Low *LKB1* Expression Results in Unfavorable Prognosis in Prostate Cancer Patients

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Source of support: Departmnetal sources

**Background:** The present study aimed to compare the expression of liver kinase B1 (*LKB1*) in prostate cancer (PCa) tissues and the paired adjacent tissues, then to evaluate the statistical relationship between *LKB1* expression and prognosis of PCa patients.

**Material/Methods:** The relative expression of *LKB1* at mRNA level was detected by quantitative real-time polymerase chain reaction (qRT-PCR). The expression of *LKB1* at protein level was measured by immunohistochemistry (IHC) method. The relationship between *LKB1* expression and clinicopathologic characteristics was estimated by chi-square test. Kaplan-Meier method was used to analyze the overall survival of PCa patients with different *LKB1* expression. Cox regression analysis was performed to estimate the significance of *LKB1* expression and clinicopathologic characteristics in the prognosis of PCa patients.

**Results:** The relative expression of *LKB1* at mRNA level was significantly lower in PCa tissues than in the normal tissues (*P*<0.001). The *LKB1* expression was proved to be affected by clinical stage (*P*=0.019) and PSA concentration (*P*=0.031) of PCa patients. Moreover, patients with negative *LKB1* expression had shorter survival than those with positive expression. Cox regression analysis confirmed that *LKB1* could be regarded as a prognostic biomarker for PCa patients (*P*=0.001, HR=3.981, 95% CI=1.698–9.336).

**Conclusions:** The expression of *LKB1* was lower in PCa tissues and might be a predictor for the prognosis of PCa patients.

**MeSH Keywords:** Prognosis • Prostatic Neoplasms • Vascular Endothelial Growth Factor Receptor-2

**Full-text PDF:** [http://www.medscimonit.com/abstract/index/idArt/894847](http://www.medscimonit.com/abstract/index/idArt/894847)
Background

Prostate cancer (PCa) is one of the most common cancers among men over 50 years old and is the sixth leading cause of cancer-related deaths world-wide [1,2]. Although its incidence is higher in developed countries than in developing counties, it is still increasing in developing areas [3–5]. There are obvious regional and ethnic differences in the pathogenesis of PCa. Currently, the diagnosis of PCa is mainly based on the combination of various procedures, and PCa is usually diagnosed as a localized disease [6,7]. Treatments for PCa predominantly include surgical castration, androgen-deprivation therapy (ADT), and radiation therapy (RT) [8,9]. However, these treatments have shortcomings and there is no effective strategy to treat metastasis and recurrence of Pca that cannot be treated by surgery or radiation therapy. Therefore, an innovative biomarker for therapies and prognosis of PCa patients is urgently needed.

Liver kinase B1 (LKB1), a serine/threonine kinase, is located at 19p 13.3 of human chromosomes [10]. It is known as a tumor suppressor and many studies have demonstrated that LKB1 plays an important role in regulating energy homeostasis, cell cycle progression, cell polarity, cell proliferation, senescence, DNA damage response, and differentiation [11–13]. It is also mutated in Peutz-Jeghers syndrome (PJS), which leads to an increasing risk of malignant tumors in multiple tissues [14]. In previous studies, LKB1 was reported to be a common mutated gene in various cancers such as non-small cell lung carcinomas, cervical cancer, breast cancer, and pancreatic carcinoma [15–18]. It was also confirmed that LKB1 was related with prostate neoplasia and could suppress proliferation and invasion of PCa [19,20]. However, its role in the progression of PCa had never been determined.

This study aimed to detect the LKB1 expression in PCa tissues and the paired adjacent normal tissues both at mRNA level and protein level. Then we attempted to further explore whether LKB1 could serve as a prognostic factor for PCa patients, so as to understand the PCa progression, provide an efficient therapy method of Pca, and increase the survival rate of PCa patients.

Material and Methods

Patients and tissues specimens

Our study included 109 patients with PCa diagnosed at the Department of Urology Surgery of The Affiliated Hospital of Jining Medical College. None of them had ever received any chemical treatment or physical therapy before surgery. The present study was approved by the Ethics Committee of The Affiliated Hospital of Jining Medical College. All participants provided signed informed written consent in advance.

The tumor tissues and the paired adjacent tissues were collected from PCa patients. All the specimens were biopsy materials and frozen in liquid nitrogen immediately, then the samples were stored at ~80°C for RNA extraction. A follow-up of 60 months was conducted. The overall survival time was defined as the time from day of surgery to the day of death. The follow-up information was obtained via a telephone or questionnaire and was updated every 2 months. Patients who died from other disease or accident were excluded from our study.

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

Total RNA from fresh PCa tissues and the paired adjacent tissues were extracted and purified using RNeasy Mini Kit (QIAGEN) according to the manufacturer’s directions. Reverse transcription was performed with a ReverTra Ace qPCR RT Kit (Toyobo Bio-Technology, Japan) according to the manufacturer’s instructions. qRT-PCR reaction was carried out in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). The GAPDH was used as endogenous control. The relative expression of LKB1 at mRNA level normalized to GAPDH was evaluated by comparative cycle threshold (CT) method. All the experiments were conducted under optimal conditions and in triplicate.

Immunohistochemistry assay

Immunohistochemistry (IHC) was used to examine the expression of LKB1 at the protein level in all tissue samples. The tumor tissues and adjacent tissues were fixed in 10% formaldehyde and embedded in paraffin. Then the paraffin sections were cut into 4-μm sections, and were dewaxed and rehydrated with xylene and graded alcohol, respectively. The sections were washed with buffer solution for 5 min and then added into the primary antibody at 4°C overnight. The second antibody was added into the sections after being washed again. Finally, coloration was performed with DAB. The results are presented as the percentage of the staining cells (0 to 100%) in tissues. Staining under 20% of the tissue cells or no staining was included in the negative group (−), while the others belonged to the positive group (+).

Statistical analysis

All data processing was carried out using SPSS 18.0 software. The difference in LKB1 expression between PCa tissues and adjacent tissues was analyzed by t test. The chi-square test was used to analyze the relationship between LKB1 expression and clinicopathological characteristics. The association
between LKB1 expression and overall survival, as well as the prognostic value of LKB1, were estimated by Kaplan-Meier and Cox regression analysis, respectively. P < 0.05 was considered to be statistically significant.

**Results**

**Low expression of LKB1 at mRNA level in PCa tissues**

QRT-PCR was used to evaluate the expression of LKB1 in PCa tissues and the adjacent tissues. The expression level of LKB1 was normalized to GAPDH. The result demonstrated that the relative expression of LKB1 at mRNA level in PCa tissues was 0.59±0.25 (mean±SD), while that in the normal tissues was 1.32±0.59 (mean ±SD). A significant decrease in the expression of LKB1 at the mRNA level was found in PCa tissues (Figure 1, P<0.001).

**Decreased expression of LKB1 at protein level in PCa tissues**

The LKB1 protein expression of all PCa tissues and a randomly selected 70 cases of adjacent tissues were assayed by IHC method. Figure 2 shows that staining degree was obviously diminished in PCa tissues compared with adjacent tissues. To obtain an exact result of protein expression level of the LKB1 gene in PCa patients, we analyzed the positive cell percentage

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**Figure 1.** Relative expression of LKB1 at mRNA level was assessed by qRT-PCR in PCa tissues and adjacent normal tissues. The LKB1 expression was normalized to GAPDH. Expression level of LKB1 was significantly lower in PCa tissues compared to the adjacent normal tissues (P<0.001).

**Figure 2.** The expression of LKB1 protein in PCa tissues and adjacent tissues. The LKB1 protein expression level was lower in PCa tissues than in adjacent tissues (P<0.001). (A) Adjacent tissue; (B) PCa tissue; (C) Positive cell percentage.
in the 2 tissues. We found that positive cell percentage was 28.4% in PCa tissues and 81.4% in normal tissues. Both the staining degree and positive cell percentage indicated that the LKB1 protein expression in PCa tissues was significantly lower than that in the normal tissues (Figure 2, \( P < 0.001 \)).

Correlation between LKB1 expression and clinicopathological characteristics

The association between LKB1 expression and clinicopathological characteristics, including age, hematuria, urine retention, creatinine (μmol/L), clinical staging, and PSA (ng/ml), were evaluated to determine whether LKB1 participates in the development of PCa. The results showed that creatinine level (\( P = 0.035 \)), advanced clinical stage (\( P = 0.019 \)), and high concentration of PSA (\( P = 0.031 \)) were all related to low LKB1 expression (Table 1). However, no clinical relevance was observed between LKB1 and age, hematuria, or urine retention (Table 1, \( P > 0.05 \)).

Association between LKB1 expression and overall survival of PCa patients

During the follow-up, 47 of 78 (60.3%) patients with negative LKB1 expression died, whereas only 7 (22.6%) patients with positive LKB1 expression died. Kaplan-Meier analysis exhibited that patients with negative LKB1 expression had significantly lower overall survival than those with positive LKB1 expression (Figure 3, log-rank test, \( P < 0.001 \)).

Table 1. The relationship between LKB1 expression and clinicopathological characteristics of PCa patients.

| Clinical features         | Case (n) | LKB1 expression | \( \chi^2 \) | \( P \) |
|---------------------------|----------|-----------------|--------------|--------|
|                           |          | Negative (n)    | Positive (n) |        |
| Age                       |          |                 |              |        |
| \( \leq 55 \)              | 39       | 26              | 13           | 0.714  |
| >55                       | 70       | 52              | 18           | 0.265  |
| Hematuria                 |          |                 |              |        |
| Yes                       | 57       | 42              | 15           | 0.212  |
| No                        | 52       | 36              | 16           | 0.137  |
| Urine retention           |          |                 |              |        |
| Yes                       | 58       | 45              | 13           | 2.212  |
| No                        | 51       | 33              | 18           | 0.137  |
| Creatinine (μmol/L)       |          |                 |              |        |
| \( \leq 110 \)             | 46       | 28              | 18           | 4.469  |
| >110                      | 63       | 50              | 13           | 0.035  |
| Clinical staging          |          |                 |              |        |
| T1+T2                     | 51       | 31              | 20           | 5.468  |
| T3+T4                     | 58       | 47              | 11           | 0.019  |
| PSA (ng/ml)               |          |                 |              |        |
| \( \leq 6 \)               | 56       | 35              | 21           | 4.645  |
| >6                        | 53       | 43              | 10           | 0.031  |

Figure 3. Kaplan-Meier analysis with the expression of LKB1 showed that patients with negative expression of LKB1 had a significantly shorter overall survival time than those with positive LKB1 expression (log-rank test, \( P < 0.001 \)).
regression analysis showed that clinical features had no significant relationship with the prognosis of PCA, but LKB1 expression (P=0.001, HR=3.981, 95%CI=1.698–9.336) was associated with the prognosis of PCA patients (Table 2). Therefore, we inferred that these clinical features could not act as markers for PCA prognosis, but LKB1 might be a novel indicator for the prognosis of PCA patients.

Discussion

PCAs is a malignant tumor that presents in the prostatic tissues of the males and is the result of disordered growth of prostatic vesicle cells. Up to now, pathogens that induce PCAs are still indefinite, which may be associated with the alteration of gene, such as the change of androgen receptor relative genes. It is usually a fatal disease for most patients diagnosed in advanced stages. Therefore, it is of great significance to explore effective diagnostic and prognostic markers for PCAs.

Several molecular markers have been investigated in PCAs tissues as predictive biomarkers [21–26]. For example, Rajal et al. reported that ERG was overexpression in PCAs and Xu et al. also verified that ERG played a prognostic role in prostatic acinar adenocarcinoma [27,28]. Zheng et al. demonstrated that SFRP1 was a potential biomarker in PCAs patients [29]. In addition, LKB1 is a tumor suppressor gene with a molecular weight of 50 KD. It contains 10 exons and consists of kinase domain, N terminal regulatory domain, and C terminal regulatory domain. It has been studied in various diseases and was confirmed to play crucial roles in different cell processes. For instance, in the study of Inge et al. the inactivation of LKB1 could sensitize NSCLC to pharmacological aggravation of ER stress, and another report by Inge et al. found that LKB1 expression was related to clinical staging and PSA concentration tightly. In addition, Kaplan-Meier analysis shod that the overall survival time of patients with low LKB1 expression was shorter than in those with high LKB1 expression. Cox regression analysis determined that there was a statistically significant relationship between LKB1 expression and the prognosis of PCAs patients, indicating that LKB1 could be a prognostic biomarker for PCAs patients.

As with other tumor suppressor genes, it is also difficult to identify the patients with or without low LKB1 expression, and potential mechanisms of LKB1 in various tumors are still unknown. Many studies have demonstrated that LKB1 functions in various cancers through the LKB1/AMPK signaling pathway [35–37]. Young-Ok Son et al. illustrated that cadmium induced autophagy through LKB1-AMPK signaling in skin epidermal cells [38]. Brown et al. showed that LKB1 expression was inhibited by estradiol-17β in MCF-7 cells [39]. Therefore, we presumed that the effect of LKB1 on PCAs might be related to the LKB1/AMPK signaling pathway or estradiol-17β approach, but this hypothesis must be verified in further research.

Conclusions

In conclusion, LKB1 is a tumor suppressor in PCAs via its decreased expression in PCAs tissues. Statistical significance was found between LKB1 expression and the prognosis of PCAs patients, suggesting that LKB1 may be a candidate prognostic marker for PCAs patients.

Table 2. Multivariate analysis for prognostic factors in prostate cancer via cox regression analysis.

| Variable            | P value | HR    | 95%CI     |
|---------------------|---------|-------|-----------|
| Hematuria           | 0.188   | 0.659 | 0.354–1.227|
| Urine retention     | 0.155   | 0.655 | 0.365–1.173|
| Clinical staging    | 0.442   | 1.255 | 0.703–2.240|
| LKB1 expression     | 0.001   | 3.981 | 1.698–9.336|

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