

An Alternatively Spliced Variant of KLK4 Expressed in Prostatic Tissue

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Introduction

Prostate cancer is the third leading cause of malignancy-related deaths in North America. Despite the availability of such good markers as the two members of the kallikrein family, prostate specific antigen (PSA), and human glandular kallikrein 2 (hK2), the necessity to find additional markers still remains to differentiate aggressive from nonaggressive forms of this cancer. In the quest of further characterizing these serine proteases, several splice variants of both PSA (1,2) and hK2 (3) have been found. Interestingly, other, recently discovered members of this gene family such as neuropsin (4) and KLK-L4 (5) also possess splice-variants. Although the significance of the alternate mRNA forms has not been investigated so far, it is already known that different splice variants of the PSA gene were noted to occur in benign prostatic hyperplasia, as opposed to prostate cancer cells (6). It is possible that the presence of various forms of PSA occurring in benign and malignant prostate tissues is the result of differential expression of the splice alternatives (7).

KLK4 (protease, KLK-L1) is another newly discovered member of the kallikrein gene family (8,9) colocalizing with PSA and the other kallikreins to chromosome 19q3.3 to 13.4. This serine protease is now known to be up-regulated by androgens in the LNCaP cell line (8) and by both androgens and progestins in the BT-474 breast cancer cell line (10). Based on Northern blotting, it was initially thought to be prostate-restricted in its expression (8). How-

ever, further investigations by reverse transcription-polymerase chain reaction confirmed its lower expression in other tissues as well, including testicular, mammary, adrenal, brain, uterine, and thyroid tissues (10,11). Although it is abundantly expressed in the prostatic epithelium, its possible diagnostic value in prostatic malignancy is yet to be determined. Here, we report a novel splice variant of KLK4, cloned from prostate tissue.

Methods

We have introduced *EcoRI* and *NotI* restriction enzyme sites into KLK4-specific primers and amplified KLK4 cDNA by polymerase chain reaction; the latter had been prepared from 2 µg of total prostate RNA as prescribed by the manufacturer (SuperScript™ Preamplification System, Life Technologies, Gaithersburg, MD, USA). The polymerase chain reaction product and pPICZαA vector (Invitrogen, Carlsbad, CA, USA) were digested with *EcoRI* and *NotI* at 37 °C for 2 h, and the products of the digest were ligated overnight at 16 °C with T4 DNA ligase, according to standard protocols (12). The construct was then transformed into chemically competent One Shot® *Escherichia coli* cells via heat shock as per the manufacturer's instructions (Invitrogen). Transformed cells were plated on Zeocin-supplemented low-salt Luria-Bertani agar and left to grow overnight at 37 °C. Next, white colonies were inoculated into 5 mL of Zeocin-supplemented low-salt Luria-Bertani medium and grown overnight on an orbital shaker at 37 °C. The recombinant plasmids were purified by a miniprep procedure (Qiagen, Mississauga, Canada) and then sequenced on an automated sequencer (ACGT Corp, Toronto, Canada) using vector-specific primers supplied by the manufacturer (Invitrogen). By using BLAST search, the KLK4 splice variant was identified by comparing the sequence against the native

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Manuscript received August 22, 2000; revised and accepted October 2, 2000

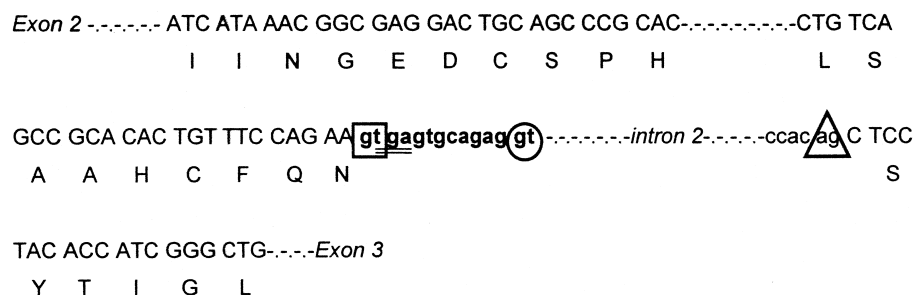


Figure 1 — Partial genomic sequence of native and splice variant KLK4, showing exon/intron organization. For complete sequence of KLK4 and nucleotide numbering see GenBank, Accession Number AF135023. The donor site used in the native KLK4 is boxed (2426–2427), while the one used by the splice variant is encircled (2438–2439). The acceptor site, which is the same for both forms of mRNA, is outlined by a triangle (2845–2846). Capital letters indicate exons, and lowercase letters denote intronic sequences. The encoded amino acids are shown underneath the exons by single-letter abbreviation. The bold intronic sequence is the extra segment found only in the splice variant (bases 2426–2437). The stop codon of the splice variant is double underlined. For more details see text.

KLK4 genomic and mRNA sequences in GenBank (accession nos.: AF135023, NM004917).

Results and discussion

The sequencing data for the KLK4 splice variant cloned into the pPICZαA expression vector is shown in Figure 1. Comparison to the native sequence reveals that this variant results from an intronic segment retention after coding exon 2. The same mechanism creates an alternately spliced form of PSA (13). This alternative splicing in the KLK4 gene results in an mRNA that is longer by 12 bases. The splicing creates a stop codon (TGA) that will lead to a premature termination of translation at amino acid 75. The truncated protein has only one amino acid of the catalytic triad and is likely biologically inactive. The alternative splicing may serve to regulate the amount of enzymatically active KLK4 protein present in the prostate. We do not have any experimental evidence that this alternative mRNA leads to protein synthesis or of its level in the prostate. Although currently not known, it would be interesting to see if this form of KLK4 protein has any diagnostic value in prostatic tissues.

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