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Gene Section
Review

KLK12 (kallikrein-related peptidase 12)

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Abstract

Review on KLK12, with data on DNA, on the protein encoded, and where the gene is implicated.

Keywords
Kallikreins; KLK12; Prostate cancer; Breast cancer; Colorectal cancer; Gastric cancer; Non-small cell lung cancer;

Identity

Other names: KLK-L5, KLKL5
HGNC (Hugo): KLK12
Location: 19q13.41
Local order: Telomere to centromere

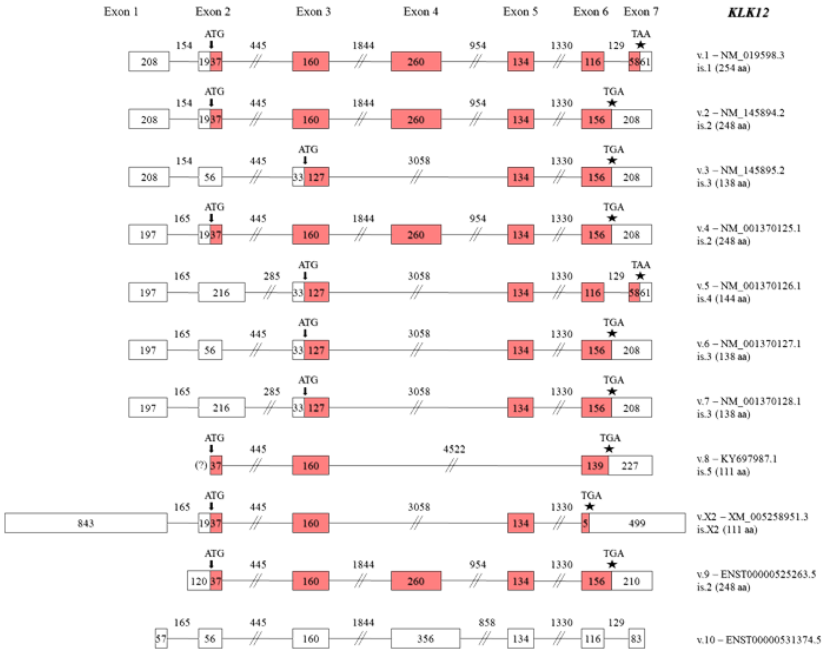

Figure 1. Schematic representation of the KLK12 gene. Exons are shown as boxes and introns as connecting lines. The coding sequences are highlighted as red, while 5' and 3' untranslated regions (UTRs) are shown in white. Numbers inside or outside boxes indicate lengths (nt) of exons and introns, respectively, while numbers in parentheses indicate lengths (aa) of protein isoforms. The question mark (?) denotes an undetermined 5' UTR. Arrows (↓) show the position of the start codons (ATG) and asterisks (*) denote the position of the stop codons (TAA or TGA). The figure is drawn to scale, except for the introns containing the (//) symbol.

DNA/RNA

Description

The KLK12 gene has a total length of 6,695 nt and comprises 7 exons and 6 intervening introns. The structure of the KLK12 gene is similar to that of the other KLK family members (Yousef et al., 2000). Four different genetic polymorphisms have been described so far: one at a splice-donor site of intron 4 (c.457+2T>C), two in exon 6 (c.618_619delTG:p.Cys206fsX72 and c.735G>A:p.Met245Ile), and one in intron 3. The c.457+2T>C polymorphism was detected at a high frequency (allele frequency:0.63), compared to the frequencies of the two polymorphisms in exon 6 (allele frequency:0.01) (Shinmura et al., 2004).

Transcription

The KLK12 pre-mRNA is subjected to alternative splicing, generating ten splice variants, nine of which are considered to encode distinct protein isoforms (Kurlender et al., 2005; Adamopoulos et al., 2018). Seven transcripts are accessible in the GenBank Reference Sequence (RefSeq) database. The predominant transcript (GenBank accession number: NM_019598.3), consisting of 1053 nt, includes all 7 exons and encodes isoform 1 precursor.

The second transcript (GenBank accession number: NM_145894.2) uses alternative splice sites in the 3' coding region, in comparison with the aforementioned transcripts, generating a protein with a distinct C-terminus compared to the isoform 1 precursor.

The third transcript (GenBank accession number: NM_145895.2)has a different open reading frame (ORF); it lacks an exon in the coding region and uses different splice sites in the 3' coding region, compared to the first transcript, generating a protein with a distinct N-terminus and the same C-terminus as isoform 2. The fourth transcript (GenBank accession number: NM_001370125.1) comprises an alternative exon in 5' untranslated region (5'-UTR) and generates the same protein isoform as transcript 2. The fifth transcript (GenBank accession number: NM_001370126.1) comprises two alternative exons in 5'-UTR, shows a different ORF and lacks an exon in the coding region, generating a protein with different N- and C-termini, in comparison with isoform 1.

The sixth transcript (GenBank accession number: NM_001370127.1) includes an alternative exon in 5'-UTR, lacks an exon within the coding region and uses alternative splice sites in the 3' coding region, generating the same protein isoform as transcript 3. The exon structure of the seventh transcript (GenBank accession number: NM_001370128.1) resembles that of the fourth one; it only lacks one exon within the coding region and generates the same protein isoform as transcripts 3 and 6.

Recently, members of our research group revealed the exon structure of a new KLK12 splice variant (GenBank accession number: KY697987.1) (Adamopoulos et al., 2018). This transcript lacks two exons within the coding sequence and uses alternative splice sites in the 3' coding region, while its 5' UTR has not been determined yet. This transcript is predicted to encode a distinct KLK12 protein isoform of 111 amino acid residues.

The sequences of seven transcripts have been deposited in the Ensembl database; only two of them have a different exon combination, compared to those of the RefSeq database. The structure of the first one (Ensembl accession number: ENST00000525263.5) is similar to the second transcript; it only lacks an exon in the 5' UTR, and has a 5' extended second exon, leading to the same protein isoform as variant 2. The other one (Ensembl accession number: ENST00000531374.5) has a distinct exon structure, which generates a premature translation termination codon.

One more splice variant (GenBank accession number: XM_005258951.3) is predicted to be encoded by the KLK12 gene, based on automatic sequence analysis of expressed sequence tags (ESTs). This transcript is similar to the third variant; it differs by having longer 5'- and 3'-UTRs, and a different ORF. The protein generated by the predicted transcript has a different C-terminus, compared to isoform 1.

KLK12 mRNA is primarily expressed in the salivary gland, stomach, uterus trachea, prostate, thymus, lung, colon, brain, breast, and thyroid gland. However, lower levels of KLK12 mRNA expression are found in other tissues too, including testis, pancreas, small intestine, spinal cord and tonsil (Yousef et al., 2000; Filippou et al., 2020).

Pseudogene

Not identified so far.

Protein

Description

Five distinct protein isoforms are generated by KLK12 transcript variants, while one more is most likely generated by the predicted transcript The main KLK12 isoform (isoform 1; GenBank accession number: NP_062544.1) precursor, designated as the classical one, consists of 254 amino acid residues and has a molecular mass of 25.3 kDa. The N-terminal signal peptide comprises 17 amino acid residues. KLK12 is a secreted trypsin-like serine protease, cleaving peptide bonds after both arginine and lysine (Memari et al., 2007; Yousef et al., 2000). KLK12 protein isoforms are synthesized as inactive precursor zymogens that are cleaved at position 22

during limited proteolysis to generate their active forms. Amino acid residues 24 and 163 represent glycosylation positions, while positions 194, 215, and 217 are substrate-binding sites. Three amino acid residues (positions: 62, 108, and 200) constitute the catalytic triad that is required for protease activity. KLK12 isoform 2 (GenBank accession number: NP_665901.1) has a distinct C-terminus compared to isoform 1, and its precursor form consists of 248 amino acid residues. KLK12 isoform 3 (GenBank accession number: NP_665902.2) has a different N-terminus compared to isoform 1 and the same C-terminus as isoform 2, and its precursor form consists of 138 amino acid residues. KLK12 isoform 4 (GenBank accession number: NP_001357055.1) has the same N-terminus as isoform 3, and a distinct C-terminus, including 144 amino acid residues. Another KLK14 precursor isoform (isoform 5; GenBank accession number: AUS91425.1) is composed of 111 amino acid residues and has a different C-terminus, in comparison with the classical isoform. One more isoform (GenBank accession number: XP_005259008.1) is predicted to be composed by 111 amino acid residues and possesses a distinct C-terminus.

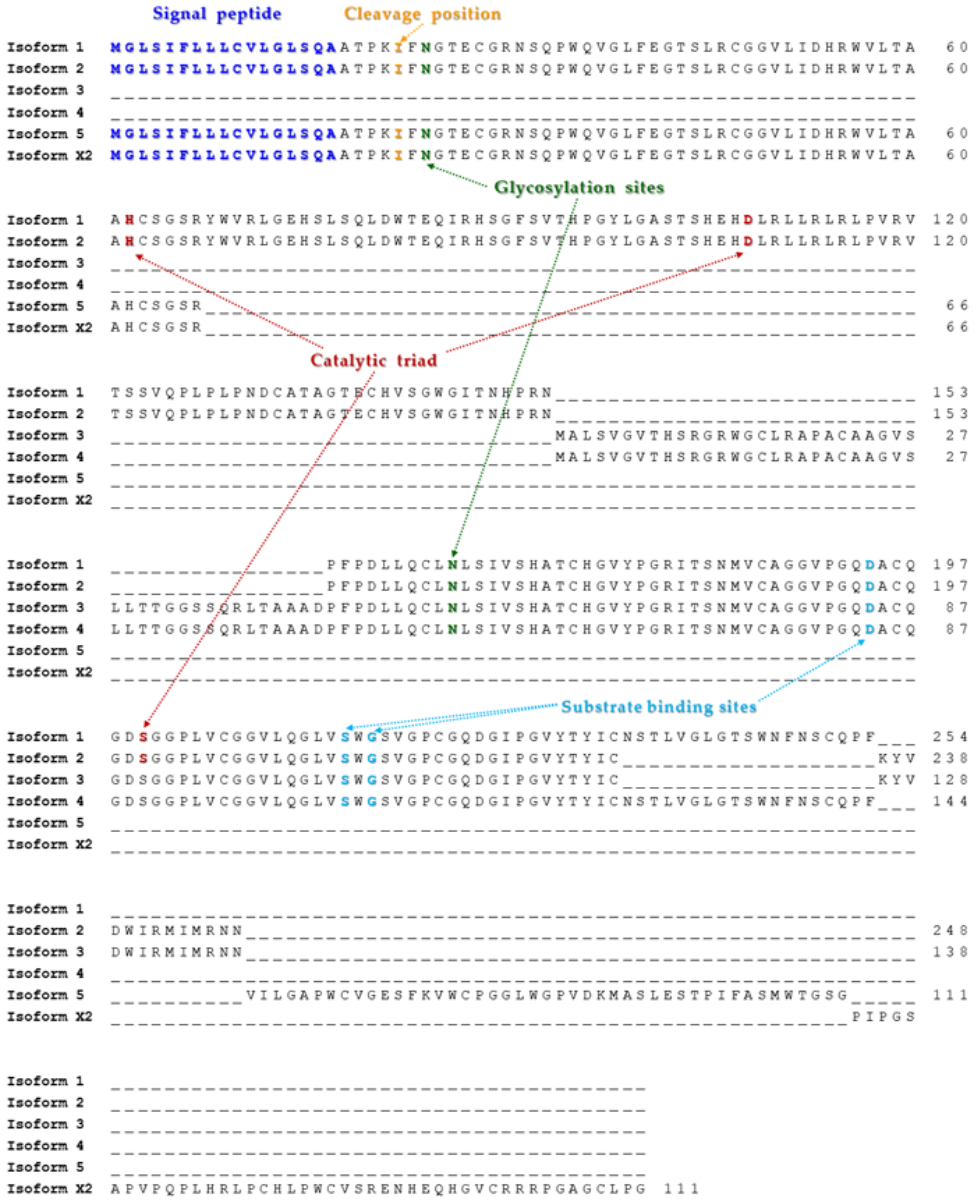

Figure 2. Alignment of amino acid sequences of the precursors of the KLK12 protein isoforms. The three amino acid residues (positions: 62, 108, and 200) constituting the catalytic triad that is required for protease activity are shown in red; the N-terminal signal peptide (positions: 1-17) is shown in blue; putative N-glycosylation sites (positions: 24 and 163) are shown in green; substrate-binding sites (positions: 197, 215, and 217) are shown in light blue; and the cleavage position is shown in orange (position 22). Numbering of amino acid residue positions is based on the sequence of the main KLK12 isoform (isoform 1) precursor.

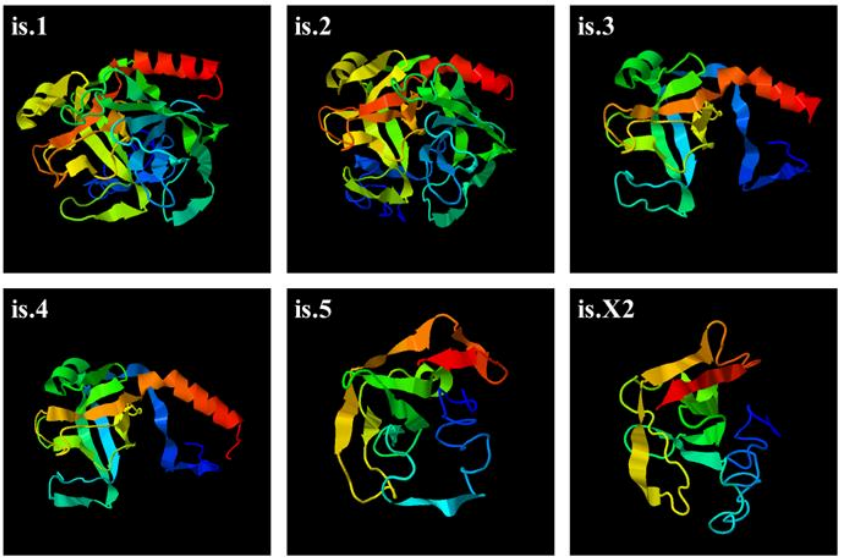

Figure 3. Predicted models of the precursors of the KLK12 protein isoforms, using the I-TASSER server (Yang and Zhang, 2015). For each protein, only the 3D structure with the highest confidence score is presented. The RasMol 'Group' color scheme color codes residues by their position in a macromolecular chain. Each polypeptide is drawn as a smooth spectrum from blue through green, yellow and orange to red. Thus, the N-termini are colored blue and the C-termini are drawn in red.

KLK12 is secreted as an inactive pro-enzyme, which can be self-activated to gain enzymatic activity. Active KLK12 shows trypsin-like activity, but quickly loses its activity due to autodegradation. KLK12 activity can also be rapidly inhibited by zinc ions and by α2-antiplasmin through covalent complex formation. It has been suggested that KLK12 participates in enzymatic cascades involving other KLKs (Memari et al., 2007). KLK12 is able to cleave all six members of the CCN family (CYR61, CTGF, NOV, WISP1, WIPS2, and WISP3) at different proteolytic sites, thus indirectly regulating the bioavailability and activity of several growth factors through processing of their CCN-binding partners (Guillon-Munos et al., 2011). In vivo substrates include also the thrombolytic zymogens plasminogen, urokinase, and plasma kallikrein. Another substrate of KLK12 secreted by the respiratory tract is influenza HA protein (Hamilton and Whittaker, 2013). Furthermore, KLK12 participates in the control of angiogenesis via a PDGFB-dependent paracrine pathway and by cleaving the extracellular matrix proteins fibronectin and tenascin. These proteins play a crucial role in endothelial cell adhesion and migration (Kryza et al., 2013; 2014; 2018). On the other hand, the proteolytic activity of KLK12 is inhibited by the action of serine peptidase inhibitor, Kazal type 6 (SPINK6) in the skin (Kantyka et al., 2011).

Expression

KLK12 mRNA is primarily expressed in the salivary gland, stomach, uterus trachea, prostate, thymus, lung, colon, brain, breast, and thyroid gland. However, lower levels of KLK12 mRNA expression are found in other tissues too, including testis, pancreas, small intestine, and spinal cord (Yousef et al., 2000).

Implicated in

Prostate cancer

KLK12 single nucleotide polymorphism (SNP) rs3865443 is significantly associated with increased risk of prostate cancer; still, this association has been described as marginal (Lose et al., 2013).

Breast cancer

KLK12 mRNA expression is downregulated in breast cancer tissues and is upregulated by steroid hormones in breast and prostate cancer cell lines (Talieri et al., 2012; Yousef et al., 2000).

Prognosis

KLK12 expression was associated with shorter disease-free (DFS) and overall survival (OS) time intervals of patients with triple-negative breast cancer (Gong et al., 2020). KLK12 variant 3 expression is downregulated in breast tumors of small size, high grade, and advanced TNM stage. Moreover, KLK12 variant 3 overexpression is associated with higher disease-free survival (DFS) rates for breast cancer patients, as well as with elevated progesterone receptor (PR) concentration (Talieri et al., 2012). KLK12 variant 3 constitutes an independent favorable marker with additional prognostic information to TNM staging of breast cancer patients. KLK12 variants 1, 2, and 3 can be

used to distinguish benign from malignant breast tissue (Papachristopoulou et al., 2018).

Colorectal cancer

KLK12 was overexpressed in HT-29 colorectal cancer cells. Silencing of KLK12 via siRNA was shown to inhibit invasion and migration of these cells. Furthermore, expression levels CDH1 were increased upon KLK12 silencing, whereas expression levels of Vimentin, SNAI1, and matrix MMP2 and MMP9 (metallopeptidases 2 and 9) decreased, thereby indicating the role of KLK12 in epithelial to mesenchymal transition (EMT). KLK12 silencing in the colorectal cancer HT-29 cells resulted in the reduction of the expression of the antiapoptotic BCL2 protein and the parallel increase in the expression levels of the proapoptotic BAX protein, thus enhancing CASP3 (Caspase3) cleavage and mediating apoptosis.

Loss of KLK12 expression increased, also, the phosphorylation of PRKAA2 (5' adenosine monophosphate 5' AMP-activated protein kinase AMPK), which in turn decreased the phosphorylation of mechanistic target of rapamycin kinase (MTOR), a central controller of cell growth and proliferation. These findings indicate that HT-29 cell viability and metastasis may be regulated by KLK12 (Li et al., 2019).

Gastric cancer

Expression of the KLK12 gene is significantly upregulated in gastric cancer tissues, as compared with normal gastric tissues, both at the mRNA and protein level. Knockdown of KLK12 expression via siRNA resulted in a reduction of proliferation and migration of MKN-45 and AGS gastric cancer cells (Zhao et al., 2012; Li and He, 2016).

Prognosis

KLK12 overexpression is significantly associated with lymph node metastasis, histological type, and TNM. Furthermore, patients with gastric tumors showing high KLK12 expression have a significantly worse five-year survival rate than those with tumors with low KLK12 expression (Zhao et al., 2012).

Non-small cell lung cancer

KLK12 levels are lower in sera from non-small cell lung cancer patients than in sera from normal controls. Serum KLK12 concentration is likely to be associated with disease stage (Planque et al., 2008).

Furthermore, CASC15, a cancer-related long non-coding RNA, was reported to contribute to proliferation and invasion of A549 and H1299 lung cancer cells through regulation of the miR-766-5p / KLK12 axis, thus increasing the expression levels of KLK12 (Bai et al., 2019).

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