NFKBIA has the Capacity to Serve as a Diagnostic Biomarker for Prostate Cancer Patient

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Abstract

Background: Prostate cancer is the the second leading cause of cancer-related death in male. *NF-κB* inhibitor alpha gene (*NFKBIA*), is found dys-expressed in the progression of tumors. The aim of this study was to investigate the expression of *NFKBIA* and its diagnostic value in prostate cancer patients.

Methods: The mRNA expression of *NFKBIA* was detected with quantitative real-time polymerase chain reaction (qRT-PCR) assay and the association of its expression with clinical characteristic was analyzed via Chi-square test. Receiver operating characteristic (ROC) curve was built to evaluate the diagnostic value of serum *NFKBIA*.

Results: The mRNA expression level of *NFKBIA* was significantly increased in prostate cancer patients compared with healthy controls (P<0.01). The high expression of *NFKBIA* was significantly associated with pathological T stage (P=0.010) and differentiation (P=0.019). ROC curve showed that serum *NFKBIA* had a high diagnostic value in differentiating prostate cancer patients from healthy individuals. The area under the ROC curve (AUC) value was 0.873 corresponding with a sensitivity of 85.6% and a specificity of 78.3%.

Conclusions: The expression of *NFKBIA* was up-regulated in serum of prostate cancer patients and it may be a useful diagnostic bio-maker for the early detection of this cancer.

Background

Prostate cancer is the most commonly diagnosed male malignancy worldwide and the second most common cause of cancer-related deaths in the United States [1, 2]. The clinical behavior of prostate tumors varies from low-grade indolent tumors to aggressive tumors that is highly malignant with rapid invasion and early bone metastasis [1, 3]. The carcinogenesis and mechanism influencing the progression and prognosis of prostate cancer is a multistep process and the incidence of the disease is continuously rising. Circulating prostate-specific antigen (PSA) is currently the most common non-invasive biomarker used for the detection of prostate cancer [4, 5]. However, to date, there are no reliable and high specificity predictors for prostate cancer detection. Therefore, the novel cancer-related genes that may serve as reliable bio-maker should be established for the prostate cancer early detection.

Nuclear factor of κB (*NF-κB*), a kind of important nuclear transcription factor, can regulate cell proliferation, apoptosis, malignant transformation, invision and metastasis [6]. Aberrant expression of *NF-κB* is reported to be involved in cancer initiation and progression [7]. In current studies, aberrant activation of *NF-κB* was found in glioblastomas and considered a tumor suppressor gene [8, 9]. Moreover, *NF-κB* is present in an inactive form when it is bound to IκBα, which is encoded by *NF-κB* inhibitor alpha gene (*NFKBIA*) [10]. It has been indicated that *NFKBIA* has identified to be involved in carcinogenesis and dysregulated in a variety of cancers, such as head and neck squamous cell carcinoma and glioma [11, 12]. However, few studies had been investigated the diagnostic performance of *NFKBIA* in prostate cancer.

In the present study, we investigated the expression level of *NFKBIA* in prostate cancer samples and healthy control samples. The relationship between *NFKBIA* expression and clinicopathological characteristics of prostate cancer patients was analyzed. Moreover, we also investigate the diagnostic value of *NFKBIA* in prostate cancer.

Methods

Patients and specimens

In the present study, blood samples were obtained from 132 patients with prostate cancer and 120 healthy controls during their hospitalization and physical examination between Huaihe Hospital of Henan University. 10 ml whole blood was collected from each participant and serum was separated by centrifugation at 12,000 g for 10 min at 4℃. Then the samples were stored at -80℃ until RNA extraction. The clinicopathological data were obtained from all cancer patients and listed in Table 1.

Table 1
Association of *NFKBIA* expression with clinicopathological features of prostate cancer patients

Parameters	Cases (n = 132)	*NFKBIA* expression		χ^2	*P*
		Low (n = 51)	High (n = 81)		
Age				0.328	0.567
< 60	74	27	47		
≥ 60	58	24	34		
Tumor size				0.005	0.941
< 2 cm	73	28	45		
≥ 2 cm	59	23	36		
Preoperative PSA				2.108	0.147
< 10 ng/mL	75	33	42		
≥ 10 ng/mL	57	18	39		
Pathological T stage				6.647	0.010
T1-T2	72	35	37		
T3-T4	60	16	44		
Metastasis				2.375	0.123
negative	77	34	43		
positive	55	17	38		
Differentiation				5.546	0.019
poor	71	34	37		
Moderate + well	61	17	44		

The study was approved by the Research Ethics Committee of Huaihe Hospital of Henan University. And all participants signed written informed consents prior to sampling.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted with from serum samples with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and dissolved in 10 ml diethypyrocarbonate-treated water. And reverse transcriptions were performed using the Reverse Transcription System (Promega, WI, USA) according to the manufacturer's instructions. QRT-PCR analysis was performed with ABI 7900 real-time PCR system (Applied Biosystems, CA, USA) according to the manufacturer's protocol. The relative expression of *NFKBIA* was determined by the

comparative $2^{\Delta\Delta Ct}$ (threshold) method and *β-actin* was used as endogenous control. Each sample was repeated in triplicate.

Statistical analysis

All statistical analyses were carried out using the software of SPSS 21.0 and GraphPad Prism 5. The difference between two groups was analyzed by student's t test. The χ^2 test was used to analyze the relationship between *NFKBIA* expression and the clinicopathological characteristics of patients. The receiver operating characteristic (ROC) curve was plotted to distinguish prostate patients from healthy individuals. The diagnostic potential of serum *NFKBIA* was evaluated by calculating the area under ROC curve (AUC), sensitivity and specificity. *P* values less than 0.05 were considered to be statistically significant.

Results

The expression of *NFKBIA* was increased in prostate cancer patients

The mRNA expression level of *NFKBIA* in 132 prostate cancer patients and 120 healthy volunteers were detected via qRT-PCR. As shown in **Figure 1**, the relative mRNA expression of *NFKBIA* was significantly higher in prostate cancer samples than that in healthy controls ($P<0.01$).

Relationship between *NFKBIA* expression and clinicopathological characteristics of prostate cancer patients

To investigate whether *NFKBIA* was involved in the development of prostate cancer, the association between *NFKBIA* expression and clinicopathological characteristics was analyzed. As shown in **Table 1**, the expression of *NFKBIA* was found to be significantly associated with pathological T stage ($P=0.010$) and differentiation ($P=0.019$). However, there was no significant association between *NFKBIA* expression and other clinical parameters including age, tumor size, preoperative PSA and metastasis (all, $P>0.05$).

Diagnostic value of *NFKBIA* in prostate cancer

ROC curve analysis was performed to investigate the correlations between *NFKBIA* dysregulation and diagnosis in prostate cancer patients. As shown in **Figure 2**, *NFKBIA* had a relatively high accuracy in differentiating prostate cancer patients from healthy individuals, with an AUC value of 0.873 (95%CI: 0.829-0.917), a sensitivity of 85.6% and a specificity of 78.3%. Moreover, the cut-off value was 1.685.

Discussion

Prostate cancer is the most common malignant tumor that presents in the prostatic tissues of the males and is the result of disordered growth of prostatic vesicle cells. Besides, it includes several phenotypes, from indolent to highly aggressive phenotypes [2, 13]. Up to now, most prostate cancer patients were diagnosed in advanced stages and it is still a fatal disease [14]. Although a mass of efforts was made to

improve the treatment of prostate cancer, the mortality remain high in the world. Therefore, it's crucial to find effective bio-markers for prostate cancer detection.

In the recent decades, several molecular markers have been investigated in prostate cancer as predictive biomarkers. For example, Lynch SM et al. investigated the expression of *miR-200c* and *miR-141* and their elevated expression may have potential as a novel biomarker or therapeutic intervention in the diagnosis and prognosis of prostate cancer [15]. Pan et al. detected *SPINK1* and *ERG* expression in initially diagnosed bone metastatic prostate cancer and found the over-expression of *SPINK1* was related to poor PSA response as well as be a useful prognostic for bone metastatic prostate cancer at the time of diagnosis [16]. Fu et al. investigated both the mRNA and protein expression of *BUB1B* in prostate cancer cell lines and indicated that high expression of *BUB1B* was an independent predictor biomarker for the diagnosis and prognosis of prostate cancer [17]. Li et al. investigated the overexpression and significance of ribosomal L1 domain containing 1 (*RSL1D1*) in prostate cancer and demonstrated that it may aid in the improvement of diagnosis, prognosis and risk stratification of patients with prostate cancer [18]. Lynch SM et al. provide evidence that *miR-24* has a tumor suppressor role in prostate cancer and may be a useful progression biomarker for prostate cancer [19].

NF-κB has been shown to play a critical role in many physiological processes including inflammation, apoptosis, and angiogenesis as well as tumor progression [20–24]. Improper activation of *NF-κB* can cause enhanced cell proliferation and evasion of apoptosis, which are the cancer hallmarks. Therefore, under normal conditions, *NF-κB* is bound to *IκBα* inhibitor when its expression is not need. *IκBα* is encoded by *NFKBIA* gene, which is also found abnormal expression involved in human cancer progression. Ata Miyar et al. found that *NFKBIA* expression was decreased in patients with high grad glioma, and the the low expression of *NFKBIA* is significantly related with poor overall survival of glioma patients [12]. Shi et al. investigated the mechanism by which HINT1 promotes the stability of inhibitor of *NF-κB α* (*IκBα*) in the cytoplasm of hepatocellular carcinoma (HCC) cells and provided new evidence that HINT1 is a regulator of *IκBα* through SCF (β-TrCP) E3 ligase [25]. In addition, Carter SL et al. implicated that *NFKBIA* is an androgen regulated gene and the protein encoded by this gene, *IκBα* is best known as an inhibitor of *NF-κB* signaling. Moreover, *IκBα* as a key mediator of the apoptotic action induced by combination of bicalutamide and vorinostat and a promising new therapeutic target for prostate cancer [26].

In this study, we investigated the expression level of *NFKBIA* via qRT-PCR analysis. The result proved that the expression of *NFKBIA* was increased compared with healthy control. And the higher expression of *NFKBIA* was tightly correlated with pathological T stage and differentiation, which indicated that *NFKBIA* participated in the development and progression of prostate cancer. The ROC curve analysis showed the high AUC, sensitivity and specificity of *NFKBIA* expression, which revealed *NFKBIA* may have diagnosis value for prostate cancer detection.

Conclusions

In conclusion, *NFKBIA* is overexpressed in prostate cancer samples than healthy control and correlated with clinical factors. *NFKBIA* may be a potential diagnostic marker of prostate cancer. What's more, further studies are needed to warrant its diagnostic utility in this malignancy.

List Of Abbreviations

NF-κB inhibitor alpha gene (*NFKBIA*)

quantitative real-time polymerase chain reaction (qRT-PCR)

area under the ROC curve (AUC)

prostate-specific antigen (PSA)

receiver operating characteristic (ROC)

ribosomal L1 domain containing 1 (*RSL1D1*)

NF-κB α (*IκBα*)

hepatocellular carcinoma (HCC)

IκBα through SCF (β-TrCP)

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.

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Authors' contributions

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

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Figures

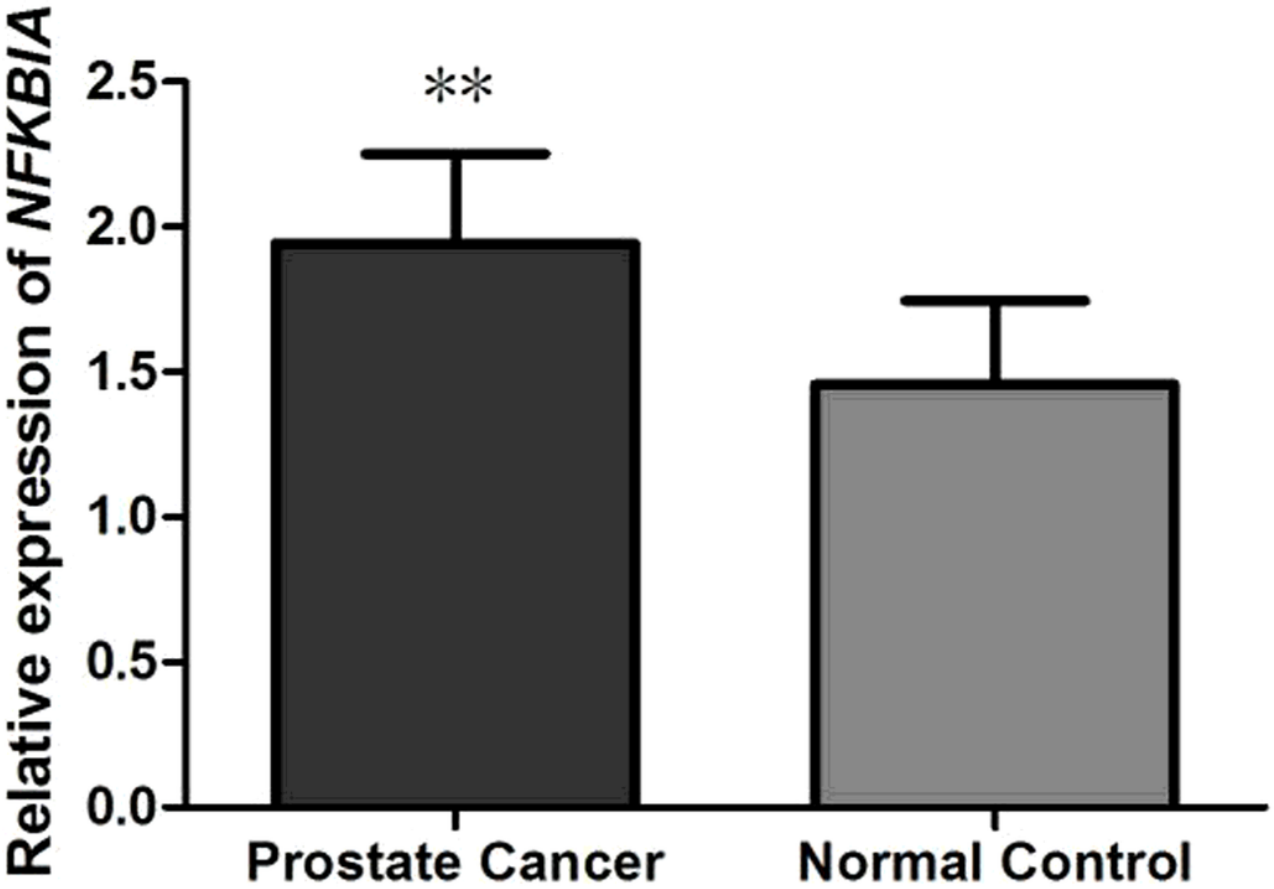

Figure 1

The mRNA expression of NFKBIA in serum samples of prostate cancer patients and healthy controls. The relative expression level of NFKBIA in prostate cancer patients was higher than in the healthy specimens (**, P<0.01).

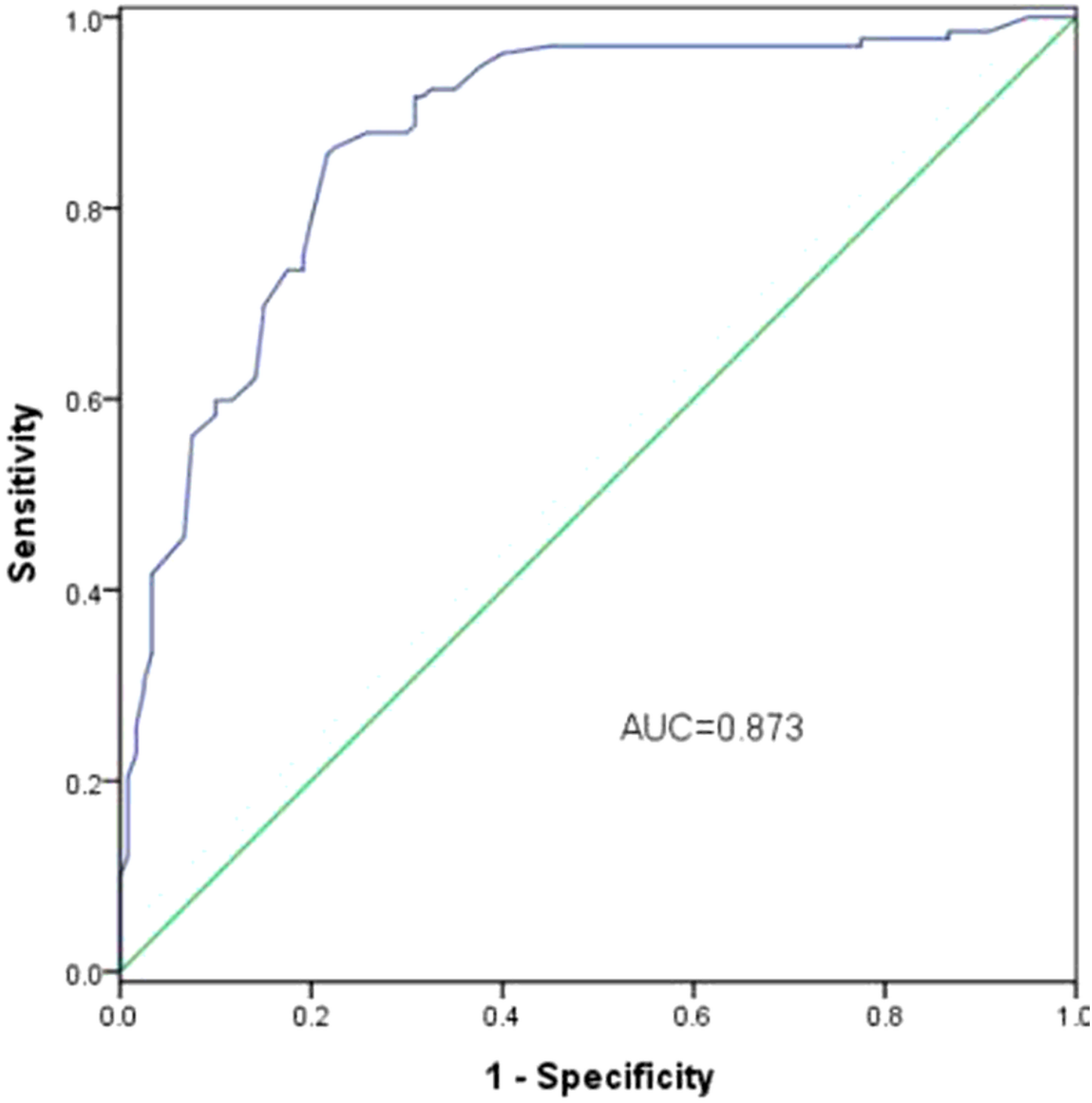

Figure 2

ROC curve analysis for the diagnostic significance of NFKBIA in prostate cancer patients.