Production of polyclonal antibodies against human tissue kallikrein 9.

Nader Memari, Greg Hussack, Shannon Cui, Linda Grass, Eleftherios P. Diamandis. University of Toronto, Toronto, ON, Canada and Mt. Sinai Hospital, Toronto, ON, Canada.

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 Pfu 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.

Copyright © 2004 American Association for Cancer Research. All rights reserved.
Citation information: Proceedings of the AACR, Volume 45, March 2004