Regular Article

Curcumin Attenuates Hydrogen Peroxide-Induced Premature Senescence via the Activation of SIRT1 in Human Umbilical Vein Endothelial Cells

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Endothelial senescence has been proposed to be involved in endothelial dysfunction and atherogenesis. Curcumin, a natural phenol, possesses antioxidant and anti-inflammatory properties. However, the effect of curcumin on endothelial senescence is unclear. This study explores the effect of curcumin on hydrogen peroxide (H_2O_2)-induced endothelial premature senescence and the mechanisms involved. Human umbilical vein endothelial cells (HUVECs) were cultured, and premature senescence was induced with $100\mu M H_2O_2$. Results showed that pretreatment with curcumin significantly attenuated the H_2O_2 -induced HUVECs' premature senescence, which was evidenced by a decreased percentage of senescence-associated β -galactosidase positive cells, improved cell division and decreased expression of senescence-associated protein p21 (all $p < 0.05$). Pretreatment with curcumin decreased oxidative stress and apoptosis in H_2O_2 -treated HUVECs. Treatment of HUVECs with H_2O_2 also down-regulated the phosphorylation of endothelial nitric oxide synthase (eNOS), decreased the level of nitric oxide in the culture medium, and inhibited the protein expression and enzymatic activity of silent information regulator 1 (SIRT1), while pretreatment with curcumin partly reversed these effects (all $p < 0.05$). Treatment with curcumin alone enhanced the enzymatic activity of SIRT1, but didn't affect cellular senescence, cell growth or apoptosis compared to the Control. The inhibition of SIRT1 using SIRT1 short interfering RNA (siRNA) could decrease the expression and phosphorylation of eNOS and abrogate the protective effect of curcumin on H_2O_2 -induced premature senescence. These findings suggest that curcumin could attenuate oxidative stress-induced HUVECs' premature senescence via the activation of SIRT1.

Key words curcumin; endothelial senescence; silent information regulator 1 (SIRT1); hydrogen peroxide

Cellular senescence is defined as an irreversible state of growth arrest and accompanied by a specific set of phenotypic changes, gene expression and cell function.¹⁾ It has been realized that senescence is induced by a variety of insults, including those causing intracellular oxidative stress, such as hydrogen peroxide (H_2O_2).²⁾ This kind of cellular senescence is called the stress-induced premature senescence (SIPS). Endothelial senescence has been proposed to be involved in endothelial dysfunction and atherogenesis.³⁾ Human studies showed that there were vascular cells exhibiting the morphological features of cellular senescence.^{4–6)}

Silent information regulator 1 (SIRT1) is a member of the sirtuin family of proteins, which are homologs of the Sir2 gene in *Saccharomyces (S.) cerevisiae*. SIRT1 has been known as an oxidized form of nicotinamide adenine dinucleotide (NAD^+)-dependent class III histone deacetylase (HDAC) and plays a key role in regulating cellular senescence, cell survival and metabolism through deacetylation of various histones and non-histone substrates.^{7,8)} SIRT1 is highly expressed in the vasculature. A growing number of studies have demonstrated that SIRT1 is involved in the process of cellular senescence, vascular aging and age-related vascular diseases, such as atherosclerosis.^{9,10)} Overexpression of SIRT1 protects human endothelium from cellular senescence, promotes cellular survival and facilitates DNA repair.^{9,11)}

Curcumin, a kind of polyphenol extracted from the yellow pigments spice plant turmeric, is commonly used as coloring agent and food additive.¹²⁾ It has been proposed that curcumin possesses multiple biological properties including anti-oxidant, anti-inflammatory and anti-microbial activities.^{13,14)} Recently, several studies found that pretreatment with curcumin sig-

nificantly improves SIRT1 activation and attenuates oxidative stress.^{15,16)} Besides, many polyphenols have been shown to activate SIRT1 directly or indirectly, decrease SIPS and therefore have therapeutic potential for age-related vascular diseases.^{17–20)} However, whether or not pretreatment with curcumin affects the activation of SIRT1 in H_2O_2 -treated endothelial cells and attenuate cellular senescence is unclear. Therefore, this study was to examine the protective potentials of curcumin on the H_2O_2 -induced premature senescence in human umbilical vein endothelial cells (HUVECs) and the possible mechanisms involved.

MATERIALS AND METHODS

Materials Curcumin, anti-human β -actin antibody, dimethyl sulfoxide (DMSO), Hank's balanced salt solution (HBSS), fetal bovine serum (FBS), endothelial cell medium (ECM), endothelial cell growth supplement, H_2O_2 solution, senescence cells histochemical staining kit and propidium iodide were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Cell Counting Kit-8 (CCK8) was purchased from Dojindo (Shanghai, China). Antibody against p21 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Antibodies against p-endothelial nitric oxide synthase (eNOS) (Ser1177) and total eNOS were purchased from Cell Signaling Technology (Danvers, MA, U.S.A.). Horseradish peroxidase (HRP) conjugated secondary antibodies against rabbit or mouse immunoglobulin G (IgG), enhanced chemiluminescence (ECL) solution were purchased from Bio-Rad (Hercules, CA, U.S.A.). Bicinchoninic acid (BCA) protein assay kit was purchased from Beyotime Institute of Biotechnology (Nanjing,

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HUVECs Culture and Treatment with Curcumin

HUVECs were purchased from ScienCell (Carlsbad, CA, U.S.A.) and cultured in complete ECM supplemented with 5% FBS, 1% endothelial cell growth supplement, 100 U/mL penicillin, and 100 μ g/mL streptomycin in a humidified atmosphere with 5% CO₂ at 37°C. All experiments were performed using 4–6 passages of HUVECs. Curcumin was stored at –20°C and dissolved in DMSO prior to treatment of cells. Concentrations of curcumin were indicated in the following experiments. The concentration of DMSO did not exceed 0.1%, at which it did not affect the experimental outcomes (data not shown).

Induction of Cellular Senescence Cellular senescence was induced with treatment with H₂O₂ as described previously.²¹⁾ HUVECs were planted at a density of 1×10^5 per well in 6-well plates and cultured overnight. Cells were starved in ECM containing 2% FBS for 12 h. Then the cells were pretreated with vehicle and curcumin (10 and 25 μ M) diluted in complete ECM for 24 h. After that, the cells were washed 3 times with ECM and treated with 100 μ M H₂O₂ diluted in ECM for 1 h. Then the cells were trypsinized, reseeded and cultured with complete ECM containing vehicle or curcumin for 10 d.

Inhibition of SIRT1 To explore the involvement of SIRT1 in the effects of curcumin pretreatment, HUVECs were transfected with SIRT1 short interfering RNA (siRNA) or control siRNA as described by Wu *et al.*²²⁾ SIRT1 and control siRNA were purchased from Life Technologies (Austin, TX, U.S.A.). HUVECs were incubated with mixture from HiPerFect Transfection kit (Qiagen, Hilden, Germany) containing 10 nM siRNA and 3.0 mL of HiPerFect reagent in 0.5 mL of culture medium. After incubation for 48 h, HUVECs were treated with 10 μ M curcumin or DMSO for 24 h and then treated with 100 μ M H₂O₂.

Cell Proliferation Assay The cell proliferation assay was performed using CCK-8. Briefly, HUVECs were seeded in 96-well plates at a density of 10^4 cells per well in 200 μ L culture medium. After starvation overnight, cells were treated with complete ECM with various final concentrations of curcumin (0, 5, 10, 25, 50 and 100 μ M) and incubated for another 48 h. At the end of the incubation, 20 μ L of the kit was added and the cells were incubated at 37°C for 1 h. Then the cells were measured for absorbance at 450 nm. Cell viability was detected by trypan blue staining. The results were presented as a percentage of control.

Senescence Associated β -Galactosidase Assay (SA- β G) SA- β G assay was used to determine the number of senescent cells according to the manufacturer's instruction. Briefly, cells were washed twice in phosphate buffered saline (PBS), fixed in 2% formaldehyde and 0.2% glutaraldehyde for 6 min at room temperature, then rinsed three times, and stained with fresh SA- β G staining solution for 18 h in darkness at 37°C without CO₂. Images of samples were taken under an inverted microscope by the observer unknown of the assignment of groups. Senescent cells were identified as blue-stained cells, and a total of 400 contiguous cells were counted to determine the percentage of SA- β G positive cells.

Cell Cycle Analysis Cell cycle was analyzed by flow cytometry as described previously.²³⁾ Briefly, After 48 h following treatment with 100 μ M H₂O₂ with or without pretreat-

ment with curcumin, the cells were harvested and washed in PBS and fixed in 70% ethanol at 4°C overnight. After washing twice in PBS, the cells were incubated with 10 mg/mL RNase A at 4°C for 1 h and then stained in 10 μ g/mL propidium iodide solution at 4°C in darkness for 1 h. Then the cells were subjected to flow cytometric analysis with FACS Calibur and CellQuest software (BD Biosciences, NJ, U.S.A.). Cell cycles were analyzed and the proportion of cells in the G₀/G₁, S and G₂/M phases was recorded.

Cellular Apoptosis Assay Initially, 10^5 cells were seeded on a 6-well plate and treated with 100 μ M H₂O₂ with or without pretreatment with curcumin. After 24 h, the cells were harvested and washed in cold PBS. Annexin V and propidium iodide (PI) staining were carried out using the Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection Kit (BD Biosciences) according to the manufacturer's instruction. After incubation in dark at room temperature for 20 min, the cells were immediately analyzed by FACS Calibur Flow Cytometry.

Reactive Oxygen Species (ROS) Production ROS generation by HUVECs was measured by 2',7'-dichlorofluorescein diacetate (DCF-DA) according to the manufacturer's protocol. Briefly, HUVECs were seeded in 6-well plates and serum-starved overnight. After washed with PBS, HUVECs were exposed to 100 μ M H₂O₂ for 1 h with or without pretreatment of 25 μ M curcumin for 30 min. Cells were then incubated with 10 mM DCF-DA for 30 min at room temperature. DCF fluorescence at an excitatory wavelength of 495 nm was recorded on a fluorescence microscopy. An aliquot of the cell suspension was lysed, and the protein concentration was determined. The generation of ROS was expressed as arbitrary absorbance units per mg protein.

Detection of Enzymatic Activity of SIRT1 The enzymatic activity of SIRT1 was detected with SIRT1 Assay Kit from Sigma-Aldrich according to the instruction as described previously.^{24,25)} The whole cell extracts were incubated with 10 μ L SIRT1 Substrate Solution in 96-well plates at 37°C for 30 min, and then mixed with Developing Solution at 37°C for 10 min. Meanwhile, the standard curve was determined. The fluorescence intensity at 450 nm was recorded and normalized to micrograms of protein. Experimental values are represented as a percentage of the control.

Western Blot Analysis After 2 and 48 h following treatment with 100 μ M H₂O₂ with or without pretreatment with 10 μ M curcumin, the cells were harvested. Protein extracts were obtained with lysis buffer according to the manufacturer's protocol (Biovision, Milpitas, CA, U.S.A.). Protein concentration was determined by the bicinchoninic acid protein assay. Equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. For immunoblotting, PVDF membranes were blocked and probed with antibodies against p-eNOS (1:1000), eNOS (1:1000), p21 (1:500), SIRT1 (1:500) and β -actin (1:2000) overnight at 4°C. After three washes, the blots were incubated with respective HRP-conjugated secondary antibodies (1:2000) for 1 h at room temperature, and then washed with TBST buffer and subsequently analyzed by an enhanced chemiluminescence dependent detection system.

Detection of the Level of Nitric Oxide (NO) in Culture Medium After 24 h following treatment with 100 μ M H₂O₂

Fig. 1. Effects of Curcumin on HUVECs Proliferation and H₂O₂-Induced HUVECs Senescence

A) Treatment with curcumin at 5, 10 and 25 μM for 48h didn't affect HUVECs proliferation. However, treatment with 50 and 100 μM curcumin statistically significantly decreased cell proliferation. B) Represented pictures of SA-βG positive cells under microscope. C) One hundred micromolar H₂O₂ effectively increased the ratio of SA-βG positive cells, while pretreatment with curcumin at 10 and 25 μM could significantly attenuate the H₂O₂-induced HUVECs senescence. *p<0.05 vs. Control group; #*p*<0.05 vs. H₂O₂ group.

with or without pretreatment with curcumin, the culture medium was collected. The level of NO was assessed indirectly by measuring the levels of oxidized forms (nitrites and nitrates) in culture medium using the Nitric Oxide Colorimetric Assay Kit (Abcam, Cambridge, MA, U.S.A.) according to the manufacturer's instruction.^{26,27} A standard curve was generated to measure levels between 1 and 100 μM of nitrite per well. The absorbance value was obtained with microplate reader at 540 nm.

Statistical Analysis Data were presented as mean± standard deviation (S.D.). Data were analyzed using one-way ANOVA followed by Newman-Keuls test. Analysis was performed with GraphPad Prism (GraphPad Software, San Diego, CA, U.S.A.). A value of *p*<0.05 was considered statistically significant.

RESULTS

Effects of Curcumin on HUVECs Proliferation and H_2O_2 -Induced HUVECs Senescence As shown in Fig. 1A, treatment with curcumin at 5, 10 and 25 μM for 48h didn't affect HUVECs proliferation. However, treatment with 50 and 100 μM curcumin statistically significantly decreased cell

proliferation ($p < 0.05$). As expected, 100 μM H_2O_2 effectively increased the ratio of SA- β G positive cells ($82.90 \pm 2.02\%$ vs. $4.02 \pm 0.66\%$, $p < 0.01$), while pretreatment with curcumin at 10 and 25 μM could significantly attenuate the H_2O_2 -induced HUVECs senescence ($54.40 \pm 3.40\%$ vs. $82.90 \pm 2.02\%$; $51.25 \pm 2.74\%$ vs. $82.90 \pm 2.02\%$, $p < 0.01$) (Figs. 1B, C).

Curcumin Attenuated H_2O_2 -Induced Oxidative Stress and Apoptosis As expected, H_2O_2 significantly increased intracellular ROS production while 25 μM curcumin alone didn't affect HUVECs ROS production. However, pretreatment of curcumin could partly restore the H_2O_2 -induced oxidative stress (Fig. 2A). Similarly, pretreatment of 25 μM curcumin could attenuate H_2O_2 -induced apoptosis ($9.67 \pm 1.26\%$ vs. $15.00 \pm 1.77\%$, $p < 0.05$, Figs. 2B, C).

Curcumin Increased the Protein Expression and Enzymatic Activity of SIRT1 in H_2O_2 -Treated HUVECs SIRT1 is reported to be involved in oxidative stress-induced premature senescence.^{19,23} As shown in Fig. 3A, 100 μM H_2O_2

Fig. 2. Pretreatment with Curcumin Attenuated the H_2O_2 -Induced Oxidative Stress and Apoptosis

A) H_2O_2 significantly increased intracellular ROS production while 25 μM curcumin alone didn't affect HUVECs ROS production. However, pretreatment of curcumin could partly restore the H_2O_2 -induced oxidative stress. B) Represented scatter plots of flow cytometry. C) Quantitative analysis showed that pretreatment of 25 μM curcumin could attenuate H_2O_2 -induced apoptosis. * $p < 0.05$ vs. Control group; # $p < 0.05$ vs. H_2O_2 group.

Fig. 3. Curcumin Increased the Protein Expression and Enzymatic Activity of SIRT1 in H_2O_2 -Treated HUVECs

A) 100 μM H_2O_2 remarkably decreased the expression of SIRT1 and pretreatment with curcumin could increase the expression of SIRT1 in H_2O_2 -treated HUVECs. B) SIRT1 activity assay showed that treatment of H_2O_2 dramatically reduced the activity of SIRT1 and treatment of curcumin alone greatly improved the enzymatic activity of SIRT1. Meanwhile, pretreatment of curcumin could also reverse the change of enzymatic activity of SIRT1 in H_2O_2 -treated HUVECs. * $p < 0.05$ vs. Control group; # $p < 0.05$ vs. H_2O_2 group; ** $p < 0.05$ vs. Control group.

remarkably decreased the expression of SIRT1 and pretreatment with curcumin could increase the expression of SIRT1 in H_2O_2 -treated HUVECs ($p<0.05$). Meanwhile, SIRT1 activity assay showed that treatment of H_2O_2 dramatically reduced the activity of SIRT1. Interestingly, treatment of curcumin alone greatly improved the enzymatic activity of SIRT1 and pretreatment of curcumin could also reverse the change of enzymatic activity of SIRT1 in H_2O_2 -treated HUVECs (both $p<0.05$, Fig. 3B).

Pretreatment with Curcumin Protected against H_2O_2 -Induced Cell Growth Arrest and Decreased the Expression of p21 To determine whether curcumin has a protective effect against H_2O_2 -induced cell growth arrest, the cell cycle was analyzed with flow cytometry. As shown in Table 1, pretreatment of 10 and 25 μM curcumin remarkably decreased the ratio of cells at G_0/G_1 stage ($60.02\pm5.22\%$ vs. $78.12\pm2.91\%$

and $56.57\pm4.15\%$ vs. $78.12\pm2.91\%$, $p<0.05$). Accordingly, the ratio of cells in S and G_2/M phases were increased in curcumin pretreated group compared to the H_2O_2 group ($p<0.05$). Meanwhile, pretreatment of 25 μM curcumin significantly decreased the expression of p21 in H_2O_2 -treated HUVECs (Figs. 4A, B).

Pretreatment with Curcumin Improved the Phosphorylation of eNOS and the Level of NO in Culture Media To explore the effect of curcumin on endothelial function in H_2O_2 -induced senescent HUVECs, the phosphorylation of eNOS at Ser1177 was investigated with Western blot analysis and the level of NO in culture medium was detected. Results revealed that pretreatment with curcumin could enhance the phosphorylation of eNOS at Ser1177 but didn't change the total expression of eNOS (Fig. 4C) and increase the level of NO in culture medium of H_2O_2 -treated HUVECs (Fig. 4D).

Fig. 4. Pretreatment with Curcumin Decreased the Expression of p21, Increased the Phosphorylation of eNOS and Level of NO in Culture Medium of H_2O_2 Treated HUVECs

A) Represented blots of p21, p-eNOS (Ser1177), eNOS and β -actin. B) Pretreatment of 25 μM curcumin significantly decreased the expression of p21 in H_2O_2 -treated HUVECs. C) Pretreatment with curcumin could enhance the phosphorylation of eNOS at Ser1177 but didn't change the total expression of eNOS. D) Pretreatment with curcumin could increase the level of NO in culture medium of H_2O_2 -treated HUVECs. * $p<0.05$ vs. Control group; # $p<0.05$ vs. H_2O_2 group.

Table 1. Effect of Curcumin on Cell Cycle Distribution of H_2O_2 -Treated HUVECs

Treatment	G_0/G_1 phase (%)	S phase (%)	G_2/M phase (%)
Control	55.67 ± 3.61	33.30 ± 5.07	11.03 ± 2.17
Curcumin 25 μM	57.14 ± 2.59	30.73 ± 4.81	12.13 ± 1.54
H_2O_2	$78.12\pm2.91^*$	$13.37\pm2.91^*$	$8.52\pm1.00^*$
Curcumin 10 μM + H_2O_2	$60.02\pm5.22^\#$	$23.35\pm4.28^\#$	$16.63\pm2.27^\#$
Curcumin 25 μM + H_2O_2	$56.57\pm4.15^\#$	$27.63\pm3.88^\#$	$15.80\pm1.69^\#$

* $p<0.05$ vs. Control group; # $p<0.05$ vs. H_2O_2 group.

Fig. 5. Inhibition of SIRT1 Abolished the Protective Effect of Curcumin Treatment on H₂O₂-Induced HUVECs Senescence

A) SIRT1 siRNA could effectively inhibit the expression of SIRT1. B) Curcumin failed to protect SIRT1 siRNA-treated HUVECs from H₂O₂-induced cellular senescence. C) Inhibition of SIRT1 could result in downregulation of eNOS and lower phosphorylation activity of eNOS, and treatment of curcumin failed to increase the phosphorylation of eNOS in H₂O₂-treated HUVECs in SIRT1 siRNA group. * $p < 0.05$ vs. Control group treated with control siRNA; ** $p < 0.05$ vs. H₂O₂ group treated with control siRNA; # $p < 0.05$ vs. Control group treated with SIRT1 siRNA; ## $p > 0.05$ vs. H₂O₂ group treated with SIRT1 siRNA.

Inhibition of SIRT1 Abolished the Protective Effect of Curcumin Treatment on the H₂O₂-Induced HUVECs Senescence As shown in Fig. 5A, treatment with SIRT1 siRNA for 48h could effectively reduce the expression of SIRT1. Meanwhile, inhibition of SIRT1 improved the ratio of SA-βG positive cells in H₂O₂-treated HUVECs and abolished the protective effect of curcumin treatment on the H₂O₂-induced HUVECs senescence ($84.82 \pm 3.00\%$ vs. $87.12 \pm 2.40\%$, $p > 0.05$, Fig. 5B). In addition, inhibition of SIRT1 could result in downregulation of eNOS and lower phosphorylation activity of eNOS, and treatment of curcumin failed to increase the phosphorylation of eNOS in H₂O₂-treated HUVECs in SIRT1 siRNA group (Fig. 5C).

DISCUSSION

Cellular senescence was first described by Hayflick and Moorhead in 1961 when they found that normal human embryonic fibroblasts can only divide for a finite number of times and cells would enter a permanent growth arrest state after serial passages in culture.^{28,29} This phenomenon was named replicative senescence, which is related to the telomere shortening.³⁰ It was found later that many kinds of stressful stimuli, such as oxidative stress, DNA damage, overexpression of certain oncogenes, could induce senescence-like state, which is called SIPS.^{31,32} Subsequent studies showed that cellular senescence was involved in aging and aging-related pathophysiology *in vivo*.^{33,34} More recently, it has been demonstrated that endothelial senescence is involved in aging related vascular disease, such as atherosclerosis.^{3,5,35,36}

Curcumin is the main constituent of curcuminoid and is the

major yellow pigment of turmeric derived from the rhizome of the herb *Curcuma longa* LINN. Curcumin has been shown to possess anti-oxidative and anti-inflammatory activities.^{13,14} Lee *et al.*³⁷ reported that treatment with curcumin could significantly extend the lifespan of two strains of *Drosophila melanogaster*. Another study demonstrated that receiving diets containing tetrahydrocurcumin, a major metabolite of curcumin, could significantly increase the average lifespan of male C57BL/6 mice.³⁸ These studies implied the potential anti-aging effect of curcumin. Indeed, a recent study showed that bisdemethoxycurcumin, another curcuminoid, could antagonize the oxidative stress-induced premature senescence in WI38 fibroblast cells through activation of SIRT1/AMP-activated protein kinase (AMPK) signaling pathway.³⁹ An animal study demonstrated that supplement with curcumin significantly ameliorated arterial dysfunction and oxidative stress with aging.⁴⁰ Our data showed that treatment with H₂O₂ significantly increased the intracellular ROS production and successfully induced cellular senescence, which was evident from the elevated ratio of SA-βG positive cells, cell cycle arrest and increased expression of p21, a key regulator in the process of cellular senescence.³⁰ Meanwhile, pretreatment with curcumin significantly decreased the ROS production and attenuate H₂O₂-induced premature senescence, apoptosis and cell growth arrest.

A growing body of evidence has shown that SIRT1 is an important modulator of cellular senescence, apoptosis, metabolism and longevity.^{9,10,41,42} Ota *et al.*⁹ showed that SIRT1 inhibition by sirtinol or SIRT1 siRNA induced premature senescence-like phenotype, increased plasminogen activator inhibitor 1 expression and decreased both protein expression

and activity of eNOS. Chen *et al.*⁴²⁾ recently demonstrated that knockdown of SIRT1 in young mesenchymal stem cells induced cellular senescence and inhibited cell proliferation, and overexpression of SIRT1 in aged mesenchymal stem cells reversed the senescence phenotype and stimulated cell proliferation. Furthermore, endothelium-specific SIRT1 overexpression inhibited hyperglycemia-induced upregulation of vascular cell senescence in diabetic mice.¹¹⁾ These studies imply that SIRT1 is a pivotal regulator of the development of cellular senescence. In our study, we found that treatment of curcumin alone could increase the enzymatic activity of SIRT1 without affecting the protein expression of it. Meanwhile, pretreatment of curcumin improved the protein expression and enzymatic activity of SIRT1 in H₂O₂-treated HUVECs. Indeed, several chemicals are reported to have anti-senescent effect *in vitro* by modulating SIRT1 pathway. These chemicals include a number of agents including resveratrol,¹⁹⁾ cilostazol,⁴¹⁾ paeonol,⁴³⁾ statins,²¹⁾ hydrogen sulfide^{23,44)} and persimmon.⁴⁵⁾ Yang *et al.*¹⁵⁾ demonstrated that pretreatment with curcumin attenuated mitochondrial oxidative damage induced by myocardial ischemia reperfusion injury through activation of SIRT1. In addition, a recent study showed that curcumin blocked the neurotoxicity of amyloid-beta_{25–35} in rat cortical neurons and activation of SIRT1.¹⁶⁾ In the present study, we found that silencing of SIRT1 decreased the expression and phosphorylation of eNOS, and abolished the protective effect of curcumin in H₂O₂-treated HUVECs. Curcumin failed to protect the SIRT1 siRNA-treated HUVECs from H₂O₂-induced senescence.

CONCLUSION

In summary, this study showed that curcumin attenuated the H₂O₂-induced premature senescence on HUVECs *via* activation of SIRT1. The results of the present study imply that curcumin could prevent against various aging-associated diseases, particularly, atherosclerosis. Further study is needed to validate the effects of curcumin on cellular senescence and aging *in vivo*.

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Conflict of Interest The authors declare no conflict of interest.

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