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Presentation Title: Production of recombinant human kallikrein 15, a potential prostate and ovarian cancer biomarker
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The human kallikrein 15 gene (*KLK15*), encoding for hK15 protein, is the most recently cloned member of the kallikrein family, a cluster of 15 serine protease genes on chromosome 19q13.4. Other members of the kallikrein family are differentially expressed in hormone-related malignancies, implicating these proteins as potential biomarkers for cancer. Prostate specific antigen (PSA), or kallikrein 3 has proven to be a useful biomarker for prostate cancer, and is considered the best serum tumor marker to date.

KLK15 is expressed in the thyroid, salivary, and adrenal glands, the prostate, colon, testis and kidneys, at both the mRNA and protein level. mRNA studies also indicate that *KLK15* is up-regulated in more aggressive forms of prostate cancer. This suggests that *KLK15* may be a potential indicator of poor prognosis, and may be useful in distinguishing tumor aggressiveness. *KLK15* is also expressed at higher levels in cancerous versus benign ovarian tumors, suggesting that *KLK15* is an unfavourable prognostic marker for ovarian cancer. Moreover, *KLK15* has been shown to be up-regulated by steroid hormones, primarily androgens. Androgens are implicated in the pathogenesis of ovarian and prostate cancer, further indicating the potential role of *KLK15* as a biomarker for these malignancies.

To determine the potential of the hK15 protein as a cancer biomarker, a sensitive and specific immunoassay for hK15 is essential. For this purpose, relatively large amounts of purified hK15 are necessary for antibody production and assay standardization. We produced pure recombinant hK15 using the *Pichia pastoris* expression system, along with chromatographic purification schemes.

cDNA for *KLK15* was obtained by reverse transcription of RNA extracted from prostatic tissues, followed by amplification using PCR with two *KLK15* specific primers. *KLK15* cDNA was cloned into the pPIC9 vector, for stable transformation into *Pichia pastoris* KM71 cells. Recombinant hK15 produced by *Pichia pastoris* is purified using cation-exchange and reverse phase chromatography, which results in a band of the expected molecular weight of approximately 28 kDa, visible by SDS-PAGE, and verified to be hK15 by tandem mass spectrometry. Higher and lower molecular weight forms of hK15 are also present, and have also been confirmed by tandem mass spectrometry. We speculate that the higher molecular weight forms are due to N-glycosylation. Recombinant hK15 is enzymatically active, and several lower molecular weight break-down products are formed as a result of hK15 self-cleavage.

The availability of pure, recombinant hK15 will facilitate the development of antibodies and ELISA assays for its quantification in tissues and biological fluids.

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