Chemico-Biological Interactions 242 (2015) 372–379

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Resveratrol induces cell death and inhibits human herpesvirus 8 replication in primary effusion lymphoma cells

Feng-Yi Tang [a], Chang-Yu Chen [b], Huey-Wen Shyu [b], Shin Hong [b], Hung-Ming Chen [b], Yee-Hsuan Chiou [c], Kuan-Hua Lin [b], Miao-Chen Chou [b], Lin-Yu Wang [b], Yi-Fen Wang [b, *]

[a] Clinical Laboratory, Yuan's General Hospital, Kaohsiung, Taiwan, ROC
[b] Department of Medical Laboratory Science and Biotechnology, Fooyin-University, Kaohsiung, Taiwan, ROC
[c] Department of Pediatrics, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, ROC

ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form
18 September 2015
Accepted 31 October 2015
Available online 10 November 2015

Keywords:
Resveratrol
Primary effusion lymphoma
Human herpesvirus 8
ROS
Apoptosis
Autophagy

ABSTRACT

Resveratrol (3,4′,5-trihydroxy-trans-stilbene) has been reported to inhibit proliferation of various cancer cells. However, the effects of resveratrol on the human herpesvirus 8 (HHV8) harboring primary effusion lymphoma (PEL) cells remains unclear. The anti-proliferation effects and possible mechanisms of resveratrol in the HHV8 harboring PEL cells were examined in this study. Results showed that resveratrol induced caspase-3 activation and the formation of acidic vacuoles in the HHV8 harboring PEL cells, indicating resveratrol treatment could cause apoptosis and autophagy in PEL cells. In addition, resveratrol treatment increased ROS generation but did not lead to HHV8 reactivation. ROS scavenger (N-acetyl cysteine, NAC) could attenuate both the resveratrol induced caspase-3 activity and the formation of acidic vacuoles, but failed to attenuate resveratrol induced PEL cell death. Caspase inhibitor, autophagy inhibitors and necroptosis inhibitor could not block resveratrol induced PEL cell death. Moreover, resveratrol disrupted HHV8 latent infection, inhibited HHV8 lytic gene expression and decreased virus progeny production. Overexpression of HHV8-encoded viral FLICE inhibitory protein (vFLIP) could partially block resveratrol induced cell death in PEL cells. These data suggest that resveratrol-induced cell death in PEL cells may be mediated by disruption of HHV8 replication. Resveratrol may be a potential anti-HHV8 drug and an effective treatment for HHV8-related tumors.

1. Introduction

Primary effusion lymphoma (PEL) also referred to as body cavity-based lymphoma, is a subtype of non-Hodgkin B cell lymphoma that mainly occurs in patients with advanced AIDS, but is also found in human immunodeficiency virus negative individuals [1,2]. All PEL cells are infected with human herpesvirus 8 (HHV8) [3,4]. HHV8, known as Kaposi's sarcoma-associated herpesvirus (KSHV), is a member of the gamma-herpesviruses and was first isolated in the AIDS-associated malignancy Kaposi's sarcoma (KS) [5]. A comprehensive review [6] reports like all herpesviruses, HHV8 can establish two different kinds of replication cycles, a productive lytic replication or a latent infection. Both phases play important roles for viral transmission and related tumorigenesis [6]. In PEL cells, HHV8 endures latent infection and only a limited set of genes, including Kaposin/K12, v-cyclin/orf72, the latency-associated nuclear antigen (LANA)/ORF73, v-FLIP/ORF71, and the viral micro-RNAs, are expressed [7–9]. The latent genes contribute to HHV8-associated malignancies by the manipulation of cell proliferation and apoptosis. Previous studies by Godfrey et al. (2005) reveal PEL cells depends upon HHV8 for survival, as loss of the HHV8 genome leads to cell death.

PEL is a very aggressive and rapidly progressing tumor and it is usually fatal. The mean survival time for PEL patients is about 2–6 months [10]. The prognosis of conventional chemotherapy for HHV8-associated PEL is poor [11]. There are no current therapies effective against the aggressive, HHV8-induced PEL. Some interesting new therapeutic strategies have been reported on the treatment of PELs, such as inhibition of NF-κB signaling [12], RNA interference against viral latent proteins [13], a small-molecule inhibitor of the p53-MDM2 interaction (Nutlin-3a) [14] or the

* Corresponding author. Department of Medical Laboratory Science and Biotechnology, Fooyin University, 151 Chie-Hsueh Road, Ta-Liao District, Kaohsiung City 83102, Taiwan, ROC.
E-mail addresses: sc071@fy.edu.tw, yfleeds@gmail.com (Y.-F. Wang).

http://dx.doi.org/10.1016/j.cbi.2015.10.025
0009-2797/

mTOR inhibitor rapamycin [15].

Antioxidant compounds present in diet have gained interest owing to their beneficial effects on health as cancer chemopreventive agents. Resveratrol, *trans*-3,4′,5,-trihydroxystilbene, belongs to stilbenoids and consists of two aromatic rings attached by a methylene bridge. It is a polyphenolic phytoalexin widely found in plants and enriched in red wine, peanuts and other sources [16]. A variety of studies have demonstrated that resveratrol has cardioprotective, antioxidant, anti-inflammatory, antibacterial, and antiviral activities [17]. In addition, resveratrol was found to inhibit proliferation and induce apoptosis in several human tumor cells [17]. Even though there are reports on the antiproliferative activity of resveratrol on different cancer cells, including leukemia [18] and lymphoma cell lines [19,20], no data are available to date in the literature concerning the effects of resveratrol on PELs (HHV8 harboring cells) and HHV8 viral replication.

In this study, we utilized the HHV8 harboring PEL cells (BCBL-1 and BC-1 cells) to test whether resveratrol could inhibit PEL cell proliferation. In addition, the effects of resveratrol on HHV8 replication have also been investigated.

2. Materials and methods

2.1. Cell lines

Four lymphoma cell lines were used in this study, they were BCBL-1 (ATCC CRL11982), BC-1 (ATCC CRL-2230), P3HR1 (ATCC HTB62) and BJAB (ATCC HB = 136). BCBL-1 (HHV-8-positive and EBV-negative) and BC-1 (HHV-8-positive and EBV-positive) cells are primary effusion lymphoma (PEL) cells, P3HR1 cells are EBV-positive Burkitt's lymphoma cells. BJAB cells are HHV8-negative and EBV-negative human lymphoblastoid cells. All the lymphoma cell lines were grown in RPMI 1640 (Invitrogen, Carlsbad, CA, USA), supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT), 2 mM L-glutamine, 100 units/ml penicillin, and 100 units/ml streptomycin (Invitrogen). Cells were grown at 37 °C in a humidified 5% CO_2.

2.2. Cell viability assays

The PEL cells suspended at 2×10^5 cells/ml were incubated with various concentrations of resveratrol (Cayman Chemical Company, Michigan, USA) and plated at a density of 2×10^5 cells per well in 24-well plates for 24 h. Cell viability was determined by trypan blue exclusion assay. DMSO was added to solvent control and did not exceed 0.1% (v/v). The untreated cells were utilized as control (considered to be 100%), and the cell viability was compared with control. Each treatment was performed in triplicate and three independent experiments were performed. Error bars represent the standard errors.

2.3. Isolation of peripheral blood mononuclear cells (PBMCs)

PBMC was isolated from laboratory volunteers using Ficoll-Paque Plus (Amersham Biosciences, USA) according to the manufacturer's protocol. The blood samples were diluted with saline (1:1) and slowly loaded on the top of the Ficoll-Paque Plus medium (2 volume diluted blood: 1 volume Ficoll). Do not to mix the two layers. After centrifugation at 400× g for 20 min, PBMCs (located at interface layer) were collected and then washed twice with PBS. PBMCs were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Cell number and viability were assessed microscopically using trypan blue exclusion assay.

2.4. Intracellular ROS measurement

The formation of ROS was measured by using a nonfluorescent probe, 2,7-diacetyl dichlorofluorescein (H_2DCFDA) that can penetrate into the intracellular matrix of cells, where it is oxidized by ROS to form fluorescent dichlorofluorescein (DCF) [21]. PEL cells or PBMCs were treated with 20 μM resveratrol for 24 h. Following the drug treatment, cells were incubated with 5 μM H_2DCFDA for 30 min at 37 °C and washed three times using PBS, and then lysed with 0.1% Triton X-100. Protein quantitation was performed on 10 μl of sample in duplicate using a BCA Protein Assay Kit (Pierce) according to the manufacturer's instructions. The remainder of the sample was used to measure the fluorescence of the oxidized product dichlorofluorescein with a Synergy HT Microplate Reader (BioTek Instruments) at 485 nm excitation and 520 nm emission. For each sample, the fluorescence units were divided by the protein content. DCF-fluorescence intensity of untreated cells (control) was set as 100%. All measurements were done in triplicate and at least three independent experiments were performed. Error bars represent the standard errors.

2.5. Caspase-3 activity assay

Caspase-3 activity was assessed using the CaspACE Assay System, Colorimetric (Promega, Madison, Wis., USA) according to the manufacturer's instructions. Briefly, PEL cells were pretreated with caspase inhibitor Ac-DEVD-CHO (5 μM) or antioxidant N-acetylcysteine (NAC, 10 mM) for 1 h or left untreated. PEL cells were further incubated with resveratrol (20 μM) for another 24 h, and lysed in cold lysis buffer provided by the manufacturer. Then 20 μg of total cell lysates were incubated with the caspase-3 substrate Ac-DEVD-p-nitroaniline (pNA) at 37 °C for 4 h. The chromophore pNA was released from the substrate upon the cleavage by caspase-3, and free pNA was monitored by a spectrophotometer at 405 nm.

2.6. Acidic vesicular organelle (AVO) staining

Acridine orange freely diffuses the membranes of cells and organelles. It is a marker of acidic vesicular organelles (AVOs) that fluoresces green in the whole cell except in acidic compartments (mainly late autophagosomes), where it fluoresces red. Development of AVOs is a typical feature of autophagy, and its formation indicates the maturation of autophagosomes and an efficient autophagic process, since only mature/late autophagosomes are acidic. Intensity of red fluorescence is proportional to number of AVO in autophagic cells [22]. Following resveratrol treatment, cell culture medium was removed from the cells and replaced with cell culture medium containing 5 ug/ml acridine orange and incubated for 10 min at 37 °C. Cells were then harvested, washed twice and examined. The fluorescence was observed using a Nikon Eclipse TE2000-U inverted fluorescent microscope, a 10×/0.30 NA Plan Fluor objective. Five visual fields of cells selected randomly, the red-colored cells and total cells were counted respectively, and the percentage of the red-colored cells was calculated.

2.7. Western blotting

BCBL-1 cells were untreated or treated with resveratrol (20 μM) for 24 h. The cells were then harvested by centrifugation and washed with cold PBS, and cell extracts were prepared in lysis buffer (0.5% Nonidet P-40, 50 mM Tris-HCl [pH 7.5], 0.25% sodium deoxycholate, 1 mM EDTA, 100 mM sodium chloride, 1× complete protease inhibitors [Roche]) for 60 min on ice. The lysate solution was spun at 12,000× *g* for 10 min at 4 °C, and supernatants were collected. Fourty micrograms of protein extract were separated by