Expression of prostate-specific antigen and human glandular kallikrein 2 in the thyroid gland

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Abstract

Prostate-specific antigen (PSA) and human glandular kallikrein 2 (hK2) are two closely related kallikreins, primarily produced by the prostate. These serine proteases are now used as biomarkers for the diagnosis and management of prostate cancer. Until recently, PSA and hK2 were thought to be strictly expressed in the prostate; however, numerous studies confirmed their presence in various biological fluids as well as in many normal and malignant tissues. Using reverse transcription-polymerase chain reaction (RT-PCR), we screened RNA extracted from 26 different normal tissues and found that both genes are expressed in the thyroid. Subsequently, we analyzed 15 RNAs extracted from thyroid tissues (10 benign and 5 malignant lesions) and found that both kallikreins were expressed in five specimens (four benign lesions and one malignant). Sequencing of the PCR products confirmed the specificity of our experiments. Immunohistochemistry localized PSA in oxyphilic cells of thyroid tissue. These data confirm expression of both PSA and hK2 in thyroid tissue and suggest that oxyphils are the source of their production. The function of these two proteases in thyroid tissue is unknown. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Kallikreins are a subgroup of the serine protease enzyme family. These kallikreins exhibit a high degree of substrate specificity and are involved in the post-translational processing of specific polypeptide precursors [1]. Three human kallikrein genes have been well-characterized; the tissue (pancreatic-renal) kallikrein (KLK1) [2], the human glandular kallikrein 2 (KLK2) [3] and prostate specific antigen (PSA or KLK3) [4]. All three human kallikrein genes have been assigned to chromosome 19q13.2-19q13.4 [5]. Even though early studies had indicated that the human kallikrein gene family might consist of several members [6–8], it is only in the past three years that new genes have been identified in the same locus. The newly discovered genes exhibit significant homologies with the classical kallikrein genes, including protease M [9] (also named zyme [10] or neurosin [11]) and the normal epithelial cell-specific 1 gene (NES1) [12].

PSA is a valuable marker for the diagnosis and management of prostate cancer [13] but its tissue specificity has been challenged by many recent reports [14]. PSA localization in extra-prostatic tissues is now well-established but its physiological role in these tissues is still under investigation [14,15]. A very recent review summarizes all known sources of extrapostatic PSA [16]. Recent studies indicate that hK2 may be a novel marker for prostate cancer, supplementing the clinical value of PSA [17–19]. The presence of hK2 and PSA in the same body fluids [20,21] supports the idea that these two kallikreins may be functionally related. Presumably, hK2 activates pro-PSA, as indicated by its ability to cleave the zymogen form of PSA in vitro [22–24].

These findings of extraprostatic sources of PSA and hK2 led us to screen a number of human tissues by RT-PCR, for the presence of PSA and hK2 mRNAs. We report here identification of the transcripts of both of these genes in a significant proportion of benign and malignant thyroid tissues.

2. Materials and methods

2.1. Tissue specimens

Total RNA isolated from 26 different human tissues was purchased from Clontech, Palo Alto, CA (Table 1). Primary thyroid tissue samples collected at the time of surgery and characterized histologically as described previously [25] were immediately frozen in liquid nitrogen and stored at $-80°C$ until extraction. The tissues were pulverized with a hammer under liquid nitrogen and total RNA was extracted as described below. Corresponding thyroid tissue was

Table 1
Detection of prostate specific antigen (PSA) transcripts in various human tissues by RT-PCR[a]

Human tissue	PSA transcript
Prostate	+ + +
Thyroid	+
Salivary glands	+
Mammary glands	+
Trachea	+
Lung	±
Testis	±
Fetal brain	−
Stomach	−
Uterus	−
Heart	−
Thymus	−
Fetal liver	−
Brain	−
Adrenals	−
Colon	−
Spleen	−
Placenta	−
Skeletal muscle	−
Cerebellum	−
Liver	−
Pancreas	−
Small intestine	−
Spinal cord	−
Kidney	−
Bone marrow	−

[a] + + +, highly positive; +, positive; ±, very weakly positive; −, negative.

fixed in 10% buffered formalin and embedded in paraffin for histopathological diagnosis [26].

2.2. Reverse transcriptase polymerase chain reaction

Total RNA was extracted from the cells using the guanidinium thiocyanate method [27]. The purity and quantity of the extracted RNA was checked spectrophotometrically at 260 and 280 nm. Total RNA (2 μg) was reverse transcribed using oligo dT primers and Superscript II reverse transcriptase (Gibco BRL, Gaithersburg, MD). The final volume was 20 μl. One μl of cDNA was then amplified by PCR with gene-specific primers. To ensure the integrity of RNA and the efficacy of the RT reaction, the housekeeping gene PGK-1 was analyzed by PCR as described previously [28]. The primers used for PSA and hK2 have been published previously by Deguchi et al. [29] and Hsieh et al. [30]

respectively, and their sequences are as follows: PSA-1: 5′-TGC-GCA-AGT-TCA-CCC-TCA-3′ and PSA-2: 5′-CCC-TCT-CCT-TAC-TTC-ATC-C-3′; hK2-1: 5′-GGT-GGC-TGT-GTA-CAG-TCA-TGG-AT-3′ and hK2-2: 5′-CAG-AAA-GCA-CAG-GTC-AGT-AGG-AC-3′. The cycling conditions for PSA were 95°C for 5 min, followed by 30 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 30 s and a final extension at 72°C for 7 min. The cycling conditions for hK2 were 95°C for 5 min, followed by 35 cycles at 94°C for 1 min, 56°C for 1 min and 72°C for 1 min and a final extension at 72°C for 10 min. Negative controls were included during the reverse transcription as well as the PCR reaction to ensure the absence of contamination. Equal amounts of PCR products were electrophoresed on 1% agarose gels and visualized by ethidium bromide staining.

2.3. Sequencing

To verify the identity of the PCR products, they were cloned into the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The inserts were sequenced from both directions using vector-specific primers, with an automated DNA sequencer (Visible Genetics Inc., Toronto, ON, Canada).

2.4. Immunohistochemistry

To localize the protein product of mRNA expression, immunohistochemistry was performed on formalin-fixed paraffin embedded tissue. Four μm-thick sections were stained with hematoxylin and eosin, and used for tissue identification and tumor classification according to accepted criteria [26]. Sequential 4 μm-thick sections were stained for PSA using a rabbit anti-human polyclonal antiserum (Dako, Denmark) (pepsin pretreatment, 1/400 dilution, 1 h incubation). The reaction product was detected with the Ultra Streptavidin detection system (Signet Laboratories, Dedham, MA) and visualized with 3, 3′-diaminobenzidine.

3. Results

We screened by RT-PCR 26 human tissues for PSA expression (Table 1). As expected, prostatic tissue exhibited the highest levels of PSA mRNA. However, a number of other tissues were also positive as follows: prostate ≫ thyroid > salivary glands > mammary gland > trachea. In these tissues, the PCR bands were clearly recognizable and when sequenced, they were all identical and matched with the published sequence of PSA mRNA (Genbank Accession # NM_001648). Traces of PSA mRNA were also seen in lung and testicular
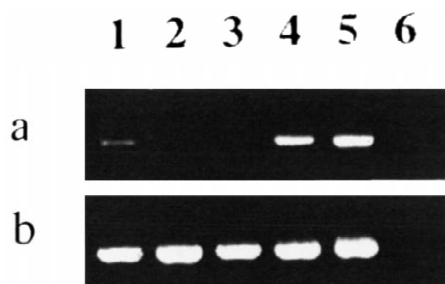
Fig. 1. RT-PCR of PSA (a) and actin (b) mRNAs extracted from 5 tissues: 1, salivary; 2, brain; 3, liver; 4, thyroid; 5, prostate; 6, negative control. Actin was used as a positive control. The conditions of amplification are described elsewhere [36].

tissues. All other tested tissues were negative for PSA mRNA (fetal brain, stomach, uterus, heart, thymus, fetal liver, brain, adrenal gland, colon, spleen, placenta, skeletal muscle, cerebellum, liver, pancreas, small intestine, spinal cord, kidney, bone marrow). A representative gel (Fig. 1) of PSA mRNA expression in five tissues demonstrates that prostate, thyroid and salivary glands are positive, while brain and liver are negative. The unexpectedly relatively high levels of PSA mRNA in thyroid tissue prompted us to examine PSA and hK2 mRNA expression in another 15 thyroid tissues, 10 from patients with benign disease or normal tissues adjacent to pathological ones (five with hyperplasia, two with thyroiditis, one with both hyperplasia and thyroiditis, and two normal) and 5 from patients with papillary thyroid carcinoma. hK2 and PSA are highly homologous kallikreins which appear to co-express in prostate and other tissues. In Fig. 2, we demonstrate detection of PSA and hK2 mRNA in the same 5 tissues (4 benign, including two normals, one from a patient with hyperplasia and one from a patient with hyperplasia and thyroiditis, and one from a patient with papillary thyroid carcinoma). The remaining 10 tissues (6 benign and 4 malignant) were negative for both PSA and hK2 mRNA. PCR-positive hK2 and PSA bands were cloned and sequenced to verify specificity. The data have
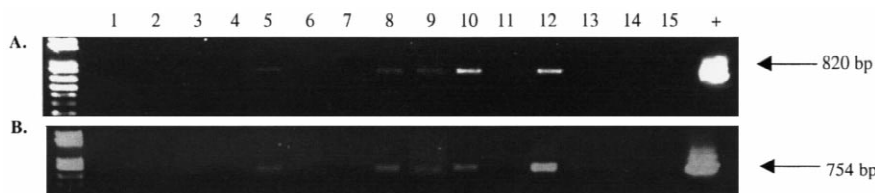

Fig. 2. RT-PCR of (A) hK2 and (B) PSA mRNAs extracted from thyroid tissues. The length of the PCR products are 820 bp and 754 bp for hK2 and PSA, respectively. Specimens 1, 2, 3, 6 and 8 were malignant, whereas the rest were benign. Prostate cDNA was used as a positive control (+).
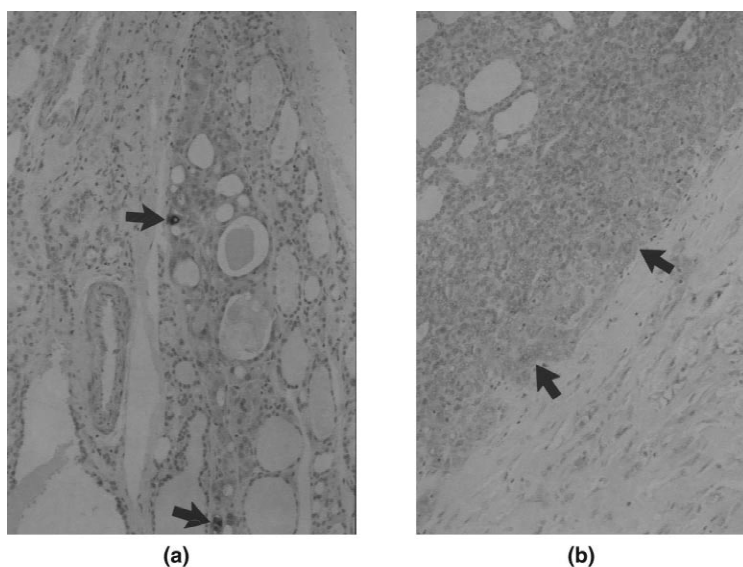
Fig. 3. Immunohistochemical localization of PSA in thyroid tissues. (a) PSA immunoreactivity in non-tumorous thyroid. The Hürthle cells of this thyroid exhibit strong cytoplasmic immunoreactivity for PSA (arrows). (b) PSA immunoreactivity in a malignant thyroid tumor. The Hürthle cell papillary carcinoma has focal strong cytoplasmic immunoreactivity for PSA; this is especially apparent at the periphery of the tumor (arrows).

shown identity of the obtained sequences with those deposited in GenBank for these two genes.

Immunohistochemistry localized PSA in the cytoplasm of both malignant and benign thyroid follicular epithelial cells, especially in areas demonstrating oncocytic metaplasia (Fig. 3).

4. Discussion

With the availability of highly sensitive techniques (time-resolved fluorometric immunoassays, polymerase chain reaction) it has become apparent that both PSA and hK2, two kallikreins thought to be prostate specific, are expressed in many non-prostatic tissues including the periurethral and perianal glands [31–33]), breast tumor extracts [34,35], breast cancer cell lines [36], endometrial tissue [37], placenta and bone marrow [38,39], breast secretions, etc. [14,15]. A detailed review of the non-prostatic PSA literature has been recently published [16]. Our finding of PSA mRNA expression in thyroid papillary carcinoma has also been reported recently by Ishikawa et al. [40]. However these authors did not examine hK2 expression.

Consistent with our previous findings [20,41], we observed either a parallel

expression or absence of PSA and hK2 in the same tissues (Fig. 2). This may be explained by the fact that these two serine proteases are regulated by similar mechanisms, as shown with breast carcinoma cell line model systems [36,42]. Both genes are up-regulated by androgens and progestins [36,42]. The simultaneous expression of PSA and hK2 allows us to speculate that these two proteins may have a functional relationship. In vitro, it has been demonstrated that hK2 can activate pro-PSA by cleavage of a few amino acids from its N-terminus [22–24]. Others have shown activation of urokinase-type plasminogen activator by hK2 [43].

Immunohistochemistry localized PSA in oxyphilic cells in the samples that were positive for mRNA. Samples devoid of oxyphils or Hürthle cells were negative. Hürthle cells are more common in inflamed thyroids with autoimmune disease (e.g. Hashimoto's thyroiditis) [26] but are also found in benign and malignant tumors of the thyroid [26–28].

The number of samples we analyzed is small and does not allow for conclusions regarding differential expression of PSA and hK2 between benign and malignant tissues. For breast cancer, we have previously shown that higher PSA expression is associated with younger patient age, more differentiated, hormone receptor positive tumors, and better patient survival rates [14,15,34,35,44,45]. In this paper, we found PSA positivity in 4/10 (40%) benign tissues and in 1/5 (20%) malignant tissues. Analysis of more samples will further reveal if indeed, PSA and hK2 expression is lower in cancerous tissue.

We did not examine the possible biological function of these two kallikreins in the thyroid. Recent literature suggests that the expanded kallikrein gene family consists of at least 14 members [46,47]. There is also compelling new evidence that at least 3 kallikreins, namely PSA, protease M, and the normal epithelial cell-specific 1 gene (NES1) may act as tumor suppressor molecules [9–12,14,47–49]. This activity may relate to their enzymatic function as serine proteases. Thus, it will be interesting to identify the biological substrates of these and other kallikreins in various tissues and fluids.

In conclusion, we demonstrated expression of two kallikreins, PSA and hK2, in thyroid tissue. It will be interesting to examine in the future, the actual biological function of these serine proteases in thyroid and other tissues and establish if there is any association between thyroid pathophysiology and kallikrein expression.

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