Relationship of common variants in WWOX gene with susceptibility and prognosis of Prostate cancer

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Research

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Abstract

Background

The aim of the study was to determine the expression of WW domain containing oxidoreductase (*WWOX*) in patients with prostate cancer (PCa) and to evaluate the correlation of *WWOX* expression and patients survival.

Methods

Immunohistochemistry (IHC) was applied to detect the *WWOX* expression. Chi-square test was adopted to evaluate the relationship between *WWOX* expression and clinical features of PCa patients. In addition, Kaplan-Meier curve was made to estimate the survival rate of PCa patients. Multivariate analysis was performed to assess the statistical significance between *WWOX* expression and prognosis of PCa patients.

Results

WWOX was weakly expressed in PCa tissues compared to the paired normal tissues by IHC. *WWOX* expression was tightly associated with Gleason score, PSA and clinical staging ($P < 0.05$), but has no relationship with age, tumor column, distant metastasis and ALP ($P > 0.05$). Survival curve demonstrated that patients with negative *WWOX* expression had lower survival rate. Finally, Cox analysis illustrated that *WWOX* was related with the prognosis of PCa patients ($P = 0.001$, HR = 4.605, 95% CI = 1.814–11.690).

Conclusion

In short, the present study showed strong evidence that *WWOX* could act as an indicator for prognosis of PCa patients.

Background

Prostate cancer (PCa) is one of the most common non-dermatologic cancers [1–3] and mainly occurs in economically developed countries [4]. And it is also one of the leading causes of cancer deaths among men all over the world. The majority of PCa were diagnosed as localized disease [5, 6]. Recently, the incidence of PCa has increased and perhaps will remain so in the foreseeable future. PCa is initiated by androgen receptor signaling pathway [7, 8]. Therapies for PCa are selected according to such factors as age, body condition and Gleason score, including transurethral prostate resection,androgen deprivation, radiotherapy and endocrine therapy [9, 10]. Because most patients are diagnosed with advanced stage, treatments on patients have little effects. Therefore, finding a biomarker for therapy and prognosis of PCa is quite important.

WW-domain containing oxidoreductase (*WWOX*) locates at chromosome 16q23.3- 24.1 and encodes a protein of 46 kD, which is an oxidoreductase containing two WW domains [11–13]. It has been determined that *WWOX* gene could spans the common chromosomal fragile site region FRA16D [14, 15]. *WWOX* is important for UV, TNF, staurosporine and p53-mediated cell deaths. Growing evidence has proved that *WWOX* played an important role on neurodevelopment, bone metabolism and tumor suppression [16–18]. Besides, restored expression of *WWOX* in lung and breast cancer cells resulted in notable caspase-mediated apoptosis, growth inhibition and blocked tumor development [19, 20]. Loss or reduced expression of *WWOX* was observed in different cancers, such as ovarian cancer, breast cancer, hepatocellular carcinoma, gastric cancer and non-small cell lung cancer [21–23]. In the present study, we detected the *WWOX* expression in PCa tissues to explore the potential biological role of *WWOX.*

Materials And Methods

Patients and specimens

A total of 101 patients who were pathologically diagnosed with PCa were selected from Urology Surgery of Beijing Tsinghua Changgung Hospital. Clinical data of patients, including age, PSA, alkaline phosphate (ALP), distant metastasis, clinical staging, Gleason score and tumor column, were obtained from the patients' initial history. Our study was approved by the Ethics Committee of Beijing Tsinghua Changgung Hospital and all patients were asked to sign the confirmed consents before surgery.

Immunohistochemistry

WWOX protein expression was determined by immunohistochemistry (IHC). The fresh tissues were continuously cut into sections of 2 μm and fixed on glass slides. The sections were dewaxed with xylene and rehydrated with graded alcohol after baking at 70℃ for 2 h. Citrate buffer (0.01M, pH = 6.0) was used for antigen retrieval. Then the primary antibody was applied to the sections overnight at 4℃. Subsequently, the sections were incubated with the second antibody at 37℃ for 2 h. Finally, staining was developed with DAB. The tissues were manually divided into two groups according to the staining percentage of cells (0 to 100%). Sections with cell staining percentages of more than 40% were clarified as positive, and the others were clarified as negative.

Statistical analysis

The relationship of clinical features and *WWOX* expression was evaluated with Chi-square. The overall survival rate of PCa patients was determined according to the Kaplan-Meier curve. Cox regression analysis was adopted to confirm the correlation between *WWOX* expression and prognosis of PCa patients.

Results

Lowexpression of *WWOX* in PCa tissues

The expression of *WWOX* was measured by IHC method in PCa tissues and the paired normal tissues. Low expression of *WWOX* was observed in the PCa tissues compared to the normal ones. The positive rates of *WWOX* were 28.7% (29 out of 101) in PCa tissues and 85.1% (86 out of 101) in the paired normal tissues. PCa tissues exhibited significantly lower *WWOX* expression than the paired normal tissues ($P < 0.001$, Table 1).

Table 1
Different expression of *WWOX* in PCa tissues and the paired normal tissues.

Tissues	Case NO.	Expression	Positive rate	*P* value
		Positive Negative		
PCa	101	29 72	28.7%	< 0.001
Normal	101	86 15	85.1%	

Relationship between *WWOX* expression and clinical features of PCa patients

We then estimated the potential relationship of *WWOX* expression and clinical features of PCa patients. The result was detailed in Table 2. *WWOX* expression shared statistical significance with the following features: Gleason score, PSA and clinical staging ($P < 0.05$). However, no correlation was found between the expression of *WWOX* and such clinical features as age, tumor column, distant metastasis and ALP ($P > 0.05$).

Table 2
Relationship of *WWOX* expression and clinical features of PCa patients.

Clinical features	Case NO.	Expression	χ2	*P*
		Negative Positive		
Age			0.395	0.530
≦ 60	37	25 12		
> 60	64	47 17		
ALP			0.954	0.329
Negative	53	43 13		
Positive	48	32 16		
Tumor column (ml)			0.847	0.358
≦ 0.5	45	30 15		
> 0.5	56	42 14		
Gleason score			5.281	0.022
≦ 7	53	43 10		
> 7	48	29 19		
PSA (ng/ml)			5.290	0.021
≦ 9	55	34 21		
> 9	46	38 8		
Distant metastasis			0.228	0.633
Yes	56	41 15		
No	45	31 14		
Clinical staging			6.324	0.012
$T_1 + T_2$	43	25 18		
$T_3 + T_4$	58	47 11		

Correlation of *WWOX* expression and the prognosis of PCa patients

Survival curve was plotted according to *WWOX* expression by Kaplan-Meier (Fig. 1). During the follow-up, 43 (59.7%) patients with negative *WWOX* expression died, while only 6 (20.7%) patients died among those who of positive *WWOX* expression. Patients with negative *WWOX* expression were more likely to die

than those with positive *WWOX* expression. Multivariate analysis revealed that *WWOX* could serve as a statistically significant prognostic factor by Cox regression analysis (*P* = 0.001, HR = 4.605, 95% CI = 1.814–11.690, Table 3).

Table 3
Multivariate analysis for prognostic factors in PCa.

Clinical feature	*P* value	HR	95%CI
Distant metastasis	0.100	0.522	0.241–1.131
PSA	0.161	1.753	0.800-3.843
Clinical staging	0.102	1.830	0.887–3.775
WWOX expression	0.001	4.605	1.814–11.690

Discussion

PCa is one of the major causes for cancer deaths of men in Europe and America. The incidence of PCa is positively related with age and is remarkably different in regions and races. The pathogenesy of PCa remains unclear. Studies from such Northern Europe countries as Denmark and Finland demonstrate that PCa derives from gene mutation to a great extent. It is also clarified that PCa might be associated with the environment.

WWOX is a newly discovered gene that participates in many processes of various cancers. Exon loss, heterozygosity absence and abnormal protein expression of *WWOX* were frequently present at different cancers, such as breast cancer, prostate cancer, ovarian cancer and esophageal cancer [24, 25]. It has been certified that *WWOX* usually behaves as a suppressor of tumor growth. In this study, we assayed its expression in PCa and evaluated the correlation between its expression and the prognosis of PCa patients.

WWOX has been confirmed to be downregulated in different cancers. Jentai Lin et al. studied that downregulation of *WWOX* was found in RCC [26]. Yachun Huang et al. revealed that *WWOX* was downregulated in human uroepithelial cells [27]. We first measured the expression of *WWOX* in PCa tissues and the paired normal tissues at protein level by HIC in this study. The result showed that the *WWOX* expression was significantly lower in PCa tissues than that in the paired normal tissues, which was in accordance with the existing literature. Then the relationship of *WWOX* expression and the clinical features of PCa patients was evaluated. Significant difference was found from the result, indicating that *WWOX* might serve as a prognostic factor for PCa patients. Based on the previous presumption, further investigations were performed to estimate the correlation between *WWOX* expression and the prognosis of PCa patients. The multivariate analysis displayed statistical significance between them, suggesting that *WWOX* was an prognostic indicator for PCa patients.

Though the correlation of *WWOX* expression and the prognosis was evaluated in this study, the concrete mechanism of *WWOX* on PCa was still unclear. In recent years, lots of studies have reported that *WWOX* functioned on various cancers by different approaches. Ekizoglu S et al. manifested that *WWOX* developed genetic alterations in breast cancer [28]. Anwen Xiong et al. explained that *WWOX* inhibited breast cancer cell growth by modulating the hedgehog-GLI1 signaling pathway [29]. In addition, Lin JT et al. demonstrated that *WWOX* suppressed the PCa cells through mediating the cell cycle with cyclin D1 [30]. All these reports can provide rationales for our future study.

Conclusions

Generally speaking, *WWOX* is a suppressor for PCa and is weakly expressed in PCa tissues. The result of univariate and multivariate analyses illustrated that *WWOX* might act as an prognostic indicator for PCa patients.

Abbreviations

WW domain containing oxidoreductase (*WWOX*)

prostate cancer (PCa)

Immunohistochemistry (IHC)

alkaline phosphate (ALP)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Beijing Tsinghua Changgung Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

S.C., Y.T. and B.X. conceived and designed the experiments; W.H. and Q.W. conceived and performed the experiments; B.S. and Y.L. prepared figures. M.F. and J.L. wrote the main manuscript text. All authors reviewed the manuscript.

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Figures

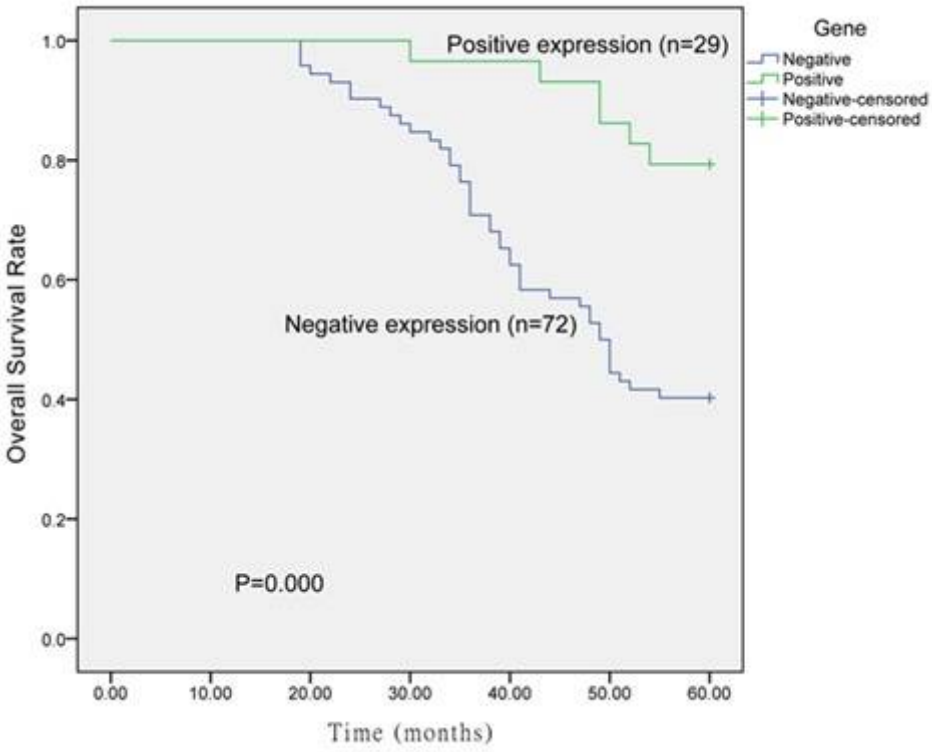

Figure 1

Kaplan-Meier survival curves were made for PCa patients. Patients with negative WWOX expression had higher mortality than those with positive expression. P value was calculated by Log-Rank test.