PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

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LIVER CANCER

Mechanism of apoptotic effects induced selectively by ursodeoxycholic acid on human hepatoma cell lines

Hui Liu, Cheng-Yong Qin, Guo-Qing Han, Hong-Wei Xu, Mei Meng, Zhen Yang

Hui Liu, Cheng-Yong Qin, Guo-Qing Han, Hong-Wei Xu, Mei Meng, Zhen Yang, Department of Gastroenterology, Shandong Provincial Hospital Shandong University, Jinan 250021, Shandong Province, China
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Correspondence to: Cheng-Yong Qin, Professor, Department of Gastroenterology, Shandong Provincial Hospital Shandong University, Jinan 250021, Shandong Province, China. h_liu13@yahoo.com.cn
Telephone: +86-531-89996339 Fax: +86-531-87068344
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Abstract

AIM: To investigate the effects of ursodeoxycholic acid (UDCA) on apoptosis and proliferation of hepatoma cell lines.

METHODS: Human hepatoma cell lines HepG2 and BEL 7402 were cultured in medium supplemented with different concentrations of UDCA, normal human hepatic line L-02 was used as control. Cell proliferation, apoptosis and gene expression were detected using methyl thiazolyl tetrazolium (MTT) assay, flow cytometry, Western blot, DNA ladder assay, electron microscopy, and immunocytochemistry.

RESULTS: Ursodeoxycholic acid inhibited the proliferation of HepG2 and BEL7402 cell lines in a dose-dependent manner. Ursodeoxycholic acid can change cell cycle distribution of HepG2 and BEL7402, the proportion of cells in G_0-G_1 phase increased whereas the proportion of S phase cells and G_2-M phase cells decreased. Ursodeoxycholic acid arrested the cell cycle in G_0-G_1 phase by down-regulating the cell cycle related proteins cyclin D_1, D_3 and retinoblastoma protein (p^{Rb}). The apoptotic rates of HepG2 and BEL7402 treated with UDCA (1.0 mmol/L) were significantly higher than those of control. In the HepG2 and BEL7402 treated with UDCA, expression of bcl-2 decreased whereas expression of Bax increased, the nuclear fragmentation and chromosomal condensed, cells shrank and lost attachment, apoptotic bodies and DNA ladders appeared. UDCA had no effect in inducing apoptosis on L-02 cell lines.

CONCLUSION: UDCA can selectively inhibit proliferation and induce apoptosis of HepG2 and BEL7402 cell lines by blocking cell cycle and regulating the expression of Bax/bcl-2 genes.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms worldwide and one of the leading causes of malignancy-related death in China[1,2]. Surgery is the most effective option, unfortunately, the majority of patients with HCC are not amenable to surgery at diagnosis. Presently, one of the main approaches to treat HCC is cytotoxic chemotherapy, but HCC is sometimes less sensitive or becomes resistant to anticancer drugs after consecutive treatment. Most studies failed to find a therapy which can produce response higher than 25% among hepatoma patients[3,4]. Therefore, it is necessary to develop new drugs to prevent and treat HCC.

The efficacy of ursodeoxycholic acid (UDCA) in primary biliary cirrhosis (PBC) has been demonstrated in several randomized clinical trials[5,6]. UDCA improved biochemical and histological parameters, prevented progression of PBC, and prolonged survival[6,7]. Recent reports suggested that bile acid derivatives induce apoptosis *via* a *p*53-independent pathway in human breast carcinoma cells[8], and UDCA inhibits the initiation and postinitiation phases of azoxymethane-induced colonic tumor development[9].

However, the effects of UDCA in human hepatoma cell lines have not been determined yet. In this study, we investigate the effect of UDCA on proliferation and apoptosis of human hepatoma cell lines and normal human hepatic cell lines and observe the mechanisms of the effects.

MATERIALS AND METHODS

Cell lines and reagents

Human primary hepatocellular cancer cell lines BEL7402 and HepG2, and normal human hepatic cell line L-02 were

purchased from Shanghai Institute of Cell Biology, the Chinese Academy of Sciences. UDCA and 5-Fluorouracil (5-Fu) were obtained from Sigma, USA. Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibcol BRL, USA.

The UDCA and 5-Fu were first dissolved in DMSO solution, then added to the culture medium with a final concentration of 0.1% DMSO (Dimethyl Sulphoxide). The monoclonal mouse anti-human bcl-2 and monoclonal rabbit anti-human Bax antibodies were obtained from Calbiochem, Cambridge, MA, USA. The Vectastain ABC kit was purchased from Vector Labs, Burlingame, CA, USA.

Cell culture

The hepatocellular cancer cell lines BEL7402 and HepG2, and the normal hepatic cell line L-02 were incubated in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin G and 100 mg/mL streptomycin at 37℃ humidified atmosphere containing 5% CO_2. The 5-Fu and DMSO were used as positive and negative controls, respectively. All studies were performed when cells were at 50% confluence.

Cell viability assay by MTT

Cells were plated at a density of 6×10^3/well in 96-well plates. Twenty-four hours later, different amounts of UDCA dissolved in DMSO were added to the medium to make a final UDCA concentration from 0.05 mmol/L to 1.0 mmol/L. Control cell cultures were treated with DMSO (0.1%) or with 5-Fu (0.4 mmol/L). At time points of 48 h, 72 h and 96 h after addition of test compounds, 20 μL methyl thiazolyl tetrazolium (MTT) was added to each well. Four hours later, 100 μL of DMSO was added to each well after the medium was removed. Finally, optical densities were measured at A570 nm and the growth inhibitory rate was calculated. Experiments were done in triplicate and IC50 (concentration of drug that inhibits cell growth by 50%) values were determined. The inhibitory rate (IR) was calculated by the following formula:

IR = (1 - average OD_{570} of treated group/average OD_{570} of DMSO control) × 100%.

Apoptosis and cell cycle distribution by flow cytometry

Four milliliters cell suspensions (HepG2, Bel7402 or L-02) at a concentration of 5×10^4/mL were incubated in 50 mL flasks. Twenty-four hours later, cells were treated with UDCA at different final concentrations from 0.1 to 1.0 mmol/L. The control cultures were treated only with the 0.1% DMSO. Forty-eight hours later, cells (1×10^6) were collected and centrifuged for 5 min at 1000 r/min, the supernatant was discarded and cells were fixed in 70% alcohol and kept at 4℃ overnight. Then cells were resuspended in 3 mL PBS and washed twice with PBS, three milliliters of citrate was added to the tube. Thirty minutes later, cells were centrifuged at 2000 r/min for 5 min and resuspended in 150 μL of Pidye and 200 μL of RNase A. The tubes were incubated at room temperature in the dark for 30 min. Cells were analyzed by flow cytometry in triplicate.

Agarose electrophoresis for DNA fragmentation

Cells (1×10^7) collected after treatment with UDCA for 48 h in 0.4 mmol/L, 0.8 mmol/L, and 1.0 mmol/L UDCA, were washed with ice-cold PBS and resuspended in 500 μL digestion buffer [100 mmol/L NaCl, 10 mmol/L Tris-HCl, pH 8, 25 mmol/L ethylene diamine tetra acetic acid (EDTA), 0.5% sodium dodecyl sulphate (SDS), 0.1 μg/mL proteinase K]. RNase was then added to make a final concentration of 20 mg/L, and incubation was continued at 37℃ for 1 h, then incubated at 50℃ overnight for complete digestion, DNA was extracted with an equal volume of phenol and chloroform, and precipitated with two volumes of isopropanol. DNA fragmentation was detected by electrophoresis in 1.5% agarose gel containing 1 mg/L ethidium bromide.

Ultrastructure of cells

Cells were rinsed in 0.1 mol/L tris-maleate buffer (pH 6.0) and 0.1 mol/L cacodylate buffer (pH 7.0), then fixed in 1% OsO4, dehydrated in graded ethanol, the embedded slices were cut into ultrathin sections by LKB-1 ultramicrotome. The sections were double stained with uranyl acetate and lead citrate, then were observed under H-800 transmission electron microscope (JEM-100CX 11/T, Japan).

Immunocytochemistry

For the immunostaining, the avidin-biotin-peroxidase complex technique, using a Vectastain ABC kit (Vector Labs, Burlingame, CA, USA), was used as described previously[10]. Cells were grown on coverslips in wells for later immunocytochemical staining. Cells on slips were fixed with acetone, dried in air, treated with 0.1% H_2O_2, blocked with normal nonimmune animal sera, then incubated with first antibodies, biotin labeled second antibody and the streptavidin-peroxidase, each for 10-20 min. After each incubation with antibodies, cells were washed with PBS for 3 times. Then cells were developed with DAB. Cells were rinsed with water and counterstained with hematoxylin, then dehydrated, transparent and counted. The negative controls were performed by replacing the primary antibody with the same dilution of normal goat serum. Immunoreactive cells were visualized using a Vectastain ABC Kit (Vector Labs, Burlingame, CA, USA). The accumulation of bcl-2 and Bax protein is indicated by a brown coloration in the cytoplasm. The labeling index was determined by calculating the number of positive cells divided by the total number of counted cells in four coverslips.

Western blot analysis

Cells were washed in PBS and lysed with TNN lysis buffer (40 mmol/L Tris, pH 8.0, 120 mmol/L NaCl, 0.5% Nonidet p-40, 0.1 mmol/L sodium orthovanadate, 2 mg/L aprotinin, 2 mg/L leupeptin, and 100 mg/L PMSF) at 4℃ for 20 min, lysates were centrifuged at 14000 r/min for 15 min to remove cellular debris. Protein concentration was determined with the BCA reagent (Pierce, Rockford, IL). Equal amounts of protein were subjected to electrophorsis on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Schleicher & Schuell) by

Table 1 UDCA induces apoptosis of HepG2, BEL7402 and L-02 cell lines (means ± SD, n = 3)

UDCA mmol/L	Apoptosis rate (%)		
	HepG2	BEL7402	L-02
0 (control)	2.58 ± 0.26	2.02 ± 0.13	3.03 ± 0.12
0.1	8.4 ± 1.6[b]	9.3 ± 2.0[b]	3.11 ± 0.21[a]
0.4	19.0 ± 3.0[b]	17.2 ± 1.7[b]	3.66 ± 0.13[a]
0.8	36.0 ± 6.0[b]	37.0 ± 5.0[b]	4.29 ± 0.23[a]
1.0	42.0 ± 6.0[b]	44.0 ± 4.0[b]	4.58 ± 0.18[a]

[a]$P > 0.05$, [b]$P < 0.01$, *vs* control.

electroblotting. The membranes were incubated with various antibodies. Monoclonal anti-p21$^{WAF1/CIP1}$ antibody was obtained from Transduction Lab (Lexington, KY). Monoclonal antibody to *p*53, and polyclonal antibodies to cyclin D1 and D3, cyclin-dependent kinase (Cdk) 2, Cdk4 and retinoblastoma protein (p^{Rb}) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Peroxidase-conjugated donkey anti-rabbit and sheep anti-mouse immunoglobulins were purchased from Amersham Life Science (Arlington Heights, IL). Protein bands were visualized with the enhanced chemiluminescence (ECL) detection system (Amersham Life Science).

Statistical analysis

Data of MTT assay were analyzed using regression analysis. Other values were expressed as mean ± standard deviation (SD). Means were compared using one way analysis of variance (ANOVA). $P < 0.05$ was considered significant.

RESULTS

Cell proliferation

Viability of HepG2 and BEL7402 cell lines was inhibited by UDCA with a concentration from 0.2 to 1.0 mmol/L. After exposure to UDCA for 48 h, the IC50 of HepG2 and BEL7402 was 0.92 mmol/L and 0.86 mmol/L respectively. The effect of inhibition was dose-dependent, cells treated with various concentrations of UDCA for 48h, the inhibitory rates in the HepG2 and BEL7402 were positively correlated with different concentrations of UDCA. In the HepG2 cell: $r = 0.96$, $P < 0.01$, 0.2 mmol/L; $r = 0.96$, $P < 0.01$, 0.4 mmol/L; $r = 0.96$, $P < 0.01$, 0.8 mmol/L; In the BEL7402 cell: $r = 0.96$, $P < 0.01$, 0.2 mmol/L; $r = 0.97$, $P < 0.01$, 0.4 mmol/L; $r = 0.98$, $P < 0.01$, 0.8 mmol/L). However, UDCA had no effect on the viability of the normal cell line L-02.

Apoptosis and cell cycle distribution

Apoptosis and cell cycle distributiion were detected 48 h after treating cells with different concentrations UDCA. Compared with control, the apoptosis rates in the groups treated with UDCA increased significantly ($P < 0.05$) and this was dose-dependent. In the UDCA groups, the cell cycle distribution changed, the proportion of cells in G_0-G_1 phase increased ($P < 0.05$) whereas the cell number in S phase and G_2-M phase decreased significantly ($P < 0.05$), indicating that UDCA arrested the HepG2 and BEL7402 cells in G_0-G_1 phase. No significant difference was observed in the apoptosis rate and cycle distribution of the cell line L-02 treated with UDCA ($P > 0.05$), (Table 1, Figure 1A and B).

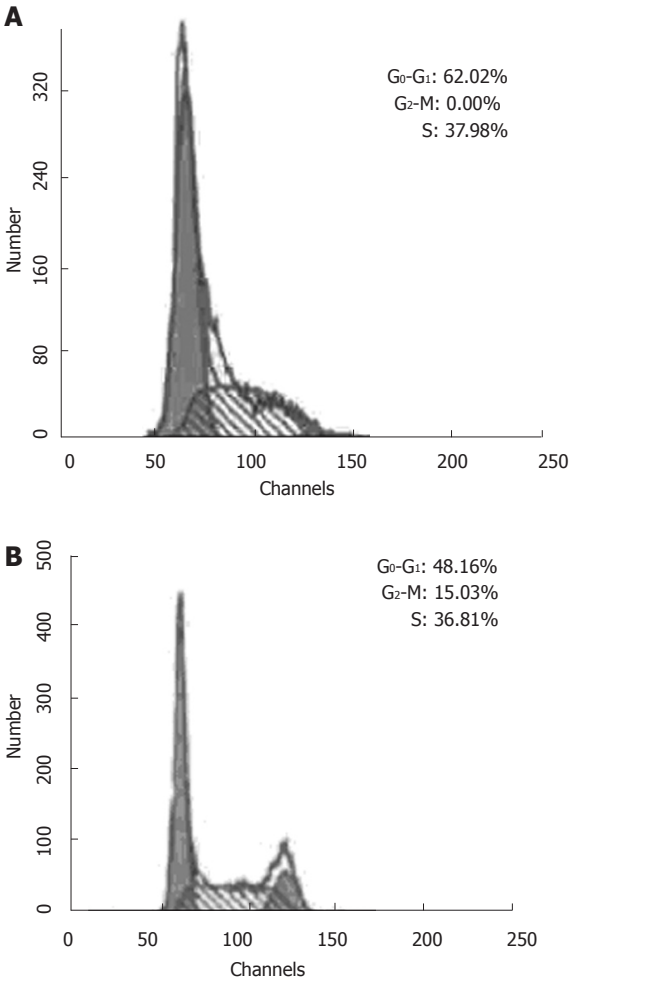

Figure 1 **A**: Cell cycle distribution of BEL7402 treated with UDCA (0.8 mmol/L) for 48 h. The cell cycle distribution changed, the proportion of G_0-G_1 phase increased significantly ($P < 0.05$) and the proportion of S phase and G_2-M phase decreased significantly ($P < 0.05$); **B**: Cell cycle distribution of L-02 cells treated with UDCA (0.8 mmol/L) for 48 h. The cell cycle distribution of L-02 cells does not change.

DNA fragmentation

Agarose gel electrophoresis of DNA extracted from cells treated with various concentrations of UDCA (0.4 mmol/L, 0.8 mmol/L, 1.0 mmol/L) revealed ladders of DNA fragmentation, this indicated the apoptosis in both HepG2 and BEL7402 cell lines (Figure 2).

Ultrastructure under electron microscope

In the UDCA treated cells, nuclear fragmentation, chromosome condensation, cell shrinkage and loss of cell-cell contact were visible. Subsequent ruffling and blebbing of the cell membrane, and formation of apoptotic bodies were also observed (Figure 3A and B).

Expression of *bcl-2* and Bax genes

The accumulations of bcl-2 and Bax proteins were indicated by brown coloration in the cytoplasm (Figure 4A and B). After cells being treated with UDCA at different

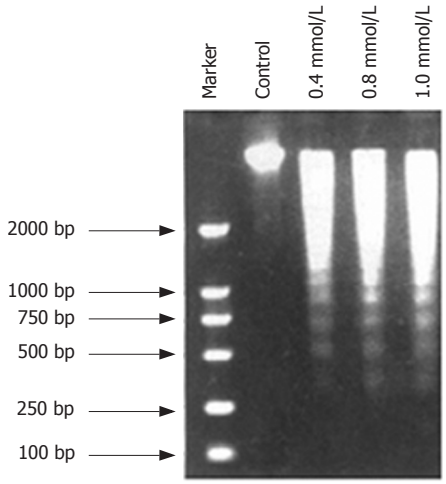

Figure 2 Induction of internucleosomal DNA fragmentation by UDCA. Cells were incubated without or with various concentrations of UDCA (0.4 mmol/L, 0.8 mmol/L, 1.0 mmol/L) for 48 h. DNA was extracted and analyzed by 1.5% agarose gel electrophoresis in the presence of ethidium bromide staining.

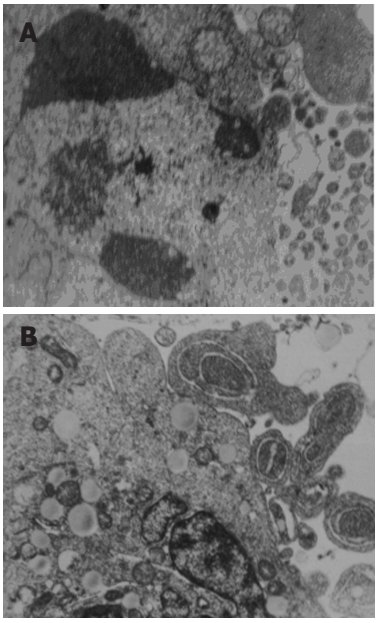

Figure 3 Morphology of HepG2 treated with UDCA (0.4 mmol/L) for 48 h (TEM, × 4000). **A**: Nuclear fragmentation, chromosome condensation, cell shrinkage and loss of cell-cell contact are visible; **B**: Subsequent ruffling and blebbling of the cell membrane, and formation of apoptotic bodies are also observed.

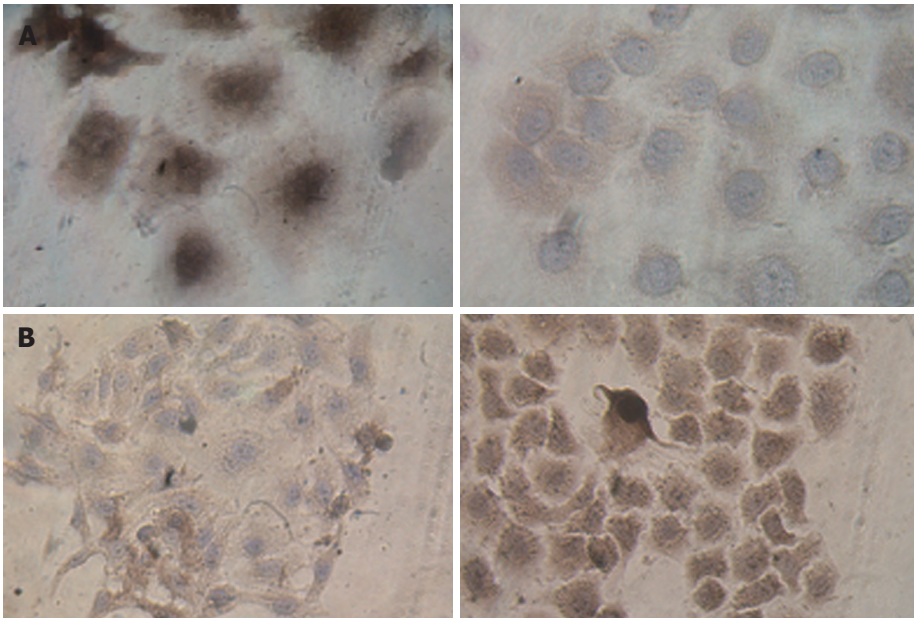

Figure 4 **A**: Effect of UDCA on expression of bcl-2 in BEL7402, immuno-cytochemical staining of bcl-2. The accumulation of bcl-2 protein is indicated by a brown coloration in the cytoplasm (× 200). Control group, expression of bcl-2 in BEL7402 cultivated for 48 h (Left), Expression of bcl-2 in BEL7402 treated with UDCA (0.4 mmol/L) for 48 h (Right); **B**: Effect of UDCA on expression of Bax in BEL7402, immunocytochemical staining of Bax. The accumulation of Bax protein is indicated by a brown coloration in the cytoplasm. Control group,expression of Bax in BEL7402 cultivated for 48 h (Left). Expression of Bax in BEL7402 treated with UDCA (0.4 mmol/L) 48 h (× 200) (Right).

Table 2 Effect of UDCA on expression of bcl-2 and Bax in HepG2, BEL7402 and L-02 (means ± SD, n = 4)

	HepG2 (%)		BEL7402 (%)		L-02 (%)	
UDCA (mmol/L)	bcl-2	Bax	bcl-2	Bax	bcl-2	Bax
0 (control)	24.3 ± 2.4	43.0 ± 5.0	21.6 ± 1.8	44.0 ± 4.0	1.0 ± 0.2	62.0 ± 4.0
0.2	19.4 ± 1.5[c]	42.0 ± 4.0[a]	20.1 ± 1.9[a]	48.4 ± 2.5[a]	1.0 ± 0.1[a]	61.0 ± 5.0[a]
0.4	14.2 ± 2.1[b]	53.6 ± 2.5[c]	13.5 ± 1.8[b]	56.0 ± 4.0[c]	0.8 ± 0.1[a]	59.0 ± 3.0[a]
0.8	10.1 ± 1.6[b]	59.0 ± 3.0[b]	11.6 ± 2.1[b]	59.0 ± 3.0[b]	0.6 ± 0.3[a]	64.0 ± 4.0[a]

[a]$P > 0.05$, [b]$P < 0.01$, [c]$P < 0.05$, *vs* control.

final concentrations (0.2, 0.4 and 0.8 mmol/L) for 48 h, the expression of Bax in the HepG2 and BEL7402 cells was higher than that in control ($P < 0.05$), and there was no significant difference in L-02 ($P > 0.05$); the expression of bcl-2 in the HepG2 and BEL7402 cells was lower than that in control ($P < 0.05$), again there was no significant difference in L-02 ($P > 0.05$). The ratio of Bax to bcl-2 was elevated along with increasing concentrations of UDCA (Table 2).

UDCA down-regulated D-type G1 cyclins

Cells were treated with various concentrations of UDCA

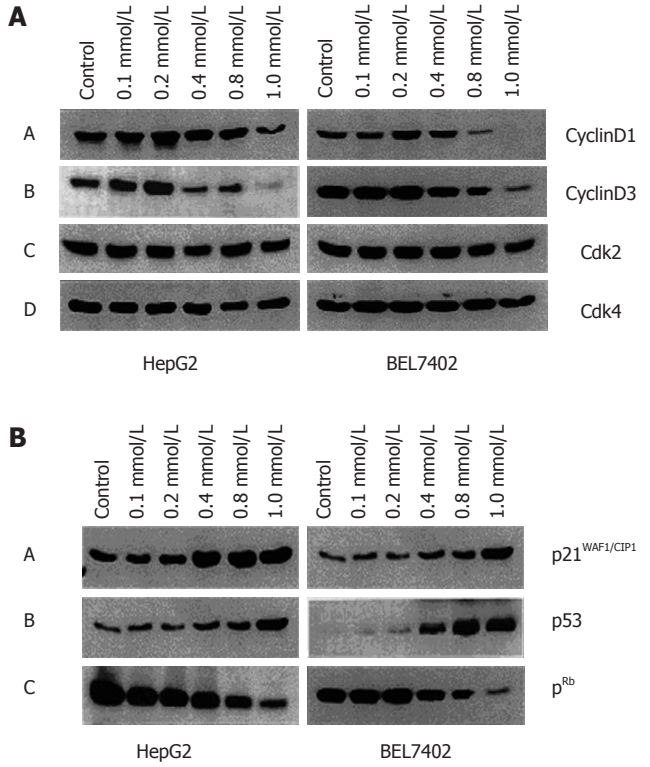

Figure 5 **A**: Effects of UDCA on production of cyclins and Cdks in HepG2 and BEL7402 cells treated with various concentrations of UDCA (0.1, 0.2, 0.4, 0.8, 1.0 mmol/L) for 48 h; **B**: Effects of UDCA treatment on protein levels of $p21^{WAF1/CIP1}$, p53, p^{Rb} in HepG2 and BEL7402. The cells were treated with 0.8 mmol/L UDCA for 48 h.

for 48 h, the expression of cell cycle regulating proteins (cyclin D1, cyclin D3, Cdk2 and Cdk4) were detected by Western blot analysis. The results showed that the intracellular protein levels of cyclin D1 and cyclin D3 were down-regulated. However, the levels of Cdk2 and Cdk4 did not change in both cell lines (Figure 5A).

UDCA increased intracelluar levels of p53 and $p21^{WAF1/CIP1}$ and inhibited phosphorylation of p^{Rb}

Treatment with UDCA resulted in a marked increase in the level of $p21^{WAF1/CIP1}$ protein in both HepG2 and BCL7402 cell lines. Meanwhile, the expression levels of *p*53 protein increased, indicating that the induction of $p21^{WAF1/CIP1}$ in both cancer cell lines was in a *p*53-dependent fashion (Figure 5B). The Rb gene product p^{Rb} is an important checkpoint protein in the G1/S phase transition of the cell cycle. Furthermore, $p21^{WAF1/CIP1}$ inhibits the phosphorylation of p^{Rb}. Therefore, experiments were carried out to examine whether the effect of decreased expression of D-type G1 cyclins by UDCA was associated with the decreased phosphorylation of p^{Rb} in cells treated with UDCA. As shown in Figure 5B, the level of expression of p^{Rb} was remarkably decreased in HepG2 and BEL7402 cells treated with UDCA.

DISCUSSION

In the present study, we demonstrate that UDCA induces apoptosis and inhibits proliferation of the human hepatoma cell lines HepG2 and BEL7402. Our results suggest that UDCA is a potential agent in the treatment of human hepatoma. Furthermore, we investigated the mechanism of action of UDCA-induced apoptosis in the two human primary hepatocellular cancer cell lines HepG2 and BEL7402.

DNA fragmentation occurs in cell apoptosis induced by a variety of agents. This cleavage produces ladders of DNA fragments that are the size of integer multiples of a nuclesome length (180-200 bp)[11]. Due to their characteristic patterns revealed by agarose gel electrophoresis, these nucleosomal DNA ladders are widely used as biochemical markers of apoptosis. A characteristic ultrastructural appearance of apoptotic cells is observed under an electron microscope. Additionally, flow cytometry analysis has been used to detect and quantify cells undergoing apoptosis[12]. The decreased cell numbers in the treatment groups compared with the control group could be due to apoptotic cell death as well as cell growth inhibition. The DNA ladder formation, characteristic of ultrastructural appearance of apoptotic cells, and the augmentation of apoptotic sub-G1 population indicate that apoptosis is a major underlying process induced by UDCA.

Cell proliferation is defined as the increase in cell number resulting from completion of the cell cycle. The cell cycle is composed of 4 phases: the gap before DNA replication (G_1), the DNA synthetic phase (S), the gap after DNA replication (G_2), and the mitotic phase (M)[13,14]. When disorder occurs in cell cycle, the cell proliferation will be inhibited and the cell apoptosis will follow[15]. In mammalian cells, D-type cyclins are synthesized during the G1 phase and rate-limiting factors for S phase entry[16-18]. In addition, Cdk inhibitors regulate cell progression by association with cyclin/Cdk complexs[19,20], which may inhibit cyclin-containing complexs that reduce the cyclin-dependent activity in damaged cells destined to apoptosis[21]. Our data showed UDCA selectively increases the intracellular protein levels of D-type cyclins, but there is no change in the levels of Cdks. On the other hand, Cdk inhibitors play important roles in cell cycle control by coordinating internal and/or external signals and impeding proliferation at several key checkpoints[22]. They fall into two categories: the CIP/KIP and the INK4 inhibitors. The CIP/KIP family members can inhibit kinase activity of Cdks during the G1/S cell cycle checkpoint. Among several Cdk inhibitors, $p21^{WAF1/CIP1}$ is an important mediator of cell cycle arrest imposed by the tumor suppressor *p*53 in response to DNA damage[23,24]. It is found that UDCA selectively increases the expression of the Cdk inhibitor $p21^{WAF1/CIP1}$ in a *p*53-dependent manner. Another important downstream target of the G1 phase cyclin/Cdk complex is p^{Rb}, which is controlled by Cdk-mediated phosphorylation[25,26], p^{Rb} is also known to regulate the transcription of a variety of genes encoding growth-regulatory factors[27,28]. The expression and phosphorylation of p^{Rb} in our study in UDCA-treated HepG2 and BEL7402 cells decreased.

In the present study, a population of cells in FCM analysis shows that cell cycle distribution changed in UDCA-treated groups. The proportion of G_0-G_1 phase increased and S phase and G_2-M phase decreased, and apoptosis rate also increased. These findings indicate

that UDCA arrests the cell cycle in G_0-G_1 phase, the mechanism of which is elucidated as above. In this phase, cell apoptosis is induced by UDCA arresting cell cycle in G_0-G_1 phase. This may be a key mechanism that UDCA inhibits proliferation of and induces apoptosis of HepG2 and BEL7402.

The bcl-2 proteins belong to a family of proteins involved in the response to apoptosis. Some of these proteins (such as Bax and Bad) are pro-apoptotic, while others (such as bcl-2 and Bcl-xl) are anti-apoptotic[29-31]. Many studies showed that overexpression of bcl-2 could inhibit apoptosis of many cells induced by various factors[32,33]. Increased expression of Bax can induce apoptosis by suppressing the activity of bcl-2[34]. It was also reported that the ratio of Bax to bcl-2, rather than bcl-2 alone, is important for the survival of drug-induced apoptosis[35]. Our study showed that UDCA induced an increased-ratio of Bax to bcl-2 associated with down-regulation of bcl-2 and up-regulation of Bax in HepG2 and BEL7402 cells and the effect of UDCA was dose-dependent. This correlated to the results that UDCA induced apoptosis of HepG2 and BEL7402 and apoptotic rate was dose- and time-dependent. This provides an evidence that increased ratio of Bax to bcl-2 is another mechanism of apoptosis of HepG2 and BEL7402 cells induced by UDCA.

The results of MTT and FCM showed that UDCA did not inhibit proliferation and induce apoptosis of L-02, which indicates that the effect of UDCA is selective. This selectivity was correlated to the effect of UDCA on the cell cycle and the expression of Bax and bcl-2 of HepG2 and BEL7402, but no effect on the L-02. This demonstrates that the UDCA is cell type specific in inducing apoptosis.

The effect of UDCA on the cell proliferation and apoptosis may involve different mechanisms, herein we demonstrated that UDCA changes cell cycle and regulates the expression of Bax and bcl-2. The relationship between proliferation and apoptosis is complicated, they are triggered by a variety of molecular signals. The molecular mechanism that regulates the balance between cell proliferation and cell apoptosis needs further investigations.

UDCA, as an endogenous tertiary bile acid in man, has several biologic effects, such as inhibition of cholesterol absorption in the intestine[36] and inhibition of bile secretion[37], stabilization of cell membrane[38] and modulation of immunologic reactions[39]. Recent reports suggested that the novel bile acid derivatives induce apoptosis *via* a *p*53-independent pathway in human breast carcinoma cells[8]. It is shown that UDCA inhibits the initiation and post-initiation phases of azoxymethane-induced colonic tumor development[9]. Thus, UDCA and its derivative may inhibit carcinogenesis *via* different mechanisms in various cell types. Our study demonstrates that UDCA may be a promising chemopreventive agent for preventing and treating human hepatoma by inducing apoptosis and proliferation inhibition.

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