Resveratrol Induces Downregulation in Survivin Expression and Apoptosis in HTLV-1-Infected Cell Lines: A Prospective Agent for Adult T Cell Leukemia Chemotherapy

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Abstract: Resveratrol, a phytoalexin found in grapes and wine, has been shown to exhibit a wide range of pharmacological properties and is believed to play a role in the chemoprevention of human cancer. Resveratrol has also been shown to induce antiproliferation and apoptosis of several leukemia cell lines. In the present study, we investigated the effect of resveratrol in adult T cell leukemia. Our present observations showed that resveratrol induced growth inhibition in all five human T cell lymphotrophic virus-1-infected cell lines examined, with 50% effective dose of 10.4–85.6 μM. In the resveratrol-treated cells, induction of apoptosis was confirmed by annexin V-based analyses and morphological changes. The most surprising observation was that resveratrol treatment resulted in a gradual decrease in the expression of survivin, an antiapoptotic protein, during cell apoptosis. These findings indicate that resveratrol inhibits the growth of human T cell lymphotrophic virus-1-infected cell lines, at least in part, by inducing apoptosis mediated by downregulation in survivin expression. In view of the accumulating evidence that survivin may be an important determinant of a clinical response in adult T cell leukemia, our present findings have led to the suggestion that resveratrol, a common constituent of the human diet, merits further investigation as a potential therapeutic agent for this incurable disease.

Introduction

The polyphenolic compound resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a naturally occurring phytochemical and can be found in a wide variety of plant species, including grapes, peanuts, and various herbs, and thus is a common constituent of the human diet. Because resveratrol is synthesized in the leaf epidermis and the skin of grapes, red wine and grapes are probably its main source in Western diets (1,2). Resveratrol was also identified as the active constituent of the dried roots of *Polygonum cuspidatum,* also called Ko-jo-kon in Japanese, used since ancient times in traditional Asian folk medicine against suppurative dermatitis, gonorrhea, favus, athlete's foot, and hyperlipidemia (3). A large number of studies demonstrated that resveratrol modulates many biological activities, including lipoprotein metabolism, eicosanoid synthesis, oxidation, and platelet aggregation (4–6). Therefore, resveratrol seems to have a very broad range of biological properties. In addition to these findings, resveratrol was recently shown to have cancer-chemopreventive (e.g., anti-initiation, antipromotion, and antiprogression) activity and to inhibit the growth of a number of human cancer cell lines (7); however, the molecular mechanism of its antitumor action has not been well clarified.

Adult T cell leukemia (ATL) is defined as a mature CD4$^+$ T cell leukemia (8,9) caused by human T cell lymphotrophic virus-1 (HTLV-1) (10–12). The clinical features of ATL constitute at least four different subtypes, i.e., acute, chronic, lymphoma, and smoldering, depending on the aggressiveness of the disease, including the serum lactate dehydrogenase and calcium levels (13,14). Because ATL is a very progressive and lethal disease (with a mean survival of only 8 mo) that shows high developmental resistance to conventional chemotherapies, new therapeutic strategies are required to improve the prognosis.

Apoptosis is regulated tightly by a number of gene products that promote cell death or extend cell survival. The mammalian Bcl-2 family of apoptosis-associated proteins consists of members that inhibit apoptosis (e.g., Bcl-2 and Bcl-X$_L$) and others that induce apoptosis (e.g., Bax), and the balance between pro- and antiapoptotic members determines

the fate of cells in many systems (15,16). The overexpression of Bcl-2 and/or Bcl-X_L is found in many human malignant tumors including ATL, and it is believed to protect the cells against apoptosis (17–19). In addition to the Bcl-2 family, another group of molecules regulating apoptosis has come to light recently. The first member of this family, defined as an inhibitor of apoptosis (IAP), was identified in the baculovirus and was characterized for its ability to blunt the host suicidal response to infection. Human IAP proteins are characterized by the presence of one to three copies of a 70-amino acid motif, the BIR domain, which bears homology to sequences found in the baculovirus IAP proteins. IAPs have been reported to inhibit apoptosis owing to their function as direct inhibitors of activated effector caspases (20,21). Survivin is a recently characterized mammalian IAP. Unique among other IAPs, survivin is selectively expressed during development and in all the most common human cancers, but not in normal adult tissues in vivo, indicating that its overexpression in cancers plays a general role in apoptosis inhibition in tumor progression (22). The high expression of survivin in cancers including ATL has been reported to be correlated with their drug resistance and/or shorter survival (23–28).

In the present study, we investigated the effect of resveratrol in ATL. To gain insights into its mechanism of action, we examined the effects of resveratrol on cell viability, apoptosis, and the levels of several apoptosis control proteins in HTLV-1-infected cell lines. Our present results demonstrate that resveratrol can inhibit the growth of HTLV-1-infected cell lines by causing apoptosis dependent on the downregulation of survivin expression and further support the use of resveratrol in clinical chemotherapy trials for ATL.

Materials and Methods

Cell Lines

The cell line KOB was established from patients' ascitic ATL cells. Cell lines KK1, SO4, and ST1 were of primary ATL cell origin. All these cell lines were interleukin-2 (IL-2) dependent and were confirmed by the concordance of the integration site(s) of the HTLV-1 proviral genome and/or the T cell receptor β-chain gene rearrangement profiles with those of the respective original leukemia cells (29,30). These ATL cell lines were maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and recombinant IL-2 (0.25 U/ml; kindly provided by Takeda Chemical Industries, Osaka, Japan). The HTLV-1-infected T cell line HUT102, established from a patient with cutaneous T cell lymphoma originally diagnosed as having Sezary syndrome but later considered lymphoma-type ATL, was cultured in RPMI 1640 medium supplemented with 10% FBS. All cells were maintained at 37°C in 5% CO_2-95% air.

Cell Growth Assay

To examine the effect of resveratrol on the viability of the cell lines, cells (2×10^5 cells/ml) were cultured in the conditions described above in 96-well tissue culture plates with 1% dimethyl sulfoxide (DMSO) or increasing doses (5–80 μM) of resveratrol (Calbiochem, Darmstadt, Germany) for 72 h, and the viability status of each cell line was then estimated by measuring the conversion of 3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2,4-sulfophenyl)-2H-tetrazolium (MTS) into water-soluble formazan (CellTiter 96 AQueous, Promega, Madison, WI). After color development, the absorbance at 490 nm (indicating the number of living cells) was measured using a Benchmark Microplate Reader (Bio-Rad, Fukuoka, Japan).

Detection of Apoptosis

In the process of early apoptosis, cells express phosphatidylserine on the outer leaflet of the cell surface membrane and consequently bind to annexin V. ST1, KK1, SO4, KOB, and HUT102 cells ($0.5–1.0 \times 10^6$/ml) cultured with 1% DMSO or the indicated concentrations (12.5–100 μM) of resveratrol for 18 h were examined for annexin V binding by flow cytometry using an annexin V-fluorescein isothiocyanate (FITC) kit (Takara Shuzo, Shiga, Japan) according to the manufacturer's protocol. In addition, ST1 cells (2.0×10^5/ml) cultured with 1% DMSO or 5, 10, and 20 μM resveratrol for 24, 48, and 72 h were examined for annexin V binding by flow cytometry.

Reverse Transcription-Polymerase Chain Reaction

Total cellular RNA was extracted from the HTLV-1-infected cell lines using ISOGEN kits (Nippon GENE, Toyama, Japan) and treated with deoxyribonuclease (Message Clean Kit, GenHunter, Nashville, TN) to remove contaminating DNA. Reverse transcription-polymerase chain reaction (RT-PCR) was performed according to the manufacturer's directions (GeneAmp RNA PCR kit, Perkin-Elmer, Foster, CA). One microgram of RNA was used for cDNA synthesis, which was carried out using Moloney murine leukemia virus RT (2.5 U/μl) and 2.5 μM oligo[d(T)$_{16}$] as primer (total 20 μl) for 20 min at 42°C. The reaction was stopped by heating the sample to 99°C for 5 min. Aliquots (1 μl) of cDNA were amplified in a 25-μl final volume with the specific primers for Bcl-2, Bcl-X_L, Bax-α, cIAP-I, and survivin, with β-actin as a control, for successful cDNA synthesis (Table 1) (31–33). The reaction was performed using *Taq* DNA polymerase (Qiagen, Tokyo, Japan) and 24–27 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min for Bcl-2, 24–27 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min for Bcl-X_L and Bax-α, 23–26 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min for cIAP-I, 22–24 cycles of 94°C 30 s, 62°C for 1 min, and 72°C for 1 min for survivin, and 24 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min for β-actin. The PCR products were separated on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. The band densities were quantified by densitometry (IP Lab Gel Imaging Sys-

Table 1. PCR Primers

Gene	Primer Sequence	PCR Product, bp
Bcl-2	Sense: 5′-GGACAACATCGCCCTGTG-3′	148[a]
	Antisense:	
	5′-AGTCTTCAGAGAGACAGCCAGGA-3′	
Bcl-X$_L$	Sense: 5′-TTGGACAATGGACTGGTTG-3′	765[b]
	Antisense:	
	5′-GTAGAGTGGATGGTCAGTG-3′	
Bax-α	Sense: 5′-CTGACATGTTTTCTGACGGC-3′	290[b]
	Antisense:	
	5′-TCAGCCCATCTTCTTCCAGA-3′	
cIAP-I	Sense: 5′-ACAAAACTGCCTCCCAAAGA-3′	824
	Antisense:	
	5′-AAATGTTCTCATTCGAGCTGC-3′	
Survivin	Sense: 5′-GCATGGGTGCCCCGACGTTG-3′	447[c]
	Antisense:	
	5′-GCTCCGGCCAGAGGCCTCAA-3′	
β-actin	Sense: 5′-TCATCACCATTGGCAATGAG-3′	154
	Antisense:	
	5′-CAGTGTGTTGGCGTACAGGT-3′	

a: Ref. 31.
b: Ref. 32.
c: Ref. 33.

tem, Signal Analysis, Vienna, VA) and normalized to those of β-actin as the internal control.

Real-Time Quantitative PCR

Real-time quantitative PCR was performed using the LightCycler Technology (Roche Diagnostic, Mannheim, Germany). For survivin mRNA amplification, 1 μl of cDNA was added in a 20-μl final volume to 2 μl of Mastermix (LightCycler DNA Master hybridization probes, Roche Diagnostics), MgCl$_2$ solution (4.5 mM), forward and reverse oligonucleotide primers (0.5 μM), and fluorescent hybridization probes (0.2 μM). The PCR program included 50 cycles (95°C for 10 s, 63°C for 5 s, 72°C for 13 s). The primers and probes used for survivin mRNA reverse-transcribed amplification were as follows: 5′-TTCTCAAGGACCACCGCATC-3′ (forward primer), 5′-ACAGAAAGGAAAGCGCAACC-3′ (reverse primer), 5′-ATCCACTGCCCCACTGAGAACGA-3′ (FITC probe), and 5′-CCAGACTTGGCCCAGTGTTTCTTCT-3′ (Red 640 probe).

Detection of the PCR product was based on fluorescence resonance energy transfer between the fluorophores. The cycle threshold was defined as the cycle number at which a significant increase in the fluorescence signal was first detected. Serial dilutions of plasmids (10–10^7 molecules per reaction) of an external standard made from a clone using the survivin-PCR fragment inserted in pGEM-T Easy Vector (Promega) were used to generate the standard curve between the cycle threshold value and the logarithm of the starting copy number of plasmids. Quantification of the unknown samples was performed using the LightCycler software. The results were expressed initially as the number of target molecules per microliter of cDNA. To standardize the results for variability

in RNA and cDNA quantity and quality, we quantified total glyceraldehyde 3-phosphate dehydrogenase (GAPDH) transcripts in each sample as internal control. GAPDH mRNA amplification was done under almost the same conditions as for survivin RT-PCR except for the MgCl$_2$ concentrations (4.0 mM). The PCR program included 40 cycles (95°C for 10 s, 60°C for 5 s, 72°C for 10 s). The primers and probes used for GAPDH mRNA reverse-transcribed amplification were as follows: 5′-TGAACGGGAAGCTCACTGG-3′ (forward primer), 5′-TCCACCCTGTTGCTGTA-3′ (reverse primer), 5′-TCAACAGCGACACCCACTCCT-3′ (FITC probe), and 5′-CACCTTTGACGCTGGGGCT-3′ (red 640 probe). A GAPDH standard curve was generated by the same methods used for survivin. Normalized levels of survivin transcripts were calculated as the ratio of the amount of survivin transcripts to the amount of GAPDH transcripts (expressed as a percentage).

Results

Resveratrol Causes Dose-Dependent Growth Inhibition in HTLV-1-Infected Cell Lines

To examine the antitumor activity of resveratrol in ATL, we investigated its effect on cell viability in five HTLV-1-infected cell lines as determined by the conventional MTS assay. Exponentially dividing cells were treated with increasing concentrations of resveratrol (0–80 μM) for 72 h. In ST1, KK1, SO4, and HUT102 cells, resveratrol markedly inhibited viability in a dose-dependent manner (Fig. 1). However, only KOB cells were relatively resistant to the inhibition (Fig. 1). On the basis of the results, the calculated 50% effective doses (ED$_{50}$) of resveratrol for ST1, KK1, SO4, KOB, and HUT102 cells were 10.4, 37.3, 25.0, 85.6, and 35.7 μM, respectively.

Resveratrol Induces Apoptosis in an HTLV-1-Infected Cell Line

To determine whether the above-mentioned growth inhibition determined by the cell viability is associated with cell apoptosis, we performed an annexin V-based apoptosis assay by flow cytometry. We first selected the ST1 cell line for the assay, because its growth inhibition was the most remarkable of all the HTLV-1-infected cell lines tested (Fig. 1). ST1 cells were treated for 18 h with 0–100 μM resveratrol. This concentration range includes a ≥10-fold higher dose than the ED$_{50}$ value (10.4 μM) of ST1. The percentage of apoptotic cells was apparent in a dose-dependent fashion at >25 μM, but not at 12.5 μM (Fig. 2A) at 18 h. In ST1 cells, however, resveratrol could markedly reduce the cell growth at lower doses (even at 5 and 10 μM; Fig. 1) at 72 h. To clarify the reason for this dose discrepancy, we examined the possibility of whether there are changes in annexin V binding after prolonged cell treatment (18–72 h) with lower resveratrol concentrations (5–20 μM). As shown in Fig. 3, enhanced an-
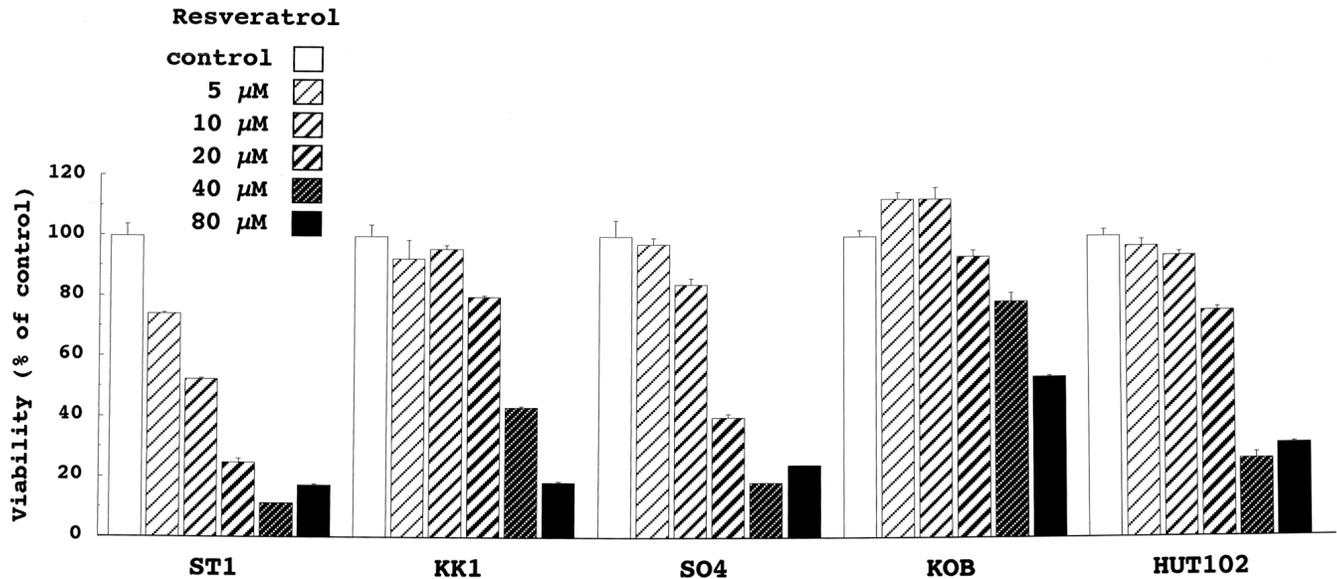
Figure 1. Growth inhibition of 5 human T cell lymphotrophic virus-1 (HTLV-1) infected cell lines after treatment with resveratrol. Each cell line (2×10^5/ml) was treated for 72 h with 1% dimethyl sulfoxide (DMSO, control) or 5–80 μM resveratrol. Viable cells were then determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2,4-sulfophenyl)-2*H*-tetrazolium (MTS) assay. Percentage of growth was calculated, with 100% representing control cells treated with 1% DMSO alone. Values are means ± SE from 3 experiments.

nexin V binding was detectable within 48 h of treatment with lower resveratrol concentrations (even at 5 μM) and gradually increased at 72 h after drug exposure. In addition to these observations, resveratrol-induced apoptosis was further supported by microscopy of the cytospin smears of May-Giemsa-stained ST1 cells, which revealed the characteristic morphological features of apoptosis (Fig. 2B). These findings indicate that resveratrol inhibits the growth of ST1 cells, at least in part, by inducing apoptosis. We therefore further examined this action in the other HTLV-1-infected cell lines as well as ST1 cells as a positive control (Fig. 4). Similarly, resveratrol (50 and 100 μM) also induced apoptosis in each of the cell lines after 18 h of treatment in a dose-dependent manner.

Modulation of Apoptosis-Related Genes in Resveratrol-Induced Apoptosis in an HTLV-1-Infected Cell Line

To further understand the molecular basis for the resveratrol-induced cell death in HTLV-1-infected cell lines, we examined whether resveratrol induced changes in the expression of apoptosis-related genes (Bcl-2, Bcl-X$_L$, Bax-α, cIAP-I, and survivin), which are commonly considered to be key players in the apoptotic pathways. ST1 cells were treated with 1% DMSO or 20–80 μM resveratrol. Total cellular RNA extracts were evaluated for these gene expressions after 10 h of RT-PCR. Resveratrol treatment did not alter the Bcl-2, Bcl-X$_L$, Bax-α, or cIAP-I expression (Fig. 5, A and B). In contrast, resveratrol caused a marked decrease in the mRNA levels of survivin expression in a dose-dependent manner.

To further substantiate this inhibitory effect, we performed real-time quantitative PCR (Fig. 5C). Again, it was

apparent that the survivin-to-GAPDH ratio in ST1 cells dramatically decreased from 1.8% to 0.5% after 10 h of resveratrol treatment (0–80 μM) in a dose-dependent manner. Moreover, in a separate set of experiments, we examined these survivin expression changes in the other HTLV-1 cell lines (Fig. 6). After 10 h of treatment with 80 μM resveratrol, the survivin mRNA levels markedly decreased in each of the cell lines. On the basis of the relative densitometric analyses, the calculated ratios (80 to 0 μM) of the survivin mRNA expression for KK1, SO4, KOB, and HUT102 cells were 0.65, 0.50, 0.45, and 0.56, respectively.

Discussion

Resveratrol is a naturally occurring trihydroxystilbene that has recently attracted considerable interest because of its remarkable multifunctional inhibitory effects on multistage carcinogenesis (7). Resveratrol has been shown to cause cell growth inhibition in some leukemic cell lines (34–41). With emphasis on the chemopreventive effect of resveratrol, we investigated its effect in ATL. In the present study, we demonstrated that resveratrol inhibited the viability of all the HTLV-1-infected cell lines examined in a dose-dependent manner (Fig. 1). We further showed that resveratrol could induce cell apoptosis in all the HTLV-1-infected cell lines examined in a dose-dependent manner (Figs. 2–4). Tsan et al. (38) and Park et al. (40) reported that resveratrol caused cell growth inhibition by inducing apoptosis in THP-1 human monocytic leukemia cells (30 μM) and U937 human monocytic leukemia cells (30–120 μM), respectively. Clément et al. (34) also demonstrated that resveratrol (16–32 μM) inhibited the growth of HL60 human promyelocytic leukemia
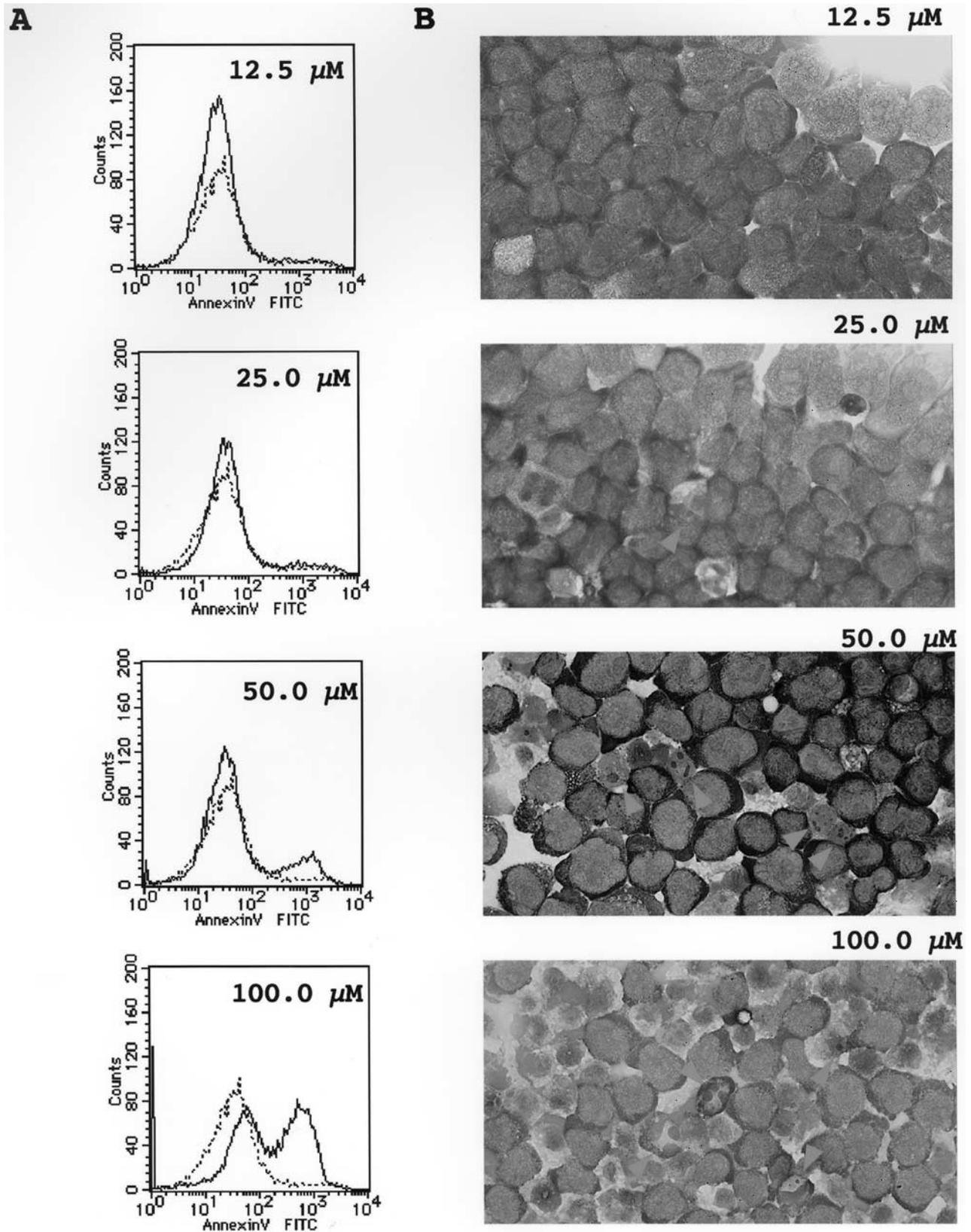
Figure 2. Resveratrol-induced apoptosis in the HTLV-1-infected cell line ST1. A: flow cytometric analysis of annexin V binding in resveratrol-treated cells. ST1 cells were exposed to 12.5–100 µM resveratrol for 18 h and examined for annexin V binding showing the process of early apoptosis by flow cytometry. Dashed lines, 1% DMSO-treated cells (control); solid lines, resveratrol-treated cells. B: morphological changes induced by resveratrol treatment in ST1 cells. Some cells examined in A were obtained for preparation of cytospin smears. Cytospin smears of May-Giemsa staining at ×1,000 original magnification are shown. Characteristic features of apoptosis, including reduction of cell size, condensation and aggregation of nuclear chromatin, and nuclear fractionation, were induced by resveratrol treatment in ST1 cells in a dosedependent manner. Red arrowheads, representative cells showing features of apoptosis.
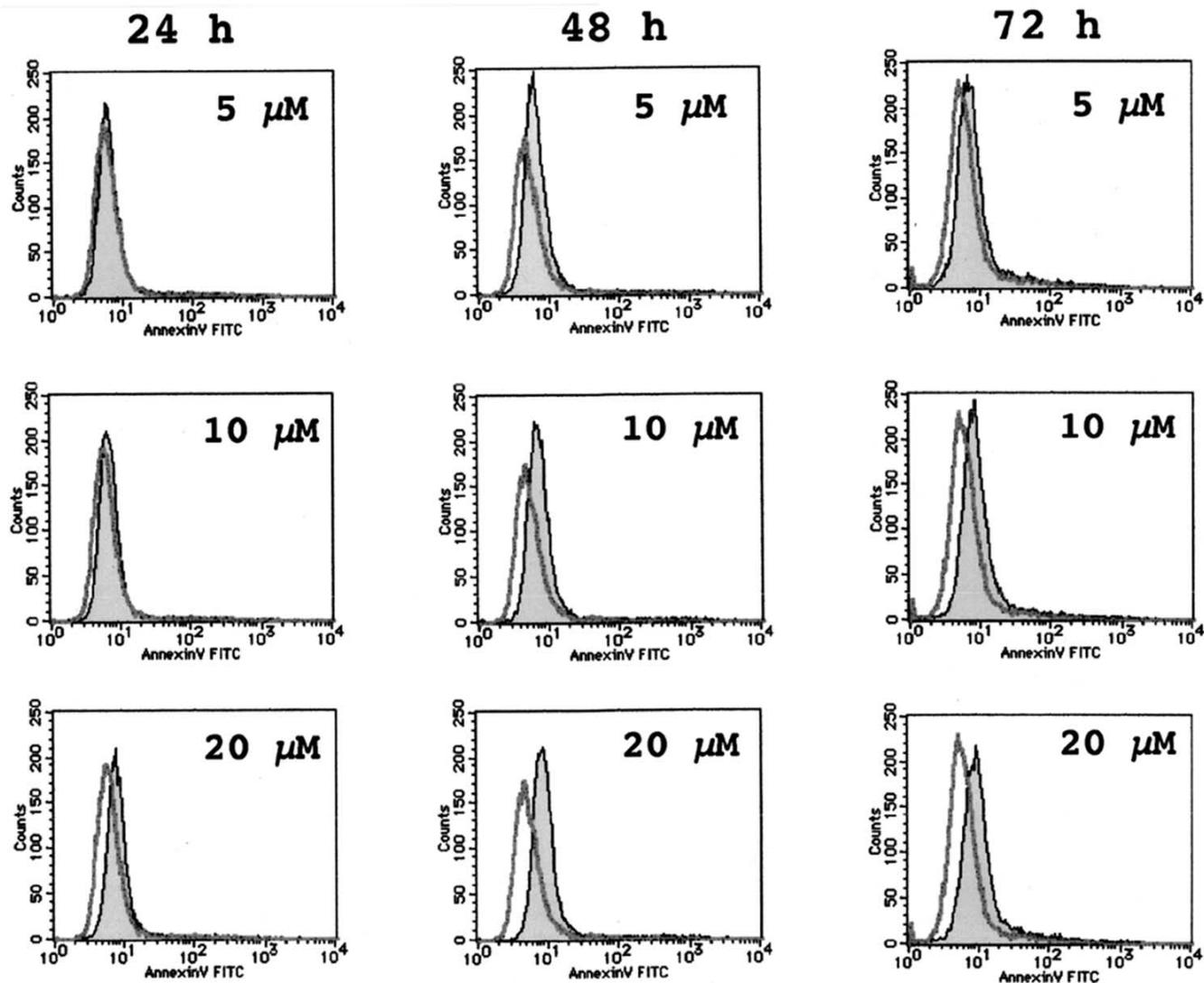
Figure 3. Annexin V binding after resveratrol treatment of ST1. ST1 cells were treated with 5, 10, and 20 μM resveratrol, and annexin V binding was analyzed by flow cytometry at 24, 48, and 72 h. Solid lines, 1% DMSO-treated cells (control); shaded histograms, resveratrol-treated cells.

cells by inducing apoptosis. In contrast, Ragione et al. (35) demonstrated that 30 μM resveratrol blocked HL60 cell proliferation by inducing S phase arrest without cell apoptosis. They further reported that the doses required to induce apoptosis were higher than those needed to induce cell cycle arrest. Thus these previous findings, together with our results, indicate that leukemia cell growth inhibition by resveratrol appears to be due to apoptotic cell death and/or its ability to induce cell cycle arrest and can vary due to leukemic cell types and experimental conditions. Although we did not evaluate the effects of resveratrol on the cell cycle kinetics, our present findings suggest that the inhibitory effect of resveratrol on HTLV-1-infected cell lines is, at least in part, mediated by the induction of apoptosis. We will next determine whether resveratrol can also induce cell cycle arrest in HTLV-1-infected cell lines.

Apoptosis is a tightly regulated process involving changes in the expression of distinct genes. Resveratrol has also been previously shown to induce apoptosis in several leukemia cell lines; however, its mechanisms for cell apoptosis were not consistent. In a study of HL60 cells, resveratrol-induced apoptosis was reported to be accompanied by a decrease in Bcl-2 expression (36). In contrast, in U937 cells, resveratrol-induced apoptosis was reported to be mediated by downregulation of cIAP-I, but not the Bcl-2 family or survivin (40). On the basis of these findings, there seem to be several plausible mechanisms for cellular apoptosis induced by resveratrol. In this study, resveratrol was found to decrease survivin, but not the Bcl-2 family or cIAP-I, expression in ST1 cells. At 80 μM resveratrol, a maximum downregulation to 28% of the initial survivin mRNA level was observed in ST1 cells (Fig. 5). Furthermore, 80 μM resveratrol also caused a marked decrease in the survivin expression in other HTLV-1 cell lines, although to a lesser extent (45–65%; Fig. 6). Inasmuch as survivin directly blocks the processing and activation of effector caspases-3 and -7,
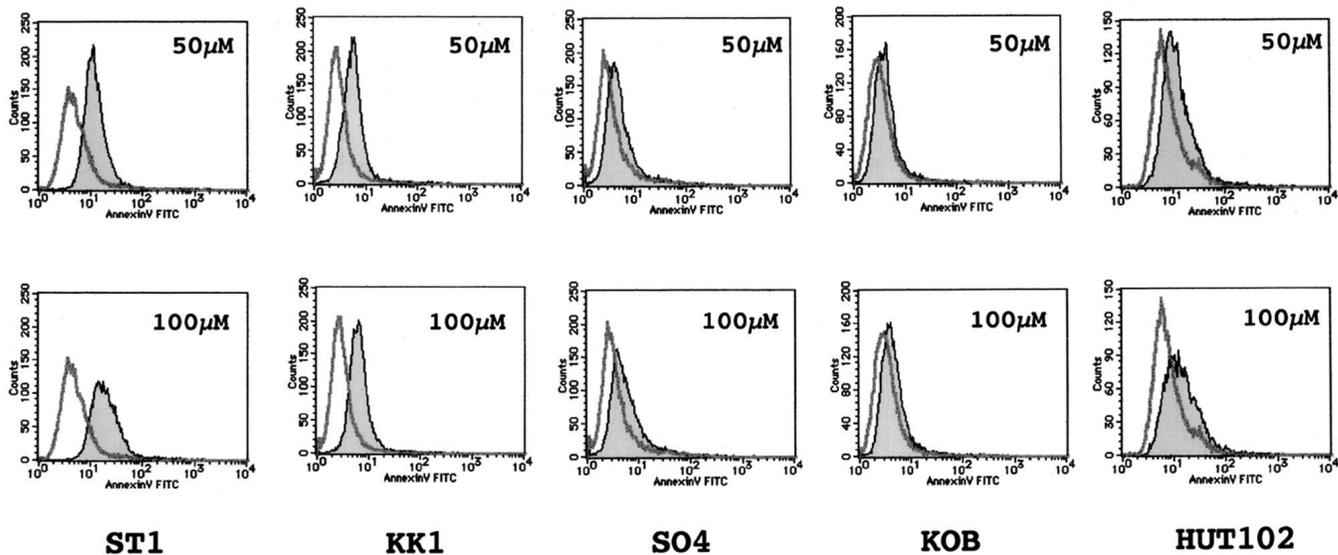
Figure 4. Resveratrol-induced apoptosis in various HTLV-1-infected cell lines. ST1 cells (positive control for apoptosis), KK1, SO4, KOB, and HUT102 cells were exposed to resveratrol for 18 h and examined for annexin V binding showing the process of early apoptosis by flow cytometry. Solid lines, 1% DMSO-treated cells (control); shaded histograms, resveratrol-treated cells.
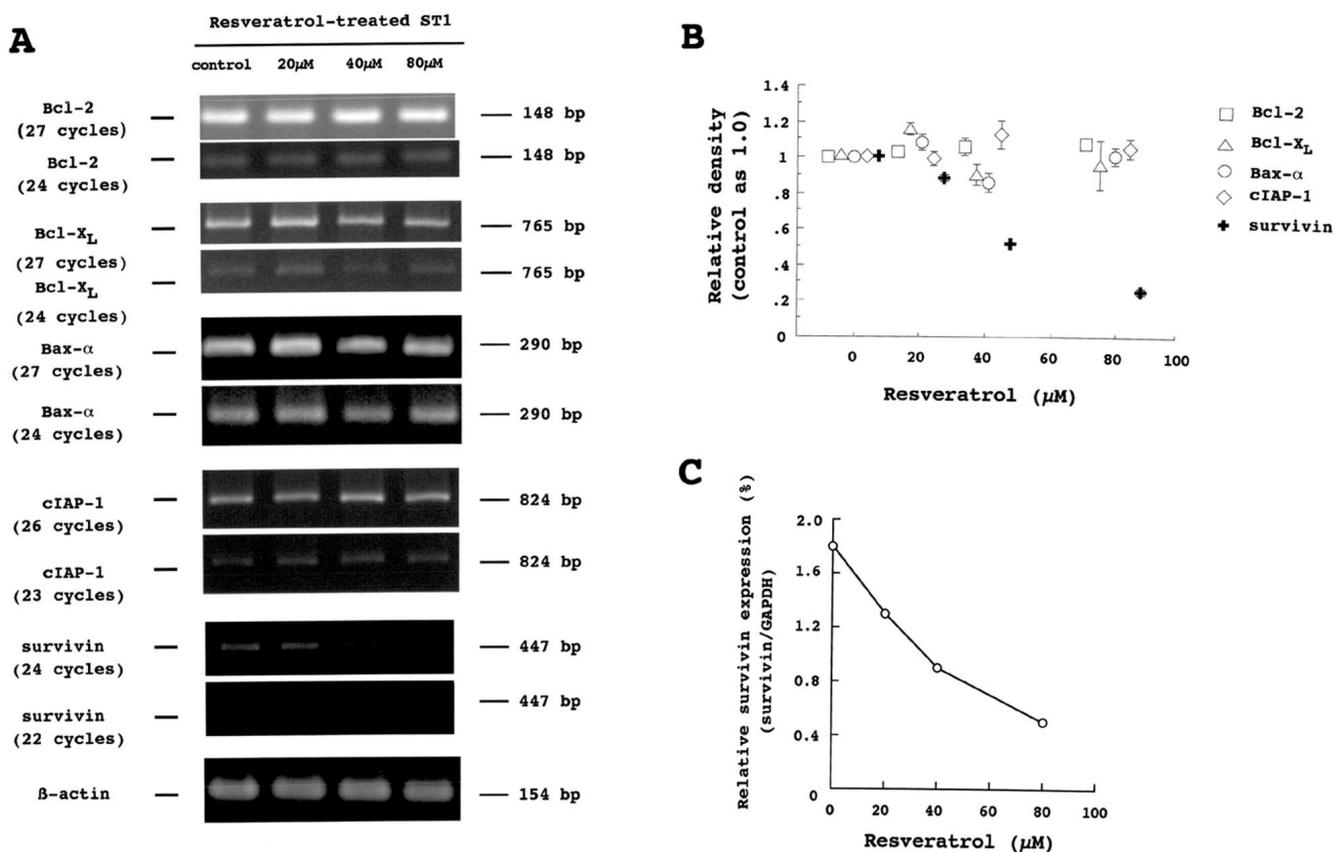

Figure 5. Expression levels of apoptosis-related genes in HTLV-1-infected cell line (ST1) after treatment with resveratrol. A: ST1 cell line was treated with 1% DMSO (control) or resveratrol for 10 h, and then total RNA was isolated, cDNA was synthesized, and amount of mRNA for Bcl-2, Bcl-X_L, Bax-α, cIAP-1, and survivin was determined by polymerase chain reaction. A representative study is shown; 2 additional experiments yielded similar results. B: relative intensity comparison of PCR products shown in A. Bands in PCR gel were scanned, and area was measured for individual bands and normalized to internal control (β-actin) using an IP Lab Gel Imaging system. Values for each control are considered 1.0. Values are means ± SE from 3 experiments. C: resveratrol-induced inhibition of survivin transcription further confirmed by real-time quantitative PCR. mRNA level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a housekeeping gene, was used as an internal standard. Normalized levels of survivin transcripts were calculated as ratio of amount of survivin transcripts to amount of GAPDH transcripts (expressed as percentage).
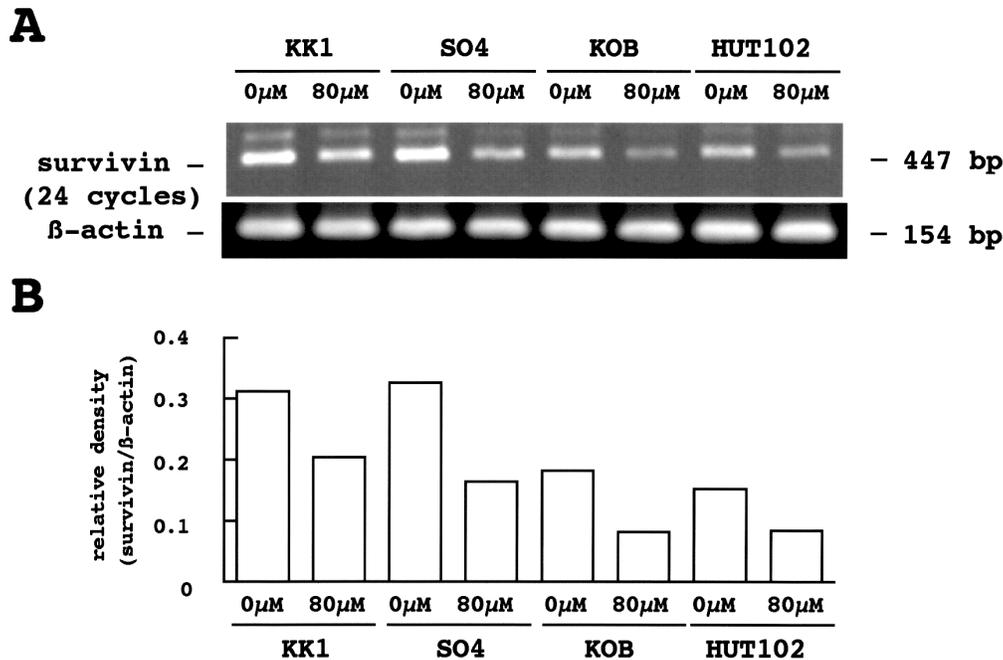
Figure 6. Expression levels of survivin gene in various HTLV-1-infected cell lines after treatment with resveratrol. A: various HTLV-1-infected cell lines (KK1, SO4, KOB, and HUT102) were treated with 1% DMSO (control) or 80 μM resveratrol for 10 h, and then total RNA was isolated, cDNA was synthesized, and amount of mRNA for survivin was determined by PCR. B: bands in PCR gel were scanned, and area was measured for individual bands and normalized to internal control (β-actin) using an IP Lab Gel Imaging system. Results show levels of survivin relative to β-actin.

which act at a common downstream part of the two major apoptotic pathways (22), it seems reasonable that resveratrol-induced apoptosis in HTLV-1-infected cell lines is mediated by a downregulation of survivin expression. Indeed, downregulation to 30% of the initial survivin mRNA expression using an antisense oligonucleotide has been reported to facilitate apoptosis in a lung cancer cell line (42). However, only KOB cells showed relative resistance to the growth inhibition by resveratrol, despite the marked suppression (45%) of survivin mRNA expression (Figs. 1 and 6). Clément et al. (34) demonstrated that resveratrol treatment enhances CD95L expression and induces CD95 signaling-dependent cell death in HL60 cells as well as in T47D breast carcinoma cells. Because only the KOB cell line was known to acquire a CD95 mutation to escape from apoptosis mediated by the CD95/CD95L systems in these HTLV-1 cell lines (43), the CD95 mutation may affect its resistance. We will also determine whether resveratrol can also enhance CD95L expression on HTLV-1 cell lines.

There is accumulating evidence that the survivin expression is upregulated in primary ATL cells compared with the normal peripheral blood mononuclear cells (28,44). ATL is associated with poor prognosis and often runs a fulminant course, and the main reason for the unfavorable prognosis is developmental resistance to most anticancer drugs. Inasmuch as survivin has also been shown to be correlated with drug resistance in several cancers (26,27), its aberrant upregulation may strongly affect potential regulating pathological manifestation, including the multiple drug resistance of ATL. Kamihira et al. (28) showed a close correlation between a high survivin expression level and shorter survival in ATL

patients. In this scenario, given our present findings, an inhibitory effect of resveratrol on survivin expression may have profound implications for ATL therapy to overcome its drug resistance. The mechanism of resveratrol-induced survivin downregulation is not clear but may represent a generalized T cell line reaction. Indeed, similar results were obtained with Jurkat cells, another human T cell leukemia line, by real-time quantitative PCR (data not shown).

The clinical implications of our studies will depend on whether resveratrol can selectively inhibit ATL cells but not normal lymphocytes. Clément et al. (34) reported that 8–32 μM resveratrol, while inducing apoptosis of HL60 cells, had no toxic effect on human peripheral lymphocytes. Gautam et al. (37) also reported that 10–80 μM resveratrol showed high selectivity toward several leukemia cell lines vs. normal hematopoietic progenitor cells with respect to growth inhibition and apoptosis. In the present study, we observed growth-inhibitory activities of resveratrol for five HTLV-1-infected cell lines as follows: ED_{50} = 10.4, 37.3, 25.0, 85.6, and 35.7 μM (Fig. 1). Thus, on the basis of previous studies, our present findings support the possibility that resveratrol can selectively inhibit ATL cells with minimal toxicity to the normal hematopoietic cells.

To the best of our knowledge, this is the first report showing a suppressive effect of resveratrol on survivin expression to induce apoptosis in ATL cell lines. In view of the accumulating evidence that survivin may be an important determinant of the clinical response in ATL, further efforts appear warranted to determine its in vivo antileukemic effect and its potential as a chemotherapeutic agent for this incurable disease.

Acknowledgments and Notes

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