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RESEARCH ARTICLE

Induction of apoptosis by total flavonoids from *Scutellaria barbata* D. Don in human hepatocarcinoma MHCC97-H cells via the mitochondrial pathway

Jie Gao · Wang-Feng Lu · Zhi-Jun Dai · Shuai Lin · Yang Zhao · Sha Li · Nuan-Nuan Zhao · Xi-Jing Wang · Hua-Feng Kang · Xiao-Bin Ma · Wang-Gang Zhang

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Abstract *Scutellaria barbata* D. Don, a traditional Chinese medicine, reportedly possesses antitumor activity against a variety of tumors. In the present study, we investigated the cytotoxic effect of total flavonoids from *S. barbata* (TF-SB) on human hepatocarcinoma cells and the underlying molecular mechanisms regarding the effect were explored. TF-SB treatment significantly reduced the cell viability of human HCC MHCC97-H cells in a dose-dependent manner. Further flow cytometric analysis showed that the apoptosis rate of MHCC97-H cells increased and the mitochondrial membrane potential (Δψm) of MHCC97-H cells decreased after TF-SB treatment. DNA ladder showed that TF-SB induced a significant increase in DNA fragmentation in MHCC97-H cells. Reverse transcription PCR and Western blot analysis revealed that the expression levels of Smac, Apaf-1, Cytochrome c, Caspase-9, and Caspase-3 were upregulated in a dose-dependent manner and after treatment with different concentrations of TF-SB for 48 h. These results suggest that TF-SB induces apoptosis in MHCC97-H cells through the mitochondrial pathway.

Keywords *Scutellaria barbata* · Hepatocarcinoma · Appptosis · Mitochondrial

J. Gao and W.-F. Lu contributed equally to this work.

J. Gao · W.-F. Lu · Z.-J. Dai (✉) · S. Lin · Y. Zhao · X.-J. Wang · H.-F. Kang · X.-B. Ma
Department of Oncology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, China
e-mail: dzj0911@126.com

J. Gao
Department of Nephrology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, China

W.-F. Lu
Department of Surgical Oncology, Shangluo Central Hospital, Shangluo 726000, China

S. Li · N.-N. Zhao
Department of Pharmacology, The Second Affiliated Hospital of Xi'an Jiaotong University, 710004 Xi'an, China

W.-G. Zhang (✉)
Department of Hematology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, China
e-mail: zwgang2013@126.com

Abbreviations

HCC	Hepatocellular carcinoma
TF-SB	Total flavones of *Scutellaria barbatae*
Δψm	Mitochondrial membrane potential
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
DMEM	Dulbecco's modified Eagle's medium
Smac	Second mitochondria-derived activator of caspase
Apaf-1	Apoptotic protease activating factor
RT-PCR	Reverse transcription polymerase chain reaction
HPLC	High-performance liquid chromatography
PI	Propidium iodide
FCM	Flow cytometry

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies with an estimated 695,900 cancer-related deaths worldwide [1]. Currently, multidisciplinary treatment significantly improves efficacy among the patients with advanced HCC. However, recurrence and metastasis are still the leading causes of patient death [2]. Multiple therapeutic approaches, including surgical resection, liver transplantation, transarterial chemoembolization, and chemotherapy, are of limited efficacy in Chinese patients with HCC [3]. Therefore, researchers have focused on the potential development of natural compounds for anticancer therapy [4]. Some extracts from Chinese herbs such as vinblastine, camptothecin, and β-elemene, have been widely used in treating a variety of human cancers [5–7].

Scutellaria barbata D. Don is one of the traditional herbs and is widely distributed in southern China [8]. *S. barbata* extract (SBE) has in vitro growth inhibitory effects on a variety of tumors, such as liver cancer, colorectal cancer, lung cancer, and breast cancer [9–12]. However, its antitumor mechanism is still unknown. The antitumor activity of herbs is closely related to apoptotic activation [13]. Apoptosis is a major pathway for maintaining the steady-state cells [14]. Recent studies have shown that apoptosis presents through a death receptor pathway, a mitochondria pathway, and an endoplasmic reticulum stress-mediated pathway [15–17], in which mitochondria play a key role in apoptosis [18]. The mitochondrial pathway initiates apoptosis through the regulation of many proteins. These regulatory proteins usually change mitochondrial membrane permeability and decrease the mitochondrial membrane potential ($\Delta\psi m$), thereby in turn inducing the release of cytochrome C and mitochondrion-dependent caspase activation as a result to apoptosis triggering [19, 20].

To date, several flavonoids, alkaloids, polysaccharides, and steroids from *S. barbata* have been characterized [21]. In this study, we focused on the mechanism underlying the anticancer effects of total flavonoids of *S. barbata* (TF-SB) on HCC cells in vitro, with the emphasis on alterations in $\Delta\psi m$ and apoptosis induction. We found that TF-SB induces the apoptosis of MHCC97-H cells through the mitochondrial dysfunction pathway.

Materials and methods

Cell line, cell culture, and reagents

HCC cell line MHCC97-H was obtained from the Shanghai Institute of Cell Biology Cells maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % *v*/*v* fetal bovine serum (FBS), and 100 μg/mL penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5 % CO_2.

DMEM and FBS were purchased from Gibco, Life Technologies (Carlsbad, CA, USA). Trypsin-EDTA, penicillin, streptomycin, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma-Aldrich (St. Louis, MO, USA). The dual-emission potential-sensitive probe 5,5′,6,6′-tetra-chloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide (JC-1) was from Beyotime Institute of Biotechnology (Shanghai, China). Reverse transcription polymerase chain reaction (RT-PCR) kit (catalog no. sc-8319) was from Ampliqon A/S (Odense, Denmark), Trizol was from Invitrogen, USA, and TIANamp Genomic DNA kit was from Beijing, China. Antibodies against second mitochondria-derived activator of caspase (Smac), apoptotic protease activating factor (Apaf-1), cytochrome c, caspase-9, caspase-3, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Peroxidase-conjugated antibodies against mouse IgG or rabbit IgG were purchased from Abgent (San Diego, CA, USA).

Preparation of TF-SB from *Scutellaria barbata* D. Don

Dried plant materials of *S. barbata* were purchased from Yi Shan Tang Chinese Herbal medicine store (Xi'an, China) and authenticated according to the descriptions found in the Chinese Pharmacopoeia. The original herb was identified as *S. barbata* D. Don (SB) by Run-Xia Liu at the Medical School of Xi'an Jiaotong University (Xi'an, China). The voucher samples, ZLK-ZY-05 (*S. barbata*), were deposited at the Department of Oncology, the Second Affiliated Hospital of Xi'an Jiaotong University.

The material was coarsely ground before extraction. A total of 300 g of the material was extracted twice with 95 % ethanol for 3 h in 50 °C. The infusion was filtered through a 1-mm pore-size filter. The leftover was collected after the combined extracts evaporated. The pH of *S. barbata* sample extract was adjusted to 2.0, and then the crude extract was chromatographed on an AB-8 macroporous adsorption resin column. After being eluted with 3 multiple column volume of distilled water and 12 multiple column volume of 70 % ethanol, the flavonoids purity increased with a recovery of 69 % [21]. The total flavonoids were stored at 4 °C for use.

High-performance liquid chromatography analysis

The components of TF-SB were identified using high-performance liquid chromatography (HPLC). An accurately weighed 100 mg TF-SB was dissolved in 50 mL methanol for HPLC analysis. For chromatographic analysis, Agilent Zorbax C18 column (5 μm, 250× 4.6 mm) with a guard column (C18, 5 μm, 4.0× 3.0 mm) was used. HPLC separation was performed using a linear gradient with a flow rate of 1.0 mL/min, while column was kept in an insulated compartment at 30 °C. The mobile phase consisted of water containing 1.0 % acetic acid (A) and acetonitrile (B) using a gradient of 28–80 % B for 0–60 min. The injection volume was 10 μL. The UV detection wave length was set at 280 nm.

Assessment of cell viability

Cells were seeded in a 96-well plate at a density of 1×10^4 cells/well and cultured with serum-free DMEM for 12 h, and then treated with different concentrations of TF-SB (0, 30, 60, 120, and 240 μg/mL) for 24, 48, and 72 h at 37 °C and 5 % CO_2. After treatment, the medium was aspirated, and 20 μL of MTT (Sigma) was added to each well and incubated for 4 h, and then, 150 μL of DMSO (Sigma) was added to each well. After shaking the plate for 10 min, the optical density (OD) was measured at 490 nm using an enzyme-labeling instrument (EX-800 type, Bio-Tek, Winooski, VT, USA). The cell viability was determined according to the following formula: Cell viability (%)= OD of sample×100/OD of control.

Measurement of apoptosis

Detection of apoptotic cells were performed using double staining of annexin V-FITC and propidium iodide (PI) (BD Biosciences, USA). MHCC97-H cells (1×10^6 cells/mL) were plated and treated with different concentrations of TF-SB in for 48 h. The cells were harvested and washed twice with 4 °C phosphate-buffered saline (PBS). The samples were resuspended with 500 μL of 1× binding buffer in the labeled tube; 5 μL of annexin V-FITC and 10 μL PI were added into the labeled tube and stained with annexin V and PI for 20 min at room temperature in the dark. The samples were analyzed using flow cytometry (FCM) analysis (BD Biosciences Clontech, USA). This experiment was repeated for three times.

DNA ladder assay

MHCC97-H cells were collected after 24 h of treatment with TF-SB (30, 60, and 90 μg/mL). DNA ladder extraction was performed using a TIANamp Genomic DNA kit (Beijing, China). The cells were lysed for 2 h in a solution containing 10 mM Tris–HCl (pH 7.8), 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM NaCl, 1 % sodium dodecyl sulfate (SDS), and 1 mg/mL proteinase K at 56 °C, and treated with 10 mg/mL RNase A for an additional 60 min at 37 °C. The lysate was extracted twice with phenol–chloroform (1:1 *v*/*v*) and precipitated overnight with ethanol at −20 °C. The precipitate was washed twice in 70 % ethanol and finally dissolved in 100 μL of TE (1 mM EDTA, 10 mM Tris–HCl, pH 8.0). The resulting DNA (10 μL) was loaded onto 2 % agarose gel and electrophoresed, and then the DNA fragments were then photographed. This assay was done triplicate.

Table 1 Primer sequences and annealing temperature of Smac, Apaf-1, cytochrome c, caspase-9, caspase-3, and β-actin in RT-PCR

Gene	Gene sequences		Size (bp)	Annealing temperature (°C)
Smac	Forward primer	5′-CTGTGACGATTG GCTTTG-3′	293	54
	Reverse primer	5′-CTCATTCCTGGC GGTTAT-3′		
Apaf-1	Forward primer	5′-TTGCTGCCCTTC TCCATGAT-3′	285	59
	Reverse primer	5′-TCCCAACTGAAA CCCAATGC-3′		
Cytochrome c	Forward primer	5′-GAGCGGGAGTGT TCGTTGT-3′	327	59
	Reverse primer	5′-GTCTGCCCTTTC TTCCTTCT-3′		
Caspase-3	Forward primer	5′-CATCCAGTCGCT TTCTGCC-3′	623	60
	Reverse primer	5′-TGCCCACAGATG CCTAAGTTC-3′		
Caspase-9	Forward primer	5′-CGAACTAACACG CAAGCA-3′	142	56
	Reverse primer	5′-CAAATCCTCCAG AACCAAT-3′		
β-actin	Forward primer	5′-CGGGACCTGACT GACTACCTC-3′	549	61
	Reverse primer	5′-GCACTCGTGATA CTCCTGCTTG-3′		

The primer pairs and the predicted sizes of the amplified PCR products and the annealing temperatures of PCR are listed. β-Actin is the housekeeping gene for RT-PCR analysis. The primer sequences were designed with Primer Premier 5.0 software

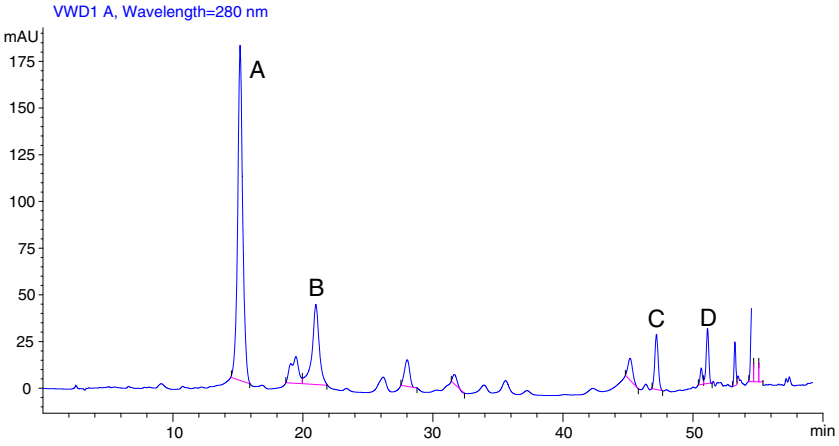

Fig. 1 HPLC analysis of TF-SB. There was a main peak in HPLC, which was identified as scutellarin (*A*). There were also some other flavonoids in TF-SB, which were identified as apigenin (*B*), baicalein (*C*), and luteolin (*D*) (wavelength=280 nm)

Measurement of mitochondrial membrane potential (Δψm)

According to the instructions of JC-1 Mitochondrial Apoptosis Detection Kit, JC-1 accumulated within intact mitochondria to form red-fluorescent J-aggregates at higher membrane potentials and green-fluorescent monomers at low membrane potentials [22]. The MHCC97-H cells were harvested with and without 48 h of TF-SB treatment. The cells were adjusted to a concentration of 1×10^5 cells/mL and incubated with 500 μL (2 mg/L) JC-1 working solution for 20 min at 37 °C. Then, the supernatant was discarded and washed twice with 1× JC-1 buffer. The samples were resuspended with 500 μL of 1× JC-1 buffer and were analyzed afterwards using FCM.

Reverse transcription polymerase chain reaction

MHCC97-H cells were seeded in six-well plate and treated with concentration TF-SB (0, 30, 60, and 90 μg/mL) separately for 48 h. Total cellular RNA was extracted using the Trizol reagent following the manufacturer's instructions. Polymerase chain reaction was carried out using specific sense and antisense PCR primers for amplification. The primer sequences were designed with Primer Premier 5.0 software (Premier Biosoft, Palo Alto, CA, USA). The primer sequences and annealing temperature of the genes are shown in Table 1. PCR product was detected on 2 % agarose gel electrophoresis and ethidium bromide staining, and was used the gray analysis by imaging system. The expression intensity of destination gene messenger RNA (mRNA) was denoted with the ratio of the photodensity of the RT-PCR products of destination gene and β-actin.

Western blot analysis

After treated with different concentrations (0, 30, 60, and 90 μg/mL) of TF-SB for 48 h, the cells were rinsed and lyzed following extract the total protein (BCA protein assay, Pierce, USA). The proteins were transferred by a SDS-polyacrylamide gel and blocked with 5 % nonfat milk in PBS for 2 h. The blotted membranes were incubated with Smac, Apaf-1, cytochrome c, caspase-9, caspase-3, and β-actin antibody (Santa Cruz, CA, USA) with an overnight incubation at 4 °C. The blots were washed three times, and then incubated with secondary antibodies conjugated with horseradish peroxidase (1:1,000; Santa

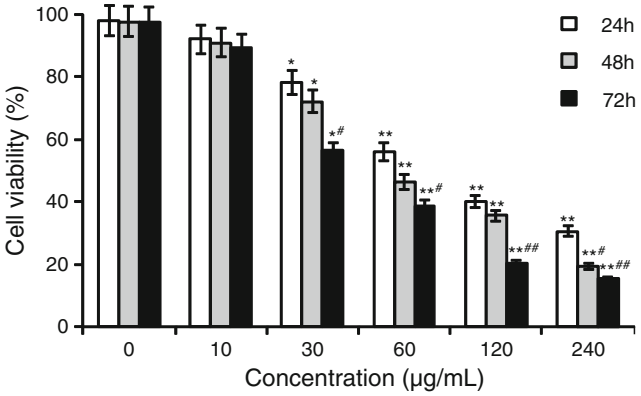

Fig. 2 TF-SB efficiently attenuated the viability of MHCC97-H cells. Cells were treated with the different concentrations of TF-SB (10, 30, 60, 120, and 240 μg/mL) of TF-SB for 24, 48, and 72 h. Cell viability was determined by MTT assay. This assay was triplicated for mean ± SEM. Dose and time dependence of cell growth could be elucidated by ANOVA analysis. *$P<0.05$, **$P<0.01$ compared with 0 μg/mL TF-SB; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with 24 h

Cruz Biotechnology). After washed three times by PBS, the immunoblotting signals were developed by ECL chemiluminescence. Photographs were taken, and optical densities of the bands were scanned and quantified with the Gel Doc 2000 (Bio-Rad).

Statistical analysis

The data are presented as the means ± SEM of at least three independent experiments and analyzed via one-way ANOVA and Student's *t* test using the SPSS 14.0 software.

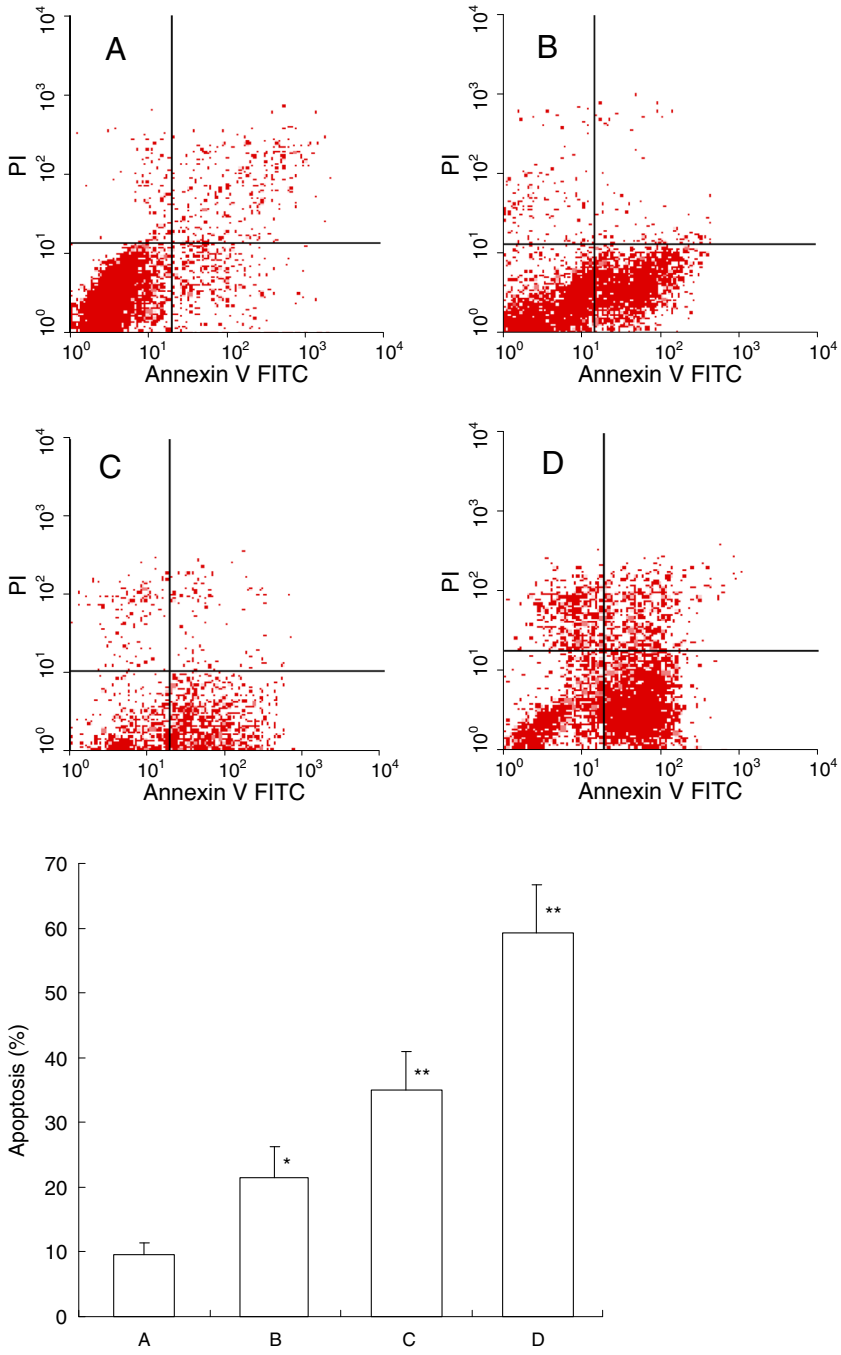

Fig. 3 TF-SB treatment induces dose-dependent apoptosis in MHCC97-H Cells. Cells treated with various concentrations of TF-SB were double-stained with annexin V and PI and analyzed by FCM. The data are represented as mean ± SEM from three independent experiments. A control group, B 30 μg/mL TF-SB group, C 60 μg/mL TF-SB group, D 90 μg/mL TF-SB group.*$P<0.05$, **$P<0.01$ compared with the control group

Differences with $P<0.05$ were considered statistically significant.

Results

Identification of TF-SB by HPLC

The components of TF-SB were identified using HPLC. As shown in Fig. 1, the main peak was identified as scutellarin (A). Other identified flavonoids in TF-SB included apigenin (B), baicalein (C), and luteolin (D). The contents of flavonoids A–D were 67.2, 8.7, 4.6, and 4.3 %, respectively.

TF-SB inhibited the cell viability of MHCC97-H cells

The cells were treated with different TF-SB concentrations (10 to 240 μg/mL) as time indicated in the figure. MTT assay was used to examine the antiproliferative effect of TF-SB. The effects of TF-SB on cell growth over 72 h are shown in Fig. 2. The MTT assay showed that TF-SB significantly decreased cell viability in a dose- and time-dependent manner in MHCC97-H cells.

Assessment of apoptosis of MHCC97-H cells using FCM

After treatment with different doses of TF-SB for 48 h, apoptosis induction was observed. According to flow cytometric analysis, apoptotic cells were separated from viable or necrotic ones by combined annexin V-FITC and PI staining. As shown in Fig. 3, in TF-SB groups, the rate of apoptotic cells was gradually increased accompanied with increasing concentrations of TF-SB. By contrast, viable apoptotic cells were rarely detected in the control group. The rate of apoptosis in the control and (30, 60, and 90 μg/mL) TF-SB groups were (9.53±2.26)%, (21.48±3.74)%, (34.92±2.17)%, and (59.22±4.68)%, respectively. The results revealed that apoptotic cells gradually increased in a dose-dependent manner.

TF-SB induced DNA degradation in MHCC97-H cells

DNA was extracted from MHCC97-H cells and subjected to DNA ladder analysis after 48 h of TF-SB treatment. As shown in Fig. 4, the results suggested that TF-SB significantly increased fragmentized DNA sequencein a dose-dependent manner.

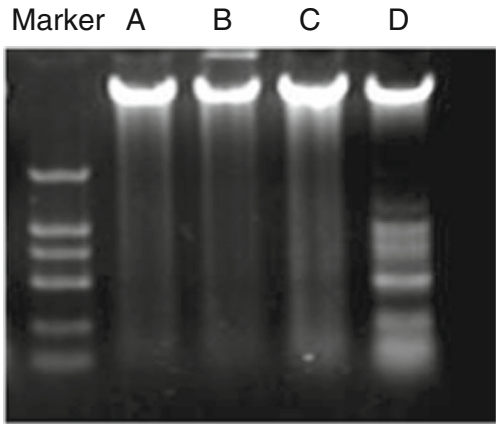

Fig. 4 DNA ladder diagram after treatment with different concentrations of TF-SB for 48 h. *A* blank control group, *B* 30 μg/mL TF-SB group, *C* 60 μg/mL TF-SB group, *D* 90 μg/mL TF-SB group

TF-SB produced a great effect on Δψm

Mitochondria play an essential role in apoptosis. JC-1 is an indicator commonly used for detecting the Δψm, and it is very sensitive to monitor the membrane potential [22]. In the present study, Δψm was detected using FCM via JC-1 staining. As shown in Fig. 5, JC-1 staining showed that Δψm decreased with increasing TF-SB concentration. After TF-SB treatment for 48 h, the percentage of depolarized cells (lower quadrants) significantly increased from 18.52 %±2.03 % to 27.31 %±1.25 %($P<0.05$) as compared with the corresponding control (6.84 %±2.27 %).

Detection of the mRNA expression of Smac, Apaf-1, caspase-9, and survivin using an RT-PCR assay

Smac plays a crucial role in the mitochondrial apoptosis pathway, and it promotes chemotherapy-induced apoptosis [23]. Apaf1 is the main component of the apoptosome and is a crucial factor in the mitochondria-dependent death pathway [24].

To confirm that TF-SB induces apoptosis via the mitochondrial pathway, the expression levels of Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3 in MHCC97-H cells were determined via RT-PCR assays. As shown in Fig. 5, treatment with 30, 60, and 90 μg/mL TF-SB significantly increased the mRNAs of Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3 in MHCC97-H cells as compared with the control group ($P<0.05$). Following TF-SB treatment for 48 h, Smac, Apaf-1, cytochrome c, caspase-

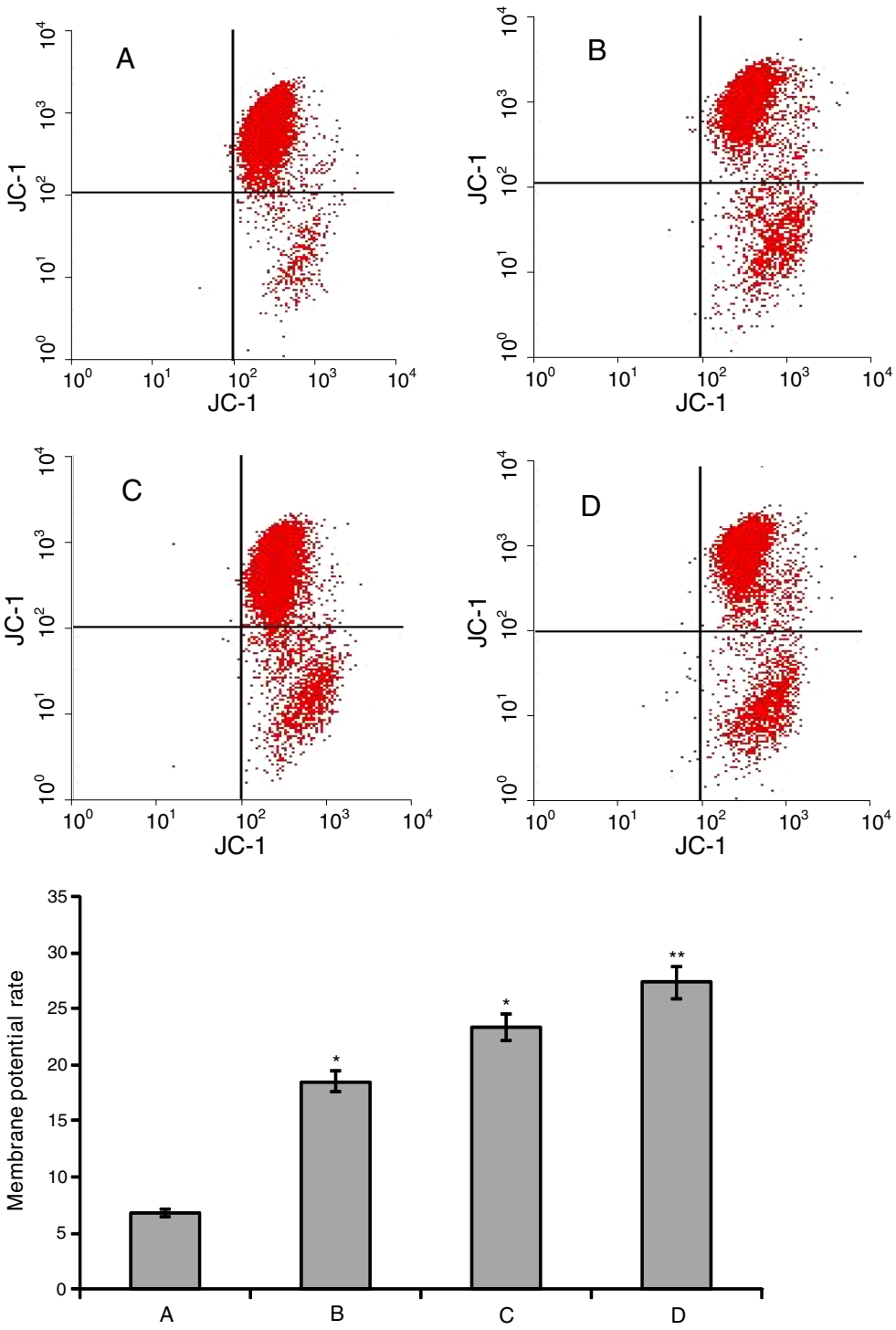

Fig. 5 The Δψm in MHCC97-H cells were odserved by FCM after treatment with TF-SB.After treated with different concentrations of TF-SB for 48 h, the Δψm were observed by FCM with JC-1 probe staining. A control group, B 30 μg/mL TF-SB group, C 60 μg/mL TF-SB group, D 90 μg/mL TF-SB group. Data are presented as the means ± SEM from three independent experiments. *P<0.05, **P<0.01 compared with the control group

9, and caspase-3 expression were upregulated in MHCC97-H cells. Furthermore, mRNAs of Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3 in the 90 μg/mL TF-SB group were increased by approximate 2.7-, 2.3-, 3.1-, 7.8-, and 5.6-fold. As shown in Fig. 6, it is showed that TF-SB markedly upregulated Smac, Apaf-1,

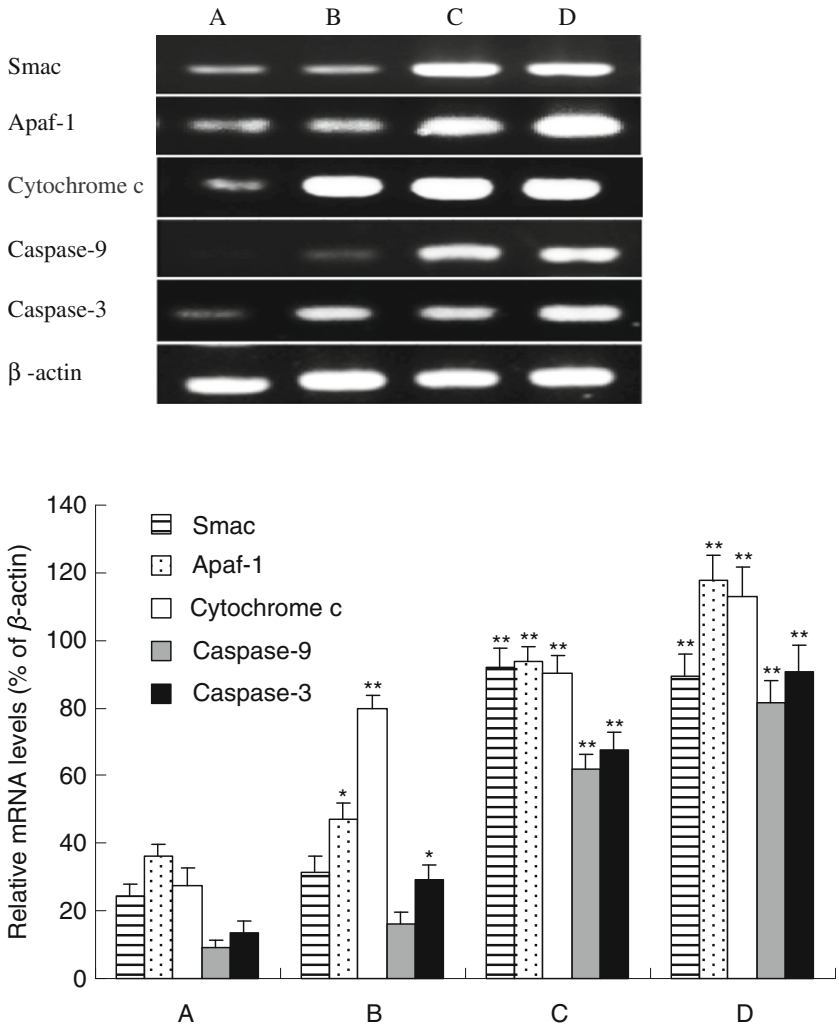

Fig. 6 The mRNA expression of Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3 in MHCC97-H cells treated with different concentrations of TF-SB. After treatment with TF-SB for 48 h, mRNA levels were detected by RT-PCR analysis. This assay was done in quintuplicate. *A* control group, *B* 30 μg/mL TF-SB group, *C* 60 μg/mL TF-SB group, *D* 90 μg/mL TF-SB group. Values represent means±SEM and were determined using the Student's *t* test. **P*<0.05, ***P*<0.01 compared with the control group

cytochrome c, caspase-9, and caspase-3 mRNA in a dose-dependent manner ($P<0.05$).

Protein expression of Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3 detected using Western blot analysis

Based on the results of the RT-PCR, we further analyzed the effects of TF-SB on protein expression in MHCC97H cells. Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3 protein were blotted against the corresponding antibodies as mentioned above in the MHCC97H cells after 48 h of treatment with TF-SB (0, 30, 60, and 90 μg/mL). As shown in Fig. 7, consistent with mRNA analysis, Western blot results showed that Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3 increased after TF-SB treatment. Furthermore, these effects were dose dependent ($P<0.05$).

Discussion

Mitochondria are the center of the cellular metabolism and productivity, as well as an adjustable fulcrum of growth and death in cells. Recent studies have shown that the mitochondria play an essential role in cell apoptosis initiation. Mitochondrial proteins directly activate cellular apoptotic programs, which triggers a series of changes before cell death called the cell death cascade that eventually leads to apoptosis [25–27]. However, the mitochondrial pathway of apoptosis is very complex and is affected by a variety of protein regulatory

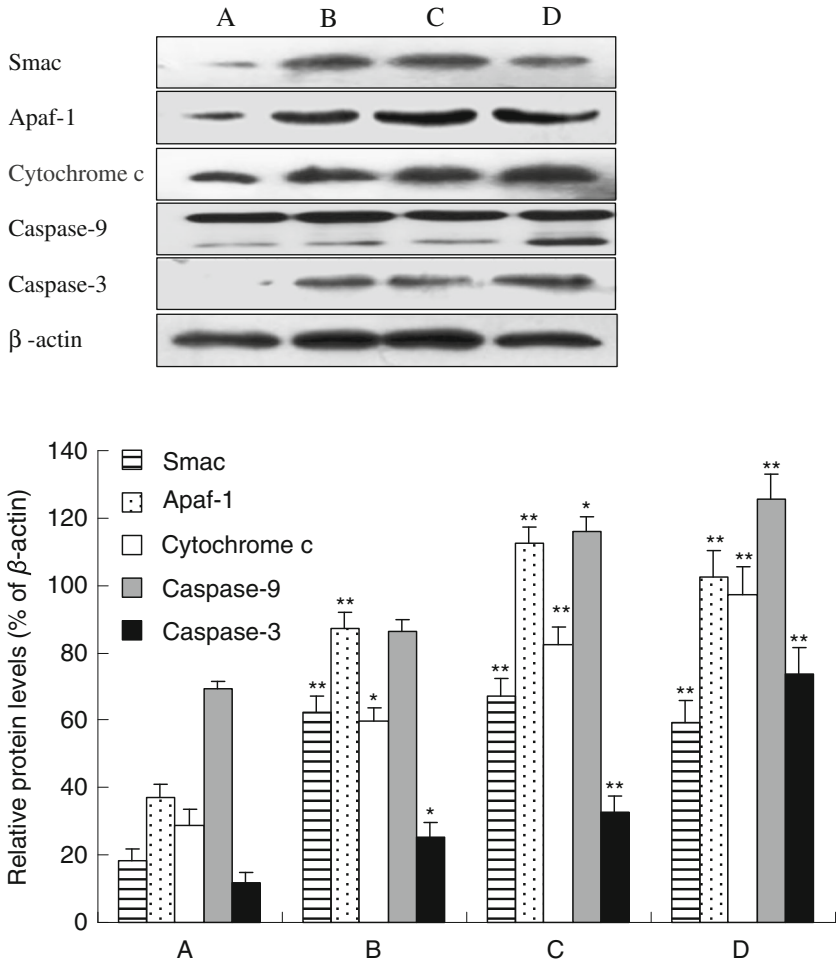

Fig. 7 The expression of Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3 in MHCC97-H cells treated with concentration span of TF-SB. After treatment with TF-SB for 48 h, protein levels were detected by Western blot. This assay was done as triplicated. *A* control group, *B* 30 μg/mL TF-SB group, *C* 60 μg/mL TF-SB group, *D* 90 μg/mL TF-SB group. Values represented means±SEM and were determined using the Student's *t* test. **P* <0.05, ***P* <0.01 compared with the control group

factors, including pro- and antiapoptotic factors. The imbalance in the apoptotic signal is closely related to the initiation of the apoptotic program [28, 29].

The signaling pathways activated by these stressors culminate in permeabilization of mitochondrial outer membrane and release of soluble proteins from the mitochondrial intermembrane space (IMS). Some soluble proteins including cytochrome c, which complexes with Apaf-1 and caspase-9 to form the apoptosom, released from the IMS have been able to trigger death by apoptosis. This results in activation of caspase-9, which orchestrates apoptosis by cleaving a subset of cellular substrates. Smac is a proapoptotic mitochondrial protein that is released from the mitochondria after apoptotic stimuli. It is combined with apoptosis inhibitor proteins BIR2 and BIR3, which activate caspase and induce apoptosis [30, 31]. The release of Smac into the cytosol from the mitochondrial inner membrane space is a key event in caspase-9 activation, which subsequently initiates a caspase cascade involving caspase-3 [32]. These two proteins are involved in regulating the mitochondrial pathway of apoptosis. Studies suggested that the release of cytochrome c is the motivator for the mitochondriainitiated apoptosis. A considerable number of cytochrome c are released from the mitochondrion to the cytosol, which form the Apaf-1/cytochrome c complex with Apaf-1. The complex activates caspase-9, which catalyzes the formation of the terminal effector caspase-3 and subsequently leads to apoptosis [33, 34].

Previous studies have shown that the antitumor activity of CE-SB is associated to cell apoptosis [35]. In the HPLC analysis, TF-SB was mainly composed of scutellarin, apigenin, baicalein, and luteolin. Scutellarin could inhibit the growth of tongue cancer cells in vitro and regulate cell adhesion [36]. Apigenin could dose and time dependently repress the proliferation and clonogenic survival of the human breast cancer T47D and MDA-MB-231 cell lines [37]. Furthermore, apigenin has apoptosis- and autophagy-

inducing effects in cancer cells [37]. Zhu et al. [38] reported that apigenin could induce apoptosis and inhibit migration and invasion ability in T24 bladder cancer cells. Apigenin leads to apoptosis via PI3K/Akt pathway, regulation of Bcl-2 family, and activation of caspase-3 and PARP. Additionally, apigenin also causes G_2/M phase arrest [38]. Takahashi et al. [39] reported that baicalein induced apoptosis through a caspase-dependent pathway in pancreatic cancer cells. The proapoptotic effect of baicalein is mediated through reducing the expression of the prosurvival protein Mcl-1 [39]. Moreover, baicalein could inhibit tumor cell invasion and metastasis via the suppression of the ERK pathway [40].

In our study, TF-SB significantly inhibited the proliferation of MHCC97-H cells in a dose- and time-dependent manner, as indicated by MTT assay. This is consistent with our previous findings, wherein CE-SB inhibited the proliferation of hepatoma H22 cells [41]. Then, we detected the apoptosis rate by the double-staining of annexin V-FITC and PI in MHCC97-H cells. After treatment with different TF-SB concentrations, the apoptotic rate increased with increasing TF-SB concentration. The degradation of DNA fragments gradually increased with increasing TF-SB concentration. Furthermore, DNA ladder analyses clearly revealed a typical ladder tape in the 90 μg/mL TF-SB group after 48 h. Thus, TF-SB induced apoptosis of MHCC97-H cells with DNA degradation. The loss of Δψm and cytochrome C release triggers apoptosis. After treatment with different TF-SB concentrations, the loss of Δψm increased in a dose-dependent manner. Therefore, TF-SB induces changes in Δψm.

To further investigate the molecular mechanism of TF-SB-induced apoptosis, we observed the mRNA and protein expression of Smac, Apaf-1, caspase-9, survivin via semiquantitative RT-PCR, and Western blot analysis. The results show that the expression of Smac, Apaf-1, cytochrome c, caspase-9 and caspase-3 were upregulated. These results suggest that TF-SB induces apoptosis in MHCC97-H cells. The mechanism may be related to the upregulation of Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3.

Conclusion

In conclusion, TF-SB induces apoptosis in HCC cell line MHCC97-H in vitro. Δψm decreases simultaneously with increasing TF-SB concentration. This effect may be associated with the expressions of Smac, Apaf-1, cytochrome c, caspase-9, and caspase-3, which are greatly dependent on the mitochondrial pathway of apoptosis.

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Conflicts of interest None

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