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ORIGINAL ARTICLE

Diagnostic Value of Peripheral Blood miR-374b-5p in Patients with Prostate Cancer

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SUMMARY

Background: **Increased evidence suggested the important role of microRNAs (miRNAs) in the tumorigenesis of prostate cancer (PCa). The aberrant expression of miRNA (miR)-374b-5p has been observed in various types of cancers. The purpose of the current study was to evaluate the relationship between miR-374b-5p expression levels and PCa and to assess the feasibility of using peripheral blood miR-374b-5p as a potential non-invasive biomarker for PCa.**

Methods: **Total RNA was isolated from the whole-blood samples of 42 PCa patients whole-blood samples, 42 benign prostatic hyperplasia (BPH) patients, and 42 healthy controls (HC). The expression of miR-374b-5p was assessed by reverse transcription quantitative polymerase chain reaction. Normalized data were subjected to the receiver operating characteristic (ROC) and Kaplan-Meier analysis.**

Results: **The expression of peripheral blood miR-374b-5p was significantly higher in PCa patients than in HC individuals and patients with BPH ($p < 0.001$). Upregulation of miR-374b-5p was observed to be related to certain parameters, including Gleason score > 7 ($p < 0.001$), and PSA > 20 ng/mL ($p < 0.01$). To further evaluate the role of miR-374b-5p in patients with PCa, ROC analysis was carried out. Our data showed that peripheral blood miR-374b-5p could screen PCa patients from HC individuals (area under the curve (AUC), 0.851; 95% CI, 0.766 - 0.936; $p < 0.001$) and patients with BPH (AUC, 0.831; 95% CI, 0.742 - 0.920; $p < 0.001$).**

Conclusions: **Increased miR-374b-5p expression in peripheral blood may serve as a potential biomarker to distinguish PCa patients from healthy controls and BPH patients.**

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KEY WORDS

miR-374b-5p, prostate cancer, BPH, diagnostic value, biomarker

INTRODUCTION

PCa is the most common malignant tumor diagnosed in males and one of the highest causes of cancer-related deaths in western countries [1]. Currently, PCa is investigated by serum prostate-specific antigen (PSA) and/or digital rectal examination (DRE). However, serum PSA is organ-specific but not tumor-specific and has low positive predictive value (PPV = 30% ~ 35%) [2], while DRE has low sensitivity [3]. The level of PSA in serum may be a consequence of different variable events, such

as prostatitis, BPH or trauma [4], which usually result in overdiagnosis and overtreatment [5]. Thus, there is an urgent search for new, preferentially non-invasive, biomarkers to allow early detection of PCa.

MicroRNAs (miRNAs) are short, non-coding, single-chain RNAs which could interfere with the expression of target genes via directly binding to the 3′- untranslated region (3'UTR) of mRNAs [6]. MiRNAs interfere with various cellular pathways such as cell proliferation, growth, differentiation, and survival [7], while cancer-related miRNAs participate in intercellular communication and disseminate through affecting their targets' expression [8]. Therefore, researchers have focused on the analysis of body fluids and have proposed miRNAs as possible biomarkers for several diseases, including PCa [9]. Maurizia et al. [10] found that PSA, miR-103a-3p, and let-7a-5p could identify both overall and clinically significant PCa better than PSA alone, even in 50 to 69 year-old men with PSA ≤ 4 ng/mL. According to Sameh et al. [11], circulating miR-21 and miR-221 could be used as specific noninvasive biomarkers for PCa due to their higher specificity and sensitivity. Bryant et al. [12] found the expression of 12 miRNAs were changed in the circulation system of 78 PCa patients compared with 28 healthy males.

In particular, miR-374 has been shown to be related to cancer development and progression. Differentially expressed miR-374 is involved in many tumors, such as stomach cancer [13], hepatocellular carcinoma [14], and breast cancer [15]. However, the expression of miR-374b-5p in PCa has not been studied.

Therefore, the current study aims to compare the expression of the promising circulating miR-374b-5p in PCa patients to subjects without cancer and evaluate their potential role as specific noninvasive molecular biomarkers for prostate cancer diagnosis.

MATERIALS AND METHODS

Patients and blood samples

All peripheral whole blood samples of healthy individuals, BPH patients, and PCa were collected from the Department of Urology, Beijing Hospital (Beijing, China) between June 2014 and January 2016. The application of patient-derived materials was approved by the Research Ethics Committee of Beijing Hospital, and written consent was obtained from all patients. Patients, who previously received androgen deprivation therapy (ADT) or radiotherapy, were excluded. The characteristics of the patients were summarized in Table 1.

RNA extraction and real-time PCR

Total RNA from the whole blood samples was extracted with Trizol (Invitrogen) according to the manufacturer's instructions. The RNA concentration and the purity of the isolated RNA samples were assayed by absorbent density analysis on OD_{260}/OD_{280}.

Stem-loop reverse transcription-polymerase chain reaction (RT-PCR) was conducted rigorously according to the manufacturer's instructions with the whole blood samples (5 mL) to detect and quantify mature miRNAs using a stem-loop antisense primer mix and M-MuLV transcriptase (NEB).

To quantify the miR-374b-5p, SYBR Green I was used for real-time PCR according to the manufacturer's instructions (TaKaRa) with the Bio-Rad iQ5 system (Bio-Rad). The relative expression level of a miRNA was normalized to an internal invariant control, U6 small nucleolar RNA. Each reaction was performed in triplicate, and analysis was performed by the $2^{-\Delta\Delta CT}$ method. Nucleotide primers used for reverse transcription were as follows (5'-3'): miR-374b-5p, GTCGTATCCAGTG CAGGGTCCGAGGTATTCGCACTGGATACGACC ACTT;

U6, TCGTATCCAGTGCAGGGTCCGAGGTATTCG CACTGGATACGACAAATATG.

The primers used for real-time PCR were as follows (5' -3'): miR-374b-5p forward, GCATATAATACAACC TGCT; U6 forward, GCGTCGTGAAGCGTTC; Universal reverse primer, GTGCAGGGTCCGAGGT.

Statistical analysis

The data are represented as mean ± standard error of the mean (SEM). Data were assayed using the ANOVA multiple comparison test (SPSS 13.0). ROC curves were used to assess the value of miR-374b-5p as a biomarker in predicting patients with PCa, and the AUC was reported. $p < 0.05$ was considered to be statistically significant.

RESULTS

Patients characteristics

Clinical and biochemical characteristics of the HC, BPH patients, and PCa patients were displayed in Table 1. The distribution of various age groups was similar ($p > 0.05$). The serum PSA levels of 42 patients with PCa were significantly higher compared to the BPH and HC groups ($p < 0.001$).

The expression of miR-374b-5p was up-regulated in peripheral blood of PCa patients.

To identify the potential role of miR-374b-5p in PCa, we first examined its expression in the peripheral blood of PCa, BPH patients, and HC subjects by real-time PCR. Compared with the BPH and HC groups, PCa patients displayed a significant increase in peripheral blood miR-374b-5p ($p < 0.001$) (Figure 1). The relative expression of miR-374b-5p was 19.8 ± 20.1 for PCa group, 1.7 ± 9.7 for BPH group, and 1.0 ± 1.0 for HC group.

Table 1. Characteristics of participants.

Variable	Prostate cancer	Benign prostatic hyperplasia	Healthy controls
Total subjects; n	42	42	42
Age, years; mean (range)	68 (56 - 81)	69 (53 - 78)	67 (51 - 81)
BMI, kg/m^2	24.2 ± 2.92	24.9 ± 3.24	24.5 ± 2.55
PSA, ng/mL; n (%)			
< 10	18 (42.8)	42 (100.0)	42 (100.0)
10 - 20	7 (16.7)	0 (0)	0 (0)
> 20	17 (40.5)	0 (0)	0 (0)
Gleason score; n (%)			
< 7 (low)	10 (23.8)	-	-
7 (intermediate)	23 (54.8)	-	-
> 7 (high)	9 (21.4)	-	-
Tumor stage; n (%)			
pT1/2	27 (64.3)	-	-
pT3/4	15 (35.7)	-	-

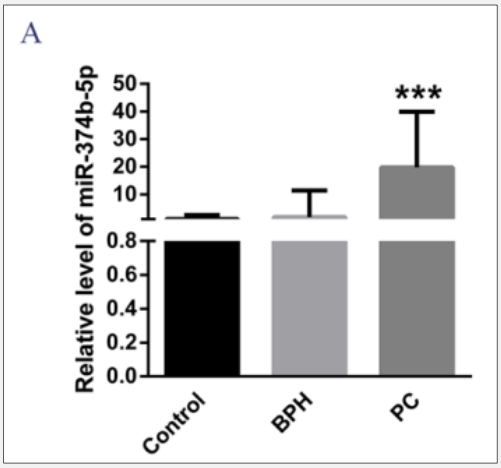

Figure 1. Increased expression of peripheral blood miR-374b-5p in patients with PCa compared with patients with BPH and HC subjects.

(A) The expression of miR-374b-5p was measured in peripheral whole-blood samples from PCa patients (n = 42), BPH patients (n = 42) and HC (n = 42). Data represent the mean ± S.D. * p < 0.05, ** p < 0.01, *** p < 0.001 (vs. BPH patients and HC).

Clinicopathological parameter changes are accompanied by increased expression of miR-374b-5p in peripheral blood of PCa patients

PCa patients with more aggressive types of tumors showed significantly elevated levels of miR-374b-5p compared to that of patients with less aggressive tumors. The relative expression of miR-374b-5p in the group of PCa patients with a Gleason score < 7 was 1 ± 0.7, and progressively increase to 2.0 ± 1.7 in PCa patients with a Gleason score of 7, and to 10.9 ± 4.0 in PCa patients with a Gleason score > 7 (Figure 2A). In addition, the expression of miR-374b-5p in patients

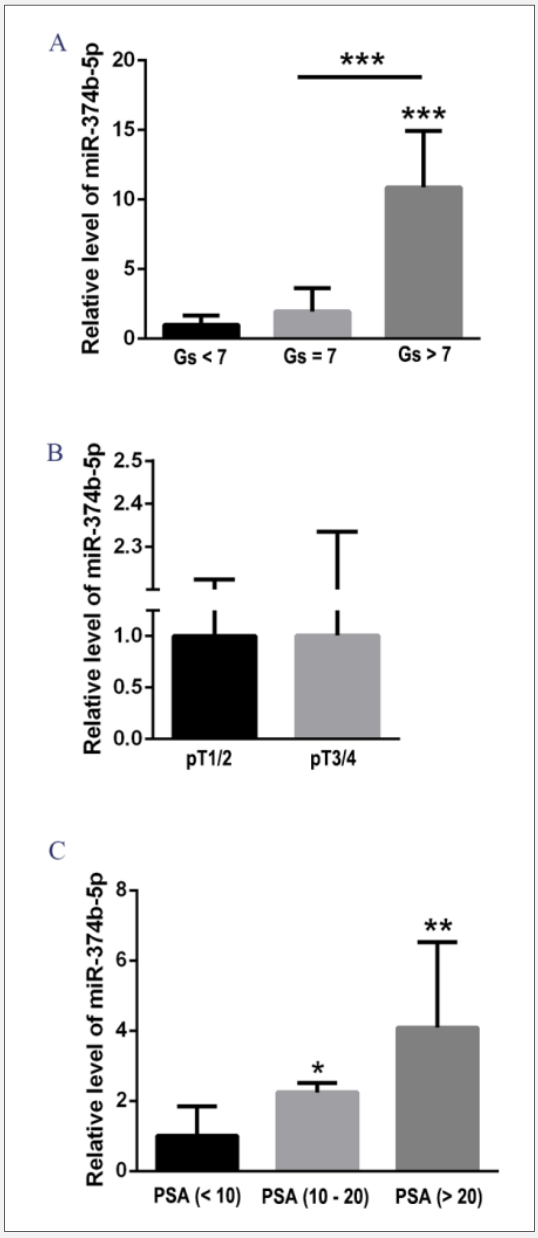

Figure 2. Clinicopathological parameter changes are accompanied by increased expression of miR-374b-5p in PCa patients whole-blood samples.

(A) The relative expression of miR-374b-5p in patients with Gs < 7, Gs = 7 and Gs > 7 (*** p < 0.001 vs. Gs < 7; *** p < 0.001 vs. Gs = 7). (B) The relative miR-374b-5p expression in stage pT1/2 and pT3/4 patients. (C) The expression of miR-374b-5p in patients with PSA < 10, PSA = 10 - 20 and PSA > 20 (* p < 0.05 vs. PSA < 10; ** p < 0.01 vs. PSA < 10). Data are presented as the mean ± S.D. Gs, Gleason score; pT, pathological tumor stage; PSA, prostate-specific antigen.

with stage pT3/4 was slightly higher than patients with stage pT1/2 ($p > 0.05$) (Figure 2B). The miR-374b-5p expression in patients with PSA < 10, PSA = 10 - 20 and PSA > 20 was respectively 1.0 ± 0.9, 2.2 ± 0.3 and 4.1 ± 2.4 (Figure 2C).

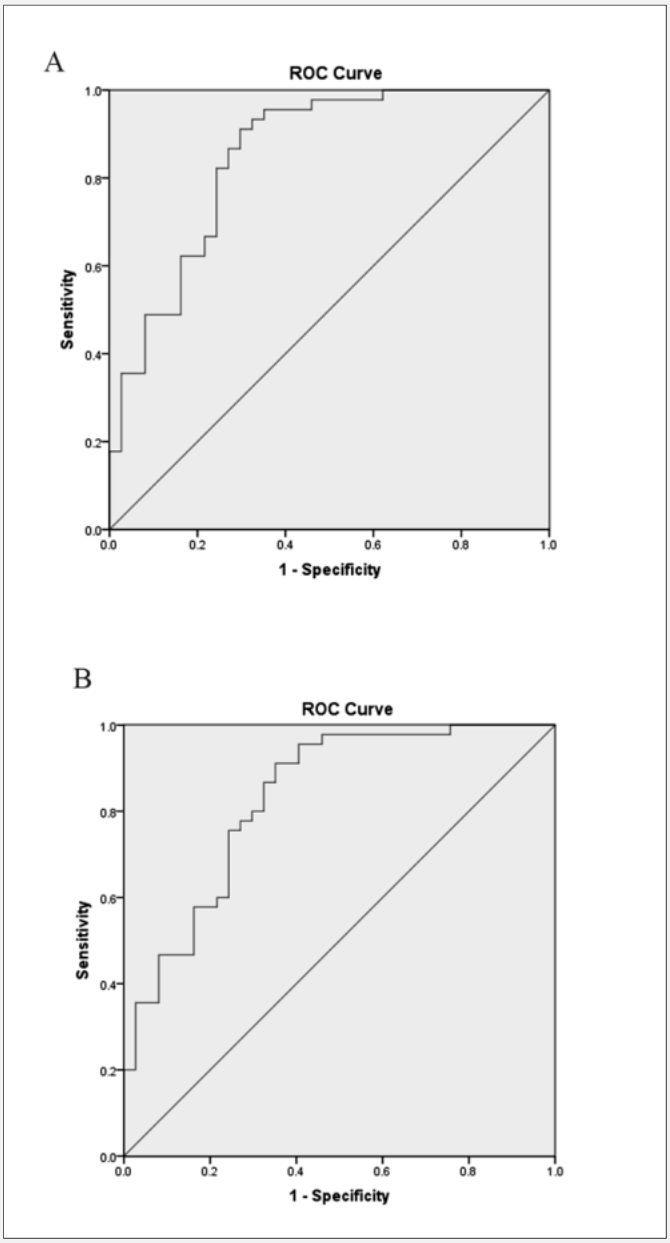

Figure 3. ROC curve analysis.

(A) The differential miR-374b-5p expression can screen PCa patients from HC (AUC, 0.851; 95% CI, 0.766 - 0.936; p < 0.001). (B) The miR-374b-5p expression level can screen PCa patients from BPH patients (AUC, 0.831; 95% CI, 0.742 - 0.920; p < 0.001). ROC - receiver operating characteristic, AUC - area under the curve, CI - confidence interval.

The miR-374b-5p level in peripheral blood may be regarded as a potential PCa biomarker

As a differentially expressed miR-374b-5p was validated in the peripheral blood of PCa patients, BPH patients, and HC subjects, the level of miR-374b-5p in peripheral blood might be identified as an indicated marker in patients with prostate cancer. Based on the analysis of ROC curves, peripheral blood miR-374b-5p was able to distinguish patients with prostate cancer from healthy controls (AUC, 0.851; 95% CI, 0.766 - 0.936; p < 0.001; Figure 3A), and BPH patients (AUC, 0.831; 95% CI, 0.742 - 0.920; p < 0.001; Figure 3B).

DISCUSSION

PCa is one of the leading causes of cancer-related death in males. The main diagnostic methods for detecting PCa are PSA testing, DRE, prostate cancer antigen 3 (PCA3), multiparametric magnetic resonance image (mpMRI), and prostate biopsy [5]. However, the diagnostic value of these tools is relatively limited. Therefore, because mature miRNAs have various advantages including easy detection of expressional alteration, possessing high stability and tissue-specific expression, they were suggested to be among the ideal biomarkers for classification of both physiological and pathological conditions [16-18].

There is growing evidence suggesting an important role for certain miRNAs in prostate carcinogenesis and linking altered miRNA expression to androgen signaling and prostate cancer aggressiveness [19,20]. In the present study, we first explored the expression of peripheral blood miR-374b-5p and confirmed that miR-374b-5p was significantly higher in PCa patients than in HC individuals and patients with BPH, which may indicate that miR-374b-5p has a strong correlation with PCa. We identified that miR-374b-5p was significantly upregulated in patients with more aggressive tumors (Gleason score > 7), suggesting a possible role in predicting the progression of PCa. ROC curve analysis showed that peripheral blood miR-374b-5p had a certain diagnostic value in patients with PCa. At the same time, the expression of miR-374b-5p was positively correlated with PSA level, which further suggested that miR-374b-5p might be involved in the unfavorable biological behavior.

miRNAs can function as a tumor suppressor or an oncogene depending on the targeted genes in different cell contexts [21]. Previous studies have shown that miR-374 may serve as either tumor suppressors or oncogenes in different types of tumor. Li et al. [14] suggested that miR-374a could promote hepatocellular carcinoma cell growth by targeting MIG-6 and activating AKT/ERK signaling pathway. Moreover, miR-374a promoted breast cancer metastasis by activating Wnt/β-catenin signaling [15]. However, Sun et al. [22] indicated that the upregulation of miR-374b-5p reduces the chemotherapeutic resistance of pancreatic cancer cells to gemcitabine by inhibiting the expression of several anti-apoptotic proteins, including BCL2, BIRC3, and XIAP. The function of a given miRNA is determined by the downstream target mRNAs, as such the same miRNA could have a dual role in different tissues, specifically tumors of different cellular origin [23]. Obviously, miR-374 is closely related to several malignant tumors.

CONCLUSION

Our study further enriched the potential carcinogenic effect of miR-374b-5p on PCa. However, the sample size of our study is not large enough, which may restrict the statistical power of the study. In the future, we will further expand the number of experimental samples to verify the expression of miR-374b-5p in patients with PCa. Furthermore, the mechanism by which miR-374b-5p is involved in PCa will be carried out through *in vitro* and *in vivo* experiments, so as to provide new ideas for the diagnosis and treatment of PCa.

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Declaration of Interest:
We declare no conflicts of interest.

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