

Original Article

Hypoxia-inducible factor 1 α (HIF-1 α) mediates the epithelial-mesenchymal transition in benign prostatic hyperplasia

Yanbo Chen*, Huan Xu*, Qiling Shi, Meng Gu, Xiang Wan, Qi Chen#, Zhong Wang#

*Department of Urology, Shanghai Ninth People's Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China. *Equal contributors and co-first authors. #Equal contributors.*

Received September 2, 2018; Accepted September 27, 2018; Epub January 1, 2019; Published January 15, 2019

Abstract: Background: Epithelial-mesenchymal transition (EMT) based cancer cell invasion and metastasis has been thoroughly studied in prostate cancer. It was well known that EMT markers which have been found in benign prostatic hyperplasia (BPH) tissues, but system descriptions have not been described. Methods: First, in order to construct the epithelial cells to mesenchymal cell transformation model, BPH-1 cells were cultured with supernatant of prostate matrix normal prostate stromal WPMY-1 cells, after obtaining the culture medium through a filter. After that, we observed the morphology of cells cultured for a period of time by microscopy, detected cell invasion ability by transwell assay, detected cell proliferation ability by MTT, and detected EMT marker expression by western. Finally, we treated the cells with anti-HIF-1 α drugs to study their effects on EMT, and then tested several related proteins simultaneously. Results: The results showed that the morphology of BPH-1 cells gradually changed to fusiform after cultured with WSCM. At the same time, E-cadherin and cytokeratin levels were significantly lower than those in normal medium. Simultaneous detection of vimentin (SMA) and Snail was positive compared to normal cultured cells. At the same time, the cells were cultured with WSCM and the invasive ability was up-regulated. After treatment with anti-HIF-1 α drug, E-cadherin and CK5/8 protein expression was up-regulated, but vimentin, α -SMA, and Snail expression was down-regulated, and in addition, p-Smad3 protein expression was also down-regulated after anti-HIF-1 α drug was added. Conclusion: The above results indicated that WSCM-1 stromal cell supernatant WSCM can induced BPH-1 cell interstitialization, and at the same time, by inducing EMT, secreting HIF-1 α activates Smad3 signaling. Our study shows that inhibition of HIF-1 α expression provides a new reference for clinical treatment of BPH.

Keywords: Hypoxia inducible factor 1, benign prostatic hyperplasia, epithelial-mesenchymal transition

Introduction

Benign prostatic hyperplasia (BPH) is a noncancerous enlargement of the prostate gland often resulting in lower urinary tract symptoms (LUTS). Prevalence of BPH increases with age, and BPH is a major disease among older males which significantly reduces the quality of their life [1-3]. It is well known that epithelial-mesenchymal transition (EMT) is a key inducing factor of aberrant prostate growth. EMT and its reverse mechanism mesenchymal-epithelial transition (MET) play important roles in the embryonic development, especially in the formation of the prostate, chronic inflammation, tissue reconstitution and cancer metastasis [4]. When epithelial cells undergo EMT, they

lose their original markers such as E-cadherin and CK8, in addition, acquiring the phenotypes of mesenchymal cells, for instance, expression of vimentin and α -smooth muscle actin [5, 6]. Until now, some clear triggers of EMT have been studied. First, cadherin switching: transitioning epithelial cells lose epithelial cadherin (E-cadherin) and express neural cadherin (N-cadherin) [7]. Secondly, apoptosis deregulation: cells lose apoptosis control and increase proliferation-to-apoptosis ratio [8]. Additionally, epigenetic regulation is also extremely significant to EMT [9-11]. Finally, the reactive stroma in the prostate microenvironment is a crucial contributor driving the EMT, it regulates androgen status and levels of multiple factors including transforming growth factor- β (TGF- β) via

HIF-1 α regulates the EMT of BPH

paracrine mechanisms [12, 13]. Recently, the microenvironment, as an important regulator of EMT has been studied extensively, and plenty of factors have been found that are involved in EMT process.

The hypoxia inducible factor 1 (HIF 1) signaling pathway promotes angiogenesis, and it contributes to the progression of numerous hyperplastic diseases, including BPH. HIF 1 is a crucial signaling factor which contributes to the activation of the 'angiogenic switch' and results in increasing expression of numerous angiogenic growth factors, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factors (bFGF) [14-17]. HIF 1 α and HIF 1 β form the heterodimeric transcription factor HIF-1, and as a master regulator of oxygen homeostasis, HIF 1 accumulates in the cytoplasmic matrix [18]. Along with translocation to the nucleus, HIF-1 activates hypoxia-sensitive genes and induces the expression of genes encoding angiogenic cytokines, such as VEGF and bFGF [19, 20]. VEGF or bFGF binds to the specific receptors on the cytomembrane of vascular endothelial cells (ECs), which regulates ECs survival, proliferation, migration, and extends the primitive vascular network [21-25]. However, so far, there is no study that shows the role of HIF-1 in the stromal microenvironment of prostate.

We hypothesized that HIF 1 α may play a significant role in inducing EMT of prostate epithelial cells through affecting stromal microenvironment, which causes BPH. To test our conjecture, we used the WPMY-1, which is a prostate stromal cell line, to simulate a stromal microenvironment for BPH-1 cells in vitro, and then we used this system to explore the influence of HIF-1 α on BPH-1 cells' EMT.

Materials and methods

Cell culture

The human BPH-1 cells were purchased from American Type Culture Collection (ATCC) (USA), the WPMY-1 cell is a human prostatic cell line, which was purchased from The Global Biore-source Center (USA). The BPH-1 cells were cultivated in RPMI-1640 fundamental medium (Invitrogen, Canada) with 10% fetal bovine serum (FBS) (Gibco, DC), but the WPMY-1 cells

were maintained culture in RPMI-1640 with 5% FBS (Gibco, USA) medium. Those cells were cultured at 37 degree in temperature incubator with 5% CO₂.

Experiment reagents

All of antibody was purchased from Abcam, including polyclone anti-HIF-1 α antibody and mouse or rabbit IgG isotype control antibody. And all of antibody was dissolved in PBS contain 5% BSA. The antibody HIF-1 α was adapted to concentration of 2 μ g/ml, and the WSCM solution was added to anti-HIF-1 α (2 μ g/ml). WPMY-1 supernatant-conditioned medium (WSCM) was collected from WPMY-1 cell culture supernatant. When WPMY-1 cells were cultured at 80% density, WSCM was obtained, and WPMY-1 cells need replaced to fresh complete medium per two days. In the first place, the supernatant was spun in centrifuge tubes at 2000 rpm and filtered through a 0.45 mm filter for concentration to a proper state. In the end, the fresh WSCM was storage at -80 $^{\circ}$.

Cell grouping and treatment

The cells were plated per 1 \times 10⁵ in 6 cm dish and maintained for 24 h, then we divided those dishes into seven groups and treatment with different stimulation: a: BPH-1 cells cultured in ordinary complete medium; b: BPH-1 cells cultured in WSCM; c: BPH-1 cells cultured in WSCM treatment with anti-HIF-1 α ; d: BPH-1 cells cultured in WSCM treatment with mouse or rabbit IgG control; e: BPH-1 cells cultured in ordinary complete medium treatment with anti-HIF-1 α antibody; f: BPH-1 cells cultured in ordinary complete medium treatment with mouse or rabbit IgG; g: WPMY-1 cells cultured in special complete medium of WPMY-1.

Immunofluorescence analysis

BPH-1 cells were collected in a 1.5 ml tube and were washed by phosphate-buffered saline (PBS), and the cells were fixed with 4% paraformaldehyde; we next used the 0.5% Triton X-100 permeabilized cells for 10 min, and washed with PBS. Finally, the cells were blocked with 5% Bovine Serum Albumin (BSA) or 2% FBS for 20 minutes at RT. We used the primary antibody rabbit anti-E-cadherin or mouse anti-vimentin (1:100; Abcam); rabbit anti-CK5/8

HIF-1 α regulates the EMT of BPH

(1:100; Cell Signaling Technology); and mouse anti- α -SMA and anti-Snail (1:100; Abcam). Cells were incubated overnight at 4°C. Next day, we used PE-labeled secondary antibody incubated with the cells (1:1000; Cell Signaling Technology) for 2 h or the cells were incubated with DAPI (Sigma) for 20 min at RT, and washed twice by PBS. Finally, the cells were observed by fluorescence microscope (PerkinElmer Life Sciences).

Western blotting assay

The cells were harvested, washed twice with cold PBS, and the total proteins were prepared from lysed cells using cell lysis buffer (Amresco, Cochran, GA) (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.5% sodium deoxycholate (SDS), 1% NP-40, 1 \times protease inhibitor cocktail, 1 mM EDTA) on ice, and supplemented with a protease inhibitor cocktail tablet (Roche, USA). We use the BCA protein assay kit detected protein concentration (Thermo fisher, USA). The cell lysates were sonicated, and resolved by 10%-12% SDS-PAGE Gels were transferred to PVDF membranes (Millipore, USA), blocked with 6% BSA, and probed using Abs directed against anti-E-cadherin and anti-vimentin (1:3,000; Cell Signaling Technology), rabbit anti-CK5/8, mouse anti- α -SMA, anti-Snail (1:4,000; Cell Signaling Technology), rabbit anti-p-Smad3 (1:1000; Abcam, USA) and GAPDH (1:1000; Abcam, USA). Secondary Abs conjugated to HRP that were used for detection included anti-rabbit (1:5,000) and anti-mouse (1:5,000; Cell Signaling Technology, Beverly, MA). In the end, the protein signals were detected by chemiluminescence systems (GE, USA), and the western blot figure was quantified using PS software.

Real-time PCR

Total RNA of cells was extracted by the RNeasy extraction kit (Qiagen, Valencia, CA), conferring to the manufacturer's instructions. cDNAs were synthesized using Reverse Transcriptase kit (ThermoFisher, USA), the genes of smad3 were detected by qPCR with SYBR® Green RT-PCR Master Mix (Roche, Switzerland) using 7500 Fast (ABI, USA). The PCR amplification process (95°C, 30 s; and 60°C, 1 min) was done for 40 cycles. The levels of gene expression relative to GAPDH were calculated with Delta-Delta-Ct (ddCt) algorithm method. All of the primers were quality checked for a single curve on melt-

ing curve, and efficiencies were 90-110%. Smad3, Forward, 5-GAGTAGAGACGCCAGTTCT-ACC-3, Reverse, 5-GGTTTGGAGAACCTGCGTC-CAT-3; GAPDH, Forward, 5-ATCTTCTCGAACCC-CGAGTGA, Reverse, CGGTT CAGCC ACTGG AGCTT.

Transwell migration assays in vitro

The BPH-1 cells were grown up to ~ 80% density and were treated with WSCM of covering with/without anti-HIF-1 α in the 24-well transwell and cultured with ordinary but FBS-free medium for 24 h. The BPH-1 cells were collected and carried through cell migration and transference assay after 24 h. In both experiments, BPH-1 cells cultured with/without WSCM were added to the lower chamber and then the cells were migrated the upper chamber. After 24 h of incubation, the migration of cells was measured through the pores using calculating under the microscope (Zeiss, Germany) using a 20 \times microscope.

The MTT assay

To detect the cell viability/proliferation curves when the cells were treated with inhibitor, BPH-1 cells were plated into 24-well plates with 5000 cells/well and grown in RPMI-1640 media or WSCM Cells. Survival was detected by MTT assay. On this measurement day, medium was carefully changed to MTT and set at 37°C for 3-5 h. After removing MTT mediums, and 200 μ l solution of DMSO was added. MTT reduction was detected absorbance at 570 nm using the EThermo Scientific Microplate Reader (BioTek, USA).

Statistical analyses

Two groups data were compared using Student's t test, and P < 0.05 was considered significant. All of the statistical significance calculations were implemented in Graphpad prism 5 software.

Results

EMT was induced by WSCM in BPH-1 cells

Noncancerous prostatic cell WPMY-1 showed a spindle shaped myofibroblast morphology. As previously researched [26], BPH-1 cells were cultured by the WPMY-1 cell culture superna-

HIF-1 α regulates the EMT of BPH

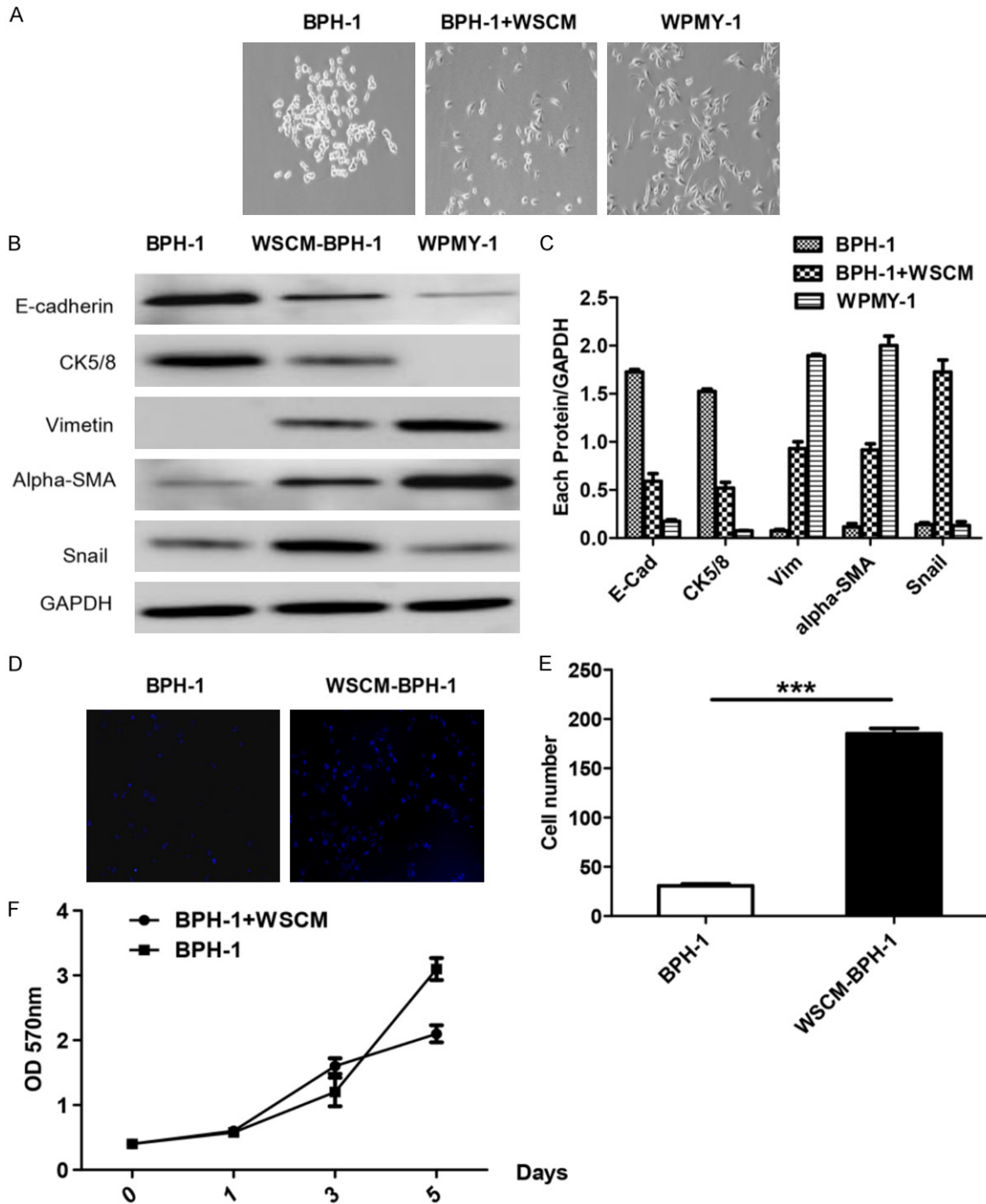


Figure 1. EMT was induced by WSCM in BPH-1 cells. BPH-1 was cultured in ordinary RPMI 1640 medium and WSCM-BPH-1 was cultured WPMY-1 supernatant. WPMY-1 cells were photographed as a control. A. Cells were photographed after cultured in different media for 5 days. B, C. Western blot analysis of EMT-related proteins in BPH cells after 7 days' culture period and relative protein levels to GAPDH. D, E. Migration assay results showed WSCM can promote BPH-1 cell migration. F. MTT assay shows the growth of BPH-1 did not alter cells after 1, 3 and 5 days of WSCM culture.

tant in vitro. In **Figure 1**, BPH-1 cells were cultured in ordinary medium (left panel), which show the representative feature of epithelial

cells; however, BPH-1 cells that were maintained with WSCM medium, which presented a spindle-shaped morphology (right panel), and

HIF-1 α regulates the EMT of BPH

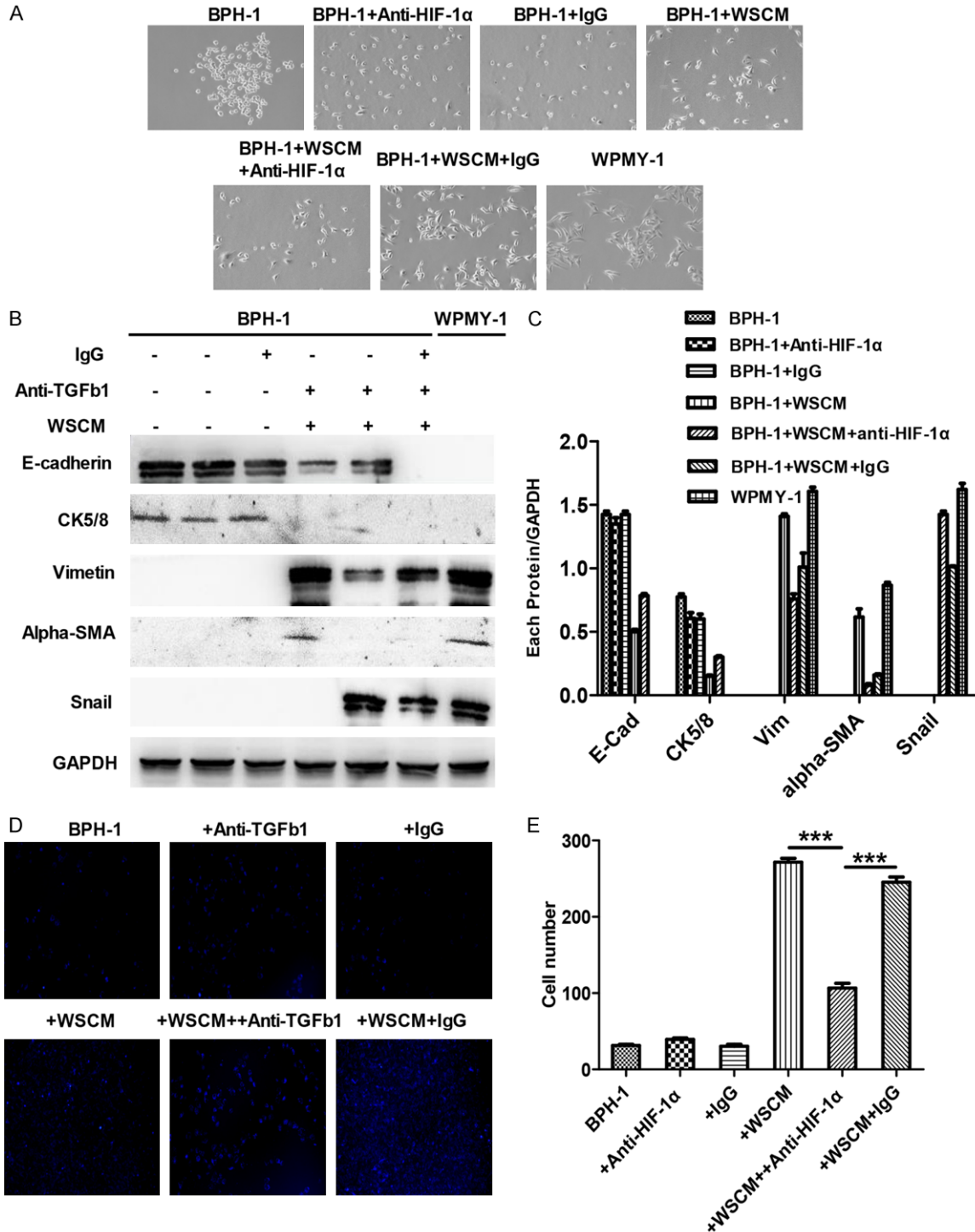


Figure 2. Anti-HIF-1 α inhibits the WSCM-induced EMT. A. Morphologic changes under different culture conditions. B, C. Western blotting analysis of EMT marker protein expression in different conditions and relative protein levels were scanned and relative density of each band was calculated as ratio to GAPDH. D, E. Migration assay. Anti-TGFb1 Ab was added into the culture system and we detected the migration capability of BPH-1 cells induced by WSCM.

BPH-1 cells had a typical spindle shaped morphology (middle panel) after maintenance in WSCM 5 days, which is similar to the prostate

normal WPMY-1 (Figure 1). To be sure of this phenotype, we detected E-cadherin and CK5/8 of the epithelial markers using western blot,

HIF-1 α regulates the EMT of BPH

and we found the protein levels of E-cadherin and CK5/8 expression were decreased in BPH-1 cells with WSCM medium in comparison to the control medium (**Figure 1B, 1C**). Notably, we also respectively detected the mesenchymal markers vimentin and α -SMA or the EMT marker Snail expression in the BPH-1 cell with WSCM medium. We found that the expression of E-cadherin and CK5/8 were decreased in WSCM cultured BPH-1 cells, but the BPH-1 cells unexpectedly acquired Vimentin, α -SMA and Snail expression by immunofluorescent staining (**Figure 1D**). Migration assay showed that the BPH-1 cells' migratory capacity was also up-regulated by treatment with WSCM medium (**Figure 1E**, right panel). Therefore, to further ensure the role of WSCM in BPH-1 cell proliferation, we implemented the MTT assay. As in **Figure 1F**, we did not detect a difference in growth rates with ordinary medium or WSCM medium cultured the BPH-1 cells, respectively. From the above results, we say that EMT can be induced in BPH-1 cells with WSCM medium.

Anti-HIF-1 α inhibit the WSCM-induced EMT

HIF-1 α is a transcription factor and belongs to Hypoxia-inducible factor 1 (HIF-1) family, and previous research reported that HIF-1 α was an EMT inducer in diverse normal or cancer cell types [27, 28]. To detect whether HIF-1 α is referred to changing of the EMT markers expression in BPH-1 cells, we evaluated its transcription factors' change on the WSCM-induced EMT by adding anti-HIF-1 α antibody with WSCM medium. As shown in **Figure 2**, BPH-1 cells had a spindle form in WSCM media; however, this morphology was reversed by anti-HIF-1 α treatment to culture medium. To further determine anti-HIF-1 α role in WSCM-induced EMT, we carried out western blotting, and we found the E-cadherin and CK5/8 expression were strongly enhanced after treatment with anti-HIF-1 α . Vimentin, α -SMA and Snail protein expression were decreased by western blot. In addition to this, we got similar results from immunofluorescence, when the BPH-1 cells was added to the HIF-1 α antibody. Results showed that vimentin, α -SMA and Snail were inhibited, but E-cadherin and CK5/8 were upregulated (**Figure 2D**). In the end, we also proved that anti-HIF-1 α could reverse the WSCM induced the BPH-1 migratory potential by using a migration assay (**Figure 2E**). These

results with anti-HIF-1 α indicated that it may play a role in WSCM cultured BPH-1 cells.

HIF-1 α affects its downstream mediator Smad3

Smad3, as a HIF-1 α signal-pathway receptor protein, has a significant role in the WSCM-induced EMT in BPH-1 cells. Hence, we investigated the function of Smad3 in the BPH-1 cells. To further investigate the interaction between HIF-1 α and Smad3, we performed the qPCR and western blotting to determine the expression of Smad3 in **Figure 3**. As shown in **Figure 3A**, we found Smad3 was phosphorylated and was up-regulated in WSCM medium cultured BPH-1 cells by comparison to the ordinary control medium. However, when we added HIF-1 α antibody into WSCM-cultured BPH-1 cells, we found that the embellishment of p-Smad3 was decreased after 7 days, and the gene of Smad3 expression was increased in mRNA level in WSCM medium cultured BPH-1 cells. We next found that if we add anti-HIF-1 α antibody into BPH-1 cells, this phenotype will be partly reversed (**Figure 3B**). These data further show that HIF-1 α may play an important role in WSCM-induced EMT in BPH-1 cells, and HIF-1 α may be coordinated by a Smad3 signal pathway.

Discussion

BPH is a well known age-related disease, in men over sixty years old [29, 30]. The main clinical manifestation of BPH is LUTS, which include increased frequency of urination, weak urine stream, urgency, hesitancy and nocturia [31, 32]. Thus, BPH reduces life quality. Several processes are involved in BPH, and the EMT process of prostate epithelial cells plays a very crucial role in BPH [33]. Numerous factors contribute to the EMT of prostate epithelial cells, and among these complicated regulators, the microenvironment provided by stromal cells is an extremely important one [7, 34]. It is already known that stromal cells play a key role in age-related tissue remodeling [35]. Compared to young men, older men have more reactive stromal cells that are characterized by up-regulation of the amount of myofibroblasts and loss of smooth muscle cells. Since myofibroblasts have more active function of secreting aberrant factors than normal fibroblasts this induces

HIF-1 α regulates the EMT of BPH

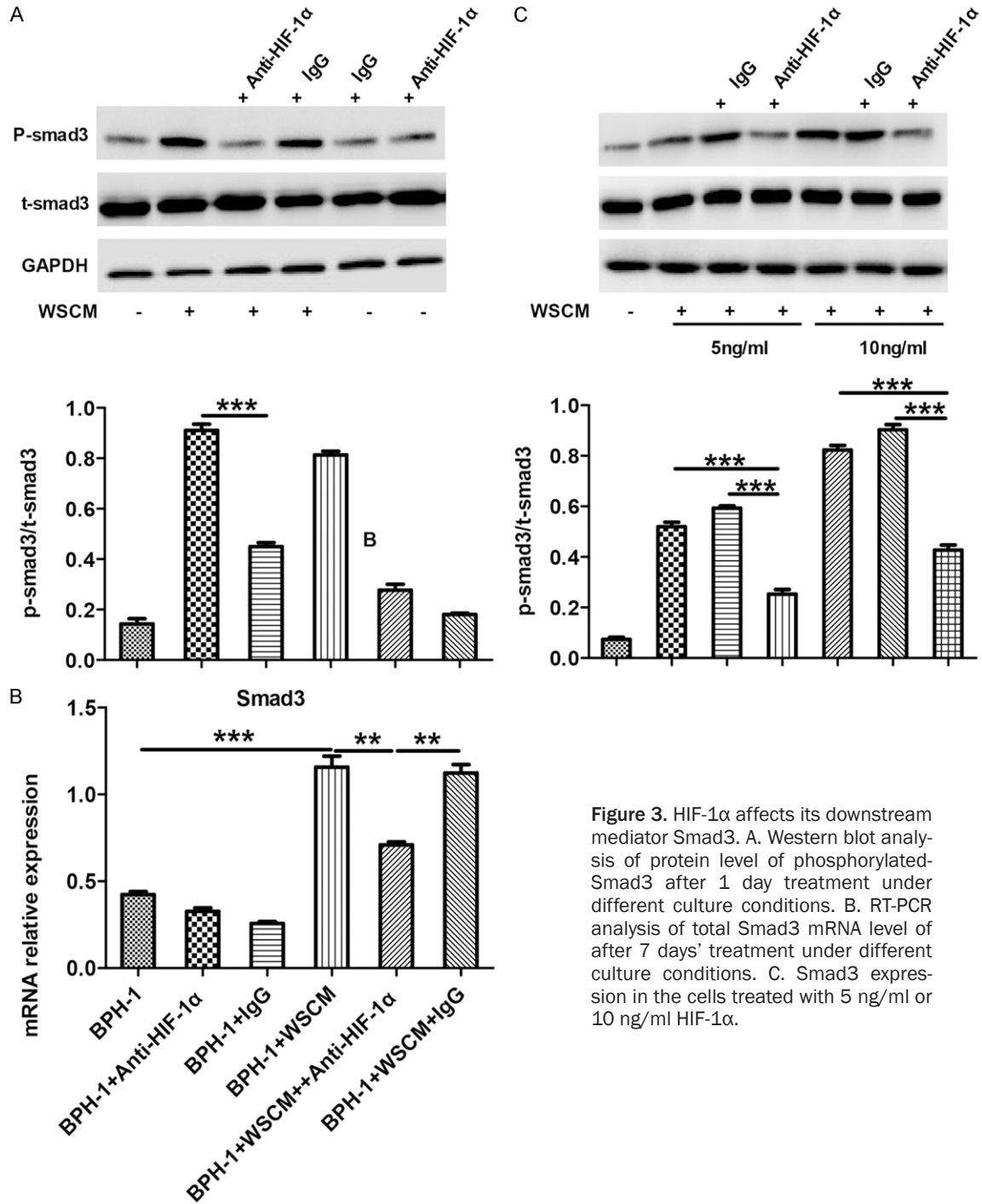


Figure 3. HIF-1 α affects its downstream mediator Smad3. A. Western blot analysis of protein level of phosphorylated-Smad3 after 1 day treatment under different culture conditions. B. RT-PCR analysis of total Smad3 mRNA level of after 7 days' treatment under different culture conditions. C. Smad3 expression in the cells treated with 5 ng/ml or 10 ng/ml HIF-1 α .

changes in nearby epithelial cells [36, 37]. Hence, these years, studies of microenvironment in BPH have attracted more and more attention. In our research, we used a BPH-1 cell line, derived from an aged male, and it has been used for the similar studies of microenvironment in BPH.

The HIF-1 α and VEGF were highly expressed with prostate hyperplasia; prostate epithelial rats cells, on the contrary, rarely have HIF-1 α expressed in normal environments [38-40]. Plenty of previous reports showed the conclusion that HIF-1 α has an active effect on the process of prostate hyperplasia; therefore, HIF-1 α

is identified as an underlying therapeutic target for BPH [38, 39]. Until now, numerous molecules are prepared for clinical use for inhibiting HIF-1 α [41]. However, a large proportion of HIF-1 α inhibitors perform their functions by targeting genes that regulate HIF-1 α expression. In addition, a HIF-1 α specific inhibitor has not been developed; thus, up to now, targeted therapy against HIF-1 α has not been established. Therefore, it is meaningful to establish a specific method to impair the function of HIF-1 α .

After treatment with WSCM, BPH-1 cells presented a stretched contour and the markers of epithelia, namely E-cadherin and CK5/8, were decreased (**Figure 1**). Additionally, the WSCM-treated BPH-1 cells expressed α -SMA, and Vimentin, both mesenchymal markers, and the Snail EMT marker (**Figure 1**). Snail is a zinc-finger transcription factor and is an EMT inducer. Snail can bind to an E-cadherin promoter E-box and represses its transcription; additionally, Snail also suppress endocytosis of transmembrane E-cadherin. In cancer cells, the high expression of Snail causes impairment of E-cadherin function and often induces EMT. In our results, we found that the microenvironment provided by stromal cells could boost EMT in BPH-1 cells by secreting EMT factors. And we discovered that WSCM could boost the BPH-1 cell migratory potential by migration assay (**Figure 1**). We also found that WSCM influence on the proliferation of BPH-1 cells was unchanged by the MTT results (**Figure 1**).

To examine the function of HIF-1 α in stromal microenvironment-induced EMT in BPH cells, we neutralized HIF-1 α by treating WSCM culture medium with HIF-1 α antibody. Then we found that anti-HIF-1 α treatment partially reversed morphologic changes induced by WSCM in comparison with the control medium. In addition, CK5/8 and E-cadherin expression were markedly increased; on the contrary, the expression of vimentin, α -SMA and Snail declined. Those results also can be supported by migration assays. Anti-HIF-1 α treatment reversed the migratory potential of BPH cells induced by WSCM.

Conclusions

In conclusion, our research shows that HIF-1 α may play a significant role in the stromal microenvironment-induced EMT process in BPH-1

cells; in addition, we established a specific method to suppress the function of HIF-1 α . Therefore, we found a novel therapeutic target for BPH.

Acknowledgements

This study was supported by Program of Shanghai City Committee of Science and Technology (15DZ1941502) and Shanghai Jiaotong University Medical and Industrial Cross Project (YG2017QN06).

Disclosure of conflict of interest

None.

Address correspondence to: Zhong Wang and Qi Chen, Department of Urology, Ninth People's Hospital, School of Medicine, Jiaotong University, Shanghai, China. Tel: +86 13301980998; Fax: +86 021 63136856; E-mail: zhongwang2001@sina.com (ZW); qiqi_chenqi@hotmail.com (QC)

References

- [1] O'Malley KJ, Dhir R, Nelson JB, Bost J, Lin Y and Wang Z. The expression of androgen-responsive genes is up-regulated in the epithelia of benign prostatic hyperplasia. *Prostate* 2014; 69: 1716-1723.
- [2] Paolone DR. Benign prostatic hyperplasia. *Clin Geriatr Med* 2014; 26: 223-239.
- [3] Khan FU, Ihsan AU, Khan HU, Jana R, Wazir J, Khongorzul P, Waqar M and Zhou X. Comprehensive overview of prostatitis. *Biomed Pharmacother* 2017; 94: 1064-1076.
- [4] van der Pluijm G. Epithelial plasticity, cancer stem cells and bone metastasis formation. *Bone* 2011; 48: 37-43.
- [5] Kalluri R and Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2014; 119: 1420-1428.
- [6] Jie XX, Zhang XY and Xu CJ. Epithelial-to-mesenchymal transition, circulating tumor cells and cancer metastasis: mechanisms and clinical applications. *Oncotarget* 2017; 8: 81558-81571.
- [7] Thiery JP, Acloque H, Huang RY and Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2014; 139: 871-890.
- [8] Kyprianou N and Isaacs JT. Activation of programmed cell death in the rat ventral prostate after castration. *Endocrinology* 2015; 122: 552-562.
- [9] Ding B, Sun Y and Huang J. Overexpression of SKI oncoprotein leads to p53 degradation

HIF-1 α regulates the EMT of BPH

- through regulation of MDM2 protein sumoylation. *J Biol Chem* 2012; 287: 14621-14630.
- [10] Naidu SR, Lakhter AJ and Androphy EJ. PIASy-mediated Tip60 sumoylation regulates p53-induced autophagy. *Cell Cycle* 2012; 11: 2717-2728.
- [11] Sciacovelli M and Frezza C. Metabolic reprogramming and epithelial-to-mesenchymal transition in cancer. *FEBS J* 2017; 284: 3132-3144.
- [12] De Wever O and Mareel M. Role of tissue stroma in cancer cell invasion. *J Pathol* 2003; 200: 429-447.
- [13] Yokobori T and Nishiyama M. TGF-beta signaling in gastrointestinal cancers: progress in basic and clinical research. *J Clin Med* 2017; 6: 23-32.
- [14] Lin CM, Chiu JH, Wu IH, Wang BW, Pan CM and Chen YH. Ferulic acid augments angiogenesis via VEGF, PDGF and HIF-1 alpha. *J Nutr Biochem* 2010; 21: 627-633.
- [15] Shi YH, Bingle L, Gong LH, Wang YX, Corke KP and Fang WG. Basic FGF augments hypoxia induced HIF-1-alpha expression and VEGF release in T47D breast cancer cells. *Pathology* 2017; 39: 396-400.
- [16] Andrikopoulou E, Zhang X, Sebastian R, Marti G, Liu L, Milner SM and Harmon JW. Current Insights into the role of HIF-1 in cutaneous wound healing. *Curr Mol Med* 2011; 11: 218-235.
- [17] Hong XY, Wang J and Li Z. AGR2 expression is regulated by HIF-1 and contributes to growth and angiogenesis of glioblastoma. *Cell Biochem Biophys* 2013; 67: 1487-1495.
- [18] Huang LE, Gu J, Schau M and Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. *Proc Natl Acad Sci U S A* 2015; 95: 7987-7992.
- [19] Semenza GL. Hydroxylation of HIF-1: oxygen sensing at the molecular level. *Physiology (Bethesda)* 2014; 19: 176-182.
- [20] Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, Teng SC and Wu KJ. Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nat Cell Biol* 2008; 10: 295-305.
- [21] Risau W. Mechanisms of angiogenesis. *Nature* 2014; 386: 671-674.
- [22] Lin J, Wei L, Xu W, Hong Z, Liu X and Peng J. Effect of hedyotis diffusa wild extract on tumor angiogenesis. *Mol Med Rep* 2011; 4: 1283-1288.
- [23] Goto F, Goto K, Weindel K and Folkman J. Synergistic effects of vascular endothelial growth factor and basic fibroblast growth factor on the proliferation and cord formation of bovine capillary endothelial cells within collagens. *Lab Invest* 2017; 69: 508-517.
- [24] Prior BM, Yang HT and Terjung RL. What makes vessels grow with exercise training? *J Appl Physiol* (1985) 2014; 97: 1119-1128.
- [25] Cavaco A, Rezaei M, Niland S and Eble JA. Collateral damage intended-cancer-associated fibroblasts and vasculature are potential targets in cancer therapy. *Int J Mol Sci* 2017; 18: 56-64.
- [26] Webber MM, Trakul N, Thraves PS, Bello-DeOcampo D, Chu WW, Storto PD, Huard TK, Rhim JS and Williams DE. A human prostatic stromal myofibroblast cell line WPMY-1: a model for stromal-epithelial interactions in prostatic neoplasia. *Carcinogenesis* 2016; 20: 1185-1192.
- [27] Nallamshetty S, Chan SY and Loscalzo J. Hypoxia: a master regulator of microRNA biogenesis and activity. *Free Radic Biol Med* 2013; 64: 20-30.
- [28] Harris AL. Hypoxia—a key regulatory factor in tumour growth. *Nat Rev Cancer* 2012; 2: 38-47.
- [29] Sausville J and Naslund M. Benign prostatic hyperplasia and prostate cancer: an overview for primary care physicians. *Int J Clin Pract* 2010; 64: 1740-1745.
- [30] Novara G, Giannarini G, Alcaraz A, Cozar-Olmo JM, Descazeaud A, Montorsi F and Ficarra V. Efficacy and safety of hexanic lipidosterolic extract of serenoa repens (Permixon) in the treatment of lower urinary tract symptoms due to benign prostatic hyperplasia: systematic review and meta-analysis of randomized controlled trials. *Eur Urol Focus* 2016; 2: 553-561.
- [31] Roehrborn CG. Male lower urinary tract symptoms (LUTS) and benign prostatic hyperplasia (BPH). *Med Clin North Am* 2011; 95: 87-100.
- [32] Oh SJ. Unsolved issues in managing benign prostatic hyperplasia. *Korean J Urol* 2013; 54: 349-350.
- [33] Huang X and Lee C. Regulation of stromal proliferation, growth arrest, differentiation and apoptosis in benign prostatic hyperplasia by TGF-beta. *Front Biosci* 2013; 8: s740-749.
- [34] Lim J and Thiery JP. Epithelial-mesenchymal transitions: insights from development. *Development* 2012; 139: 3471-3486.
- [35] Untergasser G, Madersbacher S and Berger P. Benign prostatic hyperplasia: age-related tissue-remodeling. *Exp Gerontol* 2015; 40: 121-128.
- [36] Niu YN and Xia SJ. Stroma-epithelium crosstalk in prostate cancer. *Asian J Androl* 2014; 11: 28-35.
- [37] Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD and Rowley DR. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. *Clin Cancer Res* 2012; 8: 2912-2923.

HIF-1 α regulates the EMT of BPH

- [38] Park SE, Park JW, Cho YS, Ryu JH, Paick JS and Chun YS. HIF-1 α promotes survival of prostate cells at a high zinc environment. *Prostate* 2017; 67: 1514-1523.
- [39] Yun YJ, Li SH, Cho YS, Park JW and Chun YS. Survivin mediates prostate cell protection by HIF-1 α against zinc toxicity. *Prostate* 2016; 70: 1179-1188.
- [40] Li SH, Ryu JH, Park SE, Cho YS, Park JW, Lee WJ and Chun YS. Vitamin C supplementation prevents testosterone-induced hyperplasia of rat prostate by down-regulating HIF-1 α . *J Nutr Biochem* 2010; 21: 801-808.
- [41] Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. *Trends Pharmacol Sci* 2012; 33: 207-214.