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Hypoxia activates the hypoxia-inducible factor-1α/vascular endothelial growth factor pathway in a prostatic stromal cell line: A mechanism for the pathogenesis of benign prostatic hyperplasia

Tao Zhang[a], Changlin Mao[b], Yao Chang[a], Jiaju Lyu[a], Delong Zhao[a], Sentai Ding[a,*]

[a]Department of Urology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China; [b]Department of Urology, Mindong Hospital Affiliated to Fujian Medical University, Fuan, China

Abstract

Background: The development of benign prostatic hyperplasia (BPH) is closely related to hypoxia in the prostatic stroma, and the hypoxia-inducible factor-1α/vascular endothelial growth factor (HIF-1α/VEGF) pathway has been shown to significantly activate in response to hypoxia. The underlying mechanism for activation of this pathway in the pathogenesis of BPH remains unclear.

Materials and methods: We constructed HIF-1α overexpression and knockdown BPH stromal (WPMY-1) and epithelial (BPH-1) cell lines, which were cultured under different oxygen conditions (hypoxia, normoxia, and hypoxia + HIF-1α inhibitor). Quantitative real-time polymerase chain reaction (qPCR) and Western blotting were applied to detect the expression of the HIF-1α/VEGF pathway. Cell proliferation and apoptosis were analyzed by Cell Counting Kit-8 and flow cytometry. We used the miRWalk 2.0 database and Western blotting to predict the potential miRNA that selectively targets the HIF-1α/VEGF pathway, and verified the prediction by qPCR and dual-luciferase assays.

Results: In a BPH stromal cell line (WPMY-1), the expression of VEGF was in accordance with HIF-1α levels, elevated in the overexpression cells and decreased in the knockdown cells. Hypoxia-induced HIF-1α overexpression, which could be reversed by a HIF-1α inhibitor. Moreover, the HIF-1α inhibitor significantly depressed cellular proliferation and promoted apoptosis in hypoxic conditions, assessed by Cell Counting Kit-8 and flow cytometry. However, in the BPH epithelial cell line (BPH-1), the expression level of HIF-1α did not influence the expression of VEGF. Finally, a potential miRNA, miR-17-5p, regulating the HIF-1α/VEGF pathway was predicted from the miRWalk 2.0 database and Western blotting, and verified by qPCR and dual-luciferase assay.

Conclusions: In hypoxia, activation of the HIF-1α/VEGF pathway plays a crucial role in regulating cell proliferation in a BPH stromal cell line. Regulation by miR-17-5p may be the potential mechanism for the activation of this pathway. Regulation of this pathway may be involved in the pathogenesis of BPH.

Keywords: Benign prostatic hyperplasia; Hypoxia-inducible factor-1α; Vascular endothelial growth factor; Pathogenesis; miR-17-5p

1. Introduction

Benign prostatic hyperplasia (BPH) is arguably one of the most common conditions of older men worldwide, with a prevalence of 70%–80% in men older than 80 years.[1] Studies reported that 50%–75% men in the United States of America older than 50 years will inevitably experience BPH, and 80% of patients older than 70 years will experience lower urinary tract symptoms (LUTS).[2] In China, the current prevalence is 43.68%–71.54%, with a total number of patients of more than 70 million.[3]

The diagnosis of BPH requires a combination of medical history, physical examination, and laboratory or imaging examination. A majority of patients consult the doctor because of symptoms such as frequent urination, urgent urination, or urinary retention; however, because of the slow progression of the disease, patients may get used to the symptoms and not have any complaints.[4] The auxiliary examinations, including prostate-specific antigen level, ultrasound, uroflowmetry, and urodynamic test, sometimes contribute to the diagnosis of BPH and LUTS. Recently, a novel telemonitoring home-based uroflowmetry for patients with BPH was designed to offer a constant patient low-cost follow-up, likely to revolutionize the urologic clinic landscape.[5]

Current treatments of BPH consist of watchful waiting, drug treatment, and surgical intervention,[6] and as a clinical progressive disease, most patients inevitably resort to surgical intervention. Transurethral prostatectomy is the criterion standard for the surgical treatment for general patients, but for severe LUTS caused by large prostate glands (>80 mL), current guidelines recommend simple prostatectomy or endoscopic enucleation of the prostate as the treatments of choice. Previous studies have described the comparison and preference among different surgical interventions.[7]

The prostate is identified as the only organ that rather atrophies and degenerates with aging, and undergoes embryonic reawakening

*Corresponding Author: Sentai Ding, Department of Urology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, 250021, China. E-mail address: dingsentai@126.com (S, Ding).

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with proliferation that ultimately leads to BPH. As a worldwide disease consuming enormous medical resources and causing significant patient suffering, understanding the pathogenesis and how to reverse the embryonic-reawakening process is important. However, despite years of research, the basis for the pathogenesis of BPH is still not clear.

Previous investigation has found that patients with metabolic syndromes are more prone to develop BPH,[8,9] and the relative hypoxia microenvironment of the prostate may bridge this connection.[8,10] Accumulated findings support that hypoxia induces the production of cellular growth factors in prostate stromal cells and promotes the overgrowth of the stromal component and epithelial cells within the transition–periurethral zone, finally leading to BPH.[11]

Hypoxia-inducible factor-1α is (HIF-1α) a crucial hypoxia-responding transcriptional activator and plays a significant role in oxygen transfer and distribution from hypoxia (approximately 1% O_2) to normoxia (approximately 21% O_2).[12] Through the sophisticated regulatory network, it binds to the target gene to regulate the transcription of downstream genes. Vascular endothelial growth factor (VEGF) is the core downstream regulatory gene that recruits vascular endothelial cells in avascular and hypoxia areas to induce angiogenesis and maintain oxygen homeostasis[13].

The HIF-1/VEGF pathway has been found to be involved in coronary insufficiency arrhythmia,[14] angiogenesis of diabetic retinopathy,[15] idiopathic pulmonary fibrosis,[16] and other pathophysiological processes. Its oncogenicity potency in various carcinomas has been clearly shown.[17,18] Its role in promoting the proliferation of prostate cancer has also been reported.[19,20]

In BPH, our previous study[21] delineated that the elevated expression of HIF-1α was observed in intra-acinar cells in BPH (69.5%), but not in normal prostate tissue, and HIF-lα is also an independent risk factor for acute urine retention. In addition, VEGF was also shown to be upregulated in BPH tissue and promoted the development of BPH.[22,23] As already noted, the HIF-1α/VEGF pathway is related to the adaptive responses to ischemia-hypoxia in BPH pathogenesis. However, the underlying mechanism of HIF-1α/VEGF pathway activation in hypoxic cultured stromal cells remains unclear. This study innovatively detected the expression pattern of the HIF-1α/VEGF pathway components under different oxygen conditions and further determined the potential regulating miRNA, to explore the pathogenesis of BPH.

2. Materials and methods

2.1. Cell culture and hypoxia induction

The BPH stromal cell line WPMY-1 and epithelial cell line BPH-1 were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in DMEM and RPMI-1640 medium (Keygen, Nanjing, Jiangsu, China), respectively, supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY) and 1% penicillin, in a humidified incubator maintained in 37 °C with 5% CO_2. We cultured the cells in different oxygen conditions (hypoxia, normoxia, and hypoxia + HIF-1α pathway inhibitor), with 4 groups for each culture condition: control (Con) group cultured without lentivirus transfection, negative control (NC) group transfected with nontargeting lentivirus, knockdown (KO) group transfected with HIF-1α siRNA lentivirus, and overexpression (OE) group transfected with HIF-1α OE lentivirus. Oltipraz (MCE, Monmouth Junction, NJ) was used as the HIF-1α pathway inhibitor in this study. We applied cobalt chloride for hypoxia induction in this study. Preliminary experiments determined the optimal concentration to be 500 μmol/L for the WPMY-1 cell line and 300 μmol/L for the BPH-1 cell line.

2.2. Cell transfection

One day before transfection, 0.5–2 × 10^6 cells were incubated in 1.5 mL culture medium without antibiotics, and kept at 30%–50% confluence. siRNA diluent (5 μL siRNA+ 245 μL serum-free medium) was mixed with RFect transfection reagent diluent (Excell Bio Co., Ltd., Shanghai, China; 10 μL RFect + 240 μL serum-free medium) and incubated at room temperature for 20 minutes. Then 100 μL siRNA-RFect mixture was added to the culture well and incubated at 37°C for 48 hours. The siRNA used in this study was constructed by GeneChem Co., Ltd. (Shanghai, China).

2.3. RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was extracted from cells using Trizol reagent, followed by washing from the adsorption column with 30–50 μL RNase-free water, and centrifugation at 12,000 rpm for 1 minute. The concentration and quality of the extracted RNA were determined by a NanoDrop 2000/2000c spectrophotometer (ThermoFisher, Waltham, MA). Reverse transcription was performed using HiFiScript cDNA synthesis kit, and the reactions were performed in a H9800 Real-time PCR System (Hehui, Suzhou, China) using the SYBR master mixture. The Trizol reagent, HiFiScript cDNA synthesis kit, and SYBR master mixture were all obtained from CoWin Bioscience (Beijing, China). Primers involved in the quantitative polymerase chain reaction (qPCR) test were synthesized by Genewiz (South Plainfield, NJ) and are listed in Table 1. The expression results were calculated by the 2 – ΔΔCT method.

2.4. Western blotting

The cells were collected by centrifugation (12,000 rpm, 4°C, 5 minutes) and washed in phosphate-buffered saline twice. Precooled RIPA lysis buffer (Amresco, Solon, OH) supplemented with protease inhibitors (ThermoFisher) was used to lyse cells, and the supernatant containing the cell lysate was collected by centrifugation. The protein lysate sample was then separated in 10% sodium dodecyl sulfate - polyacrylamide gel electrophoresis gels by electrophoresis (Tanon, Shanghai, China) and transferred to nitrocellulose membrane, which was then blocked with Tris Buffered Saline with Tween solution containing 5% skim milk (sealing solution) at 4°C overnight. After incubation with the primary antibodies, and followed by the secondary antibodies (cat. no. S0001 and S0002; Affinity, OH), the ECL detection reagent (GE Healthcare, Little Chalfont, Buckinghamshire) was applied to visualize the protein. GAPDH was used as the loading control in this assay. Anti–HIF-1α (cat. no. 20960-1-AP), anti–VEGF-A (cat. no. 19003-1-AP), anti-Ki67 (cat. no. 27309-1-AP), and anti-GADPH (cat. no. 60004-1-Ig) antibodies were all purchased from Proteintech (Chicago, IL).

2.5. Cell counting kit-8 assay

The cell counting kit-8 (CCK-8; MCE) was applied to assess the cell proliferation in this study. Cells in logarithmic phase were digested

Table 1

Real time-PCR primers.

Gene name	Forward (5′ > 3′)	Reverse (5′ > 3′)	Amplified fragments, bp
HIF-1A	GCTTTAACTTTGCTGGCCCC	TTTTCGTTGGGTGAGGGGAG	140
VEGF	AGGCCAGCACATAGGAGAGA	CGCGAGTCTGTGTTTTTGCA	174
MKI67	AGACCTCCCCAGAGATGGAC	TGCATGAGAACCTTCGCACT	175
GAPDH	TGACTTCAACAGCGACACCCA	CACCCTGTTGCTGTAGCCAAA	121

VEGF = vascular endothelial growth factor.

with trypsin, resuspended into cell suspensions, and subsequently laid on the 96-well plate with the cell density of 2000 cells/well. Ten microliters (5 mg/mL) of CCK-8 regent was added into each well after which the cells were incubated for 0, 24, 48, or 72 hours, and then the optical density was calculated at the wavelength of 450 nm with an Infinite F50 microplate reader (Tecan, Menedorf, Switzerland). This assay was repeated in triplicate for each group.

2.6. Flow cytometry

The cells were digested with trypsin without EDTA, washed twice with phosphate-buffered saline, and then centrifuged and collected. Binding buffer (500 μL) was added to prepare a 1×10^6 cells suspension, then 5 μL annexin V-enhanced green fluorescent protein and 5 μL propidium iodide were added to the suspension, and incubated in the dark for 10 minutes. Cell apoptosis was measured by FACSCalibur (BD Biosciences, San Jose, CA).

2.7. Dual-luciferase reporter assay

Targetscan7.2 (http://www.targetscan.org/vert_72/) was used to predict the targeted binding site of hsa-miR-17-5p to HIF-1α. The 3′ untranslated region (3′UTR) sequences of wild and mutant type HIF-1α were constructed (Tsingke, Beijing, China) and inserted to the psiCHECK-2 vector (Promega, Madison, WI). Hypoxia-inducible factor-1α–wild-type (WT) or HIF-1α–mutated (MUT) vectors and miR-17-5p mimics or miR-NC were transfected into HEK 293T cells using lipofectamine 2000 (Invitrogen, Carlsbad, CA) and then incubated for another 24 hours. Finally, the Renilla luciferase and Firefly luciferase activities were measured with the Infinite M200 fluorescence spectrophotometer (Tecan) following the manufacturer's instructions. The Dual-Luciferase Reporter Assay kit used in this assay was purchased from Promega.

2.8. Statistical analysis

SPSS 25.0 (IBM Corp., Armonk, NY) and GraphPad Prism 7.0 software were used for statistical analysis. The data are expressed as mean ± SD. All experiments in this study were conducted in triplicate, and a p value <0.05 was considered statistically significant.

3. Results

3.1. HIF-1α/VEGF pathway expression under different oxygen conditions

We applied qPCR assays to detect the mRNA expression of HIF-1A, VEGF, and MKI67 in WPMY-1 cell line (Fig. 1). The results indicated that the mRNA level of HIF-1A was elevated in the OE group and decreased in the KO group compared with the NC group under different oxygen conditions; moreover, the mRNA

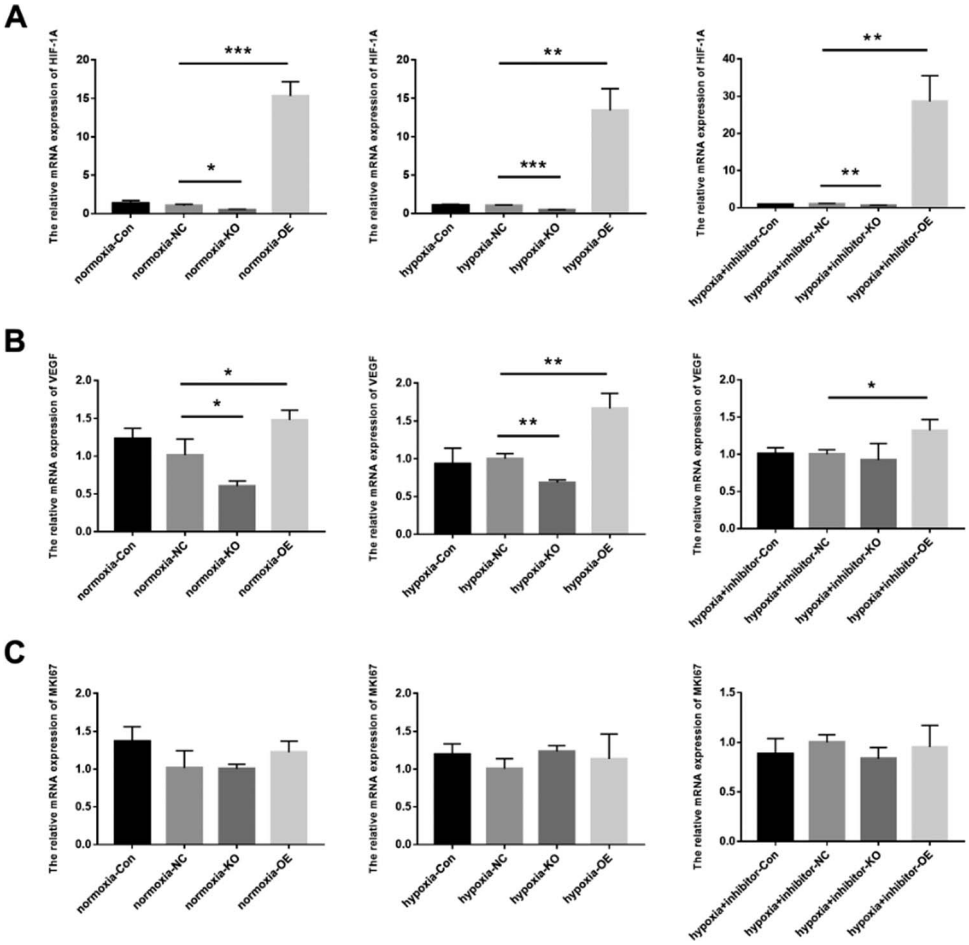

Figure 1. The qPCR results of HIF-1A, VEGF, and MKI67 mRNA expression levels in the WPMY-1 cell line. (A) The mRNA level of HIF-1A. (B) The mRNA level of VEGF. (C) The mRNA level of MKI67. (*p < 0.05, **p < 0.01, ***p < 0.001). Con = control; KO = knockdown; NC = normal control; OE = overexpression; VEGF = vascular endothelial growth factor.

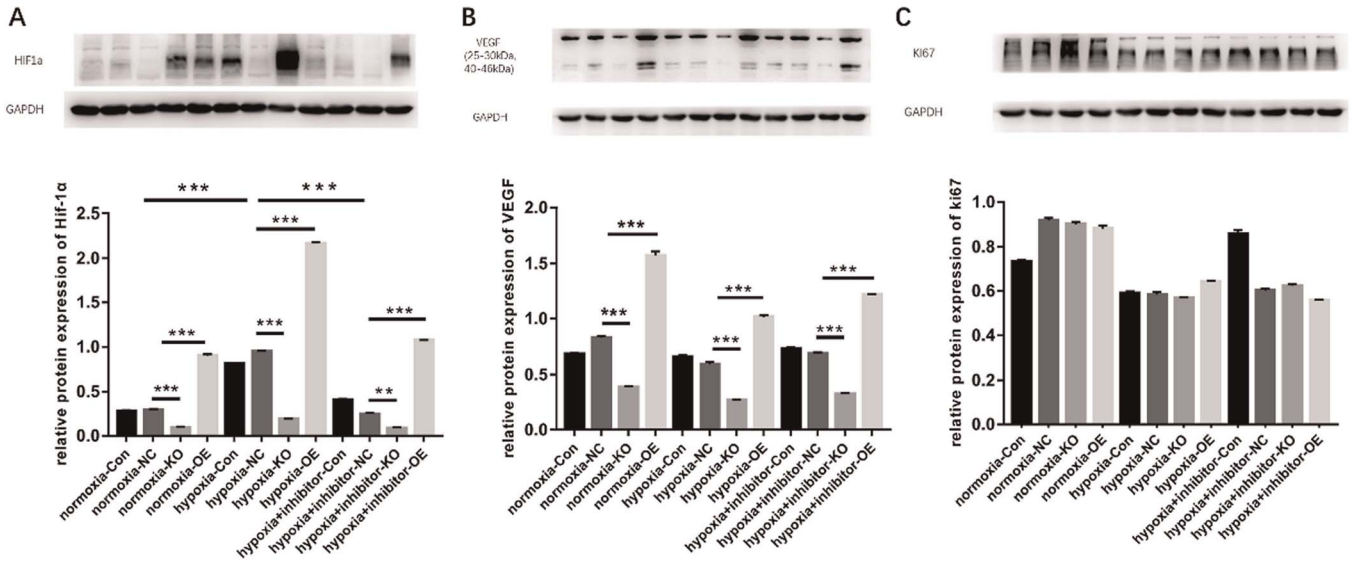

Figure 2. The Western blotting results of HIF-1α, VEGF, and Ki67 expression in the WPMY-1 cell line. (A) The expression level of HIF-1α. (B) The expression level of VEGF. (C) The expression level of Ki67. (**$p < 0.01$, ***$p < 0.001$). Con = control; HIF-1α = hypoxia-inducible factor-1α; KO = knockdown; NC = normal control; OE = overexpression; VEGF = vascular endothelial growth factor.

expression pattern of VEGF was accurately regulated by HIF-1A. However, the mRNA expression level of MKI67 demonstrated no significant differences in different groups.

The protein expression patterns of HIF-1α, VEGF, and Ki67 in the WPMY-1 cell line were assessed by Western blotting (Fig. 2). The expression of HIF-1α was upregulated in the OE group and downregulated in the KO group compared with the NC group under different oxygen conditions, and the expression pattern of VEGF paralleled the expression of HIF-1α. Furthermore, the expression of HIF-1α in the hypoxic condition was upregulated compared with the normoxic condition, which was reversed by the HIF-1α inhibitor. The expression of Ki67 demonstrated no significant differences in different groups.

Both mRNA and protein expression results from the BPH-1 cell line showed the OE of HIF-1α in the OE group and the downregulation of HIF-1α in the KO group. However, the expression pattern of VEGF did not parallel that of HIF-1α, as assessed by qPCR (Fig. 3) and Western blotting (Fig. 4).

3.2. Proliferation and apoptosis levels of BPH cells under different oxygen conditions

Cell proliferation and apoptosis were detected by CCK-8 and flow cytometry assays, respectively. In both WPMY-1 (Fig. 5) and BPH-1 (Fig. 6) cell lines, cell proliferation was upregulated in the OE group and downregulated in the KO group, and the cell apoptosis level was accordingly elevated in the KO group and decreased in the OE group, compared with the NC group. Furthermore, in the WPMY-1 cell line, the HIF-1α inhibitor further downregulated the proliferative capacity and upregulated the apoptosis level of cells under the hypoxia condition, which was not observed in the BPH-1 cell line.

3.3. Prediction and verification of potential miRNAs targeting the HIF-1α/VEGF pathway

The potential miRNAs targeting the HIF-1α/VEGF pathway was predicted using the miRWalk 2.0 Web site (http://zmf.umm.uni-heidelberg.de/mirwalk2); a total of 12 relevant databases of miRNA-mRNA interactions were included. We ranked the potential miRNAs targeting HIF-1A in order according to the number of databases supporting the interaction, and the miRNAs that were identified by more than 8 databases were chosen for further verification. These miRNAs were hsa-miR-18a-5p, hsa-miR-412-3p, hsa-miR-582-5p, hsa-miR-20b-5p, and hsa-miR-17-5p (Fig. 7A).

Hypoxia-inducible factor was overexpressed by lentivirus transfection of WPMY-1 cells, because of the natural low level of HIF-1α in the normal BPH stromal cell line. Then the HIF-1α–overexpressing WPMY-1 cell line was transfected with the miRNA mimics, and Western blotting was used to measure the expression level of HIF-1α and to determine the regulatory effect of the miRNA mimics. The results (Fig. 7B) showed that the WPMY-1 cell line transfected with miR-17-5p had the lowest HIF-1α expression, so miR-17-5p was selected as the potential targeting miRNA for further study.

The TargetScan database was used to predict the gene sequence of miR-17-5p targeting the 3′UTR of HIF-1A (Fig. 8A). The HIF1A-WT and the HIF1A-MUT plasmids were constructed and cotransfected with miR-17-5p mimic or NC mimic into HEK 293T cells, and the dual-luciferase assay was then performed. As the result indicated (Fig. 8B), the relative luciferase ratio was decreased in the HIF1A-WT + miR-17-5p cotransfection group (1.0000 ± 0.0065) compared with the HIF1A-WT + NC group (0.9175 ± 0.0058; $p < 0.05$). No significant difference was observed between the HIF1A-MUT + miR-17-5p cotransfection group (1.0000 ± 0.0223) and the HIF1A-MUT + NC group (1.0425 ± 0.0145). The results verified the regulatory effect of miR-17-5p on HIF1A in the BPH stromal cell line.

When the miR-17-5p mimic was transfected into the WPMY-1 cell line overexpressing HIF-1α, qPCR showed that the expression of HIF-1A mRNA was significantly decreased, compared with the normal control cell line ($p < 0.05$; Fig. 8C).

4. Discussion

Various hypotheses on the pathogenesis of BPH have been proposed.[24] The prostatic embryonic reawakening hypothesis, suggested that the dysregulation of prostatic stromal cells acts as the key in the pathogenesis of BPH, with the proliferation process akin to when the embryonic prostatic cells proliferates in response to stromal signaling

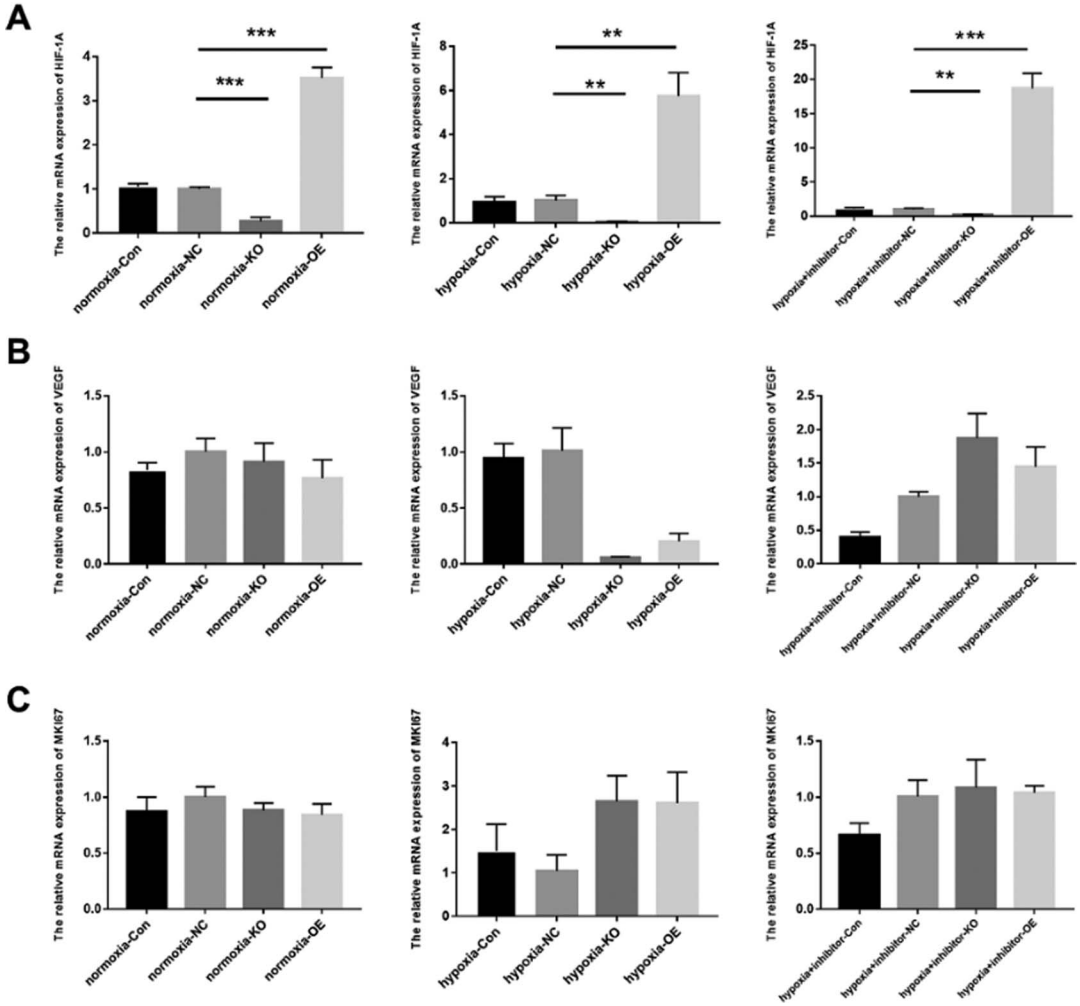

Figure 3. The qPCR results of HIF-1A, VEGF, and MKI67 mRNA expression levels in the BPH-1 cell line. (A) The mRNA level of HIF-1A. (B) The mRNA level of VEGF. (C) The mRNA level of MKI67. (**$p < 0.01$, ***$p < 0.001$). BPH = benign prostatic hyperplasia; Con = control; KO = knockdown; NC = normal control; OE = overexpression; VEGF = vascular endothelial growth factor.

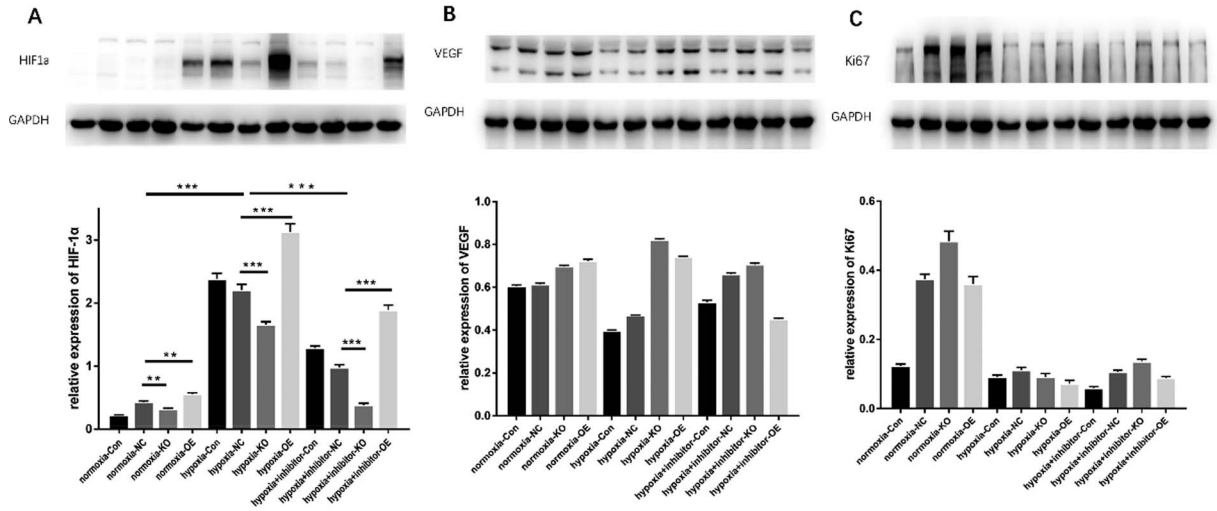

Figure 4. The western blotting results of HIF-1α, VEGF, and Ki67 expression in the BPH-1 cell line. (A) The expression level of HIF-1α. (B) The expression level of VEGF. (C) The expression level of Ki67. (**$p < 0.01$, ***$p < 0.001$). BPH = benign prostatic hyperplasia; Con = control; HIF-1α = hypoxia-inducible factor-1α; KO = knockdown; NC = normal control; OE = overexpression; VEGF = vascular endothelial growth factor.

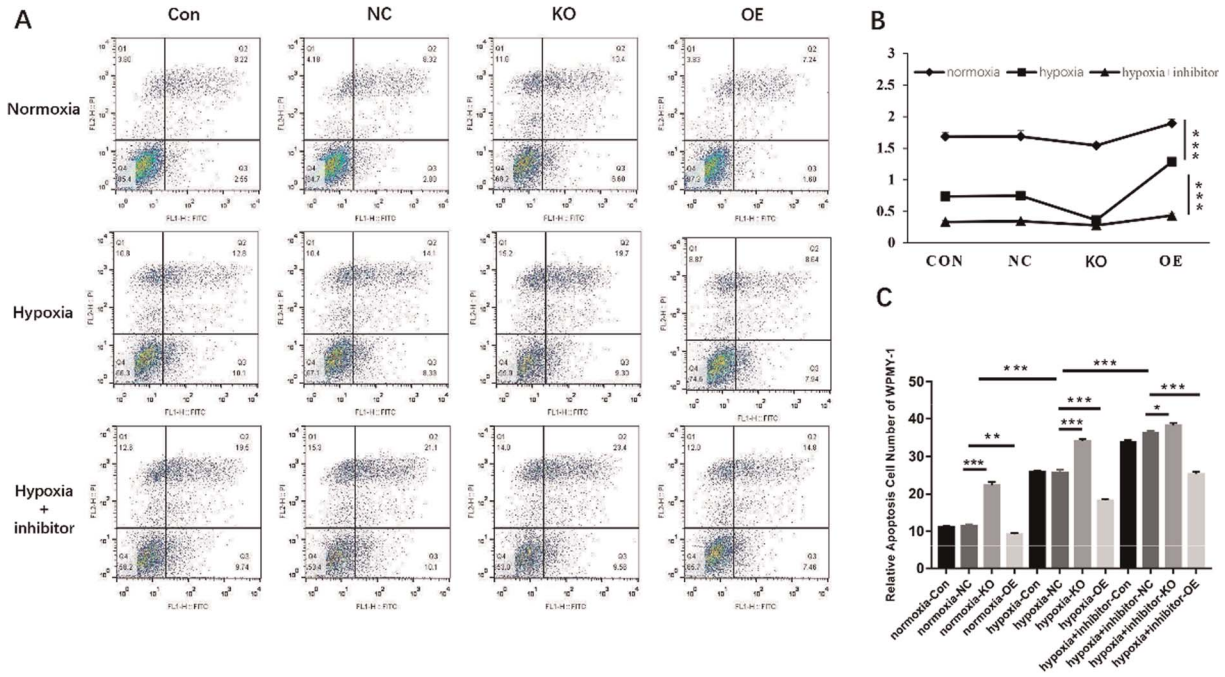

Figure 5. Cell proliferation and apoptosis results in the WPMY-1 cell line. (A) Flow cytometry assay. (B) CCK-8 assay. (C) The histogram represented the results of flow cytometry. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). CCK-8 = cell counting kit-8; Con = control; KO = knockdown; NC = normal control; OE = overexpression.

factors.[25,26] Previous research has shown an almost 1:5 epithelium to stroma ratio in the BPH tissue and proved that the stromal component dominates the principal process of BPH development.[27] A recent study that examined the different effects of stromal cells from either BPH or normal prostate on the proliferation of prostatic epithelial cells growth also supported this conclusion.[28] In the light of the aforementioned evidence, our team mainly focused on the BPH stromal cell line (WPMY-1) in this study, and the results demonstrated that the HIF-1α/VEGF pathway was activated in response to hypoxia stress and efficiently promoted cell proliferation in BPH stromal cell line. However, the results in the BPH epithelial cell line (BPH-1) did not reveal a similar regulatory role of hypoxia on the HIF-1α/VEGF pathway. The contrasting experimental results from the BPH stromal and epithelial cell lines further confirm that the pathogenesis of BPH is mainly dominated by the dysregulation of the prostatic stromal component.

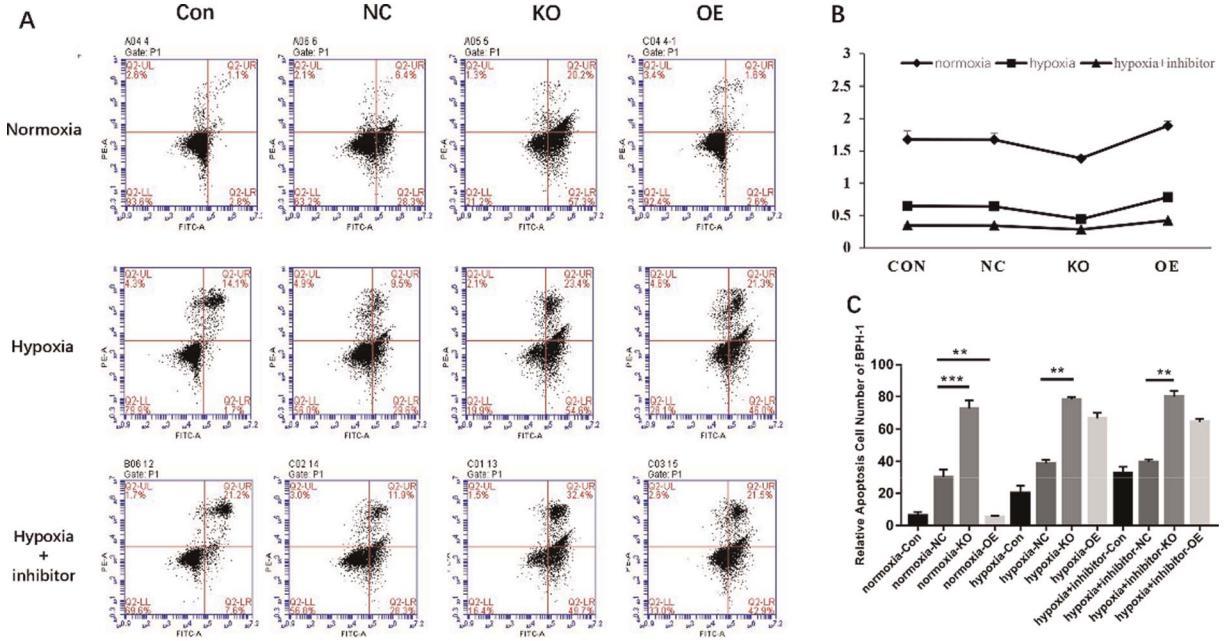

Figure 6. Cell proliferation and apoptosis results in the BPH-1 cell line. (A) Flow cytometry assay. (B) CCK-8 assay. (C) The histogram represented the results of flow cytometry. (**$p < 0.01$, ***$p < 0.001$). CCK-8 = cell counting kit-8; Con = control; KO = knockdown; NC = normal control; OE = overexpression.

A

Gene	miRNA	miRWalk	Microt4	miRanda	mirbridge	miRDB	miRMap	miRNAMap	Pictar2	PITA	RNA22	RNAhybrid	Targetscan	SUM
HIF1A	hsa-miR-18a-5p	1	0	1	0	1	1	1	1	0	1	1	1	9
HIF1A	hsa-miR-412-3p	1	0	1	0	1	1	1	1	1	0	1	1	9
HIF1A	hsa-miR-582-5p	1	0	1	0	1	1	1	1	0	1	1	1	9
HIF1A	hsa-miR-17-5p	1	0	1	0	1	1	1	1	0	1	1	1	9
HIF1A	hsa-miR-20b-5p	1	0	1	0	1	1	1	1	0	1	1	1	9

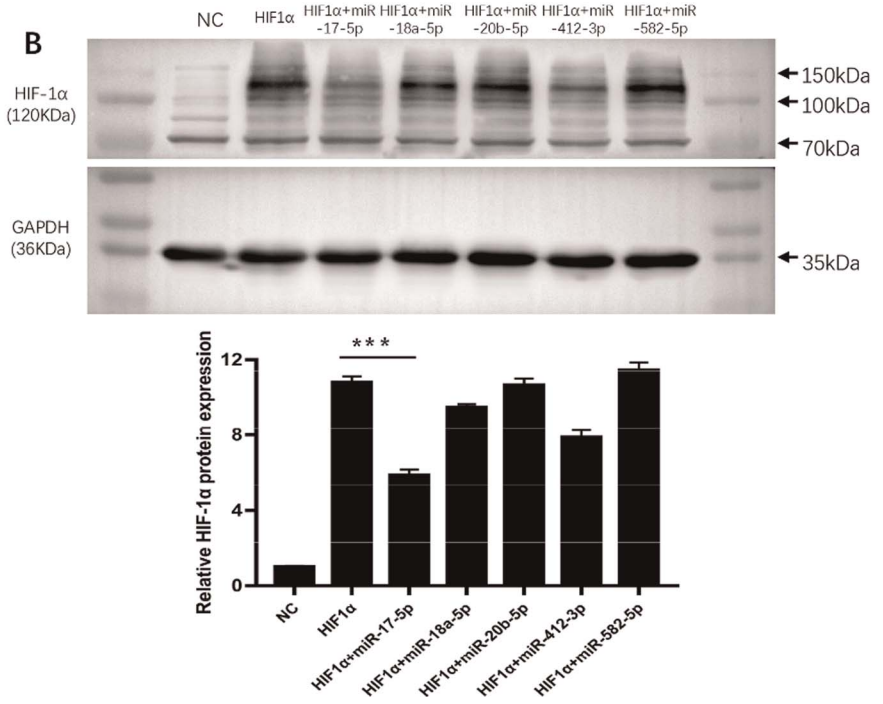

Figure 7. Potential miRNAs targeting the HIF-1α/VEGF pathway. (A) miRNAs predicted from the miRWalk 2.0 database. (B) The expression level of HIF-1α in WPMY-1 cell line (with HIF-1α overexpression) after transfected with miRNA mimics. (***$p < 0.001$). HIF-1α = hypoxia-inducible factor-1α; NC = normal control; VEGF = vascular endothelial growth factor.

As for the trigger factors that reawaken the embryonic growth potential of the prostatic stromal component, an ischemic and hypoxic microenvironment has been proved to play a significant role.[29] The dysregulation of oxygen in the prostate was reported to result in stromal structure transformation and disproportionate proliferation of stromal and epithelial components. A study conducted by Berger et al.[27] concluded that the prostatic stroma reacts to hypoxic stress by upregulating the secretion of diverse hypoxia stress factors, leading to BPH initiation. Hypoxia-inducible factor-1α, as a principal oxygen homeostasis regulator, has been shown to be overexpressed in BPH tissue and closely correlated with the development of this disease. The inhibition of HIF-1α has been shown to be a potential treatment for BPH.[30,31] Consistent with these findings, this study also found that the expression of HIF-1α was upregulated during hypoxia compared with normoxia, which was reversed by the HIF-1α inhibitor. Moreover, the HIF-1α inhibitor was able to further depress the proliferative capacity and promote the apoptosis of the WPMY-1 cell line in the hypoxic culture conditions. These results indicated that HIF-1α served as a protective factor against hypoxic stress for the BPH stromal cell line and may be involved in the pathogenesis of BPH.

Vascular endothelial growth factor, as the downstream factor of HIF-1α, with crucial proangiogenic capacity, mainly targets the endothelial cells with antiapoptotic and mitogenic effects.[32] In the WPMY-1 cell line, the expression pattern of VEGF in different groups was in accordance with HIF-1α, assessed by qPCR assay and Western blotting, which verified the regulatory effect of HIF-1α on VEGF. The relationship between BPH and VEGF-induced angiogenesis has also been addressed in previous research.[33,34] A recent study showed the amelioration of dihydrotestosterone-induced BPH by downregulation of VEGF-mediated angiogenesis for the first time.[35] Nonetheless, in this study, the expression of VEGF was not upregulated during hypoxia, compared with normoxia, in the stromal cell line, which may be ascribed to the complexity of the regulatory network of VEGF expression, with HIF-1α being only one of the upstream regulators.

Furthermore, the miRWalk 2.0 database and Western blotting were used to predict the potential miRNA targeting the HIF-1α/VEGF pathway, and the miR-17-5p was selected as the potential targeting miRNA. Previous studies have elucidated the correlation between miR-17-5p regulation and the development of prostate cancer[36,37]; however, the regulatory role of miR-17-5p and its interaction with the HIF-1α/VEGF pathway in the pathogenesis of BPH remain unclear. In this study, the results proved the regulatory role of miR-17-5p on suppression HIF-1A expression by targeting the 3′UTR region, as verified by the dual-luciferase and qPCR assays. Consistent with this result, a recent study has found that miR-17-5p suppressed retinal cell proliferation and angiogenesis by regulating the HIF-1α/VEGF

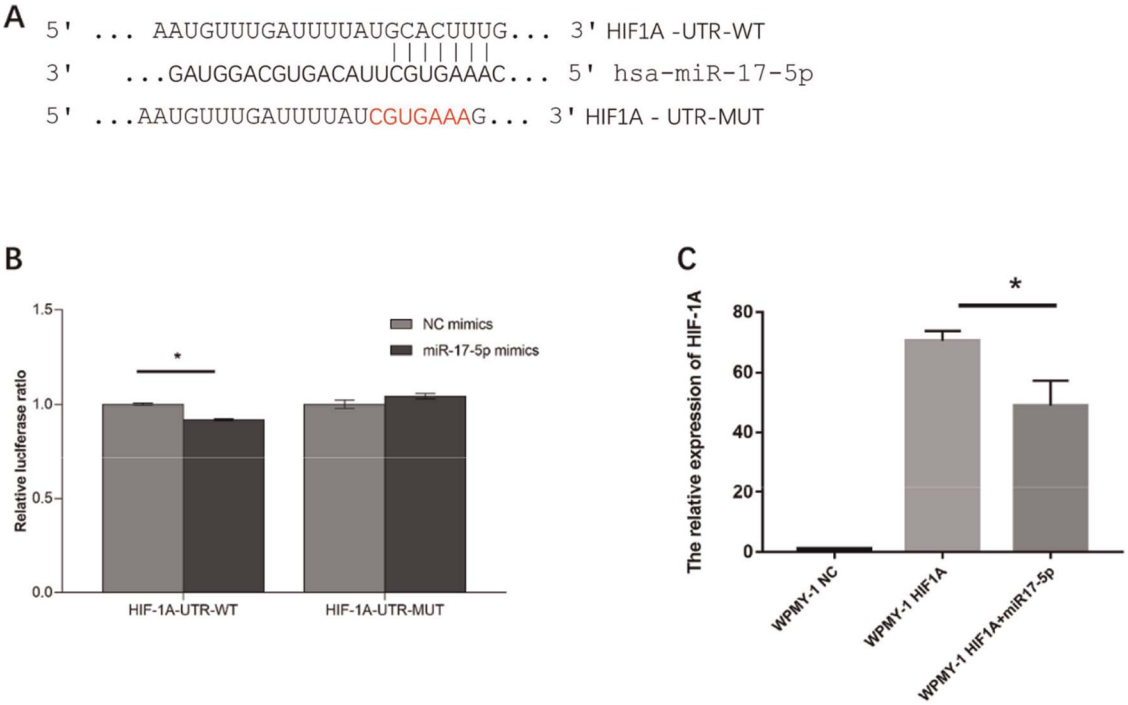

Figure 8. The regulatory role of miR-17-5p on the HIF-1α/VEGF pathway verified by the dual-luciferase reporter and qPCR assays. (A) The targeted binding sites between miR-17-5p and HIF-1A (red denotes the mutated sequences). (B) Relative luciferase activity of dual-luciferase reporter assay. (C) The qPCR result of HIF-1A mRNA expression (*$p < 0.05$). HIF-1α = hypoxia-inducible factor-1α; MUT = mutant type; NC = normal control; UTR = untranslated region; VEGF = vascular endothelial growth factor; WT = wild type.

pathway in the oxygen-induced retinopathy mouse model, and proved the targeting of miR-17-5p to the 3′UTR region of HIF-1A.[38]

This study has investigated the crucial role of HIF-1α/VEGF pathway activation in the pathogenesis of BPH and addressed the potential mechanism of this pathway. This may shed the light to the prevention and treatment of this chronic disease.

5. Conclusions

This study presented evidence that the HIF-1α/VEGF pathway was activated by the hypoxic microenvironment of the BPH stromal cell line. This had an influence on the proliferation and apoptosis of the stromal cells, and miR-17-5P was shown to be a potential regulator of the pathway. Our findings provide a framework to understand the pathogenesis of BPH and may provide new therapeutic approaches to this prevalent disease.

Acknowledgments

None.

Statement of ethics

Neither institutional review board's approval nor participants' consent was applicable for this study.

Conflict of interest statement

JL is an associate editor of *Current Urology*. SD is an editorial board member of *Current Urology*. This article was accepted after normal external review. The other authors declare that they have no competing financial interests or personal relationships that influence the work reported in this article.

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Author contributions

TZ: Methodology, writing – reviewing and editing, software;
CM: Data curation, writing – original draft preparation;
YC: Visualization, investigation;
JL: Supervision;
DZ: Software, validation;
SD: Conceptualization, funding acquisition.

Data availability

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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