Steroidal Saponin *Ypsilandra thibetica* Induces Apoptosis in Colorectal Carcinoma Cells through the Mitochondrion-dependent Reactive Oxygen Species Pathway

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Abstract

The whole plant of *Ypsilandra thibetica* Franck, a perennial herb, has the official functions in heat-clearing, detoxifying and relieving congeation. YB16, one of the steroidal saponin in *Y. thibetica*, exerts anticancer activity in prostate cancer and lung cancer. However, the bioactivity and underlying mechanisms of YB16 in colorectal carcinoma (CRC) are not understood. In the present study, by MTT, apoptosis, mitochondrial membrane potential levels and intercellular reactive oxygen species (ROS) assays, YB16 inhibited the proliferation of cells and induced apoptosis in CRC HCT116 and HCT8 cells. Meanwhile, YB16 provoked mitochondrial membrane potential depolarizing in a concentration-dependent manner. Further analysis showed that YB16 induced apoptosis via stimulating the production of intercellular ROS and the antioxidant NAC reversed the apoptosis in HCT116 and HCT8 after YB16 treatment. These data demonstrated that YB16 induced the apoptosis in CRC cell lines via mitochondrial-dependent ROS pathway and YB16 might be a potential anticancer drug to CRC. © 2019 Friends Science Publishers

Key words: YB16; Apoptosis; Colorectal carcinoma; Mitochondrion; ROS

Abbreviations: CRC, colorectal carcinoma; ROS, reactive oxygen species; TCM, traditional Chinese medicine; RPMI, Roswell Park Memorial Institute; FBS, fetal bovine serum; HRP, horseradish peroxidase; AO, Acridine orange; JC-1, fluorescence tetraethyl benzimidazoly carbocyanineiodide; DCFH-DA, dichlorofluorescin diacetate; PARP, cleaved-poly (ADP-ribose) polymerase.

Introduction

Colorectal carcinoma (CRC) is one of the most occurring epithelial malignant cancers around the world (Fidler et al., 2018; Pantoja et al., 2018). At present, the main treatments of CRC are surgery, radiation, chemotherapy and traditional Chinese medicine (TCM) treatment (Fidler et al., 2018). The TCM, used for thousands of years in clinical, is the hotspot on the researches because of the fewer side effects, life quality improvement, immune function improvement and prolongation of survival (Wang et al., 2018; Wu et al., 2018).

Traditional medical treatments involving phytotherapy have played important roles for people throughout history and modern medicine owes success to these practices. *Ypsilandra* is a small genus including only six herbaceous plant species and distributed in southwestern China and Myanmar. *Ypsilandra thibetica* Franck (Family Liliaceae), a perennial herb, is especially in Sichuan and Yunnan Provinces and the whole plant has medicinal functions, including heat-clearing, detoxifying and relieving congeation, which is used as the TCM to cure hydrops, scrofula and other symptoms (Wang et al., 2018; Wu et al., 2018). The plant materials of *Ypsilandra* were collected from Emei Mountain, Sichuan Province, China. YB16, one of the steroidal saponins in *Y. thibetica* (Fig. 1A), was isolated and kindly donated by research fellow Zhang (Xia et al., 2016a). Emerging evidence indicated that the extract of *Y. thibetica* inhibits the growth of prostate cancer, lung cancer and other cancer cells by decreasing the mitochondrial membrane potential, generating intercellular reactive oxygen species (ROS) (Xia et al., 2013; Xie et al., 2013). However, the bioavailability and molecular mechanisms of YB16 in CRC have not been confirmed. In the present study, for the first time I had been studied that YB16 exerts the functions in induction apoptosis, suppressing cell proliferation, together with decreasing the mitochondrial membrane potentia and ROS generation in human CRC cells. Therefore, YB16 can be a potential anticancer drug.

Materials and Methods

Cell Cultures

In present studies, the human CRC cells HCT116 and HCT8 were kindly donated from professor Du at School of Pharmaceutical Sciences, Sun Yat-sen University.

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Guangzhou, China. Cell lines were cultured in Roswell Park Memorial Institute (RPMI)-1640 (GIBCO-BRL, USA), combined with 10% fetal bovine serum (GIBCO-BRL) in a humidified atmosphere at 37 °C with 5% CO₂.

Cellular Viability Assay

The cellular viability assay was detected by MTT (Merck Millipore EA, Germany) (Zhao et al., 2018) on the basis of the manufacturer’s instruction. The absorbances of every well were measured in a microplate at 490 nm. The IC₅₀s were calculated by SPSS and the map of cell survival was performed by GraphPadPrism.

Western Blot

In brief, the total protein in the cells was collected in RIPA lysis buffer and the equal quantity of proteins (50 μg/sample) was electrophoretically separated by SDS-PAGE. And then, it was blotted on PVDF membranes. The membranes were probed with corresponding primary antibodies (1:1000 in volume; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Members were incubated with a horseradish peroxidase (HRP)-conjugated anti-IgG (1:5000 in volume; Bioworld Technology, MN). After washed for 30 min by PBST, they were exposed to automatic exposure machine Tanon by chemiluminescence (ECL) kit to detect the target protein.

Acridine Orange (AO) Staining

After cultured in 12-well plates overnight, HCT116 and HCT8 (3×10⁵/mL) were treated with YB16 for 24 h. Cells were stained by 10 μL of 30 mg/l AO (Beyotime Biotechnology, Shanghai, China) after discarding the supernatant. The fluorescence was detected under a fluorescence microscope (NIKON Eclipse 55i, MA, USA).

JC-1 Staining for Mitochondrial Membrane Potential (MMP)

The MMP was analyzed by JC-1 assay kit (Abcam, Cambridge, UK) according to the manufacturer’s instruction. HCT116 and HCT8 cells were plated into 12-well plates for 24 h treatment, and cells were exposed to JC-1 dye (1 μM). Finally, cells were imaged by the fluorescent microscope.

ROS Assay

The intracellular ROS were measured by flow cytometry analysis of dichlorofluorescin diacetate (DCFH-DA) fluorescence. Cells were incubated for 15 min at 37°C with 5 μM DCFH-DA. Cells were then trypsinized, centrifuged and re-suspended in cold PBS and kept on ice, protected from light. The fluorescence value was measured on the FL-1 channel of flow cytometry.

Statistical Analysis

All experiments included triplicates samples. The representative results were exhibited as mean ± SD. The statistical analysis was operated by SPSS 13.0. Student’s t-test was applied to two group comparisons and the 1-way analysis of variance (ANOVA) was used on multi group comparisons. Statistical significance * was identified as p value <0.05.

Results

YB16 Inhibits the Cell Proliferation

To reveal the effect of YB16 in CRC cell lines, first time the cell proliferation was examined by MTT. HCT116 and HCT8 cells were handled with YB16 at the concentration gradients (0.234, 0.469, 0.938, 1.875, 3.750, 7.500, 15.000 and 30.000 μM, respectively) and cell viability was examined after 48 h treatment. The results showed that YB16 significantly inhibited HCT116 and HCT8 cell proliferations in the concentration-dependent way (Fig. 1B). The IC₅₀ of HCT116 to YB16 was (1.452 ± 0.012) μM and the IC₅₀ of HCT8 to YB16 was (5.471 ± 0.011) μM. Therefore, 0.5, 1, 2 μM YB16 were used to treat with HCT116 and 1, 3, 6 μM YB16 with HCT8 for the following experiments.

YB16 Induces the Cell Apoptosis

To further prove the inhibitory ability of YB16 on CRC cell lines, the apoptosis alteration in HCT116 and HCT8 cells were analyzed. After treatment of YB16, the cells were captured by microscope, the images showed that the cells shrink gradually and die according to the concentration of YB16 increases (Fig. 2A). The apoptosis markers Bax, cleaved- caspase 3, cleaved- poly (ADP-ribose) polymerase (PARP), and Bcl-2 were detected by WB. The results indicated that the cleaved-PARP, cleaved-caspase 3 and Bax expressions were upregulated as the concentration of YB16 increased (Fig. 2B). Furthermore, the AO staining showed that after YB16 treatment, the apoptosis features rose in HCT116 and HCT8 cell lines. These results declared that YB16 lead to cell apoptosis in HCT116 and HCT8 (Fig. 2C).

YB16 Decreases the Cells Mitochondrial Membrane Potential

JC-1 was used to detect the change of mitochondrial membrane potential to evaluate whether YB16-induced CRC apoptosis is related to the mitochondrial pathway. In present study, HCT116 and HCT8 were treated with YB16 for 24 h and then stained with JC-1. By comparing to the control cells, the cell lines treated with YB16 displayed significant green fluorescence (Fig. 3A). Moreover, the green fluorescence intensity ratio dramatically increased at a concentration-dependent way (Fig. 3B and C). These results showed that YB16 generated a significant decrease in mitochondrial membrane potential on HCT116 and HCT8 cells.
YB16 Induces Apoptosis in CRC

In order to resolve whether YB16 induced the generation of ROS, HCT116 and HCT8 cells were stained with DCFH-DA for flow cytometry analysis after the YB16 treatment. The ROS levels showed that YB16 induced ROS generation in a concentration-dependent manner in CRC cells (Fig. 4A). Furthermore, the generation of ROS caused by YB16 was reversed by NAC, the inhibitor of ROS. More importantly, NAC also reversed the upregulated apoptotic markers induced by YB16 (Fig. 4B). Therefore, these results demonstrated that YB16 induced the apoptosis of CRC cells through ROS generation.

Discussion

The steroidal saponins YB16 was isolated and purified from Y. thibetica possess significant anticancer activities in many pharmacological studies (Si et al., 2014; Xia et al., 2016b). However, both cellular and molecular mechanisms of YB16 bioactivities in CRC cell lines are ambiguous. The present study found that YB16 significantly inhibited the proliferation of HCT116 and HCT8 CRC cells in a dose-dependent manner. Among HCT116 and HCT8, HCT116 was more sensitive to YB16 treatment than HCT8, demonstrating that YB16 may exhibit a relatively selective antitumor effect.

YB16 Induces Apoptosis through ROS Generation

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the antioxidant NAC, the apoptosis in HCT116 and HCT8 was significantly decreased. The results illuminated that YB16-induced apoptosis in HCT116 and HCT8 cell lines were closely associated with the generation of ROS.

Conclusion

The present study suggested that YB16 induced apoptotic cell death in HCT116 and HCT8 cell lines via triggering mitochondrial-mediated ROS generation pathways. These studies provide an insight into both the functions and molecular mechanisms of YB16 caused apoptosis in CRC cells. Hence, YB16 might become a new promising compound for the treatment of CRC.

Acknowledgement

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Reference


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