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High level of expression in the prostate of a human glandular kallikrein mRNA related to prostate-specific antigen

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Using a synthetic oligonucleotide primer complementary to human prostate-specific antigen mRNA, we found that an additional sequence possibly similar to human glandular kallikrein-1 could be read by a primer-extension sequencing technique. We were able to confirm the identity of that additional sequence with another oligonucleotide primer complementary to a specific region of the human glandular kallikrein-1 mRNA sequence. Northern blot analysis with 2 oligonucleotide probes respectively specific for prostate-specific antigen and human glandular kallikrein-1 mRNAs showed that the length of both mRNAs was similar at 1.5 kb. The level of human glandular kallikrein-1 mRNA relative to that of prostate-specific antigen could be estimated as approx. 10–20%. This study constitutes the first evidence that the human glandular kallikrein-1 gene is expressed at a high level in a human tissue.

Prostate cancer; Proteolytic enzyme, Trypsin-like enzyme; Chymotrypsin-like enzyme

1. INTRODUCTION

Before this study, the only kallikrein-related mRNA known to be expressed in the human prostate was PSA mRNA [1]. PSA is a chymotrypsin-like enzyme secreted as a major component of seminal plasma [2]. One of its possible roles is the dissolution of the seminal coagulum formed upon ejaculation [3]. This protein has generated tremendous interest in recent years and indeed has become the marker of choice to monitor therapy for prostatic carcinoma [4]. Its primary structure has been determined by both classical amino acid sequencing [5,6] and nucleotide sequencing of a cDNA clone [1]. In the latter study, the cDNA clone lacked a small portion of the 5′-terminus coding both for untranslated sequences and for the first amino acids of the signal peptide. While we were conducting studies to determine the unknown 5′-end sequences by a primer-extension technique, we obtained evidence that hGK-1 mRNA was also expressed at a high level in the human prostate. The putative kallikrein encoded by this mRNA contains structural features of trypsin-like proteases [7].

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Abbreviations: PSA, prostate-specific antigen; hGK-1, human glandular kallikrein-1; RAV2, Rous associated virus 2; 1 × Mops, 20 mM morpholinopropanesulfonic acid; 1 × SSC, 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0

2. MATERIALS AND METHODS

2.1. *Reagents*

RAV2 reverse transcriptase and [γ-^{32}P]ATP (5000 Ci/mmol) were obtained from Amersham. T_4 polynucleotide kinase was purchased from Bethesda Research Laboratories (BRL). Calf liver tRNA and *E. coli* 600 tRNA were obtained from Boehringer Mannheim.

2.2. *mRNA isolation*

Human prostatic tissues were obtained from 2 patients undergoing surgical treatment for benign prostatic hypertrophy. Other human tissues were obtained at autopsy of a 55-year-old man who died from a myocardial infarctus. Total RNA was isolated by the method of Chirgwin et al. [8]. The

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poly(A)$^+$ RNA fraction was recovered after two passages of total RNA over a small oligo-d(T)-cellulose column (BRL).

2.3. *RNA sequencing*

Specific oligonucleotide primers were chemically synthesized using a Biosearch model 8700 DNA synthesizer (New Brunswick Scientific). All primers were purified by polyacrylamide gel electrophoresis. RNA sequencing using poly(A)$^+$ RNA was performed by the primer-extension technique recently described by Geliebter et al. [9]. For each sequence reaction 5 μg poly(A)$^+$ RNA were used with 5 ng of one specific oligonucleotide primer radiolabeled with [γ-^{32}P]ATP using T_4 polynucleotide kinase. Annealing temperatures varied between 57 and 65°C depending on the specific nucleotide content according to the formula: $T(°C) = 4(G + C) + 2(A + T) - 5$.

2.4. *Northern blots with synthetic oligonucleotides*

5 μg poly(A)$^+$ RNA from human prostate, liver, kidney and testis were denatured in 6% formaldehyde and 50% formamide in 1 × Mops buffer and were applied on 1.2% agarose gels containing 2% formaldehyde and 1 × Mops. Electrophoresis was carried out at 100 V for 4–5 h. RNA was transferred and fixed on Hybond N nylon membrane (Amersham) as suggested by the supplier. Prehybridization was performed in 6 × SSC, 1 mM EDTA, 5 × Denhardt, 0.5% SDS, 100 μg/ml of MRE 600 tRNA and 20 mM Tris-HCl (pH 7.4) for at least 2 h at the same temperature as that used during the hybridization per se. Hybridization was done in fresh buffer also containing 10% dextran sulfate and one of the radiolabeled oligonucleotide probes described in section 3. These probes were labeled with [γ-^{32}P]ATP similarly to the RNA sequencing reaction. The probes were immediately purified by precipitation in the presence of 25 μg calf liver tRNA using 2.5 M ammonium acetate and 2.5 vols of 95% ethanol. Hybridization was performed at 58°C over 16–18 h with one specific oligonucleotide probe (4–6 × 10^6 cpm/ml). The filters were then washed at 54°C for 1 h in 2 × SSC, 10 mM Tris-HCl (pH 7.5), 0.5% SDS and then for 1 h more in 1 × SSC, 10 mM Tris-HCl (pH 7.5) and 0.5% SDS. Finally, the filters were air-dried for 1 min and placed between two layers of Saran wrap. Autoradiography was carried out on Kodak X-Omat AR XR2 film at −80°C.

Table 1

Oligonucleotide primers[a] complementary to PSA and hGK-1 mRNAs

Designation	Oligonucleotide primers
TrB10	5′-GGGTTGGGAATGCTTCTCGCA-3′
TrB13	5′-TTCTGAGGGTGAACTTGCGCA-3′
TrB14	5′-CCTGCCACGAGAGGCCACAAG-3′
TrB15	5′-CTTCTCAGAGTAAGCTCTAGC-3′

[a] TrB10, TrB13 and TrB14 are complementary to mRNA sequences of PSA coding respectively for amino acids 7–13 (CEKHSQP), 161–167 (AQVHPQK) and 17–23 (LVASRGR). TrB15 is complementary to the deduced mRNA sequence from the hGK-1 gene and codes for putative amino acids 161–167 (ARAYSEK). The amino acid numbering is identical to that used in [7,8]

3. RESULTS

The first experiment of this study was designed to determine the oligonucleotide sequence of the 5′-portion of human PSA mRNA which was missing from clone λ-HPSA-1 isolated and sequenced by Lundwall and Lilja [1]. For this purpose, we synthesized an oligonucleotide primer designated TrB10 (table 1) and complementary to the mRNA nucleotide sequence of PSA [1]. Primer-extension sequencing of prostatic mRNA using TrB10 (not shown) indicated that beside PSA, we could read

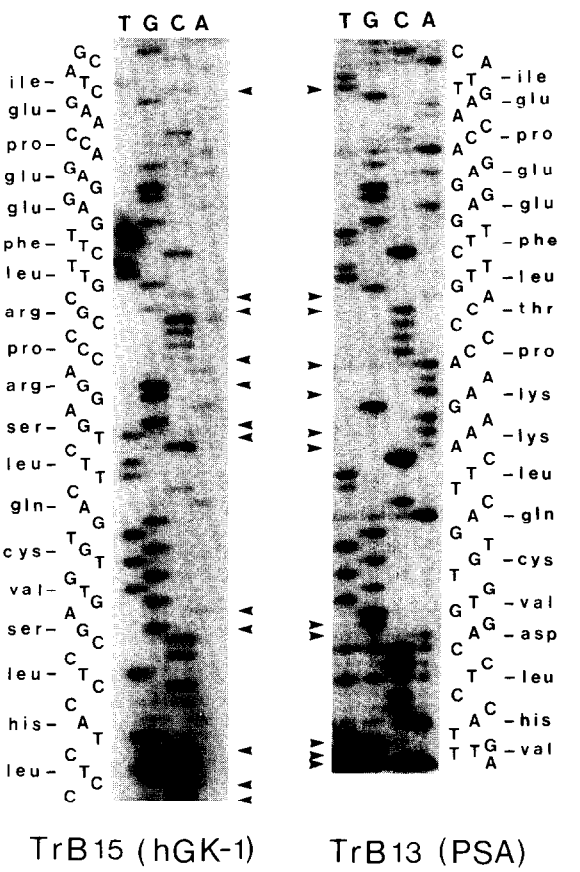

Fig.1. Primer-extension determination of the nucleotide sequence of PSA and hGK-1 mRNAs using 21-mer specific oligonucleotide primers, TrB13 and TrB15. For the sake of clarity, nucleotides corresponding to the DNA sequence are shown here instead of the observed complementary nucleotides of the primer-extended reaction. Arrowheads indicate the nucleotides which are divergent between the 2 sequences. The lengths of the 2 sequencing gels were slightly different as a result of 2 separate runs being carried out.

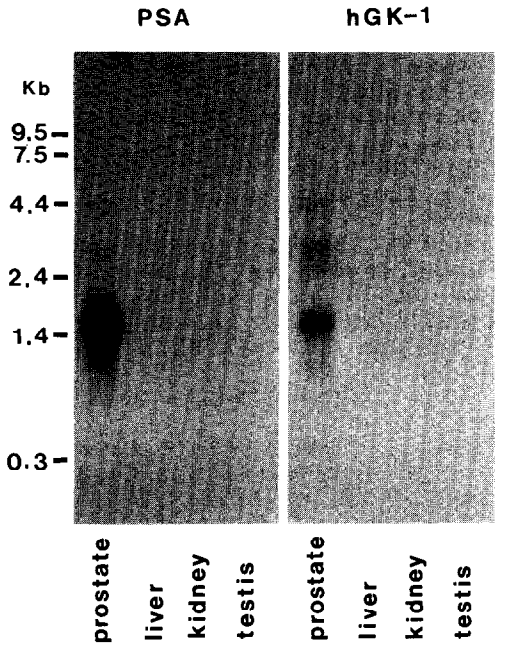

Fig.2. Northern blot analysis of PSA and hGK-1 mRNAs using oligonucleotide primers TrB13 and TrB15 as hybridization probes. The specific activity of each radiolabeled probe and the amount of radioactivity in each hybridization solution were identical. Autoradiography on X-ray films was performed for 3 h.

an additional sequence that was compatible with that recently published of the hGK-1 gene [7]. In order to confirm this result, we synthesized 2 oligonucleotide primers, TrB13 and TrB15, which were specific respectively for PSA and hGK-1 (see table 1) and performed primer-extension sequencing using prostatic poly(A)$^+$ RNA. The results are shown in fig.1. The portion of the sequencing gel shows that 12 nucleotides out of 59 were different between the 2 sequences. The whole sequences obtained with primers TrB13 and TrB15, respectively, were identical to those of PSA [1] and hGK-1 [7]. These results prompted us to determine whether both mRNAs had a similar length and what were their relative concentrations with respect to each other in the prostate and in other human tissues. Northern blot analysis using the 2 specific oligonucleotide primers as hybridization probes showed that the mRNAs for PSA and hGK-1 had an identical size of approx. 1.5 kb (fig.2). Image analysis by densitometric scanning indicated that the intensity of the hGK-1 1.5 kb band varied from 10 to 20% relative to PSA depending on the experiment. These mRNAs appear to be absent from the liver, kidney and testis.

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                                        -24                 -21
                                        met  trp  val  pro
PSA    CTTACCACCTGCACCCGGAGAGCTGTGTCACC ATG  TGG  GTC  CCG  44
hGK-1  TGCACCACCTGGCCGTGGACACCTGTGTCAGC ATG  TGG  GAC  CTG  44
                                        met  trp  asp  leu
                                        -24                 -21
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Fig.3. Comparative 5′-terminal nucleotide sequence of the DNA encoding PSA determined in this study by primer extension with TrB14 and of the DNA sequence of hGK-1 determined by Schedlich et al. [8]. Further downstream, the nucleotide sequence of PSA that could be read was identical to that of Lundwall and Lilja [7].

In order to determine specifically the unknown 5′-terminal nucleotide sequence of PSA we used another oligonucleotide primer, TrB14, complementary to PSA mRNA in a region highly divergent from the one of hGK-1 (table 1). This oligonucleotide primer was used for sequencing PSA mRNA by primer extension. The comparative 5′-terminal nucleotide sequences of PSA and hGK-1 are shown in fig.3. The 5′-untranslated region of PSA mRNA contains 32 nucleotides and has 69% homology with that of hGK-1. By contrast, the two prepro pieces are only 54% homologous (not shown).

4. DISCUSSION

This study constitutes the first evidence that hGK-1 mRNA is expressed in a human tissue. Schedlich et al. [7] have determined the structure of the hGK-1 gene, but have not studied its expression. Furthermore, the putative glandular kallikrein encoded by this gene has never been isolated from the prostate. The deduced amino acid sequence of hGK-1 [7] predicts that the encoded protein has a trypsin-like specificity whereas PSA has a chymotrypsin-like specificity [6]. There are some indications in the literature that hGK-1 may be present as a variable contaminant in preparations of PSA. Indeed, in some studies purified prostate-specific antigen showed low but detectable activity towards synthetic substrates of both trypsin and chymotrypsin [5]. In other studies, the purified preparation was active against arginine/lysine-containing substrates, but not against a chymotrypsin substrate, benzoyltyrosine ethyl

ester [2]. Finally, in one study [6], PSA had a strict chymotrypsin-like activity. For these reasons and because of high concentrations of hGK-1 mRNA in human prostate, we propose that hGK-1 may be a relatively abundant secretory protein of the prostate. This proposition has important implications for the interpretation of presently available and widely used immunoassay procedures for serum PSA in prostatic cancer patients. The specificities of the various antibodies used are not known and could be directed variably towards hGK-1 and PSA. Since the expression of various prostatic secretory proteins does not follow a parallel trend in prostatic cancer cells and since the various markers are not equivalent [10], it will become important to isolate hGK-1 and PSA uncontaminated by each other, to develop specific antibodies for both proteins and to determine their respective usefulness for the diagnostic and follow-up of prostate cancer patients.

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