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Original article

Inhibition of cell cycle progression and apoptotic activity of resveratrol in human intrahepatic cholangiocarcinoma cell lines

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Background: Cholangiocarcinoma (CCA) is a cancer of the bile duct epithelium and is characterized by a poor prognosis and unresponsiveness to conventional treatments. Effective therapeutic agents for CCA are urgently needed. Resveratrol, a phytoalexin found in several fruits, has been reported to inhibit growth of various cancer cell lines.

Objective: We investigated the effect of resveratrol on the intrahepatic CCA-derived KKU-100 and KKU-M156 cell lines.

Materials and methods: The cell lines used were established from Thai CCA patients (KKU-100, a poorly-differentiated adenocarcinoma and KKU-M156, a moderately-differentiated adenocarcinoma). The mechanisms of an antiproliferative effect of resveratrol on these cell lines were determined using sulforhodamine B assay, flow cytometry, ethidium bromide/acridine orange staining, DNA fragmentation, and Western blotting analysis.

Results: Resveratrol significantly inhibited CCA cell growth in a dose- and time-dependent manner. Resveratrol induced cell cycle arrest at the G0/G1 phase in KKU-100 by decreasing cyclin D1, cyclin E, cyclin-dependent kinase (Cdk)2 and Cdk4 levels and increasing p53, Cdk inhibitors (CDKIs) p21 and p27 levels. By comparison, resveratrol induced cell cycle arrest at the S and G2 phases in KKU-M156 cells by increasing cyclin E, Cdk2, p53, p21 and p27 levels and decreasing cyclin B1 and Cdk1 levels. Subsequently, resveratrol induced apoptosis of both cell lines by increasing the Bax/Bcl-2 ratio and apoptosis-inducing factor, and decreasing survivin and subsequent activation of caspase-9 and -3 and DNA fragmentation.

Conclusion: Our study highlighted for the first time that resveratrol had different effects on cell cycle progression and apoptosis mechanisms in different CCA cell types. Resveratrol inhibits growth of the KKU-100 and KKU-M156 cell lines by arresting different phases of the cell cycle and inducing a mitochondrial-dependent apoptosis pathway, through both caspase-dependent and -independent means. These results suggest that resveratrol could be developed as a chemotherapeutic agent against CCA.

Keywords: Apoptosis, cell cycle arrest, human cholangiocarcinoma cell lines, mitochondrial-dependent signaling pathway, resveratrol

Cholangiocarcinoma (CCA) is a malignant tumor arising from the intrahepatic or extrahepatic bile duct epithelium [1]. The highest incidence of this cancer has been reported in northeastern Thailand with age-standardized incidence rates of 94.8:100 000 in males and 39.4:100 000 in females [2]. An increasing

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incidence and mortality from intrahepatic CCA, but not extrahepatic CCA, has been reported globally [3, 4]. Due to the inability to detect or prevent early tumor formation, a majority of CCA patients presenting with advanced or unresectable disease die within 12 months. The overall 5-year survival rate of CCA patients is less than 5% [5]. To date, there is no standard chemotherapy regimen for inoperable CCA patients; therefore, effective therapeutic agents and/or adjuvant therapy for CCA are necessary.

Resveratrol (trans-3, 4', 5-trihydroxystilbene) is a polyphenolic phytoalexin found in grapes, nuts, fruits and red wine [6]. Resveratrol exhibits cancer chemopreventive activity through inhibition of tumor initiation, promotion, and progression [7]. Resveratrol appears to inhibit cell growth, induce cell cycle arrest and apoptosis in a number of cancer cell lines via several signalling pathways [8-11]. A recent study demonstrated that resveratrol inhibited cell growth and induced cell cycle arrest and apoptosis in SK-ChA-1 cell line, an undifferentiated adenocarcinoma of extrahepatic CCA [12]. Compared to extrahepatic CCA, intrahepatic CCA is the more common type of CCA in Thailand. Moreover, the incidence of intrahepatic CCA is reportedly increasing in several countries. This study therefore aimed at elucidating the anticancer activity and molecular mechanisms of resveratrol against two human intrahepatic CCA-derived cell lines (*viz.*, KKU-100 and KKU-M156 cell lines).

Materials and methods

Cell culture

The CCA KKU-100 and KKU-M156 cell lines were isolated from Thai CCA patients, previously described [13, 14]. Human peripheral blood mononuclear cells (PBMCs) were freshly isolated using the standard Ficoll-hypaque gradient centrifugation method after which they served as the normal control cells [9]. Cells were grown in RPMI 1640 (GIBCO BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL of penicillin and 100 μg/mL of streptomycin (GIBCO BRL) at 37°C in a humidified incubator containing 5% CO_2.

Cell proliferation assay

Inhibition of cell proliferation by resveratrol (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) was determined by sulforhodamine B (SRB) assay. Cells (1.9×10^4 cells/well) were seeded in 96-well plates, incubated for 24 h, and then treated with DMSO or resveratrol (0-100 μM/well) for 24, 48, and 72 h. The percentage of cell viability and the 50% inhibitory concentration (IC_{50}) were determined [15, 16].

Cell cycle analysis

Cells (1×10^6) were plated in 10-cm dishes (Corning Incorporation, MA, USA) and incubated at 37°C for 24 h. Cells were treated with DMSO or resveratrol (10, 30, and 50 μM) for 48 h. Untreated cells were also included in this experiment. After treatments, the cells were washed with cold PBS, fixed overnight in 70% ethanol at 4°C then stained for cell cycle analysis using 200 μL GUAVA® cell cycle reagent (GUAVA Technologies Inc., USA) at room temperature for 30 min in the dark, as per the manufacturer's instructions. The cell cycle distribution was analyzed in three different experiments (using 10,000 cells per analysis) by a FACSCalibur flow cytometer (Becton Dickinson Bioscience, San Jose, USA) and the data analyzed using CellQuest software.

Cytological studies

The KKU-M156 cells (2.4×10^5) were plated onto 10-cm dishes and incubated at 37°C for 24 h. Cells were treated with DMSO or 50 μM of resveratrol for 0, 12, 24, 36, and 48 h. Cytological studies were done as previously described [17]. At least 1,000 cells were counted from five randomly selected fields. The mitotic index was defined as the percentage of cells showing mitosis with condensed chromosomes.

Morphological examination

KKU-100 and KKU-M156 cells (1.9×10^3 cells/well) were grown in 96-well microtitre plates at 37°C for 24 h and treated with DMSO or $2 \times IC_{50}$ concentrations of resveratrol (60 and 48 μM, respectively) for 24 and 48 h. Morphological changes occurring in the cells were observed under a bright field inverted Nikon microscope. To accomplish the nuclear staining, the treated cells were stained with 14 μL of 100 μg/mL ethidium bromide/acridine orange (EB/AO) mixture and observed under a Nikon fluorescent microscope. Apoptotic cells with condensed chromatin or fragmented chromatin were counted and expressed in percentage from a total of 500 cells each [16, 18].

DNA fragmentation assay

The fragmented DNA was isolated according to Herrmann *et al* [19] with some modifications. Briefly, after culturing for 24 h and starving in medium containing 0.5% FBS for 24 h, both of the CCA cell lines (1×10^6) were cultured in DMSO or $2 \times IC_{50}$ concentrations of resveratrol for 24 and 36 h. The DNA was extracted and purified using a QIAamp DNA Blood Mini Kit (QIAGEN, Germany) as per the manufacturer's instructions. The DNA fragments were precipitated with ethanol, re-suspended in TE buffer, and analyzed by electrophoresis [16].

Protein extraction and Western blot analysis

After treating the cells with DMSO or $2 \times IC_{50}$ concentrations of resveratrol for 0, 12, 24, and 48 h, protein extraction and Western blotting were performed [16, 20]. After blocking, the membranes were incubated at 4°C overnight with the indicated primary antibody; including antibodies against cyclin A, cyclin B1, cyclin D1, cyclin E, Cdk1, Cdk2, Cdk4, p21, p27, Bax, Bcl-2, survivin, AIF (Santa Cruz Biotechnology, CA) or procaspase-9 and -3, activated caspase-9 (Cell Signaling, Beverly, MA) or activated caspase-3, β-actin (Sigma Chemical, St. Louis, MO) or p53 (Upstate, CA). Immunoreactive bands were then incubated with the corresponding horseradish peroxidase-conjugated goat anti-mouse IgG and goat anti-rabbit IgG antibodies (Santa Cruz Biotechnology) at room temperature for 1 h, followed by detection using an enhanced chemiluminescence kit (Pierce Biotechnology, Rockford). The intensity of the protein bands was quantified using Scion Image software.

Statistical analysis

Data were expressed as means±SEM. Differences between untreated control cells and the resveratrol-treated cells were tested for statistical significance using Student's *t*-test. Differences were considered significant at $P < 0.05$. All analyses were performed using SPSS version 10.0.

Results

Growth inhibitory effect of resveratrol on CCA cell lines

Figure 1 shows that resveratrol caused marked growth inhibition in a dose- and time-dependent manner with IC_{50} values of 74±0.18 μM and 30±0.10 μM in the KKU-100 cells at 48 and 72 h, respectively. By comparison, the IC_{50} values for the KKU-M156

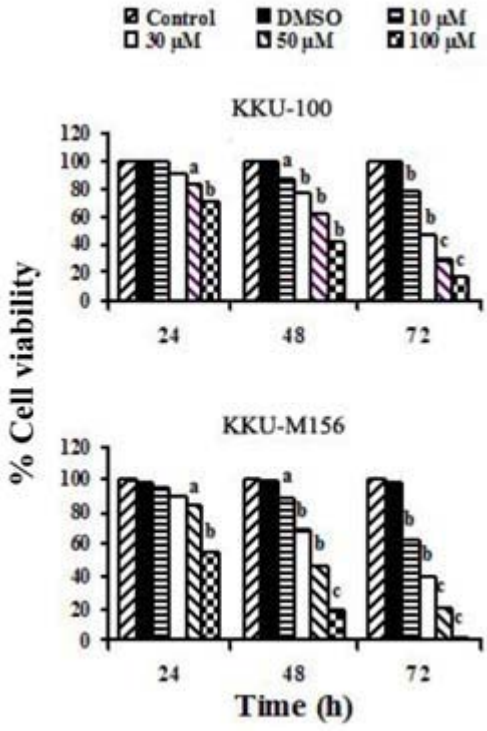

Figure 1. Growth inhibitory activity of resveratrol against KKU-100 and KKU-M156 cells. Cells were treated with DMSO or the indicated concentrations of resveratrol for 24, 48, and 72 hours. The percent cell viability was determined by SRB assay. Data are expressed as mean±SEM (n=3). $^a p < 0.05$, $^b p < 0.01$, $^c p < 0.001$ *vs.* control.

cells were 47±0.11 μM and 24±0.20 μM at 48 and 72 h, respectively. Treatment of normal human PBMCs with resveratrol resulted in very little growth inhibitory effect with an IC_{50} value of 2.70±0.09 M. Thus, the growth inhibition of the CCA cell lines induced by resveratrol was about 9×10^4 times greater than the normal PBMCs. The differences in the IC_{50} values of resveratrol against the KKU-100 and KKU-M156 cells could reflect a cell-specific alteration of the cell cycle and eventually the activation of different mechanisms of cell death.

Effect of resveratrol on cell cycle distribution

Treatment of the KKU-100 cells with resveratrol resulted in a significant dose-dependent accumulation of cells in the G0/G1 phase (from 64 to 80%) with a significant decrease in the cell population in the G2/M phase (from 29 to 12%). The cell population in the S phase did not significantly change from 7 to 8% as compared with the corresponding controls (**Figure 2A**). In the KKU-M156 cells, resveratrol induced a significant dose-dependent accumulation of cells in the G2/M phase (from 31 to 52%) and in the S

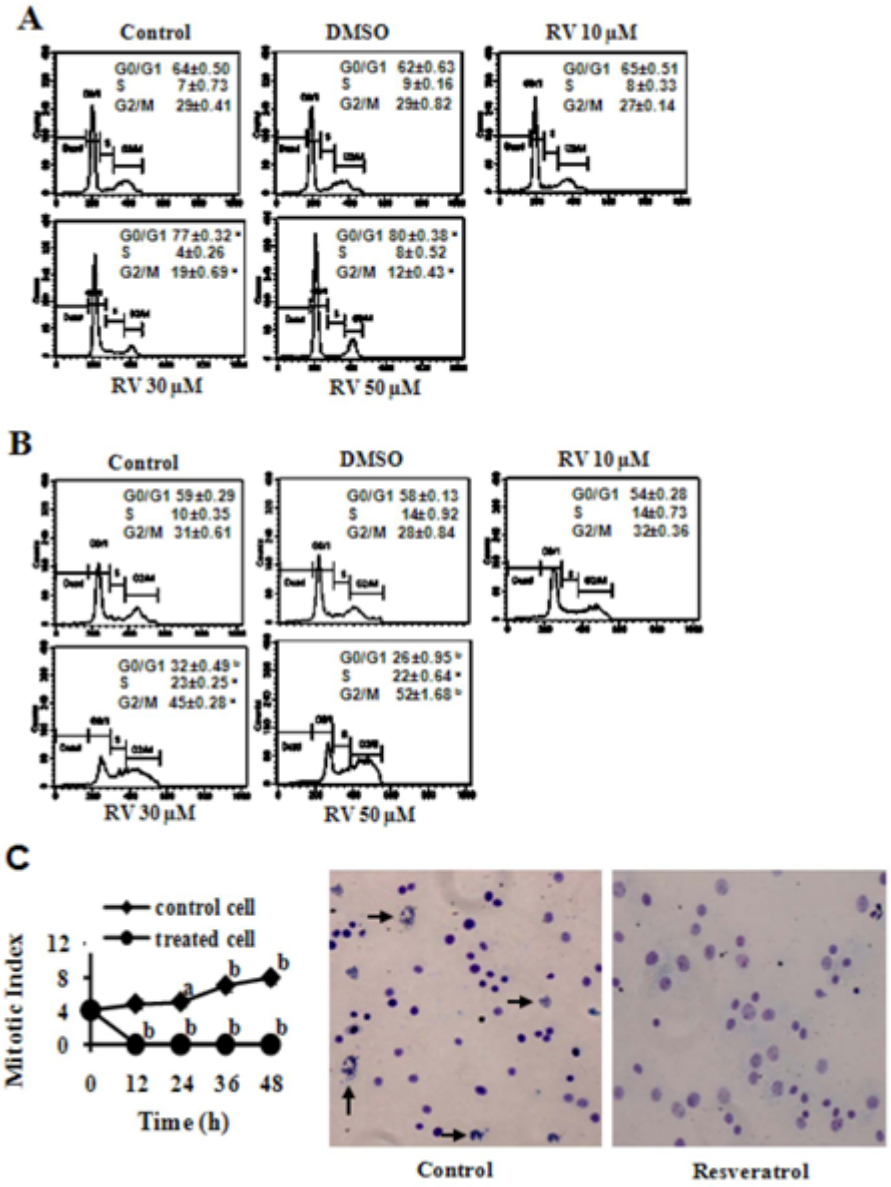

Figure 2. Effect of resveratrol on cell cycle distribution in KKU-100 **(A)** and KKU-M156 **(B)** cells. CCA cell lines were untreated or treated with DMSO or the indicated doses of resveratrol for 48 h. Cell cycle distribution of both cell lines was analyzed by flow cytometry. The percentage of cells in G0/G1, S and G2/M phases of the cell cycle are expressed as the mean±SEM (n = 3). G2 phase arrest in KKU-M156 cells **(C)**. Cells were treated with DMSO or 50 μM resveratrol for the indicated times, and stained with Giemsa Grunwald stain. The graph shows the mean±SEM of the mitotic index of KKU-M156 cells (n = 3) (left panel). Photomicrographs show mitotic cells (arrows) (middle and right panel). $^{a}p<0.05$, $^{b}p<0.01$ *vs.* control.

phase (from 10 to 22%), with a significant concomitant reduction of cells in the G0/G1 phase (from 59 to 26%) (**Figure 2B**). In our experiment, the cell cycle distribution of vehicle-treated cells was apparently not affected compared with the non-treated cells. Our results suggest that resveratrol might affect the regulatory mechanisms that are essential for cell cycle progression.

Induction of G2 or M arrest by resveratrol in KKU-M156 cell

To determine whether resveratrol induced G2 or M phase arrest, the percentage of mitotic cells among the resveratrol-treated KKU-M156 cells was determined; whereupon we found that the percentage of mitotic cells had decreased from 4% at 0 h to 0% at 12 h and completely disappeared at 48 h (**Figure 2C**).

Effect of resveratrol on the expression of cell cycle regulators

The mechanism of the resveratrol-induced G0/G1 arrest was explored in the KKU-100 cells. Immunoblot analysis revealed that resveratrol treatment of the cells resulted in a significant dose-dependent decrease in protein expressions of cyclin D1, cyclin E, Cdk4, and Cdk2 compared to the control cells (**Figures 3A** and **B**). Since both positive and negative regulators are necessary for cell cycle progression, the expression of tumor suppressor p53 and the CDKIs (p21 and p27) were also determined. As shown in **Figures 3A** and **B**, resveratrol induced a significant dose-dependent increase in p53, p21, and p27 protein expression.

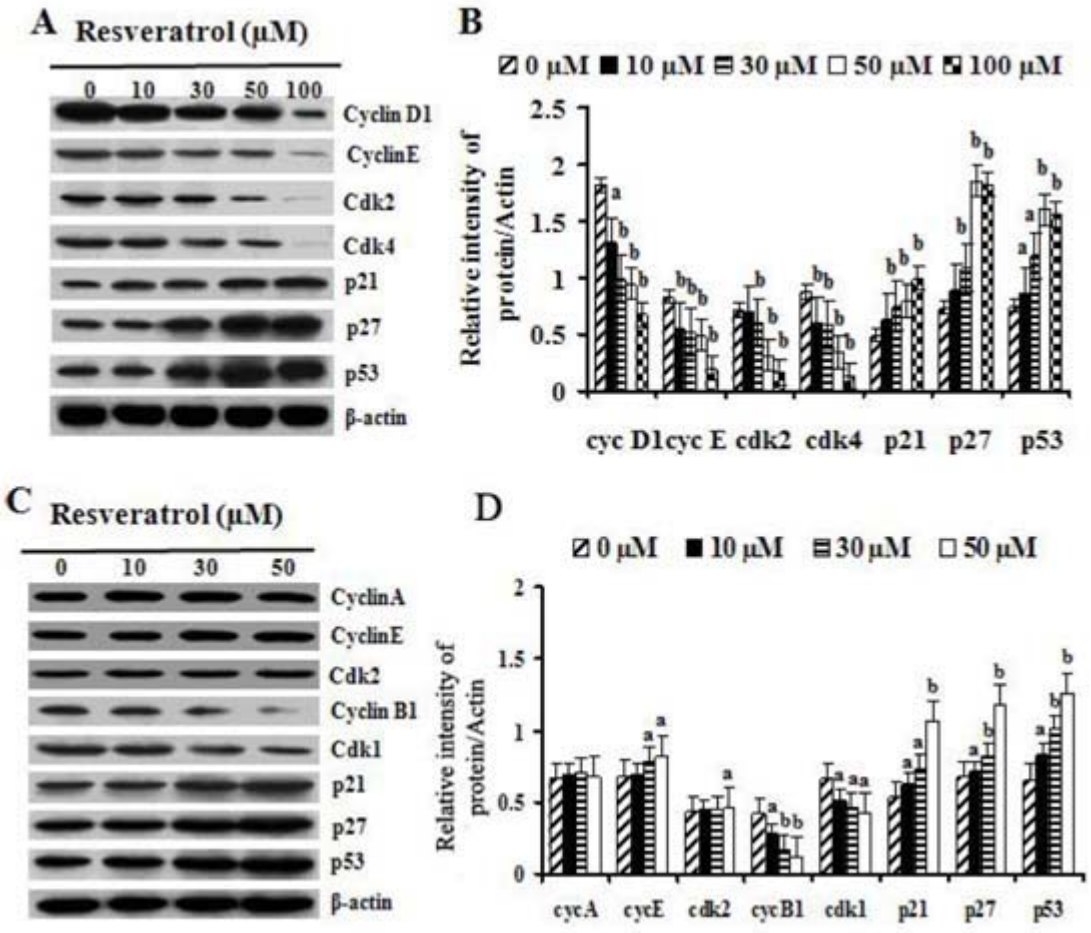

Figure 3. Effect of resveratrol on the expression of cell cycle regulatory proteins at the G0/G1 phase of the cell cycle in the KKU-100 cells **(A, B)** and at the S and G2 phases of the cell cycle in the KKU-M156 cells **(C, D)**. CCA cell lines were treated with DMSO or indicated doses of resveratrol for 48 h. Western blot analysis for each protein was performed. Shown are representative protein bands of KKU-100 **(A)** and KKU-M156 **(C)** cells. The histograms show the density of target protein normalized to β-actin protein of KKU-100 **(B)** and KKU-M156 **(D)** cells. Data are expressed as mean±SEM (n = 3). ^{a}p <0.05, ^{b}p <0.01 *vs.* control. cyclin A (cyc A), cyclin B1 (cyc B1), cyclin D1 (cyc D1), cyclin E (cyc E).

In the KKU-M156 cells, the resveratrol treatment resulted in both S and G2/M phase arrest, so it was possible to evaluate the expression of S and G2/M regulators. With regard to S phase arrest, resveratrol induced a significant dose-dependent increase in the protein expression of cyclin E and Cdk2 (**Figures 3C** and **D**), while the level of cyclin A was slightly increased at 30 μM of resveratrol. Regarding G2/M phase arrest, resveratrol significantly decreased protein expression of cyclin B1 and Cdk1, while the levels of p21, p27, and p53 were significantly increased (**Figures 3C** and **D**).

Apoptosis induction by resveratrol in CCA cell lines

In both cell lines, resveratrol treatment resulted in cell shrinkage, rounding and membrane blebbing, the typical characteristics of apoptotic cells (**Figures 4C** and **D**), whereas no corresponding effect was observed on the vehicle control cells (**Figures 4A** and **B**). By using EB/AO staining, the nuclei with homogeneous chromatin distribution could be seen in the vehicle control cells (**Figures 4E** and **F**), while the chromatin condensation and nuclear fragmentation , characteristic apoptotic features, were observed in the resveratrol-treated cells **(Figures 4G** and **H)**. It is evident that resveratrol induced apoptosis in both CCA cell lines in a time-dependent manner (**Table 1**). By way of corroboration, **Figures 4I** and **J** illustrate that resveratrol induced a typical ladder pattern of internucleosomal DNA fragmentation-a hallmark of apoptotic cell death [21].

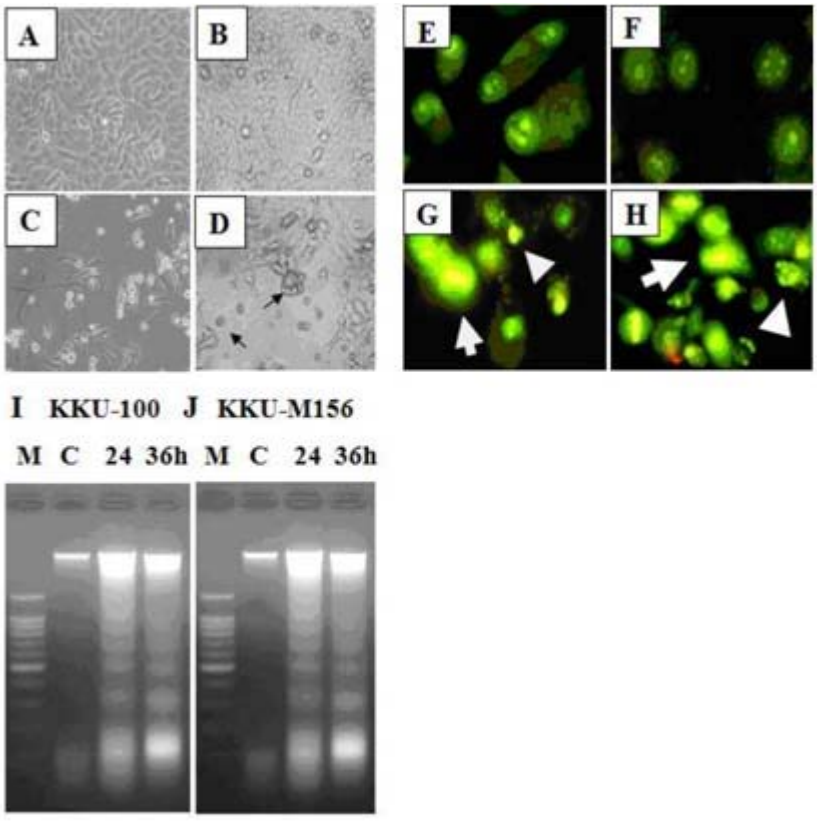

Figure 4. Induction of apoptosis by resveratrol in CCA cell lines. CCA cell lines were treated with DMSO or 2 x IC_{50} concentrations of resveratrol for 48 h. Cell morphology: photomicrographs (400x) show the DMSO treated KKU-100 **(A)** and KKU-M156 **(B)** cells or resveratrol treated KKU-100 **(C)** and KKU-M156 **(D)** cells. Cells with membrane blebbing are indicated by arrows. EB/AO staining: fluorescence photomicrographs (400x) show the DMSO treated KKU-100 **(E)** and KKU-M156 **(F)** cells and the resveratrol treated KKU-100 **(G)** and KKU-M156 **(H)** cells. Nuclei of treated cells showed chromatin condensation (arrows) and nuclear fragmentation (arrow heads). DNA fragmentation in KKU-100 **(I)** and KKU-M156 **(J)** cells. Cells were treated with DMSO or 2 xIC_{50} concentrations of resveratrol for the indicated times (24 h and 36 h). Genomic DNA was isolated and separated on 1.6% agarose gels containing 0.1 mg/mL ethidium bromide. The figures show representative results of three independent experiments. **M**, a 100 bp DNA marker; **C,** DNA of DMSO treated cells.

Table 1. Percentage of apoptotic cells of the resveratrol-treated and non-treated CCA cell lines

Compounds	% Apoptotic cells			
	KKU-100		KKU-M156	
	24 hours	48 hours	24 hours	48 hours
Control	10±0.42	18±1.11	12±0.08	23±0.24
Resveratrol	44±1.00[b]	92±0.20[b]	39±0.68[b]	87±0.86[b]

Data are means±SEM of three independent experiments. [b]p <0.01 *vs*. control

Effect of resveratrol on the expression of apoptosis-related proteins

Resveratrol treatment in both CCA cell lines resulted in a significantly increased in proteins (**Figure 5A**) of pro-apoptotic Bax but a decrease in the anti-apoptotic Bcl-2 and survivin (**Figure 5A** and **Table 2**) in a time-dependent manner, leading to an increase in the Bax/Bcl-2 ratio (**Figure 5B**).

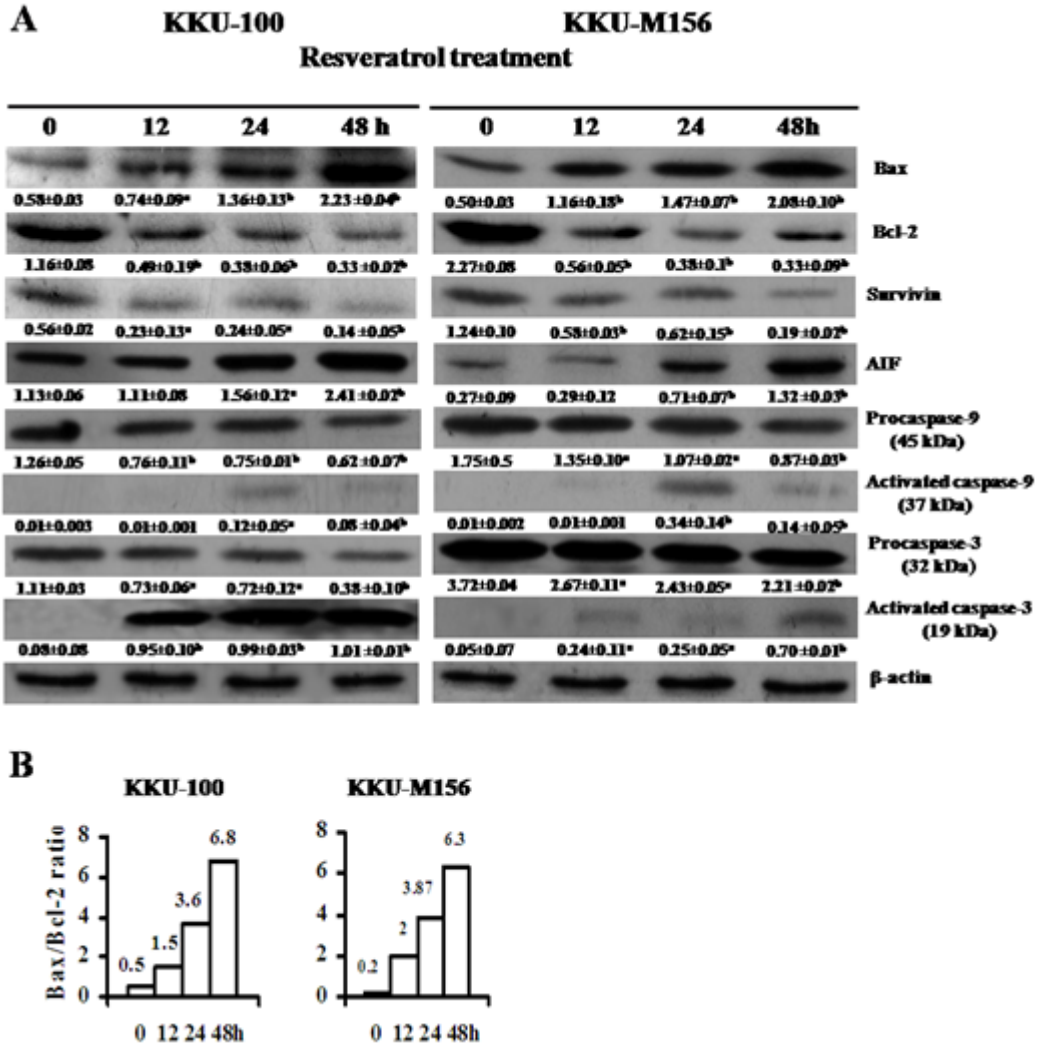

Figure 5. Effect of resveratrol on the expression of apoptosis-related proteins in CCA cell lines. KKU-100 and KKU-M156 cells were treated with DMSO or 2 x IC_{50} concentrations of resveratrol for the indicated times. The protein expression was determined by Western blotting **(A)**. The mean±SEM of density reading of target protein normalized to β-actin was expressed in the respective box (n = 3). [a]p <0.05, [b]p <0.01 *vs*. control. Bax/Bcl-2 ratio **(B)**.

Table 2. The multiple-fold decrease or increase of apoptotic related proteins of the resveratrol-treated compared to non-treated CCA cell lines.

Apoptotic proteins	KKU-100				KKU-M156			
	0	12	24	48 h	0	12	24	48 h
Activated caspase-9	1	1	12	8	1	1	34	14
Activated caspase-3	1	11	12.4	12.6	1	4.8	5	14
Survivin	1	0.4	0.4	0.3	1	0.5	0.5	0.2
AIF	1	1	1.4	2.1	1	1.1	2.6	4.9

Data are expressed as ratio of mean of protein expression level of the apoptotic related proteins in the resveratrol-treated to non-treated CCA cell lines.

Western blot analysis revealed that both CCA cell lines treated with resveratrol resulted in a significant decrease in procaspase-3 and -9 concomitant with an increase in the activated caspase-3 and -9 and AIF protein in a time-dependent manner (**Figure 5A** and **Table 2**). At 48 h of treatment, similar multi-fold increases in the AIF, activated caspase-3 and -9 were observed while there was a decrease in survivin protein expression (**Table 2**).

Discussion

Using two human CCA cell lines, with different histologic types and different drug sensitivities [13], the effect of resveratrol on cell growth, cell cycle progression and the induction of apoptosis were analyzed. As with previous reports [22] for other cancer cell lines, our results showed that resveratrol inhibited cell growth to a similar extent in both the KKU-100 and KKU-M156 cell lines in a dose- and time-dependent manner, and exhibited selective cytotoxic effects against both CCA cells in comparison with normal cell. The latter results agree with a report that resveratrol has specific cytotoxic effects toward tumor cells compared with normal PBMCs [9].

Resveratrol treatment caused a significant accumulation of cells in the G0/G1 phase of the KKU-100 cells over against the S and G2/M phases of the KKU-M156 cells. According to a previous report and our study, resveratrol was found to arrest SK-ChA-1 cells (an undifferentiated adenocarcinoma from an extrahepatic CCA) [12, 23] and KKU-100 cells (a poorly-differentiated adenocarcinoma from an intrahepatic CCA) at the G1 phase of the cell cycle; whereas KKU-M156 cell (a moderately-differentiated adenocarcinoma from an intrahepatic CCA) was arrested at the S/G2 phase of the cell cycle. These results indicate that resveratrol modulates cell cycle distribution at different phases depending on the type of cancer cells [22, 24-26].

It is now well-established that in eukaryotes, cell cycle progression is controlled by multiple cyclins, Cdks, CDKIs, and p53 protein [27, 28]. Passage through the restriction point, and transition from the G1 to the S phase, is triggered by the phosphorylation of Rb by activated Cdk4, 6/cyclin D complexes that result in its dissociation from E2F, which is then free to initiate DNA replication [27]. The kinase activity of the Cdk is negatively regulated by the binding of CDKIs; $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ [28]. As explained elsewhere, the p53 protein was found to enhance the transcriptional rate of several genes, including p21, which plays an important role in the cell cycle arrest and apoptosis [29]. Our results indicate that in KKU-100 cells, the mechanism of G1 arrest is achieved by reductions in the cyclin D1, cyclin E, Cdk4 and Cdk2 protein levels while increasing the p53, p21 and p27 protein levels. Similarly, resveratrol elicited an anti-proliferative effect by targeting cyclin D1 and Cdk4 in DU-145, which is associated with the induction of p53 and p21, in DU-145 [30] and MCF-7 [10].

Using flow cytometry on, and morphological examination of, KKU-M156 cells indicated that resveratrol induced cell cycle arrest at both the S and G2 phases, as previously shown of HepG2 cells [25]. A possible mechanism for the partial cell arrest in the S phase might be due to the elongation of the S phase, most likely a result of DNA repair mechanisms related to cell cycle restriction [31]. The cyclin E/Cdk2 complex is required for cells to make the transition from the G1 into the S phase [27]; while the cyclin A/Cdk2 complex is required for entry into, and completion of, the S phase [27]. Our results suggest that the entry of the treated cells into S phase was confirmed by an increase in cyclin E and Cdk2 protein as previously found in melanoma cells [26].

Approximately 52% of the KKU-M156 cells were found in the G2/M phase suggesting that resveratrol can partially disrupt the S/G2 transition. The G2 checkpoint was also affected by the resveratrol. Mitosis in human cells is initiated by the cyclin B1/Cdk1 complex, which is activated at the end of G2 phase [32]. Our results suggest a possible mechanism of G2 arrest in KKU-M156 cell was due to a decrease of the Cdk1 and cyclin B1 protein levels and an increase in the p53, p21, and p27 protein levels. Similar results were found in human prostate cancer cell lines [22].

Several studies indicate that apoptotic cell death, induced by resveratrol, is closely linked to modulation of a specific phase of the cell cycle in a cell-specific manner, such as G0/G1 phase arrest in HL-60 cells [33], and the S phase arrest in the human lung cancer cell line A549 [24]. In a previous study using different CCA cell line, the SK-ChA-1 cell cultured in the two-dimensional model and the three dimensional spheroids, resveratrol was found to induce cell cycle arrest at the G1/S phase leading to apoptotic cell death through an increased intracellular transglutaminase activity [12]. Our results confirm the previous studies in which the cellular morphological changes of resveratrol-treated KKU-100 and KKU-M156 cells demonstrated the typical characteristics of apoptotic cells. The induction of apoptosis in both CCA cell lines by resveratrol was through the mitochondrial apoptotic pathway; associated with the induction of p53, up-regulation of Bax protein expression, down-regulation of Bcl-2 protein expression, and activation of caspase-9 and -3, leading to DNA fragmentation [34]. A similar result was also found in MCF-7 cells [10]. Resveratrol also induced apoptosis through the mitochondrial apoptotic pathway in a caspase-independent manner through induction of AIF protein expression in both CCA cell lines [35]. An increased level of activated caspase-3 and -9, correlating with a decreased of survivin protein, indicated that down-regulation of the survivin protein maintains caspase-3 in an active state and stimulates the molecular cascade of apoptosis [36]. A similar result was shown in U937 cells [37].

These findings may lead to a future application for treatment of cholangiocarcinoma or chemo-prevention. In this study, the IC_{50} values of resveratrol in KKU-100 and KKU-M156 cells were around 24-30 μM. These concentrations were very close to the concentrations of resveratrol found in the kidney (30 μM) and liver (25 μM) of Balb/c mice after administration of resveratrol 66mg/kg [38]. As reviewed by Athar et al. [39], many studies have reported that dietary resveratrol (10 or 50 ppm) has antitumor growth and anti-metastasis effects in Donryu rats, subcutaneously implanted with an ascites hepatoma cell line as well as inhibition of ascites hepatoma in rats i.p. injected daily with 1 mg/kg of resveratrol for seven days. According to a study by Frampton et al. [40], resveratrol at a low dose (20 μM in *in vitro*, or 10 mg/kg in *in vivo*) could induce a greater sensitivity in cholangiocarcinoma cells to 5-fluorouracil. Furthermore, resveratrol, at a dose of up to 5 g/day, was found to be safe and reasonably well-tolerated with mild to moderate side effects in healthy participants [41]. Notwithstanding, a normal dietary intake of resveratrol by people seems to be less than the active dose [38]. Therefore, consumption of some dietary sources rich in resveratrol such as red wine (14.3 mg/L), rhubarb (3.9 mg/g) or red grape juices (0.50 mg/L) as food supplements may provide a health benefit to maintain a sufficient level of resveratrol for cancer chemoprevention [42].

In summary, our study highlighted for the first time that resveratrol had variable effects on the cell cycle progression and apoptosis mechanisms in different CCA cell types. Although resveratrol was able to induce apoptosis of the human CCA cell lines KKU-100 and KKU-M156, its efficacy depended on the phase of the cell cycle arrested. Based on the present findings, resveratrol may have potential as a chemotherapeutic agent in the management of human CCA. Further molecular mechanism studies are therefore required to assess the effects of resveratrol in *in vivo* models and/or in compassionate clinical trials in end-stage cholangiocarcinoma patients.

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