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The clinicopathological significance of ischemic change in benign prostatic hyperplasia using immunohistochemistry for hypoxia-inducible factor-1 alpha

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ABSTRACT

전립샘비대증에서 저산소유도인자-1α에 대한 면역조직화학염색을 이용한 허혈성 변화의 임상적, 병리학적 중요성

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목적: 전립샘비대증은 노인 남성에서 하부요로증상을 일으키는 가장 흔한 원인이다. 또한, 여러 전신 허혈성 원인 인자가 하부요로증상을 일으키거나 악화시킨다고 알려져 있다. 본 연구는 전립샘비대증에서 저산소유도인자-1α의 발현 빈도를 확인하고, 전신 허혈성 인자와의 연관성을 비교 분석하며, 면역조직화학염색을 통한 기타 다양한 인자의 변화 양상과 전립샘 조직의 조직학적 변화를 규명하고자 한다.

대상 및 방법: 2009년 1월부터 2012년 9월까지 전립샘비대증으로 경요도전립샘절제술을 시행 받은 60세 이상 남자 환자를 대상으로, 의무기록을 후향적으로 분석하였다. 혈관 위험 인자를 확인하기 위해 당뇨병, 고혈압, 현재 흡연 유무, 비만, 이상지질혈증을 조사하였다. 전립샘비대증 관련 인자로서, 국제 전립샘 증상 점수와 삶의 질 점수, 최대 요속과 잔뇨량, 혈청 전립샘 특이항원, 전립샘의 용적과 전립샘 결석의 유무, 그리고 수술 전 전립샘비대증에 대한

약물치료 상태를 조사하였다. 전립샘 조직의 병리조직학적 변화를 확인하기 위해 저산소유도인자-1α, 핵인자-κB, 성 호르몬 수용체들, 평활근 액틴에 대한 면역조직화학염색을 시행하였다. 또한, CD34 면역조직화학염색을 통해 미세혈관 밀도를 측정하였으며, Masson-trichrome 특수 염색을 통해 확산성 섬유증을 평가하였다.

결과: 본 연구에 총 101명의 환자가 포함되었으며 저산소유도인자-1α의 발현은 간질에서 56명 (55.4%), 선조직에서는 34명 (33.7%) 이었다. 이 인자와 혈관 위험 인자와의 유의한 관련성은 확인되지 않았으나, 간질에서의 저산소유도인자-1α와 미세혈관 밀도와의 유의한 상관성이 확인되었다. 또한, 간질에서의 저산소유도인자-1α와 간질에서의 안드로겐 그리고 프로게스테론 수용체와의 유의한 상관관계가 확인되었다. 핵인자-κB와의 관계에서, 선조직에서 이 인자의 활성화는 높은 미세혈관 밀도를 보였다. 확산성 섬유증과 여러 인자을 비교하였을 때, 간질에서의 저산소유도인자-1α와 평활근 액틴의 발현과 유의한 상관관계가 보였다.

결론: 전립샘의 허혈성 자극에 대한 반응으로 저산소유도인자-1α 발현이 관찰되었으며, 이들 환자에서 안드로겐 수용체의 발현률이 낮게 나타났다. 이는 안드로겐 수용체의 발현이 있으면 전립샘의 허혈성 손상에 저항력이 있다고 판단할 수 있겠다. 또한, 전립샘에 허혈성 변화가 발생하였을 때, 저산소유도인자-1α의 발현률이 증가되어 허혈성 손상에 저항성을 갖으며, 이와 다른 전립샘 조직 내에 항허혈성 경로의 활성화는 전립샘비대증에 대한 약물 치료에 불응하는 결과를 보인다고 생각한다.

색인단어: 전립샘비대증, 저산소유도인자-1α, 허혈, 면역조직화학염색

Ⅰ. INTRODUCTION

Chronic conditions, such as diabetes mellitus and hypertension, are reportedly related to various diseases, such as cardiovascular diseases, stroke, chronic renal diseases and the others. Further, metabolic syndrome, including hypertension, dyslipidemia, obesity, and hyperglycemia, has also been reported to be associated with various diseases (1-4). Therefore, managing these diseases or conditions is very important to prevent the occurrence of unexpected serious diseases and improve the quality of life (QoL) of patients. Lower urinary tract symptoms (LUTS), including hesitancy, intermittency, frequency, urgency, weak stream, residual urine sense, and nocturia, can occur owing to various diseases or conditions, such as benign prostatic hyperplasia (BPH), overactive bladder, urinary tract infections, bladder cancer, and neurogenic bladder. LUTS in elderly men are especially related to BPH, and the prevalence is increasing worldwide with demographic changes (5-7). Generally, benign prostatic enlargement, resulting from the histological changes in the prostatic tissues, can cause bladder outlet obstruction and also affect bladder functions, and these changes tend to increase with age. To date, these changes in the prostate have been reported to be caused by several mechanisms, such as interference in growth factor pathways, hormonal alterations, chronic prostatic inflammation, and chronic prostatic ischemia (8-11). Chronic

ischemia of the prostate has been especially reported to lead to changes in the structure of the prostate, including stromal fibrosis, glandular cystic atrophy, and impaired smooth muscle relaxation in some animal studies (12, 13) and widespread DNA damage as a differential reaction to hypoxia and oxidative stress in a study on cultured human prostate cells (11). Recent observational studies on the association of vascular risk factors, such as hypertension, diabetes mellitus, hyperlipidemia, and smoking, suggested that such factors play a role in the development of LUTS (14-16). In addition, medications used to manage these conditions, including sedatives, antipsychotics, antidepressants, smooth muscle relaxants, diuretics, calcium channel blockers, and nonsteroidal anti-inflammatory drugs, can affect the function of the lower urinary tracts, which can lead to drug-induced LUTS (17). Therefore, it is necessary to identify the factors affecting the ischemic changes in the prostate and to confirm the histological changes. In the current study, I investigated the association between vascular risk factors as chronic ischemia of the prostate and BPH by conducting experiments to elucidate the clinicopathological significance of the ischemic changes in BPH using immunohistochemistry stains for hypoxia-inducible factor-1alpha (HIF-1α) and the others.

II. MATERIALS AND METHODS

Study subjects and vascular risk factors

After obtaining approval from Institutional Ethics Review Board at Chosun University Hospital (No. CHOSUN 2017-07-021-002), the medical records of consecutive patients over 60 years of age who underwent transurethral resection of the prostate for BPH between January 2009 and September 2012 were reviewed. If the patients had a possibility of having prostate cancer, including the abnormal digital rectal examination findings of the prostate and abnormal prostate-specific antigen (PSA) levels (≥ 4 ng/mL), I performed the transrectal ultrasonography-guided prostate biopsy preoperatively. To identify the vascular risk factors, the presence or absence of diabetes mellitus, hypertension, current smoking, obesity (body mass index ≥ 25 kg/m^2), and dyslipidemia (total cholesterol level of ≥ 200 mg/dL or triglyceride level of ≥ 150 mg/dL) was investigated. In addition, the presence or absence of diseases that may affect bladder function, such as stroke and neurologic disorders, was investigated.

Assessment of LUTS related to BPH

As BPH-related factors, I investigated the International Prostate Symptom Score (IPSS), QoL, maximal flow rate (Qmax; mL/sec), postvoid residual volume (PVR; mL), PSA level (ng/mL), prostate volume (PV; mL), presence or absence of prostatic calculi on transrectal ultrasonography (TRUS) and preoperative medication state for BPH.

Tissue array methods

Four array blocks containing a total of 101 resected prostate tissue cores that were obtained from the patients were prepared. Briefly, the tissue cores (2 mm in diameter) were obtained from individual paraffin-embedded tissues (donor blocks) and arranged in recipient paraffin blocks, using a trephine apparatus (18). Two cores were selected from each case for analysis.

Immunohistochemical staining

Sections 4 ㎛ in thickness were deparaffinized and hydrated using a routine xylene-alcohol series. For antigen retrieval, the sections were treated with 0.01-M citrate buffer (pH, 6.0) for 5 min in a microwave oven and 3% H_2O_2 to quench endogenous peroxidase. Thereafter, they were treated with normal serum

of the host animal of the secondary antibody, to block nonspecific binding. The sections were then incubated with anti-HIF-1α (Novus Biologicals, Littleton, CO), anti-androgen receptor (AR) (Abcam, Cambridge, UK), anti-estrogen receptor (ER)-alpha (Abcam), anti-ER-beta (Abcam), anti-progesterone receptor (PR) (Ventana Medical Systems, Inc., Tucson, AZ), anti-nuclear factor-kappa B (NF-κB) p65 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-CD34 (Cell Marque, Darmstadt, Germany), and anti-smooth muscle actin (Leica, Novocastra Laboratories, Newcastle upon Tyne, UK) (Supplementary Table 1). Immunohistochemical staining was conducted following a compact polymer method, using the VENTANA benchmark XT autostainer (Ventana Medical Systems, Inc.). Visualization was performed by treatment with OPTIVIEW universal 3,3'-diaminobenzidine kit (Ventana Medical Systems, Inc.). To confirm the reaction specificity of the antibody, a negative control stain without primary antibody was employed. All immunostained sections were lightly counterstained with Mayer's hematoxylin.

Evaluation of the immunohistochemical stains

The immunoreactivities for hormonal receptors, HIF-1α (19, 20), NF-κB p65 (21), and smooth muscle actin were investigated in the nucleus. Although NF-κB p65 can be constitutively expressed in the cytoplasm, the nuclear

expression of NF-κB p65 is consistent with NF-κB activation (22). The intensities of protein expression in the immunohistochemically stained samples were scored from 0 to 3 (0 = negative; 1 = weak; 2 = moderate; and 3 = strong). The percentage of positively stained cells was categorized using a scoring system with points ranging from 0 to 4 (1 = 0-25%; 2 = 26-50%; 3 = 51-75%; and 4 = 76-100%). The immunoreactive score (IRS) was calculated by multiplying the staining intensity scores with the percentages of positively stained cells (23). The staining patterns were classified as negative (IRS: 0-4) or positive (IRS: 6-12).

Evaluation of diffuse fibrosis (DF)

To evaluate the collagenous stromal structures, Masson-trichrome special staining was performed using the common method. In this process, the collagenous stromal structures and smooth muscles were stained in blue and red colors, respectively (24).

Evaluation of the micro-vessel density (MVD)

To evaluate the MVD in the stroma, immunohistochemistry for CD34, which shows immunoreactivity to endothelial cells, was performed. Areas with highly vascularization were screened, and the three high-power magnification fields (x400) were determined. The micro-vessels were counted at each high-power magnification field, and the mean micro-vessel counts were calculated. The high and low MVD groups were divided on the basis of the median value of the mean micro-vessel counts of all patients.

Statistical analysis

For descriptive statistics, such as preoperative patients' characteristics, categorical data were expressed as numbers and percentages, and continuous data as the means ± standard deviations. Categorical variables were compared using the chi-square test and continuous variables using Student's t-test. The results were considered statistically significant at p values of < 0.05. All statistical analyses were performed using the SPSS ver. 22.0 (SPSS Inc., Chicago, IL, USA).

III. RESULTS

From January 2009 to September 2012, a total of 103 patients underwent transurethral resection of the prostate for BPH at the department of urology of Chosun University Hospital. Of these, 101 patients were included in this study (one patient diagnosed with prostate cancer and the other patient in whom enough data from the medical records were difficult to obtain were excluded). The mean age of the patients was 73.89 ± 6.21 years, and the other patients' characteristics are summarized in Table 1. The mean total score in IPSS was 23.98 ± 7.68, and the number of patients with severe symptoms was 68 (67.3%). The number of patients receiving medications for BPH, such as alpha-blockers (AB) and 5-alpha reductase inhibitors (5ARI), was 96 (95%); the other variables related to BPH are also summarized in Table 1.

The number of patients who showed HIF-1α expression in the stroma and gland was 56 (55.4%) and 34 (33.7%), respectively (Fig. 1). There was no significant association between HIF-1α expression in the stroma and gland and vascular risk factors. In addition, there was no significant difference in the presence of two or more vascular risk factors and the association of HIF-1α with three or more vascular risk factors (Tables 2 and 3). Then, the patients who showed HIF-1α expression had higher MVDs than those who did not ($p < 0.001$) (Table 4).

In the comparison with prostate-related to variables, including IPSS, QoL, uroflowmetry, PSA level, TRUS findings, previous medication for BPH, and HIF-1α expression in the stroma and gland, no significant variables were identified (Tables 5 and 6).

When the relationship between the expression of sexual hormonal receptors and the HIF-1α expression in the stroma and gland was determined, there were significant differences in the AR and PR of the stroma (AR: $p < 0.001$; PR: $p < 0.001$); however, there was no significant difference in the sexual hormonal receptors in the gland (Fig. 2). In addition, there were no significant correlations between HIF-1α expression in the gland and sexual hormonal receptors (Tables 7 and 8).

Because NF-κB can be induced by HIF-1α and AR, the impact of NF-κB activation was investigated (Fig. 3). The patients who showed NF-κB activation in the glands had higher MVDs than those who did not. This correlation was not found in the NF-κB activation in the stroma (Tables 9 and 10). In the comparison between DF and various factors, including AR, HIF-1α in the stroma and gland, smooth muscle action expression, and MVD, there were significant correlations in the HIF-1α expression in the stroma and smooth muscle actin expression (Table 11).

The patients in this study were stratified according to vulnerability to

hypoxia. First, the patients were divided into groups with high and low expressions of HIF-1α in the stroma. Second, DF and the MVD, which were consistent with the histologic findings of ischemic damage, were investigated in the stroma. Among the patients who showed HIF-1α expression, the MVD was significantly higher in the patients who showed NF-κB activation than in those who did not. In addition, DF was frequently found in the patients who showed HIF-1α expression, regardless of NF-κB activation. In addition, there was an inverse correlation between DF and smooth muscle actin expression.

IV. Discussion

BPH is a histological diagnosis that refers to the proliferation of smooth muscle and epithelial cells mainly within the prostatic transition zone (25-27). It is ubiquitous in the aging men, with its prevalence increasing with age. Currently, BPH refers to a clinical syndrome consisting of three components: hypertrophy of the prostate caused by these tissue changes; LUTS, including frequency, urgency, nocturia, weak stream, hesitancy, and residual urine sense, and bladder outlet obstruction (28). In patients with mild symptoms, closed observation with life-style modifications should be considered. However, in patients with the moderate to severe symptoms, medical therapy is considered as the initial therapy. In addition, surgery such as transurethral resection of the prostate or open prostatectomy, is usually performed to improve symptoms and decrease the progression of BPH in patients with recurrent or refractory urinary retention, recurrent urinary tract infections, and bladder stones and patients without symptomatic improvement despite medical therapies (29, 30). Histologically, prostatic hyperplasia involves an increase in the number of epithelial and stromal cells around the urethra. In this regard, various factors have been reported to date, including sexual hormones, regulation of programmed cell death, stromal-epithelial interaction, growth factors, inflammatory pathways, and genetic factors (17).

Nevertheless, AB, which reduces smooth muscle tone in the prostate and bladder neck, and 5ARI, which reduces the PV by inducing epithelial atrophy, are the most commonly used agents to ameliorate symptoms, improve the urinary flow rate, and reduces the risk of BPH progression such as acute urinary retention, and the need for surgical treatment (31, 32).

To date, there has been a continuous study of association between BPH and LUTS in relation to chronic ischemia of the prostate. Several of them suggested that certain medical conditions such as hypertension and diabetes mellitus and lifestyle behaviors, such as cigarette smoking, alcohol consumption, and physical activity, may act as independent risk factors for the development of LUTS (33, 34). Ponholzer A et al. performed an observational study to investigate the association between vascular risk factors and LUTS in 1,724 men and 812 women. They found that in men, the IPSS was identical in those with no (6.2 ± 4.1) and one (6.2 ± 4.4) vascular risk factor, yet increased by 24% (7.37 ± 5.5; (p < 0.01) in those with two or more risk factors; among the women, those with two or more vascular risk factors had an IPSS (7.0 ± 5.7, +45%) that was 2.2 points higher than that of those without a risk factor (p < 0.05). This suggests that vascular risk factors play a role in the development of LUTS in both sexes (15). Similarly, Kim et al. investigated 280 men aged more than 50 years to investigate the relationship between LUTS and risk factors for vascular diseases and obtained results similar to previous

reports. In addition, they reported that men with three or more vascular risk factors were three times more likely to have moderate to severe LUTS than men without vascular risk factors in their multiple logistic regression analysis (16). Kozlowski R et al. investigated whether chronic ischemia alters the structural and functional properties of a prostate animal model and reported that chronic prostatic ischemia causes marked changes in the prostatic structure and contractility resulting from thickening and fibrosis of the prostate; further, ischemia-induced glandular atrophy was consistently associated with decreased vascular endothelial growth factor expression (13). In addition, Azadzoi et al. showed that ischemia significantly increased prostatic tissue contraction, decreased cyclic guanosine monophosphate release, and led to capsular and stromal thickening, and epithelial atrophy with enhancement of the efficacy of doxazosin in decreasing prostatic tissue contraction by stimulators of nitric oxide synthesis and cyclic guanosine monophosphate (35).

Hypoxia-inducible factor-1 (HIF-1) is a transcription factor composed of HIF-1α and HIF-1ß subunits and is a key player in hypoxic response (19, 20). In 2016, Wu F et al. reported that HIF-1α expression is increased in highly proliferative prostate tissues, correlated with the weight of the intra-acinar prostate, and also an independent risk factor for acute urinary retention in patients with BPH (OR = 5.517, 95% CI = 2.4345 - 12.504) (36). In the current

study, HIF-1α expression in the stroma and gland was 55.4% and 33.7%, respectively; there were also no significant correlations between the vascular risk factors and HIF-1α expression in the stroma and gland, also in the patients with multiple vascular risk factors. It can be considered that most of the patients in the current study were not responding well to the medical therapy for BPH. In other words, there may be other consequences of the prostate reaction by existing medications. Forsythe JA et al. reported that stabilized HIF-1α dimerizes with HIF-ß and binds to hypoxia-response elements, stimulating expression of profuse hypoxia response genes, including those encoding erythropoietin and vascular endothelial growth factors, which stimulates erythropoiesis and angiogenesis (37). However, a significant correlation between HIF-1α and MVD was found in the current study. Kim HJ et al. reported that epithelial cells in the prostate treated with lipopolysaccharide showed high proliferation and HIF-1α levels in rats and suggested that the HIF-1/VEGF axis contributes to prostate enlargement in animal models (38). Therefore, chronic ischemic stimulation in the prostate may lead to HIF-1α expression, which causes histological changes in the prostate through various linking reactions. The current results suggested that HIF-1α/NF-κB activation could induce angiogenesis, and HIF-1α could function via NF-κB activation. In addition, the blood supply of the prostate comes from the inferior vesical artery of the branch of the internal iliac

artery. If there are no special problems with these vessels (e.g., atherosclerosis) despite presence of various vascular risk factors, ischemic stimulation, which may cause histological changes in the prostate, may not have a marked effect.

The prostate is a sexual hormone reactive organ, and the regulation of sexual steroid hormones is necessary for the gland development, maintenance, and function (39). Song L et al. observed activation of AR and PR and repression of ERα in BPH, which indicate a promotive role of AR and PR and an inhibitory role of ER-α in the pathogenesis of BPH (40). In the current study, the correlation between the sexual hormonal receptors and HIF-1α in the stroma was significantly different in the AR and PR in the stroma without HIF-1α expression in the gland. Austin et al. showed that elevated expression of AR-variant 7, but not other AR variants, was found in advanced BPH samples and was significantly correlated with the patient IPSS and TRUS volume of patients (41). Thus, chronic ischemic stimulations may lead to changes in the expression of sexual hormonal receptors in the prostate, and it may be possible to explain why it is not effective in the treatment with 5ARI, which are known to have a prophylactic effect on the progression of BPH.

The eukaryotic NF-κB transcription factor family regulates the expression of a large variety of genes involved in inflammatory and immune responses as

well as cellular growth and development (42). Vignozzi et al. reported that pretreatment with dihydrotestosterone inhibited NF-κB activation and suppressed secretion of several inflammatory/growth factors (43), and Austin et al. reported that forced activation of canonical NF-κB in human prostatic epithelial and stromal cells resulted in elevated expression of both AR full length and AR variant 7. Further, chronic activation of NF-κB induces resistance to a 5ARI (41). In the current study, no association between NF-κB activation in the gland and various factors, including AR, HIF-1α, smooth muscle actin expression, and DF with MVD, was found. It may mean that the histological changes in the prostate progress through various mechanisms and that the association with AR is not confirmed by the fact that NF-κB induced changes are involved in the expression of atypical hormonal receptors. Ischemia in the prostate with hyperplastic and hypertrophic changes can contribute to the maintenance of proliferation or ischemic damage. If the prostate has a resistant pathway against ischemia or ischemia is reversible, it can be act as a proliferative factor through NF-κB activation. In the current study, DF was frequently found in the patients who showed HIF-1α expression and NF-κB inactivation. In addition, the change in the collagenous prostatic tissues may be caused by chronic ischemic stimulation if increasing angiogenesis is failed to these stimuli. It suggests that replaced prostatic tissue to a non-functional tissue, eventually converted to a refractory BPH to pharmacotherapy.

V. Conclusion

The current study investigated patients who underwent surgical therapy for BPH despite long-term pharmacological therapy; it is defined as a serious progression of BPH in the urological field. In patients showing responses to ischemic changes of the prostate, HIF-1α expression could be confirmed; the expression of AR was significantly lower in these patients. They appear to be resistant to ischemic damage in the prostate if AR expression is present. Nevertheless, there were patients who showed high HIF-1α expression and some who did not have increased MVDs. Increased angiogenesis due to ischemia can be induced by activation of the HIF-1α/NF-κB pathway. Even if ischemic changes in the prostate are occurred, patients may be resistant to ischemic damage if HIF-1α/NF-κB pathway is activated.

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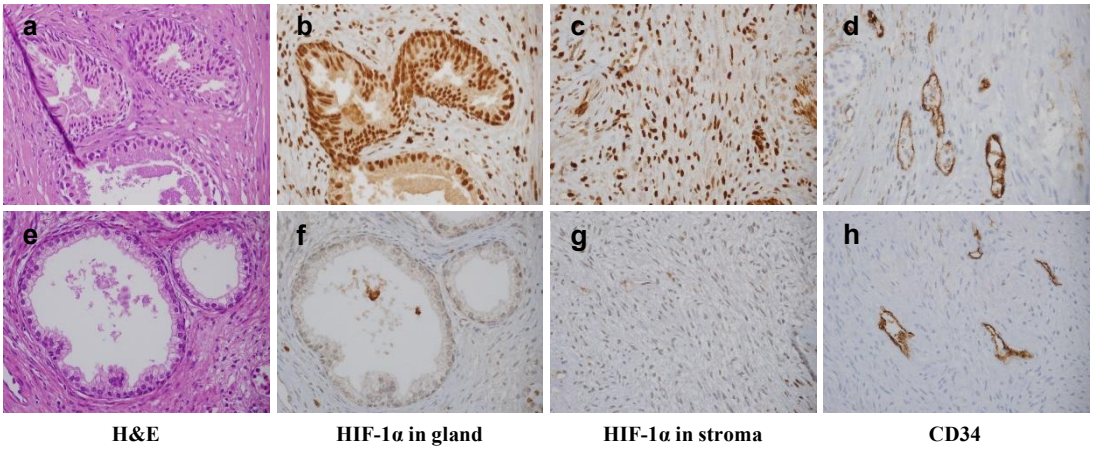

Figure 1. Representative figures for benign prostatic hyperplasia and Hypoxia-inducible factor-1alpha (HIF-1α), and CD34 immunohistochemistry. (a and e) Representative figures for hematoxylin and eosin staining. (b and f) HIF-1α immunohistochemistry in gland. (c and g) HIF-1α immunohistochemistry in stroma. (d and h) CD34 immunohistochemistry for calculating micro-vessel density (MVD). (x400).

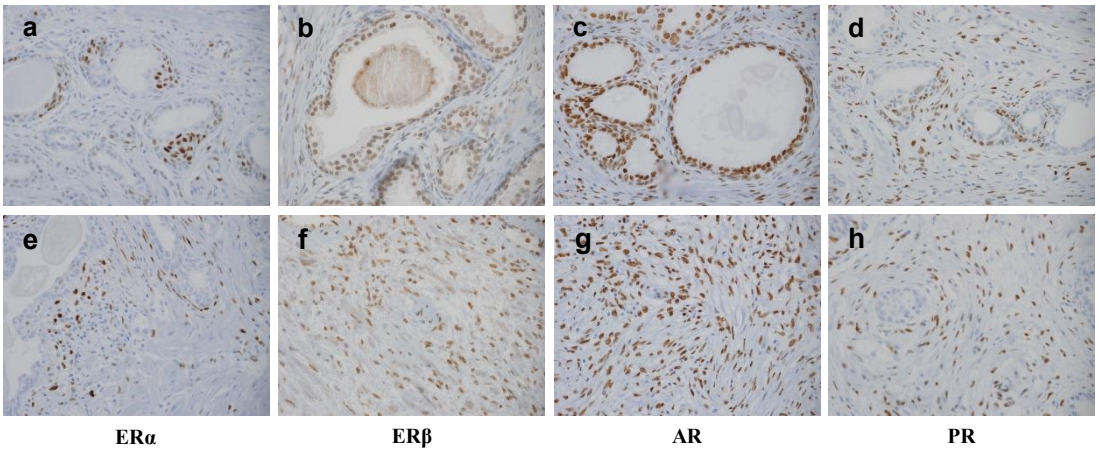

Figure 2. Representative figures for hormonal receptors including estrogen receptor-alpha and beta (ER-α and ß), androgen receptor (AR), and progesterone receptor (PR) in gland (a to d) and stroma (e to h). (x400).

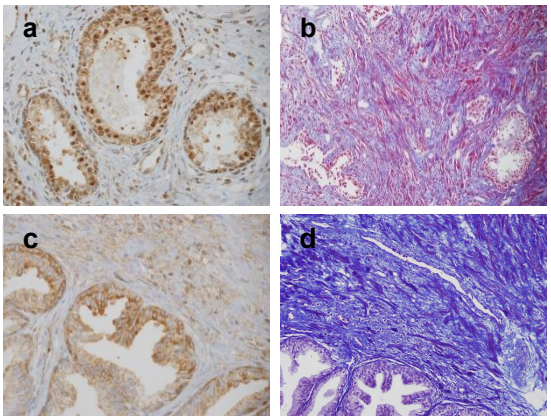

Figure 3. Representative figures for nuclear factor-kappa B (NF-κB) immunohistochemical (a and c) and Masson-trichrome special stainings (b and d). (x400).

Table 1. Patients' characteristics

Total (n = 101)	Value
Age (years)	73.89 ± 6.21
Obesity	35 (34.7)
Current smoking	14 (13.9)
Diabetes mellitus	21 (20.8)
Hypertension	41 (40.6)
Cerebrovascular accident	8 (7.9)
Dyslipidemia	28 (27.7)
Neurologic disorder	6 (5.9)
IPSS	
Total score	23.98 ± 7.68
Voiding score	13.62 ± 4.95
Storage score	10.34 ± 3.88
QoL score	4.93 ± 1.21
Mild to moderate severity	33 (32.7)
Severe severity	68 (67.3)
Uroflowmetry	
Voided volume (ml)	177.06 ± 71.31
Qmax (ml/sec)	8.24 ± 4.27
PVR (ml)	99.40 ± 106.11
PSA (ng.ml)	7.19 ± 7.52
TRUS	
Total volume (ml)	78.52 ± 36.95
Transitional zone volume (ml)	50.44 ± 29.26
Prostatic calculi	19 (18.8)
Previous medication related to BPH	
None	5 (5.0)
AB	49 (48.5)
5ARI	10 (9.9)
AB + 5ARI	37 (36.6)

Value are given as mean ± standard deviation or numbers (percentage).

IPSS: International Prostatic Symptoms Score; QoL: quality of life; Qmax: maximum flow rate; PVR: postvoid residual; PSA: prostate-specific antigen; TRUS: transrectal ultrasonography; AB: alpha-blocker; 5ARI: 5-alpha reductase inhibitor

Table 2. Correlation between hypoxia-inducible factor-1alpha (HIF-1α) in stroma and vascular risk factors

	HIF-1α expression in stroma		
	Positive	Negative	*p*-value
Total (n = 101)	56 (55.4)	45 (44.6)	
Obesity			0.502
Present	21 (37.5)	14 (31.1)	
Absent	35 (62.5)	31 (68.9)	
Current smoking			0.890
Present	8 (14.3)	6 (13.3)	
Absent	48 (85.7)	39 (86.7)	
Diabetes mellitus			0.751
Present	11 (19.6)	10 (22.2)	
Absent	45 (80.4)	35 (77.8)	
Hypertension			0.355
Present	25 (44.6)	16 (35.6)	
Absent	31 (55.4)	29 (64.4)	
Dyslipidemia			0.432
Present	19 (33.9)	12 (26.7)	
Absent	37 (66.1)	33 (73.3)	
Vascular risk factors			0.639
2-5	25 (44.6)	18 (40.0)	
0-1	31 (55.4)	27 (60.0)	
Vascular risk factors			0.944
3-5	9 (16.1)	7 (15.6)	
0-2	47 (83.9)	38 (84.4)	

Value are given as numbers (percentage).

Table 3. Correlation between hypoxia-inducible factor-1alpha (HIF-1α) in gland and vascular risk factors

	HIF-1α expression in gland		*p*-value
	Positive	Negative	
Total (n = 101)	34 (33.7)	67 (66.3)	
Obesity			0.430
Present	10 (29.4)	25 (37.3)	
Absent	24 (70.6)	42 (62.7)	
Current smoking			0.861
Present	5 (14.7)	9 (13.4)	
Absent	29 (85.3)	58 (86.6)	
Diabetes mellitus			0.316
Present	9 (26.5)	12 (17.9)	
Absent	25 (73.5)	55 (82.1)	
Hypertension			0.731
Present	13 (38.2)	28 (41.8)	
Absent	21 (61.8)	39 (58.2)	
Dyslipidemia			0.117
Present	7 (20.6)	24 (35.8)	
Absent	27 (79.4)	43 (64.2)	
Vascular risk factors			0.840
2-5	14 (41.2)	29 (43.3)	
0-1	20 (58.8)	38 (56.7)	
Vascular risk factors			0.424
3-5	4 (11.8)	12 (17.9)	
0-2	30 (88.2)	55 (82.1)	

Value are given as numbers (percentage).

Table 4. Correlation between hypoxia-inducible factor-1alpha (HIF-1α) and micro-vessel density (MVD)

	MVD		*p*-value
	High	Low	
Total (n = 101)	48 (47.5)	53 (52.5)	
HIF-1α in stroma			< 0.001*
Positive	36 (75.0)	20 (37.7)	
Negative	12 (25.0)	33 (62.3)	
HIF-1α in gland			0.625
Positive	15 (31.3)	19 (35.8)	
Negative	33 (68.8)	34 (64.2)	

Value are given as numbers (percentage). * denote $p < 0.05$.

Table 5. Correlation between hypoxia-inducible factor-1alpha (HIF-1α) in stroma and factors related to benign prostatic hyperplasia (BPH)

	HIF-1α expression in stroma		*p*-value
	Positive	Negative	
Total (n = 101)	56 (55.4)	45 (44.6)	
IPSS			
Total score	23.39 ± 7.84	24.71 ± 7.50	0.394
Voiding score	13.27 ± 5.05	14.07 ± 4.83	0.423
Storage score	10.11 ± 4.33	10.62 ± 3.26	0.497
QoL score	4.93 ± 1.17	4.93 ± 1.27	0.984
Uroflowmetry			
Voided volume (ml)	189.61 ± 72.49	161.44 ± 67.39	0.048*
Qmax (ml/sec)	8.48 ± 3.82	7.94 ± 4.79	0.524
PVR (ml)	88.08 ± 76.10	113.49 ± 134.11	0.233
PSA (ng/ml)	6.62 ± 6.89	7.90 ± 8.26	0.398
TRUS			
Total volume (ml)	73.73 ± 35.07	85.73 ± 38.34	0.079
T-zone volume (ml)	46.96 ± 30.63	54.76 ± 27.17	0.185
Prostatic calculi			0.453
Present	12 (21.4)	7 (15.6)	
Absent	44 (78.6)	38 (84.4)	
Previous medication status			0.706
5ARI (+)	27 (48.2)	20 (44.4)	
5ARI (-) or no medication	29 (51.8)	25 (55.6)	

Value are given as mean ± standard deviation or numbers (percentage). * denote $p < 0.05$.

IPSS: International Prostatic Symptoms Score; QoL: quality of life; Qmax: maximum flow rate; PVR: postvoid residual; PSA: prostate-specific antigen; T-zone volume: transition zone volume; TRUS: transrectal ultrasonography; 5ARI: 5-alpha reductase inhibitor

Table 6. Correlation between hypoxia-inducible factor-1alpha (HIF-1α) in gland and factors related to benign prostatic hyperplasia (BPH)

	HIF-1α expression in gland		*p*-value
	Positive	Negative	
Total (n = 101)	34 (33.7)	67 (66.3)	
IPSS			
Total score	23.00 ± 8.30	24.48 ± 7.36	0.363
Voiding score	13.24 ± 5.10	13.82 ± 4.89	0.576
Storage score	9.71 ± 4.35	10.66 ± 3.61	0.246
QoL score	4.74 ± 1.31	5.03 ± 1.15	0.250
Uroflowmetry			
Voided volume (ml)	175.76 ± 77.79	177.72 ± 68.40	0.897
Qmax (ml/sec)	8.45 ± 4.01	8.13 ± 4.42	0.726
PVR (ml)	98.82 ± 79.94	99.69 ± 117.75	0.969
PSA (ng/ml)	6.48 ± 6.44	7.56 ± 8.03	0.499
TRUS			
Total volume (ml)	72.56 ± 33.92	81.55 ± 38.29	0.250
T-zone volume (ml)	45.91 ± 28.28	52.73 ± 29.69	0.270
Prostatic calculi			0.831
Present	6 (17.6)	13 (19.4)	
Absent	28 (82.4)	54 (80.6)	
Previous medication status			0.940
5ARI (+)	16 (47.1)	31 (46.3)	
5ARI (-) or no medication	18 (52.9)	36 (53.7)	

Value are given as mean ± standard deviation or numbers (percentage).

IPSS: International Prostatic Symptoms Score; QoL: quality of life; Qmax: maximum flow rate; PVR: postvoid residual; PSA: prostate-specific antigen; T-zone volume: transition zone volume; TRUS: transrectal ultrasonography; 5ARI: 5-alpha reductase inhibitor

Table 7. Correlation between hypoxia-inducible factor-1alpha (HIF-1α) in stroma and hormonal receptors

		HIF-1α in stroma		*p*-value
		Positive	Negative	
	Total (n = 101)	56 (55.4)	45 (44.6)	
Stroma	ER-α			0.686
	Positive	6 (10.7)	6 (13.3)	
	Negative	50 (89.3)	39 (86.7)	
	ER-ß			0.250
	Positive	35 (62.5)	23 (51.1)	
	Negative	21 (37.5)	22 (48.9)	
	AR			< 0.001*
	Positive	12 (21.4)	26 (57.8)	
	Negative	44 (78.6)	19 (42.2)	
	PR			< 0.001*
	Positive	12 (21.4)	27 (60.0)	
	Negative	44 (78.6)	18 (40.0)	
Gland	ER-α			0.812
	Positive	11 (19.6)	8 (17.8)	
	Negative	45 (78.2)	37 (82.2)	
	ER-ß			0.981
	Positive	30 (53.6)	24 (53.3)	
	Negative	26 (46.4)	21 (46.7)	
	AR			0.551
	Positive	42 (75.0)	36 (80.0)	
	Negative	14 (25.0)	9 (20.0)	
	PR			0.691
	Positive	2 (3.6)	1 (2.2)	
	Negative	54 (96.4)	44 (97.8)	

Value are given as numbers (percentage). * denote $p < 0.05$.

ER: estrogen receptor; AR: androgen receptor; PR: progesterone receptor

Table 8. Correlation between hypoxia-inducible factor-1alpha (HIF-1α) in gland and hormonal receptors

		HIF-1α in gland		*p*-value
		Positive	Negative	
	Total (n = 101)	34 (33.7)	67 (66.3)	
Stroma	ER-α			0.184
	Positive	2 (5.9)	10 (14.9)	
	Negative	32 (94.1)	57 (85.1)	
	ER-ß			0.054
	Positive	15 (44.1)	43 (64.2)	
	Negative	19 (55.9)	24 (35.8)	
	AR			0.436
	Positive	11 (32.4)	27 (40.3)	
	Negative	23 (67.6)	40 (59.7)	
	PR			0.625
	Positive	12 (35.3)	27 (40.3)	
	Negative	22 (64.7)	40 (59.7)	
Gland	ER-α			0.197
	Positive	4 (11.8)	15 (22.4)	
	Negative	30 (88.2)	52 (77.6)	
	ER-ß			0.234
	Positive	21 (61.8)	33 (49.3)	
	Negative	13 (38.2)	34 (50.7)	
	AR			0.382
	Positive	28 (82.4)	50 (74.6)	
	Negative	6 (17.6)	17 (25.4)	
	PR			0.210
	Positive	0 (0.0)	3 (4.5)	
	Negative	34 (100.0)	64 (95.5)	

Value are given as numbers (percentage).

ER: estrogen receptor; AR: androgen receptor; PR: progesterone receptor

Table 9. Correlation between nuclear factor-kappa B (NF-κB) in stroma and various conditions of benign prostatic hyperplasia

	NF-κB expression in stroma		*p*-value
	Present	Absent	
Total (n = 101)	31 (30.7)	70 (69.3)	
AR			0.137
Positive	15 (48.4)	23 (32.9)	
Negative	16 (51.6)	47 (67.1)	
HIF-1α in stroma			0.432
Positive	19 (61.3)	37 (52.9)	
Negative	12 (38.7)	33 (47.1)	
HIF-1α in gland			0.242
Positive	13 (58.1)	21 (30.0)	
Negative	18 (41.9)	49 (70.0)	
Smooth muscle actin expression			0.767
Positive	26 (83.9)	57 (81.4)	
Negative	5 (16.1)	13 (18.6)	
Micro-vessel density in stroma			0.584
High	16 (51.6)	32 (45.7)	
Low	15 (48.4)	38 (54.3)	
Diffuse fibrosis			0.931
Positive	18 (58.1)	40 (57.1)	
Negative	13 (41.9)	30 (42.9)	

Value are given as numbers (percentage).

AR: androgen receptor; HIF-1α: hypoxia-inducible factor-1alpha

Table 10. Correlation between nuclear factor-kappa B (NF-κB) in gland and various conditions of benign prostatic hyperplasia

	NF-κB expression in gland		*p*-value
	Present	Absent	
Total (n = 101)	38 (37.6)	63 (62.4)	
AR			0.582
Positive	13 (34.2)	25 (39.7)	
Negative	25 (65.8)	38 (60.3)	
HIF-1α in stroma			0.104
Positive	25 (65.8)	31 (49.2)	
Negative	13 (34.2)	32 (50.8)	
HIF-1α in gland			0.337
Positive	15 (39.5)	19 (30.2)	
Negative	23 (60.5)	44 (69.8)	
Smooth muscle actin expression			0.903
Positive	31 (81.6)	52 (82.5)	
Negative	9 (18.4)	11 (17.5)	
Micro-vessel density in stroma			0.004*
High	25 (65.8)	23 (36.5)	
Low	13 (34.2)	40 (63.5)	
Diffuse fibrosis			0.112
Positive	18 (47.4)	40 (63.5)	
Negative	20 (52.6)	23 (36.5)	

Value are given as numbers (percentage). * denote $p < 0.05$.

AR: androgen receptor; HIF-1α: hypoxia-inducible factor-1alpha

Table 11. Correlation between diffuse fibrosis and various conditions of benign prostatic hyperplasia

	Diffuse fibrosis		*p*-value
	Present	Absent	
Total (n = 101)	58 (57.4)	43 (42.6)	
AR			0.112
Positive	18 (31.0)	20 (46.5)	
Negative	40 (69.0)	23 (53.5)	
HIF-1α in stroma			0.018*
Positive	38 (65.5)	18 (41.9)	
Negative	20 (34.5)	25 (58.1)	
HIF-1α in gland			0.840
Positive	20 (34.5)	14 (32.6)	
Negative	38 (65.5)	29 (67.4)	
Smooth muscle actin expression			0.014*
Positive	43 (74.1)	40 (93.0)	
Negative	15 (25.9)	3 (7.0)	
Micro-vessel density in stroma			0.861
High	28 (48.3)	20 (46.5)	
Low	30 (51.7)	23 (53.5)	

Value are given as numbers (percentage). * denote $p < 0.05$.

AR: androgen receptor; HIF-1α: hypoxia-inducible factor-1alpha

Supplementary table 1. Antibodies used for immunohistochemical stainings

Antibody	Corparation	Clone	Dilution	Clonality
AR	Abcam	AR-V7	1:50	Monoclonal
CD34	Cell marque	ND	1:200	Monoclonal
ER-α	Abcam	aa1-300	1:50	Monoclonal
ER-ß	Abcam	aa45-9-477	1:100	Polyclonal
HiF-1α	Novus bio.	H1alpha67	1:10	Monoclonal
NF-kB	Santa Cruz	C-20	1:100	Polyclonal
PR	Ventana	1E2	Ready to use	Monoclonal
Smooth muscle actin	Novo Castra	αsm-1	1:100	Monoclonal

ND, no description.

AR: androgen receptor; ER-α: estrogen receptor-alpha; ER-ß: estrogen receptor-beta; HIF-1α: hypoxia-inducible factor-1alpha; NF-kB: nuclear factor-kappa B; PR: progesterone receptor

Supplementary table 2. Correlation between diffuse fibrosis and conditions with vulnerability to ischemic change

	Diffuse fibrosis		*p*-value
	Present	Absent	
Total (n = 101)	58 (57.4)	43 (42.6)	
Vulnerability to ischemic change			0.047*
HIF-1α in stroma (-) or NF-κB in stroma (+)	32 (55.2)	32 (74.4)	
HIF-1α in stroma (+) and NF-κB in stroma (-)	26 (44.8)	11 (25.6)	
Vulnerability to ischemic change			< 0.001*
HIF-1α in stroma (-) or NF-κB in gland (+)	32 (55.2)	39 (90.7)	
HIF-1α in stroma (+) and NF-κB in gland (-)	26 (44.8)	4 (9.3)	

Value are given as numbers (percentage). * denote $p < 0.05$.

HIF-1α: hypoxia-inducible factor-1alpha; NF-κB: nuclear factor-kappa B

Supplementary table 3. Correlation between smooth muscle actin expression and hypoxia-inducible factor-1alpha (HIF-1α) and nuclear factor-kappa B (NF-κB) expressions in benign prostatic hyperplasia

		Smooth muscle actin expression		*p*-value
		Positive	Negative	
	Total (n = 101)	83 (82.2)	18 (17.8)	
Stroma	HIF-1α			0.119
	Positive	49 (59.0)	7 (38.9)	
	Negative	34 (41.0)	11 (61.1)	
	NF-κB			0.767
	Positive	26 (31.3)	5 (27.8)	
	Negative	57 (68.7)	13 (72.2)	
Gland	HIF-1α			0.257
	Positive	30 (36.1)	4 (22.2)	
	Negative	53 (63.9)	14 (55.6)	
	NF-κB			0.903
	Positive	31 (37.3)	7 (38.9)	
	Negative	52 (62.7)	11 (61.1)	

Value are given as numbers (percentage).

Supplementary Table 4. Correlation between micro-vessel density (MVD) and vascular risk factors

	MVD		
	High	Low	*p*-value
Total (n = 101)	48 (47.5)	53 (52.5)	
Obesity			0.068
Present	21 (43.8)	14 (26.4)	
Absent	27 (56.3)	39 (73.6)	
Current smoking			0.437
Present	8 (16.7)	6 (17.0)	
Absent	40 (83.3)	44 (83.0)	
Diabetes mellitus			0.321
Present	12 (25.0)	9 (17.0)	
Absent	36 (75.0)	44 (83.0)	
Hypertension			0.539
Present	21 (43.8)	20 (37.7)	
Absent	27 (56.2)	33 (62.3)	
Dyslipidemia			0.238
Present	12 (25.0)	19 (35.8)	
Absent	36 (75.0	34 (64.2)	
Vascular risk factors			0.301
2-5	23 (47.9)	20 (37.7)	
0-1	25 (52.1)	33 (62.3)	
Vascular risk factors			0.446
3-5	9 (18.8)	7 (13.2)	
0-2	39 (83.3)	46 (86.8)	

Value are given as numbers (percentage).

Supplementary Table 5. Correlation between micro-vessel density (MVD) and factors related to benign prostatic hyperplasia

	MVD		*p*-value
	High	Low	
Total (n = 101)	48 (47.5)	53 (52.5)	
IPSS			
Total score	23.15 ± 7.07	24.74 ± 8.19	0.301
Voiding score	13.19 ± 4.96	14.02 ± 4.95	0.402
Storage score	9.94 ± 3.72	10.70 ± 4.02	0.328
QoL score	4.96 ± 1.24	4.91 ± 1.20	0.828
Uroflowmetry			
Voided volume (ml)	17.52 ± 69.33	178.45 ± 73.70	0.838
Qmax (ml/sec)	8.63 ± 3.78	7.89 ± 4.67	0.388
PVR (ml)	88.69 ± 83.35	109.09 ± 123.18	0.337
PSA (ng/ml)	7.38 ± 7.71	7.03 ± 7.41	0.819
TRUS			
Total volume (ml)	73.94 ± 34.25	82.68 ± 39.10	0.237
T-zone volume (ml)	49.13 ± 28.36	51.62 ± 30.26	0.671
Prostatic calculi			0.315
Present	11 (22.9)	8 (15.1)	
Absent	37 (77.1)	45 (84.9)	
Previous medication status			0.893
5ARI (+)	22 (45.8)	25 (47.2)	
5ARI (-) or no medication	26 (54.2)	28 (52.8)	

Value are given as mean ± standard deviation or numbers (percentage).

IPSS: International Prostatic Symptoms Score; QoL: quality of life; Qmax: maximum flow rate; PVR: postvoid residual; PSA: prostate-specific antigen; T-zone volume: transition zone volume; TRUS: transrectal ultrasonography; 5ARI: 5-alpha reductase inhibitor