Research Article

TumorBiology

Tumor Biol 2004;25:149–156
DOI: 10.1159/000079147

Received: February 24, 2004
Accepted after revision: April 5, 2004

Differential Expression of a Human Kallikrein 5 *(KLK5)* Splice Variant in Ovarian and Prostate Cancer

Lisa Kurlender[a,b] George M. Yousef[b,c] Nader Memari[a,b]
John-Desmond Robb[b,c] Iacovos P. Michael[a,b] Carla Borgoño[a,b]
Dionyssios Katsaros[d] Carsten Stephan[e] Klaus Jung[e]
Eleftherios P. Diamandis[a,b]

[a]Department of Laboratory Medicine and Pathobiology, University of Toronto, and [b]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, and [c]Discipline of Laboratory Medicine, Memorial University, St. John's, Canada; [d]Department of Gynecology, Gynecologic Oncology Unit, University of Turin, Turin, Italy; [e]Department of Urology, University Hospital Charité, Humboldt University, Berlin, Germany

Key Words
Alternative splicing · Cancer biomarkers · Differential expression · Kallikreins · *KLK5* · Ovarian cancer · Prostate cancer

Abstract
The presence of more than one mRNA form is common among kallikrein genes. We identified an mRNA transcript of the human kallikrein gene 5 *(KLK5)*, denoted *KLK5* splice variant 1 *(KLK5-SV1)*. This variant has a different 5′-splice site, but encodes the same protein as the classical *KLK5* transcript. RT-PCR analysis of this variant transcript expression in 29 human tissues indicated highest expression in the cervix, salivary gland, kidney, mammary gland, and skin. Comparative analysis of the expression levels of *KLK5-SV1*, another splice variant named *KLK5* splice variant 2 *(KLK5-SV2)*, and the classical *KLK5* form showed that out of all three mRNA transcripts, the classical form is predominantly expressed (found in more tissues and at higher expression levels) followed by *KLK5-SV1*. *KLK5-SV1* is expressed at high levels in ovarian, pancreatic, breast and prostate cancer cell lines. *KLK5-SV1* was also found to be expressed in 9/10 ovarian cancer tissues, but it was not found in one normal ovarian tissue tested. Hormonal regulation experiments suggest that *KLK5-SV1* is regulated by steroid hormones in the BT-474 breast cancer cell line. Furthermore, this variant had significantly higher expression in normal prostate tissues compared to their matched cancer tissue counterparts. *KLK5-SV1* may have clinical utility in various malignancies and should be further explored as a potential new biomarker for prostate and ovarian cancer.

Introduction

Human tissue kallikreins are a group of serine proteases encoded by 15 structurally similar, hormonally regulated genes that tandemly localize on chromosome 19q13.4 [1]. Human kallikrein gene 5 (*KLK5*; previously known as *KLK-L2* [2] and human stratum corneum tryptic enzyme, *HSCTE*) [3] encodes a preproenzyme that contains an N-terminal signal peptide followed by an acti-

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1010–4283/04/0253–0149$21.00/0

Accessible online at:
www.karger.com/tbi

E.P. Diamandis, MD, PhD, FRCPC
Department of Pathology and Laboratory Medicine
Mount Sinai Hospital, 600 University Avenue
Toronto, M5G 1X5 (Canada)
Tel. +1 416 586 8443, Fax +1 416 586 8628, E-Mail ediamandis@mtsinai.on.ca

Table 1. Primers for PCR amplification

Primer name	Primer sequence	Primer direction	Specificity
VF	5′-TGCGGCTGAGCTGGGAGC-3′	forward	gene specific[1]
BF	5′-CTGGGGGACAGGGTGCAG-3′	forward	variant specific[2]
AF	5′-TGGGGGACAGGTGCCTGG-3′	forward	variant specific[3]
RP	5′-TGAGGTCGTTAGAGTGGCCA-3′	reverse	used with all 3 forward primers

[1] Amplifies all 3 *KLK5* mRNA forms (fig. 1).
[2] Amplifies variant *KLK5*-SV1.
[3] Amplifies variant *KLK5*-SV2.

vation peptide and the enzymatic domain. It is located adjacent to and shows a high degree of homology with *KLK4, KLK2* and *KLK3* (prostate-specific antigen, PSA) genes, that have been used as, or are candidate prostate cancer markers. *KLK5* is differentially expressed and was shown to be a potential prognostic marker for a variety of hormone-dependent malignancies, including ovarian [4], breast [5], prostate [6] and testicular [7] cancers. More recently, the hK5 protein was shown to be a potential serum diagnostic marker for ovarian and breast cancer [8].

Since 15% of mutations in the mammalian genome implicated in disease states are associated with an affected RNA splicing signal [9], it is important to study the various transcript types of genes, in comparison to their classical forms. For example, multiple forms of PSA occurring in benign and malignant prostate tissues may be the result of differential expression of the splice alternatives [10].

In the present study, we identified a novel variant of the *KLK5* gene with alternative splicing in the 5′-UTR. We characterized this variant in terms of its mRNA structure and predicted its protein sequence. This variant is denoted *KLK5*-SV1 (GenBank accession No. AY279380). We have further examined its expression pattern compared to other forms of the gene in normal tissues and provide preliminary evidence for its differential expression in prostate and ovarian cancers.

Materials and Methods

RT-PCR and Tissue Expression

Total RNA isolated from 29 different human tissues was purchased from Clontech (Palo Alto, Calif., USA). 2 μg of total RNA was reverse transcribed to a final volume of 20 μl using Superscript II (Invitrogen, Carlsbad, Calif., USA). mRNA was extracted as described below. Based on information on the expressed sequence tag (EST), we designed two sets of primers: VF/RP and BF/RP (table 1). PCR was performed using 1 μl cDNA, 100 ng of each primer, 1.25 units of Hot Star DNA Polymerase (Qiagen, Valencia, Calif., USA), 200 μ*M* deoxynucleoside triphosphates, and 10× buffer (10 m*M* Tris-HCl, pH 8.3, 50 m*M* KCl and 1.5 mmol/l Mg^{2+}). The PCR cycling conditions were 95°C for 15 min followed by 35 cycles at 94°C for 30 s, 65° for 30 s (60°C for primer set VF/RP), 72°C for 30 s, and then a final extension step of 10 min at 72°C. All PCR products were electrophoresed on a 1.5% agarose gel. PCR products were cloned and sequenced.

RT-PCR

Total RNA was extracted from ovarian, breast and prostate cancer tissues using Trizol™ reagent (Invitrogen) following the manufacturer's instructions. RNA concentration was determined spectrophotometrically.

Ovarian Cancer Tissues

Included in this study were tumor specimens from patients undergoing surgery for epithelial ovarian carcinoma at the Department of Gynecology, University of Turin, Italy. Diagnosis was confirmed by histopathology. Patients received no treatment before surgery.

Samples were shipped and stored at −80°C. They were then minced with a scalpel on dry ice and transferred immediately to 2-ml polypropylene tubes and homogenized. Total RNA was then extracted using Trizol reagent (Invitrogen) following the manufacturer's instructions. The concentration and purity of RNA were determined spectrophotometrically. 2 μg of total RNA were reverse transcribed into first-strand cDNA as described above.

Prostate Cancer Tissues

Prostate tissue samples were obtained from 23 patients who had undergone radical retropubic prostatectomy for prostatic adenocarcinoma at the University Hospital Charité, 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 Hospital. Fresh prostate tissue samples were obtained from the cancerous and non-cancerous components 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 of all the tissue samples was performed as previously described [11] to ensure that

Table 2. Cancer cell lines used to examine *KLK5*-SV1 expression

Cell line	Tissue of origin
MCF-7	breast cancer
ZR-75-1	breast cancer
BT-474	breast cancer
T-47D	breast cancer
MDA-MB-468	breast cancer
MDA-MB-453	breast cancer
SK-BR-3	breast cancer
BT-20	breast cancer
COLO 320HSR	colon cancer
HTB-75	ovarian cancer
PA-1	ovarian cancer
BG-1	ovarian cancer
ES-2	ovarian cancer
A-427	lung cancer
SK-N-BE	neuroblastoma
IMR-32	neuroblastoma
D283 Med	brain cancer
HTB-12	brain cancer
MiαPαCα-2	pancreatic cancer
HOS	osteosarcoma
SaOS	osteosarcoma
PC-3	prostate cancer
LNCaP	prostate cancer

the tissue was either malignant or benign. RNA was extracted and reverse transcribed as described above.

Breast Cancer Tissues

Ten breast cancer tissues were obtained from female patients at the participating hospitals of the Ontario Provincial Steroid Hormone Receptor Program. The normal tissues (from reduction mammoplasties) and tumor tissues were immediately frozen in liquid nitrogen after surgical resection and stored in this manner until extracted. RNA was extracted and reverse transcribed as described above.

Hormonal Stimulation Experiments

Twenty-three cancer cell lines (table 2) were purchased from the American Type Culture Collection, Rockville, Md., USA. Cells were cultured in RPMI media (Invitrogen) supplemented with glutamine (200 nmol/l) and fetal bovine serum (10%) in plastic flasks, to near confluency. The cells were then aliquoted into 24-well tissue culture plates and cultured to 50% confluency. Twenty-four hours prior to the hormonal stimulation experiments, the culture medium was changed into a medium containing 10% charcoal-stripped fetal bovine serum. For stimulation experiments in BT-474 and HTB-75 cells, various steroid hormones dissolved in 100% ethanol were added to the culture media, at a final concentration of 10^{-8} mol/l. Steroids tested were aldosterone (mineralocorticoid), dexamethasone (glucocorticoid), norgestrel (androgenic progestin), dihydrotestosterone (androgen) and estradiol (estrogen). Unstimulated cells and cells stimulated with ethanol were included as controls. The cells were cultured for 24 h, then harvested for mRNA extraction.

Our procedures have been approved by the Mount Sinai Hospital Institutional Review Board.

Results

New Transcript Identification

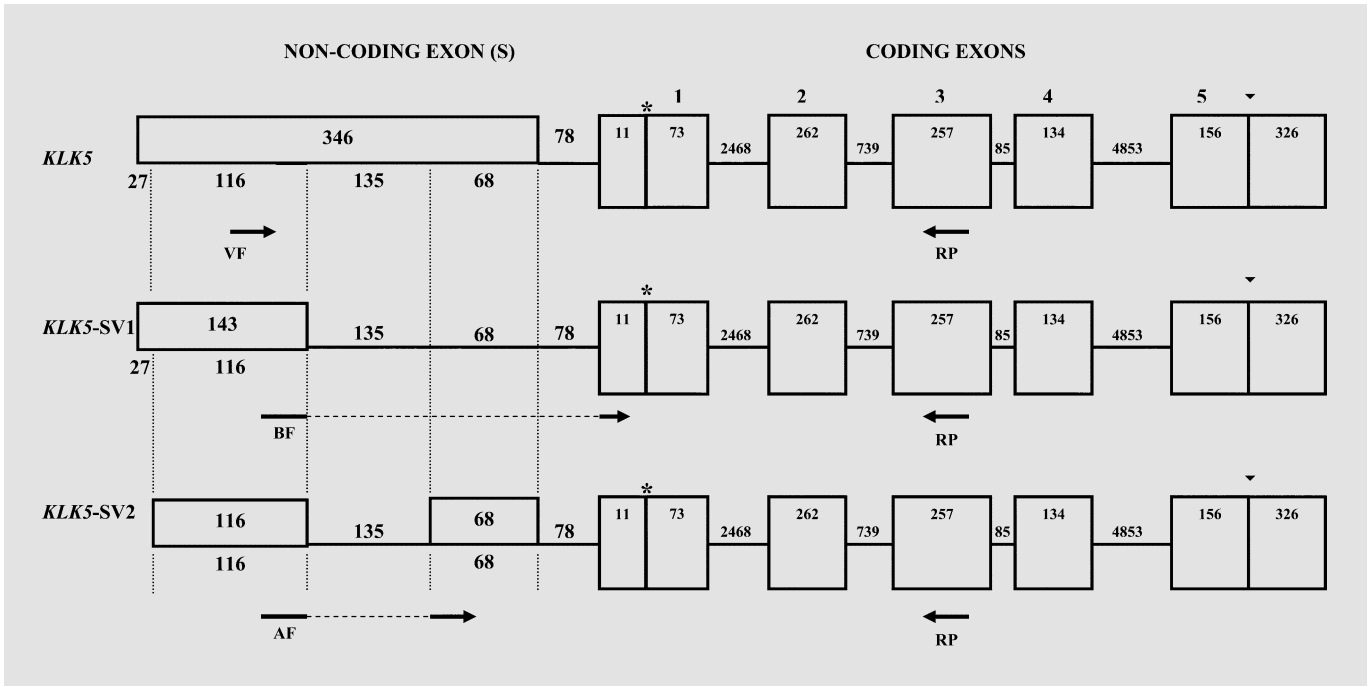
Putative variants of the *KLK5* gene were searched using the BLASTN algorithm [12] on the National Center for Biotechnology Information Web server (http://www.ncbi.nlm.nih.gov/ BLAST/) against the human EST database. Multiple sequence alignments identified 3 EST clones (GenBank accession No. BM842755, BM845065 and BE898407) that represented a unique alternative splicing that generates a truncated first non-coding exon in the 5′-UTR. Using gene-specific primers VF and RP (fig. 1) in RT-PCR experiments, an additional band was observed with molecular mass being lower than the band expected from the classical form. Sequencing of this band confirmed the existence of a novel splice variant, denoted *KLK5*-SV1. An additional 5′-UTR sequence was identified by searching the EST database. By aligning our variant with the ESTs with matched splicing in the 5′-UTR, we were able to extend the experimentally verified *KLK5*-SV1 first non-coding exon upstream by 27 bp.

Splice variant-specific primers BF and RP were designed (fig. 1), and a PCR-based protocol was developed to uniquely amplify the variant transcript *KLK5*-SV1, which generated a product of 486 bp. Primer BF will only bind to *KLK5*-SV1 as it spans both the first and second exon of this variant (fig. 1).

The predicted protein encoded by *KLK5*-SV1 is identical to the classical form of the gene since the alternative splicing in the 5′-UTR does not affect the coding region (fig. 1)

Structural Characterization of KLK5-*SV1*

The mRNA sequence for *KLK5*-SV1 is 1,362 bp and is 203 bp shorter than the mRNA of the classical *KLK5* gene. The variant is formed of five coding exons and four intervening introns. While the classical form contains the same number of exons and introns, *KLK5*-SV1 first non-coding exon starts at the same position and ends 203 bp upstream from the non-coding exon of the classical *KLK5* form (fig. 1). All coding exon/intron splice sites conform to the consensus sequence for eukaryotic splice boundaries, yet *KLK5*-SV1 has a GC replacing the more common GT splice donor site after the first non-coding exon (fig. 2).



1

Fig. 1. Schematic presentation of the structure of *KLK5*-SV1 and *KLK5*-SV2, compared to the classical form of the *KLK5* gene. Boxes represent exons and connecting lines represent introns. Numbers indicate exon and intron lengths in base pairs. The coding regions span from the start codons (*) to the stop codons (▼). The differential splicing between the classical and the variant form is in the 5′-UTR. The numbers below the 5′-UTR exons represent common base pairs among the different forms. The primer localization (table 1) is represented by arrows. Dotted lines within the arrows indicate that a single forward primer begins at the end of a particular exon and ends at the start of the next exon but does not span the intronic sequence. Vertical dotted lines were used to align common areas of the 5′-UTR.

Fig. 2. Alignment of the 5′-UTR of all three forms of the *KLK5* gene. The classical form has a transcription start site identical to the *KLK5*-SV1 form and 27 bp upstream from the *KLK5*-SV2 form. For more details see also figure 1. ATG indicates the start codon. The alternative splice site GC for the variants is underlined. The first 32 bp of the *KLK5*-SV1 (bold) and a G nucleotide at the end of non-coding exon 1 (*) are not present in a previously reported similar *KLK5* splice variant (GenBank accession No. AF435981).

```
KLK5       AATGTGCCCC AGGAGCAGTG GGTGGTTATA ACTCAGGCCC GGTGCCCAGA
KLK5-SV1   AATGTGCCCC AGGAGCAGTG GGTGGTTATA ACTCAGGCCC GGTGCCCAGA
KLK5-SV2   ---------- ---------- -------ATA ACTCAGGCCC GGTGCCCAGA

KLK5       GCCCAGGAGG AGGCAGTGGC CAGGAAGGCA CAGGCCTGAG AAGTCTGCGG
KLK5-SV1   GCCCAGGAGG AGGCAGTGGC CAGGAAGGCA CAGGCCTGAG AAGTCTGCGG
KLK5-SV2   GCCCAGGAGG AGGCAGTGGC CAGGAAGGCA CAGGCCTGAG AAGTCTGCGG

KLK5       CTGAGCTGGG AGCAAATCCC CCACCCCCTA CCTGGGGGAC AGG*GCAAGTG
KLK5-SV1   CTGAGCTGGG AGCAAATCCC CCACCCCCTA CCTGGGGGAC AGG------
KLK5-SV2   CTGAGCTGGG AGCAAATCCC CCACCCCCTA CCTGGGGGAC AGG------

KLK5       AGACCTGGTG AGGGTGGCTC AGCAGGCAGG GAAGGAGAGG TGTCTGTGCG
KLK5-SV1   ---Intron ⟶ (281 bp)------- ---------- ----------
KLK5-SV2   ---Intron ⟶ (135 bp)------- ---------- ----------

KLK5       TCCTGCACCC ACATCTTTCT CTGTCCCCTC CTTGCCCTGT CTGGAGGCTG
KLK5-SV1   ---------- ---------- ---------- ---------- ----------
KLK5-SV2   ---------- ---------- ---------- ---------- ----------

KLK5       CTAGACTCCT ATCTTCTGAA TTCTATAGTG CCTGGGTCTC AGCGCAGTGC
KLK5-SV1   ---------- ---------- ---------- ---------- ----------
KLK5-SV2   ---------- ---------- --------TG CCTGGGTCTC AGCGCAGTGC

KLK5       CGATGGTGGC CCGTCCTTGT GGTTCCTCTC TACCTGGGGA AATAAG----
KLK5-SV1   ---------- ---------- ---------- ---------- ----------
KLK5-SV2   CGATGGTGGC CCGTCCTTGT GGTTCCTCTC TACCTGGGGA AATAAG----

KLK5       ---Intron ⟶(78 bp)  (beginning of coding exon 1)
KLK5-SV1   ----------------------  (beginning of coding exon 1)
KLK5-SV2   ---Intron ⟶(78 bp)  (beginning of coding exon 1)

KLK5       GTGCAGCGGCC ATG...
KLK5-SV1   GTGCAGCGGCC ATG...
KLK5-SV2   GTGCAGCGGCC ATG...
```

2

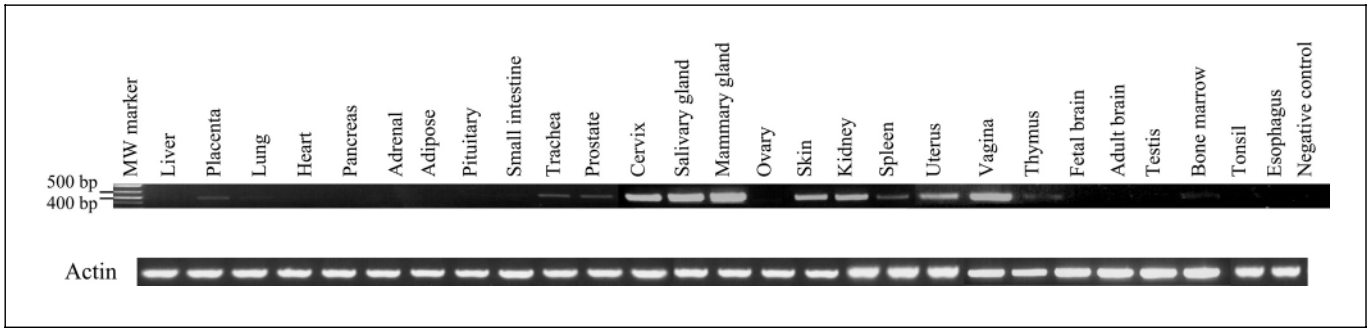


Fig. 3. Tissue expression of *KLK5*-SV1 and actin (838 bp) as determined by RT-PCR. Highest expression of the variant form was found in cervix, salivary gland, mammary gland, skin, kidney, uterus, and vagina. The primers used were BF and RP, as shown in figure 1 and table1. MW = Molecular weight.

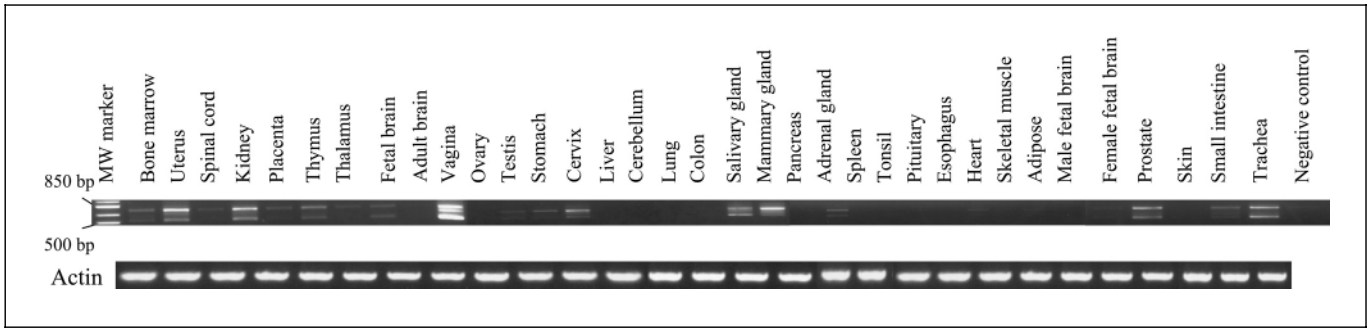


Fig. 4. Comparative tissue expression of the 3 *KLK5* mRNA forms. Expression levels of the classical *KLK5* gene (upper band; 790 bp) were usually higher than *KLK5*-SV1 (lower band; 522 bp). The middle band (590 bp) represents *KLK5*-SV2 and is weakly expressed compared to the other forms. All three bands are easily visible in the vagina. MW = Molecular weight.

Expression Pattern in Normal Tissues

KLK5-SV1 shows higher expression in the cervix, salivary gland, mammary gland, skin, kidney, skin, and vagina, and lower expression in ovary, testis, bone marrow, uterus, thymus, liver, placenta, prostate, and trachea, and no expression in the brain, tonsil, esophagus, heart, pancreas, adrenal, adipose tissue, pituitary, and small intestine (fig. 3).

As shown in figure 4, using primers VF and RP (fig. 1; these primers amplify the classical form of *KLK5* as well as *KLK5*-SV1 and *KLK5*-SV2), expression levels of the classical *KLK5* form (790-bp PCR product) are usually higher than *KLK5*-SV1 (522-bp PCR product), except in a few tissues including bone marrow, fetal brain, salivary gland, prostate, and trachea, where expression levels between the two forms were comparable. Another *KLK5* splice variant (*KLK5*-SV2, GenBank accession No. AY279381, 590-bp PCR product, fig. 1) was expressed at lower levels or not at all compared to the other two forms. All three forms of the gene are clearly visible in vagina tissue (fig. 4).

Production of KLK5-*SV1 by Cancer Cell Lines and Hormonal Regulation*

KLK5-SV1 expression was examined by splice-variant-specific RT-PCR in 23 cancer cell lines. The variant expression was significant in the ovarian cancer cell line HTB-75 and the pancreatic cancer cell line MiαPαCα-2. *KLK5*-SV1 was also expressed in 7/8 breast cancer cell lines at varying levels, in the PC-3 and LNCaP prostate cancer cell lines, and in the lung cancer cell line A-427 (fig. 5). No significant expression was found in neuroblastoma, osteosarcoma, brain and colon cancer cell lines. Hormonal regulation experiments indicate that *KLK5*-

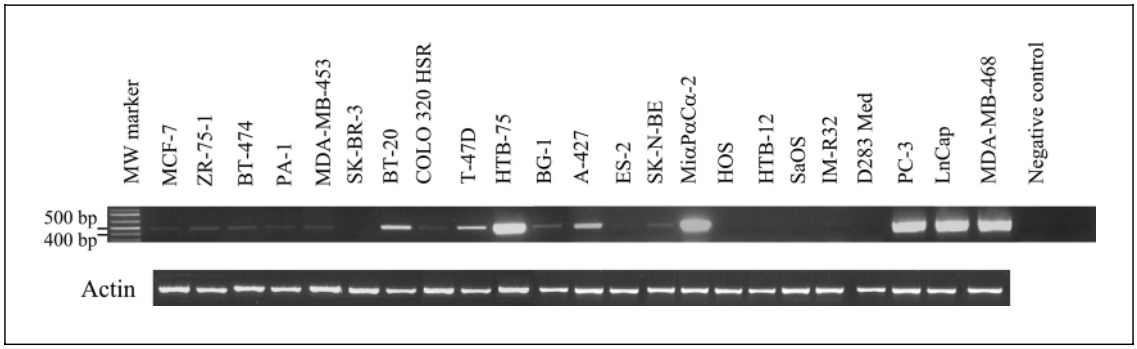


Fig. 5. Expression of *KLK5*-SV1 in cell lines (see also table 2; for discussion see text). MW = Molecular weight.

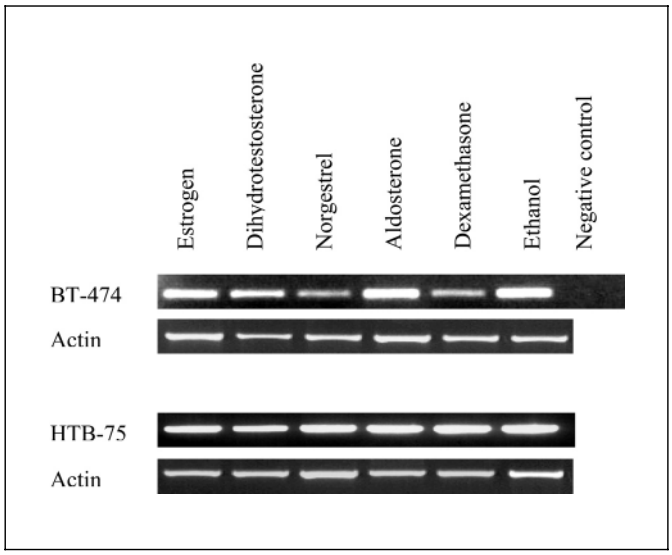


Fig. 6. Hormonal regulation in cancer cell lines. Hormonal regulation of the *KLK5*-SV1 seems to be downregulated by dexamethasone and norgestrel in the BT-474 breast cancer cell line. No hormonal regulation was noted in the ovarian cancer cell line HTB-75.

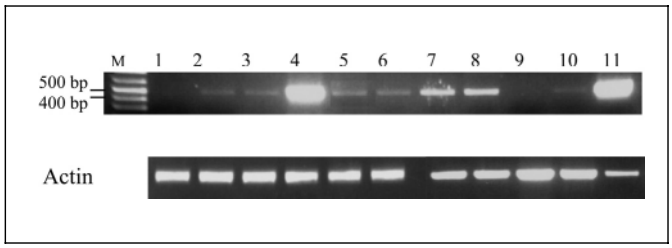


Fig. 7. Expression of *KLK5*-SV1 in normal (1) and ovarian cancer tissues (2–11).

SV1 was downregulated by dexamethasone and norgestrel in the BT-474 breast cancer cell line. No hormonal regulation was noted in the ovarian cancer cell line HTB-75 (fig. 6).

Expression in Malignant versus Normal Tissues

While no significant differential expression was found between breast cancer tissues and normal mammary gland tissue (data not shown), 9/10 ovarian cancer tissues expressed the variant whereas in one normal ovarian tissue, *KLK5*-SV1 was not expressed at all (fig. 7). Comparing 23 pairs of prostate cancer and normal prostate tissues, we found that *KLK5*-SV1 was downregulated in 14 prostate cancer tissues compared to their normal counterparts, showed no differential expression in 6 pairs and was upregulated in 3 prostate cancer tissues compared to normal prostate tissues (fig. 8).

Discussion

Splice variants are the products of differential splicing events and are prevalent in ~50% of all human genes [13]. These events are also common among the kallikreins [14]. To date, there are over 50 splice variants reported within this family. These alternative gene transcripts may possess both physiological and prognostic significance and some are emerging candidate biomarkers [15].

Here, we precisely defined the structure of *KLK5*-SV1 and found that the splicing yielded a modified first noncoding exon and an alternative GC splice donor site. This alternative donor site is known to exist in other genes [16–19]. One in every twenty alternative introns is a GC-AG intron, but to compensate for this intrinsically weak

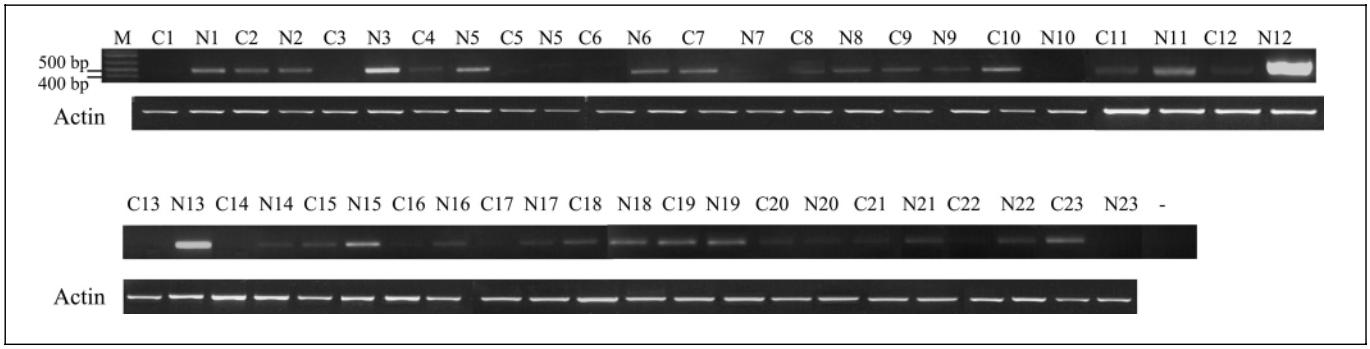


Fig. 8. Expression of the *KLK5* -SV1 in paired prostate cancer (C)/normal tissues (N) from 23 patients.

donor site, GC-AG introns always possess a consistently strong consensus sequence [20].

The alternative splicing for *KLK5*-SV1 occurs in the 5′-UTR which is common among differential transcripts [21]. The open reading frame is not affected, and we predict that the protein encoded by *KLK5*-SV1 will be identical to the classical hK5 protein (293 amino acids).

The 5′-UTR of mRNA is known to be important in post-transcriptional regulation by controlling mRNA stability, localization, and translational activity or repression [22–24]. Recent data suggest that differentiation of the 5′-UTR can be associated with the development or progression of many diseases including cancer [25]. A deletion in a critical segment in the 5′-UTR may lead to the deregulation of expression of various critical proteins involved in growth or differentiation of normal tissue [25]. This may be relevant in many alternatively spliced genes, which affect the untranslated regions. In TGF-β, an alternative transcript with a 5′-UTR truncation was found to be associated with breast cancer [26].

A single nucleotide polymorphism in the 5′-UTR of RAD51 protein that interacts with BRCA1 and BRCA2 breast cancer suppressor proteins is reported to modulate breast cancer risk [27]. The association between 5′-UTR changes and cancer is apparent in the androgen receptor gene. This event can affect the protein binding region and can result in enhanced or suppressed transcription, termination of transcription, alteration in translation or change in mRNA stability, causing an upregulation of the androgen receptor and predisposing men to developing prostate cancer [25]. Therefore, the association between differential expression of *KLK5*-SV1 in ovarian and prostate cancers and the deleted region in the 5′-UTR should be further investigated.

Since the emergence of the association of PSA, which is currently the most powerful tumor marker [28], with prostate cancer over 2 decades ago, the diagnostic and prognostic potential of other kallikrein genes is continually being explored. Recently, differential expression of kallikreins in various types of cancer suggests that splice variants are worth investigating for cancer detection. For example, preliminary studies suggest that *KLK13* variants, unlike their classical form, are not expressed in testicular tumors, and as such, have potential diagnostic and therapeutic applicability [15]. The prostate type isoform of *KLK11*/hippostasin was increased in prostate cancer and suggested to be a candidate prognostic indicator for this malignancy. Outside of the kallikrein family, various gene splice variants are implicated in cancer. An alternatively spliced version of prostate-specific membrane antigen, denoted PSMA-D, was found to have elevated expression levels in prostate cancer metastases [29]. The presence of different splice variants of CD44 (a cell surface receptor) in tumors, implicated in cancer progression and metastasis, were identified as predictors of different survival levels in cancer patients [30].

The classical *KLK5* gene is known to be under steroid hormone regulation in cancer cells and is differentially expressed in ovarian, and prostate tissues [6]. Like its classical form, *KLK5*-SV1 is upregulated in a subset of ovarian cancer tissues and downregulated in prostate cancer, compared to normal tissues. The various cancer cell lines examined appeared to have varying expression levels. *KLK5*-SV1 in the breast cancer cell line BT-474 seems to be under steroid hormone regulation.

Recently, a *KLK5* variant was cloned by Dong et al. [31] (GenBank accession No. AF435981) and was found to be overexpressed in ovarian cancer tissues and cell lines. However, expression of this variant was not exam-

ined in other malignancies. This splice variant is similar to our *KLK5*-SV1, but lacks 32 bp in the 5′-UTR and a G nucleotide at the end of the first non-coding exon (fig. 2). In addition, the 3′-end of this splice variant was not fully characterized. This variant was found to be differentially expressed in ovarian cancer, in agreement with our own data.

Acknowledgments

This work was supported by a grant to E.P.D. from the Natural Sciences and Engineering Research Council of Canada and IBEX Technologies, through a University-Industry Grant.

References

1 Yousef GM, Diamandis EP: The new human tissue kallikrein gene family: Structure, function, and association to disease. Endocr Rev 2001;22:184–204.
2 Yousef GM, Diamandis EP: The new kallikrein-like gene, KLK-L2. Molecular characterization, mapping, tissue expression, and hormonal regulation, J Biol Chem 1999;274: 37511–37516.
3 Brattsand M, Egelrud T: Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. J Biol Chem 1999;274:30033–30040.
4 Kim H, Scorilas A, Katsaros D, Yousef GM, Massobrio M, Fracchioli S, Piccinno R, Gordini G, Diamandis EP: Human kallikrein gene 5 (KLK5) expression is an indicator of poor prognosis in ovarian cancer. Br J Cancer 2001; 84:643–650.
5 Yousef GM, Scorilas A, Kyriakopoulou LG, Rendl L, Diamandis M, Ponzone R, Biglia N, Giai M, Roagna R, Sismondi P, Diamandis EP: Human kallikrein gene 5 (KLK5) expression by quantitative PCR: An independent indicator of poor prognosis in breast cancer. Clin Chem 2002;48:1241–1250.
6 Yousef GM, Scorilas A, Chang A, Rendl L, Diamandis M, Jung K, Diamandis EP: Down-regulation of the human kallikrein gene 5 (KLK5) in prostate cancer tissues. Prostate 2002;51:126–132.
7 Yousef GM, Obiezu CV, Jung K, Stephan C, Scorilas A, Diamandis EP: Differential expression of kallikrein gene 5 in cancerous and normal testicular tissues. Urology 2002;60:714–718.
8 Yousef GM, Polymeris ME, Grass L, Soosaipillai A, Chan PC, Scorilas A, Borgono CA, Harbeck N, Schmalfeldt B, Dorn J, Schmitt M, Diamandis EP: Human kallikrein 5 (hK5): A potential novel serum biomarker for breast and ovarian cancer. Cancer Res 2003;63:3958–3965.
9 Horowitz DS, Krainer AR: Mechanisms for selecting 5′ splice sites in mammalian pre-mRNA splicing. Trends Genet 1994;10:100–106.
10 Baffa R, Moreno JG, Monne M, Veronese ML, Gomella LG: A comparative analysis of prostate-specific antigen gene sequence in benign and malignant prostate tissue. Urology 1996; 47:795–800.
11 Meyer A, Jung K, Lein M, Rudolph B, Schnorr D, Loening SA: Factors influencing the ratio of free to total prostate-specific antigen in serum. Int J Cancer 1997;74:630–636.
12 Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–3402.
13 Brett D, Pospisil H, Valcarcel J, Reich J, Bork P: Alternative splicing and genome complexity. Nat Genet 2002;30:29–30.
14 Yousef GM, Diamandis EP: Human kallikreins: Common structural features, sequence analysis and evolution. Curr Genom 2003;4: 147–165.
15 Chang A, Yousef GM, Jung K, Rajpert-De Meyts E, Diamandis EP: Identification and molecular characterization of five novel kallikrein gene 13 (KLK13; KLK-L4) splice variants: Differential expression in the human testis and testicular cancer. Anticancer Res 2001; 21:3147–3152.
16 Lewin B: Genes VI. New York, Oxford University Press, 1997.
17 Erbil C, Niessing J: The primary structure of the duck alpha D-globin gene: An unusual 5′ splice junction sequence. EMBO J 1983;2: 1339–1343.
18 Wieringa B, Meyer F, Reiser J, Weissmann C: Unusual splice sites revealed by mutagenic inactivation of an authentic splice site of the rabbit beta-globin gene. Nature 1983;301:38–43.
19 King CR, Piatigorsky J: Alternative RNA splicing of the murine alpha A-crystallin gene: Protein-coding information within an intron. Cell 1983;32:707–712.
20 Thanaraj TA, Clark F: Human GC-AG alternative intron isoforms with weak donor sites show enhanced consensus at acceptor exon positions. Nucleic Acids Res 2001;29:2581–2593.
21 Mironov AA, Fickett JW, Gelfand MS: Frequent alternative splicing of human genes. Genome Res 1999;9:1288–1293.
22 Hake LE, Richter JD: Translational regulation of maternal mRNA. Biochim Biophys Acta 1997;1332:M31–M38.
23 Curtis D, Lehmann R, Zamore PD: Translational regulation in development. Cell 1995;81: 171–178.
24 Wickens M, Kimble J, Strickland S: Translational control of developmental decisions; in Hershey JWB, Mathews MB, Sonenberg N (eds): Translational Control. Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1996, pp 411–450.
25 van der Velden AW, Thomas AA: The role of the 5′ untranslated region of an mRNA in translation regulation during development. Int J Biochem Cell Biol 1999;31:87–106.
26 Arrick BA, Grendell RL, Griffin LA: Enhanced translational efficiency of a novel transforming growth factor beta 3 mRNA in human breast cancer cells. Mol Cell Biol 1994;14:619–628.
27 Blasiak J, Przybylowska K, Czechowska A, Zadrozny M, Pertynski T, Rykala J, Kolacinska A, Morawiec Z, Drzewoski J: Analysis of the G/C polymorphism in the 5′-untranslated region of the RAD51 gene in breast cancer. Acta Biochim Pol 2003;50:249–253.
28 Diamandis EP: Prostate-specific antigen – Its usefulness in clinical medicine. Trends Endocrinol Metab 1998;9:310–316.
29 Schmittgen TD, Teske S, Vessella RL, True LD, Zakrajsek BA: Expression of prostate specific membrane antigen and three alternatively spliced variants of PSMA in prostate cancer patients. Int J Cancer 2003;107:323–329.
30 Rodriguez-Rodriguez L, Sancho-Torres I, Mesonero C, Gibbon DG, Shih WJ, Zotalis G: The CD44 receptor is a molecular predictor of survival in ovarian cancer. Med Oncol 2003;20: 255–263.
31 Dong Y, Kaushal A, Brattsand M, Nicklin J, Clements JA: Differential splicing of KLK5 and KLK7 in epithelial ovarian cancer produces novel variants with potential as cancer biomarkers. Clin Cancer Res 2003;9:1710–1720.