Quantitative Analysis of Human Kallikrein 5 (KLK5) Expression in Prostate Needle Biopsies: An Independent Cancer Biomarker

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BACKGROUND: Kallikrein 5 (KLK5), a recently cloned member of the kallikrein family, codes for the secreted protein KLK5. Active KLK5 protein has a trypsin activity, and the expression of KLK5 gene seems to be regulated by steroid hormones. We performed an expression analysis and clinical evaluation of the KLK5 gene, at the mRNA level, in prostate needle biopsies.

METHODS: We examined KLK5 mRNA concentrations in 103 prostate tissue specimens. After testing of RNA quality, cDNA was prepared by reverse transcription. A highly sensitive quantitative real-time PCR (qRT-PCR) method for KLK5 mRNA quantification was developed using the SYBR Green chemistry. GAPDH was used as a housekeeping gene.

RESULTS: Specimens from patients with benign prostatic hyperplasia (BPH) showed higher levels of KLK5 mRNA expression than those from patients with prostate cancer (PCa) (P = 0.024). ROC analysis demonstrated that KLK5 expression had significant discriminatory value between BPH and PCa (AUC 0.64; P = 0.016). KLK5 mRNA expression showed a statistically significant negative correlation with the total PSA serum concentration in the PCa patients (P = 0.003). Early-stage tumors showed higher KLK5 expression than late-stage ones (P = 0.014), whereas KLK5 expression was negatively correlated to Gleason score (P = 0.005).

CONCLUSIONS: KLK5 mRNA, analyzed by quantitative PCR in prostate needle biopsies, could be an independent biomarker for the differential diagnosis and prognosis in prostate cancer.

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Prostate cancer (PCa) is the most common neoplasia in middle-aged men in the Western world and the second leading cause of cancer-related mortality, following lung cancer (1). In the US, new prostate cancer cases for 2008 have been estimated at about 218,890 (almost 29% of all cancer cases in men), with 27,050 deaths (2). Although statistics reveal its high morbidity and mortality, the etiology of prostate cancer remains generally unknown. Until now, the identified risk factors have been limited to age, race, and family history. Moreover, despite the fact that PCa is a hormone-mediated cancer, there has been little evidence that hormone concentrations may correlate with prostate cancer risk. Early diagnosis seems to be of great importance in the attempt to decrease the mortality of the disease (1). Thus, scientific efforts focus on the identification of improved tumor markers of high diagnostic specificity and sensitivity to make possible the successful screening and diagnosis of prostate cancer at an early stage.

The most established cancer biomarker is prostate-specific antigen (PSA), encoded by the kallikrein 3 gene (KLK3) (3–6). Nevertheless, PSA has limited ability to differentiate neoplastic events from noncancerous diseases of the prostate gland, such as benign prostatic hyperplasia (BPH) and prostatitis. Additionally, PSA shows low diagnostic sensitivity and specificity in early-stage cancer diagnosis (5, 6). Consequently, the demand for novel cancer biomarkers has increased.
grew larger, in spite of the fact that the discovery of different forms of serum PSA (i.e., free PSA) enhanced its specificity for PCa (5–7). Notable cancer biomarkers are believed to belong to the kallikrein family, since many KLKs seem to be related with prostate cancer progression (6–11).

Kallikreins are separated into 2 families, the tissue and plasma kallikreins. The 2 groups have nothing in common, apart from the ability to cleave kininogen (12). Human kallikrein-related peptidases are classified as a subgroup of secreted serine proteases within the S1 family of the clan PA. Of 178 human serine proteases, kallikrein-related peptidases form the largest contiguous cluster of protease genes in the human genome. Fifteen members of the human kallikrein gene family have been identified. The kallikrein locus lies in a genomic sequence of approximately 300 kb, which maps to the chromosomal region 19q13.4 (13). All genes are transcribed from telomere to centromere with the exception of KLK3 and KLK2 (3, 4).

Kallikrein mRNA transcripts encode for a single-chain serine protease preproenzyme. The signal peptide (16–30 amino acids) is cleaved from the N-terminus of the protein before secretion. Enzymatic activation is induced with the cleavage of short peptide sequences (prodomains) by other KLKs or other proteases. Pro-KLKs (except pro-KLK4) appear to be activated by cleavage after arginine or lysine residues. The KLK proteins show similar (approximately 40%–80%) amino acid sequences, in addition to the conserved protease catalytic triad residues (His, Asp, and Ser) (3).

KLKs are expressed at both mRNA and protein levels in a variety of cell types and tissues, displaying diverse physiological functions enforced through regulated proteolytic cascades. Kallikrein expression is believed to be modulated through hormonal and/or epigenetic factors (e.g., methylation, histone modification). Strong evidence suggests that the regulation of KLKs is mediated by steroid hormones (3, 4). All KLKs exhibit differential expression at the mRNA and protein levels in many cases of cancer, and thus their future as candidate cancer biomarkers seems very promising (11).

Kallikrein 5 (KLK5) is a newly discovered member of the kallikrein gene family, formerly known as kallikrein-like gene 2 (KLK-L2) (14) or human stratum corneum tryptic enzyme (SCTE) (15). KLK5 is flanked in the kallikrein locus by 2 other kallikrein genes, KLK4 and KLK6. KLK5 codes for the secreted protease KLK5, which consists of 293 amino acids and is synthesized as a preproenzyme. Enzyme activation requires cleavage of an arginine residue (Arg66–Ile67). The active KLK5 protein has a trypsin activity, and KLK5 seems to be regulated by steroid hormones (14, 15). KLK5 may be involved in a proteolytic cascade in seminal plasma along with kallikreins KLK2 and KLK3 (16). There is extensive evidence that KLK3 and KLK2 importantly contribute to the proteolytic activity in seminal fluid, cleavage of SEMG1 and SEMG2, and the consequential liquefaction of the ejaculate (17). The involvement of KLK5 in skin desquamation and skin physiology has also been established. In fact, the uncontrolled activation of KLK5, along with KLK7 and KLK14, is suspected to be the major cause of overdesquamation in a range of skin disorders (18–20).

KLK5 mRNA and/or protein are highly expressed in skin, breast, ovary, testis, and salivary gland. Lower expression is found in the prostate and the central nervous system (3, 4). KLK5 has been detected in biological fluids with the highest concentrations mainly present in breast milk, breast cyst fluid, and ovarian cancer ascites, whereas lower concentrations were measured in seminal plasma, follicular fluid, breast cancer cytosol, amniotic fluid, saliva, cerebrospinal fluid, and urine (16). Recent studies reveal that KLK5 is differentially expressed in both hormone-dependent malignancies, such as breast, ovarian, prostate, and testicular tumors, and hormone-independent cancers, such as lung and bladder carcinomas (21–28). In the present study, we analyzed KLK5 mRNA expression in cancerous and hyperplastic prostate tissue needle biopsies, followed by extensive biostatistical analysis to evaluate the expression of KLK5 gene as a potential diagnostic and prognostic tool.

Materials and Methods

Patients

We obtained 103 prostate tissue specimens from patients who had undergone transrectal ultrasound–guided needle biopsy at the Department of Urology, Attikon University Hospital, Athens. Sample collection took place from February 2002 to July 2005. The selection criteria for the specimens included the availability of sufficient tissue mass for extraction and assay. The patients represented approximately 60% of new cases of prostate cancer diagnosed and treated at the above institution during the accrual period. We recognized that the characterization of the analyzed prostate cancerous tissues may have had a relative risk due to the fact that it was unfeasible to have histologically examined the same prostate biopsy core and at the same time to have extracted total RNA from it. To cope with such a complication, urologists, throughout the 12-core transrectal ultrasound–guided biopsy scheme, removed 2 samples from the same suspected area. When the first sample was confirmed as positive for prostate cancer, according to the pathologist’s report, the second sample, used for further analysis, was defined as a prostate cancerous sample as well. If the diagnosis was
negative for cancer and the patients did not display any clinical signs for PCa 3 years after biopsy, the frozen sample, if available, was considered a BPH sample and characterized as prostate tissue deemed to contain no cancer.

Patients had not received any hormonal therapy before surgical treatment. Biopsy samples were frozen in liquid nitrogen immediately after resection and were preserved at −80 °C. Investigations were carried out in accordance with the ethical standards of the Helsinki Declaration II.

We classified cancerous samples according to the tumor/node/metastasis system. Of the 53 PCa patients, 31 (58.5%) were diagnosed with early-stage cancer (T1/T2) and 20 (37.8%) with advanced-stage cancer (T3/T4). Staging remained undetermined for 2 patients. Grade was evaluated by the Gleason scoring system: 16 (30%) patients had a Gleason’s score =6, 34 (64.2%) >6, and 3 (5.7%) unknown.

HUMAN PROSTATE CANCER CELL LINE
The human prostate cancer cell line PC3 was maintained in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 kU/L penicillin, 0.1 g/L streptomycin, 0.3 g/L 1-glutamine, and 0.85 g/L NaHCO3, in a humidified atmosphere containing 5% CO2 at 37 °C. Cells were grown as a monolayer in 75-cm2 tissue culture flasks.

TOTAL RNA EXTRACTION AND cDNA SYNTHESIS
Frozen benign and malignant tissue specimens were pulverized with a scalpel on dry ice. We extracted total RNA using TRI-Reagent (Ambion), following the manufacturer’s instructions. We assessed the concentration and purity of total RNA spectrophotometrically at 260 and 280 nm. Total RNA (1 μg) was reverse transcribed into first-strand cDNA in a 20 μL reaction volume, using M-MuLV Reverse Transcriptase RNase H– (Finnzymes).

QUANTITATIVE REAL-TIME PCR
We designed and synthesized 2 oligonucleotide primers, based on the published genomic sequence of KLK5 (GenBank accession AF135028): 5’-CCC CAA GTG CAC TTC CCT AA-3’ and 5’-TCG TGT AGA CAC CCG GTG TG-3’. Primers were specific for the mRNA molecules that code for the KLK5 active isoform and were designed to prevent contamination by genomic DNA.

Real-time PCR reaction was carried out on an ABI 7500 thermal cycler (Applied Biosystems), using the SYBR®-Green I chemistry. The quantification was performed in MicroAmp® Optical 96-Well Reaction Plates (Applied Biosystems). Detection of PCR products was accomplished by measuring the emitting fluorescence (Rn) at the end of each reaction step (reaction cycles). Threshold cycle (Ct) corresponds with the cycle number required to detect a fluorescence signal above the baseline. Calculations were done by the Sequence Detection System 1.2.3. software provided by the manufacturer (Applied Biosystems).

We performed gene expression analysis using the comparative (2−ΔΔCt) Ct method. In accordance with the method, the mRNA amounts of a target gene were normalized to an endogenous control and relatively to a calibrator. We used the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control and the PC3 cell line as a calibrator.

We performed a validation experiment to verify that gene amplification was conducted with approximately equal efficiency for both target and reference genes. Different cDNA amounts (from 100 ng to 0.01 ng) were prepared from total RNA extracted from PC3 prostate cancer cells. The normalized (2−ΔΔCt) amounts of KLK5 mRNA amounts were multiplied with the average ratio of KLK5 mRNA copies/GAPDH mRNA copies of PC3 cells, thus leading to comparable results that did not depend on the KLK5 gene expression by the PC3 cells.

AMPLIFICATION PROCESS
For each assay, we prepared a reaction mixture containing 0.4 μL cDNA, 5 μL Power SYBR-Green PCR Master Mix (2×) (Applied Biosystems), and 1 μL of each primer (75 nM) on ice and added H2O to a final volume of 10 μL. The reaction conditions included an initial step at 95 °C for 10 min (AmpliTaq Gold DNA Polymerase activation), followed by 40 cycles at 95 °C for 15 s (Melt) and 60 °C for 1 min (Anneal/Extend). Each sample was analyzed in duplicate in the PCR reaction, to estimate the reproducibility of data (Fig. 1A).

MELTING CURVE ANALYSIS
A dissociation curve analysis followed the amplification reaction for distinguishing specific from nonspecific products and/or primer-dimers. Dissociation curves display the melting temperature (Tm) of the amplicons, making possible the discrimination, since nonspecific products and/or primer-dimers correspond with lower Tm values in comparison with the specific products.

STATISTICAL ANALYSIS
Because the distribution of KLK5 expression in the BPH and PCa patients was not gaussian, the analyses of the differences between these parameters in the 2 groups were conducted with the nonparametric Mann–Whitney U-test. We constructed ROC curves for both KLK5 expression and total PSA (tPSA) con-
centration by plotting sensitivity vs 1 − specificity. The ROC curves evaluated the potential of the 2 variables to distinguish the hyperplastic prostate specimens from the cancerous ones. The areas under the curve (AUCs) were analyzed by the Hanley and McNeil method (29). The ability of the variables to predict presence of prostate cancer was studied using univariate and multivariate unconditional logistic regression analysis.

We determined the relationship between KLK5 mRNA status and qualitative variables, such as cancer stage and Gleason score, using χ² and Fisher exact test where appropriate. Any possible correlations between KLK5 expression and other continuous variables were assessed by Spearman correlation coefficient (rᵔ). A P value <0.05 was considered statistically significant.

Results

RELATIVE QUANTIFICATION OF KLK5 mRNA EXPRESSION IN PROSTATIC TISSUES

We performed validation experiments with different total RNA amounts for the target and reference genes (Fig. 1B). The results indicated an approximately equal efficiency for the KLK5 ($R^{2} = 0.9724$) and GAPDH ($R^{2} = 0.9914$) genes.

KLK5 expression in the BPH samples ranged from 0.04 to 1498.2 KLK5 mRNA copies/GAPDH mRNA copies (c/Kc) with a mean (SE) of 251.3 (56.3) c/Kc and a median of 54.6; in the PCa samples, KLK5 expression varied from 0.03 to 600.0 c/Kc, with a mean (SE) of 53.7 (56.3) c/Kc and a median of 3.50 (Table 1). The results...
were indicative of a significant increase \((P = 0.024)\) in the amounts of KLK5 mRNA for the hyperplastic specimens. The difference of the means was also found to be statistically significant, using Student \(t\)-test \((P < 0.001)\). Particularly, gene expression in the benign samples was much higher compared to the cancerous ones in all cases apart from the 10th percentile. The distribution of KLK5 expression in the 2 groups is presented in Fig. 2A.

**EVALUATION OF KLK5 mRNA EXPRESSION IN THE DIFFERENTIAL DIAGNOSIS OF PCa AND BPH**

We performed ROC analyses to show the potential of KLK5 expression for discriminating prostate cancer and benign prostatic hyperplasia. KLK5 expression \((AUC = 0.64; 95\% CI 0.53–0.75, P = 0.016)\) was found to have significant discriminatory value in the whole patient population (Fig. 2B).

We developed univariate logistic regression models to evaluate the value of KLK5 for discriminating between BPH and PCa (Table 2). These regression models demonstrated that patients with low amounts of KLK5 transcripts were at increased risk for having prostate cancer (crude odds ratio 0.66, 95\% CI 0.48–0.92, \(P = 0.014)\). In the multivariate analysis, the logistic regression models were adjusted for total PSA concentration in serum. Total PSA proved to be a significant factor for discriminating between BPH and PCa patients (crude odds ratio 1.18, 95\% CI 1.06–1.32, \(P = 0.003)\). KLK5 significantly added to the prognostic power of this multivariate model (crude odds ratio 0.69, 95\% CI 0.48–0.98, \(P = 0.041)\), indicating that KLK5 is an independent factor for the differential diagnosis of prostate cancer, improving the diagnostic significance of PSA.

To further investigate the discriminatory value of KLK5 expression in relation to PSA, we developed another logistic regression model [function combination \((FC) = -0.366 \times \log KLK5 + 0.16 \times PSA - 0.723\) and adjusted only for these 2 variables. We calculated log likelihood scores for this multivariate logistic regression model, which incorporates both of the variables, KLK5 and PSA, for each patient. For these data, the crude odds ratio, 95\% CI, and AUC were found to be 2.79, 1.58–4.92 and 0.72, respectively (Fig. 2B).

**RELATIONSHIP OF KLK5 EXPRESSION WITH OTHER CLINICOPATHOLOGICAL FEATURES**

KLK5 positivity was found more frequently in low Gleason score tumors \((P = 0.005)\) as well as in early-stage patients \((P = 0.014)\). In this analysis, we classified KLK5 values into 2 categories \((KLK5-positive and KLK5-negative)\) and analyzed associations between KLK5 status and other qualitative variables using the \(\chi^2\) and Fisher exact tests, where appropriate. We used a cutoff point equal to a median of 3.50 c/Kc (Table 3).

In particular, early-stage patients (T1 and T2) presented higher KLK5 mRNA expression \((KLK5-positive: 80.0\% and 50.0\%, respectively)\) than the advanced-stage patients \((KLK5-positive: 30.0\%)\). Thirteen patients (81.3\%) of those with a Gleason score ≤6 and only 12 (35.3\%) of those with score >6 were KLK5 positive. We found a statistically significant negative correlation between KLK5 expression levels and Gleason score using these parameters as continuous variables \((r_s = -0.412, P = 0.001)\). Significant correlation between KLK5 expression levels and patient age was not observed.

The correlation between KLK5 mRNA expression in prostate biopsies and serum tPSA concentration is shown in Fig. 3. A statistically significant negative correlation for the 2 variables \((r_s = -0.41; P = 0.003)\) appeared only for the PCa population (Fig. 3A). BPH patients showed no statistically significant correlation between KLK5 expression and serum tPSA \((r_s = 0.22, P = 0.14)\) (Fig. 3B).

Table 1. Descriptive statistics of continuous variables in serum of BPH and PCa patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BPH (n = 50)</th>
<th>PCa (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLK5, c/Kc</td>
<td>251.3 (56.3)</td>
<td>53.7 (18.2)</td>
</tr>
<tr>
<td>PSA, ng/mL</td>
<td>5.75 (0.54)</td>
<td>9.33 (0.76)</td>
</tr>
<tr>
<td>Age, years</td>
<td>69.5 (0.88)</td>
<td>65.1 (0.77)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentile</th>
<th>10th</th>
<th>25th</th>
<th>50th (median)</th>
<th>75th</th>
<th>90th</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLK5, c/Kc</td>
<td>0.14</td>
<td>1.19</td>
<td>54.6</td>
<td>390.4</td>
<td>940.0</td>
</tr>
<tr>
<td>PSA, ng/mL</td>
<td>1.20</td>
<td>2.41</td>
<td>4.91</td>
<td>7.92</td>
<td>11.5</td>
</tr>
<tr>
<td>Age, years</td>
<td>60.0</td>
<td>65.0</td>
<td>70.0</td>
<td>74.2</td>
<td>80.0</td>
</tr>
</tbody>
</table>
Prostate cancer is one of the leading causes of cancer-related deaths worldwide. Several kallikreins have turned out to be either down- or upregulated in prostate cancer compared to normal prostate tissue, which indicates their possible diagnostic and prognostic utilities (4). Kallikrein 5 (KLK5) is a novel kallikrein gene located between KLK4 and KLK6 in the kallikrein gene locus. KLK5 is expressed in various types of tissues.
holding a predominant role in skin physiology, along with KLK7. Lately, the differential expression of KLK5 in hormone-regulated cancers, at mRNA and/or protein levels, has been shown (22–27). On the other hand, KLK5 expression has also been detected in hormone-independent cancers, such as lung cancer (28).

According to our data, KLK5 mRNA was detected in both BPH and PCa tissue specimens. The amounts of gene expression were higher in hyperplastic tissues than in malignant ones (Table 1 and Fig. 2A). The observed expression pattern of KLK5 in the PCa and BPH populations affected the evaluation of the gene’s differential diagnosis potential. Regarding the ROC curve and logistic regression analysis, KLK5 showed significant potential as an independent factor in the discrimination of the 2 prostate abnormalities, in parallel with tPSA serum concentration. These observations were reinforced by the calculated negative Spearman correlation coefficient ($r_s$) for the KLK5 mRNA and serum tPSA concentrations in PCa cohort of patients (Fig. 3A). Prostate cancer is a well-known androgen-dependent malignancy. Because the expression of KLK5 seems to be downregulated by androgens, a great deal of our results could be explained this way. In addition, prostate cancer is related to the destruction of glandular architecture, and as KLKs are primarily expressed in or localized to (glandular) epithelium, the KLK proteins could flow into the blood circulation. This could explain the increased serum tPSA concentrations in PCa patients, in contrast with the lower tissue tPSA concentration, and also their negative correlation with KLK5 mRNA copies depicted in our results (30).

It is well known that mRNA measurement of the most common kallikrein-related peptidases, such as KLK3 and KLK2, in patients suffering from prostate cancer holds little potential in the discrimination of cancerous from the benign samples. However, these 2 genes, regulated at the transcriptional level by the androgen receptor, appear to correlate with other parameters of the disease and acquire an alternative clinical utility. On the other hand, KLK11 and KLK15 mRNA expression was found to have a statistically significant discriminatory value between PCa and BPH tissues (8, 31, 32).

Apart from applications in differential diagnosis, KLK5 expression was investigated for any possible prognostic value through correlations with the clinical and pathological characteristics of patients (Table 3). KLK5 expression appeared remarkably low in the late stages of the disease, compared to the earlier stages. Moreover, patients with higher Gleason scores (>6) and thus less differentiated cancer cells displayed lower KLK5 expression. There is now growing evidence that many kallikreins are related to cancer progression. PSA (KLK3) is the most established biomarker for diagnosis and management of prostate cancer (3–6), KLK15 and KLK2 were also found to have prognostic significance in prostate cancer (6, 10, 27). PSA was found to be a favorable prognostic factor for breast cancer (11). KLK13 is reported to be downregulated in breast cancer (33, 34), and KLK8 was shown to be differentially

### Table 2. Logistic regression analysis in 50 BPH and 53 PCa patients for predicting the presence of prostate cancer.

<table>
<thead>
<tr>
<th>Covariant</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude odds ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Log10 KLK5</td>
<td>0.66 0.48–0.92</td>
<td>0.014</td>
</tr>
<tr>
<td>PSA</td>
<td>1.16 1.07–1.26</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Test for trend.

### Table 3. Relationship between KLK5 expression status, patient stage, and Gleason score.

<table>
<thead>
<tr>
<th>n (%)</th>
<th>n KLK5-negative*</th>
<th>KLK5-positive*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>15 3 (20.0)</td>
<td>12 (80.0)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>16 8 (50.0)</td>
<td>8 (50.0)</td>
<td>0.014b</td>
</tr>
<tr>
<td>T3/T4</td>
<td>20 14 (70.0)</td>
<td>6 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6</td>
<td>16 3 (18.8)</td>
<td>13 (81.3)</td>
<td>0.005c</td>
</tr>
<tr>
<td>&gt;6</td>
<td>34 22 (64.7)</td>
<td>12 (35.3)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cutoff = 3.50 cKc, equal to median.  
* $x^2$ test.  
* Fisher exact test.
expressed in breast and ovarian cancer patients (35, 36). The finding that a protease gene like KLK5 is a favorable prognostic tumor marker is not new. Other proteases (e.g., pepsinogen C, PSA, KLK8, KLK13) have also been associated with favorable prognosis in different malignancies (37–40).

Fig. 3. Correlation between KLK5 mRNA levels in prostate needle biopsies and serum tPSA concentration in BPH (A) and PCA (B) patients. 

\( r_s = 0.222 \quad P = 0.14 \)

\( r_s = -0.41 \quad P = 0.003 \)
Our data propose KLK5 as a potential novel molecular biomarker for the discrimination between PCa and BPH in needle biopsies. Additionally, KLK5 could be used as a favorable marker in prostate cancer prognosis. Future directions should aim at the evaluation of KLK5 protein concentrations in hyperplastic and malignant prostate tissues using immunohistochemical and different types of immunochemical assays. Our findings would be undoubtedly reinforced if the prostate specimens were tested for the expression of certain genes, including PCA3, CRISP3, and HEPSIN, that are known to be upregulated in cancerous samples.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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