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Original Research Article

Chikusetsu saponin IVA induces apoptosis and mitochondrial dysfunction of benign prostatic hyperplasia epithelial cell line (BPH-1) by inhibiting JAK/STAT3 signaling pathway

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Abstract

Purpose: *To determine the potential effect of Chikusetsu saponin IVA (CS-IVA) on benign prostatic hyperplasia (BPH), as well as the mechanism of action.*
Methods: *Benign prostatic hyperplasia epithelial cell response to treatment by CS-IVA was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), colony formation assay, and cell cycle analysis. Flow cytometry and immunoblot assay were used to assess the effect of CS-IVA on cell apoptosis. Mitochondrial damage was examined by JC-1 staining and immunoblot, while immunoblot assay was also performed to determine the effect of CS-IVA on JAK/STAT3 pathway.*
Results: *CS-IVA inhibited the growth and cycle progression of BPH-1 cell but promoted cell apoptosis ($p < 0.01$). It also exacerbated mitochondrial damage in BPH-1 cell lines ($p < 0.01$). With regard to the mechanism of action, CS-IVA inhibited JAK/STAT3 pathway in BPH-1 cells ($p < 0.01$).*
Conclusion: *CS-IVA induces apoptosis and mitochondrial dysfunction of BPH-1 cells by inhibiting JAK/STAT3 pathway, and thus it is a potential drug for the treatment of BPH. However, in vivo studies on the effect of CS-IVA are are required to validate these results*

Keywords: *Benign prostatic hyperplasia (BPH), Chikusetsu saponin IVA (CS-IVA), BPH-1, Mitochondrial dysfunction, JAK/STAT3 pathway*

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a pathological condition featured by non-malignant enlargement of prostate tissue, which is common in elderly people [1]. It is one of the most common urological diseases in older men. Benign prostatic hyperplasia is actually a pathological diagnosis, manifested as prostatic epithelial or stromal hyperplasia [2]. Patients with BPH experience weak or difficult urination, and these lower urinary tract symptoms (LUTS) seriously affect the mental health of BPH patients. Despite multiple studies, the exact mechanisms and pathogenesis of BPH remain unclear. Inhibition of apoptosis and mitochondrial

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damage in benign prostatic hyperplasia epithelial cells (BPH-1) is a potentially effective strategy to ameliorate symptoms [3,4]. The development of new and more effective treatments is therefore essential to mitigate the symptoms of BPH.

Traditional Chinese medicine (TCM) is a key complementary as well as alternative therapy for preventing and treating various diseases, including BPH. It has been widely used in China and is known to be very safe for organisms [5]. Chikusetsu saponin IVA (CS-IVA) is the bioactive ingredient isolated from *Aralia taibaiensis*, and has been shown to have anti-diabetic, anti-hyperglycemic and anti-inflammatory properties, as well as anti-cancer properties [6]. For example, CS-IVA has been shown to reduce isoproterenol-induced myocardial fibrosis and improve cardiac function in mice by activating AMPK/mTOR/ULK1 signaling and mediating the activation of autophagy [7].

CS-IVA inhibits the growth of prostate cancer cells and induces mitochondria-regulated apoptosis by mediating the production of intracellular reactive oxygen species (ROS) [6]. CS-IVA also plays a role in the treatment of rheumatoid arthritis. Studies have shown that by inhibiting JAK/STAT3 pathway, CS-IVA reduces inflammatory factor concentration in the peripheral blood of arthritis mice, reduces bone loss and relieves arthritis symptoms [8]. However, CS-IVA has rarely been reported for the treatment of BPH, and its mechanism is unclear.

The aim of this study was to investigate the inhibitory effect of CS-VIA on the growth and cycle progression of BPH-1 cell lines, and as well as its effect on apoptosis and mitochondrial.

EXPERIMENTAL

Cell culture

Benign prostatic hyperplasia epithelial cell line (BPH-1) was bought from ATCC. The cells were incubated in RPMI 1640 containing 10 % FBS, with 5 % CO_2. They were incubated with CS-IVA at 0, 6.25, 12.5, 25, and 50 μM for 48 h.

MTT assay

To determine cell viability in BPH-1, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed. BPH-1 was briefly seeded at 2 × 10^3 cells/well into a 96-well plate for 24 h. The cells were treated with MTT solution (5 mg/ml) at 37 °C for 4 h. After adding dimethyl sulfoxide (DMSO) to the cells (without medium), the absorbance was determined spectrophotometrically at 570 nm wavelength in a microplate reader.

Cell cycle analysis

Cells were treated with the indicated drugs at different concentrations for 48 h and harvested, and then 70 % cold ethanol was added and incubated at 4 °C for 12 h. Then, fixed cells were washed with PBS (Servicebio, Wuhan, China). Finally, the system was stained with PI (Servicebio, Wuhan, China) for 30 min underfdark conditions and analysed by a flow cytometer (Annoron, Beijing, China).

Colony formation assay

BPH-1 was seeded at 400 cells/well into a 6-well plate and maintained for 14 days. The cells were then fixed and stained with crystal violet. Then cell colonies were counted manually.

JC-1 staining

To assess mitochondria damage, the cells were seeded into 12-well plates and kept for 24 h for cell adhesion. After washing with PBS, the cells were stained with 2 μM JC-1 for 15 min at 37 °C in the dark. They were washed with PBS 3 times, after which the cells were examined by a microscope.

Cell apoptosis

Apoptosis was evaluated with Annexin V/PI following the manufacturer's kit protocol ((Sigma Aldrich, USA). The cells were digested into single cells and resuspended in a reaction buffer containing Annexin V (1 ug/ml) and PI (1 ug/ml) for 5 min in a dark room. Cell apoptosis was analyzed using a flow cytometer (BD Biosciences).

Immunoblot assay

Total proteins from cells were extracted with RIPA buffer (Beyotime), and protein concentration was analyzed using a BCA kit. Then, the proteins were separated using 10 % SDS-PAGE, and transferred onto PVDF membranes, then blocked with 5 % BSA. Subsequently, the membranes were incubated with antibodies targeting cyclin D1 (1:1000, Abcam, Cambridge, UK), CDK1 (1:1000, Abcam), cyclinB1 (1:1000, Abcam), $p27^{Kip1}$ (1:1000, Abcam), cleaved caspase 3 (1:1000, Abcam), cleaved caspase 9 (1:1000, Abcam), BAX (1:1000, Abcam), bcl-2 (1:1000, Abcam), mito-cyt-c (1:1000, Abcam), COX IV (1:1000,

Abcam), cyto-cyt-c (1:1000, Abcam), p-JAK2 (1:1000, Abcam), JAK2 (1:1000, Abcam), p-STAT3 (1:1000, Abcam), STAT3 (1:1000, Abcam), and beta-actin (1:10000, Abcam). Subsequently, the membranes were incubated with specific secondary antibodies for 1 h. Finally, blots were visualised by the chemiluminescence kit (Shanghai Yuanye Biotechnology Co., LTD, Shanghai, China).

Statistical analysis

GraphPad 7.0 was used for statistical analysis and data are presented as mean ± SEM. One-way ANOVA was conducted for comparison in this study. *$P < 0.05$, **$p < 0.01$ and ***$p < 0.001$, respectively, were taken as statistically significant as applicable.

RESULTS

Chikusetsu saponin IVA (CS-IVA) inhibited cell proliferation and cell cycle

Cell viability was inhibited by 25 μM and 50 μM CS-IVA (Figure 1 B). Cell number in CS-IVA group was also reduced after treatment (Figure 1 C). *Chikusetsu* saponin IVA (CS-IVA) led to cell cycle arrest, with decrease in the number of cells in S and G2 phase, and a high percentage of cells in the G1 phase (Figure 1 D). In addition, CDK1, cyclin D1 and cyclin B1 were inhibited, awhile p27Kip1 was upregulated by CS-IVA (Figure 1 E). Thus, *Chikusetsu* saponin IVA (CS-IVA) suppressed cell proliferation and cell cycle in BPH-1.

CS-IVA induced cell apoptosis in BPH-1

CS-IVA enhanced cell apoptosis in BPH-1 (Figure 2 A). It was further revealed that CS-IVA treatment increased the expression cleaved caspase 3, cleaved caspase 9, and Bax, and reduced the expression of Bcl-2 (Figure 2 B).

CS-IVA led to mitochondria damage in BPH-1 cells

There were increased JC-1 monomers and reduced JC-1 aggregates in CS-IVA-treated cells, indicating reduced membrane potential (Figure 3 A). Moreover, CS-IVA reduced the levels of mitochondrial cyto-c, and enhanced levels of cytosol cyto-C, suggesting impaired mitochondrial membrane integrity in response to CS-IVA (Figure 3 B).

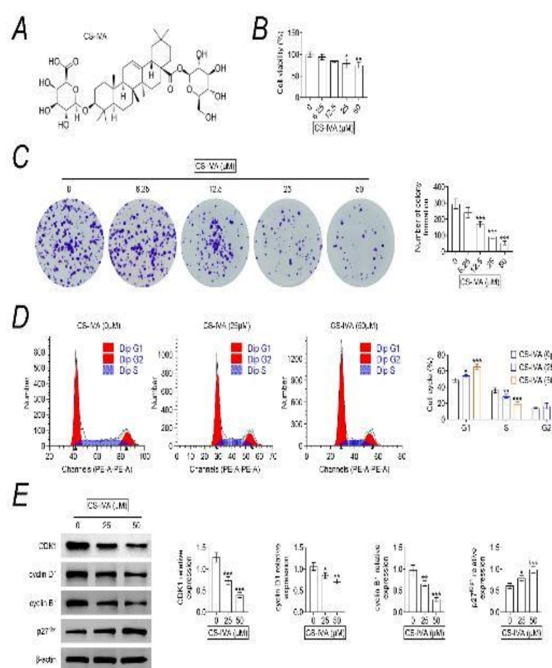

Figure 1: CS-IVA inhibited cell proliferation and cell cycle (A) Structure of CS-IVA. (B) Cell viability of BPH-1 in response to the treatment of different doses of CS-IVA. (C) Colony formation degree of BPH-1 in response to the treatment at different doses of CS-IVA. (D) Cell cycle analysis of BPH-1 in response to the treatment of different doses of CS-IVA. (E) Levels of CDK1, cyclin D1, cyclin B1 and p27Kip1 in BPH-1 in response to the treatment of different doses of CS-IVA. *$P < 0.05$, **, $p < 0.01$, ***, $p < 0.001$

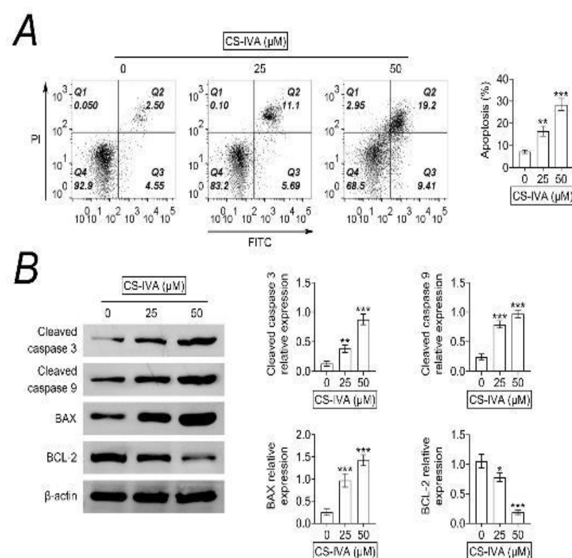

Figure 2: CS-IVA induced cell apoptosis in BPH-1 (A): BPH-1 cell apoptosis in response to CS-IVA as determined using flow cytometry. (B): Levels of cleaved caspase3, cleaved caspase 9, BAX and bcl-2. *$P < 0.05$, **, $p < 0.01$, ***$p < 0.001$

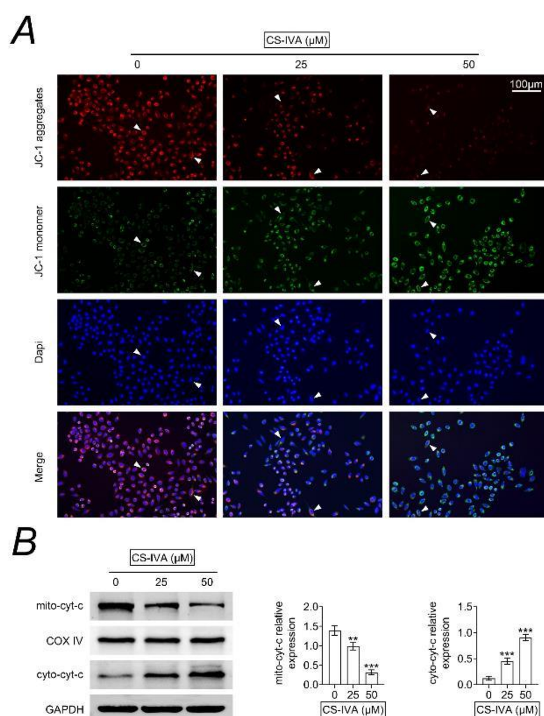

Figure 3: CS-IVA treatment led to mitochondria damage in BPH-1 cells. (A) JC-1 staining in BPH-1 in response to increasing doses of CS-IVA. (B) Levels of mitochondrial cyto-C and cytosol cyto-C in each group. One-way ANOVA was performed,$^{**}P < 0.01$, $^{***}p < 0.001$

CS-VIA increased apoptosis and mitochondrial damage by inhibiting JAK/STAT3 pathway

To reveal the mechanisms involved in the activity of CS-IVA in apoptosis and causing mitochondrial damage, JAK/STAT3 pathway was examined. Reduced levels of p-JAK2 and p-STAT3 were observed in BPH-1 treated with CS-VIA (Figure 5). CS-VIA promotes apoptosis and mitochondrial damage by inhibiting JAK/STAT3 pathway.

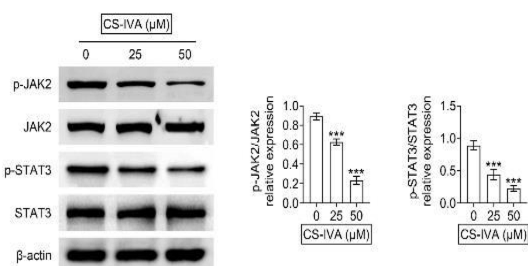

Figure 4: CS-VIA increased apoptosis and mitochondrial damage by inhibiting JAK/STAT3 pathway**.** (A) Expression levels of of p-JAK2 and p-STAT3 in CS-IVA-treated BPH-1. One-way ANOVA was performed, $^{***}P < 0.001$

DISCUSSION

The incidence of benign prostatic hyperplasia (BPH) is increasing [9,10] Other related factors include androgen and its interaction with estrogen, prostatic interstitial and glandular epithelial cells, growth factors, as well as inflammatory cells [11]. Benign prostatic hyperplasia requires the existence of functional testicles. It is generally believed that the pathological progression of BPH is related to the excessive proliferation of prostacymal and mesenchymal tissues [12]. In recent years, more and more studies have confirmed that mitochondrial function and apoptosis also play important roles in the pathogenesis of BPH. In this study, CS-IVA induced apoptosis as well as mitochondrial dysfunction in BPH-1 cells.

It was revealed that CS-IVA suppressed the growth and cell cycle progression of BPH-1 cells, and stimulated apoptosis. Therefore, CS-IVA is a promising drug for the treatment of BPH. It is knwon as a bioactive compound isolated from *Aralia taibaiensis*, and has been shown to exhibit anti-diabetic, anti-hyperglycemic and anti-inflammatory properties and anti-cancer properties. Its anti-inflammatory and osteoprotective effects of CS-IVA on rheumatoid arthritis has also been reported [13]. CS-IVA mediates cell autophagy by activating AMPK/mTOR/ULK1 pathway, consequently alleviating isoproterenol-induced myocardial fibrosis in mice as well as improving cardiac function [6]. In addition, by suppressing JAK/STAT3 pathway, CS-IVA reduced the concentration of inflammatory factors in the peripheral blood of arthritic mice, reduced bone loss and alleviated arthritis symptoms [14].

Mitochondria plays an important role in the process of prostate hyperplasia, and multiple studies have shown that intervening mitochondrial function effectively controls mitochondrial hyperplasia [15]. In the present study, CS-IVA induced mitochondrial dysfunction in BPH-1 cells, but the precise mechanism requires further study. JAK/STAT pathway serves as a critical regulator in several cellular processes and is the main signal transduction of many cytokines as well as growth factors. The activation of this pathway stimulates cell growth, differentiation, cell motility as well as apoptosis [16]. These cellular events are critical for hematopoiesis, immune system development, mammary gland development, lactation and other processes [17]. Its possible effects on mitochondrial function has been reported [18]. Several studies have also found that it plays a key role in the progression of BPH. CS-IVA

induces apoptosis as well as the mitochondrial dysfunction of BPH-1 cells via this pathway.

CONCLUSION

CS-IVA inhibits the growth and cell cycle progression of BPH-1 cell lines. In addition, it enhances the apoptosis of BPH-1 cell lines and exacerbates mitochondrial damage in BPH-1 cell lines. Furthermore, CS-IVA inhibits JAK/STAT3 pathway in BPH-1 cells, and thus is a potential drug for BPH treatment.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The authors declare that all data supporting the findings of this study are available within the paper, and any raw data can be obtained from the corresponding author upon reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jian Gao, Yuan Zhang and Lei Li designed the study and carried them out; Jian Gao supervised the data collection, analyzed and interpreted the data; and Yuan Zhang and Lei Li prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript.

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