Prostate cancer antigen-1 as a potential novel marker for prostate cancer

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Abstract

Aim: To examine the expression of prostate cancer antigen-1 (PCA-1) in prostate cancer (PCa) and to validate it as a potential marker for diagnosis of PCa.

Methods: In situ hybridization analysis of PCA-1 mRNA expression was performed on 40 benign prostate hyperplasia (BPH), 16 high-grade prostatic intraepithelial neoplasm (HG-PIN), 74 PCa and 34 other malignant carcinoma specimens. The level of PCA-1 expression was semiquantitatively scored by assessing both the percentage and intensity of PCA-1 positive staining cells in the specimens. We then compared the PCA-1 expression between BPH, HG-PIN and PCa and evaluated the correlation of PCA-1 expression level with clinical parameters of PCa.

Results: PCA-1 mRNA was expressed in the majority of both PCa and HG-PIN specimens but not in BPH and other malignant carcinoma. The expression level of PCA-1 increased along with a high Gleason score (P < 0.05), and was unrelated to other clinical parameters of PCa (all P > 0.05).

Conclusion: The data suggest that PCA-1 might be a novel diagnostic marker for PCa, and that increased PCA-1 expression might denote more aggressive variants of PCa. (Asian J Androl 2007 Nov; 9: 821–826)

Keywords: marker; prostate cancer; prostate cancer antigen-1

1 Introduction

Prostate cancer (PCa) is the most common malignancy in men in the United States and is becoming an increasingly common cancer in China. PCa carcinogenesis is related to several genetic changes. Previous studies have identified several genes that are overexpressed or underexpressed in PCa, but few specific molecular markers for PCa have been found [1, 2]. Prostate specific antigen (PSA) has been widely used as a diagnostic marker for PCa. However, elevated levels of PSA can also be found in benign prostate hyperplasia (BPH) and prostatitis [3, 4]. Therefore, there is an urgent need for
Prostate cancer antigen-1 overexpression

One or more sensitive and specific markers that can detect PCa in its early stages.

Konishi et al. [5] reported that prostate cancer antigen-1 (PCA-1) is predominantly prostate specific, and found that PCA-1 is overexpressed in PCa [5]. We further investigated the status of PCA-1 mRNA expression in different prostate samples and identify it as a potential diagnostic marker for PCa by using in situ hybridization (ISH). Moreover, we evaluated the possible correlation of PCA-1 expression level with clinical parameters of PCa.

2 Materials and methods

2.1 Tissue samples

In the present study, all the prostate tissue specimens were obtained from 130 patients of 55–78 years old at the department of Urology of the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China). They were undergoing biopsies, transuretheral resections of the prostate or open prostatectomy. None of the patients had received preoperative hormonal therapy, chemotherapy and actinotherapy. The patients were classified into 40 cases of BPH, 16 cases of high-grade prostatic intraepithelial neoplasm (HG-PIN) and 74 cases of primary PCa. In addition, there were other malignant carcinomas, including 5 cases of thyroid cancer, 9 cases of bladder cancer, 4 cases of kidney cancer, 6 cases of colon cancer, 5 cases of liver cancer and 5 cases of breast cancer. Each tissue sample was treated with 4% paraformaldehyde/0.1 mol/L phosphate-buffered saline (PBS) pH 7.4 in 0.1% DEPC for 1 h for ISH analysis. All the used samples were analyzed by two experienced urological pathologists to ensure that the samples contained predominantly epithelial cells (in BPH samples) or carcinoma cells (in PCa samples). This project was approved by the Research Ethics Committee of Zhengzhou University.

2.2 Preparation of cRNA probes

Total RNA of PCa tissue was isolated by using a total RNA isolation kit (Promega, Madison, USA). A sample of 2 µg total RNA was used in a reverse transcription reaction. Primers set for PCA-1 were: sense, 5'-GGA TCC TTT ATC GCA ATG AGA AGG-3' (BamHI site underlined); antisense, 5'-AAG GTT TGT CCG AAA GGT CAG GTT-3' (HindIII site underlined). Polymerase chain reaction (PCR) protocol (94°C 30 s, 50°C 20 s, 72°C 30 s) was run for 30 cycles and PCR product was approximately 104 bp. PCR product was separated by electrophoresis, recovered with a gel extraction kit (Omega, Doraville, USA) and ligated with pGEM-T vector. The ligate was transformed into competent Escherichia coli JM 109 cells. The correct transformant was identified by restriction enzyme analysis. Large quantities of plasmid were then harvested with a Plasmid Extraction Kit (Promega, Madison, USA). After linearization by NcoI restriction enzyme, SP6 promoter was used to drive the transcription of PCA-1-cRNA probes in vitro, and in this system Biotin labeled rUTP was introduced into PCA-1-cRNA probes. In the same way, after linearization by NotI restriction enzyme, T7 promoter was used to drive the transcription of sense PCA-1-cRNA probes in vitro, which acted as the negative control.

2.3 In situ hybridization

Five µm sections were deparaffinized in xylene (7.5 min, twice) and dehydrated in graded ethanol, then digested in pepsin solution (4 mg/mL in 3% citric acid) for 20 min at 37°C, followed by rinsing in PBS at room temperature, and processing for ISH. Digoxigenin-labeled human PCA-1 RNA probes were hybridized to the sections at 48°C overnight, under glass coverslips specific for ISH. The highest stringent post-hybridization washes were 37°C in 2 × standard saline citrate (SSC) for 10 min, 0.5 × SSC for 15 min and 0.2 × SSC for 30 min. Subsequently, the slides were incubated with biotinylated mouse anti-digoxigenin antibody at 37.5°C for 1 h followed by washing in 1 × PBS for 20 min at room temperature, and then with strepavidin-peroxidase at 37.5°C for 20 min followed by washing in 1 × PBS for 20 min at room temperature. Subsequently, a drop of 3,3'-diaminobenzidine was added and color was developed. Counterstaining was performed using hematoxylin to localize the hybridization signals. All slides were hybridized with PBS or the sense probe to substitute for the antisense probe as a negative control.

2.4 Scoring methods

The intensity of PCA-1 expression was graded on a scale of 0–3, with 3 being the highest expression observed (0, no staining; 1, mildly intense; 2, moderately intense; 3, severely intense). The staining density was quantified as the percentage of cells staining positive for PCA-1, as follows: 0, no staining; 1, < 20% of tumor cells were positive; 2, > 20% but < 50% of tumor cells were positive; 3, ≥ 50% of tumor cells were positive.
were positive; 3, > 50% of tumor cells were positive. Intensity score was multiplied by the density score to give an overall score of 0–9 [6]. The overall score for each specimen was then categorically assigned to one of the following groups: 0–1 scores, negative expression (−); 2–3 scores, weak expression (+); 4–9 scores, strong expression (++).

2.5 Statistical analysis
PCA-1 mRNA expression in HG-PIN and PCa tissues were compared by using the $\chi^2$-test. The correlation between PCA-1 expression and clinical parameters of PCa were also calculated by using the $\chi^2$-test. For all analyses, $P < 0.05$ was considered statistically significant.

3 Results

3.1 Gain of PCA-1 cDNA fragment
We successfully cloned a 104-bp fragment by reverse transcription-PCR, then the fragment was ligated with pGEM-T vector and the recombinant vector was named pGEM-T-PCA. pGEM-T-PCA and was digested by BamH I and HindIII enzymes, yielding a 3.0-kb fragment of vector and a 104-bp fragment of PCA-1 cDNA (Figure 1). We confirmed PCA-1 cDNA by sequencing, which was completely identical to the sequence in Genbank. Sequencing revealed that no base mutation occurred in the sequence of our cloned PCA-1 cDNA fragment.

3.2 PCA-1 expression in different specimens
We found that PCA-1 expression was positive in 82.4% (61 of 74) of PCa samples. Positive staining was confined to cancerous cells in PCa and was not seen either in adjacent normal cells or in BPH. Interestingly, of the 16 HG-PIN found in this series of prostate specimens, 62.5% (10 of 16) of the HG-PIN samples were found to be positive for PCA-1 staining (Table 1). PCA-1 staining was most often exhibited by atypical epithelia in HG-PIN (Figure 2). Little or no PCA-1 protein expression was observed in samples of thyroid cancer, bladder cancer, kidney cancer, colon cancer, liver cancer and breast cancer, suggesting that expression of PCA-1 is specific to PCa.

3.3 Correlation of PCA-1 expression with clinical parameters of PCa
The relationship between PCA-1 staining and clinical

<table>
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<tr>
<th>Prostate tissue</th>
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<th>Intensity × density</th>
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<th>P</th>
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<td>6</td>
<td>3.138</td>
<td>0.076a</td>
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<tr>
<td>PCa</td>
<td>74</td>
<td>13</td>
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Table 1. Comparisons of prostate cancer antigen-1 (PCA-1) expression between high-grade prostatic intraepithelial neoplasm (HG-PIN) and prostate cancer (PCa) by $\chi^2$-test. *$P > 0.05$. N, number of samples.

Figure 1. Molecular cloning of prostate cancer antigen-1 (PCA-1) cDNA fragment. pGEM-T-PCA vector was digested by BamH I and HindIII enzymes, yielding a 3.0-kb fragment of vector and a 104-bp fragment of PCA-1 cDNA: 1, DL2000 marker; 2, pGEM-T vector and fragment of PCA-1 cDNA.

4 Discussion
Alkylation is one of the mechanisms by which nucleic
Table 2. The correlation of prostate cancer antigen-1 (PCA-1) expression and clinical parameters of prostate cancer (PCa) by $\chi^2$-test.

$^aP > 0.05$, $^bP < 0.05$. N, number of samples; PSA, prostate specific antigen.

<table>
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<td>Volume (mL)</td>
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<td>16</td>
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Figure 2. In situ hybridization (ISH) staining of prostate cancer antigen-1 (PCA-1) in different prostate tissue (brown denotes positive). Benign prostate hyperplasia (BPH) glands show no positive staining in secretory cells (A, scale bar = 50 μm; × 200); PCA-1 ISH staining are cytoplasmic in some atypical glandular epithelia within high-grade prostatic intraepithelial neoplasm (HG-PIN) (B, scale bar = 25 μm; × 400) and in cancer cells within moderately and poorly differentiated PCa (C and D, scale bar = 25 μm; × 400).
acids can be altered. Alkylating agents are ubiquitous in our environment and are endogenously produced during cellular metabolism. DNA alkylation can induce mutation, inhibit replication, and is implicated in carcinogenesis [7]. This harmful modification is repaired by demethylation. DNA alkylation damage repair mechanisms are best studied in E. coli and be controlled by six genes [8]. The AlkB gene of these genes can be induced upon exposure to a sublethal dose of alkylating agents, called the adaptive response. The AlkB protein can evidently repair both ssDNA and RNA modifications generated by S-shaped methylating reagents [9, 10]. Damaged repair mechanisms would lead to carcinogenesis. Konishi N et al. [5] identify a new gene, PCA-1, which has high homology to E. coli alkB. PCA-1 cDNA transfection partially reversed the cell death of COS-7 cells exposed to an S-shaped alkylating agent, methylmethane sulfonate. Therefore, we hypothesize that PCA-1 might play a role in PCa tumorigenesis, and might serve as a marker for PCa diagnosis.

We successfully cloned a 104-bp fragment of PCA-1. The full length of PCA-1-cDNA was 1 520 bp and the cloned fragment was located in the 5′-terminal of the full length. The fragment had high specificity and nonhomology with other genes by BLAST analysis, so it was equal to probes in the screening of gene expression.

To further evaluate the finding that PCA-1 was overexpressed in PCa samples, we performed ISH analysis. The studies revealed that the positive expression rate of PCA-1 is 82.4% in PCa samples, but that there is no expression in BPH and other carcinoma tissues, suggesting that expression of PCA-1 is specific to PCa. It is well known that HG-PIN is generally accepted to be a premalignant lesion [11]. Characterization of precursor lesions is very important both for clinical diagnosis and management of PCa. Therefore, the identification of a new marker to detect the premalignant lesion is invaluable. Interestingly, the positive expression rate of PCA-1 is 62.5% in HG-PIN, and only expressed in atypical cells. These findings suggest that PCA-1 expression might be related to neoplastic transformation in the prostate. We found no significant correlation between PCA-1 expression and age, tumor volume, PSA, osseous metastasis or tumor stage. Therefore, we suggested that PCA-1 is as a sensitive and specific marker for detecting and monitoring prostate neoplasias.

We found significant correlation between PCA-1 expression and Gleason scores, and determined that an elevated level of PCA-1 expression correlates with high grade. It is suggested that PCA-1 overexpression might be an adverse predictor for clinical progression of PCa. It is well known that the biological behavior of PCa varies widely. The clinical discrepancy might be a consequence of heterogeneity of gene expression [12, 13]. The different levels of PCA-1 expression might reflect this characteristic heterogeneity in PCa. From our results, it is suggested that PCA-1 as a new marker might have a number of potential uses in the progression and prognosis of human PCa.

The cause of PCA-1 overexpression in PCa is not known. However, as a DNA repair enzyme, variant levels of PCA-1 expression among different prostate tissues might be related to various types and degrees of carcinogen exposure.

In summary, we have shown in this study that PCA-1 is overexpressed in a majority of PCa as well as HG-PIN, which correlates positively with tumor grade. Although more studies are needed to determine the role of PCA-1 in human cells and the relationship between prostate carcinogenesis and overexpression, the present results suggest that PCA-1 might be a promising molecular marker for the clinical prognosis of human PCa and a specific target for diagnosis of human PCa.

References

9. 10.
11 Sofikerim M, Tatlisun A, Karacagil M. Do all patients with high-grade prostatic intraepithelial neoplasia on initial pros-

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