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Original Contribution

Potent antiproliferative effects of resveratrol on human osteosarcoma SJSA1 cells: Novel cellular mechanisms involving the ERKs/p53 cascade

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Abstract

The chemopreventive activity of resveratrol (RSVL) has been demonstrated in several types of cancer. However, its effects and the underling mechanisms remain poorly understood. In this study, we investigated the involvement of the mitogen activated protein kinase (MAPK)/p53 signal transduction mechanism in RSVL-induced growth inhibition using a human osteosarcoma cell line. We demonstrate that RSVL reduces cell viability and growth of SJSA1 osteosarcoma cells. Morphological profiles and 4,6-diamidino-2-phenylindole nuclear staining of RSVL-treated cells indicated marked nuclear fragmentation. Cleavage of the (116-kDa) poly(ADP-ribose) polymerase protein into an 89-kDa fragment (a proapoptotic marker system) was substantially augmented by RSVL treatment. RSVL-dependent growth impairment was preceded by enhanced phosphorylation of extracellular signal-regulated kinase (ERK)1/2 (at Thr202/Tyr204). Likewise, RSVL increased the phosphorylation of p53 tumor suppressor protein (at Ser15). The effects of RSVL on ERKs and on p53 phosphorylation were abrogated by either the MAPK inhibitor PD98059 or the p53 inhibitor pifithrine-α. The present study indicates that RSVL antiproliferative effects on osteosarcoma cells are mediated by the activation of the ERKs/p53 signaling pathway and therefore identifies new targets for strategies to treat and/or prevent osteosarcoma.

Introduction

Resveratrol (*trans*-3,4′,5-trihydroxystilbene, RSVL) is a natural phytoalexin found in grapes, berries, and red wine [1]. It has been reported to confer protection against some cardiovascular diseases, a dogma that is commonly designated as “the French paradox of red wine” [2,3]. Equally important, but later discovered, was the cancer chemopreventive activity for RSVL. Studies revealed that RSVL is capable of inhibiting the three major stages of carcinogenesis, namely tumor initiation, promotion, and progression [4]. These effects are mediated through inhibition of enzymes involved in cell proliferation such as DNA polymerase and ribonucleotide reductase, which is involved in S-phase entry [5,6], or activation of proteines involved in apoptosis/growth arrest such as the cAMP/kinase-A cascade [7]. RSVL is a growth inhibitory for several human cancer cell lines such as HL60 promyelocytic leukemia cells [8], JB6 mouse epidermal cells [9], CaCo-2 colorectal cells [10,11], A431 epidermoid carcinoma cells [12], and the breast-cancer cells MCF7 [13], MDA-MB-231 [14,15], KPL-1 and MKL-F [16], and T47D cell lines [17] in addtition to animal models of carcinogenesis [4,18,19]. In this study we have evaluated the chemopreventive action of RSVL on the human osteosarcoma cell line SJSA1. Because a potent growth inhibitory effect was evident, we further checked the cellular and molecular mechanisms whereby RSVL induced these effects. We examined the involvement of the MAPK/p53 pathway in RSVL-induced growth inhibition in these cells.

Materials and methods

Antibodies and reagents

trans-Resveratrol and antiactin (clone AC-40) monoclonal antibody were obtained from Sigma (Saint Louis, MO,

Abbreviations: resveratrol, RSVL; FBS, fetal bovine serum; DAPI, 4,6-diamidino-2-phenylindole; MAPK, mitogen-activated protein kinase; ERK1/2, extracellular signal-regulated kinase 1/2; PBS, phosphate-buffered saline; PI3K, phosphatidylinositol 3-kinase; PARP, poly(ADP-ribose) polymerase; ECL, enhanced chemiluminescence.

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USA). p44/42 MAP kinase, phospho-p53 (Ser15), poly (ADP-ribose) polymerase (PARP) polyclonal antibodies and phospho-p44/42 MAPK (Thr202/Tyr204) (E10) monoclonal antibody were obtained from Cell Signaling Technology (Beverly, MA, USA). MAPK inhibitor PD98059 and p53 inhibitor pifithrine-α were obtained from Calbiochem (Ontario, Canada). All cell culture reagents were obtained from Gibco-BRL (Paisley, UK).

Cells and culture conditions

The human osteosarcoma cell line SJSA1 was kindly provided by Bohdan Wasylyk (IGBMC core facility, Strasbourg, France). The cells were maintained in RPMI1640 medium (Gibco BRL) supplemented with 5% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin in a 5% CO_2 incubator at 37 °C. The cells were passaged twice a week by harvesting with trypsin/EDTA and seeding into 25-cm^2 flasks.

Cell proliferation test

To study the effect of RSVL on cell proliferation, cells were seeded in six-well tissue culture plates at approximately 10^4 cells/well in the presence of 10% FBS for 24 h. The effects of RSVL alone or with inihibitors were tested in media containing low serum concentration (0.5%) and containing high serum concentration (10%). After 3 days of treatment with RSVL or inhibitors, the cells were harvested by treatment with trypsine and EDTA and counted with a Coulter counter (Type Z2).

Morphologic analysis

Cells were seeded in six-well tissue culture plates at approximately 10^4 cells/well in the presence of 10% FBS for 24 h. The medium was then changed using fresh RPMI-1640 supplemented with 0.5% FBS with vehicle (control) or with 100 μM RSVL. Following 48 h of incubation, the cells were examined with a Leitz phase-contrast microscope (Labovert FS) with low power magnification (×20), and they were photographed by a Leica Camera (Wild MPS52).

Cell viability analysis with trypan blue dye exclusion test

Cells were seeded in twelve-well tissue culture plates at approximately 10^4 cells/well in the presence of 10% FBS and were allowed to adhere for 24 h. They were then treated with vehicle (control) or with 1, 10, 50, 50, or 100 μM RSVL in the presence of RPMI1640 medium containing 0.5% FBS. Triplicate samples were used for each treatment. Following 48 h of treatment, the medium containing floating cells was collected and added to the adhering cells that were detached by trypsinization. Cell viability was estimated after adding an equivalent volume of a 0.125% trypan blue solution to an aliquot of the whole suspended cells and counting under the microscope the proportion of unstained versus total cells deposited in a hemacytometer. At least five counts over different fields of about 100 cells were made to evaluate the average cell viability in each group of treated cells. The viability in control cells was considered as 100% viability.

Nuclear DNA fragmentation analysis using DAPI staining

To identify the cells undergoing apoptosis we used the 4,6-diamidino-2-phenylindol staining method. Cells were seeded at 1×10^4 into 35-mm multiwell plates containing RPMI1640 medium supplemented with 10% FBS and incubated for 24 h. The cells were then treated with vehicle or with 100 μM RSVL for 48 h in the presence of 0.5% FBS. Cells were washed with PBS, fixed with 100% methanol, and then

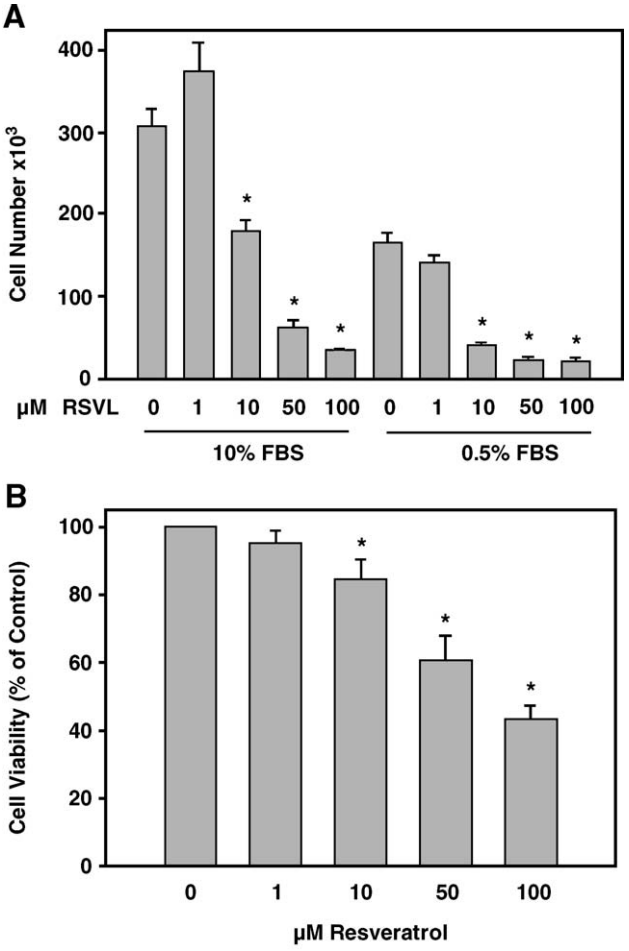

Fig. 1. Antiproliferative effects of RSVL on SJSA1 osteosarcoma cells. Cells were seeded in 6-well tissue culture plates at approximately 10^4 cells/well in the presence of 10% FBS for 24 h. The effect of RSVL was tested in medium containing low serum concentration (0.5%) and high serum concentration (10%). After 3 days of treatment with RSVL or with inhibitors, the cells were harvested by treatment with trypsin and EDTA and counted with a Coulter counter (Type Z2). (A) RSVL significantly inhibited the growth of SJSA1 cells in both high-FBS (10%) and low-FBS (0.5%) media. The inhibition was observed at 10–100 μM RSVL. Means ± SD from triplicate experiments are presented. * Significantly different from control ($p < 0.001$). (B) RSVL reduces SA1 cell viability. Cell viability determined by trypan blue exclusion is reduced in a dose-dependent manner in SA1 cells treated for 48 h with the indicated concentrations of RSVL in the presence of 0.5% FBS.