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Clinical significance of PSMA, TERT and PDEF in malignant tumors of the prostate

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Abstract. – OBJECTIVE: To investigate the clinical significance of the expression of PSMA (prostate specific membrane antigen), TERT (telomerase reverse transcriptase), and PDEF (prostate derived Ets factor) in malignant tumors of the prostate.

PATIENTS AND METHODS: The study was conducted with paraffin slices from 33 specimens of malignant tumors of the prostate and 17 of normal tissue. We found high levels of PSMA, TERT, and PDEF protein by Western blot and immunofluorescence in the malignant tumor prostate. We also detected upregulation MA, TERT, and PDEF mRNA in the malign mor of the prostate, suggesting complex re tion of these three genes in prostate cancer.

RESULTS: Variance analysis showed statis cally significant differences c g the ex pression of PSMA, TERT, and e malig nant tumor of the prostate norm issues. The high expression of P , TERT PDEF in the malignant tumor prost the important roles of hes tors in the occurrence and dev ment malignant tumors of the prosta

CONCLUSION MA, TERT, a F may serve as a refe inical diagno s and as potential targ for t ignant tumor of the prostate th peutics.

Key Wo
PSM ERT, P Malignant tumor of the prostate ression s.

Introduction

T rise in mo bidity and mortality of malig na e prostate in recent years make a y ying effective markers that enable y and accurate diagnosis and intervention[1,2]. te recent efforts, the pathogenesis of malig tumors of the prostate is still unclear, and effective treatments are no available[3]. Here, we dete the sion levels for three relevant tumor markers MA (Prostate Specific Mem Antigen), T Telomerase Reverse T ase) and PDEF rostate Derived Ets or) – by different techniques in malignant ors of the pr e and normal prostate tissue. high expres of PSMA, TERT, and PDEF i F malig tumor of the prostate at the prot A levels suggests their important roles development of malignant tumors f the prostate. This information can be relevant inical diagnosis and treatment of prostate

Patients and Methods

Collection of Tumors

We collected 33 specimens of malignant tumors of the prostate and 17 of normal prostate tissue in our hospital. They were fixed in neutral formalin (10%) for over 48 h, embedded in paraffin, and cut into the 5 μm slices. The Ethic Committee of the Third Affiliated Hospital approved this study.

Immunofluorescence

The tissue slices were de-waxed by dimethylbenzene several times and then dehydrated by ethanol gradient for antigen retrieval. After the slices were washed 3 times (5 min/time) with PBS (pH = 7.4), they were blocked in 10% bovine serum albumin (BSA) in a humidity chamber at 37°C for 30 min. Then, the slices were washed in phosphate-buffered saline (PBS) (pH = 7.4) once to remove excess of bovine serum albumin (BSA). Then, the primary antibody (1:70) was added into the slices and incubated in the humidity chamber at 4°C overnight. The next day, we

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washed the slices three times (5 min/time) with PBS and added the fluorescent secondary antibody in the dark and incubated in the humidity chamber at 37°C for 2 h. After the slices were washed with the secondary antibody, they were sealed in glycerol for observation under the fluorescence microscope. mAb anti-β-Actin, anti-PSMA, anti-TERT, and anti-PDEF, as well as fluorescence secondary antibody, were obtained from Cell Signaling Technology (Danvers, MA, USA).

RT-PCR Detection

Two samples (50 mg) from the malignant tumor of the prostate and normal tissues were rapidly transferred to 1 mL of Trizol (Tiangen Biotech, Beijing, China) and homogenized. After 5 min at the room temperature, the homogenate was centrifuged at 12,000 × g and 4°C for 15 min. The supernatant was mixed with chloroform. After 5 min at the room temperature, the mixture was centrifuged at 12,000 × g at 4°C for 15 min. Then, the same volume of isopropanol was added to the mixture. After 10 min at the room temperature, the mixture was centrifuged at 12,000 × g and 4°C for 10 min and the pellet was collected. We mixed the pellet with (75%) to wash the RNA. Then, it was dissolved in RNase-free water. The concentration of RNA and the ratio of OD_{260}/OD_{280} were determined. Finally, the mRNA for PSMA, TERT, and PDEF were amplified using the primers in Table I. The products were used for RT-PCR analysis (Tiangen Biotech, Beijing, China).

Western Blot

Two tissue samples (50 mg) were extracted from the malignant tumor of the prostate and normal tissues, washed with physiological saline, and homogenized with lysis buffer. Then, the homogenates were centrifuged at 12,000 × g at 4°C for 10 min, and the supernatant was centrifuged again under the same conditions, and then we collected the supernatant. The protein concentration was assayed by the BCA protein assay kit (Beyuntian, Beijing, China) and the samples were preserved at -80°C. The extracts were mixed with 2X loading buffer (100 µl + 4 µl-mercaptoethanol) at a volume ratio of 1:1, the mixture was boiled in boiled water for 5 min, and then preserved in refrigerator. The separating gel (5% stacking gel were prepared according to the molecular weight of the target protein. The homogenates were loaded on the gel, and the electrophoresis was performed under a constant voltage of 220 V until the bromophenol blue reached the bottom of the gel. The gel was then cut into pieces according to the molecular weight of the target proteins and placed in transfer buffer. The polyvinylidene fluoride (PVDF) membrane was cut into the shape of the gel and was placed into the transfer buffer after being soaked in methanol for 10 s. Next, the membrane transfer was performed at a constant voltage of 100 V. The PVDF membrane was blocked with 5% skim milk powder for 3 h at room temperature on the shaker. The membranes were incubated with the primary antibodies at 4°C overnight. After three washes in TTBS (10 min/time), the membrane was incubated with the secondary antibody on the shaker at a room temperature for 2 h and washed three times in TTBS (10 min/time). The mixed solution of reagents A and B in the electrochemiluminescence (ECL) assay kit were added onto the PVDF membranes for complete reaction. After 1 min of color development in the dark, the mixture was put into the gel imager and photographed using dynamic calculus pattern, and the gray analysis was performed on the protein strips using Gel-Pro 4.0.

Table I. RT-PCR primer sequence of EGF and TGF-α mRNA.

Name of gene	Primer sequence
PSMA	5'-3' ACATGGAAGCCATCACAGAC
	3'-5' AGACCGTTCAGCTGGATATTA
TERT	5'-3' ACCCCTTCCGGTCCCCGGCCC
	3'-5' GAGTTTCCGGCAGCGGCCA
PDEF	5'-3' TTGACAGCGACAAGAAGTGG
	3'-5' TCACGTCGTCCTTATGCAAG
β-Actin	5'-3' GAGCCGGGAAATCGTGCGT
	3'-5' GGAAGGAAGGCTGGAAGATG

Statistical Analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis of results. The experimental data were presented as mean ± standard deviation (Mean ± SD). *t*-test, *p*-test, and One-Way ANOVA variance analysis were performed in-group comparisons. $p < 0.05$ suggested that the differences were statistically significant.

Results

Pathological Slices

The glands were in back to back arrangement, and the original basal cells significantly disap-

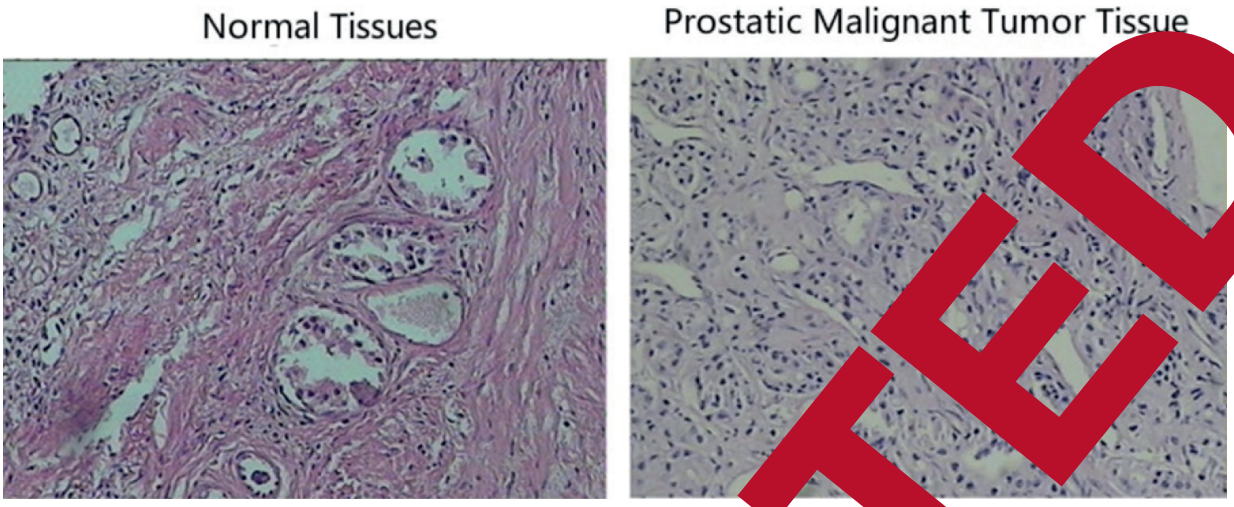

Figure 1. Pathological results of the normal prostatic tissues and the malignant tumor of th tic tissues.

peared in the malignant tumor of the prostatic tissues (Figure 1). Compared with the slices of normal prostatic tissues, gradient vacuolization of nuclei and significantly infiltrated interstitial were identified in the malignant tumor of the prostatic tissues (Figure 1). Further we found that the nuclei were mutated glands were distributed irregularly, inst of the original arrangement.

Immunofluorescence of PSMA, TERT, and PDEF in Prostatic Tis

Fluorescent expression o MA, RT, and PDEF was identified in t aligna or of prostatic tissues, but not in the normal tis- s (Figure 2). itionally, PSMA, TERT, and R were high xpressed in the malignant tu- the pros tissues. The result indicated the s of PSMA, TERT, and PDEF in malig tumor of the prostate.

Levels of PSMA, TERT, and n Prostatic Tissue

To examine the protein expression of PSMA, TERT, and PDEF by a more quantitative method, we conducted Western blot analysis. The protein levels of PSMA, TERT, and PDEF were highly elevated in the malignant tumor of

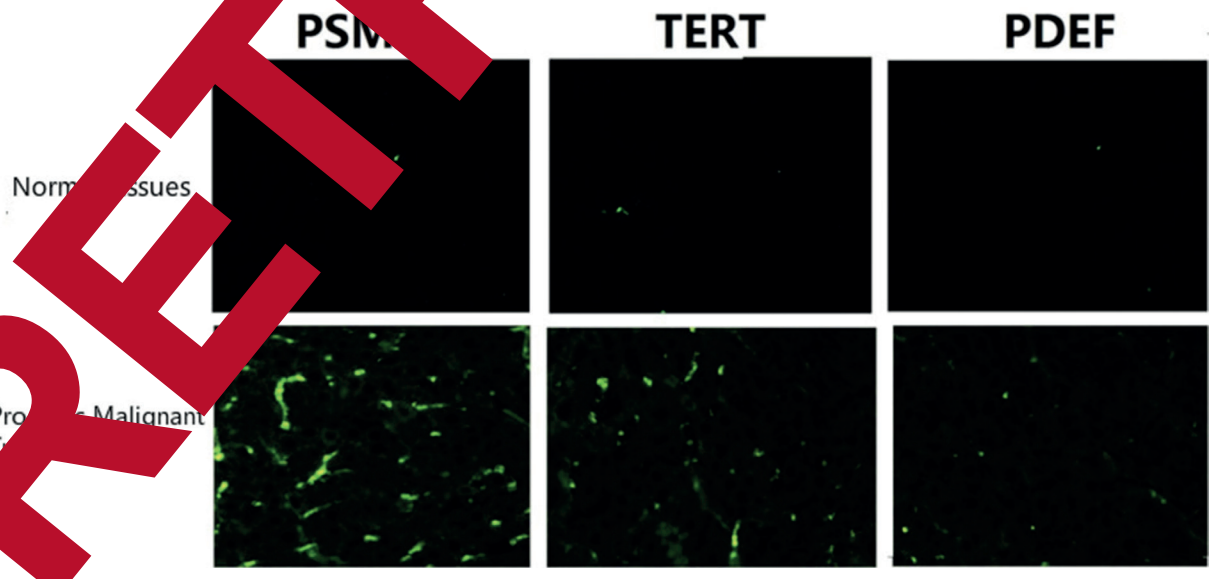

Figure 2. Expression of PSMA, TERT and PDEF in the normal tissues and the malignant tumor of the prostatic tissues (×400).

the prostatic tissues, but very lowly expressed in the normal tissues (Figure 3). Each protein was elevated more than ten times in prostate tumors compared to normal prostatic tissue, supporting the strong signal observed by immunofluorescence.

mRNA Levels of PSMA, TERT, and PDEF in Prostatic Tissue

After observing the marked upregulation of PSMA, TERT, and PDEF at the protein level, we decided to analyze the expression of PSMA, TERT, and PDEF mRNA to understand how these genes are regulated. PSMA, TERT, and PDEF mRNA were significantly increased in malignant tumor of the prostatic tissues compared to the normal prostate (Figure 4). PSMA showed the highest upregulation of the three genes analyzed (Figure 4). These observations are consistent with the elevated levels of PSMA, TERT, and PDEF at the protein level and suggest that both transcriptional and posttranscriptional mechanisms are involved in the upregulation of PSMA, TERT, and PDEF in malignant tumors of the prostate.

Discussion

Prostatic tumors are the most common [illegible] lignant tumors in males and the [illegible]nd cause [illegible] male cancer deaths[4]. Becaus[illegible] prostatic tumors have become an in[illegible]tant [illegible]h issue as the male population [illegible]nues t[illegible][5]. Unfortunately, the molecular mechanisms leading to the prostatic tumor and the developm[illegible]into malignant phenotype are mostly unk[illegible] uncertain disease course, as well [illegible]e heter[illegible] geneity of the disease, hinders [illegible] research to better understand the occurre[illegible]f prostatic tumor. Also, this situation compli[illegible]the research with clinical spec[illegible]s or stu[illegible]th progression of the pro[illegible]e tumor over [illegible] tain period[7-9]. In rec[illegible]ears, t[illegible]ncidence of prostatic diseases [illegible]ncrea[illegible] contin[illegible]ously, and their progr[illegible]sio[illegible]alignan[illegible]mors makes them a[illegible]ous [illegible]conc[illegible]. Older males repres[illegible]he largest p[illegible]of prostatic tumo[illegible]h are usua[illegible]ccompanied by symp[illegible]s li[illegible]aturia and dysuria[11]. The diagnosis of prosta[illegible]or mainly depends on imag[illegible]rostatic biop[illegible]d the pathological e[illegible]n of specimens obtained during surgery[12-14]. In recent years, most studies showed [illegible] PSMA, TE[illegible] and PDEF play important [illegible] in the de[illegible]pment of prostatic tumors. P[illegible] TERT [illegible] PDEF are expressed at low leve[illegible]ssues, but are upregulated in tumors, [illegible]uding prostatic tumor[15,16]. PSMA can serve as a therapeutic target for the immu[illegible] treatment of dendritic cells, and the [illegible] of PSMA gene can also be used as the cytotoxic reagent mediated into the prostatic tumor cells[17]. Normal cells transfected with TERT can be immortalized without any transformation in the phenotype of nuclei. TERT can maintain the length of telomeres and is believed to be

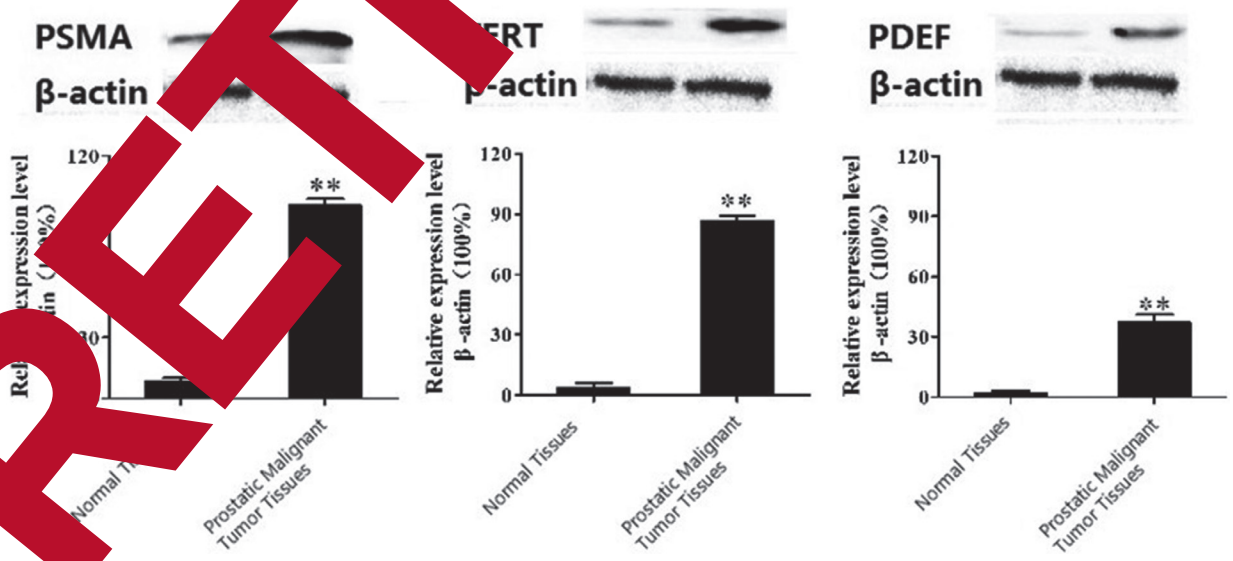

Figure 3. Expression of PSMA, TERT and PDEF protein in the malignant tumor of the prostatic tissues and normal tissues.

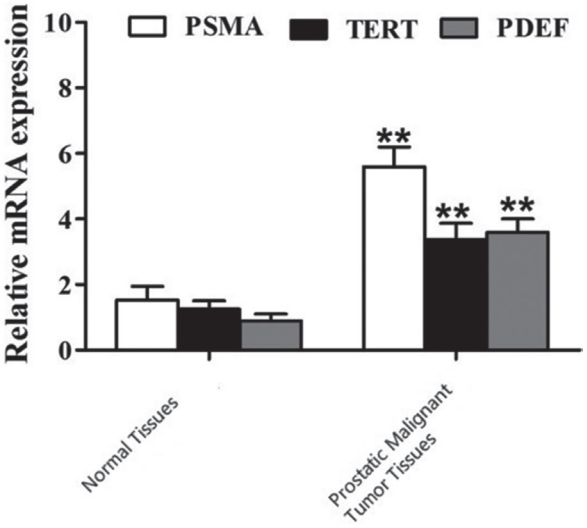

Figure 4. mRNA expression of PSMA, TERT and PDEF in the malignant tumor of the prostatic tissues and normal

associated with the aging process and tumors. The TERT expression can only be detected in cells with telomerase activity, but cannot be detected in most benign tissues[18]. TERT can endow immortality to cells, which is a key step in the transformation of malignant tumors. Thus, infiltrated tumor cells can be detected with high sensitivity using TERT from benign tissues. The role of PDEF is very important in hematopoiesis, angiogenesis, genesis, and neural connection[19]. Normal differentiation of epithelial cells in the prostate and the initial stages of the prostatic tumors are dependent on the testosterone, but the final stage of prostatic tumor becomes almost hormone independent and resistant to hormone therapies.

Conclusions

Our study showed high expression of PSMA, TERT and PDEF in the prostatic malignant tumor tissues, suggesting the important roles of PSMA, TERT, and PDEF in the development of the malignant tumor of the prostate. Therefore, PSMA, TERT and PDEF can serve as potential targets for the treatment of malignant tumor of the prostate and new reference for clinical diagnosis and treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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