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ORIGINAL PAPER

The roles of bioactive sphingolipids in resveratrol-induced apoptosis in HL60 acute myeloid leukemia cells

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Abstract
Purpose Acute promyelocytic leukemia results from a translocation between 15 and 17 chromosomes that produce PML/RARα fusion protein. PML/RARα inhibits differentiation of myeloid precursor cells at stem cell level. Resveratrol is a phytoalexin that exerts cytotoxic effects on cancer cells. Ceramides have crucial roles in cell growth, proliferation, differentiation, drug resistance, and apoptosis. In this study, we examined the possible cytotoxic effects of resveratrol on acute myeloid leukemia cells and determined the roles of ceramide-metabolizing genes in resveratrol-induced apoptosis, in addition to investigating the possibility of increasing the sensitivity of HL60 cells to resveratrol by manipulating sphingolipids.
Methods Cytotoxic effects of resveratrol, C8:ceramide, PDMP, and SK-1 inhibitor were determined by XTT cell proliferation assay. Changes in caspase-3 enzyme activity and mitochondrial membrane potential (MMP) were measured using caspase-3 colorimetric assay and JC-1 MMP detection kit. Expression levels of ceramide-metabolizing genes were examined by RT-PCR.
Results The results revealed that manipulations of ceramide metabolism toward generation or accumulation of apoptotic ceramides increased apoptotic effects of resveratrol in HL60 cells, synergistically. More importantly, gene expression analyses revealed that resveratrol-induced apoptosis via increasing expression levels of ceramide-generating genes and decreasing expression levels of antiapoptotic sphingosine kinase-1 and glucosylceramide synthase genes.
Conclusion These results showed for the first time that increasing intracellular levels of ceramides by biochemical approaches has also increased sensitivity of HL60 cells to resveratrol. We also showed that resveratrol induces apoptosis through manipulating ceramide-metabolizing genes that resulted in the accumulation of ceramides in HL60 cells.

Keywords Ceramides · Bioactive sphingolipids · Resveratrol · Acute myeloid leukemia · HL60

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Introduction

Acute promyelocytic leukemia (APL) is a subtype of leukemia that results from chromosomal translocation of t(15;17) (q21;q22), which encodes promyelocytic leukemia protein (PML)/retinoic acid receptor α (RARα) fusion protein (Alcalay et al. 1992). PML is involved in controlling of genomic stability and tumor suppression, while RARα binds to retinoic acid for differentiation of myeloid cells. PML/RARα has an aberrant protein function acting as a repressor of retinoic acid that induces differentiation of myeloid cells (Lo-Coco and Ammatuna 2006). The prevalent therapy of acute myeloid leukemia is treatment with all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) or a combination of them with some other agents (Lengfelder et al. 2000). They bind to RARα receptor and induce differentiation of myeloid cells. In addition to ATO and ATRA, DNA methylase inhibitors, rexinoids, and histone deacetylase inhibitors (HDACI) have also been used in

the clinic (Vitoux et al. 2007). The natural products that support the treatment of cancer have been given more attention in the clinic recently.

Resveratrol (3,5,4′-trans-trihydroxystilbene), is one of the these natural products found in plant constitutes such as grape skin, red wine, cranberries, blueberries, and peanuts (Lee et al. 2008). It has shown significant cytotoxic and apoptotic effect on various types of cancer cells with no harm to normal healthy cells (Pirola and Frojdo 2008). Resveratrol is synthesized from malonyl-CoA and *p*-coumaroyl-CoA molecules and yields 3,5,4′-trans-trihydroxystilbene trans-resveratrol in response to plant stress, UV radiation, injury, and fungal infection (Baur and Sinclair 2006). It is a well-known phytochemical that acts as antioxidant, anti-inflammatory, cardioprotective, and more importantly has anti-tumor activities. Resveratol inhibits tumor initiation, promotion, and progression (Jang et al. 1997; Sahin et al. 2007). However, the mechanism of resveratrol-induced cell death is not well known.

Ceramide is a crucial signaling mediator in sphingolipid metabolism, which is a key component in cell differentiation, growth, proliferation, and apoptosis (Dagan et al. 2003). It was recently demonstrated that de novo ceramide synthesis is regulated by members of the mammalian longevity assurance gene (LASS) family that are also known as ceramide synthase genes (D'Mello et al. 1994). Each gene is responsible for the generation of different lengths of ceramides having different fatty acids (Pewzner-Jung et al. 2006). While LASS1 is responsible for the synthesis of C_{18}-ceramide (Venkataraman et al. 2002), LASS2 increased the intracellular levels of C_{24}-ceramides (Mizutani et al. 2005), and LASS4 increased the levels of C_{22}-ceramides (Riebeling et al. 2003). C_{14}- and C_{16}-ceramides were synthesized by LASS5 and LASS6 genes (Weinmann et al. 2005; Xu et al. 2005). It was shown by our group and by some others that accumulation of ceramide in response to different stress conditions, such as chemotherapeutics, radiation, and environmental stress, leads to apoptosis and inhibition of cell cycle progression in various types of cancer cells, while it also increases the sensitivity of cancer cells to chemotherapeutics (Phillips et al. 2007; Schenck et al. 2007).

Glucosylceramide synthase (GCS) enzyme transfers glucose from UDP-glucose to ceramide and produces glucosylceramide (Gouaze-Andersson and Cabot 2006). Accumulation of glucosylceramide is a characteristic of some multidrug-resistant cancer cells of leukemia, breast, ovarian, and colon carcinomas (Liu and Wang 2008; Ruckhaberle et al. 2009; Xie et al. 2008). On the other hand, apoptotic ceramide can also be converted to anti-apoptotic sphingosine-1-phosphate by sphingosine kinase-1 (SK-1) (Taha et al. 2006). Glucosylceramide and sphingosine-1-phosphate promote cell growth and survival, induce angiogenesis and metastasis, and inhibit apoptosis (Ogretmen and Hannun 2004). Thus, targeting ceramide metabolism toward generation and/or accumulation of apoptotic ceramides may provide an important strategy for the treatment of different types of cancers.

In this study, we have shown that resveratrol has cytotoxic and apoptotic effects on human HL60 acute myeloid leukemia cells. More importantly, it was shown for the first time by this study that resveratrol induces apoptosis through increasing accumulation of ceramides by induction of LASS genes and repression of SK-1 and GCS genes.

Materials and methods

Chemicals

Resveratrol was obtained from Sigma-Aldrich (USA). A 10-mM stock solution of resveratrol was prepared with DMSO and stored at −20°C. C8:ceramide, *N*-(2-hydroxy-1-(4-morpholinylmethyl)-2-phenylethyl)-decanamide, hydrochloride (PDMP), and SK-1 inhibitor were obtained from Cayman Chemicals (USA), dissolved in DMSO, and stored at −20°C. The final concentration of DMSO did not exceed more than 0.1% in culture. Primers were obtained from Eurofins, Germany.

Cell lines and culture conditions

Human HL60 APL cells were kindly provided by Dr. Ali Ugur Ural from Gulhane Military Medical School, Department of Hematology. They were grown and maintained in RPMI1640 medium (Biological Industries, Israel) containing 10% fetal bovine serum and 1% penicillin–streptomycin (Invitrogen, USA) at 37°C in 5% CO_2.

Measurement of cell growth by XTT cell proliferation assay

The IC50 values (drug concentration that inhibits cell proliferation by 50%) of resveratrol, a ceramide analog, C8:ceramide, and GCS enzyme inhibitor (PDMP), as well as the IC10 value of SK-1 enzyme inhibitor were determined from cell proliferation plots obtained by XTT assay as described (Gucluler and Baran 2009). Briefly, 2×10^4 cells were seeded into 96-well plates containing 200 µl growth medium in the absence or presence of increasing concentrations of resveratrol, C8:ceramide, PDMP, or SK-1 inhibitor. After 72 h of incubation in 5% CO_2 incubator, they were treated with 40 µl of XTT reagent for 4 h. Then, the absorbances of the samples were measured under 490 nm wavelength of light by Elisa reader (Thermo Electron Corporation Multiscan Spectrum, Finland) and used for the cell proliferation plots.

Finally, IC50 values of resveratrol, C8:ceramide, or PDMP, and IC10 values of SK-1 inhibitor were calculated from cell proliferation plots. To determine the possible synergistic effects of resveratrol in combination with these ceramide metabolism-manipulating chemicals, increasing concentrations of resveratrol were applied together with IC50 value of C8:ceramide or PDMP, or IC10 value of SK-1 inhibitor. The same procedure of XTT cell proliferation assay was applied for these set of experiments as mentioned above.

Measurements of caspase-3 enzyme activity

Changes in caspase-3 enzyme activities were determined using caspase-3 colorimetric assay kit (R&D Systems, USA) as described (Baran et al. 2007). As much as 1×10^6 cells were seeded in six-well plate in 2 ml growth medium in the absence or presence of increasing concentrations of resveratrol, C8:ceramide, PDMP, or SK-1 inhibitor, or a combination of resveratrol with the others. After 72 h of incubation in 5% CO_2 incubator, the cells were collected by centrifugation at 1,000*g* for 10 min. The pellets were treated with 100 µl of cold lysis buffer (1×) to obtain the cell lysate. Then, the cell lysates were incubated on ice for 10 min and centrifuged at 14,000*g* for 1 min. Following the centrifugation, supernatants were transferred to new Eppendorf tubes. For measuring caspase-3 enzyme activity, reaction mixture including 50 µl of reaction buffer (2×), 50 µl of the samples, and 5 µl of caspase-3 colorimetric substrate was applied to the cell suspensions in 96-well plate and incubated for 2 h at 37°C in 5% CO_2 incubator. Absorbances of the samples were read under 405 nm wavelength of light via the ELISA reader (Thermo Electron Corporation Multiskan Spectrum, Finland). Then protein concentrations were measured by Bradford assay. Finally, enzyme activity levels were normalized to protein concentrations and determined by Bradford assay.

Detection of the loss of mitochondrial membrane potential

The loss of mitochondrial membrane potential was detected by JC-1 mitochondrial membrane potential (MMP) kit (Cell Technology, USA). As much as 1×10^6 cells were seeded in six-well plate in 2 ml growth medium and treated with different concentrations of resveratrol, C8:ceramide, SK-1 inhibitor, or PDMP alone, or a combination of resveratrol with the others and incubated for 72 h. Treated cells were collected by centrifugation at 1,000*rpm* for 10 min. Supernatants were removed and the pellets were dissolved in 500 µl of JC-1 dye before their incubation at 37°C in 5% CO_2 for 15 min. Then, the mixtures were centrifuged at 1,000*g* for 5 min. Pellets were resuspended in 2 ml of blank media to remove excess dye and centrifuged at 1,000*rpm* for 5 min. All pellets were resuspended in 500 µl of blank media, and 150 µl from each was added into black 96-well plate. The aggregate red form of the dye, which is found in the inner mitochondria, has an absorption/emission maxima of 585/590 nm and the monomeric green form of the dye, which is released into the cytoplasm due the loss of the MMP, has an absorption/emission maxima of 510/527 nm. The plate was read at these wavelengths by fluorescence Elisa reader (Thermo Varioskan Spectrum, Finland). Finally, green/red (510/585) values were calculated to determine the changes in MMP.

Total RNA isolation and RT-PCR

The expression levels of ceramide-metabolizing genes were determined by reverse transcriptase-polymerase chain reaction (RT-PCR). As much as 2×10^6 cells were seeded in six-well plate in 3 ml growth medium and treated with different concentrations of resveratrol for 72 h in 5% CO_2. Total RNA was extracted using a Nucleospin Total RNA Isolation kit (Machery-Nagel, USA) as described by the manufacturer. The amount and quality of RNA were measured by Nanodrop Photospectrometer (NanoDrop 1000 THERMO, USA). As much as 5 µg of total RNA was reverse transcribed into cDNA by reverse transcriptase enzyme (Fermentas, USA). After incubation at 42°C for 50 min, the reactions were stopped at 95°C for 5 min. The resulting total cDNA was then used to determine the expression levels of LASS1, LASS2, LASS4, LASS5, LASS6, SK-1, and GCS. Expression levels of *β*-actin were used as internal positive control. The primer sequences were as follows:

LASS1-F(5′-CTATACATGGACACCTGGCGCAA-3′),
LASS1-R(5′-TCAGAAGCGCTTGTCCTTCACCA-3′);
LASS2-F(5′-GCTGGAGATTCACAT-3′),
LASS2-R(5′-GAAGACGATGAAGAT-3′);
LASS4-F(5′-TGCTGTCCAGTTTCAACGAG-3′),
LASS4-R(5′-GAGGAAGTGTTTCTCCAGCG-3′);
LASS6-F(5′CCTCGAGGGATGGATTACAAGGATGA CGACGATAAGATGGCAGGG ATCTTAGCCTGG-3′),
LASS6-R(5′-CGGAATTCCGTTAATCATCCATG-GAGCAGGA-3′);
SK1-F(5′-CCGACGAGGACTTTGTGCTAAT-3′),
SK1-R(5′-GCCTGTCCCCCCAAAGCATAAC-3′);
GCS-F(5′-ATGACAGAAAAAGTA-3′),
GCS-R(5’-GGACACCCCTGAGTG-3′); and
β-actin-F(5′-CAGAGCAAGAGAGGCATCCT-3′),
β-actin-R(5′-TTGAAGGTCTCAAACATGAT-3′) (Baran et al. 2007).

Products of PCR reactions were visualized under UV after running them on 2% agarose gel electrophoresis including ethidium bromide at 90 V for 1 h.

Results

Cytotoxic effects of resveratrol, C8:ceramide, SK-1 inhibitor, and PDMP on HL60 cells

There were dose-dependent cytotoxic effects of resveratrol, C8:ceramide, PDMP, and SK-1 inhibitor on HL60 cells as measured by XTT assay. IC50 values of resveratrol (Fig. 1a), C8:ceramide (Fig. 1b), and PDMP (Fig. 1c) were calculated from cell proliferation plots and found to be 75, 45, and 61 μM, respectively. IC10 value of SK-1 inhibitor (Fig. 1d) was also calculated and found to be 5 μM.

Resveratrol in combination with C8:ceramide, PDMP, or SK-1 inhibitor showed significant synergistic cytotoxic effects on HL60 cells

There were 3, 5, 15, 29, and 45% decreases in cell proliferation of HL60 cells in response to 1, 5, 10, 20, and 50 μM resveratrol, respectively (Fig. 1a). Combination of the same concentrations of resveratrol with 45 μM C8:ceramide, which induces de novo generation of ceramides, decreased cell proliferation by 75, 76, 80, 81, and 82%, respectively, as compared to untreated controls (Fig. 2), while combination with 61 μM PDMP, which provides accumulation of ceramides, resulted in 66, 71, 76, 88, and 89% decreases in proliferation of HL60 cells, respectively (Fig. 2). Compared to untreated controls, 5 μM of SK-1 inhibitor in combination with the same concentrations of resveratrol caused 19, 28, 39, 44, and 71% decreases in cell proliferation, respectively.

Changes in caspase-3 enzyme activity showed synergistic apoptotic effects of resveratrol with ceramide, PDMP, or SK-1 inhibitor as compared to any agent alone

Caspase-3 enzyme activity was measured in HL60 cells exposed to any agent alone or combinations of resveratrol/C8:ceramide, resveratrol/PDMP, and resveratrol/SK-1 inhibitor. HL60 cells treated with 5 and 10 μM resveratrol showed 1.11, and 1.14-fold increases in caspase-3 enzyme activity as compared to untreated controls, while 45 μM C8:ceramide, 61 μM PDMP, and 5 μM SK-1 inhibitor resulted in 2.17, 1.81, and 1.71-fold increases in caspase-3 enzyme activity (Fig. 3). On the other hand, 5 μM resveratrol in combination with the same concentrations of C8:ceramide, PDMP, or SK-1 inihibitor resulted in 2.96, 2.62, and 2.45-fold increases in the enzyme activity, respectively (Fig. 3). There were 3.77, 2.68, and 2.4-fold increases in caspase-3 enzyme activity in HL60 cells incubated with a combination of the same concentrations of C8:ceramide, PDMP, or SK-1 inihibitor with 10 μM resveratrol, respectively (Fig. 3).

Effects of resveratrol alone and in combination with C8:ceramide, PDMP, or SK-1 inhibitor on loss of mitochondrial membrane potential

To confirm the caspase-3 enzyme activity results, we conducted the same set of experiments to examine the changes in mitochondrial membrane potential. The results revealed that there were 1.37 and 1.45-fold increases in cytoplasmic/

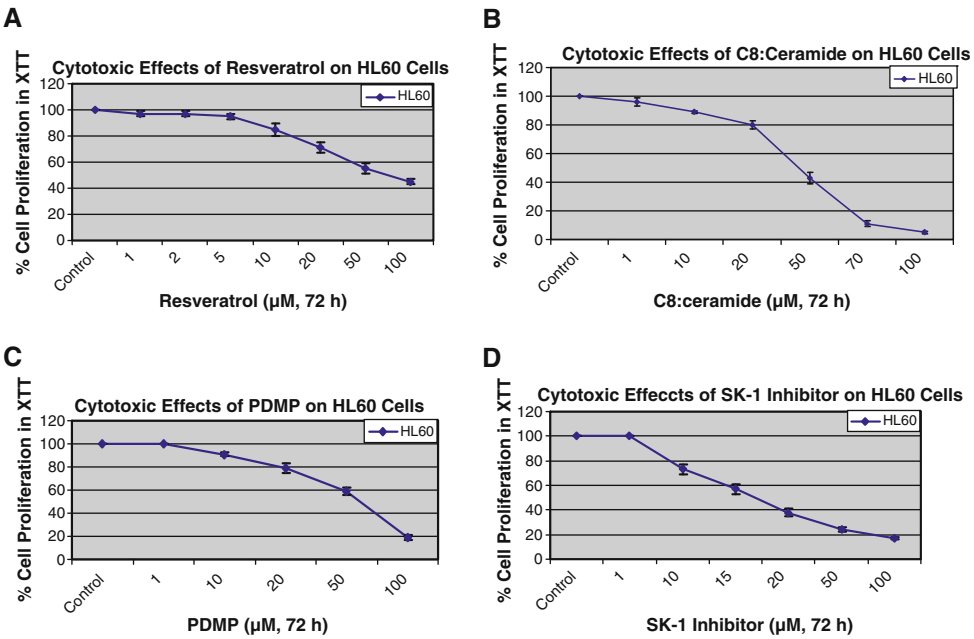

Fig. 1 Antiproliferative effects of resveratrol (**a**), C8:ceramide (**b**), PDMP (**c**), and SK-1 inhibitor (**d**) on HL60 cells. The IC50 concentrations of resveratrol, C8:ceramide, and PDMP, as well as the IC10 value of SK-1 inhibitor were calculated from cell proliferation plots. The XTT assays were performed using triplicate samples in at least two independent experiments. The *error bars* represent the standard deviations. Statistical significance was determined using two-way analysis of variance, and $p < 0.05$ was considered to be significant

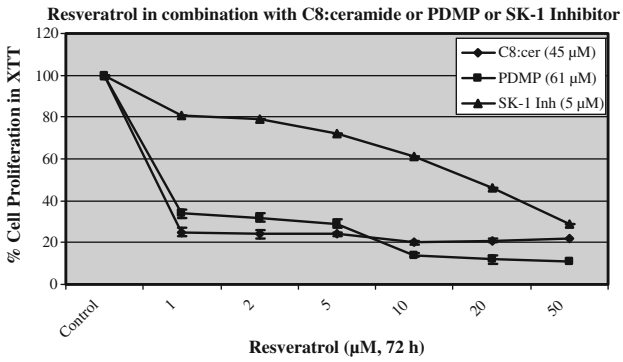

Fig. 2 Synergistic antiproliferative effects of resveratrol in combination with C8:ceramide, PDMP, or SK-1 inhibitor on HL60 cells. Cytotoxicity was determined by the XTT cell proliferation test in a 72-h culture. The XTT assays were performed using triplicate samples in at least two independent experiments. The *error bars* represent the standard deviation. Statistical significance was determined using two-way analysis of variance, and $p < 0.05$ was considered to be significant

mitochondrial JC-1 in response to 5 and 10 μM resveratrol, respectively (Fig. 4). HL60 cells treated with 45 μM of C8:ceramide, 61 μM of PDMP, or 5 μM of SK-1 inhibitor showed 25.98, 1.18, or 10.29-fold increases in cytoplasmic/mitochondrial JC-1. We applied a combination of 5 and 10 μM resveratrol together with the same doses of C8:ceramide, PDMP, or SK-1 inhibitor and observed 32.67 and 79.50-fold, 12.87 and 14.28-fold, or 1.75 and 2.41-fold increases in cytoplasmic/mitochondrial JC-1, respectively, as compared to untreated controls (Fig. 4).

Resveratrol induces de novo ceramide generation and ceramide accumulation by increasing expression levels of LASS genes and decreasing expression levels of GCS and SK-1 genes

Expression levels of ceramide-metabolizing genes in HL60 cells treated with increasing concentrations of resveratrol (1, 10, 20, and 50 μM) were determined by RT-PCR. The results revealed that there were significant increases in expression levels of LASS1, LASS2, LASS4, LASS5, and LASS6 genes and decreases in expression levels of GCS and SK-1 in a dose-dependent manner (Fig. 5). We quantified expression levels of the genes and detected 1.5, 1.98, 1.79, 1.27, and 1.96-fold increases in expression levels of LASS1, LASS2, LASS4, LASS5, and LASS6 and 0.5 and 0.6-fold decreases in expression levels of GCS and SK-1 genes, respectively, as normalized to β-actin levels and compared to untreated controls (Fig. 5).

Discussion

There are different strategies for the treatment of acute myeloid leukemia. But, significant problems are observed in patients during treatment with these chemicals. Thus, natural products that may have strong anticancer potential always attract the attention of clinicians and basic science researchers. In this study, we demonstrated anticancer potentials of resveratrol on human acute myeloid leukemia cells in addition to identifying a novel mechanism of resveratrol-induced apoptosis that involves bioactive sphingolipids. On the other hand, we increased antiproliferative and apoptotic effects of resveratrol on HL60 cells by targeting ceramide-generating and ceramide clearance genes toward generation and/or accumulation of apoptotic ceramides.

We have seen that resveratrol, C8:ceramide that induce de novo generation of apoptotic ceramides, PDMP that inhibits GCS enzyme, and SK-1 inhibitor showed cytotoxic effects of HL60 cells in a dose-dependent manner. Resveratrol application resulted in increases in caspase-3 enzyme activity and loss of mitochondrial membrane potential. Since the mitochondrial membrane potential and caspases are downstream targets of ceramides, treatment of HL60 cells with C8:ceramide, PDMP, or SK-1 inhibitor

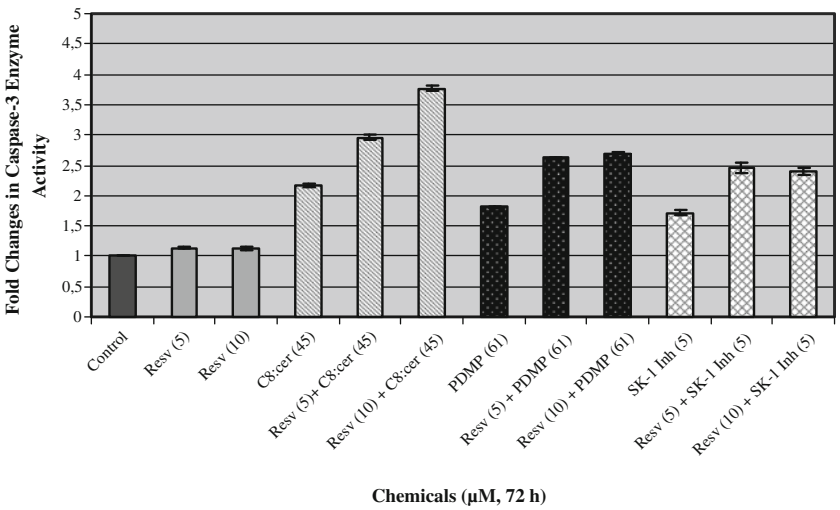

Fig. 3 Fold changes in caspase-3 enzyme activity in response to combinations of resveratrol with C8:ceramide, PDMP, or SK-1 inhibitor. The results are the means of two independent experiments. The *error bars* represent the standard deviations, and when not seen they are smaller than the thickness of the lines on the graphs. $p < 0.05$ was considered to be significant

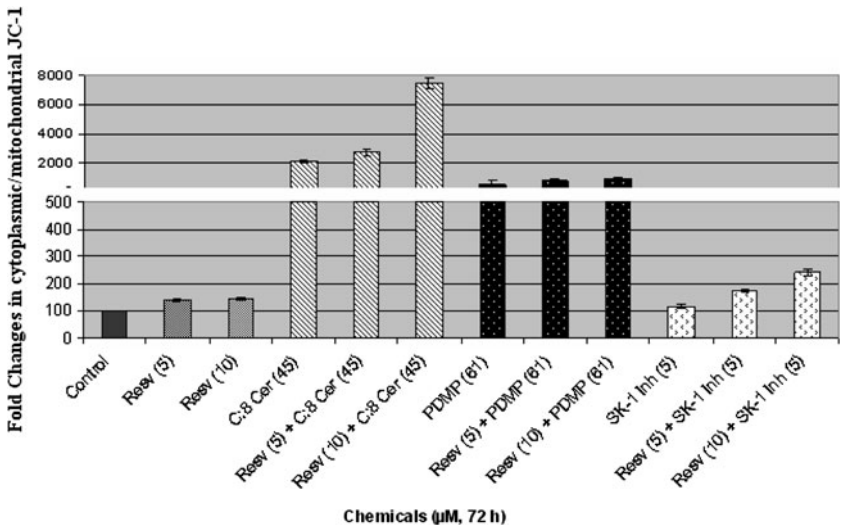

Fig. 4 Fold changes in cytoplasmic/mitochondrial JC-1 in HL60 cells exposed to combinations of resveratrol with C8:ceramide, PDMP, or SK-1 inhibitor. The results are the means of two independent experiments. The *error bars* represent the standard deviations, and when not seen they are smaller than the thickness of the lines on the graphs. $p < 0.05$ was considered to be significant

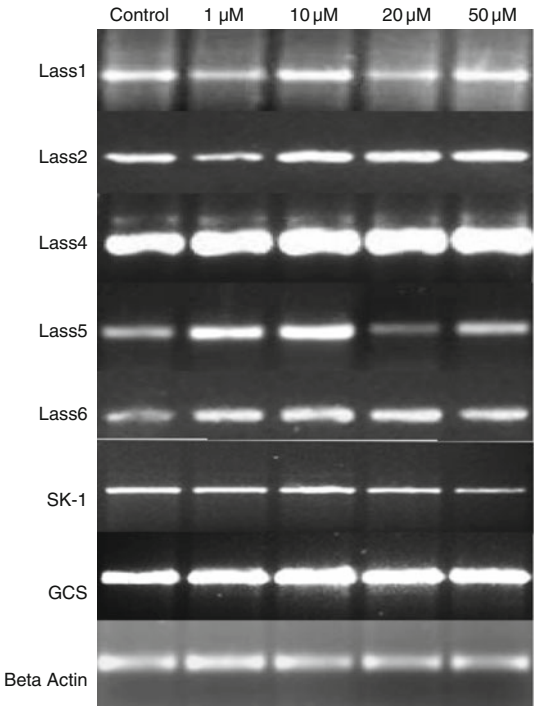

Fig. 5 mRNA levels of ceramide-metabolizing genes in response to resveratrol in HL60 cells. After 1, 10, 20, and 50 μM resveratrol were applied to HL60 cells for 72 h, expression levels of ceramide-metabolizing genes were determined by RT-PCR. Expression levels of β-actin was detected as an internal positive control

also resulted in increases in caspase-3 enzyme activity and decreases in mitochondrial membrane potential. As we manipulate the cells to increase intracellular generation or accumulation of ceramides by biochemical approaches, we observed significant synergistic cytotoxic effects of resveratrol on HL60 cells. Combinations of resveratrol with C8:ceramide, PDMP, or SK-1 inhibitor resulted in more apoptotic cells as compared to any agent alone.

These findings were in agreement with literature. It was shown that application of ceramide analogs or their mimetics induces apoptosis and/or growth arrest in various types of cancer cells (Bielawska et al. 2008; Szulc et al. 2006). It was clearly shown that there was significant inhibition of tumor growth and progression in response to ceramidoids, a recently synthesized ceramides, in head and neck squamous cancer cells and some other cancer models, in vivo (Bielawska et al. 2008; Senkal et al. 2006). Different structural analogs of ceramides have also been shown to induce apoptosis in breast cancer cells (Struckhoff et al. 2004) and drug-resistant MCF-7/Adr cells (Crawford et al. 2003). We have shown that increasing expression levels of ceramide synthase gene (LASS1) in imatinib-resistant and -sensitive K562 cells triggered apoptosis and increased sensitivity of these cells to imatinib (Baran et al. 2007).

Targeting ceramide clearance enzymes is another important strategy to increase intracellular concentrations of ceramides (Ogretmen 2006). Inhibition of SK-1 enzyme to block conversion of apoptotic ceramide to antiapoptotic S1P is a novel treatment modality for the treatment of cancers. It was shown in living animal models that inhibition of SK-1 blocked cell growth and proliferation and induced apoptosis (French et al. 2006). Inhibition of S1P receptors or application of monoclonal antibodies against S1P have also been shown to be effective protocols for the treatment of cancers (Visentin et al. 2006). Our previous data showed that partial inhibition of SK-1 by siRNA resulted in apoptosis in imatinib-resistant K562 chronic myeloid leukemia cells (Baran et al. 2007).

Glucosylceramide synthase enzyme has been shown to be involved in drug resistance in cancer. Thus, inhibition of GCS is also a novel treatment option for cancer cells. Adriamycin-resistant MCF-7 cells showed sensitivity and

underwent apoptosis in response to GCS inhibitors, while overexpression of GCS in MCF-7 cells resulted in multidrug resistance to several agents (Liu et al. 1999; Zhang et al. 1997). Moreover, combination of GCS inhibitor with some anticancer agents, such as doxorubicin, tamoxifen, vincristine, and taxol, induced apoptosis synergistically in various human cancer cells (Morjani et al. 2001; Senchenkov et al. 2001).

Interestingly, our RT-PCR results showed that there were significant increases in the expression levels of LASS1, LASS2, LASS4, LASS5, and LASS6 genes in response to resveratrol in a dose-dependent manner in HL60 cells. In parallel studies, we have also detected significant decreases in expression levels of SK-1 and GCS in a dose-dependent manner in HL60 cells. Increased expression of LASS genes (ceramide synthase) in response to chemotherapeutic stress has also been shown in different cancer cells. There was increased ceramide synthase enzyme activity in response to daunarubicine and etoposide (Bose and Kolesnick 2000; Perry et al. 2000). A camptohotecine derivative CPT-11 and hexadevylephosphocholine agents also induced apoptosis by upregulating ceramide synthase genes (Wieder et al. 1998).

Taken together, these results showed for the first time that resveratrol induces apoptosis through both increasing de novo generation of apoptotic ceramides and decreasing conversion of apoptotic ceramides to antiapoptotic sphingosine-1-phosphate and glucosylceramide. On the other, our data strongly suggest that targeting ceramide metabolism increased chemosentivity of acute myeloid leukemia cells to resveratrol. Since ceramides are the regulators of vitally important biological processes, targeting ceramide-metabolizing genes and their end products may be an important avenue for the treatment of different types of cancers.

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