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Effects of Resveratrol and Vitamin C on the Self-Renewal Capacity of Blast Progenitors in Hematologic Malignancies

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Abstract

Although a large number of phytochemicals has been shown to inhibit the growth of leukemic and cancer cells, their effects on the self-renewal (SR) capacity of the malignant stem/progenitor cells were not examined. In seven hematologic malignant cell lines including U-937, Mo7e, K-562, HL-60, U-266, Raji and Daudi, the SR capacity of blast progenitors was assessed by measuring cumulative clonogenic cells in suspension, whereas the terminal division of blast progenitors was assessed by blast colony formation (BCF) in methylcellulose. Resveratrol and vitamin C (Vit-C) with mean IC_{50} values of 41 and 514 μM for inhibiting BCF were added twice per week to cells in long-term suspension at 10 and 300 μM, respectively. Vit-C induced slight increase in senescence or necrosis rates, marked G_2/M arrest and significant inhibition of telomerase activity in two, one and five cell lines, respectively. Resveratrol, but not Vit-C, induced cell cycle arrest and/or cellular stress responses such as senescence, apoptosis, and necrosis and inhibited SR in all seven cell lines. Resveratrol coordinately induced S-phase arrest and cellular stress responses, inhibited telomerase activity and abolished SR in U-937, Mo7e and K-562 cells. Resveratrol induced marked increase in *p21* and/or *p27* transcript levels and senescence rates in U-937, K-562 and U-266 cells. Taken together, resveratrol has been shown to exert multifactorial actions and inhibit SR, whereas Vit-C by inhibition of telomerase activity alone did not affect SR.

Keywords: Leukemia; Self-renewal capacity; Terminal divisions; Resveratrol; Vitamin C

Introduction

Throughout the adult life of a mammalian organism, hematopoietic stem cells (HSCs) must replicate themselves to maintain the constant HSC pool in the marrow. The process of HSC replication through mitosis is called self-renewal (SR) [1]. Acute myeloid leukemia (AML) is organized hierarchically. Non-stem leukemia cells originate from leukemia stem cells (LSC), which represent the ultimate therapeutic target for AML [2-4]. In AML and acute lymphoblastic leukemia, LSCs arise from hematopoietic stem cells or from more differentiated and committed progenitors that acquire SR potential [5,6]. Leukemic blast progenitors are characterized as stem cells; they renew themselves and/or undergo terminal divisions with limited differentiation [7]. Therefore, the target of chemotherapy for leukemia may be leukemic blast progenitors.

In recent years, interest in the use of food plants and their products for cancer prevention has grown [8]. They act through one or more signaling pathways like NF-κB, Cox-2, STAT3, Akt, MAPK/ERK, Bcl-2, Caspases, PARP, MMP-2/-9 and Cyclin D1. Resveratrol, a polyphenol found in grape skins, has also been shown to inhibit the proliferation and induce the apoptosis in leukemia [9] and cancer [10] through signaling pathways such as STAT3 [11–13], PTEN [14,15], Akt/PI3k [14-20], mTOR [17,19], telomerase activity [21], MAPK/ERK [11,15,16,20,22-24], p38-MAPK [17,18, 23-25], JNK [22,25], MTA1/HDAC [14], H2AX [25], Wnt/β-catenin [26,27], TCF4 [24,27], SIRT1 [12,28], NKG2D ligands [29] and NFκB [13]. Anti-tumor effects of vitamin C (Vit-C) in leukemia and cancer have also been reported [30-32]. In addition, telomerase activity has been shown to be limiting the growth of human AML [33,34] and transformed cells [35]. However, in the previous studies, cell viability assay or blast colony formations (BCF) have been used to assess the growth inhibition by the two agents, and none of them determined the effects of the agents on the SR capacity.

Oxidative stress is an imbalance between pro-oxidant and anti-oxidant factors that can lead to cellular damage [36]. Tumor suppressor genes regulate diverse cellular activities, including DNA damage repair, cell cycle arrest, mitogenic signaling, cell differentiation, migration, programmed cell death and senescence. Links between tumor suppressor genes and reactive oxygen species have been uncovered.

In the present study, we investigated the effects of resveratrol and Vit-C on the growth of hematologic malignant blast progenitors in liquid suspension for up to one month. The cell cycle, type and rate of cellular stress responses, *p21* and *p27* trasncript levels and telomerase activity were also assessed.

Materials and Methods

Cell lines

The HL-60 (a gift from Dr. D.W. Golde, UCLA School of Medicine, Los Angeles, CA) is a myeloid cell line derived from an acute promyelocytic leukemia patient. The Mo7e (a gift from Dr. S. Tohda, Tokyo Medical and Dental University, Tokyo, Japan) is a subclone of the human megakaryoblastic leukemia cell line Mo7, and requires interleukin (IL)-3 for growth [37]. The K-562 is a myeloid cell line derived from a patient with chronic myelogenous leukemia in myeloid

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crisis. Daudi and Raji are B lymphoid cell lines derived from Burkitt's lymphoma patients. The U-937 is a human histiocytic, monocyte-like cell line derived from a diffuse histiocytic lymphoma patient. K-562, Daudi, Raji and U-937 cell lines were obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan). The human myeloma cell line U-266 was a gift from Dr. M. Kawano (Yamaguchi University School of Medicine, Ube, Japan).

Reagents

Iscove's modified Dulbecco's medium (IMDM), fetal calf serum (FCS) and PBS were from Gibco (Grand Island, NY). Trypan blue (TB) and L-ascorbic acid (Vit-C) were from Sigma (Tokyo, Japan). Recombinant human (rh) IL-3 was a gift from Dr. S. Clark (Genetics Institute, Cambridge, MA). Resveratrol was obtained from Biomol International LP (Farmingdale, PA).

Clonogenic assay in methylcellulose culture

BCF was done as previously reported [38].

Resveratrol and Vit-C treatments in long-term suspension cultures

Cells were cultured at a concentration of 10^4 cells/ml in 35-mm Lux tissue culture dishes (Miles Lab., Naperville, IL) in 2 ml of IMDM supplemented with 10% FCS + 0.38% DMSO (Cont media), 10% FCS + 0.38% DMSO + 10 μM resveratrol (Res media), or 10% FCS + 0.38% DMSO + 300 μM Vit-C (Vit-C media) [39]. Twice a week, to determine the plating efficiency, the cells were harvested, counted, washed and plated in 96-well plates at a concentration of 2×10^3 cells per well in 0.1 ml of IMDM with 1% methylcellulose and 10% FCS. The recovery per dish was determined by multiplying the plating efficiency by the number of cells harvested from the suspension. At each subculture, the harvested cells were transferred into the same fresh media. This procedure was repeated for each round of plating and the cumulative clonogenic cell number per dish with time was calculated. Mo7e cells were cultured in methylcellulose and suspension supplemented with 10 ng/ml rh IL-3.

Calculation of kinetic parameters for cumulative clonogenic cells

The number of cumulative clonogenic cells from the start of liquid suspension culture was serially plotted on a semi-logarithmic graph (Figures 1 and 2). Linear regression analysis using Excel was performed. The line of the fitted equation describing the plots and the significance of linearity were determined [40]. The relative slope (SLP) (rSLP) of the line defined as SLP_{agent}/ $SLP_{control}$ indicated the level of SR in the presence of agent.

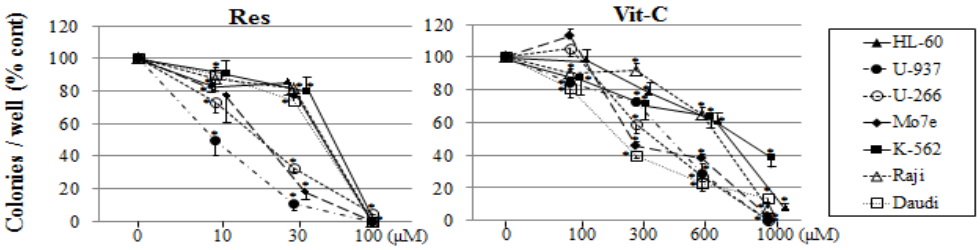

Figure 1: Effects of resveratrol and Vit-C on BCF in methylcellulose.
Full titration curves of resveratrol (Res) and Vit-C for the seven leukemia cell lines were generated. BCF per well in control culture was 100 ± 5, 68 ± 14, 125 ± 8, 129 ± 10, 115 ± 2, 70 ± 9 and 192 ± 20 in U-937, Mo7e, K-562, HL-60, U-266, Raji and Daudi cells, respectively. The data shown represent the mean ± SD of five replicate cultures. The data were normalized as the percentage of the control value in each experiment. Control medium contained 0.38% DMSO alone.
*$p < 0.05$ when compared with control cultures.

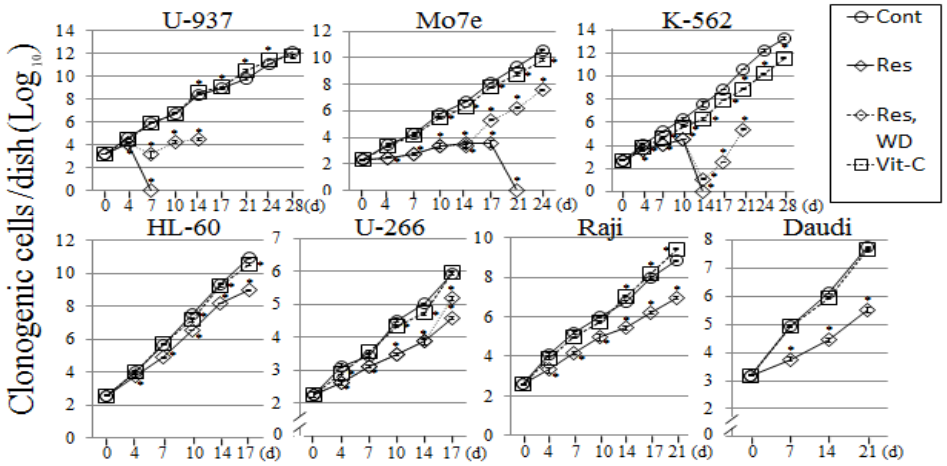

Figure 2: Effects of resveratrol and Vit-C on cumulative clonogenic cell recovery in long-term suspension cultures.
Cells were cultured in suspension with DMSO alone (Cont) or with DMSO plus resveratrol (Res) or Vit-C. Resveratrol was withdrawn at days 4, 10, 10 and 14 in U-937, Mo7e, K-562 and U-266, respectively. The data shown represent the mean ± SD of five replicate cultures. rSLP was expressed as inhibition relative to untreated cells (1.0). rSLP by Vit-C was 1.024, 0.92, 0.818, 0.963, 0.99, 1.109 and 0.967 in U-937, Mo7e, K-562, HL-60, U-266, Raji and Daudi cells, respectively. Inhibition of SR was defined as <0.8. *$p < 0.05$ when compared with control cultures. WD: withdrawal.

Telomerase activity

Telomerase activity was assessed using a Quantitative Telomerase Detection Kit (Allied Biotech, Inc., Benicia, CA). Cells were lysed and protein was isolated using the kit. The concentration of the isolated protein was determined using a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA). The telomerase activity in a cell extract was measured from its ability to synthesize telomeric repeats onto an oligonucleotide substrate. The extended product was amplified and visualized using SYBR Green. The increase in fluorescence was monitored, and the telomerase activity was determined using real-time PCR (Chromo 4TM Real-Time PCR System; Bio-Rad, Tokyo, Japan).

Trypan blue staining

Equal volumes of a cell suspension and 0.4% TB solution in 0.85% saline were incubated for 5 min at room temperature. Cells that stained blue were counted as positive. For quantification of TB-positive cells, 100 cells were counted in three separate fields using an inverted Nikon microscope, and the proportion of blue-stained cells was determined. Cells stained with specific membrane-impermeant nucleic acid dyes such as TB and propidium iodide (PI) (data not shown) were considered to be necrotic, because loss of membrane integrity is a pathognomonic feature of necrotic cell death [41].

Senescence-associated (SA)-β-galactosidase labeling

SA-β-galactosidase was detected using a Senescence Detection Kit (BioVision Inc., Milpitas, CA). Briefly, cells were fixed for 15 min with Fixative Solution and washed once with PBS. Cells were then incubated overnight at 37°C with Staining Solution Mix containing 1 mg/ml X-Gal. For quantification of SA-β-galactosidase-positive cells, 100 cells were counted in three separate fields using an inverted Nikon microscope, and the proportion of blue-stained cells was determined.

Cell cycle analysis

DNA in permeabilized cells was stained with PI using a cell phase determination kit (Cayman Chemical Company). Flow cytometry was used to detect staining and determine the percentage of cells in each phase of the cell cycle [42]. The fluorescence of PI-stained cells (10^4 nuclei) was measured within 30 min on a FACSCanto II (BD Biosciences, Tokyo, Japan) equipped with a doublet discrimination module. The apoptotic cell fraction was also determined.

Reverse transcription-PCR (RT-PCR) assay

Total cellular RNA was extracted using a High Pure RNA Isolation Kit (Roche, Mannheim, Germany). The concentration and purity of the extracted RNA were evaluated from spectrophotometric absorbance readings at 260 and 280 nm (JASCO V-530 UV-Vis spectrophotometer, Easton, MD). RT-PCR was performed with total RNA and an mRNA Selective PCR Kit Ver. 1.1 (TaKaRa, Tokyo, Japan), which prevents amplification of genomic DNA, using a PCR system (TaKaRa). The primers were 5'-ATCCCGTGTTCTCCTTT-3' (forward) and 5'-CGACCGATCTTCGG-3' (reverse) for *p21*, 5'-AACGTGCGAGTGTCTAACGG-3' (forward) and 5'-TGCGTGTCCTCAGAGTTAGCC-3' (reverse) for *p27*, and 5'-CGATGCTGGCGCTGAGTAC-3' (forward) and 5'- CGTTCAGCTCAGGGATGACC-3' (reverse) for *GAPDH*. Samples were size-fractionated by agarose gel electrophoresis along with a 50-bp molecular weight marker (Roche), and stained with ethidium bromide (Wako, Osaka, Japan).

Densitometry

Gel images were captured using a ChemiDoc XRS (Bio-Rad). A housekeeping gene (*GAPDH*) was co-amplified under the same conditions, and the expression of each target gene relative to *GAPDH* expression was determined. Specific bands and backgrounds were enclosed within the same square, and background subtraction densitometric analysis was performed using Quality One (Bio-Rad).

Statistics

Data for BCF are shown as the mean ± SD of five replicate cultures. The other assay was done in triplicate. The statistical significance was evaluated using Student's *t*-test (two-tailed) or the chi-squared test.

Results

Growth regulation in methylcellulose

In the seven cell lines, resveratrol and Vit-C inhibited BCF in a dose-dependent manner and IC_{50} ranged from 9.9 to 68 μM and 240 to 830 μM with a mean of 41 and 514 μM, respectively (Figure 1). Thus, in subsequent experiments, we cultured cells in suspension with resveratrol and Vit-C at 10 and 300 μM, respectively. These concentrations inhibited BCF by 20% ± 15% and 34% ± 17% (mean ± SD for % inhibition relative to control in seven cell lines), respectively. Three cell lines (U-937, Mo7e and U-266) were sensitive and three (HL-60, K-562 and Raji) were resistant to the two agents, and the remaining one (Daudi) showed reversed sensitivities to the agents.

Growth regulation in liquid suspension

An exponential increase in the number of cumulative clonogenic

Table 1: Inhibition of SR and telomerase activity and induction of cellular stress response and cell cycle arrest by resveratrol and Vit-C in seven cell lines.

Variable	HL-60	U-937	U-266	Mo7e	K-562	Raji	Daudi
rSLP[a]	Res (0.787)	Res (-0.378)	Res (0.666)	Res (-0.281)	Res (-0.443)	Res (0.713)	Res (0.514)
Telomerase activity[b]	Vit-C (+)	Res (+), Vit-C (+)	(-)	Res (+), Vit-C (+)	Res (+), Vit-C (+)	Vit-C (+)	(-)
Senescence[c]	(-)	Res (+++), Vit-C (+)	Res (++)	(-)	Res (+++)	(-)	Res (+)
Necrosis[c]	(-)	Res (+++)	Res (+)	Res (+++)	(-)	(-)	Res (+), Vit-C (+)
Apoptosis[d]	Res (+)	Res (++)	Res (+)	Res (++)	Res (++)	(-)	Res (+)
G_1 arrest[e]	(-)	(-)	(-)	(-)	(-)	(-)	(-)
S-phase arrest[e]	(-)	Res (++)	(-)	Res (+++)	Res (+++)	Res (++)	(-)
G_2/M arrest[e]	(-)	(-)	(-)	Vit-C (++)	(-)	(-)	Res (+)

[a] Inhibition of SR was defined as <0.8. rSLP is shown in parenthesis.
[b] No significant inhibition compared with control (-); and significant inhibition compared with control (+) by Student's *t*-test.
[c] The percentage of positive cells <10 or not significantly higher than those of control (-); and ≥10 and <20 (+), ≥20 and <40 (++), and ≥40 (+++) with values significantly higher than those of control by Student's *t*-test.
[d] % control as ≤250: (-); >250 and ≤350: (+); and > 350: (++).
[e] % control as ≤124: (-); >124 and ≤130: (+); >130 and ≤140: (++); and >140: (+++).

cells recovered per dish was observed in each of the 21 conditions (7 cell lines treated with or without each of the two agents), except for three in which growth was abolished ($p < 0.01$, r ranged from 0.984 to 0.999 by linear regression analysis) (Figure 2). Repeated addition of 10 μM resveratrol abolished SR in U-937, Mo7e and K-562 cells at days 7, 21 and 14, respectively and reduced rSLP levels to less than 0.8 in the remaining four cell lines (Table 1). Furthermore, after washout of resveratrol, rSLP returned to the control levels in four cell lines. Repeated addition of 300 μM Vit-C reduced rSLP level to less than 0.8 in none of the seven cell lines and enhanced SR in Raji cells with an rSLP of 1.109.

The sensitivities of BCF and SR to resveratrol were high in U-937 and Mo7e cells, low in HL-60 and Raji cells, and reversed in K-562 cells (Figures 1 and 2).

Cellular stress responses such as senescence, apoptosis and necrosis in liquid suspension

In U-937 cells, repeated addition of 10 μM resveratrol markedly increased the rate of senescence and necrosis (Figure 3) and the relative apoptotic ratio, determined with flow cytometry analysis (Figure 4). Resveratrol markedly increased senescence in U-266 cells, senescence and apoptosis in K-562 cells, and necrosis and apoptosis in Mo7e cells, and slightly increased apoptosis in HL-60 cells and all three in Daudi cells. Repeated addition of 300 μM Vit-C slightly increased senescence in U-937 cells and necrosis in Daudi cells.

Resveratrol increased senescence (4/7, 57%), necrosis (4/7, 57%) and apoptosis (6/7, 86%), and Vit-C increased senescence (1/7, 14%), necrosis (1/7, 14%), and apoptosis (0/7, 0%) (Table 1). Thus, resveratrol more frequently increased apoptosis than Vit-C ($p < 0.01$ by the chi-squared test).

Resveratrol withdrawal effects

In K-562 cells in liquid suspension, withdrawal of resveratrol after 4 days or after 4 days and 7 days significantly decreased necrosis and senescence when compared with the effects of repeated addition of resveratrol for 7 days or 10 days, respectively (Figure 5). Similarly, in U-937 cells, withdrawal of resveratrol after 4 days significantly decreased necrosis and senescence when compared with the effects of repeated addition of resveratrol for 14 days.

Cell cycle analysis

Repeated addition of 10 μM resveratrol induced S-phase arrest in U-937, Mo7e, K-562 and Raji cells, and G_2/M arrest in Daudi cells (Figure 6 and Table 1). Repeated addition of 300 μM Vit-C induced G_2/M arrest in Mo7e cells. Resveratrol (5/7; 71%) induced cell cycle arrest more frequently than Vit-C (1/7; 14%) ($p < 0.05$ by the chi-squared test).

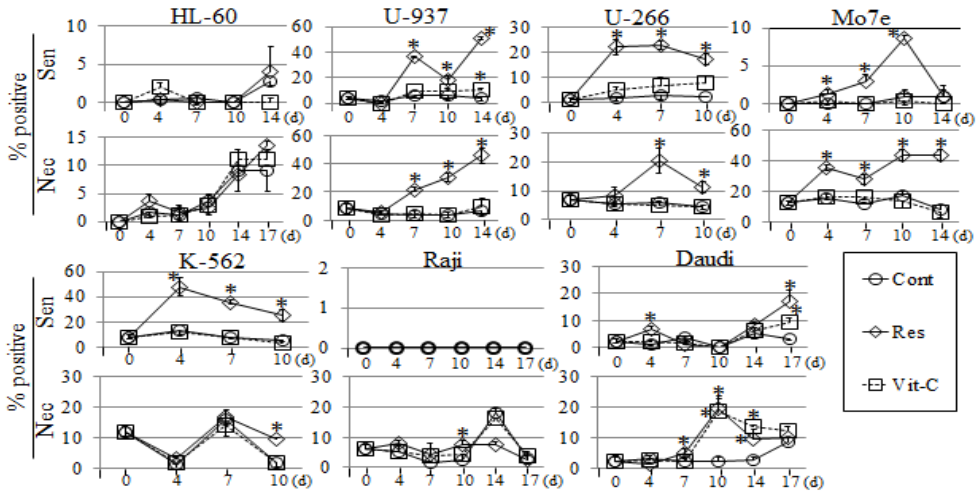

Figure 3: Senescence and necrosis in liquid suspension cultures.
Cells were treated as described in Figure 2. The percentage of cells stained with SA-β-galactosidase (Sen) or trypan blue (Nec) was determined. The data shown represent the mean ± SD of three separate measurements. *$p < 0.05$ when compared with control cultures.

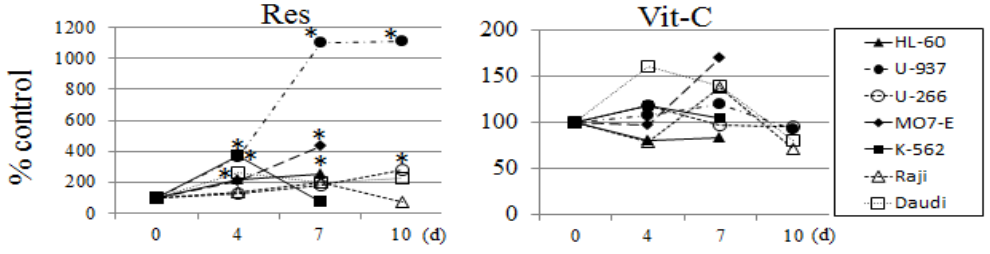

Figure 4: The relative apoptotic cell fraction determined with flow cytometry analysis.
Cells were treated as described in Figure 2. The data were normalized as the percentage of the control value in each experiment. The percentage of apoptotic cells in control culture was 0.3% ± 0.7% (n = 7), 4.8% ± 5.6% (n = 7), 3.1% ± 2.6% (n = 7) and 1.1% ± 0.6% (n = 4) at day 0, 4, 7 and 10, respectively. * Induction of apoptosis was defined as >250%. ≤250%: (-) (see Table 1).

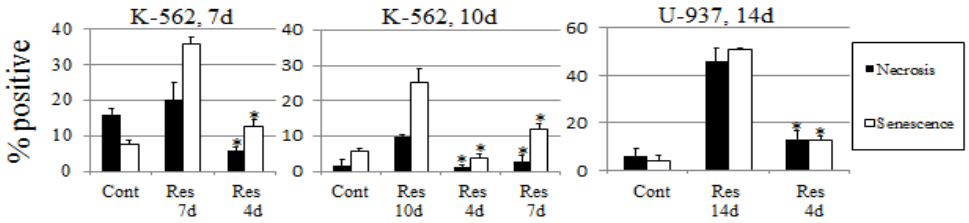

Figure 5: Effects of Resveratrol withdrawal on senescence and necrosis of leukemia cells.
K-562 and U-937 cells in liquid suspension were cultured for the indicated periods with continuous exposure to resveratrol (Res) or with exposure to resveratrol followed by resveratrol withdrawal. Control (Cont) medium contained 0.38% DMSO alone. The percentages of cells stained with SA-β-galactosidase or trypan blue were determined after culture for the indicated periods. The data shown represent the mean ± SD of three separate determinations. *$p < 0.05$ when compared with continuous exposure to resveratrol.

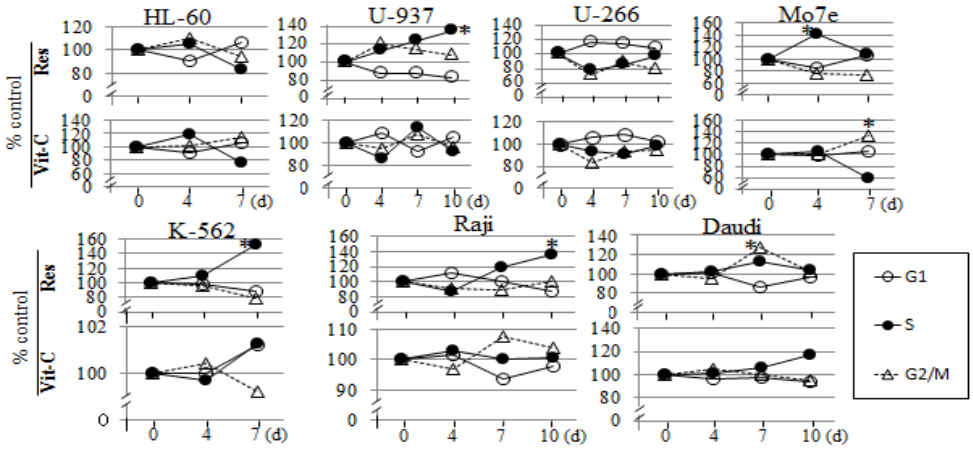

Figure 6: Cell cycle analysis.
Cells were treated as described in Figure 2. The data shown represent the mean of two or three separate measurements. The data were normalized as the percentage of the control value in each experiment. *Cell cycle arrest was defined as >124%. ≤ 124%: (-) (see Table 1).

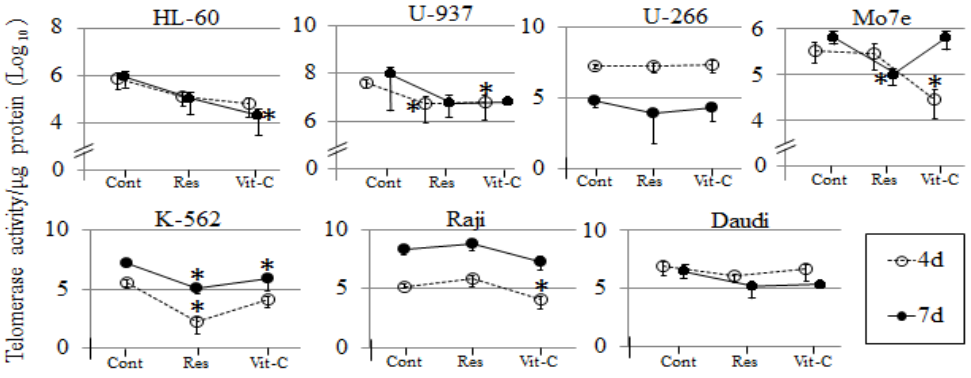

Figure 7: Effects of resveratrol and Vit-C on the telomerase activity of leukemia cells.
Cells were treated as described in Fig. 2. The data shown represent the mean ± SD of three separate measurements. *$p < 0.05$ when compared with control cultures.

Telomerase activity

Each of the two agents, by repeated addition, significantly inhibited telomerase activity in U-937, Mo7e and K-562 cells at day 4 or 7 (Figure 7). In U-266 and Daudi cells, neither of the two agents significantly inhibited telomerase activity.

CKI gene expression profiles in liquid suspension culture

In U-937 and K-562 cells, repeated addition of 10 μM resveratrol induced more than tenfold increase in *p21* levels on day 4 and the expression returned to basal level on day 7 (Figure 8). In U-266 cells, resveratrol also markedly and transiently increased *p27*, but not *p21*.

Discussion

SR inhibition by repeated addition of resveratrol was as follows:

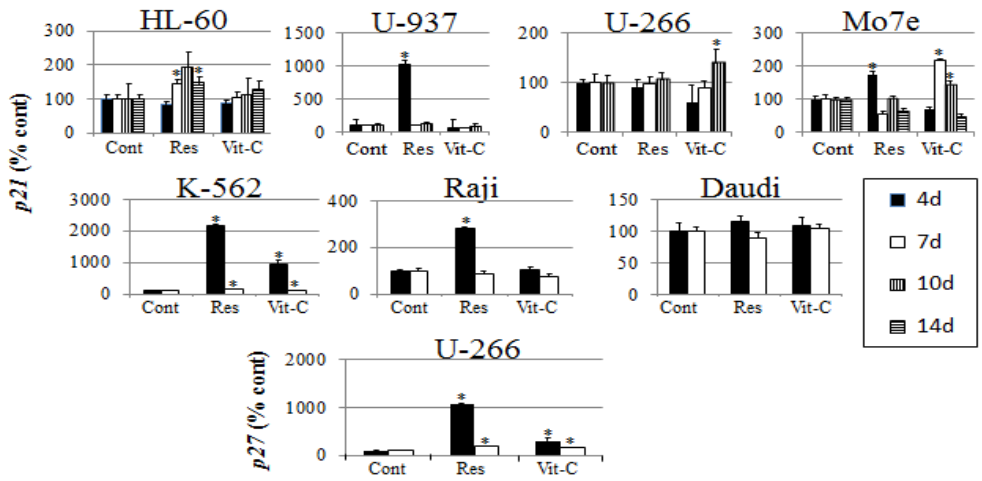

Figure 8: *p21* and *p27* gene expression profiles in liquid suspension cultures.
Cells were treated as described in Figure 2. The relative band densities were expressed as the mean ± SD of three separate determinations. * $p < 0.05$ when compared with control.

induction of S-phase arrest and cellular stress responses, inhibition of telomerase activity and abolishment of SR in U-937, Mo7e and K-562 cells; induction of G_2/M arrest and cellular stress responses, no inhibition of telomerase activity and inhibition of SR with rSLP of 0.514 in Daudi cells; and induction of either cell cycle arrest or cellular stress responses, no inhibition of telomerase activity and inhibition of SR with rSLP between 0.666 and 0.787 in the remaining three (Table 1). SR inhibition by repeated addition of Vit-C was as follows: induction of either cell cycle arrest or cellular stress responses with rSLP between 0.92 and 1.024 in three cell lines; and induction of neither cell cycle arrest nor cellular stress response with rSLP between 0.818 and 1.109 in the remaining four, including inhibition of telomerase activity in two and three cell lines, respectively.

The SR capacity of leukemic blast progenitors, assessed by measuring secondary plating efficiency or exponential growth in suspension culture [39], but not BCF, has been reported to be highly correlated with the clinical outcome in AML patients [43-45]. Competitive repopulation units (CRU) assay [46] and *in vitro* serial plating assay [47] are also available to determine the SR capacity. From this point of view, the effects of antitumor drugs on not only terminal divisions but also SR of blast progenitors should be examined to predict the effectiveness of the drugs in the therapy for malignancy. We showed that resveratrol and Vit-C preferentially inhibits SR capacity and BCF, respectively. There was no significant correlation between rSLP and % of control BCF in the seven cell lines after treatment with resveratrol (r = 0.24 by linear regression analysis) or Vit-C (r = 0.369 by linear regression analysis). This may be mainly owing to the differences in the exposure duration of the cells to the agents and the cumulative cellular damage by the agents between the two culture conditions.

In the present study, 10 μM resveratrol induced reversible inhibition of leukemia SR capacity. In HepG2 cells, lower (6.25–25 μM) and higher (≥ 50 μM) concentrations of resveratrol induced reversible S-phase arrest and irreversible apoptosis, and inhibition of PI3K and MAPK/ERK may be responsible for the induction of S-phase arrest and apoptosis, respectively [16]. However, silencing of the *p21* gene did not show any effect on resveratrol-induced S-phase arrest.

Our data indicate that the differential induction of the three types of cellular stress responses by each of the two agents depends on the type of the leukemia cell line. This may be compatible with the recent report that the cell type and the nature and intensity of the damage are the critical determinants of senescence or apoptosis induction [48].

Senescence is characterized by irreversible cell cycle arrest; overexpression of cyclin-dependent kinase inhibitors (CKIs); caspase-independent cell death; and a strong resistance to apoptosis [49]. Consistent with the notion that senescence is a tumor suppression mechanism, CKIs such as p53, pRb, p16, p19, p21 and p27 are regulators of senescence [50,51]. Resveratrol in U-937, K-562 and U-266 markedly increased *p21* and/or *p27* transcript levels and senescence rates, although *p53* and *p16* genes are absent or mutated in the cell lines [42,50,51].

Vit-C induces partial G_1/S block, inhibits telomerase activity, upregulates p53 and Bax, and inhibits Bcl-2 [52]. Resveratrol downregulates telomerase activity and cancer cell growth in vitro [21]. In cancer, the noncanonical signaling loop between telomerase reverse transcriptase (TERT) and NF-κB and between TERT and Wnt/β-catenin signaling has been proposed as a mechanism for the concomitant activation of NF-κB- and Wnt/β-catenin-dependent transcription following TERT overexpression. This drives the prolonged expression of target genes critical for the maintenance of tumor survival and proliferation [53]. However, hTERT depletion alone did not affect cancer cell survival [54].

Induction of cell cycle arrest and apoptosis, but not the inhibition of telomerase activity, was more frequent by resveratrol than Vit-C. It remains to be further clarified through which signaling pathways resveratrol, but not Vit-C, abolishes SR of blast progenitors. Compared with previous reports [39,46,47], our newly proposed assay may be quite useful to evaluate the SR capacity of blast progenitors because it indicates SR capacity as rSLP. By adding inhibitors, stimulators, ligands or siRNA to our assay, major stimulatory and inhibitory signaling pathways for SR capacity of blast progenitors may be uncovered.

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