Evaluation of Vitronectin Expression in Prostate Cancer and the Clinical Significance of the Association of Vitronectin Expression with Prostate Specific Antigen in Detecting Prostate Cancer

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Purpose: To detect the expression of vitronectin (VTN) in the tissues and blood serum of prostate cancer (PCa) patients, and evaluate its clinical significance and to evaluate the significance of the combined assay of VTN and prostate specific antigens (PSA) in PCa diagnosis.

Materials and Methods: To detect the expression of VTN as a potential marker for PCa diagnosis and prognosis, immunohistochemistry was performed on the tissues of 32 patients with metastatic PCa (PCaM), 34 patients with PCa without metastasis (PCa), and 41 patients with benign prostatic hyperplasia (BPH). The sera were then subjected to Western blot analysis. All cases were subsequently examined to determine the concentrations of PSA and VTN in the sera. The collected data were collated and analyzed.

Results: The positive expression rates of VTN in the tissues of the BPH and PCa groups (including PCa and PCaM groups) were 75.61% and 45.45%, respectively ($P = .005$). VTN was more highly expressed in the sera of the BPH patients (0.83 ± 0.07) than in the sera of the PCa patients (0.65 ± 0.06) ($P < .05$). It was also more highly expressed in the sera of the PCa patients than in the sera of the PCaM patients (0.35 ± 0.08) ($P < .05$). In the diagnosis of BPH and PCa, the Youden indexes of PSA detection, VTN detection, and combined detection were 0.2620, 0.3468, and 0.5635; the kappa values were 0.338, 0.304, and 0.448, respectively, and the areas under the receiver operating characteristic curve were 0.625, 0.673, and 0.703 ($P < .05$), respectively.

Conclusion: VTN levels in sera may be used as a potential marker of PCa for the diagnosis and assessment of disease progression and metastasis. The combined detection of VTN and PSA in sera can be clinically applied in PCa diagnosis.

Keywords: cell line; tumor; prostatic neoplasms; vitronectin; metabolism; humans; biomarkers; early detection of cancer; prostate-specific antigen.

INTRODUCTION

Prostate cancer (PCa) is a malignant tumor common among male Europeans and Americans. The incidence of this disease in China has gradually increased in recent years.[1] Prostate specific antigen (PSA) is the most widely used prostate tumor markers. Serum PSA concentrations significantly affect treatment modalities in males with PCa. However, PSA tend to increase during benign prostate hyperplasia (BPH), prostatitis, urethral catheter, and digital rectal exams.[2] Over-diagnosis and the resulting over-treatment of occult cancer are thus common. PSA detection suffers from limited sensitivity and specificity. Furthermore, the significance of PSA declines in the later stages of the disease.[3,4] The search for effective serum tumor markers for PCa is significant in improving the level of the diagnosis of PCa and determining treatment prognosis.

Vitronectin (VTN) is a glycoprotein, which is a member of the integrin family. It changes in the sera and tissues of people, and is associated with the occurrence and metastasis of tumor.[5] However, the studies on VTN and PCa are relatively rare. Hence, the present study aimed to explore the expression of VTN in the tissues and sera of PCa patients and the significance of the combined detection of VTN and PSA in the diagnosis and treatment of PCa.

MATERIALS AND METHODS

Study Patients

Patient data, blood samples, and prostate tissue samples were collected from the prostate disease patients of the urology department of the Xinjiang Medical University Affiliated Tumor Hospital between December 2010 and February 2015. All cases were examined for PSA.

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Table 1. Vitronectin expression in prostate cancer tissues and its relationship with clinicopathological factors.

Factor	Positive, no.	Negative, no.	Expression Rate, %	χ^2	*P* Value
Age, years					
< 70	16	19	45.16	0.002	.964
≥ 70	14	17	45.71		
PSA, ng/mL					
< 4	2	0	100.00	11.24	.004
4-10	11	3	78.57		
> 10	17	33	34.00		
Clinical stage					
T2	7	5	58.33	7.41	.025
T3	13	7	65.00		
T4	10	24	29.41		
Gleason score					
≤ 6	8	2	80.00	11.06	.004
7	12	8	60.00		
≥ 8	10	26	27.78		
Tumor metastasis					
Yes	9	23	28.12	7.52	.006
No	21	13	61.76		

Abbreviation: PSA, prostate specific antigen.

Pathological types were determined via biopsy or operation, whereas metastasis was determined via computed tomography (CT) scan, magnetic resonance imaging (MRI) and bone scan. Finally, 34 patients with prostate cancer without metastasis (PCa), 32 patients with prostate cancer with metastasis (PCaM), and 41 patients with BPH were included. The mean ages of the patients with PCa, PCaM, and BPH were 65.27 ± 9.07, 67.02 ± 10.02, and 64.12 ± 7.33 years, respectively.

Immunohistochemistry for VTN in Tissues

All tissue samples were collected via surgery or biopsy. Multiple 4-μm-thick sections of representative formalin-fixed, paraffin-embedded tissues were cut for immunohistochemical studies. A polymer-based immunohistochemical was used to detect VTN (Mouse Anti-Human, R&D Co, 614 McKinley Place NE Minneapolis, MN 55413, USA). All immunostained sections were examined under a light microscope (Olympus CKX41, Olympus Optical Co., Ltd., Tokyo, Japan) to evaluate VTN. VTN staining was cytoplasmic.

The standards for determining immunohistochemistry were as follows. Color intensity: 0 point; no stain, 1 point; faint cytoplasmic stain, 2 points; diffuse cytoplasmic stain, 3 points; diffuse intense cytoplasmic stain. Color area: 0 point; color area < 10%, 1 point; 10% ≤ color area < 50%, 2 points; 50% ≤ color area < 75%, 3 points; color area ≥ 75%. Color intensity was multiplied with color area; a score of 3 or more was considered as positive, whereas a score of less than 3 was considered as negative.

Western Blot Analysis of VTN in Sera

Western blot analysis was carried out according to the instruction manual, which provided guidelines regarding the preparation of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), addition of samples, electrophoresis, transfer, sealing, addition of the first antibody (Mouse Anti-Human, R&D Co., 614 McKinley Place NE Minneapolis, MN 55413, USA), cleaning, addition of the second antibody (Rabbit Anti-Mouse, Sigma Co., St. Louis, MO, USA), cleaning, chemiluminescence, exposure, development, and so on. Pictures of the chromogenic results were taken with an image analysis system, which was used to record the optical density and intensity values of the electrophoretic bands. The calculation formula for the optical density ratios was (intensity value of VTN band × optical density value of VTN band) / (intensity value of β-actin band × optical density value of β-actin band). The analysis was semi-quantitative.

Table 2. Comparison of experimental diagnosis indexes of sera with and without metastatic prostate cancer.

Test	Index			
	Sensitivity %	**Specificity %**	**Total coincidence rate,**	**% Youden index**
VTN detection	56.90	77.78	64.89	0.3468
PSA detection	76.75	49.45	59.57	0.2620
Combined parallel detection	80.23	76.12	67.12	0.5635

Abbreviations: VTN, Vitronectin; PSA, prostate specific antigen.

Determination of the Concentrations of VTN and PSA in Sera

The VTN concentrations in the sera of all the patients were detected via an enzyme-linked immunoassay (Synergy 2 multimode microplate reader, BioTek Co., USA; Enzyme-linked immunoassay kit, R&D Co., 614 McKinley Place NE Minneapolis, MN 55413, USA), whereas the PSA concentrations were detected via an electrochemiluminescence immunoassay (Cobas 6000 fully automatic electrochemistry luminescence instrument and corresponding kit, Roche Co., Grenzach, Germany). The positive results of the combined parallel detection indicated that PSA or VTN was positive in the PCa patients.

Statistical Analysis

Data analysis and processing were performed with the Statistical Package for the Social Science (SPSS Inc, Chicago, Illinois, USA) version 16.0. The immunohistochemistry results, the VTN expression in PCa tissue, and the relationship of such expression with clinicopathological factors were analyzed with the chi-square test. The Western blot results were obtained via variance analysis and the Student-Newman-Keuls (SNK) method, $\alpha = 0.05$. The evaluation indexes of VTN detection, PSA detection, and combined parallel detection in the sera from the PCa and BPH groups included kappa values, sensitivity, specificity, Youden indexes, and total coincidence rates. A receiver operating characteristic (ROC) curve was drawn using the sensitivity and specificity indexes to compare VTN detection, PSA detection, and their combined parallel detection in sera for PCa diagnosis.

RESULTS

Immunohistochemistry of VTN in Tissue Samples and its Relationship with Clinicopathological Factors

VTN protein was expressed in PCa tissues (including PCa and PCaM tissues) and BPH tissues, as shown in Figures 1 and 2, respectively. The cytoplasm was stained brown, as shown in both figures. According to the immunohistochemical results, the positive expression rates of VTN in the BPH and PCa groups (including the PCa and PCaM groups) were 75.61% and

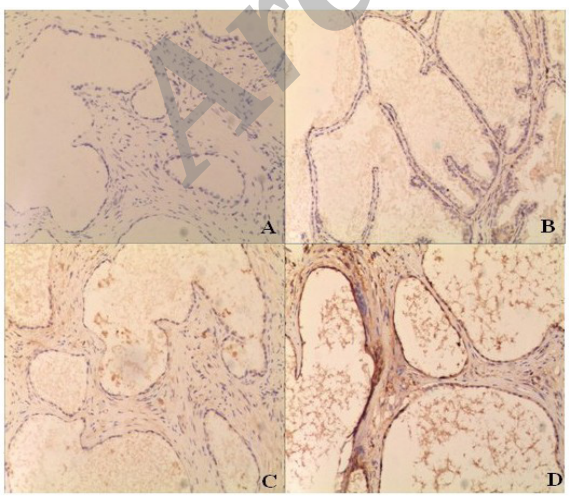

Figure 1. Vitronectin expression in tissue samples of patients with benign prostatic hyperplasia. **A)** No stain (0 point); **B)** Faint cytoplasmic stain (1 point); **C)** Diffuse cytoplasmic stain (2 points); **D)** Diffuse intense cytoplasmic stain (3 points).

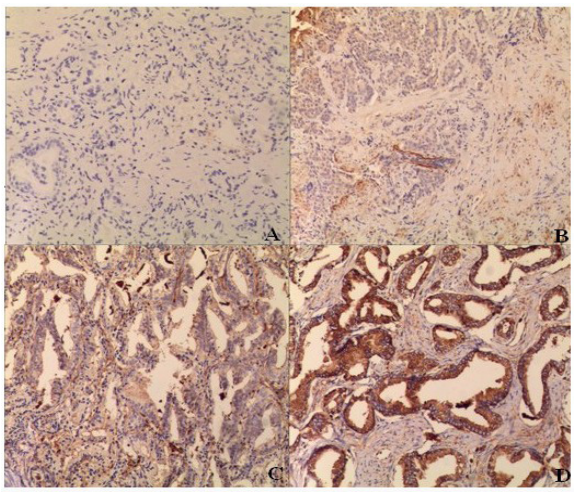

Figure 2. Vitronectin expression in tissue samples of patients with and without metastatic prostate cancer. **A)** No stain (0 point); **B)** Faint cytoplasmic stain (1 point); **C)** Diffuse cytoplasmic stain (2 points); **D)** Diffuse intense cytoplasmic stain (3 points).

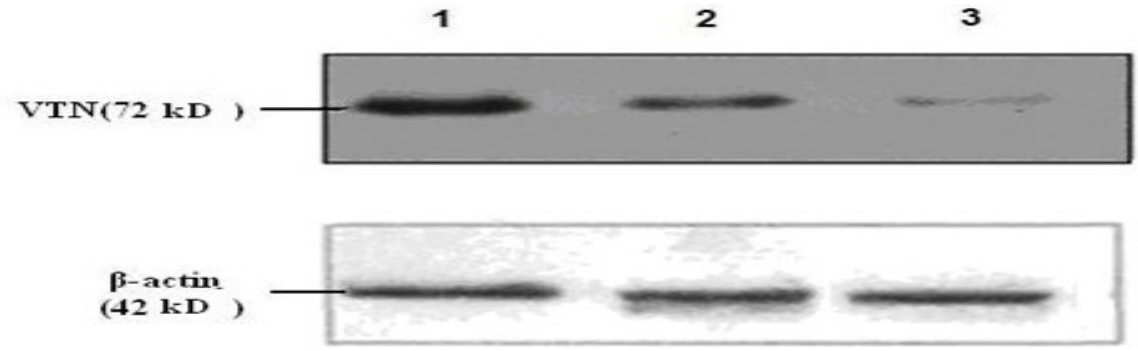

Figure 3. Vitronectin expression detected by Western blot analysis.
Figure 3 shows that vitronectin could be detected in the serum samples from the PCaM, PCa, and BPH groups. Specific bands of vitronectin appeared with a relative molecular mass of 72 kDa, and β-actin appeared with 42 kDa.
Abbreviations: BPH, benign prostatic hyperplasia; PCa, prostate cancer; M, metastasis.
1: BPH; 2: PCa; 3: PCaM.

45.45%, respectively. The VTN protein expression in the BPH group was higher than that in the PCa group (including PCa and PCaM; $P = .005$).

The VTN expression in the PCa tissues (including PCa and PCaM tissues) was related to the PSA of newly diagnosed patients, clinical stage, Gleason score, and tumor metastasis; however, it was not associated with patient age (**Table 1**).

Western Blot Analysis of VTN in Serum Samples

Figure 3 shows that VTN could be detected in the serum samples from the PCaM, PCa, and BPH groups. Specific bands appeared with a relative molecular mass of 72 kDa. The optical density ratios of VTN in the serum samples from the BPH, PCa, and PCaM groups were 0.83 ± 0.07, 0.65 ± 0.06, and 0.35 ± 0.08, respectively. The results of the variance analysis showed that the optical density ratios of VTN in the serum samples from the BPH, PCa, and PCaM groups were not totally equal ($F = 396.72$, $P = .000$). The results of the SNK method showed that VTN expressions in the three groups differed. The optical density ratios of VTN in the serum samples from the BPH group were higher than those from the PCa group. In addition, VTN expression in the PCa group was higher than that in the PCaM group (**Figure 4**).

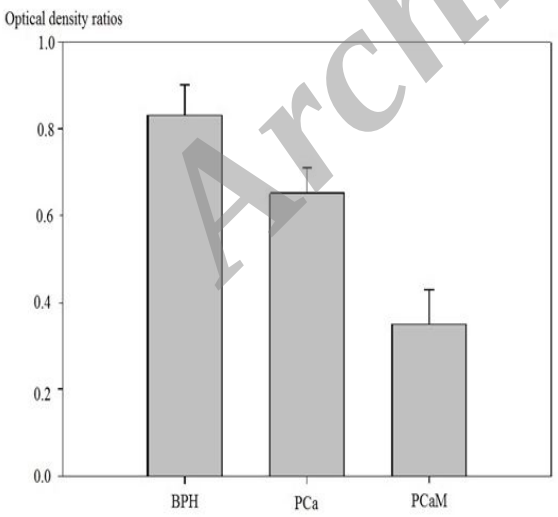

Figure 4. Optical density ratios of vitronectin for serum samples from each group.
Figure 4 shows that the optical density ratios of vitronectin in the serum samples from the BPH, PCa, and PCaM groups were 0.83 ± 0.07, 0.65 ± 0.06, and 0.35 ± 0.08, respectively. The results were statistically significant.
Abbreviations: BPH, benign prostatic hyperplasia; PCa, prostate cancer; M, metastasis.

Combined Parallel Detection of VTN and PSA for PCa and BPH Diagnosis

The VTN concentrations in the BPH, PCa, and PCaM groups were 219.63 ± 25.30, 201.72 ± 19.37, and 170.05 ± 23.80 ng/mL, respectively, with statistically significant differences ($P < .05$). The PSA concentrations in the BPH, PCa, and PCaM groups were 5.45 ± 3.48, 15.45 ± 9.66, and 65.29 ± 31.50 ng/mL, respectively, with statistically significant differences ($P < .05$).

Kappa values were used to evaluate the reliability of the diagnostic tests. The kappa values of VTN detection, PSA detection, and combined parallel detection in the PCa and PCaM groups were 0.338, 0.304, and 0.408, respectively. According to Kanidis and Koch's standards, combined parallel detection was found to be more consistent than single detection in the PCa and PCaM groups, with such consistency being moderate. It yielded a certain value for PCa diagnosis.

Sensitivity, specificity, the Youden index, and the total coincidence rate were analyzed. The calculated results showed that the single detection of PSA in PCa exhibited high sensitivity but low specificity and total coincidence rate. By contrast, the single detection of VTN yielded opposite results. In addition, the specificity of the combined parallel detection of PSA and VTN was

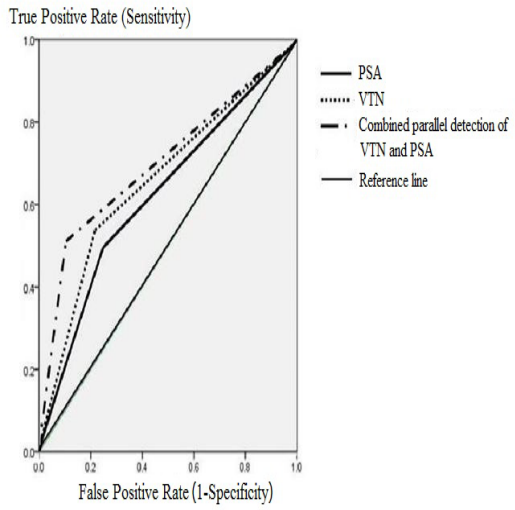

Figure 5. Receiver operating characteristic curves of prostate specific antigen detection, vitronectin detection, and combined parallel detection of vitronectin and prostate specific antigen.

Figure 5 shows that the areas under the receiver operating characteristic curves of prostate specific antigen detection, vitronectin detection, and combined parallel detection of vitronectin and prostate specific antigen were 0.625, 0.673 and 0.703 for the prostate cancer and metastatic prostate cancer groups; the difference was statistically significant ($P < .05$). Hence, the accuracy of combined parallel detection is better than that of single detection.

slightly lower than that of single PSA detection. However, the sensitivity, total coincidence rate, and Youden index were better in the combined approach (**Table 2**). The values of PSA and VTN detection for PCa diagnosis were evaluated with the ROC curve. The areas under the curves (AUCs) of PSA and VTN were 0.625 and 0.673 in the PCa and PCaM groups (**Figure 5**), respectively; the difference was statistically significant ($P < .05$). The AUC of the combined parallel detection of VTN and PSA was 0.703 in both the PCa and PCaM groups (**Figure 5**); thus, the accuracy of this combined detection method is better than that of single detection ($P < .05$).

DISCUSSION

The VTN protein is a specific ligand of the αvβ3 integrin family that is present on the surface of vascular endothelial cells.[6] because it contains the RGD peptide sequence (Arg-Gly-Asp). The interaction of VTN and αvβ3 promotes the adhesion of monocytes and endothelial cells in blood circulation. αvβ3 is widely expressed on the surface of malignant tumor cells and vascular endothelial cells in different tissues; this marker promotes malignant biological behavior, such as the occurrence, development, angiogenesis, invasion, and metastasis of malignant tumors.[7–9] The high expression of matrix metalloproteinases (MMPs) is closely related to the invasion and metastasis of malignant tumors.[10] αvβ3 can interact with MMPs and decompose fibronectin, as well as promote the invasion and metastasis of various malignant tumor cells.[11] In serum protein chips of hepatocellular carcinoma patients, VTN was degraded into small peptides with MMP-2; the outcome confirmed that small peptides of VTN could be used for the serological diagnosis of liver cancer.[12] In addition, VTN interacts with urokinase receptors (uPARs), which control cell adhesion and migration.[13]

To date, studies on the relationship between VTN and PCa are relatively rare. The present study is the first to detect VTN protein expression in PCa and BPH tissues via immunohistochemistry. According to the immunohistochemical results, the positive expression rates of VTN in the BPH and PCa groups (including PCa and PCaM groups) were 75.61% and 45.45%, respectively. The expression of VTN protein was the highest in the BPH group, followed by the PCa group. Simultaneously, the expression of VTN in patient sera was detected via Western blot analysis, the results of which are consistent with the immunohistochemistry results. This outcome showed that VTN in the serum samples from the BPH group was higher than that in the serum samples from the PCa group. Moreover, the VTN expression in the PCa group was higher than that in the PCaM group. In addition, the VTN expression in the PCa tissues (including PCa and PCaM tissues) was related to the PSA of newly diagnosed patients, clinical stage, Gleason score, and tumor metastasis. Therefore, VTN may participate in the occurrence and development of PCa via cell adhesion and migration. As a member of the integrin family, VTN promotes the adhesion of monocytes and endothelial cells in blood circulation. VTN also interacts with uPAR to adjust cell adhesion and migration, which are directly involved in PCa metastasis. VTN can interact with MMPs to promote the invasion and metastasis of PCa. With an increased expression of αvβ3, uPAR, and MMPs during the occurrence and mineralization process of PCa, VTN combines with αvβ3, interacts with uPAR, and is degraded by MMPs, thereby gradually reducing the VTN content in serum. Therefore, VTN levels in sera may be used as a potential marker of PCa for the diagnosis and assessment of disease progression and metastasis.

In addition, the analysis of PSA and VTN concentrations in sera and their ROC curves for the PCaM and PCa patients showed that the combined parallel detection of VTN and PSA achieved better diagnostic accuracy than the PCa detection method based on a single index. Therefore, combined parallel detection can be

clinically applied in PCa diagnosis.

The results of this study provide only preliminary clues. The number of samples was limited, and no follow-up was conducted after radical operation of PCa. Therefore, larger samples and long-term follow-up are needed to confirm the role of VTN detection in serum to diagnose and judge the prognosis of PCa.

CONCLUSIONS

The VTN expression in the sera and prostate tissues of the BPH patients was higher than that in the sera and prostate tissues of the PCa patients. VTN levels in sera may be used as a potential marker of PCa for the diagnosis and assessment of disease progression and metastasis. The combined parallel detection of VTN and PSA in sera can be clinically applied in PCa diagnosis.

CONFLICT OF INTEREST

None declared.

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