IMMUNOHISTOCHEMICAL LOCALIZATION AND EXPRESSION PROFILING OF HUMAN TISSUE KALLIKREIN 12 IN HUMAN PROSTATE CANCER TISSUES

Nader Memari, 1,2 Eleftherios P. Diamandis1,2, and Theodore van der Kwast 1,2

1Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada M5G 1L5
2Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada M5G 1X5

ABSTRACT

Human tissue kallikreins (genes, KLKs; proteins, hKs) are a subgroup of serine proteases involved in normal physiological and biological fluids. A number of human tissue kallikreins are established or candidate serologic biomarkers for prostate cancer. Human tissue kallikrein 12 (KLK12) has been cloned and characterized in our laboratory in many cancer types. KLK12 mRNA is expressed in a variety of human cancers including the prostate gland. Here, we report for the first time a novel human tissue kallikrein, KLK12 (classical form) and immunohistological localization of this kallikrein in normal and malignant prostate tissue. Mature form of KLK12 mRNA was detected using PCR and cloned into pET/100 TOP1 strain of E. coli. Recombinant KLK12 was purified and used as antigen in rabbits. After confirmation of antibody specificity using antibody capture assay and Western blotting, the antibody was used for immunostaining of paraffin embedded sections of human prostate tissue.

INTRODUCTION

• Human tissue kallikreins (KLKs) are a subgroup of serine proteases with diverse physiological function present in a variety of tissues and biological fluids. In our laboratory, we have been interested in the identification of new human tissue kallikreins (KLKs) and the development of specific antibodies for their detection.

• For many years the human tissue kallikrein gene family was considered to be composed of only three genes: the pancreatic/renal kallikrein (KLK1), the human glandular kallikrein 2 (KLK2), and prostatic specific antigen (KLK3).

• These three genes are now considered as the classical human tissue kallikrein. In the recent years new additional genes with significant homology to the previously known kallikreins have been identified by different investigators.

• All 15 human tissue kallikrein genes are tandemly located in a 300 kb region on chromosome 19 (19q13.3-19q13.4) and are composed of five coding exons of similar or identical size.

• The most well known member of this group of enzymes KLK3, also known as Prostate-Specific Antigen (PSA) is currently used in the diagnosis and monitoring of prostate cancer.

• In this study, we report on the cloning, expression and characterization of the human tissue kallikrein 12 (KLK12) and its cognate antibody for the first time.

• Similar to other kallikreins, KLK12 product (hK12) is predicted to be a secreted serine protease. hK12 is a pre-pro-enzyme and is then processed into the mature form with a predicted molecular weight of 24.5 KDa.

• In this study recombinant hK12 and anti-hK12 antibodies were generated. Anti-hK12 antibody was used for the immunohistochemical localization of this kallikrein for the first time, and the cellular localization of hK12 in normal and malignant prostate tissue was identified.

• Since in addition to hK2 a number of recently identified kallikreins (hK2, hK4, hK11, and hK15) have proven to be promising prostatic biomarkers, anti-hK12 antibodies generated here were further applied to a prostate cancer tissue array, and the immunohistochemical expression of hK12 in benign and malignant prostate tissues was evaluated.

METHODOLOGY

• Total lung tissue mRNA was reverse-transcribed to cDNA. PCR was conducted using the reverse-transcribing enzyme FIT and digoxigenin-dUTP primers specific to classical human KLK12.

• The amplified cDNA was cloned into pET100 TOPO plasmid vector. Plasmid DNA containing the pET-KLK12 construct was used to transform the E.coli strain BL21(DE3) cells. Protein production was detected using SDS-PAGE and western blotting.

• hK12 was purified using Ni-NTA metal affinity chromatography followed by RP-HPLC. Purified KLK12 was used as antigen to generate polyclonal antibodies. Antibody specificity was confirmed using antibody capture assay and Western blotting.

• Anti-hK12 antibody was used for immunostaining of formalin-fixed paraffin embedded prostate tissue samples (4 µm thick) using the avidine-biotin peroxidase complex technique. Rabbit-anti-hK12 antisera (1:500 dilution) and biotinylated goat anti-rabbit IgG were used as the primary and secondary antibody.

• Proteinase in tissue sections was visualized as brown color using DAB substrate solution. The sections were counterstained with hematoxylin. Prostate tissue sections treated with no primary antibody, or pre-immune rabbit serum were used as negative controls.

RESULTS:

• Intense granular supranuclear staining of hK12 in about 30% of prostate cancer samples having a Gleason grade of ¾ was observed, this intense staining was totally absent in low grade cancers or normal prostatic tissue.

• Similar to other kallikreins, KLK12 has 48% amino acid sequence identity and 57% overall similarity with hK8, 46% identity with hK10, and 38% identity with both PSA and hK2. In our cross reactivity analysis, hK12 rabbit antisera had no cross-reactivity with any of the three or any other tissue kallikreins.

• Since classical kallikreins such as hK2 and hK4 have already proved to be amongst the best prostatic biomarkers, anti-hK12 antibodies generated here were used for immunostaining of paraffin-embedded sections of human prostate tissue.

• hK12 in normal prostate tissue mainly displayed a cytoplasmic staining. With the exception of hK12 which is a prostatic luminal marker, only a minimal amount of the kallikreins tested so far can locally localize in the cytoplasm. Expression of human tissue kallikreins mainly by glandular epithelia, as well as prostate adenocarcinoma. Our results indicate localization of the major proteins identified as secreted proteins. Immunohistochemical pattern of normal prostate tissue using anti-hK12 antibody indicate that, similar to other kallikreins, hK12 is a secreted protein.

• To date, the expression of kallikrein 12 (or the antigen or hK12 level) in prostate cancer has not been investigated. Since in addition to hK2 a number of recently identified kallikreins (hK2, hK4, hK11, and hK15) have proven to be promising prostatic biomarkers, anti-hK12 antibodies generated here were further applied to a prostate cancer tissue array, and the immunohistochemical expression of hK12 in benign and malignant prostate tissue was evaluated.

• It was observed that GAPDH expression of human prostate cancer cells was not significantly different from normal prostate gland.

• The cross expression pattern is currently unknown. In high grade prostate tumors, ablation in secretory pathways involved in the transport of hK12 following its synthesis, or presence of inhibitors of proteases like serpins may prevent the secretion of hK12.

• Since high grade prostate cancer cells have high levels of intracellular hK12, this may result in the cytoplasmic accumulation of this kallikrein in prostate cancer. All four splice variants of hK12 known to date however, are predicted to encode for secreted protein.

CONCLUSION:

The production of the recombinant hK12 and anti-hK12 antibodies reported in this study provide the first tools to characterize the structure and function of this kallikrein. Utilization of hK12 specific antibodies may be valuable in prostate cancer diagnosis.

REFERENCES:


