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Development of an Immunofluorometric Assay and Quantification of Human Kallikrein 7 in Tissue Extracts and Biological Fluids

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Background: **Human kallikrein 7 (hK7), also known as human stratum corneum chymotryptic enzyme, is a chymotrypsin-like serine protease first identified in human skin extracts and predicted to be a secreted protease. The aim of this study was to develop a sensitive and specific immunoassay for hK7 and to examine the distribution of hK7 in tissue extracts and biological fluids.**

Methods: **Recombinant hK7 was produced in human embryonic kidney cells (HEK293T) and purified by a three-step column chromatographic procedure. The purified hK7 was injected into mice for antibody generation. A sandwich-type immunoassay was developed with the anti-hK7 monoclonal antibodies.**

Results: **The assay had imprecision (CV) <10% through the dynamic range of 0.2–20 μg/L and had no detectable cross-reactivity from other members in the human kallikrein gene family. Highest concentrations were found in skin, esophagus, and kidney. hK7 was also found in amniotic fluid, ascites from ovarian cancer patients, breast milk, cerebrospinal fluid, saliva, seminal plasma, serum, sweat, synovial fluid, and urine.**

Conclusions: **This study describes the first ELISA-type immunoassay for hK7 protein quantification. hK7 is found many human tissues and in various biological fluids.**

Human stratum corneum chymotryptic enzyme (HSCCE)[4] is a chymotrypsin-like serine protease that was first identified in human skin extracts *(1)* and Alzheimer disease brain (US Patent 6,093,397). Specifically expressed in keratinizing squamous epithelia, HSCCE is suggested to play a role in the turnover and/or formation of the stratum corneum *(2)*. Another possible function of HSCCE is to convert interleukin-1β (IL-1β) precursor to its active form in the epidermis *(3)*. The HSCCE gene (*KLK7*) encodes for a 253-amino acid polypeptide containing a predicted signal peptide of 22 amino acids and a propeptide of 7 amino acids *(4)*. This enzyme is predicted to be an extracellular protease.

Recently, the whole human kallikrein gene family, comprising 15 members, was assigned to chromosome 19q13.4 *(5, 6)*. Because the HSCCE gene (*a*) is located next to other members in the human kallikrein family, (*b*) has the same genomic organization and intron phase as other members of this family and (*c*) has significant homology to other members of the family in both the DNA and amino acid sequences *(7)*, HSCCE is now considered to be a member of the human kallikrein family and has been given a new name, *KLK7* for the gene and human kallikrein 7 (hK7) for the protein *(8)*.

The human kallikrein family includes hK3 [prostate-specific antigen (PSA)], which is the best biomarker for prostate cancer reported to date *(9)*. Recently, four other members of the human kallikrein family, hK5, hK6 (neurosin), hK10 (normal epithelial cell specific 1), and hK11 (trypsin-like serine protease), were found to be potential serum biomarkers for breast, ovarian, and prostate cancer *(10–13)*. In addition, many other members of the same

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[4] Nonstandard abbreviations: HSCCE, human stratum corneum chymotryptic enzyme; IL-1β, interleukin-1β; hK, human kallikrein; PSA, prostate-specific antigen; HEK, human embryonic kidney; and SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

family appear to be dysregulated in prostate, ovarian, and breast cancer *(6)*.

Although other authors have reported that *KLK7* mRNA is expressed in ovarian cancer tissues in higher amounts than in controls and reported a relationship with skin diseases *(14, 15)*, there has been no report linking hK7 protein with human disease because of a lack of methods for hK7 quantification. We report here the production of recombinant hK7 in a mammalian expression system, its purification by column chromatography, and its use as an antigen to immunize mice. Anti-hK7 monoclonal antibodies were used to develop a highly sensitive and specific immunoassay for hK7, which allowed protein quantification in tissue extracts and biological fluids.

Materials and Methods

CLONING OF FULL-LENGTH *KLK7* cDNA IN MAMMALIAN CELLS

The full-length *KLK7* cDNA was cloned by screening of a human lung cDNA library by standard techniques *(16)*. This cDNA was then subcloned into the pRc/CMV vector (BD Biosciences Clontech) to create plasmid *KLK7*/pRc/CMV. Human embryonic kidney cells (HEK293T) transfected with *KLK7*/pRc/CMV plasmid were subjected to selection by growth in G418 (400 μg/L; Invitrogen) for 3 weeks, after which time stable transformants were isolated.

PROTEIN PRODUCTION

The stable transformants containing full-length *KLK7* cDNA were cultured to near confluency in polystyrene flasks (75-cm^2) with 25 mL of DMEM (Invitrogen) with geneticin (400 μg/L), containing 100 mL/L fetal calf serum (Invitrogen) in 5% CO_2 at 37 °C. The cells from one flask were detached by use of trypsin/EDTA (Invitrogen), aliquoted with 75 mL of the serum medium, and divided into three polystyrene flasks (175-cm^2). After the cells were cultured in 5% CO_2 at 37 °C for 1 day, 25 mL of the medium in each flask was changed to 50 mL of serum-free medium (Invitrogen) with geneticin (400 μg/L). The cells were cultured in 5% CO_2 at 37 °C for 8 days, and the extracellular medium (supernatant) in each flask was then harvested.

PROTEIN PURIFICATION

Recombinant hK7 in the harvested medium was purified by a three-step column chromatographic procedure. The medium was applied to a HiTrap SP Sepharose HP cation-exchange column (bed volume, 5 mL; Amersham Biosciences) equilibrated with 10 mmol/L HEPES buffer (pH 7.4). Proteins were eluted with 10 mL each of 10 mmol/L HEPES buffer (pH 7.4) containing 350 and 500 mmol/L NaCl. After being diluted twice with 10 mmol/L HEPES buffer (pH 7.4), fractions containing hK7 were applied to a HiTrap Heparin HP affinity column (bed volume, 5 mL; Amersham Biosciences) equilibrated with 10 mmol/L HEPES buffer (pH 7.4). Proteins were eluted with 10 mL of 10 mmol/L HEPES buffer (pH7.4) containing 500 mmol/L NaCl, and 1-mL fractions were collected. Fractions containing hK7 were also applied to another affinity column, HiTrap Benzamidine FF (bed volume, 1 mL; Amersham Biosciences), equilibrated with 10 mmol/L HEPES buffer (pH 7.4) containing 500 mmol/L NaCl. The flow-through from the benzamidine column was collected. At each purification step, all fractions were monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie G-250 staining and Western blotting with a peptide antibody against hK7, produced in house. Each fraction was evaluated with the NuPAGE Bis-Tris electrophoresis system using two 4–12% gradient polyacrylamide gels (Invitrogen). One gel was stained with SimplyBlue SafeStain (Invitrogen). The proteins on the other gel were transferred to a nitrocellulose membrane (Amersham Biosciences). After blocking with 50 g/L skim milk in 0.1 mol/L Tris-HCl buffer (pH 7.5) containing 0.15 mol/L NaCl and 1 mL/L Tween 20 for 30 min at room temperature, the membrane was reacted with a rabbit anti-hK7 peptide antibody followed by goat anti-rabbit IgG conjugated with alkaline phosphatase (Jackson ImmunoResearch Inc.) in Tris-buffered saline–Tween containing 50 g/L skim milk. The secondary antibody was detected on an x-ray film by a chemiluminescent substrate (Diagnostic Products Corporation). Molecular masses on the gel were evaluated by use of image analysis software (Lab Works; Ultra-Violet Products Ltd.).

MASS SPECTROMETRY AND N-TERMINAL SEQUENCING

Positive identification and characterization of the purified recombinant hK7 were achieved by trypsin digestion and nanoelectrospray mass spectroscopy, as described previously *(17)*. Eighty pmol of the purified protein were also dot-blotted to a polyvinylidene difluoride membrane and subjected to N-terminal sequencing by the Edman degradation method in a Porton Instruments analyzer. Total protein was measured with the bicinchoninic acid method with bovine albumin as calibrator (Pierce Chemical Co.).

PRODUCTION OF MONOCLONAL ANTIBODIES

The purified hK7 was used as an immunogen to immunize mice. Briefly 100 μg of hK7 in 150 μL of solution was mixed with the same volume of complete Freund's adjuvant for the first injection and incomplete Freund's adjuvant for the subsequent injections. The mixed solution was injected subcutaneously into female Balb/c mice. Injections were repeated three times at 3-week intervals. Blood was drawn from the animals and tested for antibody generation. The splenocytes from the hK7-immunized mice were fused with the Sp2/0 myeloma cells (American Type Culture Collection) to obtain hybridomas that produced anti-hK7 monoclonal antibodies. These were identified by screening with immobilized hK7 in ELISA-type assays. The isolation, production, and purifi-

cation of anti-hK7 monoclonal antibodies were performed as previously described *(18)*.

STANDARD IMMUNOASSAY PROCEDURE

White polystyrene microtiter plates were coated with anti-hK7 monoclonal antibody 73-1; 100 μL of coating antibody solution [50 mmol/L Tris-HCl buffer (pH 7.8) containing 5 mg/L antibody] was then added to each well and incubated overnight at room temperature. The plates were washed twice with the washing buffer [10 mmol/L Tris-HCl buffer (pH 7.4) containing 150 mmol/L NaCl and 0.5 mL/L Tween 20]. hK7 calibrators or samples were added to each well (100 μL/well) after being diluted twofold in buffer A [50 mmol/L Tris-HCl buffer (pH 7.8) containing 60 g/L bovine serum albumin, 1 g/L goat globulin, 0.2 g/L mouse globulin, 10 g/L bovine globulin, and 5 mL/L Tween 20] and were incubated for 2 h with shaking at room temperature. The plates were washed six times with the washing buffer, after which 100 μL of biotinylated detection antibody solution (200 μg/L anti-hK7 monoclonal antibody 83-1 in buffer A) was added to each well and incubated for 1 h at room temperature with shaking. The plates were then washed six times with the washing buffer. Subsequently, 100 μL of alkaline phosphatase-conjugated streptavidin solution (Jackson ImmunoResearch) diluted 20 000-fold in 60 g/L bovine serum albumin was added to each well and incubated for 15 min with shaking at room temperature. The plates were washed six times with the wash buffer, and then 100 μL of diflunisal phosphate solution [0.1 mol/L Tris-HCl buffer (pH 9.1) containing 1 mmol/L diflunisal phosphate, 0.1 mol/L NaCl, and 1 mmol/L $MgCl_2$] was added to each well and incubated for 10 min with shaking at room temperature. Developing solution (100 μL; 1 mol/L Tris base, 0.4 mol/L NaOH, 2 mmol/L $TbCl_3$, 3 mmol/L EDTA) was added to each well and incubated for 1 min with shaking at room temperature. The fluorescence was measured with a time-resolved fluorometer, the Cyberfluor 615 Immunoanalyzer (MDS Nordion). Calibration and data reduction were performed automatically, as described in detail elsewhere *(19)*.

DETECTION LIMIT OF THE IMMUNOASSAY

Recombinant hK7 was used to generate the calibration curve. hK7 calibrators were prepared by diluting the purified recombinant hK7 in the general diluent. These calibrators were used to define the detection limit of the assay.

PREPARATION OF HUMAN TISSUE EXTRACTS AND BIOLOGICAL FLUIDS

The following human adult tissues were used: adrenal, axilliary lymph nodes, bone, breast, cerebellum, cervix, colon, endometrium, esophagus, fallopian tube, frontal cortex, hippocampus, kidney, liver, lung, medulla, mesentery lymph nodes, midbrain, muscle, occipital cortex, ovary, pancreas, pituitary, placenta, pons, prostate, salivary, seminal vesicle, skin, spinal cord, spleen, stomach, temporal lobe, testis, thyroid, tonsil, trachea, ureter, and uterus. Human tissue extracts were prepared as follows: Frozen human tissues (0.2 g) were pulverized on dry ice to fine powders and mixed with 1 mL of extraction buffer [50 mmol/L Tris-HCl buffer (pH 8.0) containing 150 mmol/L NaCl, 5 mmol/L EDTA, 10 g/L NP-40 surfactant, 1 mmol/L phenylmethylsulfonyl fluoride, 10 mg/L aprotinin, and 10 mg/L leupeptin]; the mixture was incubated on ice for 30 min with repeated vortex-mixing every 10 min. Mixtures were centrifuged at 15 000*g* at 4 °C for 30 min, and the supernatants were collected as tissue extracts. The biological fluids (amniotic fluid, ascites from ovarian cancer patients, breast cytosol, breast milk, cerebrospinal fluid, saliva, seminal plasma, sera, sweat, synovial fluid, and urine) were leftovers of samples submitted for routine biochemical testing. Tissue extracts and biological fluids were stored at −80 °C until use. Our procedures were approved by the Institutional Review Board of Mount Sinai Hospital.

SPECIFICITY OF THE IMMUNOASSAY

The cross-reactivities of all homologous kallikreins were investigated by use of recombinant proteins produced in house. All proteins were tested at a concentration of 1000 μg/L.

LINEARITY OF THE IMMUNOASSAY

To determine the linearity of the hK7 immunoassay, we serially diluted ascites, culture supernatant from the human keratinocyte HaCaT cell line, skin extracts, and serum in general diluent and measured the hK7 concentration with the standard assay.

RECOVERY

Recombinant hK7 was added to normal sera (from three males and three females) at different concentrations and then measured with the developed hK7 immunoassay. Recoveries were calculated after subtraction of the endogenous concentrations.

FRACTIONATION OF BIOLOGICAL FLUIDS WITH GEL-FILTRATION HPLC

To determine the molecular mass of the protein detected in biological fluids, we fractionated ascites and serum by gel-filtration chromatography, as described elsewhere *(20)*. The fractions were then collected and analyzed for hK7 in the hK7 ELISA.

Results

PRODUCTION AND PURIFICATION OF hK7

Recombinant hK7 was expressed and secreted into the medium by stably transfected HEK239T cells. Two bands (30.8 and 28.3 kDa) from hK7 in the medium were detected by Western blot analysis 3 days after culture, but the strongest signals were detected around 8 days. Partially purified hK7 from the SP Sepharose, heparin, and

benzamidine chromatography steps was detected in the 350 mmol/L NaCl eluate, the 500 mmol/L NaCl eluate, and the flow-through fractions, respectively. The 30.8-kDa band was excluded during the purification procedure. We obtained 218 μg of the 28.3-kDa purified hK7 from 450 mL of culture medium with this three-step column chromatography purification protocol (Table 1). Finally, purified hK7 was detected as a single band on a 4–12% SDS-PAGE gel stained with Coomassie G-250 solution (Fig. 1).

MASS SPECTROMETRY AND N-TERMINAL SEQUENCING

The purified band (28.3 kDa) on SDS-PAGE was confirmed to be hK7 by mass spectrometry. For example, the sequence Gly-Ser-His-Pro-Trp-Gln-Val-Ala-Leu-Leu-Ser-Gly-Asn-Gln-Leu-His-Cys-Gly-Gly-Val-Leu-Val-Asn-Glu-Arg, was detected and was identical to amino acids 39–63 of the hK7 protein (GenBank accession no. AF332583). The 30.8-kDa band was also confirmed to be hK7 (different glycosylated form). The N-terminal sequence of the 28.3-kDa purified protein was Glu-Glu-Ala-Gln-Gly-Asp-**Lys-Ile**-Ile-Asp-Gly, representing the proform of the enzyme. The activation site is between the Lys and Ile (shown in bold).

STANDARD IMMUNOASSAY PROCEDURE

A typical calibration curve for the hK7 immunoassay is shown in Fig. 2. The detection limit, defined as the concentration of analyte that could be distinguished from zero with 95% confidence, was 0.2 μg/L. We determined the detection limit by analyzing the zero calibrator 12 times and then determining the hK7 concentration that corresponded to the fluorescence of the zero calibrator + 2 SD. The upper limit of the dynamic range was 20 μg/L. The CV for the developed assay for hK7 over the dynamic range (0.2–20 μg/L) were < 10% both within runs (n = 12) and between days (n = 12 over 3 weeks), which is consistent with the precision of typical microtiter plate-based immunoassays.

No appreciable signals were obtained with recombinant kallikreins hK1–hK15 (excluding hK7) at a concentration of 1000 μg/L, suggesting no detectable cross-reactivity with these homologous proteins (data not shown). Recoveries of added recombinant hK7 (two additions; 2 and 4 μg/L) were 24–77% for male (n = 3) and female (n = 3) sera.

Table 1. Purification of recombinant hK7 produced in HEK239T cells.

Purification step	Total protein,[a] μg	hK7,[b] μg	Recovery, %	Purification, fold
Culture medium	16×10^3	1188[c]	100	1
SP Sepharose	525	458	30.7	11.8
Heparin	511	454	30.4	12.0
Benzamidine	218	218	14.6	13.5

[a] Evaluated by the bicinchoninic acid assay with albumin as calibrator (Pierce).
[b] Evaluated by the standard immunoassay or band intensities on SDS-PAGE gels.
[c] Based on a 450 mL of culture medium.

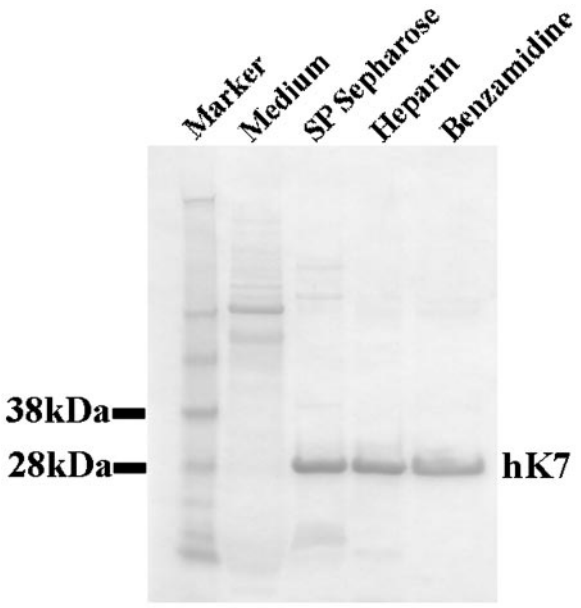

Fig. 1. SDS-PAGE of recombinant hK7 at each purification step.
Each lane was loaded with 3 μg of total protein.

hK7 IN HUMAN TISSUE EXTRACTS AND BIOLOGICAL FLUIDS

The highest detected concentrations of hK7 (ng/mg of total protein in extracts) were in skin (11.6 ng/mg), esophagus (5.1 ng/mg), and kidney (1.0 ng/mg), as shown in Fig. 3. Detected concentrations were lower in pons, spinal cord, bone, hippocampus, breast, temporal lobe, tonsil, fallopian tube, medulla, testis, axilliary lymph nodes, salivary, occipital cortex, pituitary, cerebellum, frontal cortex, ureter, lung, prostate, adrenal, ovary, endometrium, trachea, liver, thyroid, and cervix. The hK7

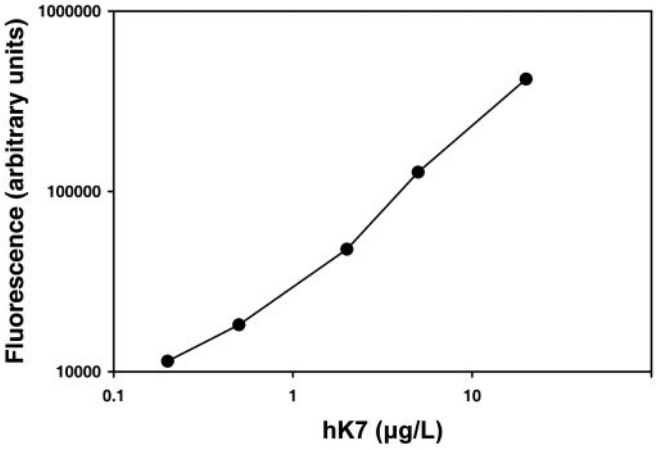

Fig. 2. Typical calibration curve for the hK7 immunoassay.
The background fluorescence (zero calibrator) was subtracted from all measurements. The dynamic range of this assay is 0.2–20 μg/L.

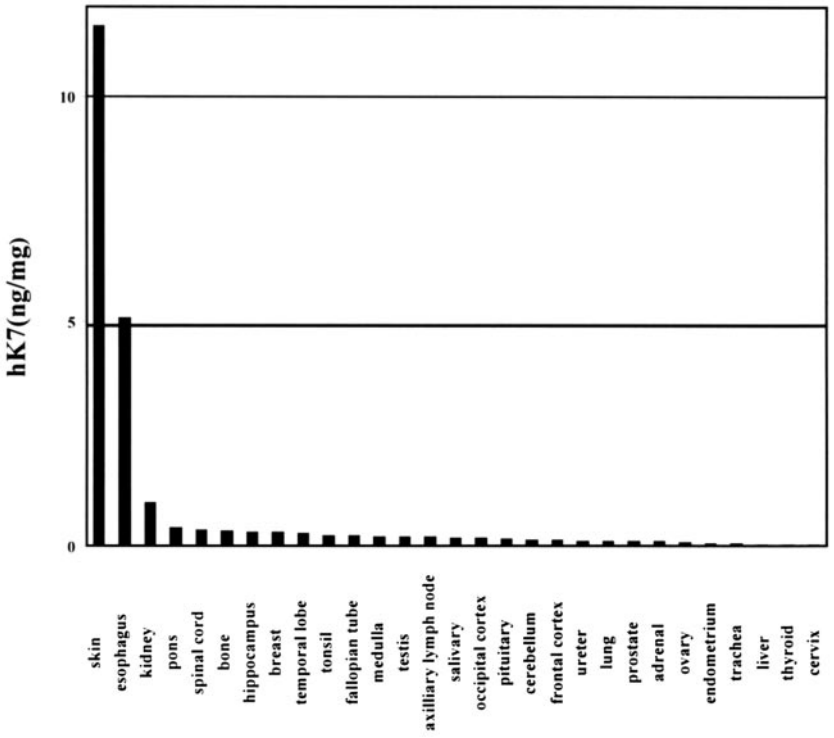

Fig. 3. Expression of hK7 in adult tissue extracts from males and females.

For a discussion, see the text. The following tissues were negative: colon, mesentery lymph nodes, midbrain, skeletal muscle, pancreas, placenta, seminal vesicle, small intestine, spleen, stomach, and uterus. All concentrations were corrected for the total protein content of the extracts and are expressed as ng of hK7 per mg of total protein.

concentration in other tested tissue extracts was below the detection limit of the assay (0.2 μg/L). hK7 was also detected in many biological fluids, with the highest concentrations in ascites from ovarian cancer patients (mean, 32 μg/L; Table 2).

HPLC

To investigate whether hK7 in biological fluids is circulating in various molecular forms, we fractionated recombinant hK7 from one serum sample and one ascites fluid with gel-filtration chromatography and analyzed all fractions with the hK7 ELISA (Fig. 4). With recombinant hK7, the detected immunoreactivity eluted predominantly as a single peak with a molecular mass of ~30 kDa, consistent with the molecular mass of free (uncomplexed) hK7. There was an additional small peak around 100 kDa in serum and ascites fluid. This peak was not positively characterized.

hK7 in serum of healthy individuals

We found a weak negative correlation between hK7 concentration and age in males ($R = -0.55$; Fig. 5A). In females, we found no correlation between hK7 concentration and age ($R = -0.13$; Fig. 5B). In both cases, hK7 concentrations in serum were <5.5 μg/L, with means of 2.9 μg/L in males and 2.3 μg/L in females (see also Table 2).

Discussion

hK7 is predicted to be translated as a pre-proenzyme that is processed and secreted as a proenzyme into the extracellular space, similarly to other extracellular proteases *(6)*. Recombinant hK7 produced in murine C127 cells containing full-length *KLK7* cDNA was secreted into the culture medium, and its amino terminus was the heptapeptide, Glu-Glu-Ala-Gln-Gly-Asp-Lys, as predicted for pro-hK7 *(4)*. Because hK7 produced in HEK cells is also secreted into the culture medium and has the same amino terminus, this result means that recombinant hK7 secreted by HEK cells is in the proform. Recombinant hK7 expressed in C127 cells as well as native hK7 is seen as two bands on SDS-PAGE gels (around 30 kDa). The molecular mass difference between the two bands may arise from difference in glycosylation at a predicted N-linked glycosylation site near the carboxy terminus *(4)*. Recombinant hK7 expressed in HEK cells and confirmed by mass spectrometry is also seen as two bands (30.8 and 28.3 kDa); the size difference likely originates from differences in glycosylation.

hK7 is a member of the human kallikrein family and has 40–60% similarity with other members at the amino acid level *(6)*. This study confirms that the developed immunoassay is highly specific for hK7 and has no detectable cross-reactivity against all other kallikreins. Previous studies have shown that hK7 transcripts are highly expressed in skin, with lower expression in brain and kidney and no detectable expression in heart, liver, skeletal muscle, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes *(4, 14)*. Other authors also found that hK7 is an active enzyme in vivo *(1)*. Our study has shown expression of hK7 protein not only in brain, kidney, and skin,

Table 2. hK7 concentration in biological fluids.

Fluid	Samples tested, n	hK7 concentration, μg/L			Positivity rate, %
		Range[a]	Mean (SD)	Median	
Amniotic fluid	7	0.0–3.2	1.1 (1.3)	0.4	71
Ascites					
Ovarian[b]	13	1.2–241	32 (64)	11	100
Nonovarian[c]	7	0.0–2.9	1.1 (1.1)	0.8	86
Breast cytosol	7	0.0	0.0 (0.0)	0.0	0
Breast milk	6	2.3–40	13 (14)	7.0	100
Cerebrospinal fluid	10	0.0–1.1	0.5 (0.5)	0.5	60
Saliva					
Male	5	13–32	16 (7.9)	14	100
Female	5	0.0–8.6	5.0 (3.2)	5.0	80
Seminal plasma	10	1.4–40	13 (14)	7.2	100
Serum					
Male	17	1.9–5.4	2.9 (0.9)	2.8	100
Female	20	1.5–4.0	2.3 (0.7)	2.1	100
Sweat	7	0.0–5.7	0.8 (2.2)	0.0	14
Synovial fluid	6	0.3–1.0	0.5 (0.3)	0.4	100
Urine					
Male	13	0.0–26	4.7 (7.5)	0.8	92
Female	12	0.0–2.0	0.4 (0.7)	0.0	25

[a] Concentrations <0.2 μg/L are shown as 0.0.
[b] Sampled from ovarian cancer patients.
[c] Sampled from non-ovarian cancer patients (nonmalignant).

but also in adrenal, axilliary lymph nodes, bone, breast, cervix, endometrium, esophagus, fallopian tube, liver, lung, ovary, pituitary, prostate, salivary, testis, thyroid, tonsil, trachea, and ureter. Because the hK4, hK6, hK8, and hK11 proteins are produced by more restricted tissues *(12, 21–23)*, hK7 might play more general roles than other human kallikreins, including the degradation of adhesion molecules between cells *(24)*. hK10 protein was also shown to be produced in many tissues *(17)*, but this

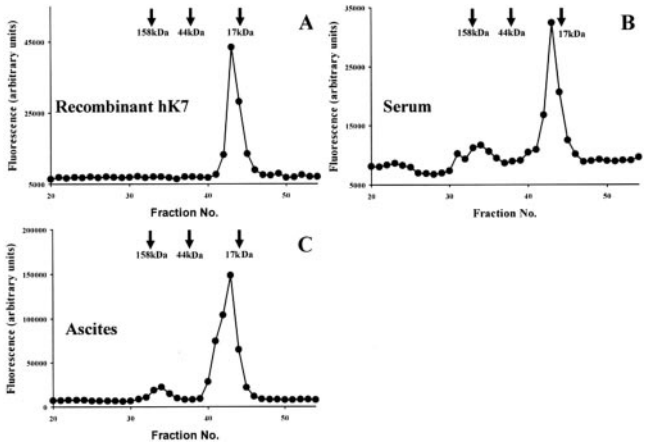

Fig. 4. HPLC separation on a gel-filtration column of recombinant hK7 (*A*), serum (*B*), and ascites fluid (*C*).

The peak around 30 kDa (*fraction 43*) represents the free form of hK7. There is another peak with a higher molecular mass (around 100 kDa) in serum (*B*) and ascites fluid (*C*). The molecular masses of three markers and their elution times are shown by *arrows*.

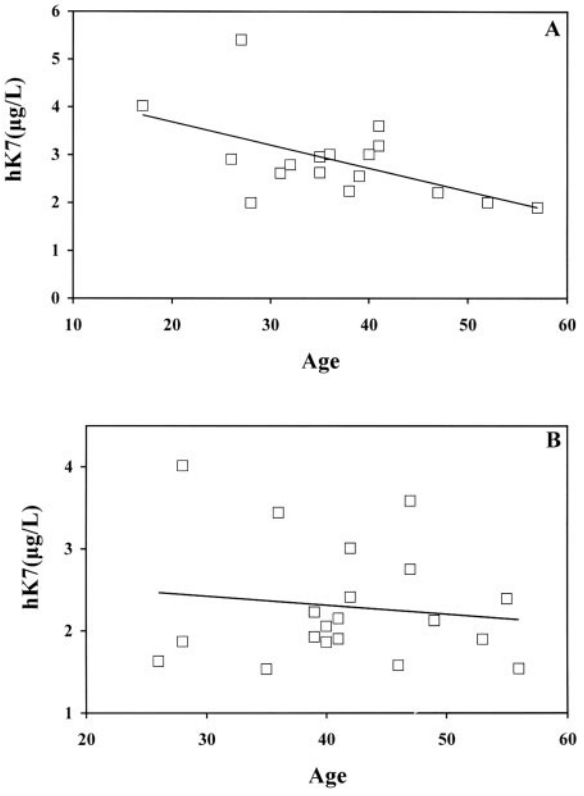

Fig. 5. Correlation between serum hK7 concentration and age in males (*A*) and females (*B*).

(*A*), $y = -0.0483x + 4.6529$ μg/L ($R = -0.55$; n = 17); (*B*), $y = -0.0109x + 2.7489$ μg/L ($R = -0.13$; n = 20). For numerical data, see Table 2.

protease has not yet been shown to have enzymatic activity *(25)*. hK7 has been detected in most analyzed biological fluids, which suggests that hK7 is an extracellular serine protease in vivo.

The data from gel-filtration HPLC suggest that hK7 is circulating in at least two forms in serum (see Fig. 4). This additional peak, speculated to be complexed hK7 (100 kDa) may well be hK7 bound to a serpin such as antichymotrypsin, which has a molecular mass of ~60 kDa, is present in serum at high concentrations, and binds PSA and other kallikreins. We did not investigate this further. In sera from prostate cancer patients, the ratio between free PSA (hK3) or complexed PSA and total PSA is used for the diagnosis and prognosis of prostate cancer *(26)*. The low recovery of hK7 from serum suggests that it may bind to inhibitors that interfere with its measurement (e.g., α_2-macroglobulin).

In the breast carcinoma cell line BT-474, the *KLK7* gene was up-regulated by estrogens and glucocorticoids, suggesting that this gene is regulated by steroid hormones *(7)*. It was previously reported that the *KLK3* (PSA) gene is regulated by steroid hormones *(27)* and that serum hK3 (PSA) gradually increases with increasing age in healthy males *(28)*. The weak negative correlation between hK7 concentration and age in males might mean that the *KLK7* gene is down-regulated by androgens.

It has already been shown that the human kallikreins hK2, hK3, hK5, hK6, hK10, and hK11 are serum biomarkers for prostate and ovarian cancer *(6, 9–13, 29–31)*. In addition, *KLK7* transcripts were found to be increased in ovarian cancer *(14)*. These reports prompt us to speculate that hK7 may be another candidate biomarker for cancer or other diseases. The measurable concentrations of hK7 in serum of healthy males and females detected by the developed immunoassay support future studies with inclusion of sera from patients with diverse diseases. The role of hK7 in tissues (particularly skin and esophagus) merits further investigation.

In conclusion, we here report for the first time the production and purification of recombinant hK7 in a human cell system. This recombinant hK7 was used to develop a highly sensitive and specific immunoassay that was subsequently used to demonstrate the presence of hK7 in biological fluids and tissue extracts. The availability of this technology could allow further investigations to establish the physiology and pathobiology of this enzyme and its possible use as a disease biomarker.

This work was supported by a University-Industry grant from the Natural Sciences and Engineering Council of Canada (NSERC) and IBEX Technologies (Montreal, Canada). The serine protease hK7, isolated from human brain, is also described in US Patent 6,093,397 [inventors, E.P. Dickson, E.M. Johnstone, S.P. Little; assignee, Eli Lilly and Company, (Indianapolis, IN)].

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