Original Article

Resveratrol Protects Human Endothelium from H₂O₂-Induced Oxidative Stress and Senescence via SirT1 Activation

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Aim: Silencing information regulator (SirT1), a NAD-dependent histone deacetylase, is an essential mediator of longevity in normal cells by calorie restriction. SirT1 has many biological functions, including transcription regulation, cell differentiation inhibition, cell cycle regulation, and anti-apoptosis. Resveratrol (RV)-induced SirT1 activation also improves endothelial dysfunction and suppresses vascular inflammation. In this study, we investigated the roles of RV-induced SirT1 activation in endothelial cells under oxidative stress.

Methods: SirT1 mRNA expression levels were examined in the endothelium layer (endothelial cells) of cardiac coronary vessels from patients receiving coronary artery bypass graft surgery (CABG) surgery and aged rats using reverse transcriptase polymerase chain reaction (RT-PCR). To further explore the effect of SirT1 activation on oxidative stress-induced aging, senescence-associated β -galactosidase (SA- β -gal) expression in RV-treated human umbilical vein endothelial cells (HUVECs) with or without H₂O₂ treatment was evaluated.

Results: SirT1 expression was decreased in aged and atherosclerotic vessels in vivo, and significantly reduced in endothelial cells purified from vessel tissues. Furthermore, SirT1 levels were dose-dependently increased in RV-treated HUVECs. The SA- β gal assay showed that RV inhibited the senescent phenotype of H₂O₂-treated HUVECs. Reactive oxygen species (ROS) production and the percentage of cells positive for SA- β gal were significantly increased in siRNA-SirT1 (knockdown of SirT1 expression)-treated HUVEC cells. Importantly, the treatment effect of RV was significantly abolished in the oxidative effects of H₂O₂-treated HUVECs by siRNA-SirT1.

Conclusion: Our data suggested that SirT1 could be a crucial factor involved in the endothelial cells of atherosclerotic CABG patients and aging rats. RV is a potential candidate for preventing oxidative stress-induced aging in endothelial cells. RV may also prevent ROS-induced damage via increased endothelial SirT1 expression.

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Key words; SirT1, NAD-dependent histone deacetylase, Resveratrol, Human umbilical vein endothelial cells, Cardiovascular dysfunction

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Introduction

Cardiovascular diseases have become a major worldwide cause of morbidity and mortality. Oxidative stress-induced damage has been proposed as a

major risk for cardiovascular disease and increased vessel wall oxidative stress is a pathogenic feature of atherosclerosis and hypertension^{1, 2}. Increased oxidative stress is a major cause of endothelial dysfunction by attenuating NO production, promoting inflammation, and through its involvement in the activation of intracellular signal transduction pathways, thus influencing ion channel activation, intercellular communication, and gene expression to, ultimately, elicit the cessation of cell division and premature senescence. Reactive oxygen species (ROS), such as H₂O₂, superoxide (O₂⁻) and the hydroxyl radical (OH·), are generated by various different pathways. Data from several studies indicate that ROS activate protein tyrosine kinases, followed by the stimulation of downstream signaling events that regulate gene expression, resulting in the modification of cardiovascular cells^{3, 4}. Redox-sensitive proteins, such as c-Src, PYK2, ERK1/2, and BMK1⁵⁻⁷, are likely critical mediators of these changes in gene expression.

Recent studies reported that the silent information regulator 2 (Sir2), a NAD⁺-dependent protein deacetylase⁸, promotes longevity in yeast and mammalian cells. Cells lacking Sir2 have a reduced replicative lifespan. Importantly, cells with an increase in Sir2 activity display a much longer lifespan than wild-type cells. This event may be regulated through hypersilencing of the formation of extrachromosomal ribosomal DNA circles in the nucleoli⁸; a known cause of senescence in yeast. Caloric restriction (CR) has already been reported as the key contributor to longevity in species from yeast and nematodes to rodents and monkeys. It is thought that CR slows the metabolic rate, generating more free NAD co-factors, resulting in greater active Sir2 deacetylase activity⁹, and an extended lifespan¹⁰. Thus, NAD⁺ dependency links Sir2 activity to metabolic changes. Furthermore, Sir2 and other related members of the sirtuin family are highly conserved from yeast to mammalian cells. The SirT1 gene encodes a member of the sirtuin family of proteins, homologs of the Sir2 gene in yeast, and contains the catalytic core domain of Sir2^{11, 12}. SirT1, which resides in the nucleus, binds and deacetylates p53, NF- κ B, and forebrain transcription factors, as well as histones¹³. SirT1 is postulated to protect against neurodegeneration and diabetes but may promote tumorigenesis¹⁴⁻¹⁶. Its role in disease progression and aging in mammals remains to be further evaluated.

The specific aim of this study was to evaluate the role of SirT1 in cardiovascular tissues from patients with coronary artery bypass graft surgery (CABG) and endothelial cells under oxidative stress. SirT1 mRNA expression levels were detected in the endothelial cells

of coronary artery from atherosclerotic vascular tissues and control vessels by quantitative real-time RT-PCR. The SirT1 mRNA expression ratio was also evaluated in the cardiac coronary vessels of rats of different ages. To investigate the role of RV in oxidative stress-induced vasculopathy, H₂O₂ was used to mimic the effect of oxidative stress in human umbilical vein endothelial cells (HUVECs) to explore how RV and SirT1 protect the endothelium from oxidative stress.

Methods

Endothelial Cell Isolation

Atherosclerotic vessels and saphenous vein were harvested from patients undergoing CABG. Endothelial cells were isolated from intact human atherosclerotic vessels and saphenous veins according to the method previously described^{17, 18}. Briefly, after removal of the connective tissue, the vein was opened longitudinally. The endothelium was isolated by digestion with 1 mg/mL Type II collagenase in PBS for 20 min at room temperature. The endothelial cells were collected by centrifugation and grown in M199 media supplemented with 10 U/mL heparin, 30 μ g/mL ECGS, 100 U/mL penicillin, 100 μ g/mL streptomycin and 10% (v/v) heat-inactivated human serum. The cells were cultured in fibronectin-coated flasks and maintained in a humidified atmosphere of 5% CO₂ in air at 37°C.

Cultivation of HUVEC Cells

HUVEC cells were purchased from ATCC cell banks (Manassas, VA, USA). These HUVECs were suspended in Dulbecco's modified Eagle's medium containing 1.0 g/L of glucose (DMEM-LG) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, 100 μ g/mL streptomycin, and 2 mM L-glutamine, and incubated at 37°C with 5% CO₂. The cells were harvested with trypsin/EDTA, suspended at 1×10^6 cells/mL in 5% dimethylsulfoxide and 30% FBS, and frozen in 1-mL aliquots in liquid nitrogen (passage 1 cells). To expand a culture, a frozen vial of HUVECs was thawed, plated in a 60 cm² culture dish, and incubated for 4 days (passage 2 cells).

In situ Staining for β -Galactosidase Activity¹⁹

Cultured cells were washed in PBS (pH 7.4) and fixed with 2% formaldehyde and 2% glutaraldehyde for 10 to 15 minutes at room temperature. After being washed twice with PBS, the cells were then incubated overnight at 37°C in freshly prepared staining buffer [40 mM citric acid/sodium phosphate (pH 6.0), 0.15 M NaCl, 2 mM MgCl₂, 5 mM potassium ferro-

cyanide, 1 mg/mL X-gal (5-bromo-4-chloro-3-indolyl β -D-galactoside)]. At the end of incubation, the cells were microscopically examined at $\times 200$ magnification (Olympus IX71, Center Valley, PA, USA).

Immunoblot Analysis

HUVEC cells were fixed, washed once in cold PBS, scraped, lysed with extraction buffer, and centrifuged at 10,000 rpm (9,730 g) for 10 minutes to remove insoluble material. Protein concentrations were determined using a protein assay kit (Bio-Rad, Hercules, CA, USA). Cell extracts in sample buffer were placed in boiling water for 5 minutes and then separated by 10% SDS-PAGE gel. After electrophoresis, the gel was transferred onto a PVDF membrane for immunoblotting. The membrane was blocked by incubation in non-fat milk at room temperature for 2 hours and incubated with anti-SirT1 antibody (1:1000 dilution, sc-15404; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 hours at room temperature, washed five times with Tris-buffered saline Tween-20 (TBST), and incubated at room temperature with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5000 dilution, sc-2004, Santa Cruz Biotechnology) for 2 hours. The membrane was washed six times with TBST, and specific bands were made visible by chemiluminescence (ECL; Santa Cruz Biotechnology). Anti- β -actin (1:10000 dilution, MAB1501; Chemicon) was used as a loading control.

Quantitative Real-Time RT-PCR

PCR reactions were performed on the ABI PRISM[®] 7900HT Sequence Detection System and the ABI Prism 5700 SDS (Applied Biosystems). The amplification mixtures (50 μ L) contained template DNA 1 μ L, 2 X SYBR Green I Master Mix 25 μ L, 0.4 μ L of each primer, and DD water. Primers were designed: SirT1 (forward): 5'-CGTCTTATCCTCTAGTTCTTGTTG-3' (Gene accession no.AF083106), SirT1 (reverse): 5'-ATCTCCATCAGTCCCAAATCC-3', GAPDH (forward): 5'-AATTGA GCCCGCAGCCTCCC-3' (Gene accession no.NM_002046), GAPDH (reverse): 5'-C CAGGCGCCCAATACGACC-3'. The conditions for amplification were as follows: one cycle of 94°C for 5 min, followed by 40 cycles of 94°C for 20 sec, 57°C for 20 sec, and 70°C for 20 sec. Reactions for each primer set were performed in triplicate, and the threshold cycle numbers were averaged.

SirT1 Activity Assay

SirT1 activity in the samples was measured using the Cyclex SIRT1 Deacetylase Fluorimetric Assay Kit according to the manufacturer's protocol (CycLex Ltd.,

Nagano, Japan) as previously described²⁰. In brief, upon NAD-dependent deacetylation of the specific substrate by SirT1, the fluoro-substrate peptide is cleaved by a lysyl endopeptidase, separating the quencher from the fluorophore. By measuring time-dependent changes in fluorescence intensity produced by the fluorophore, specific activities of SirT1 could be assessed²⁰.

H₂O₂ Treatment

Cultured HUVEC cells were washed with PBS twice and treated with 100 μ M H₂O₂ in the culture medium. After the indicated time, the medium was removed, and the cells were subjected to the subsequent experiments.

Statistical Analysis

The results are expressed as the means $\pm$ SD. Statistical analyses were performed using one-way or two-way ANOVA, followed by Tukey's test, as appropriate. A p -value < 0.05 was considered significant.

Results

Detection of SirT1 mRNA in Human and Rat Endothelial Cells

To investigate the relationship between SirT1 and atherosclerotic vessels, the expression levels of SirT1 mRNA in tissues were examined by quantitative real-time RT-PCR. The results showed that the SirT1 mRNA expression level in atherosclerotic coronary vessels from coronary artery bypass surgery (CABG) patients ($n = 18$) was significantly decreased compared to that in the same patients' partial normal vessels at the end of segments ($*p < 0.01$; **Fig. 1A**). Since the whole vessels contain many types of cells, endothelial cells were isolated from the tissues of CABG patients, and the expression levels of SirT1 mRNA were examined by qRT-PCR. The results showed that the SirT1 mRNA expression level in endothelial cells from atherosclerotic coronary vessels was significantly decreased compared to that from the same patients' partial normal vessels at the end of segments ($**p < 0.001$; **Fig. 1B**). Interestingly, qRT-PCR analysis further demonstrated a negative regression relationship between age and SirT1 mRNA expression levels in endothelial cells derived from saphenous vein harvested from patients who received CABG surgery ($p < 0.01$; **Fig. 1C**).

To further investigate the possible relationship between aging and SirT1 gene expression, the SirT1 mRNA expression ratio in the cardiac coronary vessels was evaluated in SD rats of different ages (2, 4, 6, 8,

Fig. 1. Detection of SirT1 mRNA in human atherosclerotic tissues and endothelial cells.

(A) Total RNA was extracted from atherosclerotic tissues harvested from patients undergoing CABG surgery. The amounts of SirT1 transcripts were detected by qRT-PCR. The SirT1 mRNA expression level of atherosclerotic coronary vessels was compared with the same patient's partial normal distal part of segments ($n=18$; $*p<0.01$). (B) Total RNA was extracted from endothelial cells purified from atherosclerotic tissues. The amounts of SirT1 transcripts were detected by qRT-PCR. The relative SirT1 mRNA expression level of endothelial cells from atherosclerotic coronary vessels was compared with that from partial normal vessels at the end of segments from the same patient ($n=18$; $**p<0.001$). Data shown here are the mean $\pm$ SD of three independent experiments. (C) The regression plot shows that the SirT1 level is negatively correlated with age in endothelial cells obtained from the saphenous vein harvested during bypass surgery. Data from all patients were compared with data from a 40-year-old patient.

Fig. 2. Detection of SirT1 mRNA in rat aging endothelial cells.

(A) Total RNA was extracted from the cardiac coronary vessels (whole tissue) of SD rats of different ages (2, 4, 6, 8, and 12 months, $n=3$ in each age group), and the amount of SirT1 mRNA was detected by qPCR. $*p<0.01$: 12-month-old group vs. all other groups. (B) Total RNA was extracted from endothelial cells purified from cardiac coronary vessels of SD rats of different ages (2, 4, 6, 8, and 12 months, $n=3$ in each age group), and the amount of SirT1 mRNA was detected by qPCR. $*p<0.05$: 6-month-old group vs. 2- and 4-month-old groups. $**p<0.01$: 8- and 12-month-old groups vs. 2- and 4-month-old groups. Data shown here are the means $\pm$ SD of three independent experiments.

and 12 months, $n=3$ in each age group). The results of qRT-PCR analysis showed that the relative expression level of SirT1 mRNA was not different among each group except the 12-month-old group ($*p<0.01$, **Fig. 2A**). Endothelial cells were isolated and then qRT-PCR was performed to investigate the relation between SirT1 expression level and possible aging change in endothelial cells of the rat coronary artery. **Fig. 2B** shows that the relative expression levels of SirT1 mRNA in the endothelium of cardiac coronary vessels in 8- and 12-month-old groups were signifi-

Fig. 3. Determination of SirT1 levels in HUVECs using qRT-PCR.

(A) Left: The morphology of HUVECs under normal culture conditions. Right: HUVECs were treated with 0, 10, 20, 50, and 100 μM of RV for 48 hours. Total RNA was extracted, and SirT1 mRNA levels were examined by qRT-PCR. Bar: 30 μm . (B and C) HUVECs were treated with 0, 20, 50, and 100 μM of RV for 48 hours. Total protein was extracted, and SirT1 protein levels (B) and deacetylase activity (C) were examined by Western blot and fluorimetric assay, respectively. * $p < 0.05$; 50 μM RV treatment vs. control treatment. (D) HUVECs were exposed to 100 $\mu\text{mol/L}$ H₂O₂ for 12 hours, and cells were then treated with 10, 20, and 50 μM of RV for 48 hours. Total RNA was extracted, and SirT1 mRNA levels were determined using qPCR. Data shown here are the means $\pm$ SD of three independent experiments.

cantly lower than those of 2- and 4-month-old groups (** $p < 0.001$), as measured by qRT-PCR. The SirT1 mRNA expression ratio was also significantly decreased in the 6-month-old group compared to the 2- and 4-month-old groups (* $p < 0.05$; **Fig. 2B**); however, the SirT1 mRNA expression ratio of 2-month-old cardiac vessels was not significantly different from that

of 4-month-old vessels ($p > 0.05$; **Fig. 2B**). These data suggested that the constitutive expressions of SirT1 play a crucial role in the endothelium layer and endothelial cells of cardiac coronary vessels, which were affected by aging changes as well as the atherosclerotic process.

Detection of SirT1 in H₂O₂-Treated HUVEC Cells with or without Resveratrol

To further clarify our findings and to explore the effect of RV on the endothelium under oxidative stress, H₂O₂ was used to mimic oxidative stress-induced damage to endothelial cells (**Fig. 3A**). Firstly, HUVECs were treated with different doses of RV (10, 20, 50, and 100 μ M). Using qRT-PCR (**Fig. 3A**), Western blot (**Fig. 3B**), and fluorimetric SirT1 activity assay (**Fig. 3C**), the SirT1 expression levels and activities were evaluated, and the results suggested that 50 μ M RV was the optimal concentration to stimulate the expression of SirT1 mRNA in HUVECs ($p < 0.05$; **Fig. 3A–C**). Firstly, the results demonstrated that 50 μ M RV could efficiently recover SirT1 mRNA expression levels in H₂O₂-treated HUVEC cells (100 μ mol/L for 12 hours; **Fig. 3D**). Importantly, the amount of reactive oxygen species (ROS) was significantly increased in H₂O₂-treated HUVECs compared to untreated cells ($p < 0.05$; **Fig. 4A**). Furthermore, it was observed (**Fig. 3B**) that H₂O₂ could induce senescence in HUVECs (H₂O₂ 100 μ mol/L for 12 hours, washed by PBS, and then cultured for another 14 days; culture medium was changed every 2 days) as detected by SA- β gal assay (**Fig. 4B** and **4C**). In order to study the effect of RV on the senescent phenotype in HUVECs, premature endothelial senescence was induced by the addition of H₂O₂ 100 μ mol/L for 12 hours. RV in different concentrations (10, 20, and 50 μ M) was then added to these H₂O₂-treated HUVECs for 48 hours and washed by PBS. Culture medium without H₂O₂ or RV was changed every 2 days for another 12 days. Based on the SA- β gal assay and the morphology of endothelial cells, treatment with RV inhibited the senescent phenotype. Aging cells appeared enlarged and flattened after H₂O₂ treatment (**Fig. 4B**). With H₂O₂ treatment, 80% of cells were SA- β gal-positive, versus only 37% of RV (50 μ mol/L)-treated cells under the same oxidative conditions (**Fig. 4C**). These data suggest that RV is a potential agent to rescue oxidative stress-induced damage and inhibit the senescence process in treated HUVEC cells.

Resveratrol Protects Against H₂O₂-Induced Oxidative Stress and Senescence in Human Endothelium via the Activation of SirT1

In order to study the role of SirT1 in the treatment effects of RV, the SirT1 expression in HUVEC cells was silenced using a small interfering RNA (sh-SirT1) via lentiviral delivery. The protein levels of SirT1 in HUVEC cells were significantly reduced by Sh-SirT1 (**Fig. 5A**). The role of SirT1 in modulating

Fig. 4. SA- β gal assay in ROS-treated HUVECs.

(A) HUVECs were treated with 100 μ M H₂O₂, and intracellular ROS levels were detected at the times indicated. * $p < 0.05$: 6-, 12-, 18- and 24-hour treatment groups vs. control group. (B) HUVECs were treated with 100 μ M H₂O₂ for 12 hours, and the senescent phenotype of HUVECs was detected by the SA- β gal assay after another 14 days (culture medium without H₂O₂ was changed every 2 days). SA- β gal-positive cells were observed by fluorescent microscopy (arrow). Bar: 20 μ m. (C) HUVECs were treated with 100 μ M H₂O₂ for 12 hours followed by 10, 20, or 50 μ M of RV exposure for another 48 hours, and then the senescent phenotype of HUVECs was detected by the SA- β gal assay after another 12 days (culture medium without RV or H₂O₂ was changed every 2 days). Senescent cells were identified by SA- β gal staining. Data shown here are the mean $\pm$ SD of three independent experiments.

ROS production and against the senescence process was further evaluated. The results showed that ROS production and the percentage of cells positive for

SA- β gal were significantly increased in sh-SirT1-treated HUVEC cells compared to the vector control group ($p < 0.001$; **Fig. 5B** and **5C**). Importantly, the results further demonstrated that RV can significantly inhibit ROS production and effectively block the senescence process in H_2O_2 -treated HUVEC cells ($p < 0.001$; **Fig. 5B** and **5C**). In contrast, knockdown of SirT1 by sh-SirT1 significantly reversed the anti-oxidative stress and anti-senescence effects of RV in H_2O_2 -treated HUVEC cells ($p < 0.001$; **Fig. 5B** and **5C**). Taken together, these findings indicate that RV has the capability of inferring anti-oxidative cytoprotection in endothelial cells, in part through the regulation of SirT1 expression.

Discussion

Despite the fact that many cellular events, including aging change, apoptosis, and oxidative stress, were highly correlated with SirT1^{21, 22}, no studies have examined the expression of SirT1 in atherosclerotic or aging endothelium cells. Based on the results of our study, we have shown that the mRNA expression levels of SirT1 were reduced in atherosclerotic tissues of CABG patients or aging vessels. To our knowledge, this is the first study to demonstrate that decreases of SirT1 expressions were significantly and specifically limited to the endothelium layer and endothelial cells of atherosclerotic/aging vessels. Furthermore, our study provided additional evidence that treatment with RV could effectively block the senescence process in H_2O_2 -treated HUVECs. Importantly, the anti-oxidative-stress and anti-senescence effects of RV could be reversed by SirT1 knockdown in H_2O_2 -treated HUVEC cells. These data suggested that SirT1 may be involved in the endothelium of aging and atherosclerotic vessels, and SirT1 may regulate the senescence course in oxidative stress-treated endothelial cells.

Resveratrol (trans-3,5,4'-trihydroxystilbene) is a compound found largely in the skins of red grapes, nuts, pomegranates, and red wine^{16, 23}. It possesses the ability to scavenge oxidatively generated free radicals, and prevents tumor progression by blocking NF- κ B expression, leading to cancer cell apoptosis^{16, 23-27}. RV also has promising neuroprotective and cardioprotective effects, as well as slowing aging and delaying the onset of chronic diseases^{23, 28-30}. Resveratrol supplements significantly reduced the presence of atherosclerotic lesions and lipid levels in apo E-deficient mice^{31, 32} and type 1 diabetic LDL receptor-deficient mice³³. The critical role of SirT1 in vascular endothelial homeostasis and its beneficial effects on metabolic pathways, such as regulating cholesterol homeostasis,

Fig. 5. RV protects against H_2O_2 -induced oxidative stress and senescence in HUVECs via SirT1 activation.

(A) HUVECs were transduced with either control luciferase-targeted shRNA (sh-Luc) or lentiviral vector encoding a shRNA targeting SirT1 (sh-SirT1). Total proteins were analyzed by Western blot against SirT1. (B) Intracellular ROS levels and (C) SA- β gal-positive cells of H_2O_2 -treated HUVECs exposed to RV, RV plus sh-Luc, or RV plus sh-SirT1 were detected. ** $p < 0.001$: H_2O_2 plus RV vs. H_2O_2 alone. # $p < 0.001$: H_2O_2 plus RV vs. H_2O_2 plus RV and sh-SirT1. Data shown here are the mean $\pm$ SD of three independent experiments.

suggests its therapeutic potential for the treatment of cardiovascular diseases^{34, 35}). RV, an activator of the SirT1 gene, possesses potential as a scavenger for oxidative-stress-induced free radicals and toxicity. Wallerath *et al.*³⁶) showed that resveratrol was able to increase the expression level of eNOS. Penumathsa *et al.*³⁷) and Rush *et al.*³⁸) both demonstrated the cardioprotective effect of resveratrol in hypercholesterolemic rats and spontaneously hypertensive rats, respectively. More recently, a well-designed study by Orimo and colleagues showed that overexpression of SirT1 either by virus or by resveratrol inhibited high glucose-induced senescence and dysfunction in HUVECs³⁹). In the present study, the data further showed that RV not only activated SirT1 expression levels in H₂O₂-treated HUVECs, but also aided in the recovery from the oxidative stress-induced senescence process. Moreover, ROS play a critical role in limiting the lifespan of organisms, as well as initiating the process of cellular senescence⁴⁰). Increasing levels of ROS have also been shown to restrict the lifespan of hematopoietic stem cells⁴¹). Recently, Robb *et al.* demonstrated that RV can markedly induce mitochondrial MnSOD expression and activity in MRC-5 cells, as well as in mouse brain tissues⁴²). Moreover, RV has been shown to protect against ROS-induced cell death by activating AMP-activated kinase in cardiac muscle cells⁴³). Our findings demonstrated that, even with RV treatment, knockdown of SirT1 in HUVECs increased ROS production and further promoted the development of oxidative stress-induced senescence in human endothelial cells (**Fig. 4**). Indeed, the senescence of endothelial cells leads to endothelial dysfunction and may result in advanced atherosclerotic lesions in the cardiovascular system.

In conclusion, the present results demonstrated that SirT1, a landmark gene of extended lifespan, plays an important role in the endothelial cells of vital organ vessels during the oxidative stress-induced atherosclerotic process or aging change. Furthermore, our study also indicated that RV is a potential candidate for preventing and treating oxidative stress-induced damage to endothelial cells, and its potential utility in protection against cardiovascular dysfunction should be further studied. These data show that RV may prevent ROS-induced aging and senescence via increased endothelial SirT1 expression. RV appears to be a potential anti-oxidant that may prevent or slow the process of atherosclerosis induced by ROS. The mechanism of RV-mediated SirT1 activation in the endothelium of atherosclerotic vessels needs further study.

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