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Acacetin inhibits the proliferation of Hep G2 by blocking cell cycle progression and inducing apoptosis

Ya-Ling Hsu, Po-Lin Kuo, Chun-Ching Lin*

Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, ROC

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Abstract

Flavonoids are a broadly distributed class of plant pigments, universally present in vascular plants and responsible for much of the coloring in nature. They are strong antioxidants that occur naturally in foods and can inhibit carcinogenesis in rodents. In this study, we examined acacetin (5,7-dihydroxy-4′-methoxyflavone), a flavonoid compound, for its effect on proliferation in a human liver cancer cell line, Hep G2. The results showed that acacetin inhibited the proliferation of Hep G2 by inducing apoptosis and blocking cell cycle progression in the G1 phase. Enzyme-linked immunosorbent assay showed that acacetin significantly increased the expression of p53 and p21/WAF1 protein, contributing to cell cycle arrest. An enhancement in Fas/APO-1 and its two form ligands, membrane-bound Fas ligand and soluble Fas ligand, as well as Bax protein, was responsible for the apoptotic effect induced by acacetin. Taken together, our study suggests that the induction of p53 and activity of the Fas/Fas ligand apoptotic system may participate in the antiproliferative activity of acacetin in Hep G2 cells.

Keywords: Acacetin; p53; Cell cycle; Fas/APO-1; FasL; Apoptosis

1. Introduction

Hepatocellular carcinoma (HCC), one of the most common cancers in the world, develops from transformed hepatocytes during the course of chronic liver disease. It is responsible for approximately 1 million deaths annually, mainly in underdeveloped and developing countries [1,2]. Apoptosis has been characterized as a fundamental cellular activity to maintain the physiological balance of the organism. It is also involved in immune defense machinery [3] and plays a necessary role as a protective mechanism against carcinogenesis by eliminating damaged cells or abnormal excess cells proliferated owing to various chemical agents' induction [3,4]. Emerging evidence has demonstrated that the anticancer activities of certain chemotherapeutic agents are involved in the induction of apoptosis, which is regarded as the preferred way to manage cancer [3,4].

Flavonoids are a broadly distributed class of plant pigments, universally present in vascular plants and responsible for much of the coloring in nature [5]. They are strong antioxidants that occur naturally in foods and can inhibit carcinogenesis in rodents [5,6]. Acacetin (5,7-dihydroxy-4′-methoxyflavone), a flavonoid compound, has been reported to possess antiperoxidative, anti-inflammatory, and antiplasmodial effects [7–9], and to enhance differentiation-inducing activity in HL-60 cells [10]. In addition, acacetin can also inhibit glutathione reductase, cytochrome P450, and topoisomerase I-catalyzed DNA religation [11–13]. In this study, we determined the antiproliferative activity of acacetin, and examined its effect on cell cycle distribution and apoptosis in the human liver cancer cell line, Hep G2. Furthermore, to establish the anticancer mechanism of acacetin, we assayed the levels of p53, p21/WAF1, Fas/APO-1 receptor, Fas ligand (FasL), and Bax, which are strongly associated with the signal transduction pathway of apoptosis and affect the chemosensitivity of tumor cells to anticancer agents.

* Corresponding author. Tel.: +886-7-3121101x2122; fax: +886-7-3135215.
E-mail address: aalin@ms24.hinet.net (C.-C. Lin).

Abbreviations: FasL, Fas ligand; mFasL, membrane-bound Fas ligand; sFasL, soluble Fas ligand; DMEM, Dulbecco's modified Eagle's medium; Z-IETD-FMK, *N*-benzyloxycarbonyl-Ile-Glu-Thr-Asp-fluoromethylketone; PI, propidium iodide; ELISA, enzyme-linked immunosorbent assay.

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2. Materials and methods

2.1. Materials

Fetal calf serum (FCS), penicillin G, streptomycin, amphotericin B, and Dulbecco's modified Eagle's medium (DMEM) were obtained from GIBCO BRL. Acacetin, DMSO, ribonuclease (RNase), and propidium iodide (PI) were purchased from Sigma Chemical. Sodium 3′-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene-sulfoic acid hydrate (XTT) and p53 pan enzyme-linked immunosorbent assay (ELISA) kits were obtained from Roche Diagnostics GmbH. Nucleosome ELISA, WAF1 ELISA, FasL, Fas/APO-1 ELISA, and caspase-8 assay kits, and caspase-8 inhibitor *N*-benzyloxycarbonyl-Ile-Glu-Thr-Asp-fluoromethylketone (Z-IETD-FMK) were purchased from Calbiochem.

2.2. Preparation of acacetin

Acacetin was dissolved in DMSO and stored at −20°. For all experiments, final concentrations of the tested compound were prepared by diluting the stock with DMEM. Control cultures received the carrier solvent (0.1% DMSO).

2.3. Cell line and culture

Hep G2 (American Type Culture Collection [ATCC] HB8065) was maintained in monolayer culture at 37° and 5% CO_2 in DMEM supplemented with 10% FCS, 10 U/mL penicillin, 10 μg/mL streptomycin, and 0.25 μg/mL amphotericin B.

2.4. Cell proliferation assay

Inhibition of cell proliferation by acacetin was measured by XTT assay. Briefly, cells were plated in 96-well culture plates (1×10^4 cells/well). After 24-hr incubation, the cells were treated with acacetin (0, 1, 5, 10, and 20 μg/mL) for 12, 24, 48, and 72 hr. Fifty microliters of XTT test solution, which was prepared by mixing 5 mL of XTT-labeling reagent with 100 μL of electron coupling reagent, was then added to each well. After 6-hr incubation, the absorbance was measured on an ELISA reader (Multiskan EX, Labsystems) at a test wavelength of 492 nm and a reference wavelength of 690 nm.

2.5. Cell cycle analysis

To determine cell cycle distribution analysis, 5×10^5 cells were plated in 60-mm dishes and treated with acacetin (0, 10, and 20 μg/mL) for 24 hr. After treatment, the cells were collected by trypsinization, fixed in 70% ethanol, washed in PBS, resuspended in 1 mL of PBS containing 1 mg/mL RNase and 50 μg/mL PI, incubated in the dark for 30 min at room temperature, and analyzed by EPICS flow cytometer. The data were analyzed using the Multicycle software (Phoenix Flow Systems).

2.6. Measurement of apoptosis by ELISA

The induction of apoptosis by acacetin was assayed using the Nucleosome ELISA kit. This kit uses a photometric enzyme immunoassay that quantitatively determines the formation of cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes) after apoptotic cell death. Hep G2 cells were treated with 0, 10, and 20 μg/mL acacetin, for 6, 12, 24, and 48 hr. The samples of cell lysate were placed in 96-well microtiter plates (1×10^6 per well). The induction of apoptosis was evaluated by assessing the enrichment of nucleosome in cytoplasm, and determined exactly as described in the manufacturer's protocol [14].

2.7. Assaying the levels of p53, p21, Fas/APO-1, and Fas ligands (mFasL and sFasL)

p53 pan ELISA, WAF1 ELISA, Fas/APO-1 ELISA, and FasL ELISA kits were used to detect p53, p21, Fas/APO-1 receptor, and FasL. Briefly, Hep G2 cells were treated with 0, 10, and 20 μg/mL acacetin, for 6, 12, 24, and 48 hr. The samples of cell lysate were placed in 96-well microtiter plates (1×10^6 per well) that were coated with monoclonal detective antibodies, and were incubated for 1 hr (Fas/APO-1), 2 hr (p53 or p21/WAF1), or 3 hr (FasL) at room temperature. It was necessary to determine the soluble FasL in cell culture supernatant by using FasL ELISA kit. After removing unbound material by washing with washing buffer (50 mM Tris, 200 mM NaCl, and 0.2% Tween 20), horseradish peroxidase-conjugated streptavidin was added to bind to the antibodies. Horseradish peroxidase catalyzed the conversion of a chromogenic substrate (tetramethylbenzidine) to a colored solution with color intensity proportional to the amount of protein present in the sample. The absorbance of each well was measured at 450 nm and concentrations of p53, p21/WAF1, Fas/APO-1, and FasL were determined by interpolating from standard curves obtained with known concentrations of standard proteins [15,16].

2.8. Assay for caspase-8 activity

The assay is based on the ability of the active enzyme to cleave the chromophore from the enzyme substrate, Ac-IETD-pNA. The cell lysates were incubated with peptide substrate in assay buffer (100 mM NaCl, 50 mM HEPES, 10 mM dithiothreitol, 1 mM EDTA, 10% glycerol, 0.1% CHAPS, pH 7.4) for 3 hr at 37°. The release of *p*-nitroaniline was monitored at 405 nm. Results are represented as the percent change of the activity compared to the untreated control [17].

2.9. Assay for Western blotting

Cells treated with 20 μg/mL acacetin for 24 and 48 hr were lysed and the protein concentration was determined by using a Bio-Rad Protein Assay (Bio-Rad Laboratories). For Western blotting, 50 μg of total cell lysates were subjected to SDS–PAGE. The protein was transferred to PVDF membranes using transfer buffer (50 mM Tris, 190 mM glycine, and 10% methanol) at 100 V for 2 hr. The membranes were incubated with blocking buffer (50 mM Tris, 200 mM NaCl, 0.2% Tween 20, and 3% BSA) overnight at 4°. After washing three times with washing buffer (blocking buffer without 3% BSA) for 10 min each, the blot was incubated with Bax antibody for 2–15 hr, followed by horseradish peroxidase-labeled secondary antibody for 1 hr. The membranes were washed again, and detection was performed using the enhanced chemiluminescence Western blotting detection system (Amersham).

2.10. Statistical analysis

Data were expressed as means ± standard errors. The difference between control and acacetin-treated cells was evaluated using Student's *t* test. *P* value less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of acacetin on Hep G2 cell proliferation

We first tested the antiproliferative effect of acacetin in the liver cancer cell line, Hep G2. As shown in Fig. 1, the growth inhibitory effect of acacetin was observed in a dose- and time-dependent manner. At 48 hr, the maximal effect on proliferation inhibition was observed with 20 μg/mL acacetin, which inhibited proliferation in 71.89% of Hep G2 cells. The IC_{50} value was 10.44 ± 0.08 μg/mL. The maximal proliferation effect of 76.96% was reached by 20 μg/mL acacetin at 72 hr.

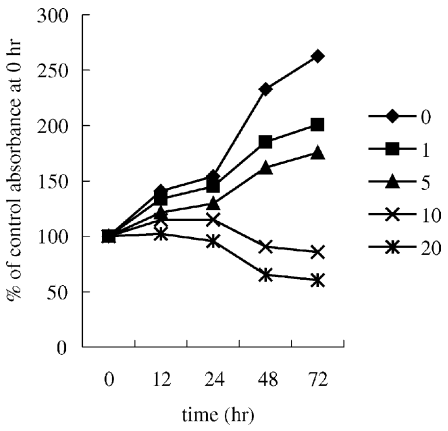

Fig. 1. Growth inhibition of Hep G2 cells by acacetin. Adherent cells that proliferated in 96-well plates (10^4 cells/well) were incubated with different concentrations (μg/mL) of acacetin for various time intervals. Cell proliferation was determined by XTT assay. Results are expressed as percent of cell proliferation of control at 0 hr. The data shown are the mean from three independent experiments, each with triplicate wells. Standard deviations are less than 10%.

3.2. Acacetin-induced cell cycle arrest and apoptosis in Hep G2 cells

The effect of acacetin on cell cycle progression of Hep G2 was determined by flow cytometry. As shown in Fig. 2,

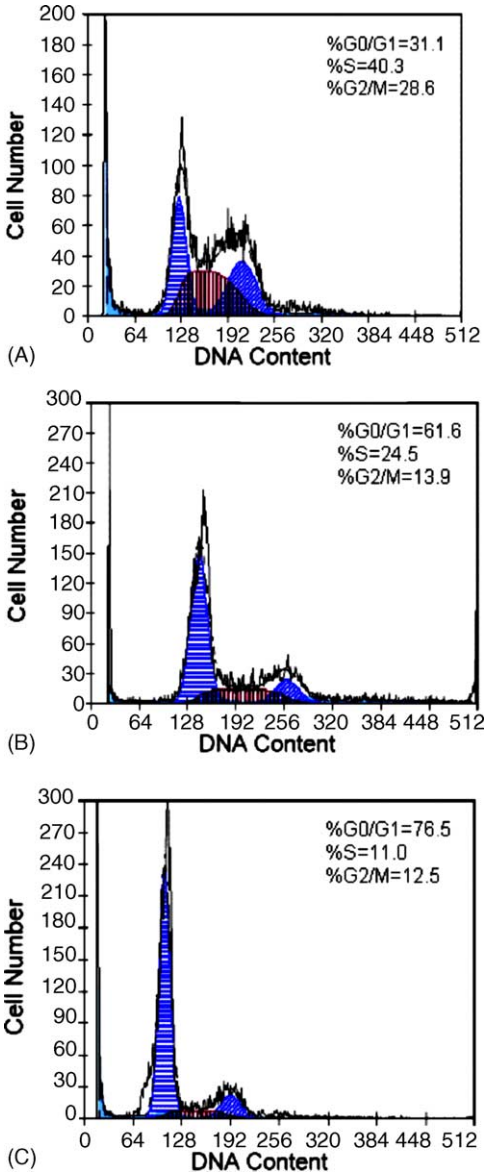

Fig. 2. Inhibition of cell cycle progress in Hep G2 cells by treatment with acacetin. (A) Cell cycle analysis of Hep G2 cells following treatment without acacetin for 24 hr. (B) Cell cycle analysis of Hep G2 cells following treatment with 10 μg/mL acacetin for 24 hr. (C) Cell cycle analysis of Hep G2 cells following treatment with 20 μg/mL acacetin for 24 hr. Cells were fixed with ethanol and stained with PI, and then cell cycle distribution was analyzed by flow cytometry. The percentage of cells in G0/G1, S, and G2/M phases were calculated using Multicycle software and are indicated on the right upper side.

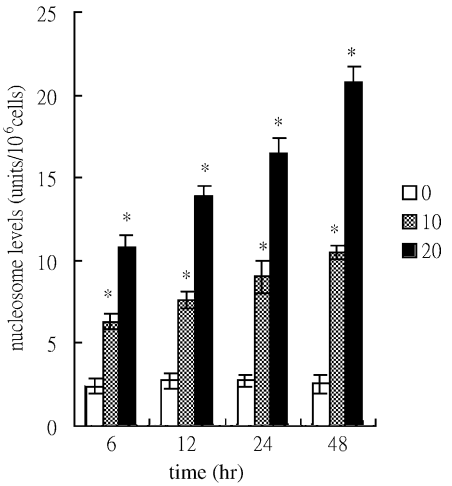

Fig. 3. Induction of apoptosis in Hep G2 by acacetin. Hep G2 cells were cultured with 0, 10, and 20 μg/mL acacetin for 6, 12, 24, and 48 hr. Cells were harvested and lysed with lysis buffer. Cell lysates that contained cytoplasmic oligonucleosomes of apoptotic cells were analyzed in the Nucleosome ELISA. The data shown are the mean ± SD of three independent experiments, each with triplicate wells. The asterisk (∗) indicates a significant difference between control and acacetin-treated cells, $^{*}P < 0.05$.

the results indicated that, compared with the control, 10 μg/mL acacetin increased the population of G1 phase from 31.1 to 61.6%. This effect was enhanced when Hep G2 cells were treated by 20 μg/mL acacetin (76.5% of the cell population in the G1 phase).

Figure 3 shows the time course of DNA fragmentation in continuous treatment with 10 and 20 μg/mL acacetin. DNA fragmentation of Hep G2 was exhibited at 6 hr and maximized at 48 hr after addition of acacetin. In contrast to the controls, when cells were treated with acacetin, the number of cells undergoing apoptosis increased from about 4-fold at 10 μg/mL acacetin to 8-fold at 20 μg/mL acacetin at 48 hr.

3.3. Acacetin increases the expression of p53 and p21/WAF1 proteins in Hep G2 cells

To determine whether tumor suppression factor p53 and its downstream molecule p21/WAF1 were involved in the acacetin-mediated antiproliferative effect of Hep G2 cells, the levels of proteins were assayed by ELISA. Marked induction of p53 protein was observed in a dose-dependent manner (Fig. 4A). The upregulation of p53 by acacetin started to increase 6 hr after treatment with acacetin, and maximum expression was observed at 12 hr. Comparison of the results between apoptotic response and induction of p53 indicated that the upregulation of p53 occurred at an early stage of acacetin-mediated apoptotic process.

Figure 4B shows that an increase in p21/WAF1 protein was apparent at 6 hr and reached maximum induction at 24 hr in acacetin-treated Hep G2 cells. Moreover, the induction of p21/WAF1 was in a dose-dependent manner. Based on these data, we suggest that acacetin-mediated cell

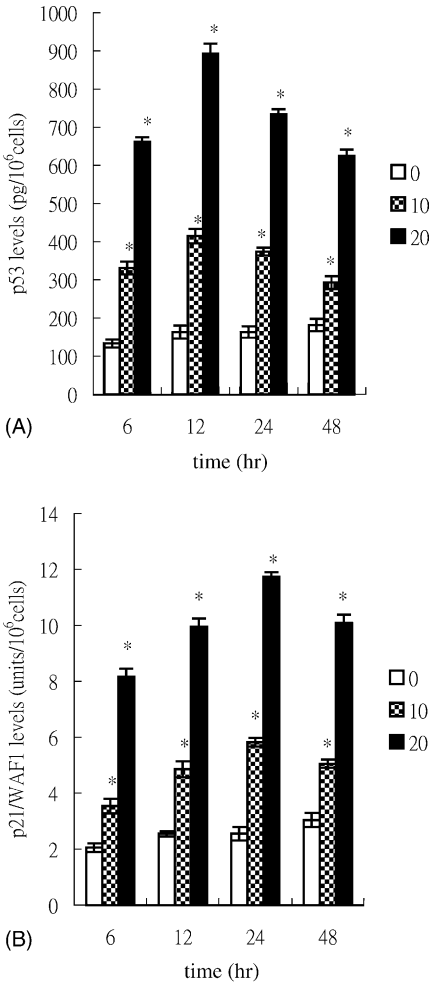

Fig. 4. Effects of acacetin on protein expression of p53 and p21/WAF1. (A) The level of p53 protein in Hep G2 cells; (B) the level of p21/WAF1 in Hep G2 cells. Hep G2 cells were treated with 0, 10, and 20 μg/mL acacetin. Lysates were prepared from these cells and p53 and p21/WAF1 levels were determined by p53 pan ELISA and WAF1 ELISA kits, respectively. The detailed protocol is described in "Section 2". The data shown are the mean ± SD of three independent experiments, each with triplicate wells. The asterisk (∗) indicates a significant difference between control and acacetin-treated cells, $^{*}P < 0.05$.

cycle arrest might operate through the induction of p21/WAF1 protein on a p53-dependent event in Hep G2 cells.

3.4. Fas/FasL apoptotic system might be a possible pathway of acacetin-mediated apoptosis

As observed in the induction of p21/WAF1, the expression of Fas/APO-1 was detected in Hep G2 cells at 6 hr after acacetin treatment. Maximum Fas/APO-1 was detected at 24 hr (Fig. 5A). It is suggested that the induction of Fas/APO-1 in acacetin-treated Hep G2 cells might be related to the activation of p53.

Results on FasL assay indicate that FasL, mFasL, and sFasL were increased in a dose-dependent manner (Fig. 5B and C). The accumulation of mFasL was observed at 6 hr after acacetin treatment, and increased progressively for up to 24 hr (Fig. 5B). A similar result was observed for sFasL

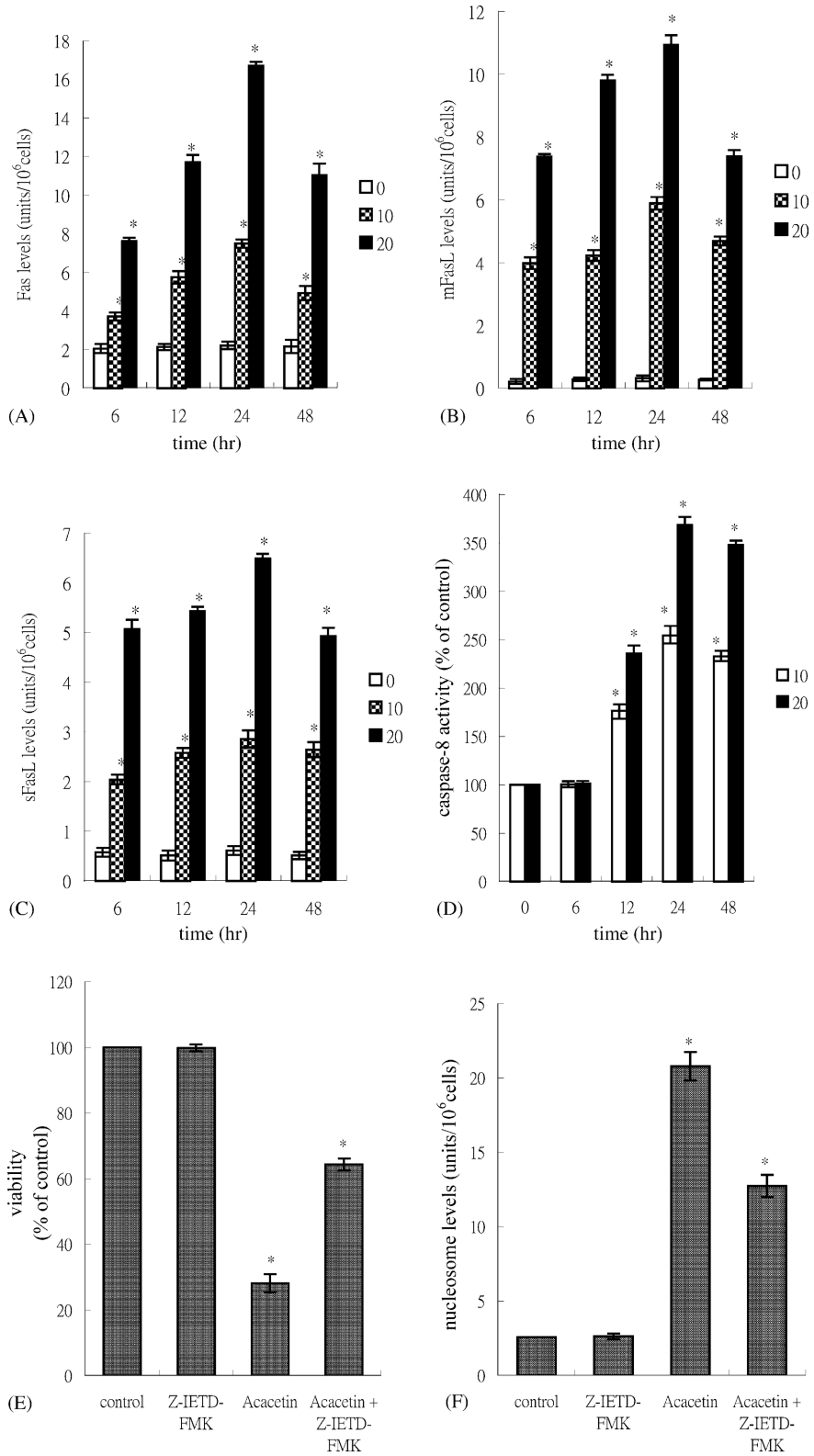

Fig. 5. The Fas/FasL system was involved in acacetin-mediated apotopsis. (A) The amount of Fas/APO-1 receptor; (B) the amount of mFasL; (C) the amount of sFasL; (D) the activation of caspase-8 in Hep G2 cells; (E) effect of caspase-8 inhibitor on acacetin-mediated antiproliferation; (F) effect of caspase-8 inhibitor on acacetin-induced apoptosis. Hep G2 cells were incubated with various concentrations of acacetin for the indicated times. The amount of Fas/APO-1 and FasL was determined by Fas/APO-1 and FasL ELISA kit. For blocking experiments, cells were preincubated with Z-IETD-FMK (10 μM) for 1 hr before the addition of 20 μg/mL acacetin. After 48-hr treatment, the cell viability and induction of apoptosis was measured by XTT and Nucleosome ELISA kit. The data shown are the mean ± SD of three independent experiments, each with triplicate wells. The asterisk (∗) indicates a significant difference between control and acacetin-treated cells, $^{*}P < 0.05$.

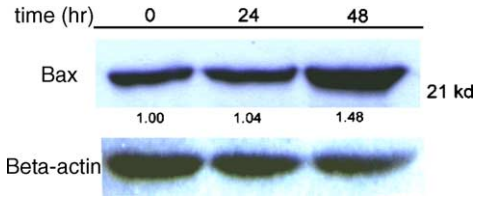

Fig. 6. Effect of acacetin on protein expression of Bax. As detailed in "Section 2", the cells were treated with 20 μg/mL acacetin. After 24 and 48 hr, the cells were harvested. Total cell lysates were separated, and 50 μg of proteins were separated *via* SDS–PAGE followed by Western blot analysis and chemiluminescence detection. The values below the figure represent change in protein expression of the bands normalized to β-actin.

(Fig. 5C). However, the amount of mFasL by acacetin was more than sFasL at all time points.

We next measured the downstream caspase of Fas/FasL system. The results showed that caspase-8 activity increased at 12 hr, and reached maximum induction at 24 hr in 20 μg/mL acacetin-treated Hep G2 cells (Fig. 5D). Furthermore, our results showed that the antiproliferative activity and induction of apoptosis by acacetin were significantly decreased in the presence of inhibitor of caspase-8 (Z-IETD-FMK) (Fig. 5E and F).

3.5. Acacetin treatment of Hep G2 cells results in an increase of proapoptotic protein Bax

The levels of Bax were investigated by Western analysis in untreated and treated Hep G2. In the case of proapoptotic Bax protein, 20 μg/mL acacetin increased Bax protein levels in contrast to the levels of the control cells, at 24 and 48 hr (Fig. 6).

4. Discussion

Normal p53 function plays a crucial role in inducing apoptosis and cell cycle checkpoints in human and murine cells following DNA damage [18]. This has been further supported by the finding that p53 is the most commonly mutated tumor suppressor gene. Moreover, the chemosensitivity of cancer cells to chemotherapy agents is greatly influenced when the function of p53 is abrogated [4]. Our results demonstrate that p53 plays an important role in acacetin-induced antiproliferative activity in Hep G2 cells. Induction of p53 by acacetin not only might cause cell cycle arrest, but may also trigger apoptosis in Hep G2 cells. This finding is supported by the following results: First, flow cytometry assay indicated that acacetin arrested the cell cycle in the G1 phase, which was attributed to the enhancement of p21/WAF1 protein that might be induced by p53. Second, both proapoptotic downstream targets of p53, Fas/APO-1, and Bax proteins were increased by acacetin. Moreover, the expression levels of these proteins were greatly increased (at 24 hr) after maximal accumulation of p53 protein (at 12 hr) in Hep G2 cells.

Fas/FasL system is a key signaling transduction pathway of apoptosis in cells and tissues [19]. Many observations have highlighted the role of the Fas/FasL system in chemotherapy-induced apoptosis of tumors by upregulation of Fas/APO-1 or its ligand [20]. Loss of Fas/APO-1 expression might be involved in the escape of liver cancer cells from the immune defense system and chemoresistance of HCC to chemotherapeutic agents [21]. Ligation of Fas by agonistic antibody or its mature ligand induces receptor oligomerization and formation of death-inducing signaling complex (DISC), followed by activation of caspase-8, then further activating a series caspase cascade resulting in cell apoptotic death [3,19]. FasL is a TNF-related type II membrane protein [22]. Cleavage of mFasL by a metalloprotease-like enzyme results in the formation of sFasL [23]. Both mFasL and sFasL can bind to Fas/APO-1 and subsequently trigger the Fas/FasL system, but sFasL has been reported to be a weaker inducer of apoptosis than mFasL [24]. Our study indicates that Fas ligands, mFasL and sFasL, increase in acacetin-treated Hep G2 cells. Also, the level of Fas/APO-1 and the activity of caspase-8 are simultaneously enhanced in FasL-upexpressing Hep G2 cells. Furthermore, cell growth inhibition and apoptotic induction of acacetin decreases in Hep G2 cells with caspase-8 inhibitor treating. Thus, our results have demonstrated that the Fas/FasL system plays an important role in acacetin-mediated Hep G2 cellular apoptosis.

The Bcl-2 family is a key regulator of apoptosis [3,25]. It is not surprising that the level of Bax expression was found to increase in acacetin-treated Hep G2 cells. Wild-type p53 is known to be the upstream regulator of the Bax protein, which can antagonize the anti-apoptotic activity of Bcl-2 [18]. The highest increase of Bax expression in Hep G2 after 48-hr incubation might result from the accumulation of p53, which reached its highest peak earlier (after 12-hr treatment).

In summary, our study demonstrates that the induction of p53 and activity of the Fas/FasL apoptotic system may participate in the antiproliferative activity of acacetin in Hep G2 cells. Our study has clearly demonstrated that acacetin may be a promising chemopreventive agent for treating liver cancer.

References

[1] Seow TK, Liang RC, Leow CK, Chung MC. Hepatocellular carcinoma: from bedside to proteomics. Proteomics 2001;1:1249–63.
[2] Kern MA, Breuhahn K, Schirmacher P. Molecular pathogenesis of human hepatocellular carcinoma. Adv Cancer Res 2002;86:67–112.
[3] Hengartner MO. The biochemistry of apoptosis. Nature 2000;407:770–6.
[4] Brown JM, Wouters BG. Apoptosis, p53, and tumor cell sensitivity to anticancer agents. Cancer Res 1999;59:1391–9.
[5] Cody V. Crystal and molecular structures of flavonoids. Prog Clin Biol Res 1988;280:29–44.
[6] Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. Nutr Cancer 1993;20:21–9.

[7] Cholbi MR, Paya M, Alcaraz MJ. Inhibitory effects of phenolic compounds on CCl_4-induced microsomal lipid peroxidation. Experientia 1991;47:195–9.
[8] Liao YH, Houghton PJ, Hoult JR. Novel and known constituents from Buddleja species and their activity against leukocyte eicosanoid generation. J Nat Prod 1999;62:1241–5.
[9] Kraft C, Jenett-Siems K, Siems K, Jakupovic J, Mavi S, Bienzle U, Eich E. In vitro antiplasmodial evaluation of medicinal plants from Zimbabwe. Phytother Res 2003;17:123–8.
[10] Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M. Effect of citrus flavonoids on HL-60 cell differentiation. Anticancer Res 1999;19: 1261–9.
[11] Zhang K, Yang EB, Tang WY, Wong KP, Mack P. Inhibition of glutathione reductase by plant polyphenols. Biochem Pharmacol 1997;54:1047–53.
[12] Doostdar H, Burke MD, Mayer RT. Bioflavonoids: selective substrates and inhibitors for cytochrome P450 CYP1A and CYP1B1. Toxicology 2000;144:31–8.
[13] Boege F, Straub T, Kehr A, Boesenberg C, Christiansen K, Andersen A, Jakob F, Kohrle J. Selected novel flavones inhibit the DNA binding or the DNA religation step of eukaryotic topoisomerase I. J Biol Chem 1996;271:2262–70.
[14] Kikuchi S, Hiraide H, Tamakuma S, Yamamoto M. Expression of wild-type p53 tumor suppressor gene and its possible involvement in the apoptosis of thyroid tumors. Jpn J Surg 1997;27:226–33.
[15] Mediavilla MD, Cos S, Sanchez-Barcelo EJ. Melatonin increases p53 and p21WAF1 expression in MCF-7 human breast cancer cells in vitro. Life Sci 1999;65:415–20.
[16] Castaneda F, Kinne RK. Apoptosis induced in HepG2 cells by short exposure to millimolar concentrations of ethanol involves the Fas-receptor pathway. J Cancer Res Clin Oncol 2001;127:418–24.
[17] Oh SH, Lee BH. Induction of apoptosis in human hepatoblastoma cells by tetrandrine via caspase-dependent Bid cleavage and cytochrome *c* release. Biochem Pharmacol 2003;66:725–31.
[18] May P, May E. Twenty years of p53 research: structural and functional aspects of the p53 protein. Oncogene 1999;18:7621–36.
[19] Nagata S, Golstein P. The Fas death factor. Science 1995;267:1449–56.
[20] Jiang S, Song MJ, Shin EC, Lee MO, Kim SJ, Park JH. Apoptosis in human hepatoma cell lines by chemotherapeutic drugs via Fas-dependent and Fas-independent pathways. Hepatology 1999;29:101–10.
[21] Lee SH, Shin MS, Lee HS, Bae JH, Lee HK, Kim HS, Kim SY, Jang JJ, Joo M, Kang YK, Park WS, Park JY, Oh RR, Han SY, Lee JH, Kim SH, Lee JY, Yoo NJ. Expression of Fas and Fas-related molecules in human hepatocellular carcinoma. Hum Pathol 2001;32:250–6.
[22] Suda T, Takahashi T, Golstein P, Nagata S. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell 1993;75:1169–78.
[23] Kayagaki N, Kawasaki A, Ebata T, Ohmoto H, Ikeda S, Inoue S, Yoshino K, Okumura K, Yagita H. Metalloproteinase-mediated release of human Fas ligand. J Exp Med 1995;182:1777–83.
[24] Schneider P, Holler N, Bodmer JL, Hahne M, Frei K, Fontana A, Tschopp J. Conversion of membrane-bound Fas(CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity. J Exp Med 1998;187:1205–13.
[25] Antonsson B, Martinou JC. The Bcl-2 protein family. Exp Cell Res 2000;256:50–7.