

Resveratrol induces apoptosis MH7A human rheumatoid arthritis synovial cells in a sirtuin 1-dependent manner

Hiroshi Nakayama · Takahiro Yaguchi ·
Shinichi Yoshiya · Tomoyuki Nishizaki

Received: 23 March 2010 / Accepted: 28 July 2010 / Published online: 10 August 2010
© The Author(s) 2010. This article is published with open access at Springerlink.com

Abstract Resveratrol, a phytoalexin, reduced the viability of MH7A cells, a human rheumatoid arthritis synovial cell line. In the apoptosis assay, resveratrol increased TUNEL-positive cells and stimulated H2A.X phosphorylation. Resveratrol disrupted mitochondrial membrane potentials in MH7A cells and stimulated cytochrome c release from the mitochondria to the cytosol. Resveratrol activated caspase-3 and caspase-9 but not caspase-8 in MH7A cells. Resveratrol upregulated the expression of the NAD-dependent deacetylase sirtuin 1 mRNA and down-regulated the expression of the Bcl-X_L mRNA, and resveratrol-induced MH7A cell death, mitochondrial damage, and caspase-3/-9 activation were prevented by sirtinol, an inhibitor of sirtuin 1. The results of the present study show that resveratrol induces MH7A cell apoptosis by activating caspase-9 and the effector caspase-3 along mitochondrial disruption as a result of reduced Bcl-X_L expression, allowing cytochrome c release from the mitochondria into the cytosol, in a sirtuin 1-dependent manner. This suggests that resveratrol could suppress hyperplasia of synovial cells, a critical factor of rheumatoid arthritis.

Keywords Resveratrol · MH7A human synovial cell · Apoptosis · Sirtuin 1 · Rheumatoid arthritis

Introduction

Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic inflammation of multiple joints, with disruption of joint cartilage. Lines of evidence have pointed to inflammatory cytokines inducing hyperplasia of synovial cells in joints as an etiology for rheumatoid arthritis. Of cytokines, tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) have been highlighted, since high concentrations of TNF- α and IL-1 are found with the synovial fluid and the plasma for patients with rheumatoid arthritis [1, 2] and those cytokines produce matrix metalloproteinases or activate osteoclasts, causing irreversible damage to soft tissues and bones [3]. TNF- α inhibitors, anti-TNF antibodies, a soluble TNF receptor fusion protein, and an IL-1 receptor antagonist have been attempted for treatment of rheumatoid arthritis, but their side effects such as serious infections and inducible malignant tumors remain not to be resolved [4].

Resveratrol, a phytoalexin that is present in grape skin and red wine, exerts a variety of actions to reduce superoxides, suppresses carcinogenesis and angiogenesis, prevent diabetes mellitus, inhibit inflammation, or prolong life span [5]. Furthermore, resveratrol decreases plaque formation relevant to neurodegenerative diseases such as Alzheimer disease and Huntington disease [6]. Of particular interest is that resveratrol is potent and specific inhibitor of NF- κ B activation induced by TNF- α or IL-1 β , and therefore, resveratrol might be available for therapy of rheumatoid arthritis [7–9].

The present study aimed at understanding the mechanism of resveratrol-induced apoptosis in MH7A cells, a human rheumatoid arthritis synovial cell line. We show here that resveratrol induces MH7A cell apoptosis by activating caspase-9 and the effector caspase-3 in response

H. Nakayama · T. Yaguchi · S. Yoshiya · T. Nishizaki (✉)
Division of Bioinformation, Department of Physiology,
Hyogo College of Medicine, Nishinomiya 663-8501, Japan
e-mail: tomoyuki@hyo-med.ac.jp

H. Nakayama · S. Yoshiya
Department of Orthopedic Surgery, Hyogo College of Medicine,
Nishinomiya, Japan

to mitochondrial damage in a sirtuin 1-dependent manner. This supports the possibility for resveratrol as a beneficial drug for the treatment of rheumatoid arthritis.

Materials and methods

Cell culture

MH7A cells were obtained from Riken cell bank (Ibaraki, Japan). Cells were cultured in a culture medium; RPMI-1,640 (Wako, Osaka, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (final concentration, 100 U/ml), and streptomycin (final concentration, 0.1 mg/ml), in a humidified atmosphere of 5% CO₂ and 95% air at 37°C.

Cell viability

Cell viability was evaluated by the method using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) as previously described [10].

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

MH7A cells were fixed with 4% paraformaldehyde. After removing inactivate endogenous peroxidase with methanol containing 0.3% H₂O₂, a permeabilization buffer was applied to cells and stood on ice for 5 min. Then, a Labeling Reaction Mixture was added and incubated in a humidified chamber at 37°C for 60 min. Reactive cells were stained with 3% methyl green and detected with a light microscope.

H2A phosphorylation assay

MH7A cells were incubated in a chemiluminescence detection assay kit (Upstate, Charlottesville, Virginia, USA) and reacted with an anti-phospho-H2A.X (Ser139) followed by an anti-mouse-HRP conjugate. Phosphorylation of H2A.X at Ser139 was stained with a chemiluminescent HRP substrate LumiGLO, and signals were

detected with a microplate luminometer (ARVO mx/light, Perkinelmer, Waltham, MA, USA).

Assay of mitochondrial membrane potentials

Mitochondrial membrane potentials were measured using a DePsipher™ kit as previously described [10]. Briefly, MH7A cells were untreated and treated with resveratrol (100 μM) in the absence and presence of sirtinol (10 μM) for 24 h. After washing with cold phosphate-buffered saline (PBS), cells were incubated in a DePsipher™ solution at 37°C for 20 min. Then, cells were washed with 1 ml of a reaction buffer containing a stabilizer solution. The fluorescent signals were observed with a laser scanning microscopes (LSM 510, Carl Zeiss Co., Ltd, Germany) equipped with an epifluorescence device using a fluorescein long-pass filter (fluorescein and rhodamine) at an absorbance of 590 nm for red aggregations and at an absorbance of 530 nm for green aggregations.

Reverse transcription–polymerase chain reaction (RT–PCR)

Total RNAs of MH7A cells before and after treatment with resveratrol (100 μM) were purified by an acid/guanidine/thiocyanate/chloroform extraction method using a Sepasol-RNA I Super kit. After purification, total RNAs were treated with RNase-free DNase I (2 unit) at 37°C for 30 min to remove genomic DNAs, and 10 μg of RNAs were resuspended in water. Then, oligo dT primers, dNTP, 5× first-strand buffer, and SuperScript III RNase H-reverse transcriptase were added to the RNA solution and incubated at 65°C for 5 min followed by 60°C for 1 min, 56°C for 60 min, 58°C for 60 min, 85°C for 5 min to synthesize the first strand cDNA. Subsequently, 1 μl of the reaction solution was diluted with water and mixed with 10 × PCR reaction buffer, dNTPs, MgCl₂, oligonucleotide, dimethylsulfoxide [final concentration, 5% (v/v)], and 1 unit of Taq polymerase (Fermentas, St. Leon-Roth, Germany) (final volume, 20 μl). RT–PCR was carried out with a Takara Thermal cycler Dice (Takara Co. Ltd., Shiga, Japan) programmed as follows: the first one step, 94°C for 2 min and the ensuing 30 cycles, 94°C for 1 s, 62°C for 15 s, and 72°C

Table 1 Primers used for RT–PCR

Gene name	Sense primer	Anti-sense primer	Base pair
Bad	CTGGGGCTGTGGAGATCCGGAGTCGCC	TCACTGGGAGGGGGCGGAGCTTCCCC	320
Bak	GAGCCCATTCACCATTCTACCT	AGAGAGGAAGGGAGAGAAGCTGAGAGGAC	381
Bax	GGGAGACACCTGAGCTGACC	GGACTCCAGCCACAAAGATGG	404
Bcl-2	GAAGTGGGGGAGGATTGTGGCC	TCGACGTTTTGCCTGAAGACTGTAA	486
Bcl-X _L	AGGGAGGCAGGCGACGAGTTT	TGAAGAGTGAGCCAGCAGAACCA	421
Sirtuin 1	ATTACTGAAAAACCTCCACGAACACAAAA	GCCTACTAATCTGCTCCTTTGCCACTCT	379

for 30 s using primers shown in Table 1. PCR products were stained with ethidium bromide and visualized by 2% (w/v) agarose electrophoresis.

Western blotting

After treatment with resveratrol (100 μ M) for 6–24 h, MH7A cells were harvested and centrifuged at $600\times g$ for 10 min. After washing out with 1 ml of PBS, the pellet was resuspended in 50 μ l of a buffer A (20 mM Hepes, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 250 mM sucrose, pH 7.5) and homogenized. The lysate was centrifuged at $1,000\times g$ for 10 min, and the supernatant was further centrifuged at $10,000\times g$ for 1 h. The pellet and supernatant were used as the mitochondria- and cytosol-enriched fraction, respectively. Each fraction was loaded on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. Blotting membranes were blocked with TTBS (150 mM NaCl, 0.05% Tween20, and 20 mM Tris, pH 7.5) containing 5% BSA and subsequently reacted with an anti-cytochrome c antibody (1:400) (Chemicon, Billerica, MA, USA), followed by an HRP-conjugated goat anti-mouse IgG antibody. Immunoreactivity was detected with an ECL kit (GE Healthcare, NJ, USA) and visualized using a chemiluminescence detection system (FUJIFILM, Tokyo, Japan). Signal density was measured with an Image Gauge software (FUJIFILM, Tokyo, Japan).

Enzymatic assay of caspase activity

Caspase activation was measured using a caspase fluorometric assay kit (Ac-Asp-Glu-Val-Asp-MCA for a caspase-3 substrate peptide; Ac-Ile-Glu-Thr-Asp-MCA for a caspase-8 substrate peptide; and Ac-Leu-Glu-His-Asp-MCA for a caspase-9 substrate peptide) as previously described [10].

Results

Resveratrol induces apoptosis MH7A cells

In the MTT assay, resveratrol reduced MH7A cell viability in a concentration (1–200 μ M)- and treatment time (24–72 h)-dependent manner (Fig. 1a), suggesting that resveratrol induces MH7A cell death. To examine whether it is due to apoptotic cell death, we carried out TUNEL assay and H2A phosphorylation assay. Resveratrol increased TUNEL-positive cells in a concentration (100–200 μ M)-dependent

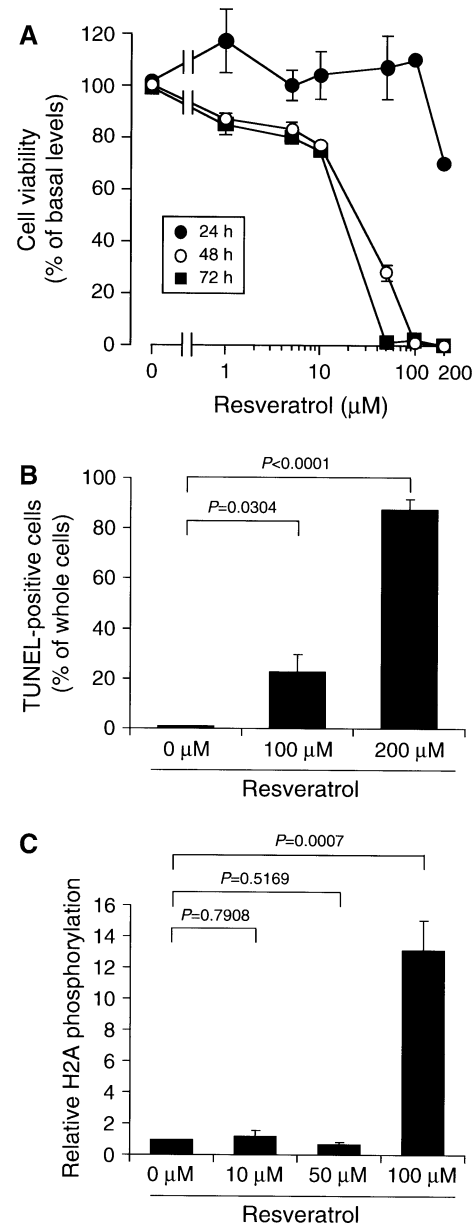


Fig. 1 Resveratrol induces apoptosis in MH7A cells. **a** MH7A cells were treated with resveratrol at concentrations as indicated for 24–72 h in serum-free culture medium, and cell viability was quantified with an MTT assay. In the graph, each point represents the mean (\pm SEM) percentage of basal levels (MTT intensities for cells untreated with resveratrol) ($n = 8$). **b** Cells were treated with resveratrol at concentrations as indicated for 24 h in serum-free culture medium, and TUNEL-positive cells were counted. In the graph, each column represents the mean (\pm SEM) percentage of basal levels (TUNEL-positive cell numbers without resveratrol treatment) ($n = 3$ –6). P values, unpaired t -test. **c** Cells were treated with resveratrol at concentrations as indicated for 24 h in FBS-free culture medium, and H2A.X phosphorylation was quantified. In the graph, each column represents the mean (\pm SEM) ratio against basal levels (H2A.X phosphorylation without resveratrol treatment) ($n = 4$). P values, unpaired t -test

manner (Fig. 1b), indicating that resveratrol induces MH7A cell apoptosis. DNA damage or apoptosis is recognized to stimulate phosphorylation of histone H2A.X. Resveratrol (100 μ M) significantly enhanced H2A.X phosphorylation, the extent reaching approximately 13 folds of control levels (Fig. 1c). This provides further evidence for resveratrol-induced MH7A cell apoptosis.

Resveratrol disrupts mitochondrial membrane potentials in a sirtuin 1-dependent manner

Resveratrol is shown to regulate mitochondrial functions or calories by interacting with NAD-dependent deacetylase sirtuin 1 [11, 12]. Interestingly, resveratrol-induced MH7A cell death was inhibited by sirtinol (10 μ M), an inhibitor of sirtuin 1 (Fig. 2), suggesting that resveratrol induces MH7A cell death in a sirtuin 1-dependent manner. Moreover, the resveratrol effect was inhibited by trichostatin A (30 nM), and inhibitor of histone deacetylase (HDAC) (Fig. 2). This may account for the implication of sirtuin 1-regulated apoptosis-related gene transcription in the resveratrol effect.

To see whether resveratrol-induced MH7A cell apoptosis is mediated via the mitochondria, mitochondrial membrane potentials were monitored. For untreated cells, orange-red fluorescent signals alone were found (Fig. 3a, b). In contrast, resveratrol (100 μ M) accumulated green fluorescent signals without orange-red fluorescent signal (Fig. 3c, d), indicating that resveratrol disrupts mitochondrial membrane potentials in MH7A cells. The resveratrol effect on mitochondrial membrane potentials was abolished by sirtinol (10 μ M)

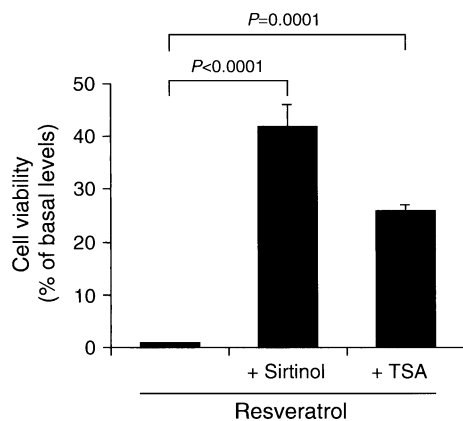


Fig. 2 Resveratrol-induced MH7A cell death is inhibited by a sirtuin 1 inhibitor or an HDAC inhibitor. MH7A cells were treated with resveratrol (100 μ M) in the absence and presence of sirtinol (10 μ M) or trichostatin A (TSA) (30 nM) for 24 h in serum-free culture medium, and cell viability was quantified with an MTT assay. In the graph, each column represents the mean (\pm SEM) percentage of basal levels (MTT intensities for cells untreated with any drug) ($n = 4$). P values, unpaired t -test

(Fig. 3e, f). This implies that resveratrol disrupts mitochondrial membrane potentials in MH7A cells under the control of sirtuin 1. Taken together, these results indicate that resveratrol induces MH7A cell apoptosis by damaging the mitochondria in a sirtuin 1-dependent manner.

In the RT-PCR analysis, resveratrol (100 μ M) increased the expression of the sirtuin 1 mRNA in MH7A cells in a treatment time (20–60 min)-dependent manner (Fig. 4a). This points to sirtuin 1 as a significant target in resveratrol-induced MH7A cell death. Accumulating evidence has shown that resveratrol upregulates or downregulates the expression of the Bcl-2 family that includes Bcl-2 and Bcl-X_L, to prevent from mitochondrial damage, and Bad, Bax, and Bak, to induce mitochondrial damage [13]. Resveratrol (100 μ M) downregulated the expression of the Bcl-X_L mRNA in MH7A cells from 1-h through 3-h treatment

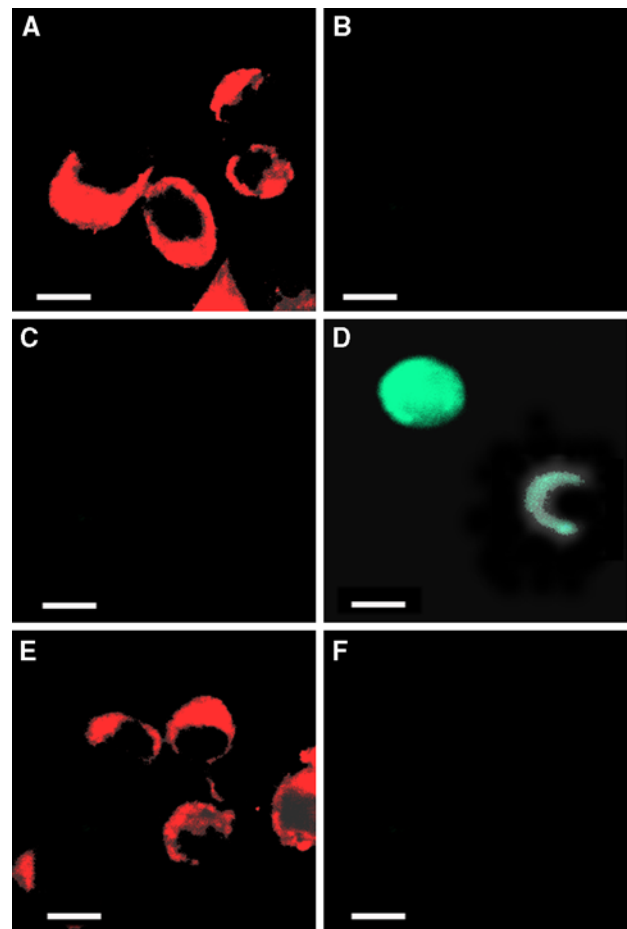
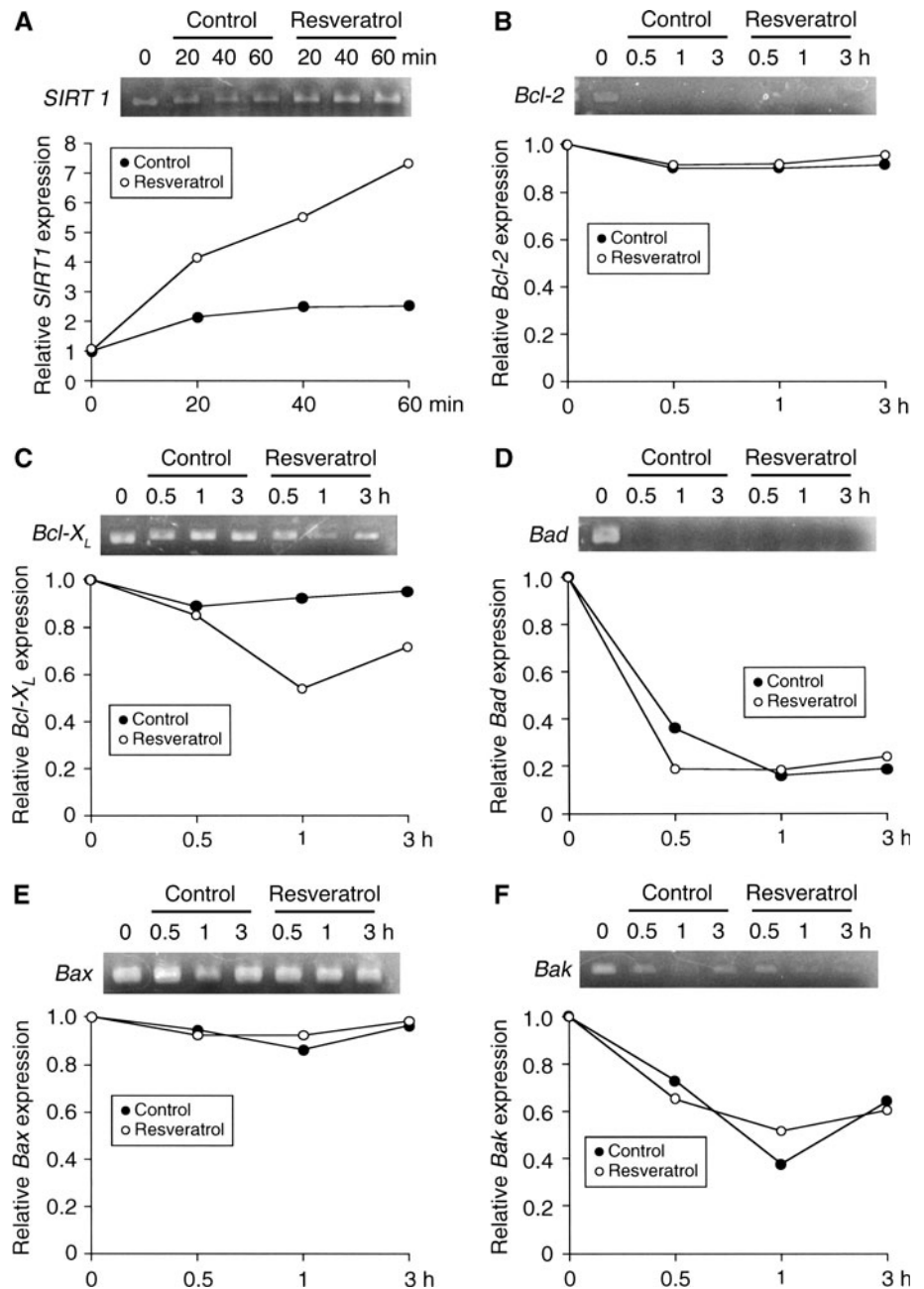


Fig. 3 Resveratrol disrupts mitochondrial membrane potentials in a SIRT1-dependent manner. MH7A cells were treated with resveratrol (100 μ M) in the absence and presence of sirtinol (10 μ M) for 24 h in serum-free culture medium, and mitochondrial membrane potentials were monitored. a, c, e Orange-red fluorescent images at an absorbance of 590 nm. b, d, f Green fluorescent images at an absorbance of 530 nm. Note that similar results were obtained with 3 independent experiments

Fig. 4 Resveratrol upregulates the expression of the sirtuin 1 mRNA and downregulates the expression of the Bcl-X_L mRNA. MH7A cells were untreated (Control) and treated with resveratrol (100 μM) for 20–60 min or 0.5–3 h in serum-free culture medium, and then RT-PCR was carried out. In the graph, *each point* represents the ratio against the intensity at 0 min/h regarding as 1. Note that similar results were obtained with 3 independent experiments



(Fig. 4c), while it had no effect on expression of mRNAs for Bcl-2 (Fig. 4b), Bad (Fig. 4d), Bax (Fig. 4e), and Bak (Fig. 4f). Collectively, resveratrol may disrupt mitochondrial membrane potentials by reducing Bcl-X_L expression through a pathway relevant to sirtuin 1-mediated transcription.

Resveratrol activates caspase-3 and caspase-9 through mitochondrial damage in a sirtuin 1-dependent manner

Mitochondrial damage allows release of apoptosis-related factors including cytochrome c. In the Western blot

analysis using the mitochondrial and cytosolic component from MH7A cells, resveratrol (100 μM) increased the presence of cytosolic cytochrome c in parallel with a treatment time (3–24 h)-dependent decrease in the presence of mitochondrial cytochrome c (Fig. 5). This explains that resveratrol stimulates release of cytochrome c from the mitochondria into the cytosol.

In the enzymatic assay of caspase activity, resveratrol (100 μM) significantly activated caspase-3 and caspase-9, but no activation of caspase-8 was obtained (Fig. 6). Resveratrol-induced activation of caspase-3 and caspase-9 was inhibited by sirtinol (10 μM) (Fig. 6). Consequently, the

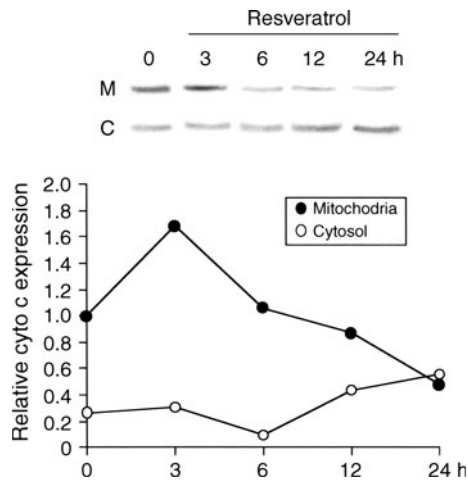


Fig. 5 Resveratrol stimulates cytochrome c release from the mitochondria. MH7A cells were treated with resveratrol (100 μ M) for 3–6 h in serum-free culture medium, followed by fractionation into the mitochondrial (M) and cytosolic component (C), and Western blotting was carried out. In the graph, each point represents the ratio against the immunoreactive intensity at 0 h for the mitochondrial component regarding as 1. cyto c, cytochrome c. Note that similar results were obtained with 3 independent experiments

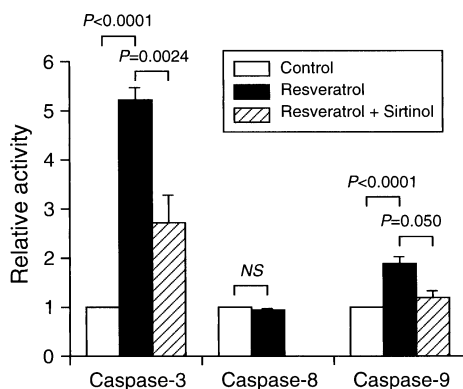


Fig. 6 Resveratrol activates caspase-3 and caspase-9 in a SIRT1-dependent manner. MH7A cells were treated with resveratrol (100 μ M) in the absence and presence of sirtinol (10 μ M) for 24 h in serum-free culture medium, and caspase activities were assayed. In the graph, each column represents the mean (\pm SEM) ratio against basal levels (caspase activities for cells untreated with any drug) ($n = 4$ –6). P values, unpaired t -test. NS not significant

results indicate that resveratrol activates caspase-9 and the effector caspase-3 in association with mitochondrial damage allowing cytochrome c release in a sirtuin-dependent manner, to induce MH7A cell apoptosis.

Discussion

In the present study, resveratrol reduced the viability of MH7A cells, a human rheumatoid arthritis synovial cell line. Resveratrol increased TUNEL-positive cells and

stimulated H2A.X phosphorylation, which confirms resveratrol-induced MH7A cell apoptosis. Resveratrol disrupted mitochondrial membrane potentials in MH7A cells, stimulated cytochrome c release from the mitochondria into the cytosol, and activated caspase-3 and caspase-9. For a mitochondria-mediated apoptotic pathway, damaged mitochondria releases apoptosis-related proteins such as cytochrome c, apoptosis-inducing factor (AIF), Smac/DIABLO, Omi/HtrA2, and endonuclease G into the cytosol, and released cytochrome c activates caspase-3 by forming an apoptosome complex together with apoptosis proteases activating factor-1 (Apaf-1) and caspase-9 [14]. Resveratrol, accordingly, appears to induce MH7A cell apoptosis by activating caspase-9 and the effector caspase-3 in concert with mitochondrial disruption allowing cytochrome c release from the mitochondria into the cytosol.

Amazingly, resveratrol upregulated the expression of the sirtuin 1 mRNA in MH7A cells, and resveratrol-induced MH7A cell death, mitochondrial damage, and caspase-3/-9 activation were prevented by a sirtuin 1 inhibitor. This confirms that sirtuin 1 plays a critical role in resveratrol-induced MH7A cell apoptosis. Sirtuin 1 may regulate apoptosis-related gene transcription mediated by FOXO or NF- κ B [16, 17]. The Bcl-2 family keeps mitochondrial integrity and controls release of mitochondrial proteins such as cytochrome c from the mitochondria. In the present study, resveratrol downregulated the expression of the Bcl- X_L mRNA in MH7A cells, although expression of mRNAs for Bcl-2, Bax, Bad, and Bak was not affected. This, taken together with the finding that resveratrol-induced MH7A cell death was attenuated by an HDAC inhibitor, raises the possibility that resveratrol perturbs mitochondrial membrane potentials by downregulating Bcl- X_L gene transcription in a sirtuin 1-dependent manner, followed by mitochondrial apoptotic events.

In the previous study, resveratrol-induced apoptosis in fibroblast-like synoviocytes derived from patients with rheumatoid arthritis by activating caspase-8 as a primary target, which cleaves Bid, causing mitochondrial damage that triggers the activation of caspase-9 and the effector caspase-3, without affecting the levels of Bax, Bcl- X_L , and Bcl-2 [15]. The resveratrol action there is completely distinct from the action found in the present study. For resveratrol-induced MH7A cell apoptosis, sirtuin 1, that downregulates expression of the Bcl- X_L mRNA, was a primary target of resveratrol, and no activation of caspase-8 was obtained. This may represent an additional resveratrol action on apoptosis in human rheumatoid arthritis synovial cells. The reason for difference in the resveratrol actions between fibroblast-like synoviocytes and MH7A cells, however, remains to be explored.

In summary, the results of the present study show that resveratrol disrupts mitochondrial membrane potentials as

a result of decreased Bcl-X_L expression, allowing cytochrome c from the mitochondria into the cytosol, thereby activating caspase-9 and the effector caspase-3, to induce MH7A cell apoptosis in a sirtuin 1-dependent manner. This suggests that resveratrol is capable of preventing hyperplasia of synovial cells. Resveratrol, therefore, could be developed as a promising drug for the treatment of rheumatoid arthritis.

Acknowledgments We thank Dr. Miyazawa (Kissei Pharmaceutical Co., Ltd.) for his help in dealing with MH7A cells.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Eastgate JA, Symons JA, Wood NC, Grinlinton FM, di Giovine FS, Duff GW (1988) Correlation of plasma interleukin-1 levels with disease activity in rheumatoid arthritis. *Lancet* 2:706–709
- Saxne T, Palladino MA Jr, Heinegard D, Talal N, Wollheim FA (1998) Detection of tumor necrosis factor- α but not tumor necrosis factor- β in rheumatoid arthritis synovial fluid and serum. *Arthritis Rheum* 31:1041–1044
- Olsen NJ, Stein CB (2004) New drugs for rheumatoid arthritis. *N Engl J Med* 350:2167–2179
- den Broeder A, van de Putte L, Rau R, Schattenkirchner M, Van Riel P, Sander O, Binder C, Fenner H, Bankmann Y, Velagapudi R, Kempeni J, Kupper H (2002) A single dose, placebo controlled study of the fully human anti-tumor necrosis factor- α antibody adalimumab (D2E7) in patients with rheumatoid arthritis. *J Rheumatol* 9:2288–2298
- Elliott PJ, Jirousek M (2008) Sirtuins: novel targets for metabolic disease. *Curr Opin Investig Drugs* 9:371–378
- Karuppagounder SS, Pinto JT, Xu H, Chen HL, Beal MF, Gibson GE (2009) Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease. *Neurochem Int* 54:111–118
- Elmali N, Baysal O, Harma A, Esenkaya I, Mizrak B (2007) Effects of resveratrol in inflammatory arthritis. *Inflammation* 30:1–6
- Molnar V, Garai J (2005) Plant-derived anti-inflammatory compounds affect MIF tautomerase activity. *Int Immunopharm* 5:849–856
- Penberthy WT (2007) Pharmacological targeting of IDO-mediated tolerance for treating autoimmune disease. *Curr Drug Metab* 8:245–266
- Yasuda Y, Saito M, Yamamura T, Yaguchi T, Nishizaki T (2009) Extracellular adenosine induces apoptosis in Caco-2 human colonic cancer cells by activating caspase-9/-3 via A_{2a} adenosine receptors. *J Gastroenterol* 44:56–65
- Kaerberlein M, McDonagh T, Heltweg B, Hixon J, Westman EA, Caldwell SD, Napper A, Curtis R, DiStefano PS, Fields S, Bedalov A, Kennedy BK (2005) Substrate-specific activation of sirtuins by resveratrol. *J Biol Chem* 280:17038–17045
- Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α . *Cell* 127:1109–1122
- Shakibaei M, Harikumar KB, Aggarwal BB (2009) Resveratrol addiction: to die or not to die. *Mol Nutr Food Res* 53:115–128
- Wang X (2001) The expanding role of mitochondria in apoptosis. *Genes Dev* 15:2922–2933
- Byun HS, Song JK, Kim YR, Piao L, Won M, Park KA, Choi BL, Lee H, Hong JH, Park J, Seok JH, Lee YJ, Kang SW, Hur GM (2008) Caspase-8 has an essential role in resveratrol-induced apoptosis of rheumatoid fibroblast-like synoviocytes. *Rheumatology* 47:301–308
- Giannakou ME, Partridge L (2004) The interaction between FOXO and SIRT1: tipping the balance towards survival. *Trends Cell Biol* 14:408–412
- Salminen A, Kaarniranta K (2009) Regulation of the aging process by autophagy. *Trends Mol Med* 15:217–224