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Original Article

Qianliening Capsule Promotes Mitochondrial Pathway Mediated the Apoptosis of Benign Prostatic Hyperplasia Epithelial-1 Cells by Regulating the miRNA-181a

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SUMMARY

Background: Our previous studies reported that Qianliening capsule (QC) has a significant therapeutic effect on BPH. Therefore, we investigated the effect QC on apoptosis of human prostatic hyperplasia epithelial-1 cells (BPH-1).

Methods: The BPH-1 cells were treated with various concentrations of QC *in vitro*. Morphology of BPH-1 cell was observed, and the cell viability was determined by the 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) assay. The levels of Cytochrome C, caspase-9 and caspase-3 were detected using the flow cytometry and colorimetric assay respectively. The Bax mRNA and the miRNA-221, -222, -15a, -16, -181a was determine by Real-time PCR analysis.

Results: The apoptosis of BPH-1 cells treated with QC increased than that of untreated cells, as evidenced by loss of plasma membrane asymmetry, the nuclear condensation and fragmentation, collapse of mitochondrial membrane potential in a dose depended manner. The levels of Cytochrome C and caspase-9, caspase-3 in the cells treated with QC increased using the flow cytometry and colorimetric assay respectively. The mRNA and protein expression of Bax and the expression of miRNA-181a in the cells treated with QC increased in a dose dependent manner.

Conclusion: QC could induce BPH-1 cells apoptosis by regulating miRNA-181a mediated mitochondrial dependent apoptosis pathway, which may be one of the important mechanisms that QC treated benign prostatic hyperplasia.

1. Introduction

Multiple factors contribute to the pathogenesis of benign prostatic hyperplasia (BPH).[1–4] Proliferation and apoptosis of prostatic cells play an important role in the processes of BHP.[5] The caspase cascading pathways are very important in regulating the apoptosis of prostatic cells.[6–9] Mitochondria mediated apoptosis pathway play an important role in the internal pathway of cell apoptosis, which involves the release of cytochrome C, the activation of caspase-3/-9 and the abnormal expression of Bcl-2 and Bax, etc. Futhermore, many miRNAs, such as miR-15a, -16, -221, -222, -181a regulate the activation of mitochondrial signaling pathway in prostatic cells.[10–14] Qianliening capsule (QC) as a traditional Chinese medicine from the People's Hospital of Fujian University of Traditional Chinese Medicine was revealed that QC could inhibit the proliferation of prostate and suppress the EGF/EGFR signaling pathway and regulate the expression of sex hormones as well as their receptors to treat the BPH in vivo,[15,17–19] meanwhile QC could promote the apoptosis of BPH-1 cells in vivo and *in vitro*.[15,16] However, the precise mechanism of QC on the apoptosis has remained to be fully elucidated. In this study, we

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investigated whether promotes mitochondrial pathway mediated the apoptosis of BPH-1 cells by regulating the miRNA-181a.

2. Materials and methods

Drugs preparation. QC (Food and Drug Administration approval no. Z20110009), consisting of 5 herbs: *Radix et Rhizoma Rhei, Hirudo, Radix Astragali, Radix Achyranthis Bidentatae* and *Semen Cuscutae* in a 5:1:4:3:2 ratio, was provided by the Academy of Pharmacology of Fujian University of Traditional Chinese Medicine (Fujian, China).[14,15] The culture medium diluted into 10 mg/ml filtered and then matched into the final concentration of 0, 1.25, 2.5, 5 mg/ml when used. All materials and methods were performed in compliance with international ethical guidelines and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, The experiments were approved by the Institutional Animal Care and Use Committee of Fujian University of Traditional Chinese Medicine (Fuzhou, China).

2.1. Cell line

The BPH-1 cell line was provided by the Institute for Molecular Biology, College of Life Sciences, Nankai University, Tianjin, China.

2.2. Culture of BPH-1 cells

The BPH-1 cells were cultured in RPMI-1640 medium containing 10% (v/v) FBS, 100 U/ml penicillin and 100 μg/ml streptomycin (HyClone, SV30010; GE Healthcare Life Sciences, Little Chalfont, UK), at 37 °C in a humidified incubator with 5% CO_2. The cells were subcultured at 80–90% confluency.

2.3. Determination of cell viability by MTT assay

BPH-1 cells seeded into 96-well plates at a density of 1×10^5 cells/ml in 100 μl medium. The cells were treated with various concentrations of QC (0, 1.25, 2.5, 5 mg/ml) for 24 h, and then incubated with 100 μl 0.5 mg/ml methyl thiazolyl tetrazolium (MTT) at 37 °C for 4 h. The medium in each well was removed, and 100 μl dimethyl sufoxide (Sigma-Aldrich) to resolve the crystals prior to incubation at room temperature for 10 min. The absorbance (A) was determined using a microplate reader (Model ELX800; BioTek, Winooski, VT, USA) at 570 nm. The survival rate was calculated as follows: Survival rate (%) = A experiment/A control × 100%.

2.4. Cell morphology observation

BPH-1 cells seeded into 6-well plates at a density of 2×10^5 cells/ml in 2 ml medium. The cells were treated with various concentrations of QC for 24 h and cell morphology was observed using a DP70 phase-contrast microscope (Olympus Corporation, Tokyo, Japan). Images were captured at a magnification of × 100.

2.5. Detection of cell apoptosis

Apoptotic cell rates were determined by flow cytometry, the cells were trypsinized (0.25% trypsin without EDTA) and a cell suspension in RPMI-1640 was prepared. Aliquots (1 ml) of the cell suspension were washed three times in PBS at 4 °C. Following the final wash, the cells were resuspended in 500 μl binding buffer (Beyotime Institute of Biotechnology, Shanghai, China). Subsequently, 5 μl Annexin V-FITC and 5 μl propidium iodide (PI) were added and the cells were incubated at room temperature for 15 min. The cells were then analyzed using a FACScalibur flow cytometer (BD Biosciences).

2.6. Detection of cytochrome C by flow cytometry

BPH-1 cells seeded into 6-well plates at a density of 1×10^6 cells/ml in 2 ml medium, and treated with various concentrations of QC for 24 h, The supernatant was removed, add 2 ml clear liquid GENMED (Reagent A), and removed Reagent A, added Trypsin EDTA mixture (GMS12024) at 37 °C incubated for 1 min, added 3 ml complete cell culture medium (GMS12052) and followed by the following operation according to the manufacturer's instructions (GENMED SCIENTIFICS INC. U.S.A). In brief, added Clear liquid, Blocking fluid, Cytochrome C antibody, Staining Solution, counter stain respectively. Finally, Cytochrome C was analyzed by flow cytometry (FACSAria).

2.7. Detection of mitochondrial membrane potential by flow cytometry

Changes in mitochondrial membrane potential (MMP) were measured using the JC-1 Mitochondrial Potential Assay Kit (Nanjing KeyGen Biotech. Inc. Jiangshu, China). BPH-1 cells were seeded into 6-well plates at a density of 2×10^5 cells/ml, After cultured for 24 h, and treated with various concentrations of QC for 24 h. The cells were

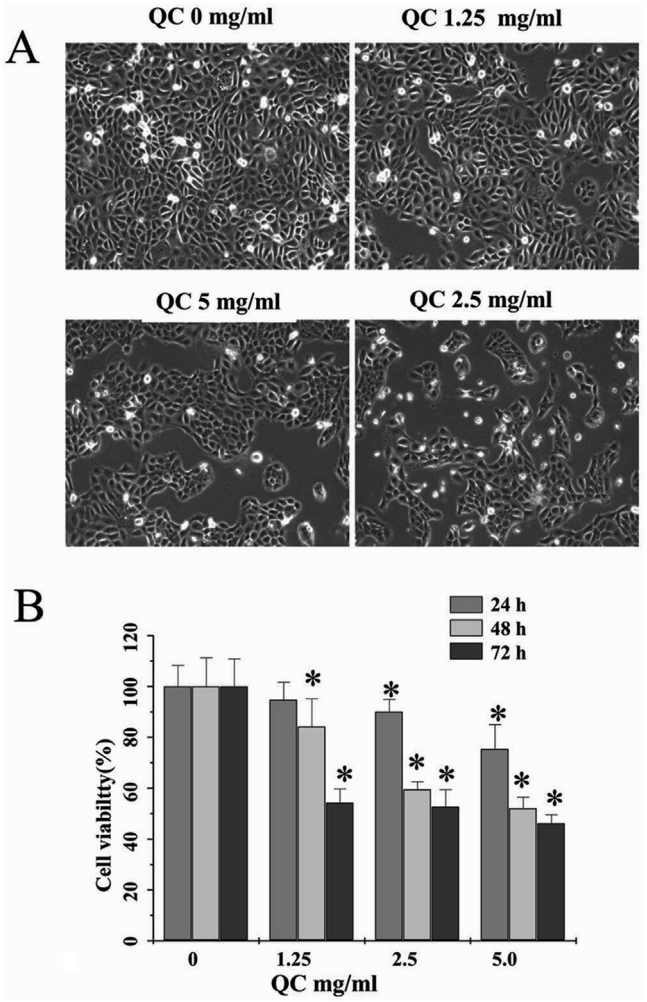

Fig. 1. Effect of QC on the morphology and viability of the BPH-1 cells. A. The cells were observed under a phase-contrast microscope. Images are representative photographs captured at a magnification of × 100; The BPH-1 cells were treated with various concentrations (0, 1.25, 2.5, 5 mg/ml). B. The cells were treated with various concentrations (0, 1.25, 2.5, 5 mg/ml) and the cell detected viability by MTT assay.

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