

ORIGINAL ARTICLE

Solanine inhibits proliferation and promotes apoptosis of the human leukemia cells by targeting the miR-16/Bcl-2 axis

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Summary

Purpose: Leukemia causes tremendous human mortality especially in children and young adults. This study was undertaken to investigate the anticancer effects of Solanine against the normal human NCI-H526 and human leukemia AML-193 cell lines.

Methods: Cell proliferation was determined by MTT assay. DAPI and annexin V/propidium iodide (PI) assays were used for the determination of apoptosis. The expression analysis was done by qRT-PCR. Protein concentrations were determined by western blot analysis.

Results: DAPI staining showed that Solanine causes nuclear morphological changes. The annexin V/PI staining showed

that Solanine increased the leukemia apoptotic cell death dose-dependently. The expression of Bax was increased while of Bcl-2 was decreased. The qRT-PCR analysis showed that microRNA (miR)-16 was significantly ($p < 0.05$) downregulated in leukemia AML-193 cells as compared to normal NCI-H526 cells.

Conclusion: Taken together, these results showed that Solanine inhibits the proliferation of leukemia cells via induction of apoptosis and modulation of miR-16/Bcl-2 axis.

Key words: leukemia, apoptosis, microRNA, solanine, proliferation

Introduction

As per the estimates of American Cancer Society, leukemia is reported in 0.315 million people and around 0.215 people die of it annually. Although leukemia constitutes just 3% of all cancers, because of its high mortality rate and high incidence in children and persons below 40 years of age, it is considered one of the devastating malignancies [1,2]. Only few efficient chemotherapeutic agents are available for leukemia and they provide severe side effects [3]. With this background, the identification of novel chemotherapeutics may enable the proper management of leukemia. Plants are sessile organisms and to cope up with the changing environmental conditions, they synthesize a huge number of natural compounds [4]. These natural compounds are the products of primary and sec-

ondary metabolism and thus named accordingly as primary and secondary metabolites [5]. The primary metabolic pathways are highly conserved throughout the whole plant kingdom and include the generalized pathways of photosynthesis and respiration. However, the secondary metabolism is highly sophisticated and the production of a particular secondary metabolite may be limited to even only a particular plant species [6]. Nevertheless, the plant secondary metabolites are classified in some well-defined classes. Alkaloids comprise the most dominant class of plant secondary metabolites [7]. Besides their minimal role in plant growth and development, the alkaloids help the plants interact with the abiotic and biotic components of the environment [8]. Solanine is a glycoalkaloid generally

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found in the species of genus *Solanum* [9]. Solanine has been reported to exhibit anticancer effects against different types of cancers [10]. However, its anticancer activity has not been reported against the human leukemia cells. This study was therefore designed to investigate the anticancer effects of Solanine against the human leukemia cells.

Methods

Cell proliferation estimation assay

The normal NCI-H526 and leukemia AML-193 cells were seeded at 1×10^6 cells/well in 96-well plates for 12 h and then treated with different concentrations of solanine B for 24 h. A total of 20 μ l MTT solution (2.5 mg/ml) was then added to each well for 24 h. Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, St. Louis, Missouri, USA) was removed and 500 μ l dimethyl sulfoxide was added to each well to dissolve formazan crystals. Optical density was recorded using an ELISA microplate reader at 570 nm.

DAPI staining assay

AML-193 cells were cultured at a density of 2×10^5 cells/well in 6-well plates and were subsequently treated with 0, 5, 20 or 40 μ M Solanine for 24 h. Cells were then stained with DAPI for 20 min at room temperature. The cells were then fixed with 70% methanol at -20°C overnight, and observed using fluorescence microscopy. A similar procedure was followed for Annexin V-fluorescein isothiocyanate (FITC)/PI (Sigma-Aldrich, USA) staining; cells were stained with annexin V/PI and investigated using a flow cytometer, (BD Biosciences, San Jose, CA, USA).

RT-PCR based expression analysis

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). This was followed by DNase I treatment and cDNA synthesis using High-Capacity cDNA Archive™ Kit following the manufacturer's protocol. With human GAPDH gene as internal control of gene expression, RT-PCR-based expression analysis was performed using the SYBR Green method. The amplifica-

tion conditions were 10 min initial denaturation at 95°C followed by 40 cycles of 30s denaturation at 95°C , 30s of primer annealing at 58°C and 20s of extension at 72°C .

Transfection of cancer cells

RiboBio (Guangzhou, China) was used for synthesis of miR-NC and miR-16 mimics. pCDNA3.1 was used for overexpression of Bcl-2 and RNAi-based method was used for JAM-2 gene silencing. Transfection of Capan-2 cancer cells was performed using Lipofectamine 2000 reagent (Invitrogen) following the manufacturer's protocol. When 80% of cell confluence was attained post transfection, the cells were washed phosphate buffered saline (PBS), followed by treatment with 0.25% trypsin to obtain a homogeneous cell suspension.

Western blotting

Following treatment with various concentrations of Solanine, cells were harvested and lysed in radioimmunoprecipitation lysis buffer (20 mM HEPES, 350 mM NaCl, 20% glycerol, 1% Nonidet P 40, 1 mM MgCl_2 , 0.5 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 1 mM, phenylmethane sulfonyl fluoride, protease inhibitor cocktail and phosphatase inhibitor cocktail). The protein concentration was determined by bicinchoninic acid (BCA) assay. A total of 20 μ g protein/lane was separated on 10% SDS-PAGE gel. Proteins were then transferred to nitrocellulose membranes, blocked with 5% bovine serum albumin (BSA) for 45 min at room temperature and probed with the following primary antibodies overnight at 4°C . Proteins were then incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (sc-2357-CM) for 1 h overnight at 4°C . WEST-SAVE Up™ luminal-based enhanced chemiluminescent reagent was then used to visualize bands (ABFrontier, Co., Ltd., Seoul, Korea).

Statistics

The mean and standard deviation (SD) values were calculated from the data obtained from the experimental setups and the final representation was made as mean \pm SD. Graphpad Prism 7 software was used to perform *t*-test. P value ≤ 0.05 was taken as an indicator of statistically significant difference.

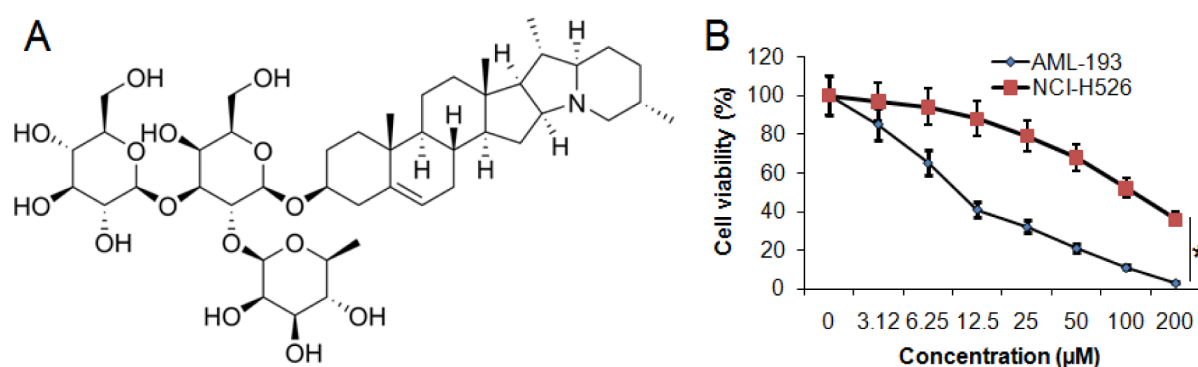


Figure 1. A: Structure of Solanine. **B:** MTT assay showing the effects of Solanine on the viability of normal NCI-H526 and leukemia AML-193 cells. The experiments were performed in triplicate and expressed as mean \pm SD (* $p < 0.05$).

Results

Leukemia cell growth was inhibited by Solanine

To determine the antiproliferative ability of Solanine (Figure 1A) against the human leukemia cells, the normal cells (NCI-H526) and leukemia cells (AML-196) were treated with 0 to 200 μM concentrations of Solanine. The proliferation curve plotted from the absorbance values using MTT assay, showed a greater decline in cell growth of leukemic cells upon increasing Solanine doses but the effects were not much severe on the normal lung cells (Figure 1B). The IC_{50} concentration of Solanine was 10 μM against the AML-196 leukemia cells, as compared to the IC_{50} of 125 μM against the normal NCI-H526 cells.

Solanine treatment induced apoptosis in leukemia cells

To deduce the effects of Solanine in promoting apoptotic cell death in leukemia cells, the AML-196 cells treated with 0, 5, 10 or 20 μM of the molecule were processed for DAPI staining. The nuclear morphology was analyzed through fluorescent microscopy. The DAPI-stained cells showed significant decrease in nuclear viability and the effects were more significant at higher treatment concentrations (Figure 2). Annexin V/PI staining showed that Solanine increased the apoptosis in a concentration-dependent manner (Figure 3).

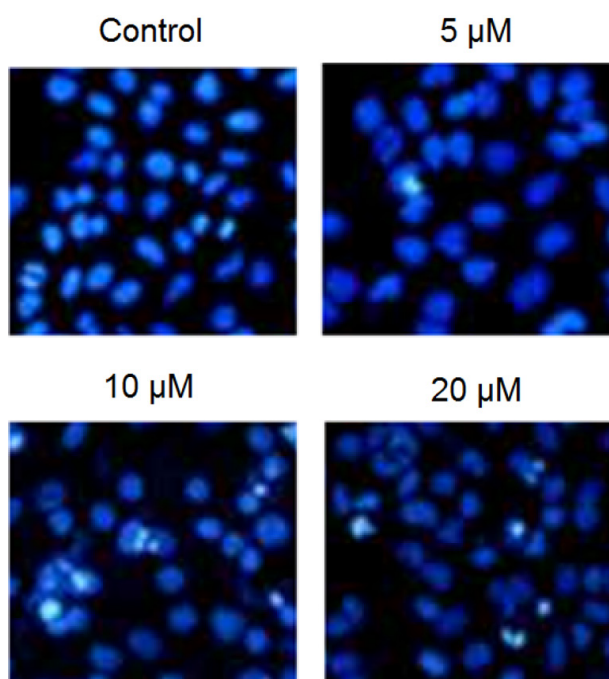


Figure 2. DAPI staining showing the effects of different concentrations of Solanine on the nuclear morphology of AML-193 cells. The experiments were performed in triplicate.

Solanine increased the Bax/Bcl-2 ratio

To further confirm the role of Solanine in inducing apoptosis, western blotting study was performed.

Solanine increased the Bax protein expression and decreased the Bcl-2 concentration-dependently (Figure 4). Together, the results indicate the positive role of Solanine in inducing the apoptosis in leukemia cells.

Solanine exerts its effects by targeting miR-16/Bcl-2

The expression of miR-16 was significantly downregulated in human leukemia AML-196 cells (Figure 5A). The TargetScan analysis showed that miR-16 targets Bcl-2 (Figure 5B). Interestingly, Solanine caused upregulation of miR-16 expression (Figure 5C) as well as downregulation of Bcl-2 (Figure 5D).

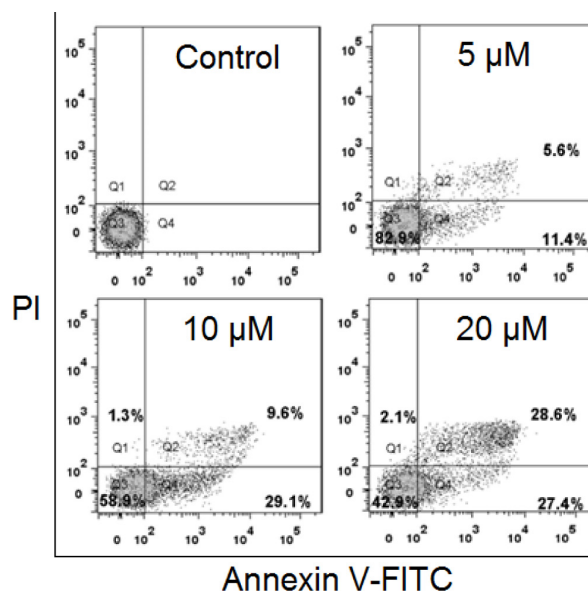


Figure 3. Annexin V/PI staining showing the effects of different concentrations of Solanine on the percentage of apoptotic AML-193 cells. The experiments were performed in triplicate.

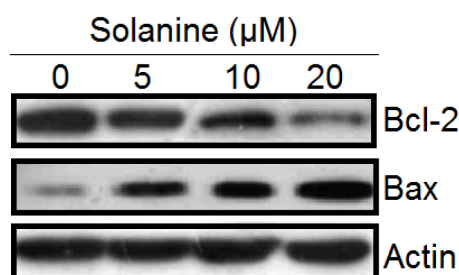


Figure 4. Western blotting showing the effects of different concentrations of Solanine on the expression of Bax and Bcl-2 in AML-193 cells. The experiments were performed in triplicate.

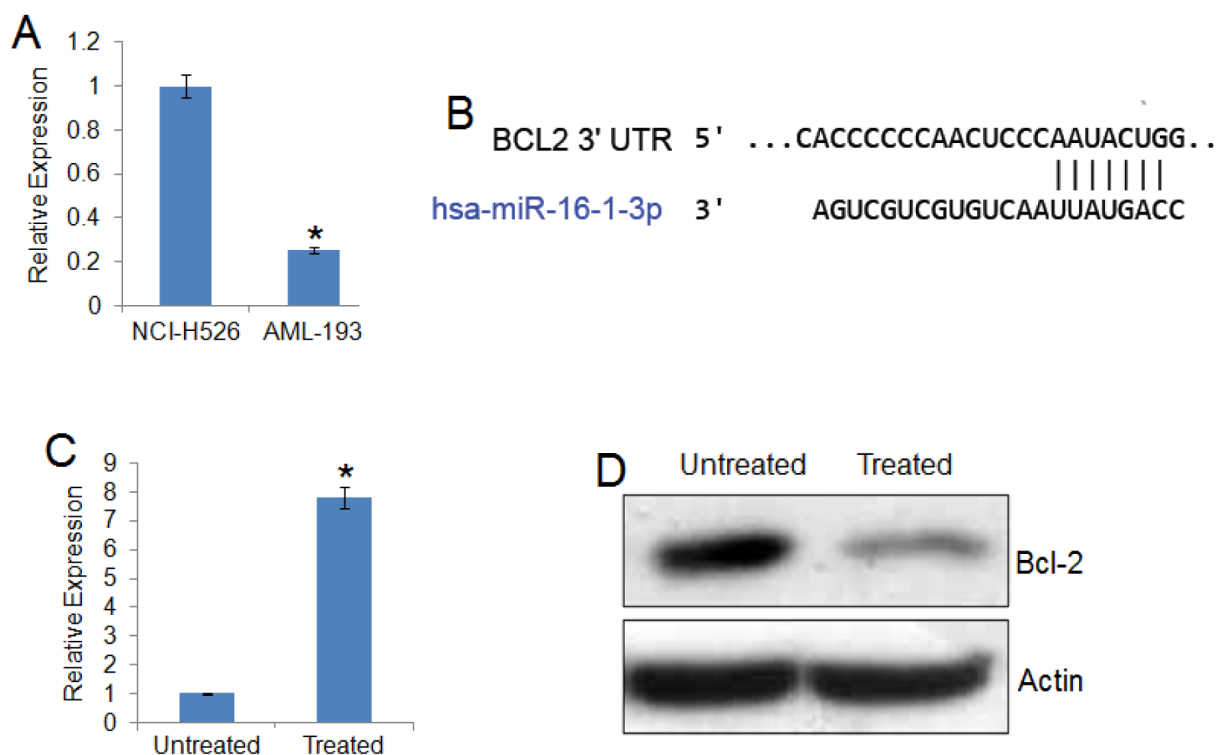


Figure 5. A: Expression of miR-16 in NCI-H526 and AML-193 cells. **B:** TargetScan analysis showing miR-16 targets Bcl-2. **C:** Expression of miR-16 in untreated and Solanine-treated AML-193 cells. **D:** Expression of Bcl-2 in untreated and Solanine-treated AML-193 cells. The experiments were performed in triplicate and expressed as mean \pm SD (* p < 0.05).

Discussion

Natural compounds have shown great potential to combat human diseases. Among natural products, alkaloids have shown great capability to halt the growth of cancer cells [11]. For example, vinca alkaloids have been reported to significantly suppress the proliferation of cancer cells [12]. Solanine is also an important alkaloid. Sun et al reported that Solanine exerts growth inhibitory effects via activation of mitochondrial apoptosis [13]. Solanine has also been shown to suppress the growth of human lung adenocarcinoma cells [14]. Solanine was reported to target microRNAs to suppress the leukemia growth. Hasanain et al also reported the activation of ROS-mediated autophagy [15]. Wang et al have also reported that Solanine regulates the radiosensitivity of esophageal cancer cells [16]. Meng et al showed that Solanine suppressed the growth of human hepatocellular carcinoma [17]. In this study, we examined the anticancer effects of Solanine against the human leukemia cells and found that this molecule inhibits the growth of cancer cells by activating the apoptotic cell death. Annexin V/PI staining showed that Solanine increased the apoptotic cell death in a dose-dependent manner. The Bax and Bcl-2 are

the main marker proteins of apoptosis [18]. Herein we observed that Solanine caused a significant increase in the Bax and decrease in the Bcl-2 expression, further confirming the induction of apoptosis. This molecule has also been shown to target microRNAs suppressing leukemia cells. Herein, we also found that Solanine upregulated the expression of miR-16. The TargetScan analysis showed that miR-16 targets Bcl-2. Additionally, Solanine caused significant suppression of Bcl-2 inducing apoptosis. Taken together, Solanine may prove beneficial in the treatment of human leukemia.

Conclusion

The results of the present study indicate that Solanine suppresses the growth of human leukemia cells via induction of apoptosis. The apoptosis induction is due to the capacity of Solanine to cause upregulation of miR-16 which in turn causes reduction of Bcl-2. Therefore, this molecule may prove beneficial in the treatment of human leukemia.

Conflict of interests

The authors declare no conflict of interests.

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