The auxiliary diagnostic value of prostate-specific antigen and α-methylacyl-CoA racemase in prostate cancer

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Abstract. Prostate cancer (PCa) is one of the most common types of malignant tumor, which places a major burden on the health of men, worldwide. A prerequisite to ensure good treatment outcomes for patients with PCa is an accurate diagnosis. The present study aimed to investigate the diagnostic value of prostate-specific antigen (PSA) and α-methylacyl-CoA racemase (P504S) in PCa, using the tumor-associated immunolabels. In total, clinical data was collected from 125 patients undergoing prostate biopsy or surgery between January 2015 and September 2019, and stratified into: PCa (45), benign prostatic hyperplasia (BPH) (60) and unconfirmed diagnosis (20). Immunohistochemistry analysis was performed to assess PSA and P504S expression levels in each group compared with that in the controls (the normal tissue in each group was the internal control). The results demonstrated that the expression level of P504S was significantly higher in the PCa group compared with that in the BPH group. Furthermore, no significant association was observed in the PCa group between PSA and P504S expression levels, and the Gleason grading groups. A total of 20 unconfirmed diagnoses was verified via PSA/P504S. Taken together, the results suggest that combination PSA and P504S have a positive effect in identifying prostate cancer. However, PSA and P504S still have limitations in their diagnosis and the final results need to be carefully and comprehensively analyzed, thus further studies are required to determine their diagnostic values.

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Abbreviations: PSA, prostate-specific antigen; P504S, α-methylacyl-CoA racemase; BPH, benign prostatic hyperplasia; PCa, prostate cancer

Key words: prostate cancer, PSA, P504S

Introduction

Prostate cancer (PCa) is one of the most common types of tumor and its incidence rate has increased in recent years worldwide. In the United States, >170,000 new prostate cancer cases were reported in 2019, and the number of new prostate cancer cases in China had exceeded 60,000 in 2015 (1-3). Notably, early detection, diagnosis and treatment can result in improved patient outcomes, such as improving the 5-year survival rate (4). The early clinical symptoms of PCa are difficult to detect, and the sensitivity of the serum marker prostate-specific antigen (PSA) is low, which can result in false negative test results (5,6). Prostate biopsy is considered the 'gold standard' for the diagnosis of prostate cancer (7). The pathological diagnosis of PCa is the result of a comprehensive analysis of cytological features (nucleus enlargement, nucleolus prominence and deep dyeing of chromatin), structural changes (sieve glands, solid nest gland, and strip and single gland) and analysis of relevant tumor markers, such as PSA, P504S and high molecular weight keratin (8). Typically, benign prostatic hyperplasia (BPH) or neoplastic changes of prostate can be easily identified (8); however, in several cases the diagnosis of benign hyperplasia or cancer of the prostate is difficult, and may be accompanied by basal cell hyperplasia and transparent cells. Basal cell hyperplasia can be mistaken for benign lesions, and transparent cell changes lose the morphological features of conventional tumor cells, such as enlarged or deep staining, which makes diagnosis difficult to determine from cytological or structural features alone (9). Thus, cellular biomarkers, such as PSA and α-methylacyl-CoA racemase (P504S) may be used for further diagnostic confirmation of ambiguous lesions (10,11). PSA is a glycoprotein secreted by prostate columnar and glandular epithelial cells, and is extensively used for the early diagnosis of PCa (12). Furthermore, it is organ-specific and can be used to distinguish secondary adenocarcinomas that invade the prostate; however, it is less specific to tumors, thus, it is not used alone for the diagnosis of PCa (12). According to a previous report, P504S is also highly sensitive and specific in the diagnosis of PCa (13). It is expressed in the mitochondria and peroxisomes, and participates in the β-oxidation process of both fatty acids and

their derivatives, which is a process associated with tumorigenesis (14-16). Thus, the present study aimed to investigate the diagnostic values of PSA and P504S by measuring their expression levels in PCa tissues using immunohistochemistry (IHC) analysis, particularly for PCa tissues that cannot be characterized by cytological features alone.

Materials and methods

Patient information. The present retrospective study was approved by the Medical Ethics Committee of Qianjiang Central Hospital (Qianjiang, China; approval no. 201912001) and verbal informed consent was obtained from all patients prior to the start of the study. Prostate specimens were collected from 125 patients (mean age, 64 years; age range, 42-78 years) admitted to the Department of Pathology at Qianjiang Central Hospital (Hubei, China) between January 2015 and September 2019. The inclusion criteria were as follows: i) Tissue sample had no obvious necrosis, cystic changes or pigmentation, defects; ii) tissue sample was paraffinized and able to be analyzed using IHC analysis and hematoxylin and eosin (H&E) staining; iii) complete pathological information and iv) complete patient information. The H&E stained slides were reviewed by pathologists at the Department of Pathology of Qianjiang Central Hospital (Qianjiang, China) and stratified into three groups; BPH (n=60), PCa (n=45) and unconfirmed diagnosis (n=20).

IHC to detect PSA and P504S. IHC analysis was performed to determine PSA and P504S expression levels in prostate tissue samples. Tissues were fixed in 4% neutral formaldehyde for 24 h at room temperature, dehydrated and paraffin-embedded. The paraffinized samples from each group were cut into 4-μm-thick sections and mounted onto adhesive slides. Samples were deparaffinized in xylene at room temperature and rehydrated in a descending ethanol series (95, 75, 50 and 25%, respectively). Sections were incubated with citrate buffer (ProteinTech Group, Inc.) under high pressure at 121˚C for 3 min to retrieve the antigen, prior to being blocked with 10% goat serum (ProteinTech Group, Inc.) for 30 min at 37˚C and incubated with 0.3% H_2O_2 for 30 min at 37˚C to inhibit endogenous peroxidase activity. Tissue sections were incubated with rabbit polyclonal anti-PSA (cat. no. 10501-1-AP) and polyclonal anti-P504S (cat. no. 15918-1-AP) overnight at 4˚C (both 1:100 and from ProteinTech Group, Inc.). Subsequently, membranes were incubated with goat anti-rabbit IgG antibody-HRP multimer (1:100; cat. no. PV-0023; BIOSS) for 20 min at 37˚C. The slides were subsequently stained with 3,3'-diaminobenzidine for 5-10 sec at room temperature, prior to counterstaining with hematoxylin for 5 min at room temperature. The slides were dehydrated with ethanol (95, 75, 50 and 25%, respectively), mounted in neutral resins and observed under a light microscope (magnification, x100).

Evaluation of IHC results. PSA is predominantly expressed in the cytoplasm of cells; however, it is also expressed in the cell membrane and stains deeper in the cytoplasm near the luminal edge. Similarly, P504S is predominantly expressed in the cytoplasm of cells. In the present study, the presence of light-yellow, brownish-yellow and tan staining of the cell membrane and cytoplasm of prostate cells was considered positive, whereas no staining or focal staining was considered negative. The percentage positivity was graded from 0+ to 3+ according to the percentage of cells stained, as follows: 0% (0+, no expression); 1-25% (1+, mild); 26-50% (2+, moderate) and >50% (3+, strong). Results were considered positive if they exceeded 1+.

Gleason grading groups for PCa. The data for patients with PCa was stratified into five levels (1-5) as follows: Level 1, single round acinus with uniform shape; level 2, single round acinus with slightly irregular shape; level 3, non-fused single acinus with significant morphological differences; level 4, fusion-sieve acinus; level 5, solid or single infiltrating tumor cells, or accompanied by necrotic tissue, according to the 2014 version of the Gleason grading system (17). The evaluation of the disease is based on the degree of differentiation of the gland, with the most differentiated cases at level 1 and the least differentiated cases at level 5.

Statistical analysis. Statistical analysis was performed using SPSS software (version 23.0; IBM Corp.). PSA and P504S immunostaining results were analyzed using a χ^2 test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

PSA/P504S expression levels in BPH and PCa groups. Regarding the BPH group, 80.00% of all cases (48/60) stained positive for PSA expression, while 93.33% of all cases (56/60) reported negative expression in P504S (Fig. 1; Table I). Regarding the PCa group, the positive rate of P504S (88.89%; 40/45) was significantly higher compared with that in the BPH group ($P<0.001$), while no significant difference was observed in PSA expression between the PCa and BPH groups ($P>0.05$; Table I).

Association between PSA/P504S expression levels and PCa amongst Gleason grading groups. PCa was stratified into five groups according to the Gleason grading system for assessing differentiation of the disease and patient prognosis. The positive rate of P504S increased, while the positive rate of PSA slightly decreased from groups 1-5. However, no significant differences were observed in PSA and P504S expression levels between any of the grading scores (Fig. 2; Tables II and III).

PSA/P504S diagnosis of unconfirmed cases. Regarding the 20 ambiguous cases, it was difficult to accurately identify morphology of PCa using H&E staining; however, three predominant structural features were observed; Atrophy (nine cases), basal cell hyperplasia (six cases) and clear cell degeneration (five cases) (Table IV). Of the nine cases of atrophy, five were positive for PSA/P504S expression and identified as cancer, while four cases were negative for P504S expression and identified as BPH. All six cases of basal cell hyperplasia were identified as BPH. One of six cases of clear cell degeneration was diagnosed as prostatic intraepithelial neoplasia (the morphology is between normal and tumor tissues, which has the potential to turn into cancer). In total, seven of the 20 ambiguous cases were considered PCa by PSA and P504S.

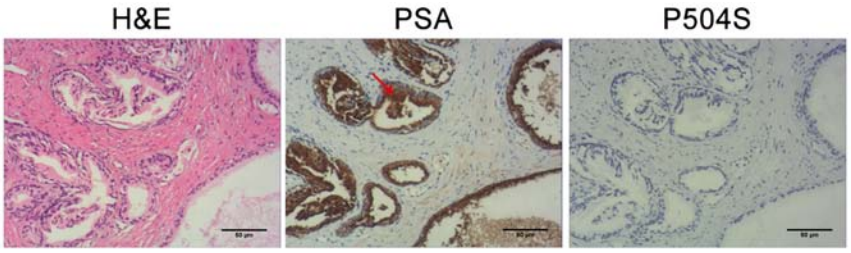

Figure 1. PSA/P504S expression in the benign prostatic hyperplasia group. P504S expression was negative. The yellow-brown areas in each IHC image indicate positive expression. The red arrow highlights the positive expression (magnification, x100). PSA, prostate-specific antigen; P504S, α-methylacyl-CoA racemase; H&E, hematoxylin and eosin.

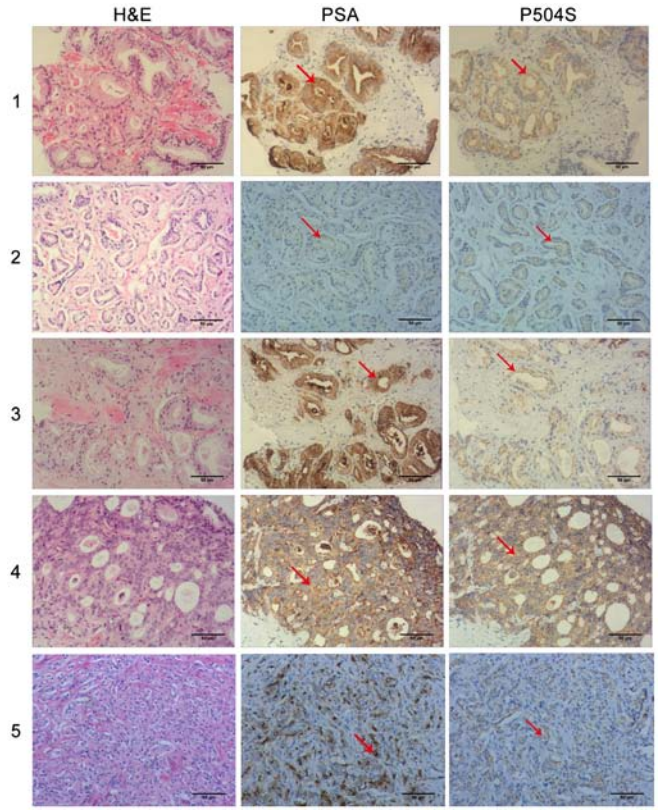

Figure 2. Representative images of PSA/P504S expression and prostate cancer according to Gleason grading. The positive rate of P504S increased, while the positive rate of PSA slightly decreased from groups 1-5. The yellow-brown areas indicate positive expression. The red arrow highlights the positive expression (magnification, x100). PSA, prostate-specific antigen; P504S, α-methylacyl-CoA racemase; H&E, hematoxylin and eosin.

Discussion

PCa is a malignant tumor that predominantly affects older men, particularly those >60 years and the incidence rate in the United States increased by 0.1% in 2019 (1,18). When patients present with suspected PCa symptoms, such as dysuria, hematuria and frequent urination (10), the most common diagnostic method is to screen for serum PSA, as elevated PSA is associated with PCa (the normal value is generally <4 ng/ml, and PSA is often >10 ng/ml when prostate cancer occurs) (19); however, this test is only indicative and the results are not absolute. Prostate biopsy can directly identify the tumor morphology under the microscope, which is one of the most common screening measures (20). Prostate biopsy typically relies on the cytological and structural features of the tissue under the microscope for diagnosis; however, routine diagnosis is difficult as several cases of PCa mimic the morphology of benign prostate gland (8). Thus, it is essential to identify tumor markers that can distinguish between benign and malignant tumor types.

PSA is a chymotrypsin-like serine protease produced by prostate epithelial cells (12). When the prostate is stimulated, PSA expression increases and this can be detected in PCa tissues using IHC analysis; however, there is no differential expression between benign and malignant prostate tissues (21), which is consistent with the results of the present study. Notably, PSA is an organ-specific marker, thus possesses diagnostic applications (9). It can be used as a specific indicator to identify the tumor origin, particularly with metastatic tumors (12). Furthermore, high PSA expression levels are indicative of PCa, although it is not expressed, or only partially expressed, in a few high-grade cases of PCa, cases of PCa with neuroendocrine differentiation and other tissues, such as poorly differentiated adenocarcinoma and squamous cell carcinoma of the prostate (22). Thus, according to the PSA test results, diagnosis of prostate tumors requires a combination of assessing clinical symptoms and other positive markers, such as P504S and high molecular weight cytokeratin, rather than individual assessments (10).

P504S is predominantly expressed in the cytoplasm of affected cells and is a marker for PCa, with a sensitivity of 82-100% (9,14,23), and is not expressed in healthy prostate tissue. In the present study, P504S was expressed predominately in tumor tissues; however, the degree of expression was not associated with the Gleason grading groups of the assessed tumors, suggesting that P504S has little effect on the prognosis of PCa.

Typically, diagnosis of positive high-grade prostate tumors is dependent on the morphological features (single-cell layer and nuclear atypia) identified by H&E staining. However, certain ambiguous cases, such as glandular basal cell hyperplasia, gland atrophy, clear cell degeneration and squamous metaplasia require confirmation via immunolabeling (9,24). In the present study, ambiguous cases with positive PSA and P504S expression (particularly P504S) were identified as cancer, while cases with no P504S expression were classified as benign hyperplasia. Lack of basal cells is a key feature of PCa; however, in >40% of renal adenomas cases and >60% of renal tubular hyperplasia and partial atrophy cases, basal cells are reduced or absent (25,26). Thus, the interpretation

Table I. PSA/P504S expression in the BPH and PCa groups.

	BPH (n=60)		PCa (n=45)		
Antibody	Negative (%)	Positive (%)	Negative (%)	Positive (%)	P-value
PSA	12 (20.00)	48 (80.00)	11 (24.44)	34 (75.56)	0.58
P504S	56 (93.33)	4 (6.67)	5 (11.11)	40 (88.89)	[a]2.90×10^{-17}

[a]P<0.001, comparison of P504S in PCa and BPH. PSA, prostate-specific antigen; P504S, α-methylacyl-CoA racemase; BPH, benign prostatic hyperplasia; PCa, prostate cancer.

Table II. PSA expression in the prostate cancer group, divided according to the Gleason grading system (n=45).

Gleason grading groups	Number of patients, n	PSA expression	
		Negative, n	Positive, n
1	7	3	4
2	4	1	3
3	13	5	8
4	12	4	8
5	9	3	6

PSA, prostate-specific antigen.

Table III. P504S expression in the prostate cancer group, divided according to the Gleason grading system (n=45).

Gleason grading groups	Number of patients, n	P504S expression	
		Negative, n	Positive, n
1	7	1	6
2	4	1	3
3	13	2	11
4	12	1	11
5	9	0	9

P504S, α-methylacyl-CoA racemase.

Table IV. Final diagnosis following immunohistochemistry analysis of PSA/P504S expression in the ambiguous cases (n=20).

Category	Number of Patients, n	Final diagnosis		
		BPH, n	PCa, n	PIN, n
Atrophy	9	4	5	0
Basal cell hyperplasia	6	6	0	0
Clear cell degeneration	5	2	2	1

BPH, benign prostatic hyperplasia; PCa, prostate cancer; PIN, prostatic intraepithelial neoplasia.

of basal immunostaining results should be considered carefully, as they can support the diagnosis of PCa, following appropriate investigation of H&E morphology and detection of PSA/P504S expression (27). A previous study reported that the final diagnosis of PCa must be supported by the positive staining of specific immunolabels, particularly P504S, based on the morphology of H&E staining (28).

It is indisputable that each diagnostic method has certain limitations. For example, regarding biopsy, there may be cases where the specimen cannot be obtained, particularly when the tumor is very small. Similarly, antibodies that are associated with tumors, including PSA are not 100% expressed in the right place on every occasion, which decreases their sensitivity (10). However, with the development of genetic testing technology, such as the use of next generation sequencing of urine exfoliated cells to detect microRNAs (29), such as miR-21-5p, miR-141-3p, miR-375 and miR-574-3 in prostate cancer, studies have indicated that this technique may be used as an auxiliary diagnostic technique, and a method to assess long-term prognosis (30,31).

In summary, according to a detailed understanding of the clinical symptoms and assessment of morphological features identified using H&E staining, the use of PSA and P504S serves a key roles in improving the diagnostic rate of PCa, particularly for ambiguous cases. However, immunolabels present several limitations, such as insufficient sensitivity and specificity, and incorrect expression of antibodies, thus they can only be considered as auxiliary tools for diagnosis at present, and further investigations are required to determine their involvement in diagnosing PCa.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

DJY, XRZ, XS and QXC designed the present study. YL and XS performed the literature analysis and participated in data analysis and interpretation. XS wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This retrospective study was approved by The Medical Ethics Committee of Qianjiang Central Hospital (Qianjiang, China; approval no. 201912001) and verbal informed consent was obtained from all patients prior to the study start.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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