

Early diagnostic role of PSA combined miR-155 detection in prostate cancer

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Abstract. – OBJECTIVE: As a kind of malignant tumor in the male genitourinary system, prostate cancer exhibits significantly increased occurrence. Prostate-specific antigen (PSA) expression can be seen in the prostate cancer, prostatitis, and other diseases, therefore, lack of diagnostic specificity. The miR-155 expression is abnormally increased in the tumors. Therefore, this study aims to explore the clinical significance of PSA combined miR-155 detection in the early diagnosis of prostate cancer.

PATIENTS AND METHODS: A total of 86 patients diagnosed with prostate cancer were enrolled in this study. PSA and miR-155 gene expression in tumor tissue were detected by using Real-time PCR. The serum levels of PSA were measured by using enzyme-linked immunosorbent assay (ELISA). The correlation of PSA and miR-155 expression with age, body mass index (BMI), tumor volume, tumor-node-metastasis (TNM) stage, lymph node metastasis (LNM), and other clinicopathological features were analyzed, respectively.

RESULTS: Serum PSA expression and PSA gene in tumor tissue were significantly higher compared to that in adjacent tissues ($p < 0.05$). PSA gene and protein increased significantly with the clinical stage of TNM and decreased following the increase of grade ($p < 0.05$). The miR-155 level was significantly elevated in the tumor tissue compared with para-carcinoma tissue ($p < 0.05$). PSA and miR-155 expressions were positively correlated with TNM stage, tumor volume, and LNM, and negatively correlated with grade ($p < 0.05$).

CONCLUSIONS: PSA and miR-155 were closely related to the clinicopathological features of prostate cancer. Combined detection is helpful for the early diagnosis of prostate cancer.

Key Words:

Prostate cancer, PSA, miR-155, TNM stage, Gene detection.

Introduction

Prostate cancer is a type of malignant tumor with high morbidity in the male genitourinary system around the world^{1,2}. In recent years, following the era of population aging, and the diet and lifestyle changes, the incidence of prostate cancer patients increased year by year with younger trends^{3,4}. In developing countries, the early screening rate of prostate cancer is low, leading to patients advanced when diagnosed and can't receive radical surgery. Therefore, the survival and treatment effects were poor^{5,6}. Due to lifestyle and eating habits, the incidence of prostate cancer in developed countries is also at a high level⁷. As the prostate cancer itself has the characteristics of concealing, most patients are at an advanced stage, so the treatment is very complicated⁸. The pathogenesis of prostate cancer is complex, and its metastasis and invasion mechanisms are affected by many factors. Therefore, early diagnosis of prostate cancer is the key to improve the prognosis of patients⁹.

Prostate-specific antigen (PSA) is a single-stranded polypeptide belonging to the serine protease family with tissue-specific and chymotrypsin-like functions^{10,11}. PSA can decompose the main collagen in semen, therefore playing the role of semen dilution¹². PSA can be synthesized in both normal and cancerous epithelial cells¹³. PSA has tissue specificity that only exists in human prostate acinar and ductal epithelial cell cytoplasm instead of other cells. However, it is not tumor-specific, thus can be elevated by prostatitis, benign prostatic hyperplasia, and prostate cancer^{14,15}. MicroRNA exists in a variety of forms with a wide range of mechanisms. They participate in the regulation of growth and development,

so as to enhance the ability to adapt the environment¹⁶. It is showed that miR-155 is overexpressed in prostate cancer, breast cancer, and other tumors^{17,18}. Therefore, this study aims to explore the clinical significance of PSA combined miR-155 in the early diagnosis of prostate cancer.

Patients and Methods

General Information

A total of 86 patients with prostate cancer who were diagnosed by histopathological examination from January 2015 to December 2016 were enrolled in the Affiliated Nanhua Hospital, University of South China (Hengyang, China). All patients were treated by surgery with mean age at 55.1 ± 6.6 (45-77) years old. The inclusion criteria were listed as the followings⁵: primary prostate cancer confirmed by the pathology, without surgery, chemotherapy, radiotherapy or other treatment. The exclusion criteria were listed as the followings⁵: recurrent prostate cancer, treated by surgery, radiotherapy, or chemotherapy previously, combined with other diseases, such as infectious diseases, malignant tumors, severe liver and kidney disease, pulmonary fibrosis, bone metabolic diseases, secondary renal hypertension, systemic immune diseases, and malignant tumor complications. The tumor tissue was collected during the operation for the pathological diagnosis, classification, and staging. The tumor tissues and adjacent tissues were collected intraoperatively and stored at -80°C . Another 40 patients with benign prostatic hyperplasia at the same period were selected as control with mean age at 53.1 ± 5.4 (41-70) years old. This study was approved by the Academy of Medical Ethics Committee. All the enrolled subjects had signed the informed consent.

Clinical Data Collection

The clinical data were collected, including name, age, family history, family history of prostate cancer, body mass index (BMI), tumor size, tumor type, tumor grade, clinical stage, tumor metastasis, pathological stage.

Main Reagents and Instruments

TRIzol reagents were purchased from Invitrogen/Life Technologies (Carlsbad, CA, USA). PSA enzyme-linked immunosorbent assay (ELISA) kit was purchased from R&D Systems (Minneapolis, MN, USA). RNA extraction kit and reverse transcription kit were purchased from Axygen (Tewksbury, MA, USA). LabSystem Version 1.3.1

Microplate reader was purchased from Bio-Rad Laboratories (Hercules, CA, USA). ABI 7700 Fast Fluorescence Quantitative PCR was derived from ABI (Foster City, CA, USA). DNA amplifier was provided by PE Gene Applied Biosystems (Foster City, CA, USA). Other common reagents were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China).

Specimen Collection

Tumor tissue and adjacent tissue (within 5 cm from tumor tissue) were collected intraoperatively and stored at -80°C . A total of 5 ml peripheral venous blood was extracted before operation using heparin sodium anticoagulant blood collection tube and centrifuged at 2000 r/min for 10 min. The supernatant was stored at -20°C .

Real-time PCR

Total RNA was extracted from tumor tissue and adjacent tissue, and reverse transcribed to complementary DNA (cDNA). The primers were designed by Primer 6.0 and synthesized by Invitrogen/Life Technologies (Carlsbad, CA, USA). PSA, forward, 5'-CATGGTATGATGGACC-3', reverse, 5'-TTCGAAGTCGCACAGCAGCT-3'; miR-155, 5'-CGATGGTATATGGACC-3', reverse, 5'-TTCGAGCCGATACAGCAGCT-3'; GAPDH, forward, 5'-ACCAGGTATCTGCTGTGTT-3'; reverse, 5'-TAACCATGATGTCAGCGTGGTT-3'. Real-time PCR was performed at 55°C for 1 min, followed by 35 cycles of 55°C for 1 min, 92°C for 30 s, and 58°C for 45 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was selected as internal reference. The relative expression of mRNA was calculated by $2^{-\Delta\text{Ct}}$ method.

ELISA

The expression of human epidermal growth factor receptor 2 (HER2) in the serum was detected by ELISA. The supernatant was collected by centrifuging peripheral blood collected from each group according to the ELISA kit (R&D Systems, Minneapolis, MN, USA). The optical density (OD) of each well was measured by the microplate reader to establish the standard curve. The corresponding sample concentration was calculated.

Statistical Analysis

All data analyses were performed on SPSS 22.0 software (SPSS Inc., Armonk, NY, USA). All data were presented as mean $\pm$ standard deviation and compared by Student's *t*-test or one-way ANOVA. Tukey's post-hoc test was used for com-

Table I. Clinical information.

	Cases	%
Age (year)		
≤ 60	38	44.19
> 60	48	55.81
BMI (kg/m ²)		
≤ 23	36	41.86
> 23	50	58.14
Family history of prostate cancer (%)		
With	42	48.83
Without	44	51.17
Tumor size		
T1 (< 2 cm)	23	26.74
T2 (2-5 cm)	17	19.76
T3 (> 5 cm)	18	20.93
T4 (neoplasm invasiveness)	28	32.57
Histological grade		
Well	33	38.37
Moderate	25	29.07
Poor	28	32.56
TNM stage		
I-II	32	37.21
III-IV	54	62.79
LNM		
Without	34	39.53
With	52	60.47

paring measurement data between groups. Correlation analysis was performed using Pearson. $p < 0.05$ was depicted as statistically significant.

Results

Clinicopathological Characteristics

The clinicopathological features of prostate cancer patients were listed in Table I.

PSA Gene Expression in Prostate Cancer Patients

Real-time PCR was used to detect the expression of PSA gene in tumor tissues of patients with prostate cancer. The results showed that the expression of PSA gene in prostate cancer tissues was significantly higher than that in the adjacent tissues and the control group ($p < 0.05$, Figure 1).

Serum Levels of PSA in Prostate Cancer Patients

The level of PSA in the serum of patients with prostate cancer was detected by ELISA. The results demonstrated that the expression of PSA protein in the serum of patients with prostate cancer was significantly higher compared to that of normal control group ($p < 0.05$, Figure 2).

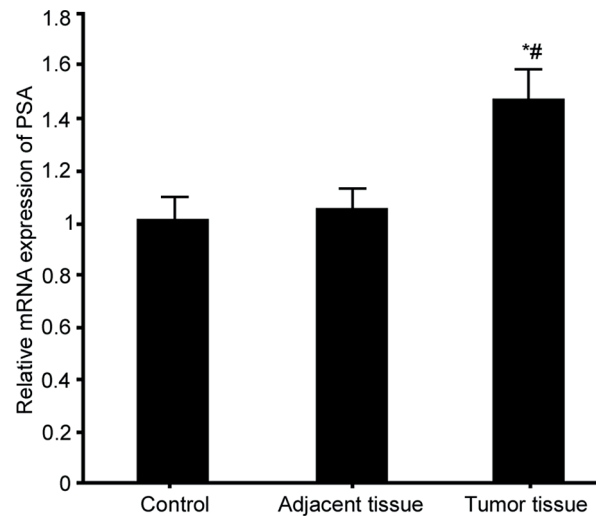


Figure 1. PSA gene expression in prostate cancer patients. * $p < 0.05$, compared with control, # $p < 0.05$, compared with adjacent tissue.

Analysis of miR-155 Expression in Patients with Prostate Cancer

Real-time PCR was adopted to analyze the expression of miR-155 in tumor tissues of patients with prostate cancer. The expression of miR-155 was significantly higher in prostate cancer tissues compared to that in adjacent tissues and controls ($p < 0.05$) (Figure 3).

PSA Gene and Protein Expressions in Prostate Cancer Patients with Different TNM Stages

The expression of PSA gene and protein in tumor tissue and serum of prostate cancer patients

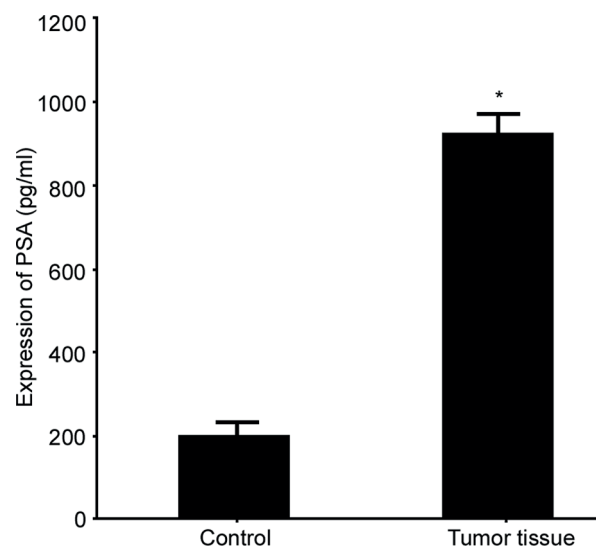


Figure 2. Serum PSA expression in prostate cancer patients. * $p < 0.05$, compared with control.

Table II. Correlation analysis of PSA and miR-155 with clinicopathological features.

	TNM stage	Tumor size	LNM	Histological grade
PSA	0.639*	0.521*	0.652*	-0.611*
miR-155	0.725*	0.421*	0.512	-0.361*

* $p < 0.05$.

with different TNM stages were detected by Real-time PCR and ELISA. The results exhibited that the expression of PSA gene and protein in tumor tissue and serum increased following TNM upstage ($p < 0.05$, Figure 4).

MiR-155 Expression in Prostate Cancer Patients with Different TNM Stages

Real-time PCR was selected to test the expression of miR-155 in tumor tissue and serum of prostate cancer patients with different TNM stages. The results revealed that the expression of miR-155 in tumor tissue and serum of prostate cancer patients were enhanced following with the increase of TNM staging ($p < 0.05$, Figure 5).

Correlation Analysis of PSA and miR-155 with Clinicopathological Features

PSA expression was positively correlated with TNM stage, tumor volume, and LNM, while negatively correlated with tissue grade ($p < 0.05$). The expression of miR-155 was positively correlated with TNM stage, tumor volume, and LNM, whereas negatively correlated with histological grade ($p < 0.05$, Table II).

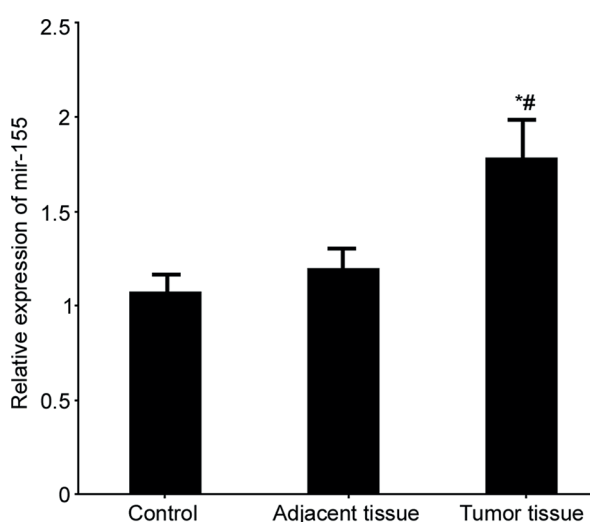


Figure 3. MiR-155 expression in prostate cancer patients. * $p < 0.05$, compared with control, # $p < 0.05$, compared with adjacent tissue.

Discussion

Prostate cancer is a malignant epithelial tumor that occurs in the prostate. It has become the most common cancer in male skin cancer and accounts for the second as the cancer death factor in male¹⁹. However, since prostate cancer is lack of evident symptom in the early stage, it is difficult to make the diagnosis²⁰. Serum PSA is a specific marker

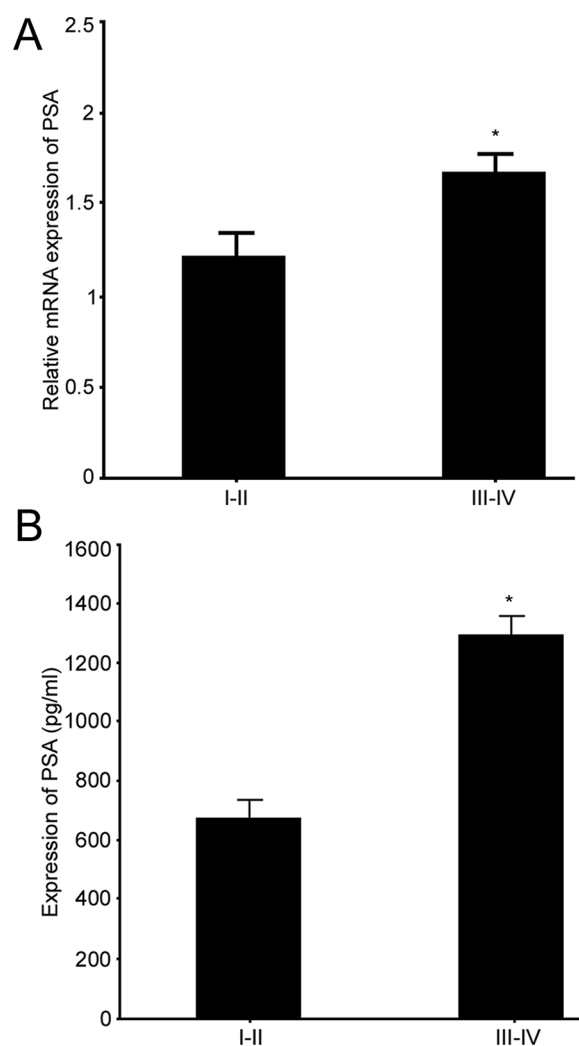


Figure 4. PSA gene and protein expressions in prostate cancer patients with different TNM stages. **A**, PSA gene expression. **B**, PSA protein expression. * $p < 0.05$, compared with stage I-II.

of prostate tissue. PSA is a serine protease and glycoprotein secreted by prostate epithelial cells that is secreted directly into the prostate ductal system^{21,22}. The normal function of PSA is to help semen clot hydrolysis and liquefaction, so as to regulate the male fertility²³. There is a blood-epithelial barrier around the normal prostate duct system, which prevents the PSA from entering the blood directly from the prostate epithelium, thereby maintaining a low concentration of PSA in the blood²⁴. Prostate cancer destroys the blood-epithelial barrier and increases the secretion of PSA, resulting in PSA directly entering the blood. The higher degree of malignancy, the greater damage on the normal and higher serum PSA²⁵. PSA rises in most of the prostate tissue with clinical significance. It is also one of the most important indicators for the early detection. Although PSA is the most commonly used method for the detection of prostate cancer, PSA-positive results also appear in benign prostatic hyperplasia and prostatitis. Moreover, it is still lack of specificity for the early diagnosis of prostate cancer²⁶.

MiRNAs are abnormal in the development of tumor and involved in the regulation of tumor²⁷. The specific expression of miRNA in peripheral blood can reflect the occurrence, progression, and severity of the disease. MiRNA has the characteristics of stability that cannot be degraded by RNase easily. In addition, it can be repeated the freezing and thawing for long-term storage. Moreover, it is stable in the plasma and serum, so that miRNA can be used as a disease serological molecular marker^{28,29}. It was shown that miR-155 overexpressed in prostate cancer, breast cancer, and other tumors^{17,18}. Therefore, this study explored the role of combined PSA and miR-155 in the early diagnosis of prostate cancer. We tested PSA gene and protein, and miR-155 expressions in patients with prostate cancer to analyze their correlations with clinicopathological features. TNM staging analysis exhibited that the expressions of PSA and miR-155 increased following TNM upstaging, suggesting that PSA and miR-155 were associated with the pathological features of prostate cancer. Further analysis revealed that PSA and miR-155 expressions were not related to age, BMI, family history, pathological type; positively correlated with clinical TNM stage, tumor size, and LNM; and negatively correlated with tissue grade, indicating that PSA and miR-155 detection can be used as a molecular target for the diagnosis and prognosis of prostate cancer. These results demonstrated that

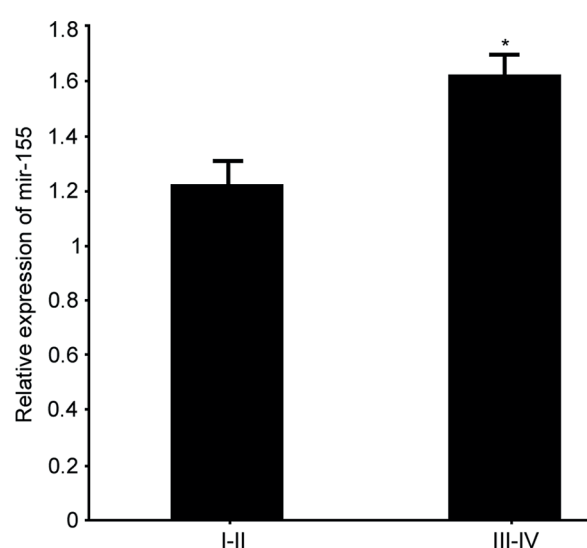


Figure 5. MiR-155 expression in prostate cancer patients with different TNM stages. * $p < 0.05$, compared with stage I-II.

the combination of PSA and miR-155 are early diagnostic indicators of prostate cancer. Further in-depth investigations are needed to discuss their mechanism in prostate cancer.

Conclusions

PSA and miR-155 are closely related to the clinicopathological features of prostate cancer. Combined detection is helpful to the early diagnosis of prostate cancer. Therefore, this study provides a new theoretical basis for the early diagnosis of prostate cancer.

Acknowledgments

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Conflict of Interest

The Authors declare that they have no conflict of interest.

References

- 1) HERSHMAN DL, UNGER JM. Adverse health effects of intermittent vs continuous androgen deprivation therapy for metastatic prostate cancer-reply. *JAMA Oncol* 2016; 2: 686-687.

- 2) ZHU BP, GUO ZQ, LIN L, LIU Q. Serum BSP, PSA-DT and Spondin-2 levels in prostate cancer and the diagnostic significance of their ROC curves in bone metastasis. *Eur Rev Med Pharmacol Sci* 2017; 21: 61-67.
- 3) McDONALD AM, JONES JA, CARDAN RA, SAAG KS, MAYHEW DL, FIVEASH JB. Combining computed tomography-based bone density assessment with FRAX screening in men with prostate cancer. *J Clin Densitom* 2016; 19: 430-435.
- 4) REPKA MC, GULERIA S, CYR RA, YUNG TM, KONERU H, CHEN LN, LEI S, COLLINS BT, KRISHNAN P, SUY S, DRITSCHILLO A, LYNCH J, COLLINS SP. Acute urinary morbidity following stereotactic body radiation therapy for prostate cancer with prophylactic alpha-adrenergic antagonist and urethral dose reduction. *Front Oncol* 2016; 6: 122.
- 5) LIAUW SL, KROPP LM, DESS RT, OTO A. Endorectal MRI for risk classification of localized prostate cancer: Radiographic findings and influence on treatment decisions. *Urol Oncol* 2016; 34: 416 e415-421.
- 6) BOURGUIGNON LY, SHIINA M, LI JJ. Hyaluronan-CD44 interaction promotes oncogenic signaling, microRNA functions, chemoresistance, and radiation resistance in cancer stem cells leading to tumor progression. *Adv Cancer Res* 2014; 123: 255-275.
- 7) ZHANG T, TSENG C, ZHANG Y, SIRIN O, CORN PG, LINING-TAPIA EM, TRONCOSO P, DAVIS J, PETTAWAY C, WARD J, FRAZIER ML, LOGOTHETIS C, KOLONIN MG. CXCL1 mediates obesity-associated adipose stromal cell trafficking and function in the tumour microenvironment. *Nat Commun* 2016; 7: 11674.
- 8) BASU S, MAJUMDER S, BHOWAL A, GHOSH A, NASKAR S, NANDY S, MUKHERJEE S, SINHA RK, BASU K, KARMAKAR D, BANERJEE S, SENGUPTA S. A study of molecular signals deregulating mismatch repair genes in prostate cancer compared to benign prostatic hyperplasia. *PLoS One* 2015; 10: e0125560.
- 9) WANG J, PARIS PL, CHEN J, NGO V, YAO H, FRAZIER ML, KILLARY AM, LIU CG, LIANG H, MATHY C, BONDADA S, KIRKWOOD K, SEN S. Next generation sequencing of pancreatic cyst fluid microRNAs from low grade-benign and high grade-invasive lesions. *Cancer Lett* 2015; 356: 404-409.
- 10) CHENG Z, CHOI N, WANG R, LEE S, MOON KC, YOON SY, CHEN L, CHOO J. Simultaneous detection of dual prostate specific antigens using surface-enhanced raman scattering-based immunoassay for accurate diagnosis of prostate cancer. *ACS Nano* 2017; 11: 4926-4933.
- 11) CALOPEDOS RJS, CHALASANI V, ASHER R, EMMETT L, WOO HH. Lutetium-177-labelled anti-prostate-specific membrane antigen antibody and ligands for the treatment of metastatic castrate-resistant prostate cancer: a systematic review and meta-analysis. *Prostate Cancer Prostatic Dis* 2017; 20: 352-360.
- 12) BURROWS T, RAY AA, HARTSBURG C. Prostate cancer screening practices amongst physicians in the North Simcoe Muskoka Local Health Integration Network. *Can J Urol* 2017; 24: 8734-8739.
- 13) SU XJ, ZENG XT, FANG C, LIU TZ, WANG XH. Genetic association between PSA-158G/A polymorphism and the susceptibility of benign prostatic hyperplasia: a meta-analysis. *Oncotarget* 2017; 8: 33953-33960.
- 14) SOORIAKUMARAN P, NYBERG T, AKRE O, WIDMARK A, HAMDY F, GRAEFEN M, CARLSSON S, STEINECK G, WIKLUND NP. Survival among men at high risk of disseminated prostate cancer receiving initial locally directed radical treatment or initial androgen deprivation therapy. *Eur Urol* 2017; 72: 345-351.
- 15) SHEN P, ZHAO J, SUN G, CHEN N, ZHANG X, GUI H, YANG Y, LIU J, SHU K, WANG Z, ZENG H. The roles of prostate-specific antigen (PSA) density, prostate volume, and their zone-adjusted derivatives in predicting prostate cancer in patients with PSA less than 20.0 ng/mL. *Andrology* 2017; 5: 548-555.
- 16) JUNG M, SCHAEFER A, STEINER I, KEMPENSTEFFEN C, STEPHAN C, ERBERSDOBLER A, JUNG K. Robust microRNA stability in degraded RNA preparations from human tissue and cell samples. *Clin Chem* 2010; 56: 998-1006.
- 17) JI H, LI Y, JIANG F, WANG X, ZHANG J, SHEN J, YANG X. Inhibition of transforming growth factor beta/SMAD signal by MiR-155 is involved in arsenic trioxide-induced anti-angiogenesis in prostate cancer. *Cancer Sci* 2014; 105: 1541-1549.
- 18) MCINNES N, SADLON TJ, BROWN CY, PEDERSON S, BEYER M, SCHULTZE JL, MCCOLL S, GOODALL GJ, BARRY SC. FOXP3 and FOXP3-regulated microRNAs suppress SATB1 in breast cancer cells. *Oncogene* 2012; 31: 1045-1054.
- 19) DI FRANCO CA, JALLOUS H, PORRU D, GILIBERTO GL, CEBRELLI T, TINELLI C, ROVERETO B. A retrospective comparison between transrectal and transperineal prostate biopsy in the detection of prostate cancer. *Arch Ital Urol Androl* 2017; 89: 55-59.
- 20) YUKSEL M, KARMIK K, ANIL H, ISLAMOGLU E, ATEŞ M, SAVAS M. Factors affecting surgical margin positivity in robotic assisted radical prostatectomy. *Arch Ital Urol Androl* 2017; 89: 71-74.
- 21) CHEN Y, LIN Y, NIE P, JIANG W, LIU Y, YUAN R, LI M, ZHAO S, LIN H, LI P, ZHANG J, HU Z, XU J, ZHU X. Associations of prostate-specific antigen, prostate carcinoma tissue Gleason score, and androgen receptor expression with bone metastasis in patients with prostate carcinoma. *Med Sci Monit* 2017; 23: 1768-1774.
- 22) WU J, CHENG G, ZHANG C, ZHENG Y, XU H, YANG H, HUA L. Long noncoding RNA LINC01296 is associated with poor prognosis in prostate cancer and promotes cancer-cell proliferation and metastasis. *Onco Targets Ther* 2017; 10: 1843-1852.
- 23) NAJEEB MA, AHMAD Z, SHAKOOR RA, MOHAMED AMA, KAHRAMAN R. A novel classification of prostate specific antigen (PSA) biosensors based on transducing elements. *Talanta* 2017; 168: 52-61.
- 24) LI S, SHI M, ZHAO J, ZHANG L, HUANG Y, ZHAO S. A highly sensitive capillary electrophoresis immunoassay strategy based on dual-labeled gold nanoparticles enhancing chemiluminescence for the detection of prostate-specific antigen. *Electrophoresis* 2017; 38: 1780-1787.

- 25) SITU J, ZHANG H, LU L, LI K, HU C, WANG DJ. Clinical significance of PSMA, TERT and PDEF in malignant tumors of the prostate. *Eur Rev Med Pharmacol Sci* 2017; 21: 3347-3352.
- 26) NAKAI Y, TANAKA N, ANAI S, MIYAKE M, HORI S, TATSUMI Y, MORIZAWA Y, FUJII T, KONISHI N, FUJIMOTO K. Transperineal template-guided saturation biopsy aimed at sampling one core for each milliliter of prostate volume: 103 cases requiring repeat prostate biopsy. *BMC Urol* 2017; 17: 28.
- 27) BAUTISTA-CARO MB, ARROYO-VILLA I, CASTILLO-GALLEGO C, DE MIGUEL E, PEITEADO D, PLASENCIA-RODRIGUEZ C, VILLALBA A, SANCHEZ-MATEOS P, PUIG-KROGER A, MARTIN-MOLA E, MIRANDA-CARUS ME. Decreased frequencies of circulating follicular helper T cell counterparts and plasmablasts in ankylosing spondylitis patients Naive for TNF blockers. *PLoS One* 2014; 9: e107086.
- 28) LAI NS, YU HC, CHEN HC, YU CL, HUANG HB, LU MC. Aberrant expression of microRNAs in T cells from patients with ankylosing spondylitis contributes to the immunopathogenesis. *Clin Exp Immunol* 2013; 173: 47-57.
- 29) GUO ML, GUO LL, WENG YQ. Implication of peripheral blood miRNA-124 in predicting acute myocardial infarction. *Eur Rev Med Pharmacol Sci* 2017; 21: 1054-1059.