Cloning of a New Member of the Human Kallikrein Gene Family, KLK14, Which Is Down-Regulated in Different Malignancies

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ABSTRACT

Kallikreins (KLKs) belong to the serine protease family of proteolytic enzymes. Human pancreatic/renal KLK (KLK1) encodes for an enzyme that is involved in posttranslational processing of polypeptide precursors. The function of the other members of this gene family is currently unknown, but growing evidence suggests that many KLKs are implicated in carcinogenesis. By using the positional candidate approach, we were able to identify a new human KLK-like gene, KLK14 (also known as KLK-L6). This new gene maps to chromosome 19q13.3–q13.4 and is formed of seven exons (two untranslated and five coding exons) and six intervening introns. KLK14 was defined as a KLK gene based on structural and mapping criteria, in relation to other known KLK genes. KLK14 is expressed in a variety of tissues, but the highest levels of KLK14 are found in the central nervous system, including brain, cerebellum, and spinal cord. Our preliminary results show that KLK14 is down-regulated, at the mRNA level, in breast, testicular, prostatic, and ovarian cancer.

INTRODUCTION

New gene discovery has been significantly accelerated with the availability of the EST database (dbEST) and the development of powerful bioinformatics software for gene prediction (1). KLKs are a subgroup of serine proteases with diverse physiological functions (2). The human tissue KLK gene family was first, until recently, thought to consist of only three members: (a) the pancreatic/renal KLK (KLK1); Ref. 3; (b) the glandular KLK (KLK2; Ref. 4); and (c) PSA (PSA or KLK3; Ref. 5). However, in the last 2–3 years, many new members of the human KLK gene family have been identified (6). Recent advances in this field facilitated a better understanding of the role of KLKs in diverse physiological and pathological conditions related to the cardiovascular, hematological, endocrine, sensory, and muscular systems (7).

In addition, growing evidence suggests a role of human KLK genes in cancer. PSA testing has revolutionized the diagnosis and management of patients with prostate cancer (8). hK2 (encoded by the KLK2 gene) is also gaining popularity as an adjuvant diagnostic marker for prostate cancer (9). The normal epithelial cell-specific 1 gene (NES1 or KLK10; Ref. 10), zyme/protease M/neurosin/KLK6 (11), neurosin/TADG14 (tumor-associated differentially expressed gene 14)/KLK8 (12), and the stratum corneum chymotryptic enzyme (HSCC/ KLK7; Ref. 13) are differentially expressed in various malignancies (6).

In our efforts to identify new KLK-L genes that might be involved in malignancy, we have examined a 300-kb genomic region around human chromosome 19q13.3–q13.4, which is known to contain the KLK gene locus and cloned a new gene, KLK14. Here, we describe the cloning of the new gene, its genomic and mRNA structure, its precise location in relation to other known KLKs, and its tissue expression pattern. KLK14 is highly expressed in tissues of the CNS including the brain, cerebellum, and spinal cord. Our preliminary results suggest that this gene is down-regulated, at the mRNA level, in breast, testicular, ovarian, and prostate cancer.

MATERIALS AND METHODS

Analysis of Genomic Sequences around the Human KLK Gene Locus.

We have obtained sequencing data of approximately 300 kb of nucleotides on chromosome 19q13.3–q13.4 from the web site of the Lawrence Livermore National Laboratory. This sequence was in the form of nine contigs of variable lengths. Restriction analysis study, long PCR reactions, bacterial artificial chromosome and PI-derived artificial chromosome library screening, and end sequencing were used to construct a contiguous (~300-kb) region representing the putative human KLK locus. Computer programs were used to predict the presence of putative new genes in the genomic area of interest, as described previously (14).

EST Searching. The predicted exons of the putative new gene, KLK14, were subjected to homology search using the BLASTN algorithm (15) on the National Center for Biotechnology Information web server against the human EST database, dbEST. A clone with 99% identity was obtained from the I.M.A.G.E. consortium (16) through Research Genetics Inc. (Huntsville, AL). The clone was propagated, purified, and sequenced from both directions with an automated sequencer, using insert-flanking vector primers. Tissue Expression. Total RNA isolated from 27 different human tissues was purchased from Clontech (Palo Alto, CA). We prepared cDNA as described below and used it for PCR reactions. Tissue cDNAs were amplified at various dilutions. Due to the high degree of homology with other human KLKs, and to exclude nonspecific amplification, the PCR products of the Klk14 gene were cloned into the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The inserts were then sequenced from both directions using vector-specific primers, with an automated DNA sequencer.

RT-PCR. Total RNA was extracted from normal and malignant breast tissues using Trizol reagent (Life Technologies, Inc., Gaithersburg, MD) following the manufacturer’s instructions. RNA concentration was determined spectrophotometrically. Two µg of total RNA were reverse-transcribed into first-strand cDNA using the Superscript premplification system (Life Technologies, Inc.). The final volume was 20 µl. Based on the combined information obtained from the predicted genomic structure of the new gene and the EST sequences, two gene-specific primers were designed (Table 1), and PCR was carried out in a reaction mixture containing 1 µl of cDNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 µM deoxynucleoside triphosphates, 150 ng of primers, and 2.5 units of AmpliTaq Gold DNA polymerase (Roche Molecular Systems, Branchburg, NJ) on a Perkin-Elmer 9600 thermal cycler. The cycling conditions were 94°C for 9 min to activate the Taq Gold DNA polymerase, followed by 43 cycles of 94°C for 30 s, 63°C for 1 min, and a final extension step at 63°C for 10 min. Equal amounts of PCR products were electrophoresed on 2% agarose gels and visualized by ethidium bromide staining. All primers for RT-PCR spanned at least two exons to avoid contamination by genomic DNA.

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The abbreviations used are: EST, expressed sequence tag; KLK, kallikrein; KLK-L, KLK-like; PSA, prostate-specific antigen; RT-PCR, reverse transcription-PCR; CNS, central nervous system; hK, human KLK (protein).

http://www.bsc.ahu.edu/genome/genome.html.


Normal and Malignant Breast Tissues. Normal breast tissues were obtained from women undergoing reduction mammoplasties. Breast tumor tissues were obtained from female patients at participating hospitals of the Ontario Provincial Steroid Hormone Receptor Program. The normal and tumor tissues were immediately frozen in liquid nitrogen and stored in this manner until extracted. The tissues were pulverized with a hammer under liquid nitrogen, and RNA was extracted as described above, using Trizol reagent.

Normal and Breast Cancer Cell Lines. The MCF-12A normal breast cell line and the BT-474 and T-47D breast cancer cell lines were purchased from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI 1640 (Life Technologies, Inc.) supplemented with glutamine (200 mmol/liter), bovine insulin (10 mg/liter), fetal bovine serum (10%), antibiotics, and antimycotics in plastic flasks to near confluence. The cells were then aliquoted into 24-well tissue culture plates and cultured to 50% confluence. Twenty-four h before the experiments, the culture media were changed into phenol red-free media containing 10% charcoal-stripped fetal bovine serum.

Normal and Malignant Prostate Tissues. Prostate tissue samples were obtained from 10 patients who had undergone radical retropubic prostatectomy for prostatic adenocarcinoma at the Charite University Hospital (Berlin, Germany). The patients did not receive any hormonal therapy before surgery. The use of these tissues for research purposes was approved by the Ethics Committee of the Charite Hospital. Fresh prostate tissue samples were obtained from the cancerous and noncancerous parts of the same prostates that had been removed. Small pieces of tissue were dissected immediately after removal of the prostate and stored in liquid nitrogen until analysis. Histological analysis from all of the tissue pieces was performed as described previously (17) to ensure that the tissue was either malignant or benign. The tissues were pulverized with a hammer under liquid nitrogen, and RNA was extracted using RNeasy Mini Spin columns (Qiagen Inc., Valencia, CA) following the manufacturer’s instructions. Total RNA concentration and quality were determined spectrophotometrically.

Normal and Malignant Testicular Tissues. Ten pairs of normal and cancerous testicular tissues were obtained from 10 patients who had undergone radical orchiectomy for testicular tumors at the Charite Hospital. All patients had a histologically confirmed diagnosis of primary testicular cancer and received no treatment before surgery. Malignant and normal tissues were obtained from the cancerous and noncancerous parts of the same testis. Histological analysis from all of the tissue pieces was performed as described previously (17) to ensure that the tissue was either malignant or benign. The tissues were pulverized with a hammer under liquid nitrogen, and RNA was extracted using RNeasy Mini Spin columns (Qiagen Inc.) following the manufacturer’s instructions. Total RNA concentration and quality were determined spectrophotometrically.

Normal and Malignant Ovarian Tissues. Normal ovarian cDNA was obtained from Gene Pool cDNA collection (Invitrogen). Ovarian tumor tissues were obtained from patients who had undergone surgery for primary ovarian carcinoma at the Department of Obstetrics and Gynecology, Gynecological Oncology Unit, University of Turin (Turin, Italy). Tissue specimens with different types of ovarian tumors were confirmed histologically, and 50–100 mg of tumor tissue were pulverized on dry ice, followed by total RNA extraction using the Trizol method (Life Technologies, Inc.). After measurement of total RNA concentration by spectrophotometry, 4 μg of total RNA from each tissue were used to carry out first-strand cDNA synthesis using the SuperScript Preamplification System, as prescribed by the manufacturer (Life Technologies, Inc.). To test the success of cDNA synthesis, 1 μl of the reverse transcription product was amplified using PCR with primers specific for actin. Product was visualized on a 2% agarose gel stained with ethidium bromide.

Table 1  Primers used for RT-PCR analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer name</th>
<th>Sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLK14</td>
<td>L6-F1</td>
<td>TCA-GGT-TGC-CCT-GGC-CAA-GGC</td>
<td>647 (with L6-R1)</td>
</tr>
<tr>
<td></td>
<td>L6-F2</td>
<td>GCA-CCT-AGC-TGG-CAC-CA-CGC</td>
<td>1133 (with L6-R1)</td>
</tr>
<tr>
<td>PSA</td>
<td>PSAAS</td>
<td>TGC-GCA-AUG-TCA-CACA-TCA</td>
<td>754</td>
</tr>
<tr>
<td></td>
<td>PSAS</td>
<td>CCG-TCT-CCT-ATC-GTC-CTG</td>
<td>372</td>
</tr>
</tbody>
</table>

* All nucleotide sequences are given in the 5′→3′ orientation.

Fig. 1. Genomic organization and partial genomic sequence of the KLK14 gene. Intronic sequences are not shown except for short sequences around the splice junctions. The 3′-untranslated region is shown in capital non-bold letters. For full sequence, see GenBank accession number AF161221. The start and stop codons are underlined, and the exon-intron junctions are underlined. The translated amino acids of the coding region are shown under a single-letter abbreviation. The catalytic residues are boxed. Putative polyadenylation signals are shown in bold. The 3′-end of the gene has been verified for only 110 bases after the stop codon.

Structure Analysis. Multiple alignment was performed using the Clustal X software package and the multiple alignment program available from the Baylor College of Medicine (Houston, TX). Phylogenetic studies were performed using the Phylip software package. Distance matrix analysis was performed using the Neighbor-joining/UPGMA program, and parsimony analysis was done using the Protpars program. The hydrophobicity study was performed using the Baylor College of Medicine search launcher programs. Signal peptide prediction was performed using the SignalP server. Protein structure analysis was performed by SAPS (structural analysis of protein sequence program).

RESULTS

Identification of the KLK14 Gene. The genomic area encompassing the putative human KLK gene locus was subjected to different gene prediction programs as described above. We predicted a new gene formed of at least five exons. Screening of the human EST...
database dbEST revealed an EST clone (GenBank accession number A935298) with 99% identity with our predicted exons. The full-length sequence of the EST was compared with the genomic sequence of the putative gene and showed 100% identity with three areas (exons 5–7, according to our GenBank submission number AF161221; the new gene was originally designated KLK-L6 in our GenBank submission). The three exons were separated by introns of variable length. The presence of two conserved amino acids of the catalytic triad suggested that this new gene encodes for a novel serine protease. Sequence homology and phylogenetic analysis further revealed that this new gene is closely related to the KLK subfamily of serine proteases (as discussed below). These three exons were further experimentally verified by RT-PCR analysis using primers L6-F1 and L6-R1 and brain cDNA as template (Table 1). Exon 4 was identified based on: (a) a consensus exon/intron splice site (AG...GT); (b) preservation of intron phases of the KLK genes (see “Discussion” for details); (c) the presence of the histidine residue of the catalytic triad (His\(^67\)) surrounded by a well-conserved peptide motif just before the end of this exon (Fig. 1); and (d) comparable exon length to other KLK genes. Furthermore, exon 4 was experimentally verified by RT-PCR of brain cDNA, followed by sequencing of the PCR product.

To obtain the full structure of the gene, several RT-PCR reactions were performed with cDNA from different tissues, under different reaction conditions, using the reverse primer (L6-R1) together with different forward primers located in upstream putative exons predicted by the computer programs. Only one of these primers (L6-F2; Table 1) was able to produce a PCR product of \(~1.1\) kb. This PCR product was fully sequenced. By aligning the sequence obtained from the PCR reaction with the genomic sequence of the putative gene, we were able to characterize the full genomic structure of the gene.
Because five other new KLK genes were previously identified in this region (6, 14, 18, 19), this novel gene was named KLK-L6. With the new human KLK gene nomenclature, this gene was designated the official symbol KLK14. Translation of the mRNA of the gene in all reading frames indicated that there is only one reading frame that produces a full-length polypeptide chain without interrupting in-frame stop codons. Further support was obtained by the observation that this was the only frame that will preserve the three amino acid residues essential for serine protease activity (the catalytic triad) and the conserved motifs surrounding them (Fig. 1). Two putative in-frame methionine start codons were found in positions 2803 and 3716 of the genomic sequences (numbers refer to our GenBank submission number AF161221). The first is most likely the actual start codon of the gene because (a) it has an adenine nucleotide in position 23, an observation that was found to be conserved in 97% of vertebrate mRNAs (20); (b) in all other known KLKs, the start codon is located in the exon preceding the exon containing the histidine residue of the catalytic triad (6); and (c) this start codon is located at a comparable distance from the end of the first coding exon, which always ends with an intron phase of I, in all known KLK genes. A stop codon was found 153 bp downstream from the start of the last coding exon that harbors the serine residue of the catalytic triad, at a distance that is almost identical in all KLK genes (Fig. 1).

Structural Characterization of the KLK14 Gene. The KLK14 gene, as presented in Fig. 1, is formed of seven exons and six intervening introns, spanning an area of 6349 bp of genomic sequence on chromosome 19q13.3–q13.4. The lengths of the exons are 110, 135, 62, 172, 254, 137, and 263 bp, respectively (the 3' untranslated region may be longer). The intron/exon splice sites (mG T.... AGm) and their flanking sequences are closely related to the consensus splicing sites (-mGTAAGT . . . CAGm-) (21).

As is the case with some other KLK genes, the first two exons are untranslated (22, 23), and the start codon is located at a distance of 22 bp from the start of the first coding exon. The predicted protein-coding region of the gene is formed of 756 bp encoding a deduced 251-amino acid polypeptide chain with a predicted molecular weight of 27,500. The 3'-end of the gene is not yet fully verified; however,
Fig. 3. A comparative hydrophobicity analysis of the hK14 polypeptide, which are consistent with a globular protein, similar to several evenly distributed hydrophobic regions throughout the hK14 polypeptide, which is known to have chymotrypsin-like activity (Fig. 2; Ref. 6).

Fig. 6. Tissue expression of the KLK14 gene as determined by RT-PCR (top panel). Actin (bottom panel) is a control gene. KLK14 is expressed at the highest levels in the CNS. For further discussion, see the text.

Two putative polyadenylation signals (AATAAA) are located at positions 8060 and 8073 of the genomic sequence, at a comparable distance from the stop codon to that of the PSA and KLK2 genes. The presence of aspartate (D) at position 198 suggests that KLK14 possesses a trypsin-like cleavage pattern similar to that in most of the other KLKs but different from that in PSA, which has a serine (S) residue in the corresponding position, and is known to have chymotrypsin-like activity (Fig. 2; Ref. 6).

Fig. 7. Expression of the KLK14 gene in normal and cancerous breast tissues and cell lines. The gene is expressed in all 3 normal tissues (N1, N2, and N3) but is not expressed at all in 21 tumors, has lower than normal expression in 3 tumors, and has an expression level comparable to that in normal tissue in 1 tumor (T. tumor). Whereas the gene was expressed in the normal breast cell line (MCF-12A), it was not detected in the two breast cancer cell lines examined (BT-474 and T-47D), as seen by negative PCR control. For data interpretation, see the text. The actin gene (bottom panel) was used as a control.

Serine proteases [i.e., histidine (VITAAHC), serine (GDSGGP), and aspartate (DLMLL); Refs. 25 and 26; Fig. 2], also show other amino acid residues that are completely conserved between KLKs and KLK-L proteins and shows an 11-amino acid loop characteristic of the classical KLKs (PSA, hK1, and hK2) that is not found in hK14 or other members of the KLK multigene family (18, 19, 22–24).

Sequence analysis of eukaryotic serine proteases indicates the presence of 29 invariant amino acids (27). Twenty-seven of them are conserved in the hK14 polypeptide. One of the nonconserved amino acids (Val132 instead of Leu) is also found in the KLK-L2 (hK5) protein, and according to protein evolution studies, this amino acid change represents a conserved evolutionary substitution to a protein of the same group (27, 28). The remaining amino acid (Asn156 instead of Pro) is not conserved among all other human KLKs (Fig. 2). Twelve cysteine residues are present in the mature hK14 protein; 10 of them are conserved in all KLKs, and the remaining 2 (Cys136 and Cys238) are present in most of the other KLK-L proteins but not in the classical KLKs, and they are expected to form an additional disulfide bridge (Fig. 2).

To predict the phylogenetic relatedness of the hK14 protein, the amino acid sequences of the KLK proteins and some other serine proteases were aligned together using the Clustal X multiple alignment program, and a distance matrix tree was predicted using the Neighbor-joining/UPGMA and Protpars parsimony methods. Fig. 4 shows the phylogenetic analysis that separated the classical KLKs (hK1, hK2, and PSA) and clustered hK14 with zyme and KLK-L4 proteins in a separate group away from other serine proteases, consistent with previously published studies (19, 29) and indicating that this group of genes probably arose from a common ancestral gene by gene duplication.

Mapping and Chromosomal Localization of the KLK14 Gene. Alignment of the KLK14 gene and the sequences of other known KLK genes within the 300-kb area of interest enabled us to precisely localize all genes and to determine the direction of transcription, as shown in Fig. 5. The KLK1 gene is the most centromeric, transcribing from telomere to centromere, followed by PSA and then by KLK2, which transcribe in the opposite direction. All other KLK-L genes transcribe in the same direction as KLK1. KLK14 resides 12,897 bp telomeric to KLK-L4, and 43,187 bp centromeric to a novel Siglec gene (GenBank submission number AF135027). The Siglec gene is not a serine protease and has no homology to any of the KLK gene family members.

Tissue Expression of the KLK14 Gene. As shown in Fig. 6, the KLK14 gene is primarily expressed in tissues of the CNS, including the brain, cerebellum, and spinal cord. As is the case with other KLKs, lower levels of expression are found in many other tissues including breast, thyroid, kidney, colon, spleen, bone marrow, uterus, small intestine, and placenta. To verify the RT-PCR specificity, the PCR products were cloned and sequenced.

Expression of KLK14 in Normal and Malignant Tissues. We studied the differential expression of KLK14 in different normal and malignant tissues. To characterize the extent and frequency of expression of the KLK14 gene in breast tumors compared to normal breast
tissues, we used cDNA derived from 3 normal and 25 malignant breast tissues. KLK14 gene expression was detectable in all three normal tissues. Of the 25 tumors, KLK14 gene expression was undetectable in 21 tumors, lower than normal in 3 tumors, and comparable to that of the normal tissues in 1 tumor (Fig. 7). We also compared KLK14 gene expression in the MCF-12A normal breast cell line and two breast cancer cell lines (BT-474 and T-47D). KLK14 was highly expressed in the normal breast cell line but was undetectable in both breast cancer cell lines examined (Fig. 7).

Matched pairs of normal and malignant tissues from 10 patients were also examined to study the differential expression of KLK14 in both prostate and testicular cancer. In prostatic tissues, the expression of the gene was absent or lower in eight tumor tissues compared with their normal counterparts and was higher than normal in only two tumor tissues (Fig. 8). KLK14 was also found to have lower or no expression in 7 of 10 testicular tumor tissues examined (compared with their normal counterparts), a comparable level of expression in 1 tumor, and higher than normal expression in 2 tumors. Whereas KLK14 expression was easily detectable in the normal ovarian cDNA examined, it was expressed in only 1 of the 15 ovarian cancer tissues examined. In all cases, the actin gene was used as a control gene to check for the quality and quantity of the cDNA used. These preliminary results suggest that this gene is down-regulated, at the mRNA level, in diverse malignancies.

**DISCUSSION**

KLKs are a subgroup of serine proteases that play important roles in diverse physiological processes (2, 7). A number of structural and localization criteria have been proposed to classify putative KLKs into this multigene family (30, 31) As shown in Fig. 9, KLKs are characterized by the following common structural features: (a) all genes are formed of five coding exons and four intervening introns [some genes have extra 5′-untranslated exon(s) (22, 23, 32)]; (b) the coding exon lengths are usually comparable or identical between family members; (c) the intron phases are always conserved (I-II-I-0; see the Fig. 9 legend for definition of intron phases); (d) these genes are clustered in the same chromosomal region, without any intervening non-KLK-L genes (Fig. 5); and (e) the histidine residue of the catalytic triad of serine proteases is located near the end of the second coding exon, the aspartate residue is located in the middle of the third coding exon, and the serine is located at the beginning of the fifth coding exon. As shown in Fig. 9, all these criteria apply to the newly identified KLK14 gene. Thus, KLK14 should be considered as a new member of the KLK multigene family.

The wide range of tissue expression of KLK14 should not be surprising because by using the more sensitive RT-PCR technique instead of Northern blot analysis, many KLK genes were found to be expressed in a wide variety of tissues including the salivary glands, kidney, pancreas, brain, and tissues of the reproductive system (uterus, mammary gland, ovary, and testis; Ref. 2).

Our preliminary finding that the KLK14 gene is down-regulated at the mRNA level in different malignancies is in accord with findings for other KLKs [e.g., PSA, NES1, and protease M (6)]. There is now growing evidence that many of the KLKs and KLK-L genes are related to malignancy. PSA is a useful biomarker for prostate cancer (8). A recent report provided evidence that PSA has antiangiogenic activity and that this activity is due to its action as a serine protease (33). This study also suggested that other serine proteases, including new members of the KLK multigene family of enzymes, should also be evaluated for potential antiangiogenic action. Recent reports suggest that hK2 could be another useful diagnostic marker for prostate cancer (9). NES1 appears to be a tumor suppressor gene (10). The zymase/protease M/neurosin gene is differentially expressed in primary breast and ovarian tumors (11), and the human stratum corneum chymotryptic enzyme (HSCCE) has been shown to be expressed at abnormally high levels in a subset of ovarian cancers (13). Another recently identified KLK-L gene, tumor-associated differentially ex-
pressed gene 14 (TADG14/neuropsin), was found to be overexpressed in about 60% of ovarian cancer tissues (12). Prostate/KLK-L1, another newly discovered KLK-L gene, is speculated to be linked to prostate cancer (29). A newly discovered KLK, KLK-L4, was found to be down-regulated in breast cancer (34). Thus, extensive recent literature suggests multiple connections of many KLK genes to various forms of human cancer (reviewed in Ref. 6).

Many KLKs seem to play important physiological roles in the CNS. In mouse, neuropsin appears to have an important role in neural plasticity, and the amount of neuropsin mRNA is related to memory retention after a chemically induced ischemic insult (35). The human neuropsin gene was first isolated from the hippocampus (23). Another KLK, the zymoprotease M/neurosin gene, was isolated from Alzheimer’s disease brain and shown to have amyloidogenic activity (25, 26). Zyme was also found to be localized in perivascular cells and microglial cells in human Alzheimer’s disease brain (25). Trypsin-like serine protease (TLSP), another newly discovered KLK gene, was isolated from brain hippocampus cDNA and is thought to play a role in brain plasticity (36). Finally, the KLK-L2 gene is also expressed at high levels in the brain (19). Thus, we speculate that the KLK14 gene, being expressed at high levels in all examined CNS tissues, might have a role in brain physiology and pathobiology.

In conclusion, we have cloned a new KLK gene and describe its genomic structure, chromosomal localization, and tissue expression pattern. Our preliminary results suggest that the gene is down-regulated in diverse cancer tissues. This gene should be examined as a potential tumor marker because it is predicted to be secreted and is likely present in the circulation. Also, the high level of expression of the KLK14 gene in the CNS suggests that this gene may have an important function in brain physiology.

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