THE ROLE OF NF-KB IN THE PROLIFERATION, APOPTOSIS, ANTI-APOPTOSIS AND AUTOPHAGY LEVELS OF PROSTATE HYPERPLASIA CELL LINE BPH1

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ABSTRACT

Objective*: The role of nuclear factor kappa B (NF-κB) in proliferation, apoptosis, anti-apoptosis and autophagy in BPH1 cells was explored in this paper.*

Methods: *BPH1 cells were cultured in vitro and randomly divided into the control group (normal complete medium), NF-κB inhibitor group (100 μmol/L PDTC reagent complete medium) or NF-κB activator group (100 μg/L PMA reagent complete medium). The CCK-8 method was used to detect cell proliferation and the apoptosis rate was detected using flow cytometry. The expression of NF-κB p65, apoptosis protein Bcl-2, caspase-3 and autophagy protein LC-3II were detected using the western blot method.*

Results: *While the proliferation rate of the NF-κB inhibitor group was significantly lower, the apoptosis rate of the NF-κB inhibitor group was significantly higher than both the control group and the NF-κB activator group (P<0.05). The expression of NF-κB p65 protein in the NF-κB inhibitor group was significantly lower than the control group at 8h, 12h and 24h (P<0.05). The expression of NF-κB p65 protein in the NF-κB activator group was significantly higher than the control group at 4h, 8h, 12h and 24h (P<0.05). The expression of Bcl-2 protein in the NF-κB inhibitor group was significantly lower, while the expression of the caspase-3 protein was significantly higher than the control group and the NF-κB activator group (P<0.05). The expression of LC-3 II protein in the NF-κB activator group was significantly lower than in the control group (P<0.05).*

Conclusion: *NF-κB can significantly inhibit the proliferation and apoptosis of BPH cells and inhibit the autophagy level. The results of the present study represent a new research direction for prostate proliferation related target gene therapy and drug therapy.*

Keywords: *NF-κB, prostatic hyperplasia cells, proliferation, apoptosis, anti-apoptosis, autophagy.*

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Introduction

Benign prostatic hyperplasia (BPH) is a progressive disease occurring more often in middle-aged and elderly men. An outcome of the hyperplasia of glands, fibers and muscle tissues, it is caused by disordered sex hormone metabolism found most commonly in middle-aged and elderly people; its incidence gradually increases with age[1]. Early symptoms of BPH patients are not typical. As lower urinary tract obstruction worsens, patients may experience a series of symptoms such as frequent urination, dysuria and urinary retention that seriously affects their quality of life and sleep; such symptoms are particular of middle-aged and elderly men in our country[2]. At present, there are many studies on the pathogenesis of BPH, and the balance of epithelial and mesenchymal cell proliferation and apoptosis is widely considered as a leading cause. Among related factors, age and functional testis are necessary conditions for the onset of BPH[3].

Presently, clinical treatment is based on a three-step treatment approach of symptom relief, drug treatment and surgical treatment. However, since drug therapy can only delay the progression of the disease and surgical treatment has many related

complications, clearer understanding of the cause of BPH is essential for improving clinical treatment.

Nuclear transcription factor kappa B (NF-κB) is a type of transcriptional activator that has recently been receiving extensive research attention for its role in supporting healthy immune response. Widely present in various eukaryotic cells[4], previous studies show that it interacts with the body's immune response, embryogenesis, inflammation, apoptosis, virus infection and is related to many other life-sustaining processes[5]. Interested in the potential for manipulation of NF-κB to improve clinical treatment of BPH, the present study explored the role of NF-κB in cell proliferation, apoptosis, anti-apoptosis and autophagy by culturing prostate hyperplasia cell lines in vitro.

Materials and methods

Experimental reagents and instruments

The following reagents were utilized:

• BPH-1 cells (purchased from Shanghai Guandao Bioengineering Co., Ltd.);

• RPMI1640 medium (purchased from Wuhan Chunchun Biotechnology Co., Ltd.);

• Shuangkang solution (purchased from Shanghai Ziqi Biotechnology Co., Ltd.);

• EDTA digestive solution (purchased from Beijing Kairuiji Biotechnology Co., Ltd.);

• PDTC reagent (purchased from Shanghai Xinyu Biotechnology Co., Ltd.);

• PMA reagent (purchased from Shanghai Hengfei Biotechnology Co., Ltd.);

• An ECL chemiluminescence kit (purchased from Shanghai Yuanye Biotechnology Co., Ltd.);

• A BCA protein quantitative kit (purchased from Shanghai Hengfei Biotechnology Co., Ltd.);

• NF-κB p65, Bcl-2, Caspase-3 and LC3 Rabbit anti-human polyclonal antibody (purchased from US Cell Signaling).

The following experimental instruments were utilized:

• Multifunctional enzyme marker (purchased from Meigu Molecular Instrument Shanghai Co., Ltd.);

• A table-type low speed centrifuge (purchased from Sichuan Shuke Instrument Co., Ltd.);

• A cell culture box (purchased from Shanghai Fuze Trading Co., Ltd.); a vortex oscillator (Guangzhou Haohan Instrument Co., Ltd.);

• A super low temperature refrigerator (Shanghai Tashi Trading Co., Ltd.);

• Flow cytometry (purchased from Shanghai Ranzhe Instrument Equipment Co., Ltd.);

• An electrophoresis instrument (purchased from Shanghai Fuze Trading Co., Ltd.);

• A pressure steam sterilizer (purchased from Shanghai Xinyu Biotechnology Co., Ltd.);

• A constant temperature water bath box (purchased from Shanghai Yihui Biotechnology Co., Ltd.);

• An electric constant-temperature blast-drying oven (purchased from Beijing Taize JIAYE Technology Development Co., Ltd.).

Cell culturing, transfection and grouping

BPH-1 cells were cultured in a DMEM high-glucose medium that contained 10% fetal bovine serum and 1% green streptomycin double antibody solution. The cells were cultured in a 5% CO_2 incubator at 37°C and digested with 0.25% trypsin.

Cells were then passaged as they grew to 80% - 90% of the bottom wall of the culture dish, and as cell growth covered 70% of the culture dish bottom, PBS was used to wash the culture dish twice. Cells were randomly divided into control group, NF-κB inhibitor group or NF-κB activator group, with eight dishes in each group. A normal complete medium was added to dishes in the control group, while the NF-κB inhibitor group received a 100 μmol/L PDTC reagent complete medium, and the NF-κB activator group, a100 μg/L PMA reagent complete medium.

Detection method

• The CCK-8 method was used to detect the cell proliferation of each group. First, BPH-1 cells with logarithmic growth were digested by adding 1 ml of trypsin digestion solution; single-cell suspension was then prepared by blowing evenly. Next, the concentration was adjusted to 10×104/ml, and 100 μl, with 1×104/cell per well, and was inoculated into a 96-well plate for cultivation. Around 48 hours following culturing, a 5 μl CCK-8 solution was added into each well and cultured at 37°C and 5% CO_2. Following incubation in an incubator, the absorbance value of the 96-well plate was determined using an enzyme-labeled instrument below 450 nm wavelength; the cell proliferation rate was then calculated.

• Flow cytometry was used to detect the apoptosis rate of each group. First, BPH-1 cells with logarithmic growth were digested in a 0.25% trypsin digestion solution without EDTA. The cells were

then blown off using a pipette. Next, cell suspension was placed in a sterile centrifuge tube. Following centrifugation, the supernatant was discarded and PBS was added to resuspend the cells, they were then centrifuged again. Lastly, DNA staining was added at 4°C and measured after 30 minutes of dark treatment. The apoptotic peak was determined as the subploid peak before the diploid peak, and the apoptotic rate indicated the apoptotic state of the cells.

• The western blot method measured protein expression. First, BPH-1 cells with logarithmic growth were selected and washed with a pre-cooled PBS buffer; residual PBS was discarded. Next, cells were centrifuged in a 4°C pre-cooled high-speed centrifuge. The supernatant was detected using the BCA method. The 5×SDS PAGE protein loading buffer was added with a ratio of 4:1, then denatured at 100°C for 5-10 minutes. The separation gel and concentrated glue were then prepared. Next, polyacrylamide gel electrophoresis was carried out, and then transferred to a PVDF membrane added to 5% skim milk powder with 90 minutes of closure. 1:1000 of diluted antibody was then added, followed by overnight incubation in a refrigerator at 4°C; the corresponding secondary antibody was then added, followed by incubation for two hours at room temperature before exposing the ECL chemiluminescence reagent for development. The OD value of the protein region was recovered using ImageJ launcher software.

Statistical methods

All measurement data in this study were expressed by $\bar{x}\pm s$. Independent sample t-tests were used to compare treatment group means against the control group, and $P<0.05$ indicated statistical significance. Data were analyzed using SPSS 20.0.

Results

Effects of NF-κB transfection on proliferation and apoptosis in prostate proliferating cell lines

The proliferation rate of the NF-κB inhibitor group was significantly lower than those of the control group and of the NF-κB activator group ($P<0.05$). The apoptosis rate of the NF-κB inhibitor group was significantly higher than both the control group and the NF-κB activator group ($P<0.05$).

There was no significant difference in cell proliferation rate or in apoptosis rate between the control group and the NF-κB activator group ($P>0.05$), as shown in Table 1.

Group	Sample size	Increase rate	Apoptosis rate
Control	8	3.42±1.89	0.12±0.05
NF-κB inhibitor	8	1.57±0.32*#	0.17±0.06*#
NF-κB activator	8	3.39±2.03	0.11±0.03

Table 1: Effects of NF-κB transfection on proliferation and apoptosis in prostate proliferating cell lines.
*Note: *P<0.05 compared with the control group; #P<0.05 compared with the NF-κB activator group.*

Effects of NF-κB transfection on the expression of NF-κB p65 in prostate proliferating cell lines

Western blot analysis showed that expression of NF-κB p65 protein in the NF-κB inhibitor group was significantly lower than in the control group at 8h, 12h and 24h ($P<0.05$). Furthermore, p65 protein expression in the NF-κB activator group was significantly higher than in the control group at 4h, 8h, 12h and 24h ($P<0.05$), as seen in Figure 1 and Table 2.

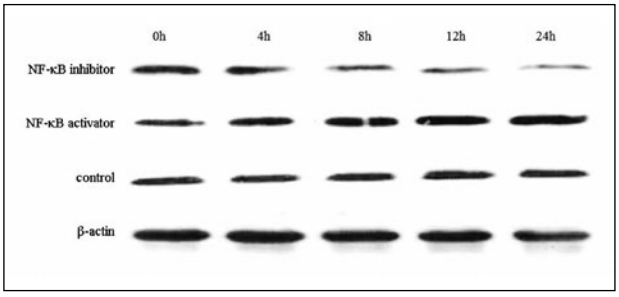

Figure 1: Western blot analysis of NF-κB p65 protein expression in each group.

Group	Sample size	0h	4h	8h	12h	24h
Control	8	0.52±0.08	0.50±0.06	0.53±0.07	0.51±0.05	0.55±0.06
NF-κB inhibitor	8	0.49±0.03	0.47±0.11	0.30±0.04*	0.25±0.01*	0.21±0.02*
NF-κB activator	8	0.50±0.06	0.63±0.10*	0.65±0.09*	0.62±0.11*	0.68±0.13*

Table 2: Comparison of NF-κB p65 protein expression in each group.
*Note: *P<0.05 compared with the control group.*

Effects of transfection of NF-κB on the expression of Bcl-2 and caspase-3

Results of the western blot method showed that expression of Bcl-2 protein in the NF-κB inhibitor group was significantly lower than in both the control group and the NF-κB activator group ($P<0.05$).

Furthermore, protein expression of caspase-3 was also significantly higher in the NF-κB inhibitor group than in the control group and in the NF-κB activator group. There was no significant difference in expression of Bcl-2 or caspase-3 in the NF-κB

activator group (P<0.05) or in the control group (P>0.05), as seen in Figure 2 and Table 3.

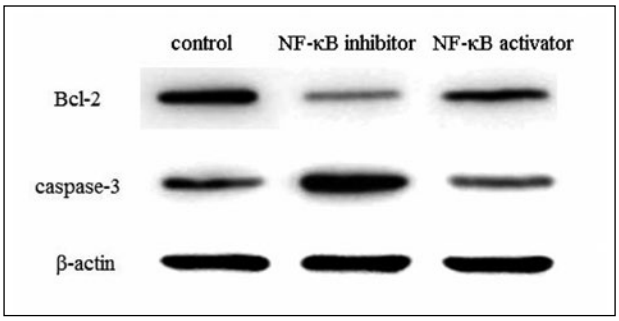

Figure 2: Western blot analysis of Bcl-2 and caspase-3 protein expression in each group.

Group	Sample size	Bcl-2	caspase-3
The control group	8	0.48±0.07	0.39±0.05
NF-κB inhibitor group	8	0.31±0.05*#	0.51±0.07*#
NF-κB activator group	8	0.50±0.08	0.40±0.09

Table 3: Comparison of the expression of apoptotic proteins Bcl-2 and caspase-3 by group.
*Note: *P<0.05 compared with the control group; #P<0.05 compared with the NF-κB activator group.*

Effects of transfection of NF-κB on autophagy protein LC-3II expression in proliferating cell lines

Western blot results showed that the expression level of LC-3II protein in the NF-κB activator group was significantly lower than the control group (P<0.05), as seen in Figure 3.

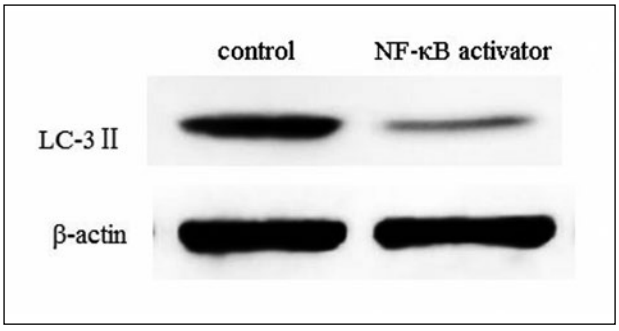

Figure 3: Western blot analysis of LC-3IIprotein expression in each group.

Discussion

Benign prostatic hyperplasia (BPH) is characterized by bladder outlet obstruction with hyperplasia of prostatic stroma and glandular components, and with increasing age, males are at high risk. The incidence rate of prostate hyperplasia in the world has become a serious health threat in the elderly[6]. In recent years, evidence suggests that simple hormone endocrine theory is insufficient to explain the pathogenesis of BPH, owing to the extensive factors involved[7]. Androgen is closely related to the occurrence of benign prostatic hyperplasia, which can promote prostate cell proliferation and inhibit prostate cell apoptosis. However, some scholars believe that the regulation of androgen in cell proliferation and apoptosis is related to some common gene regulation procedures[8]. It is clear that BPH is a disorder of cell proliferation apoptosis balance, which is prone to occur in middle-aged and elderly men, and that increased risk of cell apoptosis with age is owing to aging tissues and organs. Reducing the risk of apoptosis will therefore require strengthening the self-defense and protection mechanisms of cells[9, 10].

NF-κB, a transcription regulator, controls the transcription of many genes that can bind to the promoter nucleotide sequences of some characteristic genes in cells. A heterodimer composed of P50 and p65, it encodes acute phase proteins, cytokines, chemokine receptors and adhesion molecules by regulating genes; therefore, it plays an important role in regulating many immune and inflammatory reactions. The stimulation of bacteria, toxins and proinflammatory cytokines can cause the activation of NF-κB[11, 12]. NF-κB exists in various types of cells and has a variety of biological functions such as cell growth, differentiation and apoptosis.

An important transcription activator, it is widely studied in inflammatory diseases, cancer and anti-aging diseases. Since activation of NF KB does not require regulation of new translation proteins, when cells are damaged, NF KB can react quickly to regulate the expression of various proteins in cells to cope with the damage and maintain cells' life activities[13]. The signal pathways of apoptosis are principally endogenous apoptosis and exogenous apoptosis. Altered mitochondrial outer membrane permeability is key to activation of the endogenous apoptosis pathway, which leads to Caspase-3 activation. The Bcl-2 superfamily can control the integrity of mitochondria, and the anti-apoptotic protein Bcl-2 is capable of inhibiting apoptosis[14]. Autophagy is the mechanism for the circulation, digestion and utilization of intracellular substances, and is involved in many physiological activities such as cell growth and development. Some scholars have found that activation of NF-κB can inhibit autophagy, and as MTOR protein is a key negative regulatory site in autophagy, speculate that its mechanism may be realized by activating the mTOR

signaling pathway[15]. Results showed that the NF-κB inhibitor group could significantly inhibit cell proliferation and promote apoptosis. Furthermore, western blot analysis showed that the expression of Bcl-2 protein in the NF-κB inhibitor group was significantly lower than in the control group and the NF-κB activator group ($P<0.05$), while caspase-3 protein expression was significantly higher in the NF-κB inhibitor group than the control group and NF-κB activator group ($P<0.05$). In this study, the relationship between NF-κB and autophagy was observed by detecting the LC-3IIprotein level. The expression of LC-3IIprotein in the NF-κB activator group was significantly lower than the control group ($P<0.05$), suggesting that activation of NF-κB can significantly inhibit autophagy.

In conclusion, NF-κB can significantly inhibit the proliferation and apoptosis of BPH cells by suppressing the autophagy level to some extent. The results of the present study provide a new research direction for prostate proliferation-related target gene therapy and drug therapy.

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