Original Article

Resveratrol Induces Apoptosis and Autophagy in T-cell Acute Lymphoblastic Leukemia Cells by Inhibiting Akt/mTOR and Activating p38-MAPK*

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Abstract

Objective To explore the effects of resveratrol-induced apoptosis and autophagy in T-cell acute lymphoblastic leukemia (T-ALL) cells and potential molecular mechanisms.

Methods The anti-proliferation effect of resveratrol-induced, apoptosis and autophagy on T-ALL cells were detected by using MTT test, immunofluorescence, electronic microscope, and flow cytometry, respectively. Western blotting was performed for detecting changes of apoptosis-associated proteins, cell cycle regulatory proteins and state of activation of Akt, mTOR, p70S6K, 4E-BP1, and p38-MAPK.

Results Resveratrol inhibited the proliferation and induced apoptosis and autophagy in T-ALL cells in a dose and time-dependent manner. It also induced cell cycle arrest at G0/G1 phase via up regulating cyclin-dependent kinase (CDK) inhibitors p21 and p27 and down regulating cyclin A and cyclin D1. Western blotting revealed that resveratrol significantly decreased the expression of antiapoptotic proteins (Mcl-1 and Bcl-2) and increased the expression of proapoptotic proteins (Bax, Bim, and Bad), and induced cleaved-caspase-3 in a time-dependent manner. Significant increase in ratio of LC3-II/LC3-I and Beclin 1 was also detected. Furthermore, resveratrol induced significant dephosphorylation of Akt, mTOR, p70S6K, and 4E-BP1, but enhanced specific phosphorylation of p38-MAPK which could be blocked by SB203580. When autophagy was suppressed by 3-MA, apoptosis in T-ALL cells induced by resveratrol was enhanced.

Conclusion Our findings have suggested that resveratrol induces cell cycle arrest, apoptosis, and autophagy in T-ALL cells through inhibiting Akt/mTOR/p70S6K/4E-BP1 and activating p38-MAPK signaling pathways. Autophagy might play a role as a self-defense mechanism in T-ALL cells treated by resveratrol. Therefore, the reasonable inhibition of autophagy in T-ALL cells may serve as a promising strategy for resveratrol induced apoptosis and can be used as adjuvant chemotherapy for T-ALL.

Key words: Resveratrol; Apoptosis; Autophagy; T-cell acute lymphoblastic leukemia; Akt/mTOR; p38-MAPK

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INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignant tumor arising from T-cell progenitors. T-ALL accounts for 15% of newly diagnosed ALL cases in children and 25% in adults. The relapse in patients with T-ALL was about 30% and the outcome remained extremely poor[1]. Therefore, search for new treatment is an urgent need from these patients.

Resveratrol (34 4',5-trihydroxystilbene), a naturally occurring phytoalexin enriched in enormous dietary products, particularly in the skin of red grapes, was first reported in 1997 as a potential anticancer agent[2]. Over the past 15 years, many studies have illustrated a variety of effects of resveratrol, including anti-proliferation, inhibition of angiogenesis and release of invasive and metastatic factors[3], as well as inhibition of tumorigenesis. The anticancer effects of resveratrol have been observed in different cancers, and its mechanisms involved were cell type-dependent.

It is well-known that the survival and aggressiveness of cancer cells are closely associated with abnormal cell death. Programmed cell death (PCD) can be divided into two main forms: apoptosis and autophagy[4]. Apoptosis, a cell-intrinsic suicide mechanism, is controlled by some specific genes and multiple cellular signaling pathways. Autophagy, primarily as a type of cellular catabolic degradation response to nutrient starvation or metabolic stress, is considered as a survival mechanism induced in adverse conditions to maintain cell integrity, or conversely, and it is also involved in a particular mode of cell death called autophagic cell death[5]. Recently, increasing evidences have demonstrated that apoptosis and autophagy may be triggered by common upstream signals and thus affect cancer development and therapy[6-7]. However, the precise cross talk between apoptosis and autophagy to seal the fate of cancer cells are sophisticated, and sometimes contradictory.

The abnormal activation of Akt/mammalian target of rapamycin (mTOR) signaling cascade, which promotes tumor cell growth, proliferation, survival and resistance to drug-induced apoptosis[8-9], is a common occurrence in cancer cells[10-11]. Currently, reports have also demonstrated the important role of PI3K/Akt/mTOR signal pathway in regulating autophagy[12-14]. In addition, studies have shown that resveratrol can exert its apoptosis[15] and autophagy[16] inducing effects through altering the p38-mitogen-activated protein kinase (MAPK) activation, however, no report about how p38-MAPK was integrated into resveratrol-induced apoptosis and autophagy in T-ALL cells was made available.

In the present study, we used resveratrol to treat four T-ALL cell lines and observed that resveratrol induced apoptosis and autophagy in T-ALL cells via Akt/mTOR/p70S6K/4E-BP1 inhibition and p38-MAPK activation. The relationship between apoptosis and autophagy was also explored.

MATERIALS AND METHODS

Cell Culture

The T-ALL cell lines, Molt-4 (glucocorticoid (GC) resistant) and Jurkat (GC resistant) were kindly provided by Dr. Stephan W. Morris (St. Jude Children's Research Hospital). CEM-C1-15 (GC resistant) and CEM-C7-14 (GC sensitive) were kindly provided by Dr. E. Brad Thompson (University of Texas Medical Branch, Galveston, TX). All cells were maintained in RPMI 1 640 containing 10% FBS, 2 mmol/L L-glutamine, and 100 U/mL penicillin/ streptomycin at 37 °C in a 5% CO_2 humidified atmosphere.

Reagents and Antibodies

Resveratrol, p38 inhibitor SB203580, autophagy inhibitor 3-methyladenine (3-MA) and monodansyl-cadaverine (MDC) were purchased from Sigma. Annexin V-FITC Kit was purchased from Bipec Biopharma Corporation (USA). Antibodies to Bad, Mcl-1, Bim, caspase-3, Cyclin A, p21, and p27 as well as horseradish-peroxidase(HRP)-conjugated secondary anti-rabbit and anti-mouse antibodies were obtained from Santa Cruz Biotechnology. Antibodies to phospho-Akt, phospho-mTOR, phospho-p70S6K, phospho-4E-BP1, phospho-p38, CyclinD1, Bax, Bcl-2, Beclin1, and LC3 (microtubule-associated protein1 light chain 3) were purchased from Cell Signaling Technology. Anti-GAPDH antibody was obtained from Kangcheng Bio-Tech (Shanghai, China).

Measurement of Cell Proliferation

Cell proliferation was determined by MTT assay. Cells in logarithmic growth phase were seeded in 96-well plate (2×10^4 cells per well) and incubated with different concentrations of resveratrol for indicated time spans. Then, 20 μL of MTT (5 mg/mL) was added to per well and incubated for 4 h. The cell plate was centrifuged at 1 000 rpm for 10 min. The supernatant was gently removed from cell pellet and

150 μL of dimethyl sulfoxiode (DMSO) was added in each well. The plate was gently shaken for 10 min and the absorbance at 570 nm was measured with an ELISA reader. Three separate experiments were conducted and each was performed in triplicate. The ratio of growth inhibition was calculated based on the formula: Inhibition rate (%) = (1-absorbance of the experimental group/absorbance of the control group) × 100%.

Apoptosis Measurement by Flow Cytometry

The cells were treated as indicated and then were collected, washed twice with cold PBS, resuspended at 1×10^6 cells/mL in 400 μL binding buffer. After 5 μL of Annexin V-FITC was added, the cells were incubated for 15 min at 4 °C in the dark. Then, 10 μL of PI was added and cells were incubated for another 5 min at 4 °C in the dark. The apoptosis was detected by flow cytometry (FASC Aria, BD Bioscience, San Jose, CA) within 1 h and at least 1×10^4 cells were collected.

Analysis of Autophagy

MDC was recently introduced as a specific autophagolysosome marker to analyze the autophagic process at the molecular level. Cells were treated with resveratrol for indicated time spans and then incubated with 0.05 mmol/L MDC in PBS at 37 °C for 10 min. After incubation, cells were washed three times with PBS and immediately analyzed with a fluorescence microscope (BX-50; Olympus) equipped with a filter system (V-2A excitation filter: 380/420 nm, barrier filter: 450 nm) .

Electronic Microscopy

The cells treated with resveratrol were collected and fixed in PBS containing 0.1% glutaraldehyde (pH 7.4) for 2 h at room temperature, post-fixed in 1% OsO4 in water for one hour. Ultra-thin sections of cells were then analyzed under a transmission electronic microscope (Jeol J EM-1200EX, Japan).

Cell Cycle Analysis

For each analysis, cells were treated with resveratrol for 24 h. After treatment, cells were collected, washed, fixed with 70% ethanol and stored overnight at -4 °C followed by washing and staining with 5 μg/mL PI in the presence of DNase free RNase (Sigma). Cells were used to analyze DNA content using the flow cytometry (Beckman Coulter, Inc., Miami, FL, USA).

Western Blot Analysis

Whole cell lysates, quantitation, electrophoresis and transmembrane were manipulated according to our previous report[17]. GAPDH was used as the loading controls.

Statistical Analysis

All of the experiments were repeated at least three times and data were expressed as means±SD (standard deviation). Statistical software SPSS15.0 was used for the assessment. The Student's *t* test was used to compare means of two groups and One-Way ANOVA was used for comparing means of multiple samples. $P<0.05$ was considered as statistically significant.

RESULTS

Resveratrol Inhibited the Growth and Induced Apoptosis in T-ALL Cells

In order to evaluate the potential therapeutic effect of resveratrol for T-ALL, four T-ALL cell lines, the GC-resistant CEM-C1-15, Jurkat, Molt-4, and GC-sensitive CEM-C7-14, were used in our experiment. According to the preliminary study, resveratrol concentration of 200 μmol/L was used for subsequent studies. As shown in Figure 1A, after 48 h of treatment with 200 μmol/L resveratrol, the inhibition rate of CEM-C1-15, Jurkat, Molt-4, and CEM-C7-14 cells was 51.5%±5.4%, 68.8%±5.5%, 58.2%±4.1%, 42.2%±4.8%, respectively. The growth inhibition of resveratrol on CEM-C1-15 cells was manifested in a concentration- and time-dependent manner (Figure 1B).

To define whether resveratrol-induced cell death is associated with apoptosis, Annexin V/PI double staining and flow cytometry were used to detect apoptosis. As shown in Figure 1C, the cells treated with 200 μmol/L resveratrol for 48 h exhibited a significant apoptosis compared with those in the control group.

To further verify apoptosis, electronic microscopy was used to visualize resveratrol induced apoptotic characteristics, such as cell membrane asymmetry, chromatin condensation and margination, nuclear fragmentation and apoptotic bodies (Figure 1D).

The Bcl-2 family proteins are known to be closely related to apoptosis. We performed a time-course study to examine the changes of Bcl-2

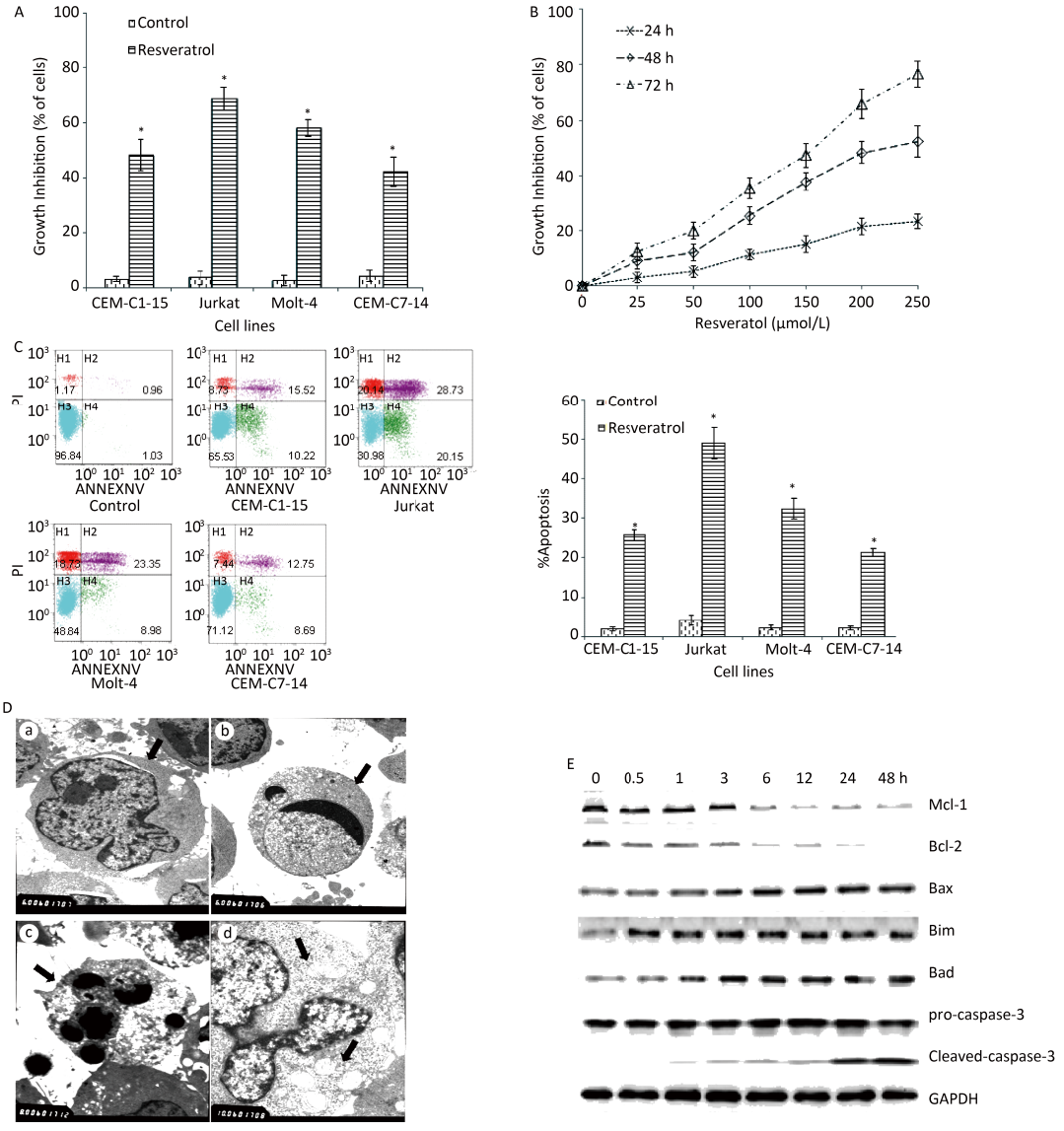

Figure 1. Resveratrol inhibited the growth and induced apoptosis of T-ALL cells. A. Four T-ALL cell lines (glucocorticoid sensitive CEM-C7-14 and glucocorticoid resistant CEM-C1-15, Jurkat, Molt-4) were treated with resveratrol (200 μmol/L) for 48 h, and the growth inhibition of the cells were evaluated by MTT assay. $^{*}P<0.05$, resveratrol versus control. B. Resveratrol induced growth inhibition in CEM-C1-15 cells presented in a time and concentration-dependent manner. Cells were exposed to different concentrations of resveratrol (25, 50, 100, 150, 200, and 250 μmol/L) for 24, 48, and 72 h. The inhibition rate was analyzed by MTT assay. C. Apoptosis of T-ALL cells induced by resveratrol was detected by Annexin V-FITC/PI double staining and flow cytometry. The cells of four T-ALL cell lines were treated with 200 μmol/L of resveratrol for 48 h (the control group of Jurkat, Molt-4 and CEM-C7-14 was similar to that of CEM-C1-15 and data not shown). Data were presented as mean±SD. $^{*}P<0.05$, resveratrol versus control. D. Electronic microscope photographs of CEM-C1-15 cells treated with 200 μmol/L of resveratrol for 12 h. The black arrows point to (a): tumor cells showed large nucleoli, nuclear pleomorphism, and the nucleus cytoplasm ratio imbalance. (b): nuclear shrinkage (pyknosis), chromatin condensation, and blebbing. (c): nuclear fragmentation (karyorrhexis) and apoptotic bodies. (d): The mitochondria, ribosome and cell organ were complete. E. The expression of apoptosis associated proteins in T-ALL cells treated with resveratrol for different times. CEM-C1-15 cells were treated with 200 μmol/L of resveratrol for 0, 0.5, 1, 3, 6, 12, 24, and 48 h and then Western blot were performed. The level of total GAPDH protein was used as the loading control.

family proteins and caspase-3 cleavage in resveratrol treated T-ALL cells. As shown in Figure 1E, the expression of antiapoptotic proteins (Mcl-1 and Bcl-2) was declined in CEM-C1-15 cells after resveratrol treatment, while the proapoptotic proteins (Bax, Bim, and Bad) and cleaved-caspase-3 were increased significantly in a time-dependent manner. These results indicated that resveratrol induced apoptosis through up regulating proapoptotic proteins and down regulating antiapoptotic proteins in T-ALL cells.

Resveratrol Induced Autophagy in T-ALL Cells

As resveratrol triggered apoptosis in T-ALL cells, we next assessed whether it also induced autophagic cell death. MDC, a specific autophagolysosome marker, mainly accumulated in mature autophagic vacuoles (AVs), provides reliable autophagy detection[18]. When cells with autophagy were observed under a fluorescence microscope, AVs stained by MDC appeared as distinct dot-like structures distributed within the cytoplasm or localized in the peri-nuclear regions. As shown in Figure 2A, the MDC-labeled vesicles were increased in CEM-C1-15 cells after treatment with 200 μmol/L resveratrol for 3 h, with the maximum effect appeared at 12 h, then decreased dramatically at 24 h. Similar results were obtained in Jurkat, Molt-4 and CEM-C7-14 cells (data not shown). These results indicated that resveratrol induced the formation of autophagic vacuoles in T-ALL cells. Autophagy could also be detected by electronic microscopy. The focal degradation of cytoplasmic areas sequestered by the phagophore (a specialized type of smooth, ribosome-free double membrane) was one of the hallmarks of autophagy. As shown in Figure 2B, autophagic vacuoles of T-ALL cells were observed under electronic microscopy after resveratrol treatment.

Autophagy of T-ALL cells induced by resveratrol was also judged by the ratio of LC3-II/LC3-I and the expression of Beclin1, two well-validated biomarkers of autophagy. As shown in Figure 2C, the obvious increase in the ratio of LC3-II/LC3-I and Beclin1 were observed after CEM-C1-15 cells were treated with 200 μmol/L of resveratrol for different time spans. These results indicated that, in addition to inducing apoptosis, resveratrol also induced autophagy in T-ALL cells.

Resveratrol Induced Cell Accumulation at G1 Phase of Cell Cycle in T-ALL Cells

To examine whether resveratrol affected cell cycle, we performed the cell cycle detection by flow cytometry. As shown in Figure 3A, significant increase in percentage of G1 phase in T-ALL cells was observed after the treatment with resveratrol for 24 h when compared with the control group. Moreover, both the cyclin A (which is required for DNA replication in S phase) and the cyclin D1 (which promotes cells to pass G1/S checkpoint and enter S phase) were attenuated in resveratrol treated T-ALL CEM-C1-15 cells, while p21 and p27, two important cyclin-dependent kinase inhibitors, were obviously increased (Figure 3B). These results suggested that resveratrol induced cell cycle arrest at G0/G1 phase in T-ALL cells through down-regulating cyclin A and cyclin D1 and up-regulating p21 and p27.

Akt/mTOR/p70S6K/4E-BP1 Suppression and p38-MAPK Activation Were Involved in Resveratrol-induced Apoptosis and Autophagy

To investigate whether the Akt/mTOR/p70S6K/4E-BP1 and p38-MAPK signaling pathways were involved in resveratrol-induced apoptosis and autophagy, Western blotting was performed to detect the activated state of associated proteins. As shown in Figure 4A, resveratrol inhibited the phosphorylation of Akt, mTOR, p70S6K and 4E-BP1 in a time-dependent manner. On the contrary, the phosphorylation of p38 was significantly increased, reaching the peak in 1 h after CEM-C1-15 cells were treated with 200 μmol/L resveratrol.

To further explore whether the activation of p38 was specific and how it played a role in the interaction between apoptosis and autophagy, we examined the effects of SB203580 (a specific inhibitor of p38) and 3-MA (specific inhibitor of autophagy) on resveratrol-induced apoptosis and autophagy in CEM-C1-15 cells. As shown in Figure 4B, C, and D, pretreatment of CEM-C1-15 cells by p38 inhibitor SB203580 reduced the resveratrol-induced apoptosis, autophagy and cleaved-caspase-3, slightly decreased up regulation of proapoptotic proteins Bim and Bax, but significantly attenuated the down regulation of antiapoptotic Bcl-2 and Mcl-1. Moreover, when compared with the resveratrol treatment alone, the specific autophagic proteins (LC3-II and Beclin1) were also declined in CEM-C1-15 cells pretreated with SB203580. These results indicated that the suppression of p38-MAPK attenuated resveratrol induced apoptosis and autophagy.

To test the impact of resveratrol induced autophagy on apoptosis of T-ALL cells, 3-MA, the specific autophagy inhibitor (preventing autophagy at an early stage) was used. The CEM-C1-15 cells were treated with 10 mmol/L 3-MA or 200 μmol/L resveratrol alone, or in combination with both agents. As shown in Figure 4E, 3-MA significantly inhibited the resveratrol induced autophagy. 3-MA also increased resveratrol induced apoptosis (Figure 4F) through further upregulating Bim, Bad, Bax, and cleaved-caspase-3, and down regulating Bcl-2 and Mcl-1 (Figure 4G), suggesting that 3-MA could enhance the resveratrol-induced apoptosis of T-ALL cells by inhibiting the autophagy.

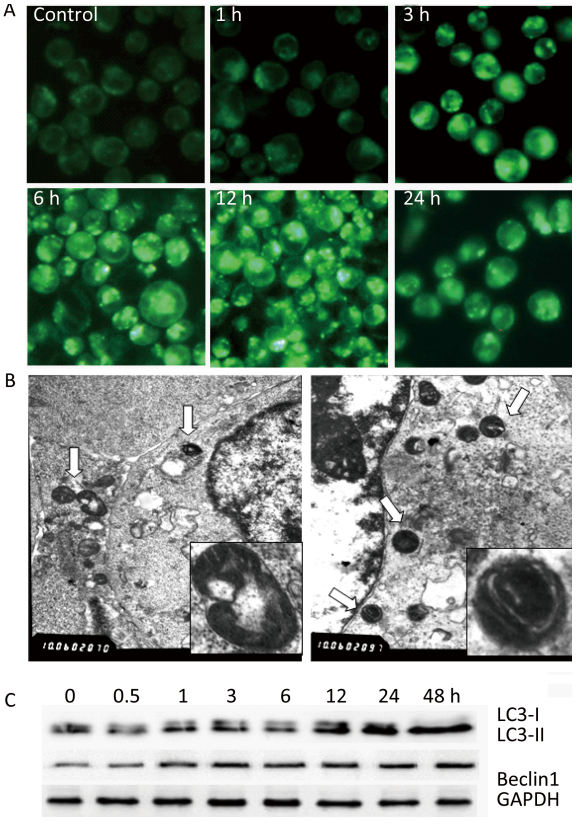

Figure 2. Resveratrol induced autophagy in T-ALL cell lines. A. MDC-labeled vesicles in CEM-C1-15 cells treated with 200 μmol/L of resveratrol for different time spans. Cells were treated with 200 μmol/L of resveratrol for 1, 3, 6, 12, and 24 h. Fluorescence particles in the cytoplasm indicate autophagic vacuoles (magnification × 400). B. Electronic microscope photographs of CEM-C1-15 cells treated with 200 μmol/L of resveratrol for 6 h. The white arrows pointed to autophagic vacuoles and autolysosomes. C. Western blots of LC3 and Beclin1. CEM-C1-15 cells were treated with 200 μmol/L of resveratrol for 0, 0.5, 1, 3, 6, 12, 24, and 48 h. The level of total GAPDH protein was used as the loading control.

DISCUSSION

Resveratrol Induces Growth Inhibition, Apoptosis and G1 Accumulation in T-ALL Cells

Resveratrol, a natural occurring plant antibiotic, has been shown to have multiple potential chemoprotective activities in many kinds of cells and animal models[19-20]. Resveratrol acts through suppressing proliferation and inducing apoptosis in a variety of cancer

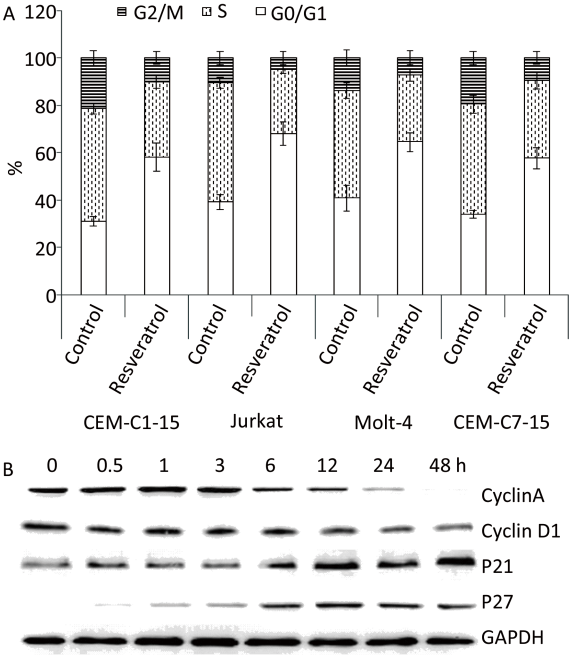

Figure 3. Resveratrol induced cell cycle arrest at G1 phase and up regulation of p21 and p27 while down regulation of cyclin A and cyclin D1. A, Cell cycle analysis of CEM-C1-15, Jurkat, Molt-4 and CEM-C7-14 cells. Cells were treated with 200 μmol/L of resveratrol for 24 h, and then PI staining and flow cytometry were performed. Values represent the mean±SD, of three independent experiments. B, Cell cycle regulating proteins (cyclin A, cyclin D1, p21 and p27) in CEM-C1-15 cells treated with 200 μmol/L of resveratrol for different time spans were detected by Western blot. The level of total GAPDH protein was used as the loading control.

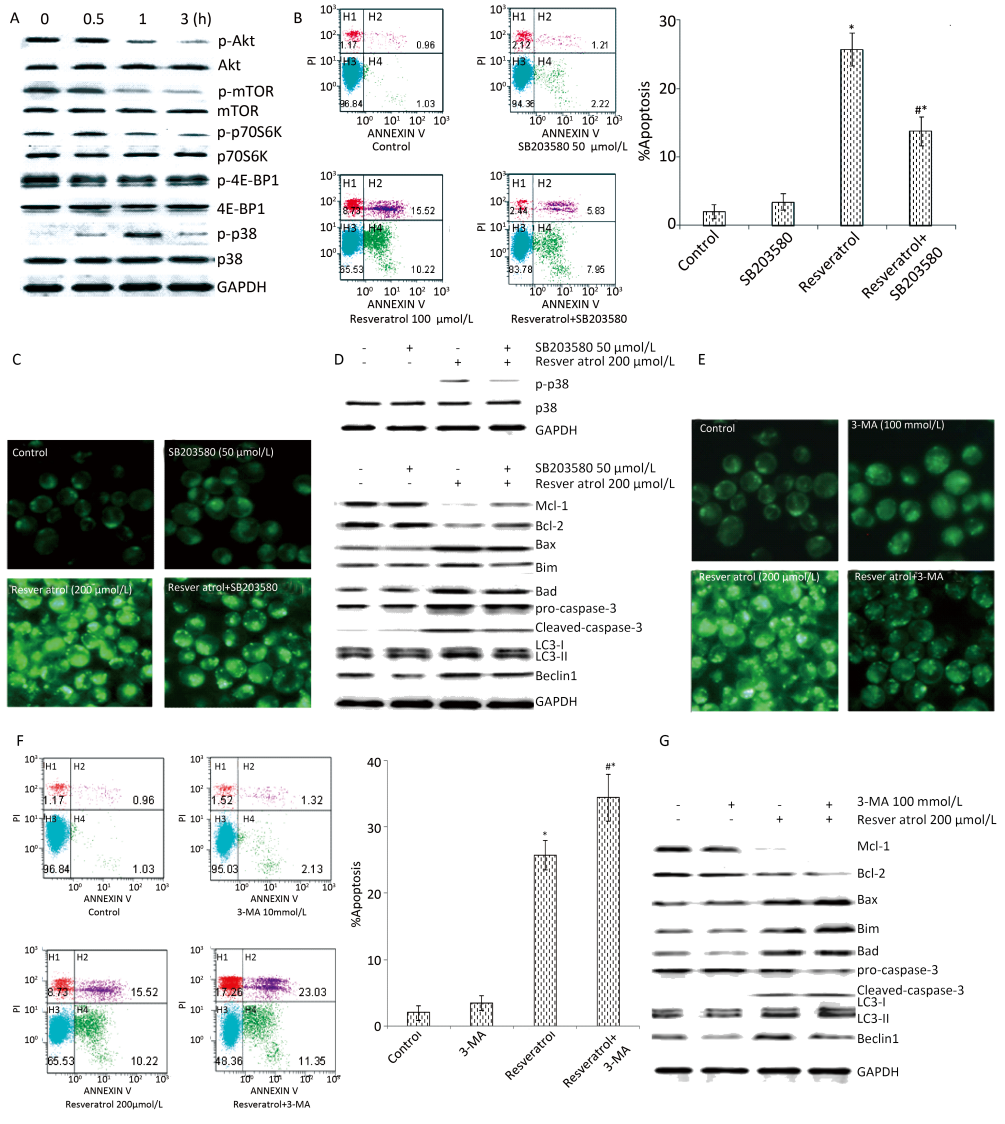

Figure 4. Signal transduction pathways of Akt/mTOR/p70S6K/4E-BP1 and p38-MAPK were involved in resveratrol-induced apoptosis and autophagy in GC-resistant T-ALL cells. A. Activity changes of Akt/mTOR/p70S6K/4E-BP1 and P38-MAPK signaling pathways in CEM-C1-15 cells treated with 200 μmol/L of resveratrol for 0, 0.5, 1, and 3 h. B. Specific p38-MAPK blocker SB203580 inhibited the apoptotic cell death induced by resveratrol. CEM-C1-15 cells were treated with 50 μmol/L SB203580 or 200 μmol/L resveratrol alone, or with both agents for 48 h. Apoptosis detection was measured by flow cytometry with Annexin V-FITC/PI double staining. Data are presented as mean±SD. $^{*}P<0.05$ as compared with the control group. $^{\#}P<0.05$, resveratrol + SB203580 versus resveratrol. C. SB203580 inhibited the autophagy in CEM-C1-15 cells induced by resveratrol. CEM-C1-15 cells were treated with 50 μmol/L SB203580 or 200 μmol/L resveratrol alone, or with both agents for 12 h. The autophagic vacuoles were observed with MDC (magnification × 400). D. Changes of phosphorylated p38-MAPK, apoptosis and autophagy-related proteins in CEM-C1-15 cells were treated with resveratrol or resveratrol plus p38-MAPK inhibitor SB203580. Cells were pretreated with or without 50 μmol/L SB203580 for 1 h, and then were treated with 200 μmol/L of resveratrol for another 1 h (p-p38) or 24 h (apoptosis and autophagy-related proteins). The cells were collected, lysed and Western blot was performed. E. Specific autophagy blocker 3-MA inhibited the autophagy in CEM-C1-15 cells induced by resveratrol. The cells treated with 10 mmol/L 3-MA or 200 μmol/L resveratrol alone, or combination with both agents for 12 h. The autophagic vacuoles were stained with MDC and observed under fluorescence microscope (magnification × 400). F. 3-MA increased the apoptosis in T-ALL cells induced by resveratrol. CEM-C1-15 cells were treated with 10 mmol/L 3-MA or 200 μmol/L resveratrol alone, or combination with both agents for 48 h. Apoptosis was measured by Annexin V-FITC/PI double staining and flow cytometry. Data are presented as mean±SD. (n=3). $^{*}P<0.05$ as compared with control group. $^{\#}P<0.05$, resveratrol + 3-MA versus resveratrol. G. Changes of apoptosis-related proteins in CEM-C1-15 cells treated with 3-MA or resveratrol alone, or combination with both agents. Cells were pretreated with or without 10 mmol/L 3-MA for 1 h, and then were treated with 200 μmol/L of resveratrol for another 24 h. The cells were collected, lysed and Western blot was performed.

cell lines. However, there have been few reports about resveratrol induced apoptosis of ALL cells despite a number of reports on solid tumors. Dörrie et al. showed that resveratrol induced extensive apoptosis by depolarizing mitochondrial membranes and activating caspase-9 in ALL cells[21]. Cecchinato et al. reported that resveratrol induced apoptosis in human T-ALL Molt-4 cells through inhibiting the Notch and PI3K/Akt pathways and activating the p53 and GSK3-beta pathways[22].

The present study demonstrated that resveratrol induced remarkable growth inhibition and extensive apoptosis in four T-ALL cell lines, among which three were GC resistant. The sensitivity to resveratrol was different in three GC-resistant T-ALL cells, with Jurkat cells being the most sensitive, followed by Molt-4 cells, while CEM-C1-15 C cells were relatively the least sensitive. It was interesting to note that the GC-sensitive cell line, CEM-C7-14 cells, was less sensitive to resveratrol than other three GC-resistant cell lines. The molecular mechanisms which were involved in resveratrol induced apoptosis turned out to be cell type-dependent.

In our study, resveratrol induced cleaved-caspase-3 and up-regulation of proapoptotic protein Bim, Bad and Bax, while down-regulation of antiapoptotic Bcl-2 and Mcl-1, as an indicator to the resveratrol induced apoptosis, might be mainly through the activation of mitochondrial pathway.

The further study on signal transduction pathways involved in resveratrol induced apoptosis verified that resveratrol suppressed Akt/mTOR signal pathway by dephosphrylation of Akt, mTOR, p70S6K and 4E-BP1, whereas p38-MAPK signal pathway was activated by phosphorylation of p38 (Figure 4A). The specific p38 inhibitor SB203580 significantly suppressed resveratrol induced down regulation of Bcl-2 and Mcl-1 in CEM-C1-15 cells, but only slightly inhibited resveratrol induced up regulation of Bim, Bax and Bad (Figure 4D), suggesting that, in addition to p38-MAPK signal pathway, other signal pathways were involved in resveratrol induced apoptosis, such as reported p53 and Notch[22].

Sustained growth arrest is an important mechanism to counteract tumor growth *in vivo*. Resveratrol has been reported to induce cell cycle arrest at G0/G1 phase in many cancer cell lines[23]. Several studies also demonstrated that resveratrol induced growth arrest and subsequent induction of apoptosis via downregulation of cyclin D1[24-25] and upregulation of p21 and p27[26]. The findings of the present study were consistent with those of previously reported studies.

Resveratrol Induced Autophagy in T-ALL Cells

Autophagy is an evolutionarily conserved, multistep lysosomal degradation process in which a cell destroys long-lived proteins and damaged organelles. Autophagy may play a crucially regulatory role in many pathological processes, most notably in cancer, and might be a target pathway of anti-cancer therapeutic agents. Resveratrol has been proposed as a potential agent for cancer chemoprevention and treatment. In addition to inducing apoptosis, resveratrol also induces autophagy in cancer cells[27].

Beclin1 and LC3 are two specific markers of cell autophagy and both of them are strongly involved in autophagic process, especially in its early stages. Beclin1 was initially isolated as a Bcl-2-binding protein[28] and the first human protein is shown to be indispensable for autophagy. Beclin1 and hVps34 (class III PtdIns 3-Kinase) form a complex in charge of autophagic nucleation in mammals. Beclin1 promotes autophagy and inhibits proliferation of cancer cells by forming and activating the autophagy promoting complex Beclin1-hVps34[29], and the suppression of Beclin1 expression impairs autophagy. LC3, associated with the formation of the autophagic vacuole[30], is currently considered as a specific molecular marker for autophagosomes in mammals. LC3 proteins can be divided into two forms: LC3-I (18kDa) and LC3-II (16kDa). The amount of LC3-II correlates with the extent of autophagosome formation and is an autophagosomal marker[31]. In the present study, the resveratrol was shown to induce formation of MDC labeled autophagolysosome, up-regulation of LC3-II and Beclin1 in T-ALL cells (Figure 2C), suggesting that resveratrol not only induced apoptosis, but also induced autophagy in T-ALL cells.

Bcl-2 homologues lie at the interface between apoptosis and autophagy, regulating these two critical cellular signaling pathways[32]. Although Bcl-2 family proteins were initially characterized as cell death regulators, it has recently been accepted that the Bcl-2 family plays a dual role in the regulation of apoptosis and autophagy. For example, proapoptotic proteins such as Bad and Bim can induce autophagy[33], while antiapoptotic proteins Bcl-2[34] can inhibit autophagy by constitutively binding to Beclin1 and sequestering Beclin1 away from Vps34[35]. The another Bcl-2 homologue Mcl-1 is also

a key autophagy regulator in which the Mcl-1 null neurons die in an autophagy-dependent manner[32]. In our present study, resveratrol induced up-regulation of Bim and Bad and meanwhile induced down-regulation of Bcl-2 and Mcl-1 in T-ALL cells, indicating that resveratrol might regulate apoptosis and autophagy in a coordinated way through inducing the changes of Bcl-2 homologues.

The signal transduction pathways involved in resveratrol induced autophagy are complex and have not yet been clarified. The study that resveratrol can induce apoptosis by activating p38-MAPK has been reported in breast cancer cells[36]. However, the effect of resveratrol induced p38-MAPK activation on autophagy has not been determined, and also there is no report on resveratrol inducing autophagy in T-ALL cells. The previous study has shown that triterpenes induced autophagy through the inhibition of p38-MAPK in colon cancer[37], but in our study, resveratrol induced autophagy in T-ALL cells through activation of p38-MAPK, and the inhibitor for p38-MAPK not only inhibited apoptosis, but also attenuated the expression of Beclin1 and the ratio of LC3-II/LC3-I (Figure 4D), indicating that activation of p38-MAPK was also involved in resveratrol induced autophagy of T-ALL cells.

Inhibition of Autophagy Potentiated Resveratrol Induced Apoptosis in T-ALL Cells

As different forms of programmed cell death, autophagy and apoptosis have a complex mutual relationship, depending on different cell types and stresses. The induction of autophagy and apoptosis can occur in a sequential, simultaneous or independent manner after cells are subject to various stresses[38]. In some instances, autophagy can act as an initiating factor for apoptosis-induction, and interruption of autophagy will delay and attenuate apoptosis. However, in other cases, autophagy can antagonize apoptosis, and blockage of autophagy will sensitize cells to apoptosis. Autophagy and apoptosis can also exist independently, and inhibition of either of them will promote cells to opposite program cell death. Hence, the relation between autophagy and apoptosis has been increasingly noted, but the exact molecular mechanism is unclear.

The findings of the present study showed that resveratrol not only induced autophagy, but also induced apoptosis in T-ALL cells. The blockage of autophagy by 3-MA increased resveratrol induced apoptosis (Figure 4F, G), suggesting that resveratrol-induced autophagy might act as a protective mechanism to promote T-ALL cell survival. In other words, resveratrol-induced autophagy negatively modulates apoptosis and may contribute to chemo-resistance in T-ALL cells.

Taken together, our study provides evidences that resveratrol induced cell growth inhibition, autophagy and apoptosis in T-ALL cells (especially in GC-resistant cells) via the inhibition of Akt/mTOR/p70S6K/4E-BP1 and the activation of p38-MAPK. These findings support the possibility of resveratrol as a potential drug for the treatment of T-ALL, especially for GC-resistant T-ALL, and the reasonable inhibition of autophagy in T-ALL cells may be a promising strategy for resveratrol induced apoptosis and can be used as adjuvant chemotherapy for T-ALL.

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DECLARATION OF INTERESTS

The authors declare that they have no conflict of interests.

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