

5257 Production of polyclonal and monoclonal antibodies against human tissue kallikrein 12.

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

Human kallikreins are a group of 15 genes, tandemly located on chromosome 19, which are predicted to encode for serine proteases. Growing evidence indicates that many kallikreins are involved in malignancy. Classical kallikreins such as *KLK2* and *KLK3* (also known as prostate specific antigen, PSA) are currently used as serological biomarkers in diagnosis and monitoring of prostate cancer. In addition, numerous reports indicate that mRNA of many of the newly identified kallikreins is differentially expressed in ovarian, breast and prostate cancer. No rich natural source for many of the kallikreins is currently available; consequently the production of recombinant kallikreins for antibody generation and functional studies is highly pursued. Human kallikrein12 (*KLK12*) is one of the newly identified members of the kallikrein gene family. Our earlier results indicate that *KLK12* is hormonally regulated and its expression at the mRNA level is down regulated in breast cancer patients. Similar to other kallikreins, *KLK12* protein (*hK12*) is predicted to be a secreted serine protease. Currently, no method is available to detect and quantify *KLK12* expression at the protein level. Here we report the production of the first polyclonal and monoclonal antibodies against this kallikrein. Recombinant *hK12* (*rhK12*) was produced using an *E.coli* protein expression system. Commercially available total lung tissue mRNA was reverse-transcribed to cDNA. Polymerase chain reaction using primers specific to the mature form of human *KLK12* was conducted. The PCR product was cloned into pET/100 TOPO plasmid vector and the construct was used to transform Top10 *E.coli* strain BL21(DE3) for protein production. The identity of the *rhK12* produced was confirmed by mass spectroscopy. *rhK12* was purified to homogeneity using nickel-nitrilotriacetic metal affinity chromatography, followed by reversed-phase high performance liquid chromatography. Highly pure *rhK12* was used as immunogen for the production of antibodies in New Zealand White rabbits and female BALB/c mice. The antigen was diluted in complete Freund's adjuvant for the first subcutaneous injection and in incomplete Freund's adjuvant for subsequent injections. Rabbit polyclonal and two monoclonal anti-*hK12* antibodies were produced. Specific affinity of *rhK12* antibodies was tested by Western blotting and antibody capture assays. These are the first antibodies generated against this kallikrein to-date. These antibodies were used to develop a sandwich ELISA capable of measuring the level of *hK12* in normal and cancerous tissues and various biological fluids.

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