RETRACTED ARTICLE: The value of \( \text{FGF9} \) as a novel biomarker in the diagnosis of prostate cancer

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ABSTRACT

**Background:** Fibroblast growth factor 9 (FGF9) is reported to be associated with the pathogenesis of cancers. However, its clinic significance in prostate cancer (PCa) had not yet to be elucidated. The aim of this study was to investigate the diagnostic value of FGF9 in PCa.

**Methods:** Quantitative real-time polymerase chain reaction (qRT-PCR) and western blot analyses were used to detect the expression of serum FGF9 at mRNA and protein level in 90 PCa patients, 48 prostatic benign diseases (PBD) patients and 30 normal individuals. The association between FGF9 and clinicopathological features was determined by Chi-square test. Receiver-operator characteristic (ROC) was established to evaluate the diagnostic performance of FGF9 and PSA.

**Results:** Serum FGF9 expression was significantly elevated in PCa patients \((p < .001)\) and was obviously decreased after surgery \((p < .001)\). FGF9 expression was also associated with lymph node metastasis \((p = .010)\). The diagnostic value of FGF9 was higher than the conventional tumor marker PSA with a AUC of 0.846 combined with a sensitivity of 83.3% and a specificity of 81.1%.

**Conclusions:** Serum FGF9 may be employed as a potential diagnostic biomarker of PCa.

Introduction

Prostate cancer (PCa) is the second common type of malignancy in males worldwide [1]. Its incidence in China is increasing with years [2]. There are many risk factors for PCa such as age, race/ethnicity, PSA velocity, comorbidities, and family history [3,4]. The early diagnosis is crucial for the increase of disease-free survival (DFS) and the decrease of mortality [5]. As the most commonly used biomarker for PCa, the PSA has greatly improved the management of PCa during the past two decades, especially at early detection, improving the chances of curative treatment for PCa [6,7]. However, PSA remains a questionable diagnostic or prognostic marker because of the large difference between decreasing disease aggressiveness and the improvement of treatment levels, leading to the overdiagnosis and overtreatment of PCa [7–9]. Therefore, new effective biomarkers for the detection of PCa are required immediately.

Fibroblast growth factor 9 (FGF9) is a member of human FGF family which is a family of polypeptide growth factors and takes important effects on various of biological functions such as embryonic development, tissue repair, and tumorigenesis [10–12]. FGF9 was originally discovered in the secretions from human glioma MCF-G1 cell line, acting as a gli-activating factor [13]. Findings have suggested that FGF9 is important for the cell signaling (epithelial to mesenchymal) in embryonic development [14], moreover, its expression has also been identified in different tumours, including colon cancer, gastric cancer and lung cancer [15–17], indicating FGF-9 is involved in the tumourgenesis. With respect to in PCa, FGF9 was found to be up-regulated and its over-expression could promote the progression of PCa [18,19]. However, whether FGF9 could be a potential biomarker for the diagnosis of PCa has not yet been investigated.

In the present study, the aim was to detect the expression of serum FGF9 and its relationship with the development of PCa. Besides, we explored its diagnostic value in PCa patients by establishing a ROC curve.

Materials and methods

**Patients and samples**

A total of 168 individuals including 90 patients who were diagnosed with PCa (pathologically confirmed as PCa) and 78 controls were recruited from Yanzhou Branch of Jining Medical University in this study. None of the patients had received any radiotherapy or chemotherapy before sampling. Among the control cohorts, 48 patients including 4 with benign adenomas, 30 with prostatitis and 14 with prostatic benign hyperplasia were taken as benign controls while the other 30 healthy individuals were regarded as healthy controls. The study was approved by the Ethics Committee of...
the hospital and written informed consents were obtained from all participants in advance.

5 ml blood samples were extracted from the patients with PCa, benign prostatic diseases and healthy controls, respectively. Besides, the postoperative blood of patients with PCa was obtained, too. Then all the samples were severely centrifuged for 20 min at 3000 rpm and the supernate was stored at \(-80^\circ\text{C}\) until use. The clinicopathologic characteristics of each patient were recorded in a database.

**RNA extraction and qRT-PCR analysis**

Total RNA extraction from serum samples was performed using TRIzol reagent (Invitrogen, Carlsbad, USA), and the cDNA was synthesized with the First-Strand cDNA Synthesis Kit (Thermo, Waltham, USA) by random priming according to the manufacturer’s protocol. The reaction product was quantified by RT-PCR. β-actin was taken as the internal controls. The sequence of primers for FGF9 was as follows: FGF9, forward-5’-GTGGATCCATGGCTCCCTTAGGTGAAGTTG-3’ and reverse-5’-TCGAATTCAACTTTGGCTTAGAATATCCTTA-3’. The relative mRNA expression of FGF9 was quantified using \(2^{-\Delta\Delta Ct}\) method. Each sample was performed in triplicate.

**Western blot analysis**

The total proteins were extracted from all serum samples by TPER Protein Extraction Reagent (Pierce, Rockford, IL), respectively. Subsequently, the protein samples were separated by 10% SDS–polyacrylamide gel electrophoresis (PAGE), and then the brands were transferred onto a nitrocellulose membrane (Invitrogen, Renfrew, Scotland). The membranes were incubated with mouse anti-human FGF-9 mAb in PBS at 4°C for overnight after being clocked by 5% non-fat milk. Finally, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse antibodies at room temperature. The relative expression of FGF-9 protein was visualized using a chemiluminescent detection with a luminescent image analyzer LAS-1000 (Fuji Photo Film GmbH, Duesseldorf, Germany).

**The measurement of prostate-specific antigen (PSA)**

The concentration of PSA in the serum samples was determined using an automated immunoassay analyzer with ARCHITECT i2000SR\textsuperscript{R} (Abbott Diagnostics, Abbott Park, IL, USA) according to the manufacturer’s instructions.

**Statistical analysis**

All statistical analyses were carried out with SPSS 18.0 software (SPSS Inc., Chicago, IL) and the figures were designed by GraphPad prism 5.0 (GraphPad, CA). Student’s t-test and Chi-square test were respectively used for the comparison of the variables in two and more than two groups. The diagnostic value of FGF9 was evaluated by establishing the Receiver operating characteristic (ROC) curve. The differences were considered to be statistically significant when \(p < .05\).

**Results**

**The expression of serum FGF9 was increased in patients with PCa**

The relative mRNA and protein expression levels of serum FGF9 in patients with PCa, benign controls, and healthy controls were detected by qRT-PCR and western blot analysis, respectively. As shown in Figure 1(a), the mRNA expression of serum FGF9 in patients with PCa was significantly higher than that in benign controls and healthy controls (2.24 ± 0.57 vs. 0.62 ± 0.53 vs. 0.81 ± 0.46) \((p < .001)\). Meanwhile, the protein expression level of FGF9 was also found to be increased in patients with PCa compared to that in benign controls and healthy controls \((p < .001, \text{Figure 1b) (Supplementary Figure 1)})\). Additionally, the expression level of FGF9 in the preoperative serum of patients with PCa was significantly higher than that in postoperative serum both at mRNA and protein level (Figure 2, \(p < .001\) (Supplementary Figure 2)).

**Relationship between serum FGF9 expression and clinicopathologic features**

To further detect the potential role of FGF9 expression in the development of PCa, the patients were divided into two groups according to the serum FGF9 expression. Then the relationship between serum FGF9 expression and clinical factors of patients with PCa was analyzed. The result exhibited that the increased expression of serum FGF9 in patients with
PCa was significantly associated with lymph node metastasis \((p = .010)\) while there was no association between age \((p = .408)\) and clinical stage \((p = .139)\) (Table 1).

**The diagnostic significance of serum FGF9 in patients with PCa**

To investigate the diagnostic value of serum FGF9, we built an ROC curve with the expression of it and compared the difference between FGF9 and traditional tumour marker PSA in acting as a diagnostic marker. The outcome showed that the AUC of FGF9 was 0.846 with a sensitivity of 83.3% and a specificity of 96.2% while the AUC of PSA was 0.771 with a sensitivity of 96.2% and a specificity of 56.7% (Figure 3). These results revealed that the diagnostic value of FGF9 was higher than the traditional tumour marker PSA for the patients with PCa. Furthermore, we evaluated whether the expression levels of FGF9 was correlated with PSA. However, there was no significant difference observed between serum FGF9 expression and PSA levels \((r^2 = .077, Figure 4)\).

**Discussion**

In the present study, we detected the expression of FGF9 both at mRNA and protein level using qRT-PCR and western blot analysis, respectively. The outcome showed at both two levels, the expression of FGF9 was significantly elevated in patients with PCa compared to that in controls. Furthermore, we provided evidence that FGF9 could be a diagnostic marker for PCa for the first time.

Actually, FGFs, produced by stromal cells or epithelial cells, have been reported to play an important role in the growth of prostate very early [20–22]. As a member of FGF family, high FGF9 expression is found to play important roles in various human cancers such as lung cancer and glioma [17,23–25]. In PCa, over-expression of FGF9 has been reported to promote the progression and metastasis [19]. In our study, we detected the expression of serum FGF9 and showed that it was significantly higher in patients with PCa than that in benign controls and normal controls, which was consistent with the previous study. This result revealed that serum FGF9 might be an oncogene in PCa. In addition, the results were strengthened by our findings of a significant decline of FGF9 expression in postoperative serum of PCa patients. These observations suggested that alterations of FGF9 might be implicated in the tumourigenesis of PCa. In the previous study, the high FGF9 expression had been proved to be associated with increased tumour stage and lymph node metastasis [24]. In our study, in order to analyze the relationship between FGF9 expression and clinical factors of patients with PCa, the overexpression of FGF9 was found to be significantly associated with lymph node metastasis, which indicated that FGF9 was involved in the development of PCa.

![Figure 2](image-url)  
**Figure 2.** The expression of FGF9 in preoperative serum of patients with PCa was significantly higher than that in postoperative serum both at mRNA (a) and protein (b) level. *p < .001 represented the significant difference between the compared two.

![Figure 3](image-url)  
**Figure 3.** ROC curve showed the diagnostic significance of FGF9 and PSA in the patients with PCa. The diagnostic value of FGF9 (AUC = 0.846) was better than of PSA (AUC = 0.771).

**Table 1.** The association between serum FGF9 expression and the clinicopathological parameters of patients with PCa.

| Variables               | Cases \((n = 90)\) | Low \((n = 47)\) | High \((n = 43)\) | \(p\) value |
|-------------------------|--------------------|-----------------|-----------------|-------------|
| Age \(\geq 50\)         | 44                 | 25              | 19              | .408        |
| Age \(< 50\)           | 46                 | 22              | 24              |             |
| Clinical stage          |                    |                 |                 | .139        |
| I + II                 | 46                 | 28              | 18              |             |
| III + IV               | 44                 | 19              | 25              |             |
| Lymph node metastasis  |                    |                 |                 | .010        |
| Absent                 | 51                 | 33              | 18              |             |
| Present                | 39                 | 14              | 25              |             |
Early screening for tumours has made a great contribution to the decreased mortality of the disease, and currently, PCa is most often diagnosed through PSA-screening. PSA is still the available tumour marker in PCa now, but it remains unreliable as a diagnostic or prognostic marker for PCa [26,27]. PSA expression is usually elevated in the prostate with inflammation [28], benign prostate hyperplasia (BPH) [29], or infections other than PCa, leading to the low specificity and sensitivity. Moreover, it often implicates age, potential comorbidities and therapeutic consequences of patients [30], and for all this, a variety of patients diagnosed with PCa are often “overtreated”. In the present study, ROC analysis indicated that FGF9 had considerable diagnostic efficiency for PCa with a high AUC of 0.846. Thus, FGF9 could act as a potential available diagnostic biomarker for PCa.

Furthermore, to compare the difference between the diagnostic value of FGF9 and PSA, we measured the expression both of them and compared their AUC as well as sensitivity and specificity. The outcome showed that although the difference of them was not significant, their diagnostic value was different obviously from each other. And the diagnostic information of FGF9 did not overlap with the PSA.

In summary, our findings confirm that the expression of FGF9 is up-regulated in PCa and it is involved in the development of this cancer. Moreover, FGF9 acts as a biomarker for the diagnosis of PCa, and shows superior performance compared to PSA. However, future studies need to be performed on other large-scale clinical specimens.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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