting that these zymogens are activated by enzymes with trypsin-like activity [15]. The majority of KLK proteins (KLK1–2, KLK4–6, KLK8, and KLK10–14) have an Asp residue in their binding pocket (or Glu for KLK15) suggesting that they possess trypsin-like substrate specificity. Other kallikreins, such as PSA, KLK7, and KLK9, possess chymotrypsin-like activity [16]. It has recently been shown that pro-KLK proteins can serve as substrates for activated KLKs, thereby setting the stage, potentially, for a proteolytic cascade, whereby differentially expressed kallikreins within the tissue microenvironment proteolytically activate other kallikrein proenzymes, with the entire array of activated species subsequently acting on extracellular substrates to either mediate physiological functions or contribute to disease progression [17–20]. Cascade models are currently being applied to skin desquamation and semen liquefaction, and may have relevance in tumor invasion and metastasis.

Kallikreins may have clinical utility in serving as targets for a number of novel therapeutic approaches currently under investigation. There is increasing evidence that a group of serine protease inhibitors (collectively known as serpins) may play a role in blocking KLK activity. The design of specific kallikrein serpins exploits the flexible reactive-site loop (RSL) of the inhibitors which is implicated in the interaction with the putative protease [21–23]. Binding of the enzyme and cleavage of the serpin leads to covalent bond formation between the two proteins, irreversibly trapping the protease in a non-reactive state. The specificity of serpin inhibition depends on both the amino acid sequence and length of the RSL. Several serpin inhibitors of kallikrein activity have been identified; but many of these lack specificity for the kallkrein family or specific members, which adversely affects their therapeutic potential. In particular, phage display technology has been used in conjunction with amino acid substitutions in the RSL of α_1-antichymotrypsin (ACT) to construct novel KLK2 specific inhibitors. Several potential serpin inhibitors were identified and tested against other serine proteases including chymotrypsin, PSA and urokinase Plasminogen Activator, with only one showing KLK2-specific inhibition [24,25]. The same technology is being used to discover additional kallikrein-specific serpin inhibitors.

Other therapeutic strategies have taken advantage of kallikrein activity or tissue specificity (e.g., PSA in prostate) to deliver tissue-specific toxic genes and induce active immunotherapy using KLK-based vaccines. Using an adenoviral or non-viral/liposomal vector delivery system containing a cell suicide gene, under the regulation of prostate-specific *PSA* promoter and enhancer elements, it is possible to selectively stimulate gene expression within PSA-producing prostate cancer cells, resulting in prostate