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4452 Production of polyclonal antibodies against human tissue kallikrein 9.

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Human tissue kallikreins (KLKs) are a group of 15 genes, tandemly located on chromosome 19 with considerable similarity at the DNA and amino acid level. Two of the kallikreins namely, *KLK2* and *KLK3* (also known as prostate specific antigen, PSA) are currently used as serological biomarkers of prostate cancer. Human tissue kallikrein 9 (*KLK9*) is one of the newly identified members of the kallikrein gene family. Similarly to other kallikreins, the *KLK9* gene is regulated by steroid hormones. *KLK9* expression in BT-474, MCF-7, and T-47D breast cancer cell lines and BG-1 ovarian cancer cell line is up-regulated by steroid hormones. Recent reports indicate that *KLK9* mRNA is differentially expressed in ovarian and breast cancer. Ovarian and breast cancer patients with *KLK9*-positive tumors have longer progression-free and overall survival compared to those who are *KLK9*-negative. Due to lack of a sensitive method for the detection of *KLK9* protein (*hK9*), the prognostic significance of this kallikrein in cancer, at the protein level, remains elusive. Here we report for the first time production of recombinant *hK9* (*rhK9*) and generation of polyclonal antibodies against this kallikrein. Total prostate tissue mRNA was reverse-transcribed to cDNA. Polymerase chain reaction with P*fu* polymerase and primers specific to the mature form of *KLK9* was performed. The amplified cDNA was cloned into pET/200 TOPO plasmid vector containing an N-terminal polyhistidine (6xHis) tag. The pET-*KLK9* construct was used to transform the *E.coli* strain BL21(DE3) for protein production. The identity of *rhK9* was confirmed by mass spectroscopy. *rhK9* was mainly produced in "inclusion bodies". The inclusion bodies were purified and then dissolved with guanidine hydrochloride. *rhK9* was purified to homogeneity using nickel-nitrilotriacetic (Ni-NTA) metal affinity chromatography, followed by reversed-phase high performance liquid chromatography. *rhK9* was used as immunogen for production of antibodies in New Zealand White rabbits and female BALB/c mice. Specific affinity of *rhK9* polyclonal antibodies was tested using antibody capture assays and Western blotting. These antibodies will be used to develop a sandwich ELISA capable of measuring *hK9* in normal and cancerous tissues.

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