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Resveratrol: A Candidate Drug for Treating Rheumatoid Arthritis

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1. Introduction

Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic inflammation of multiple joints, with disruption of joint cartilage. Accumulating evidence has pointed to inflammatory cytokines inducing hyperplasia of synovial cells in joints as an etiology for rheumatoid arthritis. High concentrations of cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) are found in synovial fluid and plasma from patients with rheumatoid arthritis (Eastgate et al., 1988; Saxne et al., 1998) and those cytokines produce matrix metalloproteinases or activate osteoclasts, causing irreversible damage to soft tissues and bones (Olsen & Stein, 2004). Challenges for rheumatoid arthritis treatment, therefore, have been attempted using TNF- α inhibitors, anti-TNF antibodies, a soluble TNF receptor-fusion protein, or an IL-1 receptor antagonist. A concern with these therapies, however, are side effects such as serious infections and inducible malignant tumors (den Broeder et al., 2002).

Resveratrol (Fig. 8), a phytoalexin that is present in grape skin and red wine, exerts a variety of actions to reduce superoxides, suppress carcinogenesis and angiogenesis, prevent diabetes mellitus, inhibit inflammation, and prolong life span (Elliott & Jirousek, 2008). Furthermore, resveratrol decreases plaque formation relevant to neurodegenerative diseases such as Alzheimer's disease and Huntington's disease (Karuppagounder et al., 2009). Of particular interest is that resveratrol is a potent and specific inhibitor of NF- κ B activation induced by TNF- α or IL-1 β , and therefore, resveratrol might be a potential therapy for rheumatoid arthritis (Elmali et al., 2007; Molnar & Garai, 2005; Penberthy, 2007).

2. Materials and methods

2.1 Cell culture

MH7A human rheumatoid arthritis synovial cells were obtained from the Riken cell bank (Ibaraki, Japan). Cells were cultured in a culture medium; RPMI-1640 (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.

2.2 Cell viability

Cell viability was evaluated by the method of Mosmann (1983) using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT).

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

MH7A cells were fixed with 4% paraformaldehyde. After inactivating endogenous peroxidase with methanol containing 0.3% H₂O₂, a Permeabilization Buffer (Takara Bio Inc., Otsu, Japan) was applied to cells and stood on ice for 5 min. Then, a Labeling Reaction Mixture (Takara Bio Inc.) 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.

2.4 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 identified by staining with chemiluminescent HRP substrate LumiGLO, and signals were detected with a microplate luminometer (ARVO mx/light, PerkinElmer, Waltham, MA, USA).

2.5 Assay of mitochondrial membrane potentials

Mitochondrial membrane potentials were measured using a DePsipher™ kit. 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 microscope (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.

2.6 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 (Nacalai Tesque, Kyoto, Japan). 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 Bio Inc.) 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 for

30 s using primers shown in Table 1. PCR products were stained with ethidium bromide and visualized by 2% agarose electrophoresis.

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

Table 1. Primers used for RT-PCR

2.7 Real-time RT-PCR

Total RNA was isolated from MH7A cells with Sepasol RNA I super kit (Nacalai Tesque, Kyoto, Japan). Total RNA (5 µg) was reverse-transcribed to cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Tokyo, Japan), and quantitative real-time RT-PCR was performed with Applied Biosystems 7900HT real-time PCR system (Applied Biosystems, Foster City, CA, USA) using the SYBR Green realtime Master Mix (TOYOBO, Osaka, Japan) protocol. Primers used for real-time RT-PCR are shown in Table 2. The PCR cycling conditions were 95°C for 4 min, followed by 40 cycles at 95°C for 15 s and 62°C for 15 s and 72°C for 45 s. A standard curve was made by amplifying 0.5, 1, 2, 4, and 8 µl of the GAPDH mRNA diluted at 1:250. The mRNA quantity for each target gene was calculated from the standard curve using an SDS 2.1 Software (Applied Biosystems, Foster City, CA, USA).

Gene name	Sense primer	Anti-sense primer	Base pair
AIF	TCACAAAGACACTGCGATTCAAACAGT	GTTGCTGAGGTATTCGGGGAGGAT	491
FOXO-1	TAIGGACACITIGCGITTCATTATTAGGATAAC	GGCAGAAGGGAGAATGAGATGAAGTATG	366
FOXO-3	TGGATGCTGATGGGTTGGATTTTAA	TGGTGTGAGGGGTGCTGTCC	415
P21	ACCGAGTGGGGGCATCATCAA	GGATGGGGTGGATGAGGAAGGTC	534
P27	CGTGGCTCGTCGGGGTCTGT	CCCTTCTCCACCTCTTGCCACTC	393
P53	GCCATCTACAAGCAGTCACAGCACAT	GGCACAAACACGCACCTCAAAGC	348
GAPDH	GACTTCAACAGCGACACCCACTCC	AGGTCCACCACCCTGTTGCTGTAG	128

Table 2. Primers used for real-time RT-PCR

2.8 Western blotting

After treatment with resveratrol (100 µM) for 6-24 h, MH7A cells were harvested and centrifuged at 600 x g for 10 min. After washing-out with 1 ml of PBS, the pellet was resuspended in 50 µl of buffer A (20 mM Hepes, 10 mM KCl, 1.5 mM MgCl₂, 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 1000 x g for 10 min and the supernatant was further centrifuged at 10000 x g for 1 h. The pellet and supernatant were regarded as the mitochondria- and cytosol-enriched fractions, respectively. Each fraction was loaded on 12% sodium dodecylsulfate 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 Image Gauge software (FUJIFILM, Tokyo, Japan).

2.9 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).

3. Results

3.1 Resveratrol induces MH7A cell apoptosis

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.

Resveratrol increased TUNEL-positive cells in a concentration (100-200 μ M)-dependent 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 fold of control levels (Fig. 1C). This provides further evidence for resveratrol-induced MH7A cell apoptosis.

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

Resveratrol is shown to regulate mitochondrial functions or energy metabolism by interacting with NAD-dependent deacetylase sirtuin 1 (Kaeberlein et al., 2005; Lagouge et al., 2006). 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 tricostatin A (30 nM), an 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. 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.

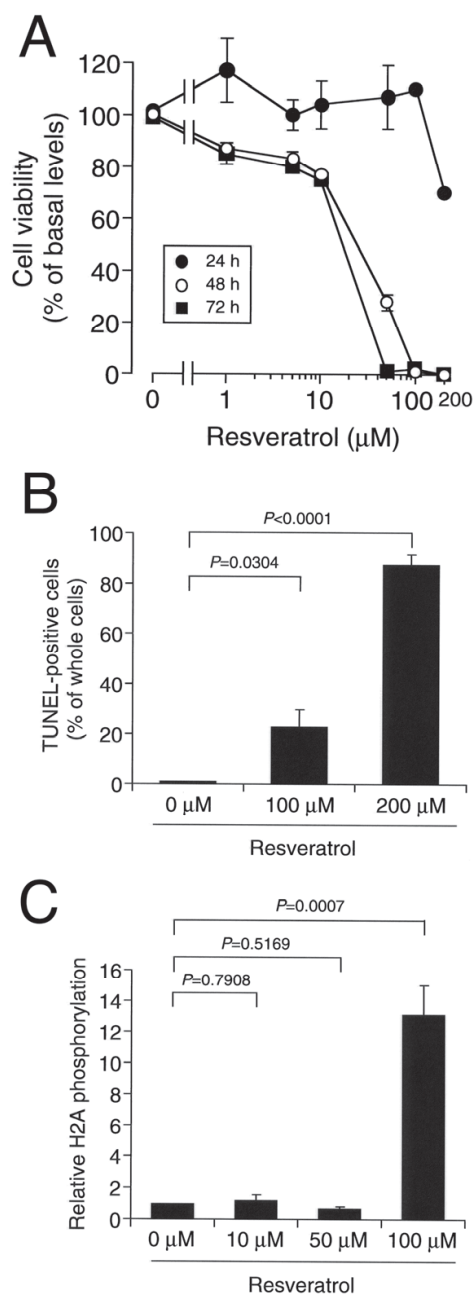


Fig. 1. Resveratrol induces apoptosis in MH7A cells. **(A)** MH7A cells were treated with resveratrol at concentrations as indicated for 24-72 h in serum-free culture medium, and cell viability was quantified with an MTT assay. In the graph, each point represents the mean (\pm SEM) percentage of basal 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.

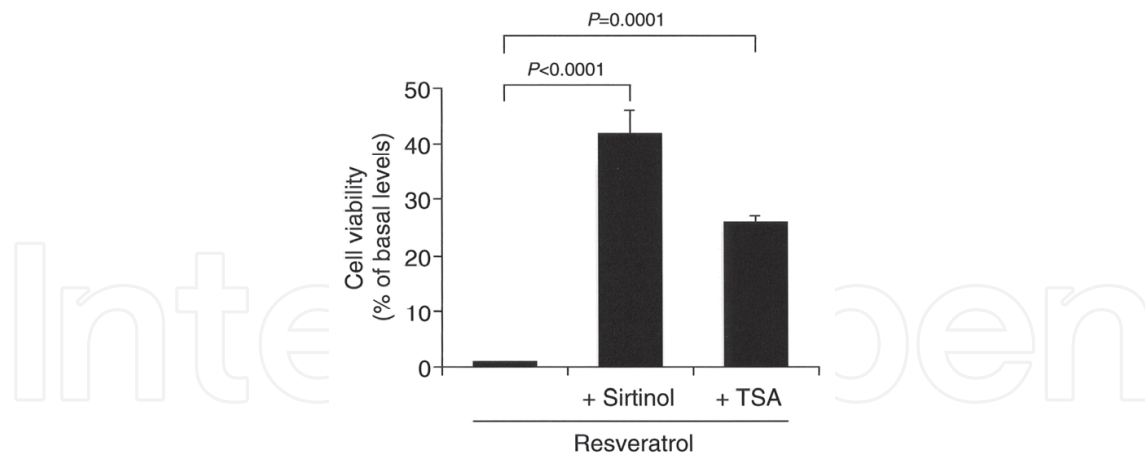


Fig. 2. Resveratrol-induced MH7A cell death is inhibited by a sirtuin 1 inhibitor or an HDAC inhibitor. MH7A cells were treated with resveratrol (100 μ M) in the absence and presence of sirtinol (10 μ M) or tricostatin A (TSA) (30 nM) for 48 h in serum-free culture medium, and cell viability was quantified 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.

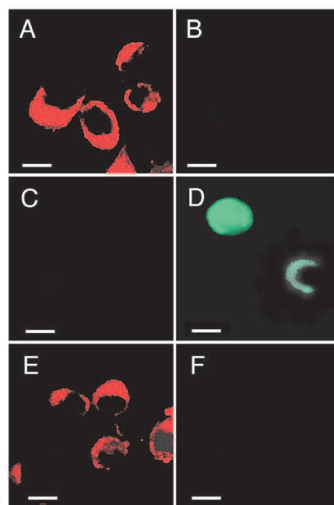


Fig. 3. Resveratrol disrupts mitochondrial membrane potentials in a sirtuin 1-dependent manner. MH7A cells were treated with resveratrol (100 μ M) in the absence and presence of sirtinol (10 μ M) for 24 h in serum-free culture medium, and mitochondrial membrane potentials were monitored. (A,C,E) Orange-red 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.

In the RT-PCR analysis, resveratrol (100 μ M) increased 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 being a significant target in resveratrol-induced MH7A cell death. Accumulating evidence has shown that resveratrol upregulates or downregulates 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 (Shakibaei et al., 2009). Resveratrol (100 μ M)

downregulated expression of the Bcl-X_L mRNA in MH7A cells from 1-h through 3-h treatment (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.

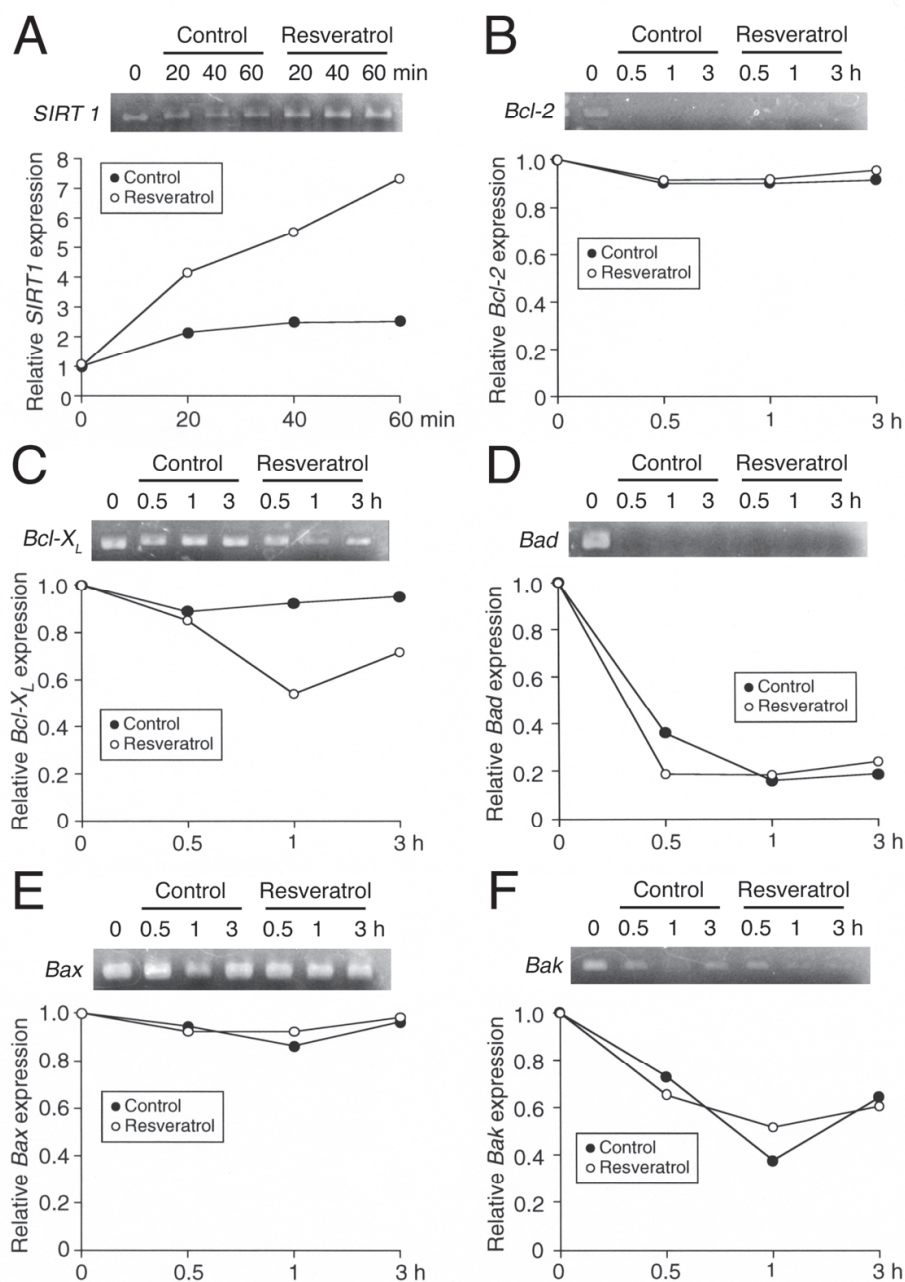


Fig. 4. Resveratrol upregulates expression of the sirtuin 1 mRNA and downregulates expression of the Bcl-X_L mRNA. MH7A cells were untreated (Control) and treated with resveratrol (100 μ M) for 20-60 min or 0.5-3 h in serum-free culture medium, and then RT-PCR was carried out. In the graphs, each point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

3.3 Resveratrol activates caspase-3 and -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 μ M) increased 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 suggests that resveratrol stimulates release of cytochrome c from the mitochondria into the cytosol.

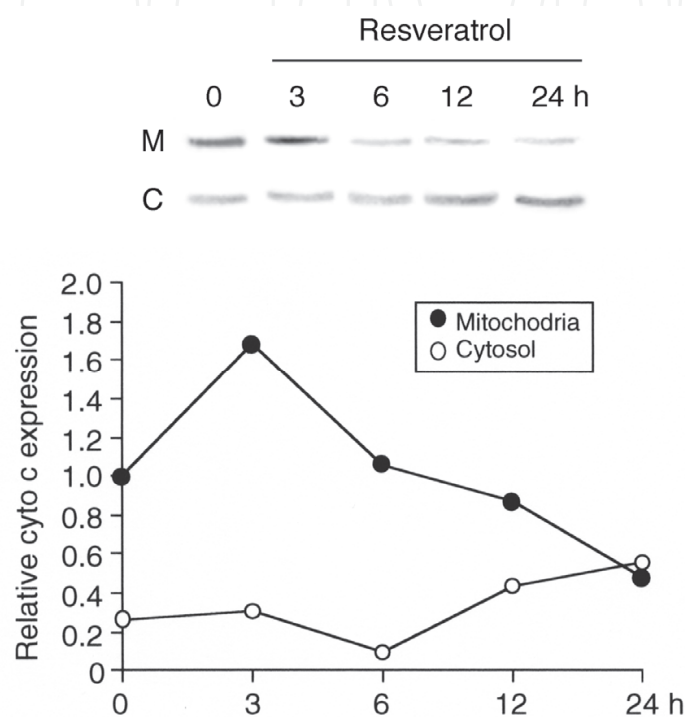


Fig. 5. Resveratrol stimulates cytochrome c release from the mitochondria. MH7A cells were treated with resveratrol (100 μ M) for 3-24 h in serum-free culture medium, followed by fractionation into the mitochondrial (M) and cytosolic component (C), and Western blotting was carried out. In the graph, each point represents the ratio against the immunoreactive intensity at 0 h for the mitochondrial component regarded as 1. cyto c, cytochrome c. Note that similar results were obtained with 3 independent experiments.

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

Overall, resveratrol appears to downregulate Bcl-X_L expression in a sirtuin 1-dependent manner, which promotes Bax-Bax complex, causing disruption of mitochondrial membrane potentials allowing cytochrome c release from the mitochondria. This is followed by activation of caspase-9 and the effector caspase-3, which, in turn, are responsible for apoptosis in MH7A human rheumatoid arthritis synovial cells (Nakayama et al., 2010) (Fig. 7).

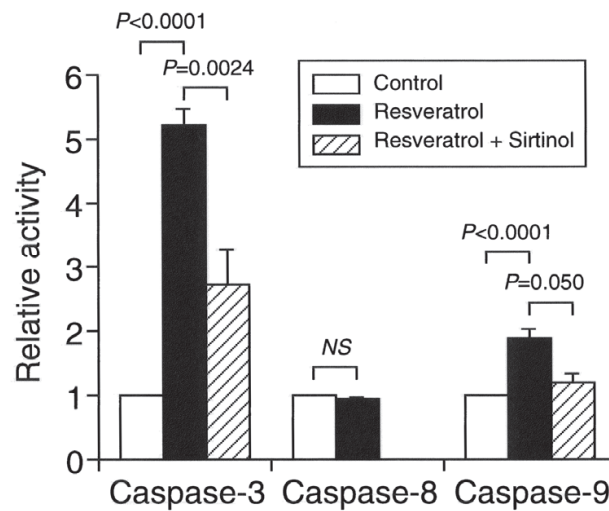


Fig. 6. Resveratrol activates caspase-3 and -9 in a sirtuin 1-dependent manner. MH7A cells were treated with resveratrol (100 μ M) in the absence and presence of sirtinol (10 μ M) for 24 h in serum-free culture medium, and caspase activities were assayed. In the graph, each column represents the mean (\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.

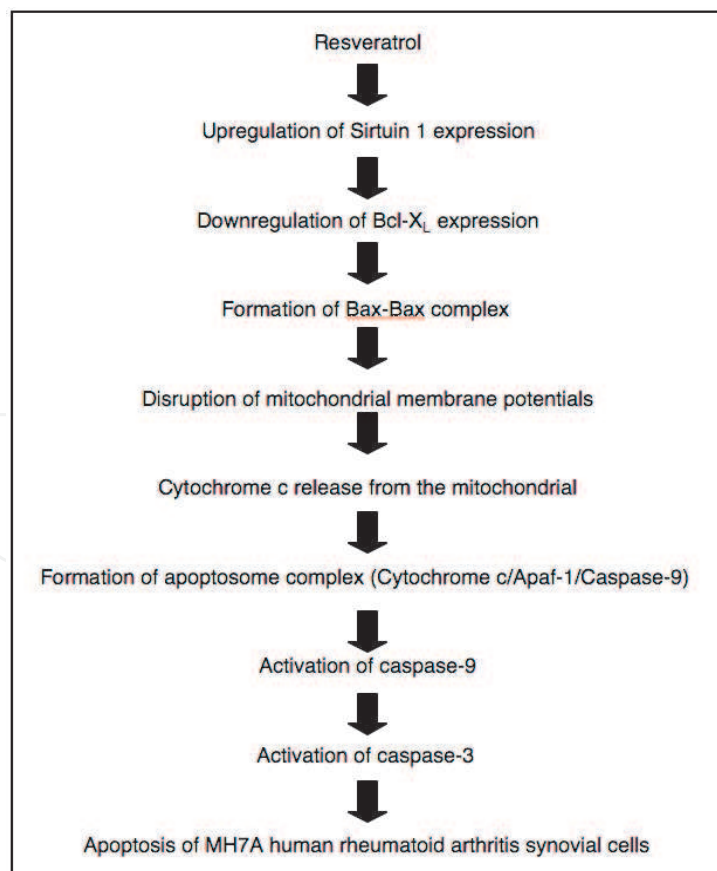


Fig. 7. Pathway underlying resveratrol-induced apoptosis in MH7A human rheumatoid arthritis synovial cells.

3.4 Resveratrol induces MH7A cell apoptosis with higher potency

We examined the effect of other polyphenols such as piceatannol, rhapontin, (-)-catechin, (+)-catechin, (-)-epicatechin, (-)-gallocatechin, (-)-epigallocatechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-gallocatechin gallate, and (-)-epigallocatechin gallate on MH7A cell death (Fig. 8). Of these, piceatannol, rhapontin, (-)-gallocatechin, (-)-epigallocatechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-gallocatechin gallate, and (-)-epigallocatechin gallate induced MH7A cell death in a concentration (10-100 μ M)- and treatment time (24-72 h)-dependent manner, but with lesser potency than resveratrol, whilst no effect was observed for (-)-catechin, (+)-catechin, or (-)-epicatechin (Fig. 9). This indicates that resveratrol is capable of inducing apoptosis in MH7A human rheumatoid arthritis synovial cells, with much higher potency than other polyphenols.

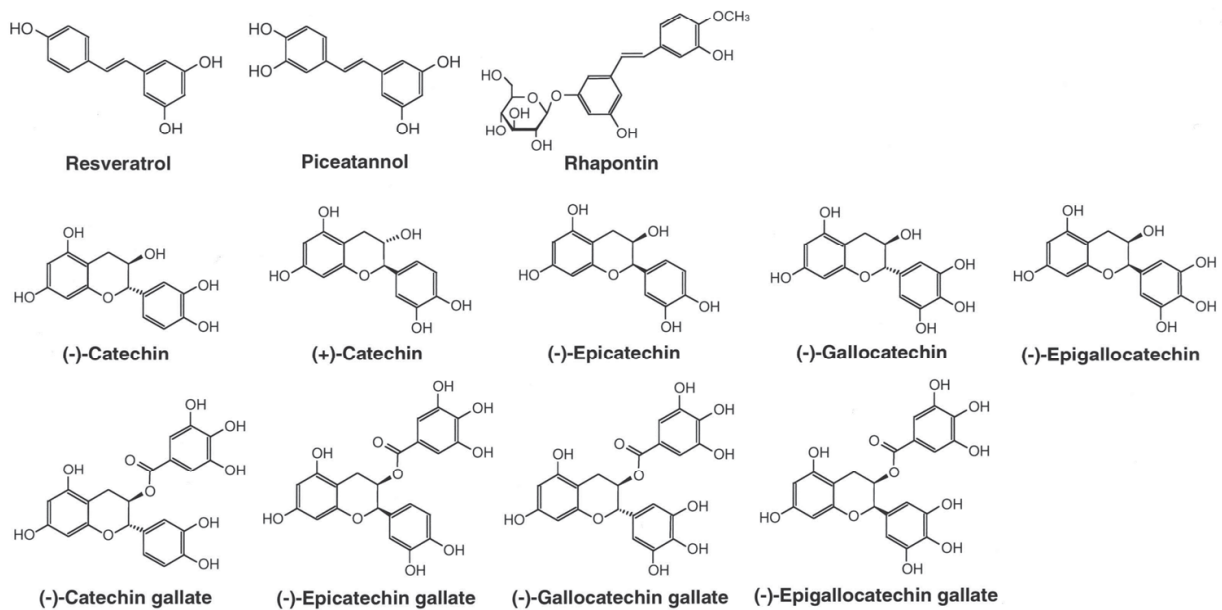


Fig. 8. Chemical structures of resveratrol and a variety of polyphenols.

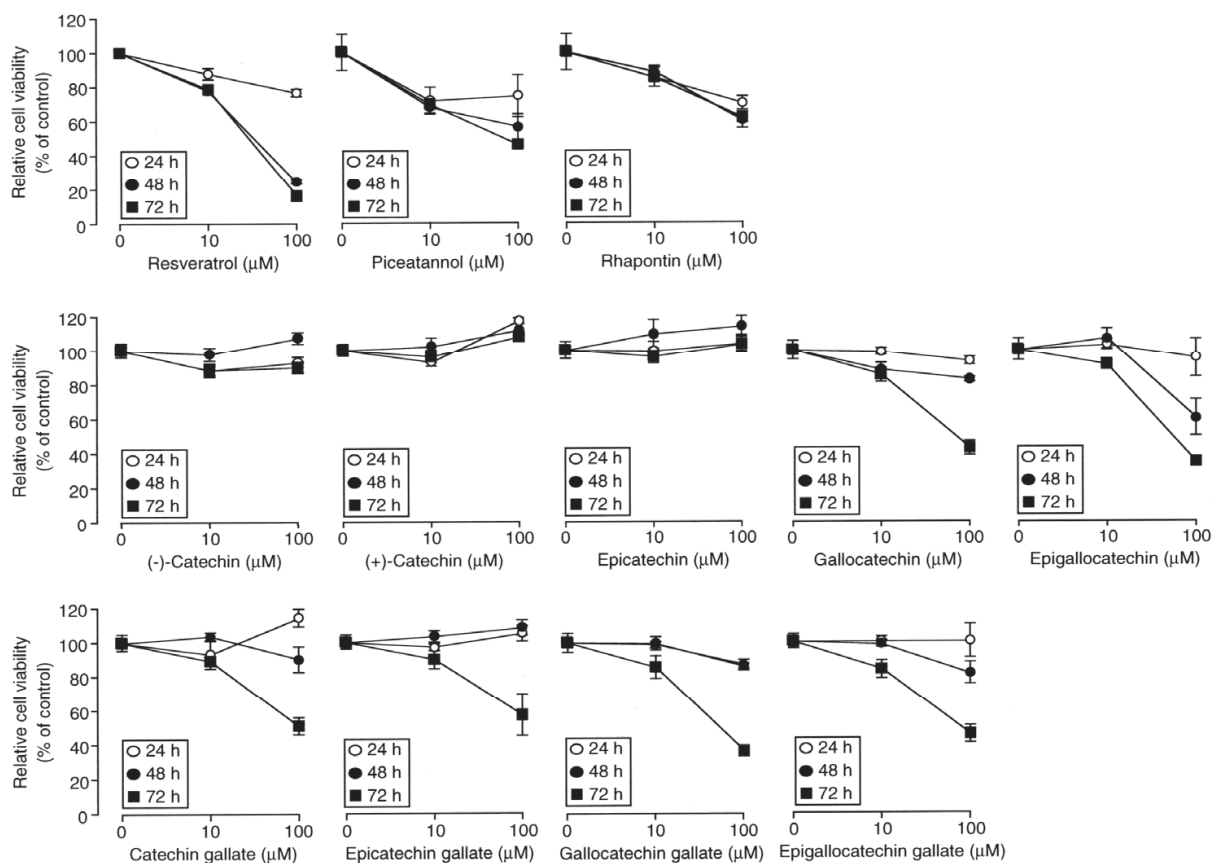


Fig. 9. Effects of resveratrol and a variety of polyphenols on MH7A cell death. MH7A cells were treated with resveratrol and polyphenols as indicated at concentrations ranging from 10-100 μM for 24-72 h in serum-free culture medium, and cell viability was quantified with an MTT assay. In the graphs, each point represents the mean (\pm SEM) percentage of basal levels (MTT intensities for cells untreated with resveratrol or polyphenols) ($n=8$).

3.5 Resveratrol increases expression of mRNAs for FOXO-1, FOXO-3, p21, p27, and AIF in MH7A cells

Sirtuin 1 regulates apoptosis-related gene transcription mediated by FOXO or NF- κB (Giannakou & Partridge, 2004; Salminen & Kaarniranta, 2009). In support of this, resveratrol induces growth arrest and apoptosis by activating FOXO in prostate cancer cells (Chen et al., 2010). In the real-time RT-PCR analysis, resveratrol (100 μM) increased expression of mRNAs both for FOXO-1 and FOXO-3 in MH7A cells in a bell-shaped treatment time (20-60 min)-dependent manner, with the peak at 20-min treatment (Fig. 10A), indicating involvement of FOXO in resveratrol-induced MH7A cell apoptosis.

Lines of studies have shown that resveratrol induces apoptosis in a variety of cells by activating p53 (Huang et al., 1999; Kuo et al., 2002; She et al., 2001). Resveratrol (100 μM) here, however, had no effect on expression of the p53 mRNA in MH7A cells (Fig. 10B). This suggests that resveratrol induces MH7A cell apoptosis in a p53-independent manner.

Resveratrol is shown to upregulate expression of p21 and p27 (Ganapathy et al., 2010; Ragione et al., 2003). Resveratrol (100 μM) increased expression of mRNAs both for p21 and p27 still in MH7A cells in a bell-shaped treatment time (20-60 min)-dependent manner, with

the peak at 40-min treatment (Fig. 10B). This raises the possibility that resveratrol could suppress MH7A cell proliferation and growth by inhibiting cyclin-dependent protein kinases (Cdks) via control of p21 and p27.

Apoptosis-inducing factor (AIF) is released from damaged mitochondria and causes chromatin condensation and large-scale (~50 kbp) DNA fragmentation, leading to caspase-independent apoptosis. Interestingly, resveratrol induces apoptosis in human lung adenocarcinoma ASTC-a-1 cells through mitochondria-mediated AIF release (Zhang et al., 2011). In the present study, a huge increase in the expression of the AIF mRNAs in MH7A cells was found after 40-min treatment with resveratrol (100 μ M) (Fig. 10C), suggesting that AIF also participates in resveratrol-induced MH7A cell apoptosis.

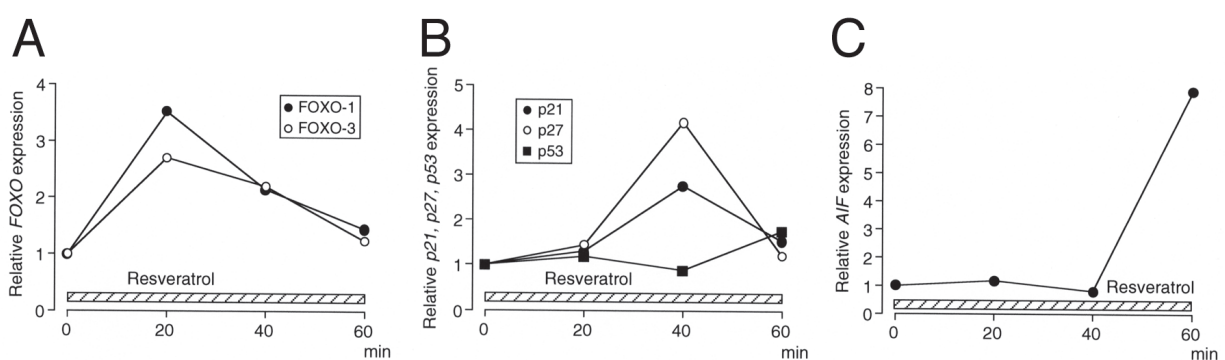


Fig. 10. Effects of resveratrol on mRNAs for FOXO-1, FOXO-3, p21, p27, p53, and AIF. MH7A cells were treated with resveratrol (100 μ M) for periods as indicated in serum-free culture medium, and then real-time RT-PCR was carried out. The mRNA quantity for each gene was calculated from the standard curve made by amplifying different amounts of the GAPDH mRNA, and normalized by regarding the average of independent basal mRNA quantity at 0 h as 1. In the graphs, each point represents the mean (\pm SEM) ratio ($n=3$ independent experiments).

4. Discussion

Accumulating evidence has shown that resveratrol induces apoptosis or suppresses cell growth and proliferation by interacting with FOXO, NF- κ B, and p53 involving wide-range of gene transcriptions such as those for apoptosis-related proteins, p21, p27, and AIF (Chen et al., 2010; Ganapathy et al., 2010; Huang et al., 1999; Kuo et al., 2002; Ragione et al., 2003; She et al., 2001). Notably, resveratrol is shown to increase expression of sirtuin 1, a mammalian NAD⁺-dependent deacetylase (class III HDAC) (Franco et al., 2010). In the present study, resveratrol upregulated expression of mRNAs for FOXO-1, FOXO-3, p21, p27, AIF, and sirtuin 1 in MH7A cells but otherwise downregulated expression of the Bcl-X_L mRNA, without affecting expression of mRNAs for p53, Bcl-2, Bad, Bax, or Bak. FOXO belongs to the O subclass of the forkhead family of transcription factors, which includes FOXO-1, FOXO-3, FOXO-4, and FOXO-6. Evidence has pointed to the role of sirtuin 1 in the regulation of apoptosis-related gene transcription mediated by FOXO or NF- κ B (Giannakou & Partridge, 2004; Salminen & Kaarniranta, 2009). FOXO and/or sirtuin 1,

therefore, may be a primary target of resveratrol-regulated apoptosis-related gene transcription.

Resveratrol induced MH7A cell apoptosis in a concentration (1-200 μ M)- and treatment time (24-72 h)-dependent manner. Two major pathways for apoptosis are well-recognized, i.e., oxidative stress-induced mitochondria-mediated apoptotic pathway and endoplasmic reticulum (ER) stress-induced apoptotic pathway. For the former pathway, the Bcl-2 family such as Bcl-2, Bcl-X_L, Bad, and Bax plays a central role; Bcl-2 and Bcl-X_L protect the mitochondria by capturing Bax, but Bad otherwise disrupts mitochondrial membrane potentials by releasing Bax from a Bcl-2/Bax complex or a Bcl-X_L/Bax complex. Oxidative stress disrupts mitochondrial membrane potentials by making a Bax/Bax pore, thereby damaging the mitochondria to allow release of apoptosis-related proteins such as cytochrome c, AIF, Smac/DIABLO, Omi/HtrA2, and endonuclease G into the cytosol (Wang, 2001). Subsequently, released cytochrome c activates caspase-3 by forming an apoptosome complex together with apoptosis proteases activating factor-1 (Apaf-1) and caspase-9, leading to apoptosis (Wang, 2001). Resveratrol disrupted mitochondrial membrane potentials, stimulated cytochrome c release from the mitochondria into the cytosol, and activated caspase-3 and -9 in MH7A cells. This, taken together with the finding that resveratrol downregulated the Bcl-X_L mRNA, accounts for mitochondria-mediated caspase-dependent pathway in resveratrol-induced MH7A cell apoptosis. Of particular interest is that resveratrol-induced MH7A cell apoptosis, mitochondrial damage, and caspase-3/-9 activation were prevented by a sirtuin 1 inhibitor or an HDAC inhibitor. This confirms that sirtuin 1 is required for resveratrol-induced MH7A cell apoptosis.

Findings by Byun et al. (2008) suggest that resveratrol induces 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 activation of caspase-9 and the effector caspase-3, without affecting the levels of Bax, Bcl-X_L, and Bcl-2. This observation contrasts with our finding that resveratrol does not activate caspase-8 in MH7A cells (Nakayama et al., 2010). Thus, resveratrol-induced MH7A cell apoptosis may be mediated via a novel apoptotic pathway.

In contrast, resveratrol upregulated expression of the AIF mRNA in MH7A cells. AIF induces apoptosis by causing chromatin condensation and DNA fragmentation. This suggests that resveratrol could induce MH7A cell apoptosis via an additional pathway, i.e., mitochondria-mediated caspase-independent pathway.

Resveratrol also increased expression of the p21 and p27 mRNAs in MH7A cells. p21 and p27 are recognized to inhibit cyclin E/Cdk2 that proceeds cell growth at the G₁ phase of cell cycling. Resveratrol, consequently, could suppress MH7A cell growth by inhibiting cyclin E/Cdk2 under the control of p21 and/or p27.

Like resveratrol, some other polyphenols induced apoptosis in MH7A human rheumatoid arthritis synovial cells. Of polyphenols examined here resveratrol induced MH7A cell apoptosis with the highest potency. This implies that, of the polyphenols, resveratrol could be the best target for the development of new drugs for treating rheumatoid arthritis.

In summary, the results of the present study show that resveratrol upregulates expression of FOXO and sirtuin 1 relevant to apoptosis-related gene transcription and its regulation in MH7A human rheumatoid arthritis synovial cells. Resveratrol downregulates expression of

the Bcl-X_L mRNA, possibly mediated by FOXO, which causes disruption of mitochondrial membrane potentials, allowing cytochrome c release from the mitochondria into the cytosol, leading to activation of caspase-9 and the effector caspase-3 in a sirtuin 1-dependent manner. This represents a mitochondria-mediated caspase-dependent apoptotic pathway. Upregulation of the AIF mRNA by resveratrol, alternatively, suggests resveratrol-induced MH7A cell apoptosis via a mitochondria-mediated caspase-independent pathway. Moreover, upregulation of the p21 and p27 mRNAs by resveratrol may account for resveratrol-induced inhibition of MH7A cell growth.

5. Conclusion

Resveratrol induces apoptosis in MH7A human rheumatoid arthritis synovial cells, and does so with much higher potency than other polyphenols. It achieves this effect by decreasing Bcl-X_L expression, which disrupts mitochondrial membrane potentials, allowing cytochrome c release from the mitochondria into the cytosol, thereby activating caspase-9 and the effector caspase-3 in a sirtuin 1-dependent manner, which induces apoptosis. In addition, AIF, p21, and p27 may also participate in resveratrol-induced MH7A cell apoptosis and growth inhibition. These findings imply that resveratrol may be capable of preventing hyperplasia of synovial cells in human rheumatoid arthritis. Resveratrol, thus, could be developed as a promising drug for treatment of rheumatoid arthritis.

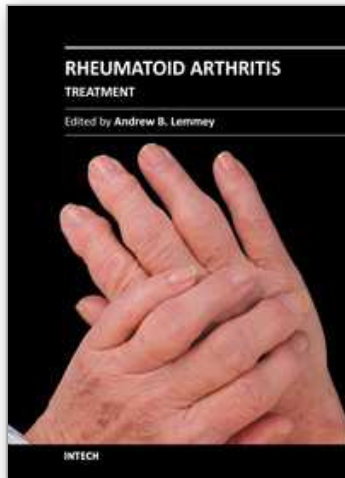
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The purpose of this book is to provide up-to-date, interesting, and thought-provoking perspectives on various aspects of research into current and potential treatments for rheumatoid arthritis (RA). This book features 17 chapters, with contributions from numerous countries (e.g. UK, USA, Canada, Japan, Sweden, Turkey, Bosnia and Herzegovina, Slovakia), including chapters from internationally recognized leaders in rheumatology research. It is anticipated that Rheumatoid Arthritis - Treatment will provide both a useful reference and source of potential areas of investigation for research scientists working in the field of RA and other inflammatory arthropathies.

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