Upregulation of miR-1199-5p is associated with reduced type 2 5-α reductase in benign prostatic hyperplasia

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Abstract

Background

The 5-α reductase inhibitors (5-ARIs) are the first-line drug managing benign prostatic hyperplasia (BPH). Unfortunately, some patients showed no responses to 5-ARIs therapy, even suffering from worse symptoms. Although the decreased expression of 5-α reductase type 2(SRD5A2) in BPH tissues might explain the 5-ARIs therapy failure, the mechanisms underlying SRD5A2 decreased remained unelucidated.

Objectives

To investigate the mechanisms of microRNA regulating the variable expression of SRD5A2 resulting in treatment failure of 5-ARIs.

Materials and methods

The expression of SRD5A2 and microRNAs in BPH tissues and prostate cells were detected by immunohistochemistry, western blotting, and quantitative real-time PCR (qRT-PCR). Dual-luciferase reporter assay was performed to confirm that microRNA directly combine to SRD5A2 mRNA. The apoptosis of prostatic cells was detected by flow cytometry.

Results

13.6%, 28.8%, and 57.6% of BPH tissues showed negative, weak, and strong positive SRD5A2 expression, respectively. Normal human prostatic epithelial cell line RWPE-1 strongly expressed SRD5A2, whereas the immortalized human prostatic epithelial cell line BPH-1 weakly expressed SRD5A2. miR-1199-5p expression level in BPH-1 was remarkably higher than that in RWPE-1(P□0.001), and miR-1199-5p expression was significantly upregulated in BPH tissues with negative SRD5A2 expression than those with positive SRD5A2 expression. After miR-1199-5p mimics transfection, SRD5A2 expression was decreased markedly in RWPE-1 cells, whereas after miR-1199-5p inhibitor transfection, SRD5A2 expression increased in BPH-1 cells. Dual-luciferase reporter assay showed that miR-1199-5p could bind the 3’ untranslated region of SRD5A2. Additionally, miR-1199-5p could decrease the sensibility of finasteride (100 μM) on RWPE-1 cells.

Conclusion

Our results demonstrate that SRD5A2 expression varied in BPH tissues and miR-1199-5p might be one of the several factors contributing to differential SRD5A2 expression in BPH patients.

1. Introduction

Benign prostate hyperplasia (BPH) is histologically characterized by aberrant proliferation of epithelial and stromal cells in the prostatic transition zone[1]. BPH incidence increases with age; approximately 50% of men in their 50s show pathological manifestations of BPH and more than 80% in their 80s[2, 3]. The overgrowth character of the prostate indicates that approximately 25% of men will develop BPH clinical symptoms in their whole life[4]. BPH deteriorates life quality of older men because of lower urinary tract symptoms (LUTSs), including urination and retention[1, 5].

Watchful waiting, drug therapy, and surgery are the BPH therapies at present[6]. Most patients receive medical therapy when LUTSs first occur, mainly including α-adrenergic blockers and 5-α reductase inhibitors (5-ARIs)[7]. However, only 5-ARIs effectively reduce the prostate size by approximately 20–30% in 4–6 months[8]. Targeting 5-α reductase (SRD5A), 5-ARIs block the transformation of testosterone (T) to dihydrotestosterone (DHT), which has a higher affinity to androgen receptors (ARs). Reduced DHT concentration in the prostate induces apoptosis and necrosis of AR-dependent cells and eventually reduces the prostate size[8, 9]. Finasteride and dutasteride are the two primary 5-ARIs drugs that target different 5-AR isotypes. Finasteride specifically inhibits 5-α reductase type 2(SRD5A2), mainly expressed in the prostate[10], whereas dutasteride inhibits both 5-α reductase 1 and SRD5A2; however, both these inhibitors show similar therapeutic efficacy.

Although 5-ARIs were considered the first-line therapy in BPH management, approximately 30% of patients complained of no improvement in symptoms after the 5-ARIs therapy, suffered from worsened symptoms, and eventually required surgery[8, 11]. At present, the mechanisms of 5-ARIs treatment failure are inadequately understood, making it impossible to predict the effectiveness of 5-ARIs therapy on an individual. Thus, some patients must undergo ineffective long-term treatment, associated adverse effects, and unnecessary expenditure. Therefore, exploring the mechanisms resistant to 5-ARIs therapy is especially important.

Some investigations reported significant variability in SRD5A2 protein expression among the BPH samples, and 10 -36.5% of BPH samples did not express SRD5A2 protein, which was adjacent to the proportion of clinical patients resistant to ARIs[6, 8, 12]. These indicated that decreased SRD5A2 expression in BPH tissues might lead to 5-ARIs therapy failure. Therefore, SRD5A2 is expected to be a specific molecular marker for distinguishing whether individuals are responsive to 5-ARIs. However, the mechanisms inducing the decreased SRD5A2 expression in prostate tissues are not fully understood.

miRNA, a group of extremely conserved short non-coding RNAs, ranging from 19 -22nt, regulates protein expression by binding to specific mRNA sequences[13]. Like DNA methylation and histone modification, miRNA plays critical epigenetic roles in initiating and progressing many diseases[14, 15]. A single miRNA can target several mRNAs to affect the expression of many genes[16]. miR-1199-5p, located on chromosome 19p13.12, is an epithelial-mesenchymal transition (EMT)-regulatory miRNA that inhibits

EMT and tumor cell invasion[17]. Here, we demonstrate the decreased SRD5A2 protein in 40.6% of the prostate samples. Additionally, miR-1199-5p could decrease the expression of SRD5A2 in prostatic epithelial cells and competitive inhibition of miR-1199-5p could effectively rescue the expression of SRD5A2 protein in epithelial cells expressing weak SRD5A2. And importantly, dual-luciferase report assay showed that miR-1199-5p could bind the 3'UTR of *SRD5A2* gene directly.

2. Materials And Methods

2.1 Patients

Fifty-nine BPH specimens of transition zone were collected from patients undergoing transurethral resection of the prostate at the Beijing Chaoyang Hospital, Capital Medical University. Patient characteristics are shown in Table 1. All human specimens were acquired under the approval of the Institutional Review Board of Beijing Chaoyang Hospital, Capital Medical University (2017-KE-6, Beijing, China). The research was executed according to the World Medical Association Declaration of Helsinki, and each patient signed written informed consent. The mean age of the BPH patients was 70 years, ranging from 56 -91 years. Before being evaluated for the expression of SRD5A2 protein, these specimens were paraffin-embedded and proven to be BPH and non-cancerous through routine histological analysis by pathologists.

2.2 Immunohistochemical analysis of SRD5A2

Immunohistochemistry (IHC) was performed as previously described by Lin *et al*[9]. Briefly, specimen sections were incubated with SRD5A2 primary antibody (Novus Biological Inc., Centennial, CO, USA NBP1-46510) following the manufacturer's recommendations at a concentration of 1/1500. Negative controls were used throughout the immunostaining protocol. Three representative areas from each sample were randomly selected under 40× magnification to assess immunoreactivity by two genitourinary pathologists. A hundred cells selected randomly from the epithelium were manually counted from each representative section. Each cell was scored on a 0–3 scale according to the intensity of the staining. Then, a visual score was generated for each sample, ranging from 0 to 300. A score of 0–100 was defined as weak expression, and a score of 101–300 indicated strong expression.

2.3 Cells and culture condition

The immortalized human prostatic epithelial cell line BPH-1 and HEK293T were obtained from the Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Normal human prostatic epithelial cell line RWPE-1 was acquired from Shanghai Zhong Qiao Xin Zhou Biotechnology Co.,Ltd. BPH-1 were cultured in RPMI 1640 medium (Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Melbourne, Australia) and 1% penicillin - streptomycin (HyClone, Logan, UT, USA). 293T were cultured in Dulbecco's Modified Eagle Medium (Gibco, Rockville, MD, USA) supplemented with 10% FBS and 1% penicililin -streptomycin. RWPE-1 was

cultured in Keratinocyte Medium with a keratinocyte growth supplement. Cells were incubated at 37℃ with 5% CO2. All in vitro experiments were repeated three times.

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

Total RNA was extracted using TRIzol reagent (Invitrogen Inc.,Carlsbad, CA, USA). The quality and quantity of extracted RNA were assessed using NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcriptase was used to produce the first-strand complementary DNA (TIANGEN, Beijing, China) according to the manufacturer's instructions. miRNA Real-Time PCR Assay kit was used to detect miRNA expression level (TransGen, Beijing, China). mRNA expression was normalized to GAPDH expression, whereas miRNA was normalized to U6. The relative RNA expression was calculated as the inverse log of the delta/delta CT. Specific primers are shown in Table 2.

2.5 Cell transfection

miR-1199-5p mimics, miR-100-5p inhibitor, and miRNA negative control (miR-NC) were synthesized by Suzhou GenePharma Co., Ltd. (Suzhou, China) and transfected into cells by siRNAmate Suzhou GenePharma Co., Ltd. (Suzhou, China) according to the manufacturer’s instructions. The RNA was extracted for qRT-PCR after transfection for 24h, and the protein was extracted for western blotting after transfection for 48h.

2.6 Western blotting

Cells were lysed for extracting total protein with RIPA buffer containing a mixture of protease and phosphatase inhibitors. The protein concentration was detected by the Pierce^{TM} BCA protein assay reagent (Thermo, Rockford, USA). 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel was used to separate the protein lysates. Then, they were transferred to poly (vinylidene fluoride) (PVDF) membranes (Merck KGaA, Darmstadt, Germany). The membranes were blocked and incubated with primary antibody of SRD5A2(1:1000) or GAPDH (1:2000) at 4 ℃ overnight. Subsequently, the PVDF membranes were washed three times with TBST and then incubated with horseradish peroxidase-conjugated secondary antibodies (1:2000; Abcam, Cambridge, UK) at room temperature for 1 h. Then, the membranes were washed again three times for 5 mins each with TBST. Enhanced chemiluminescence (ECL) western blotting substrate was used for visualization and detection.

2.7 Dual-luciferase reporter assay

For the dual-luciferase reporter assay, 293T cells were seeded in 24-well plates (50 000 per well) and co-transfected with a PGL-3 control plasmid, wild-type or mutant SRD5A2 3′UTR vector, and miR-1199-5p mimics or control (Suzhou GenePharma Co., Ltd, Suzhou, China). After 24 h, the cells were harvested, and Firefly/Renilla luciferase activities were analyzed using the dual-luciferase reporter assay kit according to the manufacturer's protocol (Promega, Madison, WI, USA).

2.8 Flow cytometry analysis assay

Annexin V-FITC and PI Detection Kit (BD Biosciences, NJ, USA) was used to determine cell apoptosis. 2×10^5 RWPE-1 or BPH-1 cells treated with finasteride/DMSO and mimics/inhibitor were harvested, resuspended in 100μl flow cytometry binding buffer, and then stained with 5μl Annexin V/FITC and 5μl PI following manufacturer's instructions. Determining BPH-1 and RWPE-1 cells apoptosis using flow cytometry (BD FACSCanto™ II, NJ, USA).

2.9 Statistical analysis

All statistical analyses were performed using SPSS 23 software (SPSS, Chicago). Comparisons between datasets from qRT-PCR, western blotting and fluorescence analysis were carried out using a t-test. All tests were two tailed, and $P < 0.05$ was considered statistically significant.

3. Results

3.1 Diversity of SRD5A2 expression in different BPH tissues and cells

To evaluate SRD5A2 expression in different prostate tissues, a total of 59 BPH specimens were collected. Immunohistochemical staining showed that SRD5A2 was expressed primarily in epithelial cells of the prostate transition zone, and a small amount of SRD5A2 was expressed in stromal cells, which was consistent with previous studies[6, 7, 18]. In our study, SRD5A2 expression varied in different patients. Eight cases (13.6%) showed negative SRD5A2 expression, 17 cases (28.8%) showed weak expression and 34 cases (57.6%) showed strong positive expression (Fig. 1A, B). Meanwhile, SRD5A2 expression in BPH-1 and RWPE-1, two classical cell lines of prostatic epithelium, were detected by western blotting. We found that SRD5A2 was slightly expressed in BPH-1, whereas strongly expressed in RWPE-1(Fig. 1C).

3.2 miR-1199-5p is expressed differently in different BPH tissues and cells

We used two databases, miRWalk[19] and miRDB[20], to predict potential miRNAs that might bind the 3'UTR of SRD5A2. Among them, miRWalk predicted 1334 results and miRDB predicted 83 results, with a total of 39 intersections (Fig. 2A). We selected nine miRNAs from the 39 intersections with high prediction scores in the miRDB database as candidate miRNAs (Table 3), namely: miR-4666a-5p, miR-3907, miR-548m, miR-146b-5p, miR-4448, miR-3174, miR-6751-3p, miR-1199-5p and miR-5591-5p.

To identify miRNAs that could affect SRD5A2 expression, we detected the relative expression values of nine candidate miRNAs through qRT-PCR in BPH-1 and RWPE-1. The results showed that miR-1199-5p expression was significantly higher in BPH-1 than in RWPE-1 (Fig. 2B). To understand the interrelation between miR-1199-5p and SRD5A2 expression in clinical samples, we analyzed miR-1199-5p expression in tissues with negative SRD5A2 expression, weak SRD5A2 expression, and positive SRD5A2 using qRT-

PCR. The results showed that miR-1199-5p expression was significantly upregulated in the tissues with negative and weak SRD5A2 expression than those with positive SRD5A2 expression (Fig. 2C).

3.3 Mir-1199-5p Regulate The Srd5a2 Expression And Decrease The Apoptosis Of Rwpe-1

To verify whether miR-1199-5p could regulate SRD5A2 expression, we transfected miR-1199-5p mimics into RWPE-1 cells that strongly expressed SRD5A2 and miR-1199-5p inhibitors into BPH-1 cells that weakly expressed SRD5A2. qRT-PCR and western blotting were used to detect the changes in SRD5A2 mRNA and protein expression levels after transfection. The results showed that the expression of SRD5A2 mRNA (Fig. 3A) and protein (Fig. 3B) were significantly decreased after RWPE-1 cells were transfected with miR-1199-5p mimics. After being transfected with miR-1199-5p inhibitor, the protein level of SRD5A2 significantly elevated in BPH-1 cells (Fig. 3C). Additionally, we used the RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/)[21]to predict the binding sites of miR-1199-5p to SRD5A2. According to the specific binding sites, we constructed two SRD5A2 3'UTR luciferase reporter constructs, one containing the species conserved seed sequence and the other carrying a mutated version of the seed sequence with seven nucleotides exchanged (Fig. 3D). Transient transfection of miR-1199-5p mimics and the SRD5A2 3'UTR wild-type reporters in 293T cells revealed a significant decrease in luminescence, which was not observed in the mutant version of the reporter (Fig. 3E). These results confirmed that miR-1199-5p can bind to SRD5A2 3'UTR and inhibit its expression.

We also detected the influence of finasteride on BPH-1 and RWPE-1 after mimics and inhibitors transfection by flow cytometry. As we previously reported, finasteride could not induce apoptosis of BPH-1, which expressed decreased SRD5A2[9]. However, increasing SRD5A2 expression in BPH-1 cells could not develop the sensibility of finasteride (100 μM) on BPH-1 cells after miR-1199-5p inhibitor transfection (Fig. 3F). Notably, finasteride (100 μM) could accelerate the apoptosis process of RWPE-1 and decreasing the expression of SRD5A2 could inhibit its progression after miR-1199-5p mimics transfection (Fig. 3G). Different AR expressions might explain this phenomenon. Particularly, RWPE-1 cells express AR, whereas BPH-1 cells lack AR[22, 23].Thus, these data indicated that miR-1199-5p could decrease SRD5A2 expression and influence prostate cells apoptosis.

4. Discussion

The 5-α reductase is the key enzyme in transforming T into DHT, the major steroid hormone for AR, and SRD5A2 is the main subtype distributed in prostate tissues. BPH, androgenic alopecia, and prostate cancer are associated with high levels of DHT produced by SRD5A2 because of excessive AR signaling. Currently, 5-ARI therapy is one of the most important medical treatments in managing BPH by inhibiting SRD5A2 to hinder the transformation of T into DHT[24]. Additionally, there are some herbs for BPH treatment targeting SRD5A2. Jin BR *et al*. found that baicalin ameliorated pathological prostate enlargement by inhibiting SRD5A2 activity and androgen-dependent apoptosis[22]. Song KH *et al*. reported

that extracts of Phyllostachys pubescens leaves repressed SRD5A2 promoter activity and enhanced BPH in a rat model. However, SRD5A2 expression varied in BPH tissues. In our present study, we observed that 27% of BPH tissues had weak SRD5A2 expression and 13.6% of tissues had nearly no SRD5A2 expression, which is close to 10% reported by Lin[9], and lower than 30% reported by Niu[6] and Wang[25]. Kang *et al*. reported that the reduction of SRD5A2 in prostate tissue in some patients might be responsible for 5-ARIs treatment failure[8]. Understanding the reasons behind reduced SRD5A2 in some prostate tissues might help identify patients insensitive to 5-ARIs treatment and further avoid ineffective over treatment with adverse effects.

DHT is indispensable during the formation of male genitalia, prostate, urethra, and development of secondary sexual characters during puberty. Small prostate and female pseudohermaphroditism occur when a male human lacks SRD5A2 expression during fetal period[8, 26]. Therefore, epigenetic modifications during adulthood should account for the decreased SRD5A2 expression in some BPH patients. Epigenetic modifications induced reversible and heritable changes, which promoted differences in gene expression without altering DNA sequence, including DNA methylation, histone modifications, and miRNA[27]. There are several research about the variability of SRD5A2. Austin DC et al. found that inflammation could increase SRD5A2 expression by activating NF-κB, resulting in BPH progression and 5-ARIs resistance[7]. However, Ge R et al. reported that inflammatory mediators could decrease SRD5A2 expression by increasing DNMT1 expression, which methylated SRD5A2 promoter[12]. Xue B et al. suggested that inflammatory mediators and saturated fatty acid could facilitate SRD5A2 promoter methylation by stimulating macrophages[11]. Previous studies have identified that the methylation of the promoter region of the SRD5A2 gene was closely related to the decrease in SRD5A2 protein expression and increasing age and inflammatory mediators were associated with high methylation of SRD5A2 promoter[6, 8, 9, 11, 12]. However, 10% of BPH tissues with negative SRD5A2 expression showed approximately no methylation in the promoter region of SRD5A2[9], suggesting the occurrence of other underlying mechanisms adjusting SRD5A2 expression in the prostate.

As a small non-coding RNA widely expressed in various cells, miRNA could bind to the 3'UTR of mRNA to form a silencing complex, causing mRNA degradation or repressing translation[28]. There are several reports about miRNA in prostate cancer, but only a few on BPH investigation. Zhang *et al*. identified 26 dysregulated miRNAs between BPH and healthy men, suggesting that aberrantly expressed miRNAs might be involved in BPH development[29]. Wang *et al*. reported that long noncoding RNA RNM3OS inhibited miR-361 and miR-29a/29b, which suppressed the expression of COL3A1 and TGF-β1, leading to BPH development by promoting TGFβ1-induced prostate stromal cells transformation into myofibroblasts[30]. However, there are no relevant studies on miRNA and SRD5A2 at present. In our study, we found miR-1199-5p expression in BPH tissues with negative and weak SRD5A2 expression was significantly higher than those with positive SRD5A2 expression. Additionally, SRD5A2 expression decreased in RWPE-1 transfected with miR-1199-5p mimics, whereas SRD5A2 expression was rescued in BPH-1 cells when transfected with miR-1199-5p inhibitors. We further verified that miR-1199-5p adjusted SRD5A2 expression by binding its 3'UTR through luciferase assay.

After SRD5A2 expression decreased, the mechanisms promoting BPH were still under discussion. We found decreased SRD5A2 expression in RWPE-1 could increase the insensitive to finasteride (100 μM) treatment after miR-1199-5p mimics transfection. Besides, Wang *et al*. reported an androgen to estrogen switch in the absence of SRD5A2 resulting from promoter methylation in prostate tissues, followed by estrogen playing the dual role of proliferation or inhibition via ERα or ERβ separately[25]. Although testicles could secrete small an amount of estrogen, 75–90% of circulating estrogens in men were converted by aromatase[31]. Chen *et al*. identified that estrogen promoted the proliferation of primary stromal cells and increased CCR3, CD40L, CXCL9, IL-10, and IL-17 in BPH tissues[32]. We detected estrogen levels in ten pairs of prostate tissues with weak or strong SRD5A2 expression. The results showed that the estrogen level of prostate tissues with weak SRD5A2 expression significantly increased (P = 0.0002, the result is not shown), suggesting that SRD5A2 was inversely related to estrogen in prostate tissue.

To the best of our knowledge, our study is the first to explore the correlation between miRNA and SRD5A2 expression, which is innovative to a certain extent. miR-1199-5p may serve as a marker to screen patients with different SRD5A2 expression levels in clinical settings and conduct personalized treatment for them. However, there are a few limitations. Due to the relatively limited number of tissues, there is no definite difference in miR-1199-5p expression between tissues with negatively and weakly expressed SRD5A2. Thus, further research with a large sample size of BPH tissues is required. Besides, the BPH tissues collected in this study did not exclude patients treated with 5-ARIs before surgery; hence, it was unclear whether 5-ARIs could affect SRD5A2 expression. Therefore, further research is needed to support our hypothesis that miR-1199-5p interferes SRD5A2 expression and its clinical implications on personalized management of BPH.

Abbreviations

5-ARIs: 5-α reductase inhibitors

BPH: benign prostatic hyperplasia

SRD5A2: 5-α reductase type 2

LUTSs: lower urinary tract symptoms

T: testosterone

DHT: dihydrotestosterone

ARs: androgen receptors

EMT: epithelial-mesenchymal transition

IHC: Immunohistochemistry

FBS: fetal bovine serum

qRT-PCR: quantitative real-time PCR

ECL: Enhanced chemiluminescence

Declarations

Ethics approval and consent to participate

The research was carried out according to the World Medical Association Declaration of Helsinki, and each patient has signed written inform consent. The research was approved by the Institutional Review Board of Beijing Chaoyang Hospital, Capital Medical University (2017-KE-6, Beijing, China).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are available on reasonable request from the corresponding author.

Competing interests

The authors declare no competing interests.

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Authors' contributions

The authors listed below substantially contributed to the intellectual content of the paper in various sections. YNN and YC designed and supervised the study. ZLL and ZML designed, prepared, drafted, and revised the manuscript and performed the statistical analysis. ZML and ZLL collected and tested prostate tissues. FC and MXJ performed cell testing. All authors read and approved the final manuscript.

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Tables

Table 1. Principal cohort characteristics (n = 59)

Variables	Mean	Standard deviation	Min	Max
Age, years	70.5424	7.43552	56	91
IPSS	22.5333	6.94676	12	33
PSA, ng/ml	6.489	6.59175	0.21	34.43
BMI	23.7269	2.68572	17.99	29.74
TPV, ml	75.9058	29.08218	24.52	146.89

IPSS, international prostate symptom score; PSA, prostate-specific antigen; TPV, total prostate volume.

Table 2. Primer sequences used in reverse transcription quantitative-polymerase chain

Gene	Direction	Primer sequence (5'- 3')
miR-4666a-5p	F	CGCGATACATGTCAGATTGTATGCC
miR-3907	F	TATATAGGTGCTCCAGGCTGGC
miR-548m	F	CGCGCAAAGGTATTTGTGG
miR-146b-5p	F	GTCCAGTTTTCCCAGGAATCCC
miR-4448a	F	GGCTCCTTGGTCTAGGGGTA
miR-3174	F	CCTAGTGAGTTAGAGATGCAGAGCC
miR-6751-3p	F	ACTGAGCCTCTCTCTCTCCAG
miR-1199-5p	F	TATATATACCTGAGCCCGGGCC
miR-5591-5p	F	GATGCCCATGCCGATTCTT
U6	F	CTCGCTTCGGCAGCACA
	R	AACGCTTCACGAATTTGCGT
SRD5A2	F	ACTGCTCAATCGAGGGAGG
	R	CACCCAAGCTAAACCGTATGTC
GAPDH	F	GAACGGGAAGCTCACTGG
	R	GCCTGCTTCACCACCTTCT
β-actin	F	GAGCGGGAAATCGTGCGTGACATT
	R	GATGGAGTTGAAGGTAGTTTCGTG

Table 3. Prediction scores of miRNA

miRNA	Prediction score
miR-4666a-5p	90
miR-3907	87
miR-548m	85
miR-146b-5p	81
miR-4448	78
miR-3174	77
miR-6751-3p	75
miR-1199-5p	75
miR-5591-5p	73

Figures

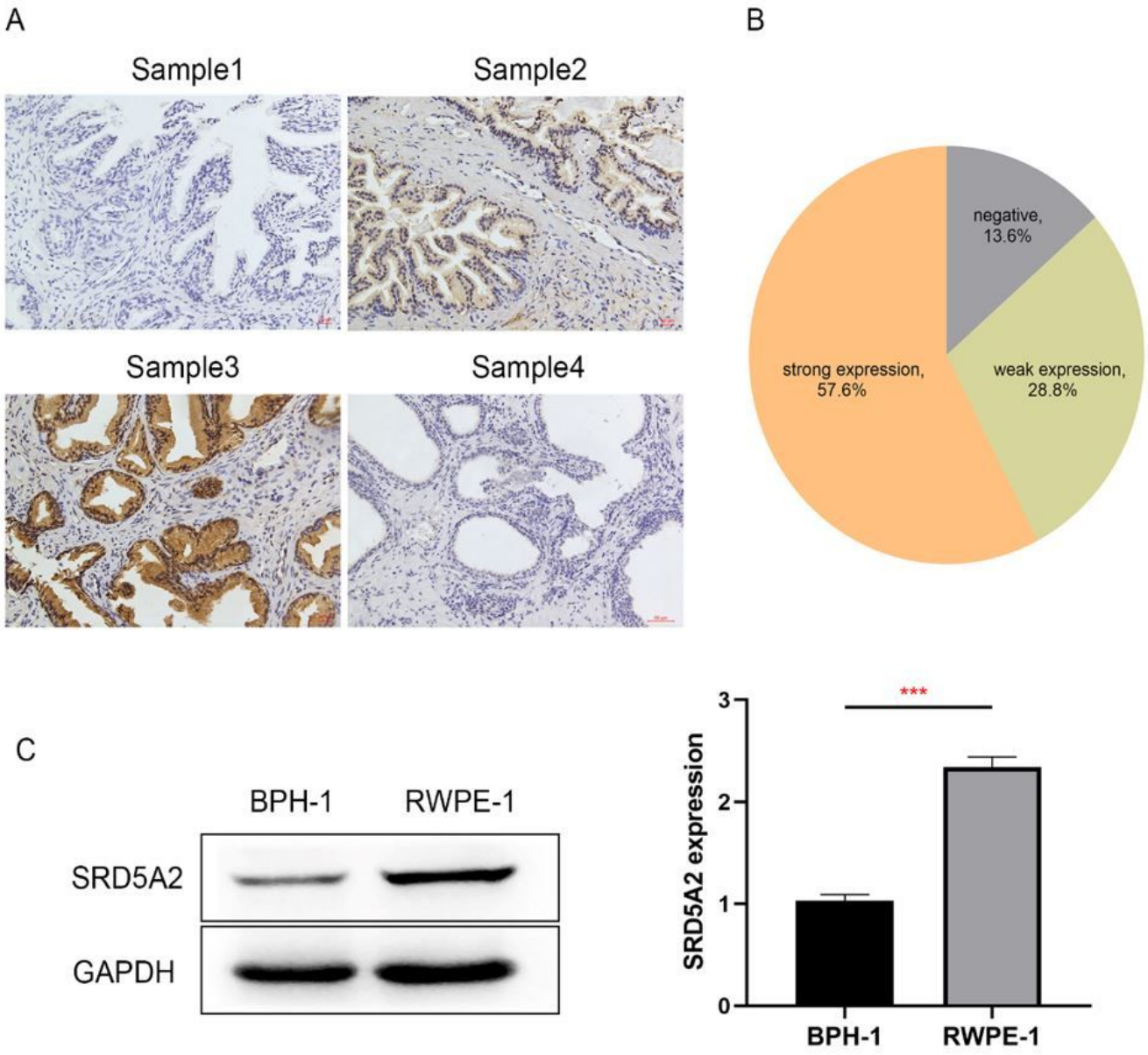

Figure 1

Diversity of SRD5A2 expression of different BPH tissues and cells. (A) Immunohistochemical staining showed that the expression of SRD5A2 was varied in different BPH tissues. Sample 1: negative SRD5A2 expression. Sample 2: weak SRD5A2 expression. Sample 3: strong SRD5A2 expression. No antibody: negative control without incubation with a primary antibody. (B) Varied SRD5A2 expression in BPH tissues. (C)Different SRD5A2 expression in BPH-1 and RWPE-1 cells. Scale bars = 50 μm; $^{***}p \leq 0.001$.SRD5A2: 5-α reductase type 2; BPH: benign prostate hyperplasia; BPH-1: immortalized benign prostatic hyperplasia epithelial cells; RWPE-1: normal prostate epithelial cell line. Full length blots/gels are presented in Additional file 1: Fig S1 - Additional file 2: Fig S2.

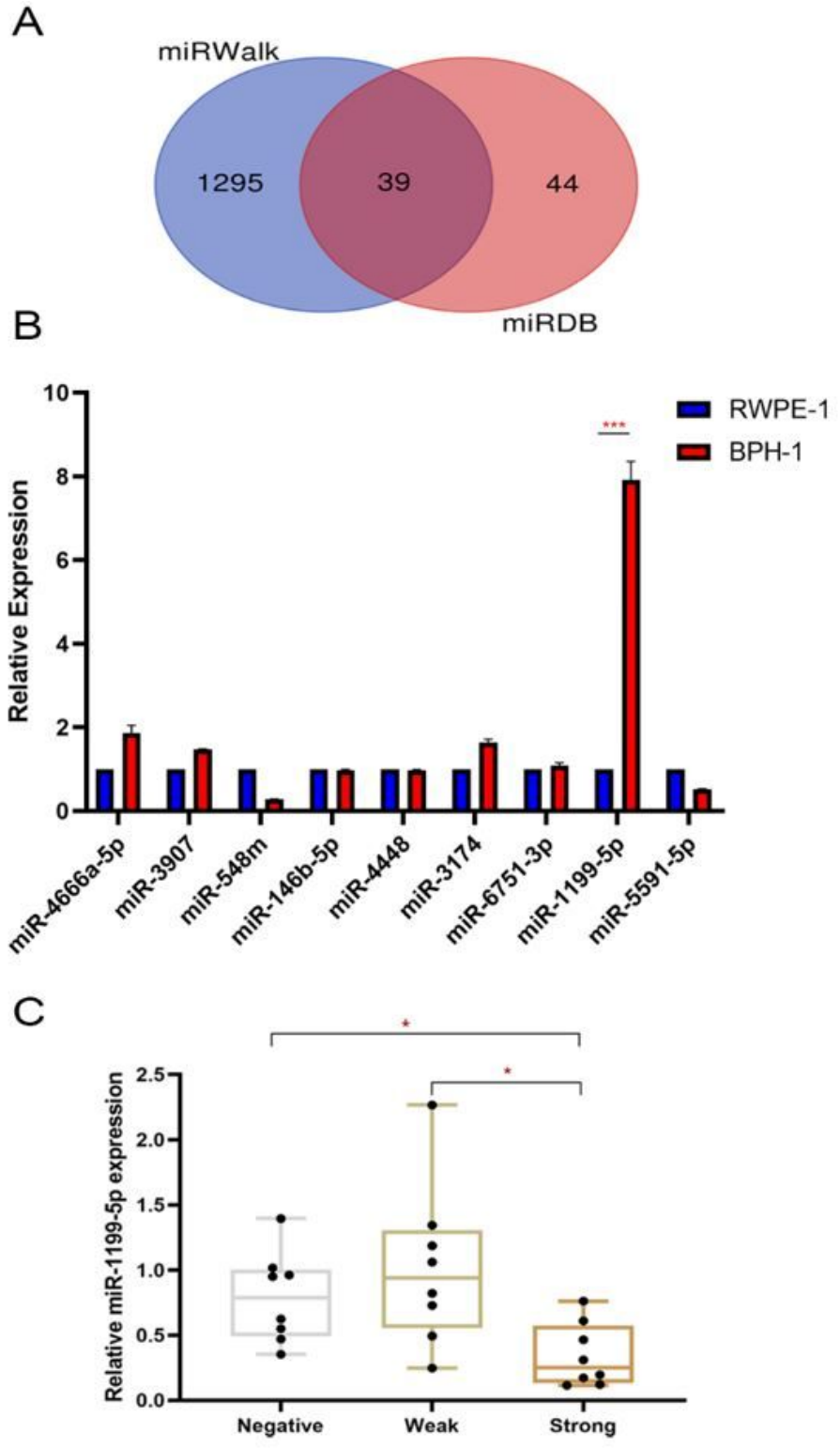

Figure 2

Prediction and screening of miRNA. (A) Venn diagram showed that 39 miRNAs were predicted to binding 3'UTR of SRD5A2 by miRWalk (http://mirwalk.umm.uni-heidelberg.de/) and miRDB (http://mirdb.org) database. (B)The RT-qPCR results showed that only the expression of miR-1199-5p in BPH-1 was significantly higher than that in RWPE-1. (C) The relative expression of miR-1199-5p in tissues with negative SRD5A2 expression, weak SRD5A2 expression, and positive SRD5A2. *P□0.05;***P□0.001;miRNA:

microRNA; SRD5A2: 5-α reductase type 2; BPH: benign prostate hyperplasia; BPH-1: immortalized benign prostatic hyperplasia epithelial cells; RWPE-1: normal prostate epithelial cell line.

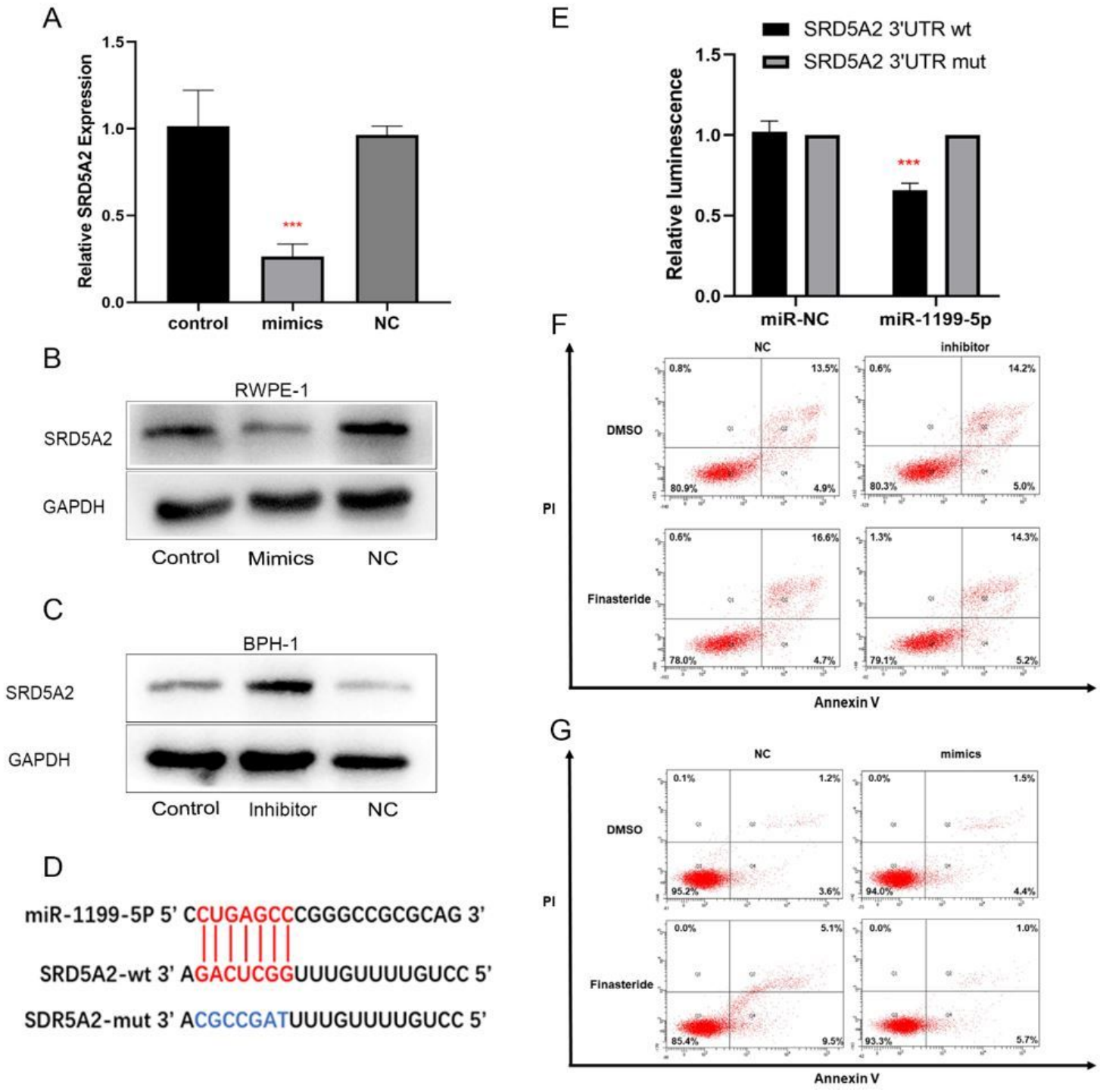

Figure 3

miR-1199-5p regulating the expression of SRD5A2. (A)The RT-qPCR results showed that SRD5A2 mRNA was significantly downregulated after transfection with miR-1199-5p mimics in RWPE-1 cells. (B-C) The Western Blotting results showed that miR-1199-5p mimics decreased the expression of SRD5A2 in RWPE-1 cells and miR-1199-5p inhibitors rescued the expression of SRD5A2 in BPH-1 cells. (D) Schematic

representation of the mature miR-1199-5p sequence, putative miR-1199-5p target site in the 3'UTR of SRD5A2 mRNA. (E) Overexpression of miR-1199-5p markedly decreased the relative luciferase activity in the WT 3'UTR of SRD5A2 mRNA, while the mutated 3'UTR of SRD5A2 was insensitive to miR-1199-5p overexpression. (F) The apoptosis of BPH-1 treated with finasteride (100 mM)/DMSO after transfection with miR-1199-5p inhibitor/ inhibitor-NC. (G) The apoptosis of RWPE-1 treated with finasteride (100 mM)/DMSO after transfection with miR-1199-5p mimics/mimics-NC. ***P□0.001; SRD5A2: 5-α reductase type 2; BPH-1: immortalized benign prostatic hyperplasia epithelial cells; RWPE-1: normal prostate epithelial cell line. Full length blots/gels are presented in Additional file 3: Fig S3 - Additional file 6: Fig S6.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1GAPDH.tif
- FigureS2SRD5A2.tif
- FigureS3GAPDH.tif
- FigureS4SRD5A2.tif
- FigureS5GAPDH.tif
- FigureS6SRD5A2.tif