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Minireview

An update on human and mouse glandular kallikreins

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Abstract

Human glandular kallikreins are secreted serine proteases, involved in many biological processes. Recently, the complete organization of the human and mouse genomic loci has been elucidated. These loci harbor the largest clusters of serine proteases within the human and mouse genomes. Mouse orthologs to all human kallikrein genes, except for *KLK2* and *KLK3* genes, have now been identified. Here, we describe an update of the genomic organization of these families in human and mouse, and provide some thoughts for future research directions.

Keywords: Glandular kallikreins; Human; Mouse

Introduction

Why should clinical chemists be interested in glandular kallikreins?

Glandular kallikreins are secreted serine proteases. Their relatively small size (approximately 30 kDa), secretion into various biological fluids, presence in serum, and high specificity of some tissues for glandular kallikrein expression qualifies them as candidate biomarkers of human disease. The best known member of the human glandular kallikrein family is prostate-specific antigen (PSA). The official name for this gene is KLK3 and of its encoded protein, human kallikrein 3 (hK3). The clinical value of PSA for screening, diagnosis, and monitoring of prostate cancer is unquestionable. PSA is the best cancer biomarker to date [1]. Another member of the human glandular kallikrein family, human glandular kallikrein 2 (KLK2; encoding for hK2 protein), has also showed potential as a biomarker for prostate cancer. Combined with PSA, it enhances the discrimination between benign hyperplasia and cancer [2–4]. Another serine protease, plasma kallikrein, is encoded by a gene located on chromosome 4q35. This enzyme is structurally different from the tissue kallikreins and is involved in the blood coagulation cascade [5]. It will not be discussed further in this commentary.

Human glandular kallikreins

The human glandular kallikrein family was, until recently, thought to consist of 3 genes, KLK1 (also known as pancreatic/renal/tissue kallikrein), KLK2, and KLK3 [6]. These are also known as "the classical human glandular kallikreins." Over the last 4 years, we and others have cloned many new genes that have similarities to the three classical human glandular kallikreins and are located at the same locus. These new developments have been reviewed elsewhere [7–9]. An examination of the human glandular kallikrein gene locus on chromosome 19q13.4 (close to the telomere of the long arm of human chromosome 19) reveals that the locus is composed of 15 genes encoding serine proteases, which are tandemly localized, without any intervention by other genes. The 15 genes at this locus were included in one family that is now known as the "expanded human tissue kallikrein gene family" [7–10]. The sequence produced by the human genome project enabled the identification of all genes in this region and it was confirmed that the originally proposed organization of the genes at this locus is correct.

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Recent reports further suggest that in addition to PSA and hK2, many other members of the expanded human glandular kallikrein family are promising new biomarkers for ovarian, prostate, and breast cancer (reviewed in Ref. [8]). For example, hK5, hK6, hK7, hK8, hK10, and hK11 may be new biomarkers for ovarian cancer, hK4, hK11, and hK15 for prostate cancer, and hK5 and hK10 for breast cancer. New knowledge in this field is rapidly emerging.

Despite the identification of all genes within the human glandular kallikrein locus, the function of most of the encoded kallikrein enzymes is still elusive. The tandem localization of all these genes, their proximity, the same direction of transcription (for all except the genes encoding PSA and hK2), their transcriptional regulation by steroid hormones, and the parallel expression of many glandular kallikreins in the same tissues [7] has prompted us to speculate that this family might be coordinately expressed through a ''locus control region.'' Furthermore, these enzymes may participate in a cascade enzymatic pathway that could involve proenzyme activation, enzyme degradation, and enzyme binding to specific proteinase inhibitors [11]. These proposals need experimental verification. Already, it has been reported that some members of this family can activate the pro-forms of other members, for example, hK2, hK4, and hK15 can activate pro-PSA [12,13]. Such cascade pathways involving serine proteases are already well-known, for example, in coagulation, fibrinolysis, wound healing, and digestion.

Mouse glandular kallikreins

Until recently, it was thought that the glandular kallikrein gene family in mouse consisted of approximately 24 genes, of which 14 were assumed to be functional (the rest being pseudogenes). The completion of the mouse genome project (http://www.ncbi.nlm.nih.gov/genome/guide/mouse/) allowed identification of all mouse kallikrein genes on chromosome 7 and the comparison of the locus in the mouse with that found in humans [14]. The new data suggest that the total number of glandular kallikrein genes in the mouse is 37, of which 11 are pseudogenes. Interestingly, among the new genes that were identified in the mouse, there are 12 genes that represent the orthologs of the 12 newly identified human kallikrein genes (KLK4–KLK15). A schematic representation of the glandular kallikrein loci in human and mouse is shown in Fig. 1. Among all human glandular kallikrein genes, KLK2 and KLK3 are the only ones that do not have mouse orthologs. Instead, the homologous region, occupied in humans by KLK3 and KLK2, is occupied by a pseudogene (ψmGK25) of reverse

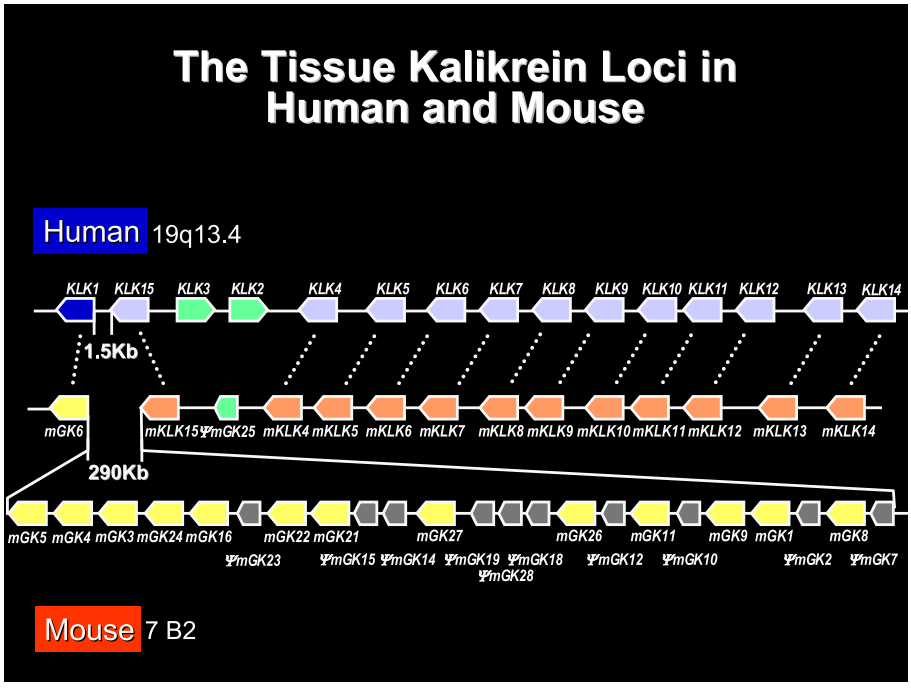

Fig. 1. Schematic representation of human and mouse tissue kallikrein genes. The boxes represent genes with arrows pointing to the direction of transcription. The three classical human glandular kallikrein genes are represented in dark blue (KLK1) and green (KLK2, KLK3). The newly identified human glandular kallikrein genes are represented in light blue and their orthologous genes in the mouse in orange (the orthologous genes are connected by dotted lines). The mouse ortholog of KLK1 is mGK6. The region occupied in humans by KLK2 and KLK3 genes corresponds to a region harboring the mouse pseudogene ψmGK25. The classical mouse glandular kallikrein genes are represented by yellow (those that are expressed) and gray boxes (pseudogenes). Names of all genes are shown above (human) or below (mouse) the boxes. For the human genes, the official nomenclature has been used [15]. For the mouse genes, those orthologous to the human are shown with the same names but with a prefix m (mouse). For the classical mouse glandular kallikrein genes, we used the originally proposed nomenclature with the prefix mGK (mouse glandular kallikrein).

orientation in the mouse genome. The distance between KLK1 and KLK15 genes in humans is only 1.5 kb, while in the mouse, the distance is 290 kb and contains all the classical mouse kallikrein genes (denoted as mouse glandular kallikreins, mGKs, in Fig. 1).

Implications

The large number of genes in humans and mouse, their co-localization, and the homologies between human and mouse glandular kallikrein genes call for a phylogenetic analysis that would further delineate the evolutionary origin of these genes. Currently, this analysis can be summarized with the following statements [14]:

1. Regarding the newly identified 12 human glandular kallikrein genes (KLK4–KLK15; shown as light blue boxes in Fig. 1) and their mouse orthologs (mKLK4–mKLK15; shown as orange boxes in Fig. 1), it seems that these genes, phylogenetically, are not closely related to the classical human or mouse glandular kallikrein genes, respectively. A more detailed phylogenetic analysis of the origin of these genes would be highly interesting.
2. The three classical human kallikrein genes, KLK1, KLK2, and KLK3, are phylogenetically well separated from the murine classical glandular kallikrein genes (shown as yellow boxes in Fig. 1). This suggests that these genes have evolved independently in the two species, through duplication of the tissue kallikrein gene, after the separation of the two lineages.
3. An examination of the map of Fig. 1 reveals that the location occupied in the human locus by KLK2 and KLK3 genes is occupied in the mouse by the pseudogene ψmGK25. These two human genes are the only ones without a mouse ortholog, and their direction of transcription is opposite to all other human and mouse glandular kallikreins. Thus, the concept that mice are lacking PSA and hK2 is still valid.

Conclusions

The human glandular kallikrein gene family is the largest cluster of serine proteases within the whole human genome. The best known cancer biomarker, PSA, and the emerging prostatic biomarker, hK2, do not seem to have mouse orthologs. Thus, the generation of knockout mice for these genes to delineate their function is not possible, and other approaches are needed to obtain a more complete picture of their function in different tissues.

The newly identified human kallikreins (KLK4–KLK15), many of which were shown to be dysregulated in ovarian, prostate, and breast cancer, have mouse orthologs (mKLK4–mKLK15). Generating knockout mice for these genes may provide important clues for the function of the human genes. A thorough evaluation of the tissue expression specificity of the human and the orthologous mouse genes is warranted. This would be essential when studying the possible enzymatic pathways in which all these genes are participating. More research is necessary to elucidate the biological and physiological role of the glandular kallikreins.

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