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Original Article

Role of miR-4428 in the Early Diagnosis and Risk Assessment of Senile Prostatic Hyperplasia

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SUMMARY

Background: Benign prostate hyperplasia (BPH) is a common disease in elderly men. The purpose of this study is to evaluate the clinical value of miR-4428 in senile BPH, aiming to identify a novel and effective biomarker for BPH.
Methods: The study included 120 patients with BPH and 120 healthy individuals (controls). Expression levels of miR-4428 were measured with RT-qPCR. The risk factors of senile BPH were conducted by logistic regression analysis, and ROC curves were utilized to determine the diagnosis value of miR-4428 in of BPH. CCK8 and flow cytometric were used to study the proliferation and apoptosis of prostate cells. The oxidative stress response of BPH cells was evaluated by measuring malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH) levels.
Results: miR-4428 is upregulated in senile BPH patients and exhibits the ability to distinguish BPH from healthy individuals, demonstrating high sensitivity (83.33%) and specificity (71.67%). Upregulation of miR-4428 may be a predictive marker in senile BPH. Furthermore, a significant increase in miR-4428 was observed in BPH-1 cells than in WPMY-1 cells. This trend was reversed upon transfection with miR-4428 inhibitors, resulting in decreased proliferation rate and increased apoptosis rate of cells. Notably, BPH induces oxidative stress, which leads to increased levels of MDA and decreased levels of SOD and GSH, which can be reversed by transfection with miR-4428 inhibitors.
Conclusion: miR-4428 can differentiate between senile BPH patients and healthy individuals with high sensitivity and specificity, making it a valuable a diagnostic biomarker for BPH.

1. Introduction

Benign prostatic hyperplasia (BPH) is a common diseases of the urinary system, and age is an important risk factor.[1] The main cause of the disease is the body's sex hormone metabolism disorders, and then leads to the prostate gland, fiber, and muscle tissue appearing in different degrees of hyperplasia, and finally leads to urinary dysfunction.[2] BPH incidence increases with age, approximately 13.8% in men aged 40–49 years, more than 40% in men aged 50–79 years, and more than 80% in men aged 80 years and older.[3] The clinical manifestations of BPH include renal impairment, urinary retention, and dysuria which seriously deteriorates the life quality of older men.[4] Among the aging population, BPH has become a major clinical issue. Although there are a lot of medications for BPH, long-term use can produce serious physical dependence and adverse side effects, limiting their effectiveness.[5,6] Previous studies have shown that miRNAs may play an important role in BPH.[7] However, the precise role of miRNAs in BPH are still not fully understood because of the complex interaction of miRNAs with gene expression levels.

miRNAs are 22 nucleotides encoding single-stranded noncoding RNAs, that regulate gene expression by targeting mRNAs for cleavage or translation.[8] They are crucial for processes including biogenesis, expression regulation, biological function, epigenetics and cell-cell communication.[9] Dysregulation of miRNAs may result in the diseases such as prostate hyperplasia, autoimmune diseases, and cancer.[10] Therefore, many miRNAs are utilized clinically as biomarkers for disease diagnosis or targeted therapy. For example, miR-182 is upregulated in prostate cancer and represents a potentially useful diagnostic and prognostic biomarker.[11] The diagnostic accuracy of miR-30a-5p combined with miR-654-5p as biomarkers in the diagnosis of heart failure showed an increasing trend.[12] miR-200a-3p can protect intestinal epithelial cells from limited partner injury by inhibiting RIPK1-mediated inflammation and necrosis, which provides a possible target for necrotizing enterocolitis.[13] miR-4428 is a non-coding RNA associated with disease. Multiple studies confirm that miR-4428 is significantly upregulated in malignant tumor tissues, such as lung adenocarcinoma, non-small cell lung cancer, and papillary thyroid carcinoma.[14–16] However, there are few research reports on the relationship between BPH and miR-4428.

In the present study, the relationships between the level of miR-4428 expression in the serum of BPH patients and early diagnosis and risk assessment were investigated. Additionally, the effects of transfection with miR-4428 inhibitor on proliferation and apoptosis rates, and the changes level of oxidative stress indices were investigated in both healthy individuals and BPH patients. The aim of this study was to develop a new diagnostic biomarker for BPH.

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2. Materials and methods

2.1. Study subjects

A total of 120 senile BPH patients treated at Zhangjiakou First Hospital from January 2022 to December 2023 were included in BPH group. Fasting blood samples were taken from these subjects. Samples were centrifuged at 4,000 rpm for 10 min to obtain serum and then centrifuged at 12,000 rpm for 15 min to remove cellular debris. The obtained samples were stored at -80 °C for reserve. Another 120 healthy subjects were included in the control group. This study was conducted with the approval of the Ethics Review Committee of Zhangjiakou First Hospital.

2.2. Collection and testing of general data

General data such as age and body mass index (BMI) of patients and healthy individuals were collected and recorded through medical records retrieval. The prostate volume (PV) was measured by ultrasonic diagnostic instrument (GE LOGIQ E9, USA). Patients were asked to drink 500–1000 mL of water, and their urine is drained into urograph (Laborie, Canada) to record maximum flow rate (Qmax). Prostate-specific antigen (PSA) was determined using the patient's serum by ELISA kit (Abcam, UK). The patient's conditions were recorded according to the international prostate symptom score (IPSS) and quality of life score (QOL).

2.3. Cell culture

The human prostate stromal cell line WPMY-1 and the human BPH epithelial cell line BPH-1 were obtained from the cell bank of the Chinese Academy of Sciences (Beijing, China). The cells were cultured in DMEM medium at 37 °C and 5% CO_2.

2.4. Cell transfection

Cells were plated in a growth medium without antibiotics 24 h before transfection. Transient transfection with the miR-4428 inhibitor or the inhibitor negative control (inhibitor NC) was performed with Lipofectamine 2000 (Invitrogen). Cells were inoculated into 24-well plates, incubated for 6 h, and then replaced with a complete medium substrate. Cells were available 48 h after transfection.

2.5. RNA extraction

Samples were blended with Trizol reagent (Thermo, USA) and chloroform, mixed with rapid shock, and left on ice for 5 min. Then, centrifuge 12,000 rpm for 10 min. The supernatant was mixed with isopropyl alcohol and centrifuged at 4 °C at 12,000 rpm for 10 min. After 75% alcohol washing, ddH_2O was added to the precipitate to obtain total RNA. Concentration and purity were assessed using a NanoDrop2000 (Thermo, USA), with OD260/280 values between 1.8 and 2.0 being deemed suitable for subsequent experiments.

2.6. Real-time quantitative PCR

cDNA was synthesized with MiScript Reverse Transcription Kit (Qiagen, Germany). Then, the amplification was carried out on a real-time qPCR system (Bio-Rad, USA) using SYBR Green (Invitrogen, USA). The reaction conditions were 95 °C for 10 min, 95 °C for 15 s, 60 °C for 1 min, 40 cycles. The relative expression was calculated by the$2^{-\Delta\Delta ct}$ method and normalized to U6.

2.7. Assessment of cell proliferation

Cells were inoculated in 96-well plates with 3 repeats per well. CCK8 (Dojindo Molecular Technologies, Japan) reagent was added after incubation at 37 °C for 24, 48 and 72 h. After 3 h of incubation, the OD450 in each well was determined by microplate Reader.

2.8. Flow cytometry

A total of 2×10^6 cells from each group were taken and resuspended in 1 × binding buffer containing fluorescent dye-coupled Annexin V. The mixture was incubated at room temperature for 15 min, add 5 μL of propidium iodide staining solution, and apoptotic cells were detected by flow cytometry.

2.9. Oxidative stress detection

The concentration of malondialdehyde (MDA), activity of superoxide dismutase (SOD), and expression levels of glutathione (GSH) were used to indirectly evaluate oxidative stress of BPH cells. The cultured cells were determined according to the instructions of MDA, SOD and GSH kits (JIAN CHENG TECHNOLOGY, China).

2.10. Statistical analysis

SPSS 27.0 or GraphPad Prism 9.0 were used to analyze all data, and the mean ± SD (n = 3). Differences between groups were assessed using Student's t-test or one-way ANOVA. A chi-square test was used to evaluate the relationship between the expression of miR-4428 and the clinicopathologic parameters. The significance of miR-4428 in differentiating BPH from healthy subjects was assessed by ROC analysis. Statistical significance was considered by $p < 0.05$.

3. Results

3.1. General information of BPH patients and healthy controls

The healthy control group was 120 cases, with an average age of 68.93 ± 5.50 years. Similarly, 120 cases of BPH, with an average age of 69.86 ± 5.80 years. Age and BMI were not significantly different from patients with BPH ($p > 0.05$, Table 1). While BPH patients showed a higher prostate volume (PV, 46.27 ± 8.80 vs. 35.85 ± 6.00 mL) and prostate-specific antigen (PSA, 5.41 ± 1.46 vs. 2.30 ± 1.22 ng/mL) than that of healthy individuals. The maximum flow rate (Qmax, 9.89 ± 2.68 vs. 16.01 ± 4.21 mL/s) of BPH patients is lower than healthy individuals ($p < 0.05$, Table 1).

Table 1
General information of the study subjects.

	Healthy control (n = 120)	BPH patients (n = 120)	p value
Age (years)	68.93 ± 5.50	69.86 ± 5.80	0.206
BMI (kg/m^2)	23.56 ± 3.20	24.07 ± 4.11	0.282
PV (mL)	35.85 ± 6.00	46.27 ± 8.80	< 0.001
Qmax (mL/s)	16.01 ± 4.21	9.89 ± 2.68	< 0.001
PSA (ng/mL)	2.30 ± 1.22	5.41 ± 1.46	< 0.001
IPSS score	-	17.24 ± 8.87	-
QOL score	-	3.72 ± 1.54	-

BMI, body mass index; IPSS, international prostate symptom score; PSA, prostate-specific antigen; PV, prostate volume; Qmax, maximum flow rate; QOL, quality of life score.

3.2. Relationships of miR-4428 with BPH patients' clinicopathological features

By analyzing the expression level of miR-4428 in patients with prostate hyperplasia, the relationships between clinicopathological characteristics and expression level of the patients were estimated. It was divided into high group and low group according to the average expression value of miR-4428. The significant association of senile BPH degree with PV (p = 0.018), Qmax (p = 0.018), PSA (p = 0.013), IPSS score (p = 0.006), QOL score (p = 0.045), suggesting that they are involved in developing senile BPH (Table 2).

3.3. Analysis of risk factors of senile BPH

The results of logistic regression analysis showed that miR-4428 (p < 0.001), Qmax (p = 0.012), and PSA (p < 0.001) were identified as risk factors for BPH in the elderly (Table 3).

3.4. Expression and significance of miR-4428 in BPH

In senile BPH patients, miR-4428 expression levels were elevated compared to healthy individuals, and the difference was statistically significant (p < 0.001, Figure 1A). Meanwhile, miR-4428 has high sensitivity (83.33%) and specificity (71.67%) for distinguishing BPH patients from healthy individuals (AUC = 0.8479, Figure 1B) via ROC curve.

Table 2
Association between clinicopathological features and miR-4428 expression levels in BPH patients.

Variable	Total (n = 120)	miR-4428		p value
		Low (n = 59)	High (n = 61)	
Age (years)				0.279
< 69	53	29	24	
≥ 69	67	30	37	
BMI (kg/m^2)				0.364
< 24.07	58	31	27	
≥ 24.07	62	28	34	
PV (mL)				0.018
< 46.27	60	36	24	
≥ 46.27	60	23	37	
Qmax (mL/s)				0.013
< 9.89	74	43	31	
≥ 9.89	46	16	30	
PSA (ng/mL)				0.006
< 5.41	52	33	19	
≥ 5.41	68	26	42	
IPSS score				0.045
< 17.24	60	35	25	
≥ 17.24	60	24	36	
QOL score				0.067
< 3.72	61	35	26	
≥ 3.72	59	24	35	

BMI, body mass index; IPSS, international prostate symptom score; PSA, prostate-specific antigen; PV, prostate volume; Qmax, maximum flow rate; QOL, quality of life score.

Table 3
Analysis of risk factors of senile prostatic hyperplasia.

	OR	(95% CI)	p value
miR-4428	0.115	0.057–0.231	< 0.001
Age (years)	1.017	0.511–2.022	0.962
BMI (kg/m^2)	1.015	0.512–2.011	0.967
PV (mL)	0.502	0.244–1.030	0.060
Qmax (mL/s)	2.423	1.217–4.825	0.012
PSA (ng/mL)	0.156	0.077–0.318	< 0.001

BMI, body mass index; OR, odds ratio; PSA, prostate-specific antigen; PV, prostate volume; Qmax, maximum flow rate.

3.5. Effect of miR-4428 on BPH cell

miR-4428 was significantly upregulated in BPH-1 cells compared to WPMY-1 cells (Figure 2A). The expression of miR-4428 in various types of BPH-1 cells, and the expression of co-transfection of its inhibitor was significantly decreased after transfection (Figure 2B). Obviously, inhibition of miR-4428 could significantly decrease cell proliferation (Figure 2C) and increase apoptosis rate (Figure 2D).

3.6. Effects of miR-4428 on oxidative stress of BPH cells

The untreated BPH-1 cells showed relatively higher concentrations of MDA (Figure 3A), and the decreasing activity of SOD (Figure 3B) and GSH (Figure 3C) in comparison with WPMY-1 cells (p < 0.01). Transfection with miR-4428 inhibitor reversed this trend, decreasing MDA (Figure 3A) concentration and increasing SOD (Figure 3B) and GSH (Figure 3C) concentration (p < 0.01).

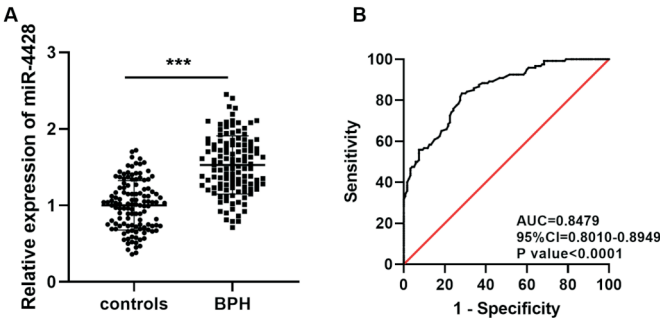

Figure 1. The expression and significance of miR-4428 in BPH. (A) miR-4428 was upregulated in BPH patients compared with healthy controls. (B) miR-4428 can distinguish between BPH patients and healthy individuals with high sensitivity and specificity. AUC, area under curve; BPH, benign prostatic hyperplasia. *** p < 0.001.

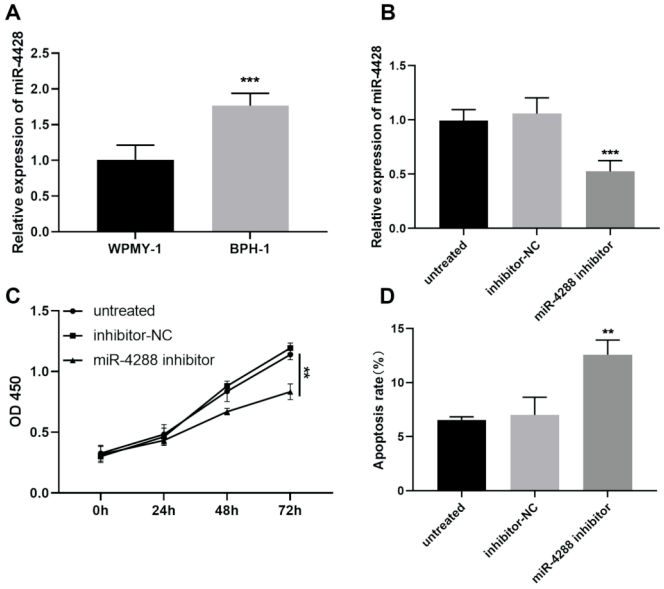

Figure 2. Effect of miR-4428 on proliferation and apoptosis in BPH-1 cells. (A) The expression in BPH-1 cells was higher than in WPMY-1 cells of miR-4428. (B) Transfection with miR-4428 inhibitor decreased miR-4428 expression. (C) Transfection with miR-4428 inhibitor decreased cell proliferation. (D) Transfection with miR-4428 inhibitor accelerated apoptosis. NC, negative control. ** p < 0.01, *** p < 0.001.

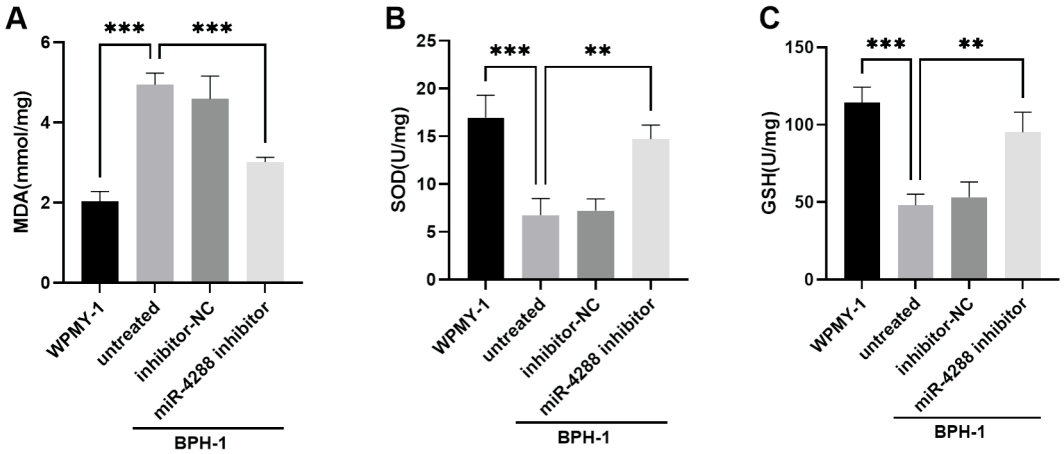

Figure 3. Effects of miR-4428 on oxidative stress of BPH cells. (A) BPH induced the increase of MDA concentration, and transfected miR-4428 inhibitor decreased this trend. (B, C) BPH induced the decrease of SOD and GSH concentrations, and transfection with miR-4428 inhibitor reversed this trend. GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase. ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

BPH generally has a long course of disease, and its clinical symptoms generally become more and more serious with the increasing age of patients.[17] miRNA is an endogenous non-coding RNA small molecule, mainly composed of 21–25 nucleotides, which has functions of regulating gene transcription and translation.[18] There have been extensive reports on the role of miRNAs in prostate cancer, but very few studies have been done on miRNAs in BPH. Currently, a variety of different miRNAs have been found clinically, which can be used as markers of disease prognosis.[19] Zhang et al. found many miRNAs related to BPH when studying MicroRNA expression profiles in benign prostatic hyperplasia, including miR-4428.[20] However, studies on the relationship between miR-4428 and BPH, and other aspects have not been carried out. Therefore, this study focused on evaluating the function of miR-4428 in early diagnosis and risk assessment of senile BPH, with a view to developing new potential clinical biomarkers.

It has been previously reported that miR-4428 interacts with various factors to play different functions in different diseases, and has important applications value in the early diagnosis, treatment, and prognosis of diseases.[21] miR-4428 is a downstream target gene of RGMB-AS1 to regulate PBX1, and miR-4428 is negatively correlated with the expression of both RGMB-AS1 and PBX1, which may be an effective way to develop therapeutic approaches for patients with cervical cancer.[22] Additionally, ACTA2-AS1 interacts with miR-4428 and negatively regulates the expression of BCL2L11 to inhibit the progression of colon adenocarcinoma.[23] It has been reported that circCSNK1G1 can regulate miR-4428/FUT2 signaling pathway to participate in Osteoarthritis progression, which could present a new target for clinical of Osteoarthritis in the clinical setting.[24] Our study found that miR-4428 was highly expressed in senile BPH patients, consistent with previous reports.[20] This suggests that high expression of miRNA-4288 is related to the occurrence of BPH. Meanwhile, ROC curve analysis for the miR-4428 has a high AUC between groups of BPH patients and controls. The results demonstrated that the aberrant expression of miR-4428 was detectable with sensitivity and specificity and was able to distinguish between BPH patients and normal subjects. It also further confirmed that miRNA-4288 may be used as a biomarker for detection of BPH.

Our study found that the expression of miR-4428 in BPH-1 cells was significantly increased compared with WPMY-1 cells, aligning with previous reports.[20] Further research found that, after transfection with miR-4428 inhibitor, the expression of miR-4428 in BPH-1 cells was restored, cells proliferation rate was decreased, and apoptosis rate was accelerated. These results indicate that miR-4428 may inhibit the occurrence of BPH. Some dysregulation molecules are thought to regulate inflammatory responses and oxidative stress. It is possible to reflect the degree of oxidative stress in the body by detecting dynamic changes in oxidative stressors. The level of resistance may be evaluated by measuring the activity of antioxidant enzymes.[25] SOD is an important antioxidant enzyme in vivo, which has the function of clearing ROS. As the final product of lipid peroxidation in cells during oxidative stress, MDA is also a key indicator of lipid oxidation.[26] Study has shown that the prostatic hyperplasia patients is in a state of oxidative stress, and the level of MDA increases.[27] SOD 2 activity may interfere with the development of prostate cancer by induction of NF-κB pathway.[28] GSH, an enzyme that catalyzes the breakdown of hydrogen peroxide.[29] They are essential for cellular defense. In this study, MDA concentration in BPH-1 cells was higher and SOD and GSH concentration was lower, which were reversed by miR-4428 inhibitor transfection. These results indicate that miR-4428 plays an important role in regulation of senile BPH. The specific interaction mechanism between miR-4428 and BPH needs further study.

In summary, this study found that miR-4428 was significantly overexpressed in senile BPH patients, and could effectively distinguish between BPH and healthy individuals. Transfection with miR-4428 inhibitor reduced cell proliferation and accelerated cell apoptosis. Our study suggests that miR-4428 may be a novel biomarker for diagnosis of BPH.

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