ELSEVIER

DECREASED CONCENTRATIONS OF PROSTATE-SPECIFIC ANTIGEN AND HUMAN GLANDULAR KALLIKREIN 2 IN MALIGNANT VERSUS NONMALIGNANT PROSTATIC TISSUE

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ABSTRACT

Objectives. To study quantitatively the relative expression of human glandular kallikrein 2 (hK2) and prostate-specific antigen (PSA) in paired (from the same patient) cancerous and noncancerous prostatic tissue to evaluate whether these proteins are overexpressed or underexpressed in cancer.

Methods. We studied 14 patients who underwent radical retropubic prostatectomy for prostate cancer. Cancerous and adjacent normal tissues were excised and then extracted to prepare cytosolic extracts. The extracts were analyzed for total protein, and for hK2 and PSA using sensitive and specific immunofluorometric procedures.

Results. PSA was present in the prostatic extracts at about 50 to 100 times higher amounts than hK2. The correlation between PSA and hK2 values was good. Both prostate kallikreins were expressed more in noncancerous than in cancerous prostatic tissue.

Conclusions. Our results demonstrated that both PSA and hK2 are down-regulated in prostate cancer compared with noncancerous tissue. The degree of down-regulation was higher for PSA than for hK2. The mechanism and physiologic consequences of this down-regulation are unknown. UROLOGY **56:** 527–532, 2000. © 2000, Elsevier Science Inc.

Prostate-specific antigen (PSA) is now used widely for the early diagnosis of prostate cancer (PCa) and to monitor the response to therapy.[1] However, the expression of PSA is not specific to cancer, and its serum concentration is also frequently elevated in benign prostatic hyperplasia (BPH). To improve the clinical value of PSA, different approaches have been developed, including PSA density, PSA velocity, and age-specific reference ranges. Furthermore, it has been shown that measurement of the different molecular forms of serum PSA helps to discriminate between PCa and BPH. Recent studies indicate that the free/total PSA ratio in serum is lower in patients with PCa than in patients with BPH, thus enhancing the clinical usefulness of PSA testing in PCa screening programs.[2]

Although increased serum concentrations of PSA are associated with PCa, the situation appears to be different within the prostate. Immunohistochemical analyses with anti-PSA antibodies,[3,4] measurements of intraprostatic concentrations of PSA,[5] assessment of PSA mRNA in prostatic tissue specimens,[3,6] and in situ hybridization techniques[3,7] have demonstrated that the amount of PSA protein or PSA transcripts is not increased in PCa compared with BPH. Rather, advanced PCa is generally associated with lower levels of PSA gene expression.[3,5,7] The results of a very recent study[8] demonstrated that patients with PCa with low tissue-PSA (T-PSA) levels developed progressive disease and that none of the patients with high T-PSA levels did. The investigators concluded that T-PSA is superior to other hitherto routinely used markers for the prediction of outcome of patients with newly diagnosed PCa who underwent hormonal therapy.

Human glandular kallikrein 2 (hK2) belongs to the same family of serine proteases as PSA (human

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TABLE I. ***Descriptive statistics for PSA and hK2 concentrations in noncancerous and cancerous prostatic tissues from 14 patients***

	Mean (μg/mg TP)	Standard Deviation (μg/mg TP)	Median (μg/mg TP)	*P* Value*
PSA				
No cancer	16.2	12.3	15.4	
Cancer	7.8	7.4	6.7	0.005
Difference (%)†	52	—	56	
hK2				
No cancer	0.19	0.14	0.16	
Cancer	0.11	0.095	0.091	0.016
Difference (%)†	37	—	43	

KEY: PSA = prostate-specific antigen; hK2 = human kallikrein 2; TP = total protein.
** Calculated by the Wilcoxon signed rank test.*
† Calculated by assuming that value in noncancerous tissue is 100%.

kallikrein gene family) and displays a strong structural homology to PSA.[9,10] These two kallikreins also share a close relationship in terms of prostate localization, function, and regulatory features.[9] Recent findings that hK2 cleaves proPSA (244 residues) to generate enzymatically active PSA (237 residues) suggest a physiologic role of hK2 in the regulation of PSA.[11–13] Furthermore, recent studies suggest a potential role of hK2 as an additional prostate tumor marker.[14,15]

Darson *et al.*,[16,17] using immunohistochemical techniques, first provided evidence of increased hK2 expression in cancerous tissue compared with adjacent normal tissue. However, to our knowledge, no report has as yet quantitatively compared the levels of PSA and hK2 in cancerous and normal tissue from paired specimens obtained from the same patients. We report the analysis of PSA and hK2 in paired tumor/normal prostatic tissue extracts from 14 patients and provide evidence that both kallikreins are generally down-regulated in PCa, with PSA being more markedly suppressed than hK2.

MATERIAL AND METHODS

Study Group

Included in this study were 14 patients who had undergone radical prostatectomy for PCa at the Charité University Hospital, Berlin. The use of tissue specimens for research purposes was approved by the Ethics Committee of Charité University Hospital, Berlin. Patient age ranged from 57 to 71 years (median 65). The cancer of each patient had been classified according to the TNM system, and the histologic grade had been classified as grade 1, 2, or 3, as previously described in detail.[18] The number of patients in each classification according to pathologic stage and grade was as follows: pT1N0M0, 1 patient; pT2N0M0, 10 patients; and pT3N0M0, 3 patients; and G1, 2 patients; G2, 9 patients; and G3, 3 patients.

Tissue Samples

Prostatic tissue samples were obtained from the cancerous and noncancerous portions of the same prostates that had been surgically removed by radical retropubic prostatectomy. The cancerous specimens generally originated from the peripheral parts of the dorsal/dorsolateral prostate, located near the prostate capsule, and the noncancerous samples were usually taken from the periurethral region of the central zone of the prostate. These matched pairs were used for further analysis. Small pieces of tissue were dissected immediately after removal of the prostate and stored in liquid nitrogen until analysis. The cut edges within the prostate were marked so that the dissected pieces could easily be assigned to the adjacent prostatic tissue examined histopathologically. Histologic analysis from all tissue pieces used was performed as previously described[18] to ensure that the material used was either malignant or benign (data not shown).

Tissue Extract Preparation

Tissue samples (~30 to 50 mg) were thawed, cut into small pieces, and homogenized in 100 μL of 10 mmol/L sodium phosphate buffer, pH 7.46, containing 2.5 mL/L Triton X-100, with a Wheaton glass homogenizer by 10 strokes on ice. The extracts were then sonicated. The homogenates were transferred into 1.5-mL tubes. The homogenizer was then rinsed twice with 100 μL of the extraction solution. This 300-μL mixture was centrifuged at 23,000*g* for 15 minutes at 4°C. The supernatants were recovered and subsequently used for assay of PSA and hK2. To investigate the effectiveness of the extraction procedure, the pellets were resuspended and the extraction was repeated several times. The recommended final extraction procedure consisted of three extractions with Triton solution and the measurement of the analytes in the combined supernatants. More details on the validation and the recovery of the extraction procedure can be found elsewhere.[19] All supernatants were stored at −80°C until analysis.

Immunoassays

The concentrations of PSA were determined using an in-house method, previously described and validated.[20] A new, time-resolved immunofluorometric assay, recently developed in our laboratory, was used to measure the serum hK2 concentrations.[21] The PSA and hK2 assays have detection limits around 0.001 μg/L. The hK2 assay has less than 0.2% cross-reactivity to PSA, and the PSA assay is free from cross-reactivity with hK2. To measure hK2 and PSA in tissue extracts, the supernatants were thawed and diluted 5000-fold and 50,000-fold, respectively, with 60 g/L bovine serum albumin solution.

Total Protein Assay

The total protein concentrations in the tissue extracts were determined with the Coomassie Brilliant Blue assay reagent, using bovine serum albumin as the calibrator.

Statistical Analysis

Statistical analysis was performed with Statistical Analysis System software (SAS Institute, Cary, NC). The analyses of the differences between hK2 and PSA in noncancerous and cancerous tissue were performed with the nonparametric McNemar test. The relationships between the different variables were assessed by Spearman's correlation and the Wilcoxon and Kruskal-Wallis tests.

RESULTS

We analyzed 14 pairs of prostatic tissue extracts (cancer/normal) to determine the concentrations of hK2 and PSA in benign and malignant tissue (Table I). The distribution of the PSA concentrations in benign (noncancerous) and malignant (cancerous) tissues is shown in Figure 1A. The PSA values ranged from 0.63 to 36.3 μg/mg protein in noncancerous tissue (mean 16.2; 95% confidence interval [CI] 9.7 to 22.7) and from 0.24 to 29.1 μg/mg protein in cancerous tissue (mean 7.8; 95% CI 3.8 to 11.7). Figure 1B shows the paired values of PSA in noncancerous and cancerous tissue for every patient. Eleven of the 14 patients had higher PSA levels in the noncancerous tissue, 1 patient had higher PSA levels in the cancerous tissue, and 2 patients had about the same levels of PSA in both tissue specimens. Analysis by the McNemar test indicated that the differences between the noncancerous and cancerous tissue were statistically significant ($P = 0.006$). Similar results were obtained by analysis of the data using the Wilcoxon signed rank sum test (Table I).

The distribution of hK2 concentrations in the noncancerous and cancerous tissue is shown in Figure 2A. The hK2 values ranged from 0 to 0.44 μg/mg protein in the noncancerous tissue (mean 0.19; 95% CI 0.18 to 0.20) and from 0 to 0.32 μg/mg protein in the cancerous tissue (mean 0.12; 95% CI 0.11 to 0.13). Figure 2B shows the paired values of hK2 in the noncancerous and cancerous tissue specimens for every patient. Nine of the 14 patients had higher hK2 levels in the noncancerous tissue, 2 patients had higher hK2 levels in the cancerous tissue, and 3 patients had about the same levels of hK2 in both tissue specimens. Analysis by the McNemar test indicated that the differences between the noncancerous and cancerous tissues were of borderline statistical significance ($P = 0.039$). Similar results were obtained by analysis of the data with the Wilcoxon signed rank sum test (Table I).

A positive correlation was found between the PSA and hK2 concentrations in both noncancerous (Spearman's rank correlation coefficient = 0.69, $P < 0.01$) and cancerous tissues (Spearman's rank correlation coefficient = 0.62, $P = 0.024$) (Fig. 3). However, one cancerous sample was excluded from the analysis because it was an outlier (Fig. 3B).

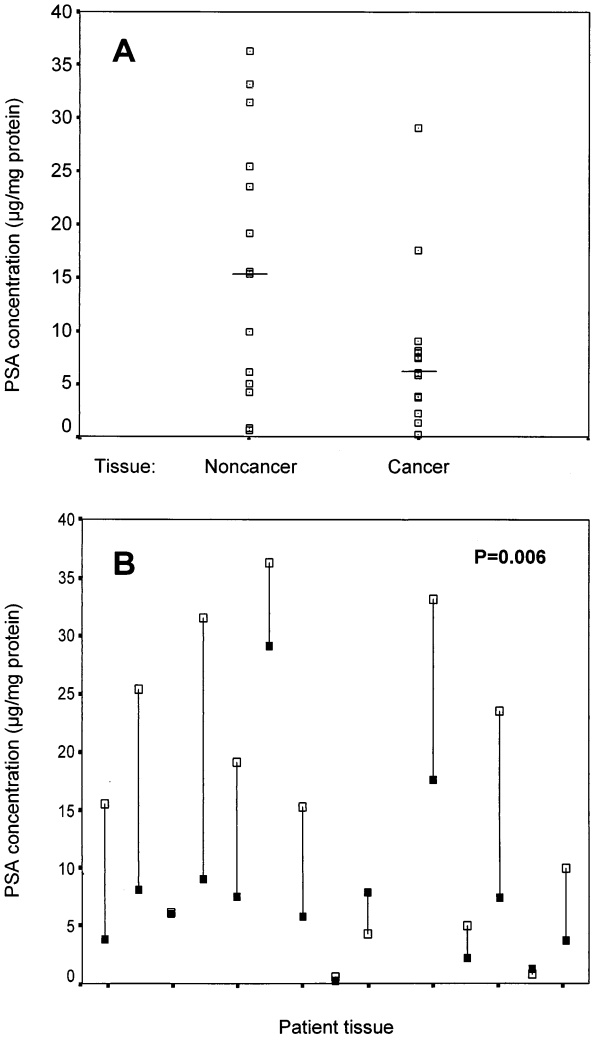

FIGURE 1. *(A) Distribution of PSA concentrations in noncancerous and cancerous prostatic tissue. The bold horizontal line represents the median. (B) Paired values of PSA concentrations in noncancerous (white squares) and cancer (black squares) tissue for every patient. The* P *value was calculated by the McNemar test.*

COMMENT

Pretlow *et al.*[5] were the first to use quantitative PSA analysis of benign and malignant prostatic tissues and monoclonal antibodies for immunohistochemical studies. They found that PSA is expressed at a lower level in PCa than in BPH, but a broad overlap existed between these two conditions. Similar results were reported by Qiu *et al.*,[7] who also investigated the distribution of cathepsin-D and pS2 in BPH and cancer specimens and attempted to

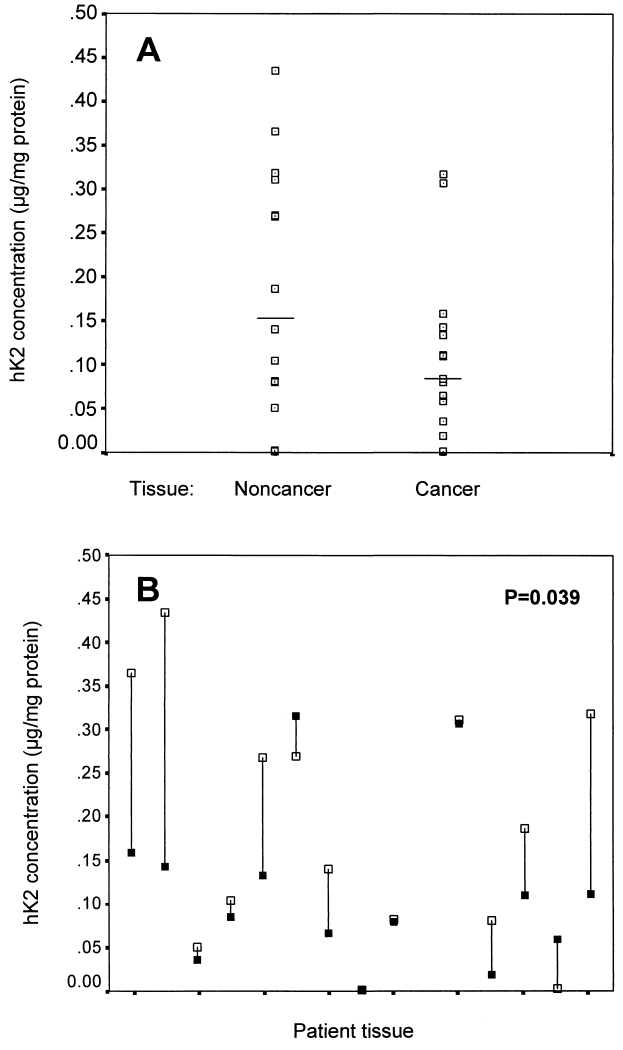

FIGURE 2. *(A) Distribution of hK2 concentrations in noncancerous and cancerous prostatic tissues. The bold horizontal line represents the median. (B) Paired values of hK2 concentrations in noncancerous (white squares) and cancerous (black squares) tissue for every patient. The* P *value was calculated by the McNemar test.*

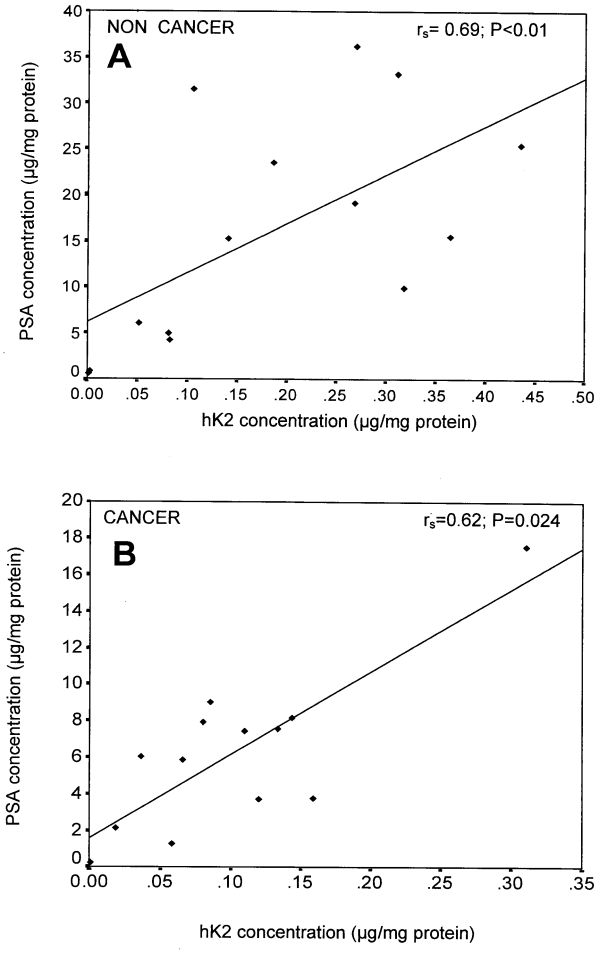

FIGURE 3. *Correlation between PSA and hK2 concentrations in (A) noncancerous and (B) cancerous prostatic tissue from 14 and 13 patients, respectively. Spearman's rank correlation coefficient (r_s) was used to determine the correlation between the two parameters.*

link their concentrations with the histopathologic tissue features, the PSA levels, and the androgenic status of the gland. PSA mRNA was found to be more abundant in BPH tissue than in adjacent cancerous tissue, within the same specimen, as shown by in situ hybridization. Furthermore, BPH tissue consistently gave more intense staining than cancerous tissue using immunohistochemistry.[3]

Recent findings[22,23] suggest that PSA may act as a tumor suppressor, a role that may well explain the reduced levels of PSA detected in PCa tissue. More specifically, Heidtmann *et al.*[23] have recently demonstrated that PSA is able to convert Lys-plasminogen to biologically active angiostatin-like fragments, which were able to inhibit the proliferation and tubular formation of human umbilical vein endothelial cells with the same efficacy as angiostatin (a potent inhibitor of angiogenesis, tumor growth, and metastasis).

Because of the many similarities between hK2 and PSA,[9] the former is regarded as a promising new serum marker for PCa. Apart from its identification in the serum of patients with PCa, recent studies have indicated that the hK2/free PSA ratio may supplement the role of PSA and percent free PSA, providing additional specificity for cancer detection, especially when the total PSA level is 2 to 4 µg/L.[14,15]

The concentration of hK2 protein in the prostate had been evaluated so far only by immunohistochemical techniques. Darson *et al.*[16] reported expression of hK2 and PSA in the benign and malignant prostate. They found that hK2 was expressed in a greater percentage of cells in adenocarcinoma than in benign tissue, with the most intense staining occurring in the highest grades of cancer. PSA

was also expressed in all cases, but the expression in cancer was less than in the benign epithelium. In a later study,[17] the same group evaluated the expression of hK2, pro-hK2, and PSA in benign and malignant prostate specimens and in lymph node metastases using immunohistochemistry. All samples tested displayed intense immunoreactivity for hK2 and pro-hK2; hK2 was again expressed in a greater percentage of cells in adenocarcinoma than in benign tissue.

In our study, we used highly sensitive immunoassays, specific for PSA and hK2, to measure the concentration of the two proteins in noncancerous and adjacent cancerous prostatic tissue within the same specimen. To our knowledge, this is the first report of the quantitative analysis of hK2 and PSA proteins in such samples. Our results clearly demonstrated that PSA is present in the prostatic extracts at about 50 to 100 times higher amounts than is hK2 (Table I). Similar differences in the concentration of the two analytes were seen in seminal plasma and male serum.[21,24,25] We found a good correlation between the PSA and hK2 values in the prostatic extracts (Fig. 3), in accord with data for serum[21,24,25] and nonprostatic fluids such as breast cyst fluid, breast milk, and nipple aspirate fluids.[26]

The findings that both PSA and hK2 concentrations correlate with each other and are present at higher concentrations in benign than in malignant prostatic tissue allow us to hypothesize that these two kallikreins may be regulated by similar mechanisms, a situation that appears to be true in breast carcinoma cell lines.[27,28] Although we had information on the tumor stage and Gleason score, we could not establish any statistically significant association between these parameters and either PSA or hK2 expression, because of the insufficient sample size ($n = 14$). Future studies should re-examine this question using more patients.

Our finding that hK2 is expressed at higher levels in benign tissue than in cancerous tissue is in contrast with previous data generated by immunohistochemistry studies.[16,17] Although the reason for the discrepancy is obscure, our data are strengthened because in the same extracts, we did confirm the well-known down-regulation of PSA, which, in this case, served as internal quality control parameter. Furthermore, the similar pattern of hormonal regulation between PSA and hK2[27,28] suggests that the factors that affect PSA expression in the prostate may, at the same time, affect hK2 expression in the same direction. We did however, see a lower percentage of down-regulation of hK2 than PSA (Table I). This difference may be responsible for the slightly higher increase in serum concentrations of hK2 (compared with PSA) evident in PCa compared with BPH.[15,29]

In conclusion, our results indicate that PSA and hK2 are expressed at higher levels in noncancerous than in cancerous prostatic tissue. The physiologic consequences and the mechanism of this down-regulation require further investigation.

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