Human tissue kallikrein 9: Production of recombinant proteins, antibody generation and ELISA development

Nader Memari1,2, Linda Grass1, Ece I. Karakuş1, Julie L.V. Shaw1,2, and Eleutherios P. Diamandis2

Department of Laboratory Medicine and Pathobiology, University of Toronto and Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada

ABSTRACT

Human tissue kallikreins (genes, KLKs; proteins, hKs) are a subgroup of hormonally regulated secretory serine proteases. The human kallikrein gene family is comprised of 15 members. Two of the classical kallikreins, hK2 and hK3, currently have utility in cancer diagnostics. Numerous reports indicate that the mRNA of many of the newly identified kallikreins is differentially expressed in human primary tumors. The production of recombinant kallikreins such as hK6, hK10, and hK11 in recent years and consequent generation of antibodies against these kallikreins not only has provided new insights into their physiological and pathological role but also resulted in generation of highly sensitive serological assays. These assays confirm the potential utility of these kallikreins as cancer biomarkers at the protein level.

INTRODUCTION

Human tissue kallikrein gene family is a group of 15 closely related genes, located on chromosome 19 (q13.4) in tandem, which encode for secreted serine proteases with various physiological functions. The human kallikrein gene family (KLKs), human glandular kallikreins (2-12) and prostatic-specific antigen (PESA, PSA) are amongst the first tissue kallikreins identified. In addition to these “classical” tissue KLKs, in recent years, 12 additional tissue kallikrein genes have been identified in the same locus (Borgono and Diamandis, 2004; Clements et al., 2004).

Human tissue KLK gene family has attracted significant attention mainly due to their association with a number of common diseases in addition to possible novel biomarkers (Okuslu and Diamandis, 2005). KLK2, KLK3, and KLK11 are established or candidate serologic biomarkers in early diagnosis and monitoring of prostate cancer. In addition to prostate cancer, many of the newly identified kallikreins are differentially expressed in ovarian (Ni & al., 2004), breast (Millikan & al., 2000), cervical (Samin et al., 2004), and colorectal (Ogawa & al., 2005) cancer. No rich natural source of mRNA is currently available for the newly identified kallikreins. The production of recombinant tissue kallikreins for antibody generation and functional studies in recent years is highly pursued.

Human tissue kallikrein 9 (KLK9) was originally identified in our laboratory in the year 2000. The full sequence of the gene (GenBank accession # AF153006) and its precise chromosomal localization have been characterized and confirmed (Yousef & al., 2004). Similar to other kallikreins, hK9 is predicted to be synthesized as a prohormone which is processed into a mature hormone (229 amino acids). hK9 harbors a signal peptide of 19 amino acids and a 3-amino acid pro-segment. hK9 mRNA, by RTPCR analysis, is found in a wide variety of tissues. hK9 mRNA is differentially expressed in ovarian and breast cancer.

Among the fifteen members of the KLK gene family, KLK9 is the only kallikrein for which production of recombinant protein has not been reported to date. In this study, we describe the production of recombinant hK9 and generation of antibodies against this kallikrein.

METHODOLOGY

Total testis tissue mRNA was reverse-transcribed to cDNA. Polymerase chain reaction (PCR) was used to amplify the full length mRNA using primers specific to the pro-form human KLK9. The PCR product was cloned into pET/200 TOPO plasmid vector (Promega) for protein production. hK9 was purified to homogeneity using nickel-NTA affinity chromatography followed by reversed-phase high performance liquid chromatography (HPLC).

Highly purified hK9 was used as immunogen for antibody production. Rabbit anti-hK9 serum displayed no cross-reactivity with other kallikreins and could specifically recognize E. coli and CHO-derived hK9 in Western blots. Full-length KLK9 cDNA was cloned in pPDNA3.1 vector and was transfected into Chinese hamster ovary (CHO) cells as well as human embryonic kidney (HEK)-293 cells.

Stable cell lines secreting pro-hK9 with no tag were generated. CHO-derived hK9 was purified using FPLC and reversed-phase HPLC and was used as immunogen to generate polyclonal and monoclonal antibodies.

An immunoequivalency with no cross-reactivity with other members of the kallikrein gene family was developed. hK9-ELISA could detect the hK9 generated in E. coli as well as CHO and HEK-293 derived hK9. Our preliminary results indicate that hK9 is present in a variety of tissues including liver, muscle, testis and seminal vesicle as well as in biological fluids such as human breast milk, amniotic fluid, and seminal plasma. The reagents generated here will help define the physiological role of this kallikrein and its involvement in human disease.

RESULTS

Figure 4. Detection of hK9 fusion protein in bacterial cell pellets and during purification steps, as detected by SDS-PAGE (Coomassie stain). Lane 1) Mark-12 molecular mass marker; Lane 2) BL21 cell pellet [no insert]; Lane 3) BL21 cell pellet [containing hK9 insert] in the absence of IPTG, at 4 hrs; Lane 4) BL21 cell pellet [containing hK9 insert] 4 hours post IPTG stimulation; Lane 5) Purified hK9 after Ni-NTA chromatography.


Figure 5. Western blot to test for cross-reactivity of hK9 rabbit antiserum with CHO-derived hK9 and HEK-293 derived hK9. The identity of the purified mammalian hK9 was confirmed by mass spectroscopy. Using Western blotting, the hK9 rabbit antiserum specifically recognized the mammalian hK9 with no cross-reactivity from other kallikreins. We further used the CHO-derived hK9 to generate additional anti-hK9 polyclonal and monoclonal antibodies in rabbit and mouse. Utilizing these antibodies, an immunoequivalency with sensitivity of 0.5 nM/ml was developed.

Figure 6. Detection of hK9 fusion protein in bacterial cell pellets and during purification steps, as detected by SDS-PAGE (Coomassie stain). Lane 1) Mark-12 molecular mass marker; Lane 2) BL21 cell pellet [no insert]; Lane 3) BSA; Lane 4) Blank; Lane 5) Purified hK9 fusion protein.


CONCLUSION

The production of the different forms of hK9 reported in this study provide the research tools required to characterize the structure and function of this kallikrein. Utilization of hK9 specific polyclonal and monoclonal antibodies for functional studies in mammalian cell lines of specific tumour cell lines has shown promise.

REFERENCES


