# *SOX9* stands for a bio-marker for prostate cancer detection

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## Abstract

# Background

Prostate cancer is one of common cancers around the world, and in our country the incidence and mortality of PCa are both increasing. More and more reports have revealed that *SOX9* is involved in various human cancers. In this study, we aimed to explore the relationship between *SOX9* expression and diagnostic value of PCa patients.

# Methods

In this study, quantitative real-time PCR (qRT-PCR) was performed to determine the expression of *SOX9* of the 131 PCa patients and 74 healthy volunteers. And receiver operating characteristic (ROC) curve was used to determine the diagnostic value of *SOX9* for PCa patients.

# Results

The results of qRT-PCR showed that the expression of serum *SOX9* in PCa patients was higher than that in healthy controls ($P < 0.05$). And the expression of *SOX9* was significantly associated with PSA ($P = 0.001$), differentiation ($P = 0.000$), and lymph node metastasis ($P = 0.000$). Besides, the area under the ROC curve (AUC) was 0.966 with the sensitivity of 93.2% and specificity of 87.8% respectively. The optimal cutoff value of *SOX9* was 2.34.

# Conclusions

Our results found that *SOX9* is a novel oncogene for PCa, and may be a novel and effective biomarker for the diagnosis of patients with PCa.

## Background

Prostate cancer (PCa) is one of the most common cancers in male genitourinary system. And in the worldwide, the incidence of PCa is at the second of all the malignant tumors only after lung cancer in incidence of elderly men [1]. PCa is a clinically heterogeneous multifocal disease, and the incidence is continuously rising. The carcinogenesis and mechanisms of PCa is multistep process. Because there are no obvious characteristics at the early stages, most PCa patients have already been at advanced stages or accompanied by metastasis when they are diagnosed. Moreover, although prostate-specific antigen (PSA) has been used for the earlier detection of clinically localized PCa, the PSA testing has low specificity for PCa [2–5]. Thus there are no reliable predictors of PCa behavior and aggressiveness. So finding effective biomarkers to achieve early diagnosis and treatment has become very important for PCa patients.

*SOX9* belongs to the SRY-related HMG box (SOX) family of developmental transcription factors [6]. As DNA binding proteins, SOXs widely exist in multiple tissues in the process of embryonic development [7, 8]. In mammals, *SOX9* is been widely studied. *SOX9* has a decisive role for sex determination and the development and maturation of bone, testicular, kidney, heart, brain and pancreas [9–14]. The regulation of *SOX9* can occur in multiple links, including transcription, translation, posttranslational modification, nuclear transport and the interaction of coenzyme.And In addition, it was reported that *SOX9* was associated with several human cancers. For example, gastric cancer, non-small cell lung cancer and pancreatic cancer [15–17] What's more, *SOX9* has also been reported in prostate cancer, that it was required for cancer initiation and contribute to PCa tumor growth and invasion [18]. However, *SOX9*there are few reports about the correlation of *SOX9* expression with the diagnosis of PCa patients.

In the current study, we aimed to detect the serum expression level of *SOX9* in PCa patients with qRT-PCR method and to investigate the association between *SOX9* expression and diagnosis of PCa patients.

# Methods

Patients and specimens collection

All of 131 PCa cancer patients confirmed by pathologists were finally recruited in the study from Huaihe Hospital of Henan University. 74 healthy volunteers were collected as the control group, and no one had been diagnosed with any malignancy in the group. Our study was approved by the Ethics Committee of Huaihe Hospital of Henan University, and the written consents were also obtained from all participants. The clinicopathological features of the PCa patients were listed in Table 1, including age, tumor size, PSA, differentiation, TNM stage, and lymph node metastasis. The serum samples were taken from all the 131 PCa patients and 74 healthy volunteers, and the samples were collection before patients accepting any surgery and therapy. Then all the collected serum samples were put into blood collection tube of EDTA and stored at -80℃ until use.

Table 1
Association of *SOX9* expression with clinicopathological features of PCa patients

| Characteristics | Number (n = 131) | *SOX9* Expression | | $\chi^2$ | *P* value |
|---|---|---|---|---|---|
| | | Low(n = 60) | High(n = 71) | | |
| Age (years) | | | | 0.525 | 0.469 |
| ≥ 65 | 61 | 30 | 31 | | |
| < 65 | 70 | 30 | 40 | | |
| Tumor size (cm) | | | | 0.073 | 0.787 |
| ≥ 3 | 66 | 31 | 35 | | |
| < 3 | 65 | 29 | 36 | | |
| PSA (ng/ml) | | | | 13.817 | 0.001* |
| ≤ 10 | 50 | 22 | 28 | | |
| 10–20 | 39 | 10 | 29 | | |
| > 20 | 42 | 28 | 14 | | |
| Differentiation | | | | 17.185 | 0.000* |
| Well | 54 | 14 | 40 | | |
| Moderate | 41 | 21 | 20 | | |
| Poor | 36 | 25 | 11 | | |
| TNM stage | | | | 0.486 | 0.486 |
| I + II | 72 | 31 | 41 | | |
| III + IV | 59 | 29 | 30 | | |
| Lymph node metastasis | | | | 15.030 | 0.000* |
| Negative | 79 | 47 | 32 | | |
| Positive | 52 | 13 | 39 | | |

RNA extraction and qRT-PCR

Total RNA was isolated with Trizol (Invitrogen) reagent. Then the first-strand cDNA was synthesized by Primescript™ RT reagent Kit (Takara, Japan). In this study the expression of *SOX9* was determined using the SYBR Green® Realtime PCR Master Mix (QPK-201, Toyobo Co, Ltd, Osaka, Japan). The primer sequences of *SOX9* and *β-actin* were as follows: *SOX9* forward: 5'-AGCGAACGCACATCAAGAC-3' and reverse: 5'-GCTGTAGTGTGGGAGGTTGAA-3', *β-actin* forward: 5'-GGTGGCTTTTAGGATGGCAAG-3', reverse:

5’-ACTGGAACGGTGAAGGTGACAG-3’. *β-actin* was used as an internal control. The relative expression level of *SOX9* was normalized to *β-actin* and calculated using the $2^{-\Delta\Delta Ct}$ method.

# Statistical analysis

All statistical analyses were performed with software SPSS 19.0 and GraphPad Prism 5. The *SOX9* expression level was presented as mean ± SD. Student’s t-test was used to analyze the expression differences between two groups. The associations between serum *SOX9* expression and various clinicopathological characteristics were assessed using Chi-square tests. To determine the diagnostic performance of serum *SOX9* expression in distinguishing PCa patients from healthy controls, we performed receiver operating characteristic (ROC) analysis. $P < 0.05$ was considered as statistically significant.

# Results

Increased expression of *SOX9* in PCa patients

To examine the *SOX9* expression in 131 PCa patients and 74 healthy controls, qRT-PCR was performed. The results in Fig. 1 showed that *SOX9* expression was significantly higher in PCa tissues than that in healthy volunteers ($P < 0.05$).

The correlation of *SOX9* expression with clinicopathologic features of PCa patients

In this study, Chi-square test was used to detect the association between *SOX9* expression level and the clinicopathological data of PCa patients. The results were shown in Table 1. *SOX9* expression was significantly associated with PSA ($P = 0.001$), differentiation ($P = 0.000$), and lymph node metastasis ($P = 0.000$). However, there was no significant association with the age, tumor size and TNM stage of PCa patients (all $P > 0.05$).

Diagnostic value of *SOX9* expression in PCa patients

ROC curves in our study showed that *SOX9* could be used to distinguisht PCa patients from the healthy volunteers with the area under the curve (AUC) value of 0.966 (Fig. 2) and sensitivity of 93.2%, specificity of 87.8% respectively. And the optimal cutoff value for *SOX9* expression level is 2.34.

# Discussion

PCa is the most prevalent cancer in men and as one of the malignant tumors of male genitourinary system, the biological behaviour of PCa is the most complex and the incidence is the higher in developed countries [19]. In our country, the PCa incidence of upward trend is obvious in recent years. Based on the data of center for disease control and prevention of Shanghai for recent 20 years, the incidence of PCa sharp increased and the advanced metastasis PCa is currently incurable [20]. For PCa, carcinogenesis

and the mechanisms affecting the progression and prognosis are multistep processes [21]. Although the previous reports have shown that many factors including *lncRNA HCG11*, *miR-129*, as well as *TMPRSS2-ERG* fusion gene have affected the development and prognosis of PCa [22–24], the prediction and diagnosis of PCa patients was still poor. Thus it is important to find a novel biomarker to improve the diagnostic efficiency of PCa.

Human SOX family has several members, and the functions of the gene family are various. For example, *SOX* genes regulate development and differentiation of many cell types. *SOX* genes within the same subgroup can counteract the function of the genes in another subgroup and the same *SOX* gene could regulate different stages of development in one cell type [25, 26]. *SOX9* is a member of *SOX* family, which is a developmental transcription factor playing a key role in the regulation of sex determination, cartilage development, intestinal differentiation [27]. At the 7.5 weeks of pregnancy, the expression of *SOX9* can be found in embryonal tissue [28]. In the existed studies, *SOX9* acts as an important role in many tissues and organs [9]. In recent years, researchers have found that as a transcription factor [29, 30], Abnormal expression of *SOX9* is associated with the occurrence of many diseases, including many human cancers. For example, Guo et al. found that the expression of *SOX9* was higher in HCC tissues than adjacent tissues and the overexpression of *SOX9* in HCC tissues is of great predictive value on tumor progression and poor prognosis [31]. Shao et al. found that the expression of *SOX9* in gactric cancer was also higher than that in adjacent non-cancerous tissues. The expression of *SOX9* was associated with the growth, invasion, and metastasis of gastric cancer, as well as the prognosis [32].

Moreover, *SOX9* was also identified as one of the earliest molecular expressed in the primordial prostate. The conditional deletion of *SOX9* demonstrated a requirement for *SOX9* in ventral prostate differentiation and development. The study of Huang et al. reported that *SOX9* expression played an important role in prostate development and the initiation of PCa [18]. The study of Qin et al. showed that the combination of *HIVEP3* and *SOX9* help to predict the tumor progression and prognosis of PCa patients [33]. However, the diagnostic value of *SOX9* in PCa remains still unclear and is an active focus of our current research.

In our study, we found that the expression level of *SOX9* was higher in PCa patients than that in healthy group. The similar result was in agreement with the previous studies. In addition, we also focused on the relationship between the expression level of *SOX9* and the clinicalpathological features of PCa patients. The results showed that the expression level of *SOX9* was significantly correlated with PSA, differentiation, and lymph node metastasis. And the result of ROC curves analysis indicated that the expression of *SOX9* could be used as a diagnostic biomarker for PCa patients.

# Conclusions

In conclusion, our current data may provide a novel biomarker for the diagnosis of PCa, but the further investigation should be implement about the *SOX9* acting on PCa patients.

# List Of Abbreviations

quantitative real-time PCR (qRT-PCR)

receiver operating characteristic (ROC)

area under the ROC curve (AUC)

Prostate cancer (PCa)

SRY-related HMG box (SOX)

# Declarations

### Ethics approval and consent to participate

This study was supported by the Ethics Committee of Huaihe Hospital of Henan University 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.


### Funding

Not applicable.


### Authors’ contributions

H.Z., W.T. design of the work; L.G., Z.Y. the acquisition, analysis, X.B., X.L. interpretation of data; H.Z., W.T. the creation of new software used in the work; X.B., X.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.


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# Figures

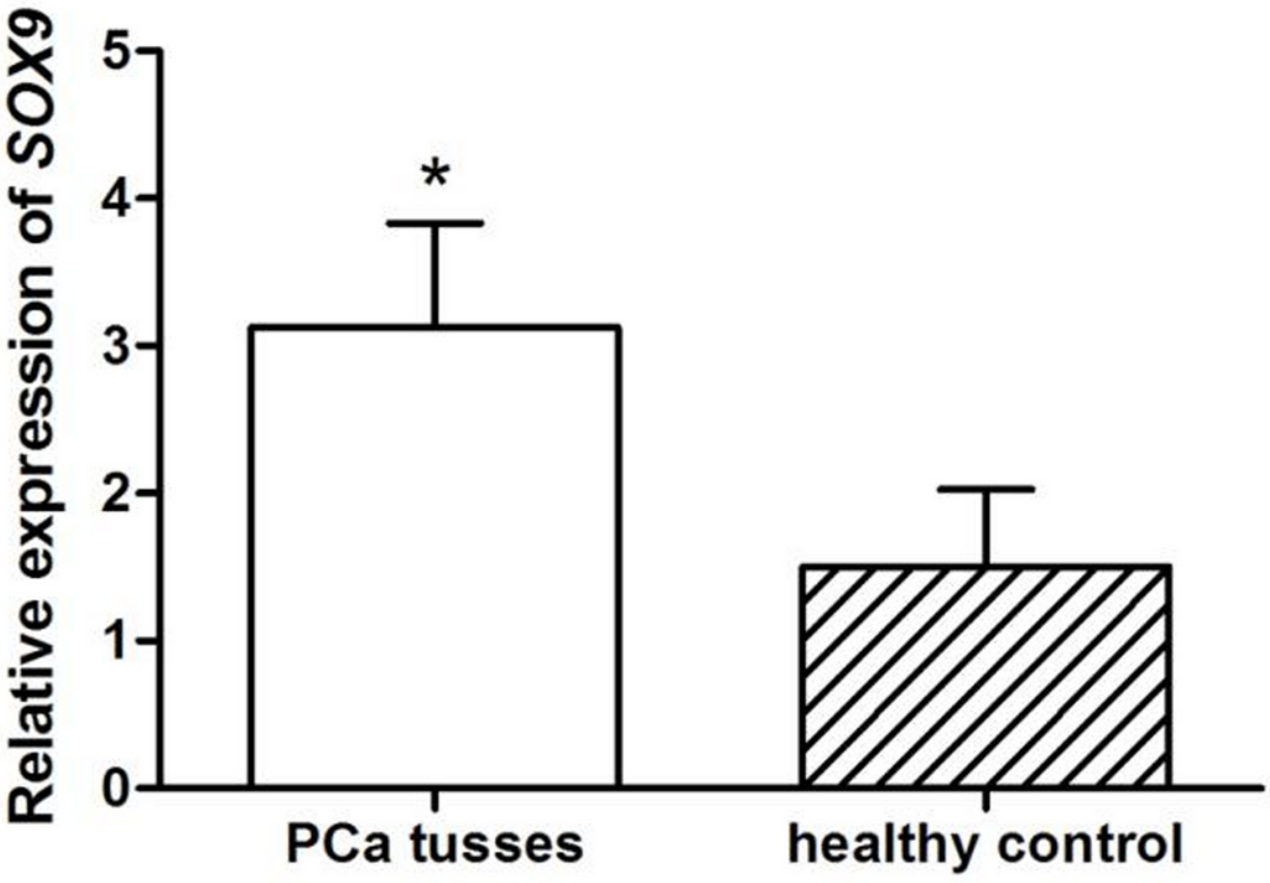
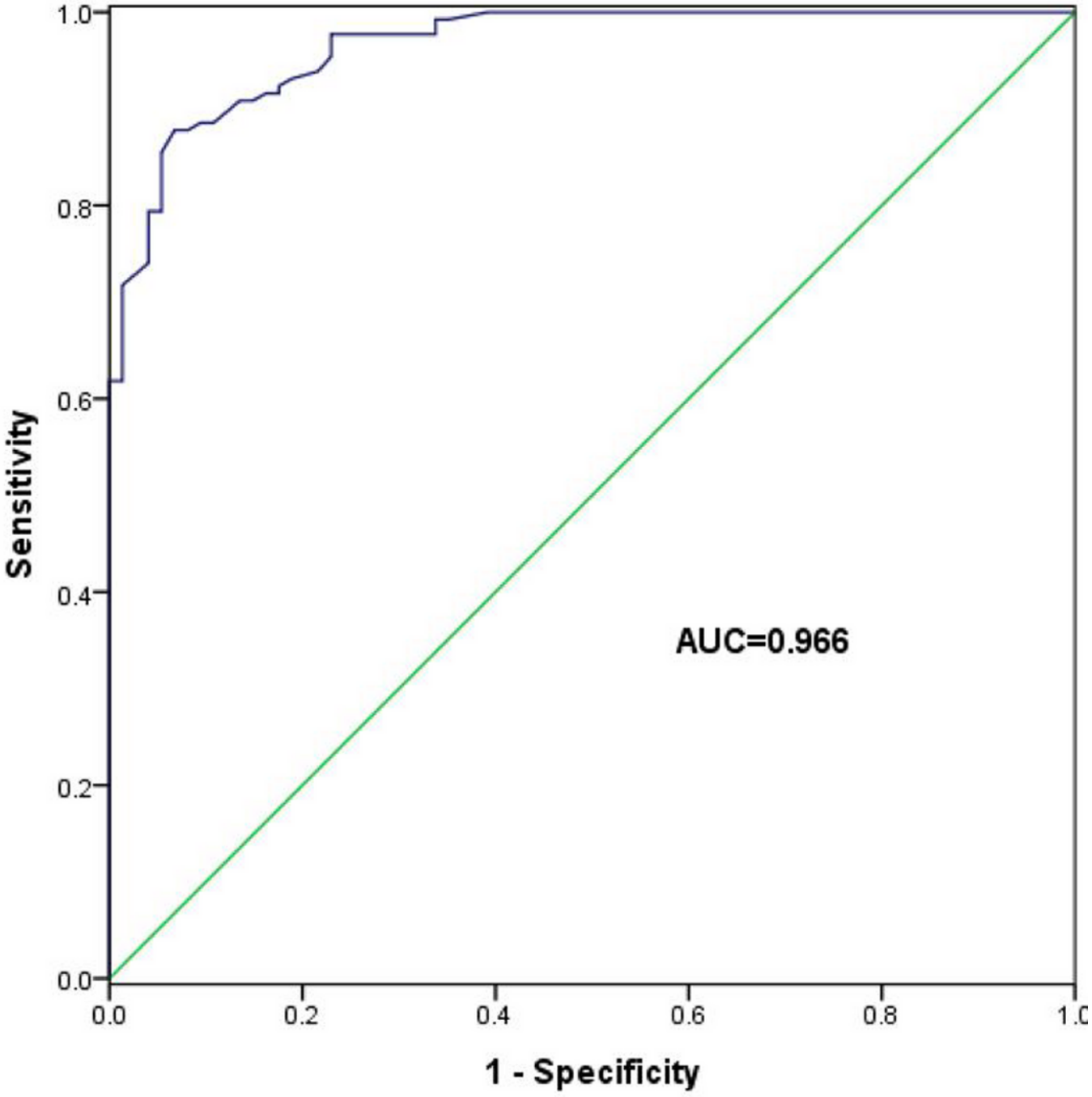



**Figure 1**

Serum SOX9 expression levels evaluated by qRT-PCR in PCa patients and healthy controls (*, P<0.05).




## Figure 2

ROC analysis for evaluation of the accuracy of serum SOX9 to distinguish PCa patients from healthy volunteers.