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Lycopene alleviates oxidative stress-induced cell injury in human vascular endothelial cells by encouraging the SIRT1/Nrf2/HO-1 pathway

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ABSTRACT

Background and objective: Epidemiological research have displayed that dietary intake rich in lycopene, an antioxidant, is negatively correlated with the risk of cardiovascular disease (CVD). This study aimed to investigate whether the intervention with different concentrations of lycopene could attenuate H_2O_2-induced oxidative stress injury in human vascular endothelial cells (VECs).

Methods: The human VECs HMEC-1 and ECV-304 were incubated with a final concentration of 300 µmol/L H_2O_2, followed by they were incubated with lycopene at doses of 0.5, 1, or 2 µm. Subsequently, cell proliferation, cytotoxicity, cell adhesion, reactive oxygen species (ROS) contents, adhesion molecule expression, oxidative stress levels, pro-inflammatory factor production, the apoptosis protein levels, and the silent information regulator-1 (SIRT1)/nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway protein levels were tested by CCK-8 kit, lactate dehydrogenase (LDH) kit, immunofluorescence labeling, cell surface enzyme immunoassays (EIA), enzyme-linked immunosorbent assay (ELISA), as well as Western blot assays, respectively.

Results: Under H_2O_2 stimulation, HMEC-1 and ECV-304 cell proliferation and the SIRT1/Nrf2/HO-1 pathway protein expression were significantly reduced, whereas cytotoxicity, apoptosis, cell adhesion molecule expression, pro-inflammatory and oxidative stress factors production were apparently encouraged, which were partially countered by lycopene intervention in a dose-dependent manner.

Conclusion: Lycopene alleviates H_2O_2-induced oxidative damage in human VECs by reducing intracellular ROS levels, inflammatory factor production, cell adhesiveness, and apoptosis rate under oxidative stress conditions through activation of the SIRT1/Nrf2/HO-1 pathway.

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Cardiovascular disease; lycopene; oxidative stress; the SIRT1/Nrf2/HO-1 pathway; apoptosis

Introduction

Cardiovascular disease (CVD) is a class of common diseases that seriously threaten human health, including coronary artery disease, hypertensive heart disease, and stroke, among others (1). It is expected that the number of patients with severe cardiovascular diseases such as myocardial infarction (MI) and stroke will increases straightforwardly in the next 20 years as the world's population ages, urbanizes, and the prevalence of cardiovascular risk factors rises (2). Oxidative stress refers to one type of cytopathological change that occurs when cells are exposed to high concentrations of oxygen or chemical derivatives of oxygen (3). Oxidative stress is widespread in all blood vessels, and its overproduction by reactive oxygen species (ROS) is a high-risk factor for the progression of several CVDs, such as MI, atherosclerosis (AS), and diabetes (4–6). When the organism occurs injury or the oxidation/antioxidant level is imbalanced, it will cause intracellular ROS excess and then lead to the occurrence of oxidative stress (7,8). Oxidative stress has been proven to play a significant role in the occurrence of CVD (9). Therefore, the search for drugs that inhibit oxidative stress is crucial for the treatment of CVD.

The vascular endothelium is the innermost structure covering the walls of arteries, capillaries, and veins, and is able to actively control the degree of vasodilation/constriction, the extravasation of solutes, fluids, hormones, macromolecules, platelets, and blood cells (10). In addition to serving as a protective barrier between blood and vascular walls, they are also important cells in maintaining the integrity of the vascular system and the homeostasis of the internal environment (11). The vascular endothelial cells (VECs), a single-cell layer directly covering the intimal surface of blood vessels, produce antiproliferative and anti-inflammatory cytokines and modulate the coagulation cascade via a balanced production of anticoagulant and procoagulant factors (12). The function of the VECs is impaired in patients with cardiovascular risk factors or CVD (13). Therefore, targeting the protection of VECs is a fundamental approach in cardiovascular diseases, and this is associated with increased oxidative stress and impaired NO balance, which contribute to the progression of the disease. It has been found that the dysfunction of VECs is associated with increased oxidative stress and impaired NO balance, resulting in increased endothelial permeability, platelet aggregation, leukocyte adhesion, which contribute to the

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progression of CVD, such as AS, hypertension, heart failure, and cerebral thrombosis (14–17). VEC apoptosis caused by oxidative stress is an initiating link in the occurrence of cardiovascular diseases (18). Therefore, VECs have an important role in studying cardiovascular diseases. Endothelial cell injury model is an important tool for elucidating the mechanism of endothelial injury and screening endothelial protective drugs.

Lycopene is a widely used antioxidant that can repair oxidative damage to both animal organisms and cells (19). Lycopene has the greatest antioxidant potential among carotenoids, and it quenches singlet oxygen with a rate constant of ten times that of tocopherol, 47 times that of β-carotene, and 100 times that of vitamin E (20). Previous studies have discovered that lycopene exerts antioxidant effects by directly scavenging free radicals in cells or indirectly increasing endogenous cellular antioxidant capacity through the activation of related signaling pathways (21). Lycopene has been reported to have preventive or therapeutic effects for many types of diseases, such as cardio-cerebrovascular, epilepsy, and cancer, among others. For instance, Lycopene via upregulation of the Wnt/β-Catenin pathway promotes neurogenesis in the hippocampus and subventricular and alleviates cognitive dysfunction in amyloid-induced Alzheimer's Rats (22). Lycopene ameliorates neurochemical deficits, oxidative stress, apoptosis, and physiological abnormalities in Parkinson's mice, providing a promising strategy for the treatment of Parkinson's disease (23). Further, lycopene supplementation attenuated the cardiac remodeling process and improved cardiac diastolic function after myocardial infarction (24). HE *et al.* found that lycopene may reduce cardiomyocyte apoptosis after myocardial infarction by suppressing the NF-κB pathway (25). Lycopene attenuates H_2O_2-activated oxidative injury in endothelial cells and contributes to the prevention of atherosclerotic cardiovascular disease via inhibiting apoptosis by decreasing p53 and caspase-3 mRNA expression in injured cells (26). However, at present, the specific mechanism of lycopene involved in cardiovascular diseases remains to be further explored.

This study aimed to analyze the effect and underlying mechanism of lycopene on oxidative damage in human VECs under H_2O_2 stimulation and to provide a theoretical basis for the antioxidant effect of lycopene in the prevention and treatment of CVD.

Materials and methods

Cell culture and treatment

Human monocyte cell line U937, human microvascular endothelial cell line HMEC-1, and human umbilical vein endothelial cell line ECV-304 (ATCC, USA) were incubated at 37°C in a 5% CO_2-saturated humidified cell culture incubator with 10% FBS (Sigma-Aldrich, USA). Each type of cell was randomly divided into five groups, including control, H_2O_2 (Sigma-Aldrich, USA), H_2O_2+0.5 μM lycopene, H_2O_2+1 μM lycopene, and H_2O_2+2 μM lycopene. The concentrations of lycopene were chosen referring to previous studies (27–29). For the control group, no medication was added. For the H_2O_2 group, a final concentration of 300 μmol/L H_2O_2 was added to the cells for incubation for 24 h. For the H_2O_2+0.5 μM lycopene group, 0.5 μM lycopene was incubated under H_2O_2 stimulation for 24 h. For the H_2O_2+1 μM lycopene group, 1 μM lycopene was incubated under H_2O_2 stimulation for 24 h. For the H_2O_2+2 μM lycopene group, 2 μM lycopene was incubated under H_2O_2 stimulation for 24 h.

Cell proliferation assay

The treated cells from each group were seeded in 96 well culture plates at a cell number per well of 3×10^3, and 5 replicate wells were set in each group. Cell culture supernatants were removed at 0 h, 24 h, 48 h, and 72 h after seeding, and 10 μl of CCK-8 solution was added to each well according to the CCK-8 cell proliferation activity assay kit instructions (Sigma-Aldrich, USA). After incubation at 37°C in 5% CO2 for 1 h, the OD value at 450 nm was measured by using a microplate reader (bio rad, USA), and the cell growth curve was plotted.

Enzyme-linked Immunosorbent Assay (ELISA)

The levels of monocyte chemoattractant protein-1 (MCP-1), macrophage colony-stimulating factor (M-CSF), C-X-C motif chemokine ligand 10 (CXCL-10), tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), malondialdehyde (MDA), and glutathione (GSH) were measured by ELISA kits (Shanghai lianshuo biotech Co., Ltd, Shanghai, China).

Cell Surface Enzyme Immunoassays (EIA)

The cells after five different treatments were collected separately. Subsequently, intracellular vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and E-selectin levels were assessed using a commercial EIA kit following the instructions provided by the supplier (Shanghai lianshuo Biotech Co., Ltd, Shanghai, China).

Lactate Dehydrogenase (LDH) Assay

Five replicate wells were set for each group of cells with different treatments and then placed in a 37°C incubator for culture. Subsequently, 96 well plates were removed after incubation for 48 h, and the precipitates were dislodged by centrifugation at 1000 r/min for 5 min after pipetting the medium from each well. The content of LDH in the culture fluid of each group was tested by using the LDH detection kit following the instructions (Sigma-Aldrich, USA).

Intracellular Reactive Oxygen Species (ROS) Level

Five groups of differently treated cells were collected. The cells were rinsed three times by using D-Hank's solution at room temperature and then incubated in the dark for 20 min at 37°C after the addition of 10 μM dihydroethidium (DHE) fluorescent probe (Sigma-Aldrich, USA). The cells were then rinsed 3 times with D-Hank's medium and incubated with DAPI stain

for 30 min. Finally, the fluorescence intensity was determined by using a fluorescence spectrophotometer.

Cell adhesion assay

A total of 5.0×10^5 calcein-labeled (Sigma-Aldrich, USA) human monocytes U937 were added to 6-well plates culturing 5 different groups of treated human umbilical vein endothelial cells at 1 ml per well. Nonadherent monocytes were carefully washed out with Hanks' balanced solution. Cells were fixed with 200 μm paraformaldehyde in each well, and then adherent monocytes were counted by using an inverted high-power microscope.

Western blotting

Total cell protein was extracted with RIPA lysate, and the protein concentration was applied by using a BCA protein assay kit (Thermo Fisher, USA) in a microplate reader. After denaturation for 10 min with the addition of a loading buffer, 50 μg protein samples were subjected to SDS-PAGE and transferred onto PVDF membranes. The membrane was blocked with a blocking solution (5% nonfat dry milk) for 2 h and subsequently washed three times using TBST. Specific primary and secondary antibodies were next added separately, followed by incubation on a shaker. The information of antibodies was listed in Supplementary Table S2. Image J software was applied to detect and analyze the gray values of protein bands on the membrane.

RT-Qpcr

Five groups of differently treated cells were collected, and then Trizol (ThermoFisher, USA) was added to extract total cellular RNA, and total cellular RNA was reverse transcribed into cDNA by using a reverse transcription kit. RT-qPCR was performed by using cDNA as template, and then PCR products were detected with a real-time PCR system (ThermoFisher, USA) with three replicate wells set for each group. The relative expression levels were calculated by using the $2^{-\Delta\Delta CT}$ method. The primers were listed in Supplementary Table S1. We used GAPDH as endogenous control.

Luciferase reporter assays

p3×ARE/Luc was transfected into ECV-304 cells by using Lipofectamine 3000 (Sigma, USA) according to the manufacturer's instructions. Subsequently, luciferase activity was determined by using luciferase substrate solution (Promega, Beijing, China) after the lysis of the cells.

Statistical analysis

SPSS 22.0 and GraphPad prism 7.0 were used for data analysis and mapping. Student's t-test was used for the comparison of two sample means, and the analysis of variance was used for the comparison of means of multiple groups, with $P < .05$ considered significant.

Results

Lycopene attenuates endothelial cell injury induced by H_2O_2

To evaluate the effect of lycopene on H_2O_2-activated human VEC injury, we incubated the human microvascular endothelial cell HMEC-1 and the human umbilical vein endothelial cell ECV-304 with the final concentration of 300 μmol/L H_2O_2. Next, they were incubated with lycopene at doses of 0.5, 1, or 2 μm based on the pretreatment of the H_2O_2 group. CCK-8 results displayed a significant decrease in HMEC-1 and ECV-304 cell proliferation after H_2O_2 treatment, whereas the cell proliferation rate was restored in a dose-dependent manner after lycopene intervention (Figure 1a,b). Next, we evaluated cytotoxicity using LDH assay. The cytotoxicity of HMEC-1 and ECV-304 was obviously increased after H_2O_2 treatment, while lycopene incubation was able to reduce the cytotoxicity caused by H_2O_2 in a dose-dependent manner (Figure 1c,d). Additionally, whether lycopene has an effect on H_2O_2-induced human vascular endothelial cell apoptosis was measured by detecting the expression of apoptosis marker proteins Bcl-2, Bax, and cleaved caspase 3. The results revealed that H_2O_2 treatment could cause the downregulation of Bcl-2 level and the increase in Bax and cleaved caspase 3 protein levels in HMEC-1 and ECV-304 cells, which were countered by lycopene treatment (Figure 1e). The above results suggested that lycopene partially counteracted the promotion of HMEC-1 and ECV-304 cytotoxicity and apoptosis and the inhibition of cell proliferation caused by H_2O_2 in a dose-dependent manner.

Lycopene reduces H_2O_2-activated monocyte endothelial adhesion and the expression of adhesion molecules in endothelial cells

Adhesion of blood-borne monocytes to the vascular endothelium and entry into the intima has been demonstrated to be one of the key events in CVD, such as AS. Subsequently, fluorescently labeled U937 monocytes were applied to perform monocyte endothelial adhesion experiments. The results displayed that H_2O_2 intervention significantly increased the adhesion of monocytes to HMEC-1 and ECV-304 cells, whereas this adhesion rate was substantially decreased after lycopene treatment, as indicated by attenuated green fluorescence (Figure 2a). We next evaluated adhesion molecule expression in HMEC-1 and ECV-304 cells to further check the effect of lycopene on endothelial cell adhesion. Western blotting and EIA findings showed that H_2O_2 apparently up-regulated the protein levels and contents of adhesion molecules E-selectin (Figure 2b,c), VCAM-1 (Figure 2b-d), and ICAM-1 (Figure 2b-e) in HMEC-1 and ECV-304 cells, which were down-regulated after lycopene intervention in a dose-dependent manner.

Lycopene can lighten H_2O_2-induced inflammatory responses in endothelial cells

Next, the production of chemokines and proinflammatory factors within the HMEC-1 or ECV-304 cells of each group was further

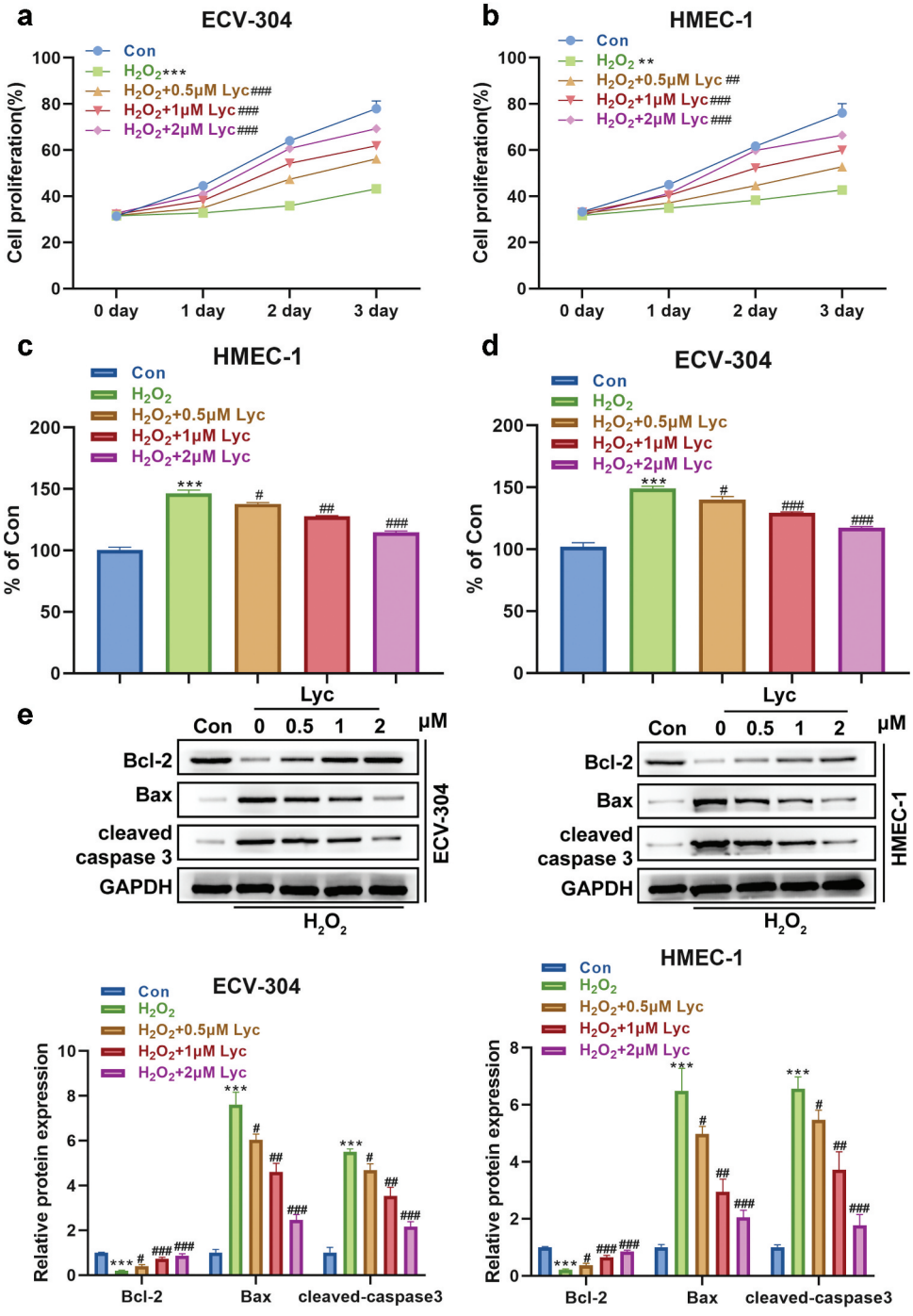

Figure 1. Lycopene attenuates endothelial cell injury induced by H_2O_2. a: CCK8 assay detection of lycopene on cell proliferation of ECV-304 cells under H_2O_2 stimulation. b: CCK8 assay detection of lycopene on cell proliferation of human microvascular endothelial cells HMEC-1 under H_2O_2 stimulation. c: LDH assay detection of lycopene on the cytotoxicity of ECV-304 cells under H_2O_2 stimulation. d: LDH assay detection of lycopene in the cytotoxicity of human microvascular endothelial cells HMEC-1 under H_2O_2 stimulation. e: The protein levels of Bcl-2, Bax, and cleaved caspase 3 in HMEC-1 and ECV-304 cells intervened with lycopene under H_2O_2 stimulation were applied by Western blotting. $^{**}P<0.01$, $^{***}P<0.001$ versus the control group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ versus H_2O_2 group. $n=3$

analyzed by using ELISA assays to confirm the effect of lycopene on H_2O_2-induced inflammatory responses. Results as shown in Figure 3, the production contents of chemokines, including MCP-1, M-CSF, and CXCL-10, and proinflammatory cytokines, including TNF-α and IL-1β, in HMEC-1 and ECV-304 cells were remarkably increased after H_2O_2 stimulation. Moreover, the production of MCP-1, M-CSF, CXCL-10, TNF-α, and IL-1β gradually decayed after intervention with lycopene at doses of 0.5, 1, or 2 μM.

Lycopene ameliorates H_2O_2-induced oxidative stress in HMEC-1 and ECV-304 cells

Multiple studies have shown that oxidative stress can affect endothelial cell growth and apoptosis, oxidatively modify lipoproteins, and promote inflammation, which in turn affect cardiovascular disease processes such as atherosclerotics. Therefore, suppression of endothelial cell damage by oxidative stress has great significance in the prevention of CVD. Next, we used

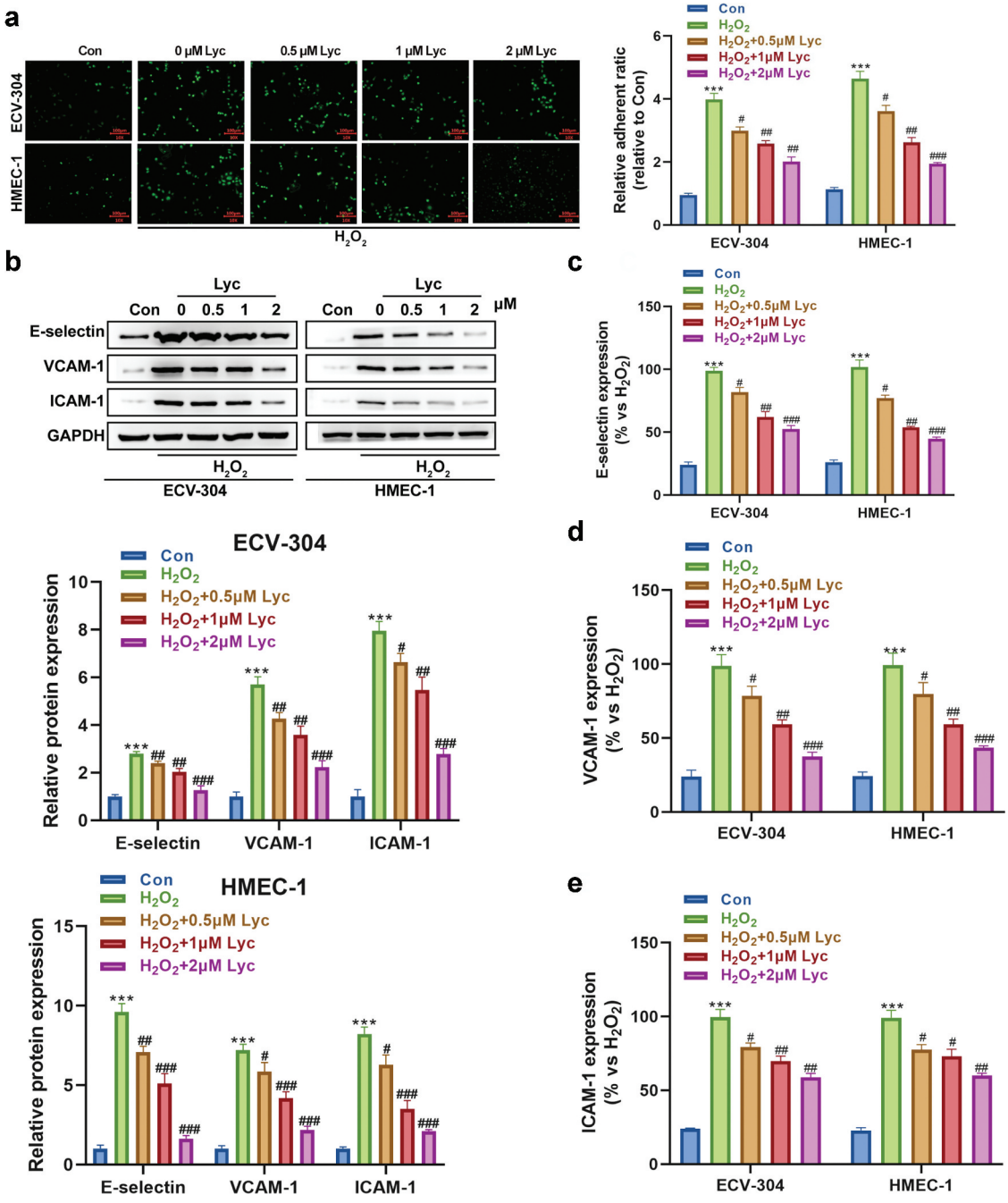

Figure 2. Lycopene reduces H_2O_2-induced monocyte endothelial adhesion and adhesion molecule levels in endothelial cells. a: Immunofluorescence labeling was applied to check the adhesion rate between HMEC-1 or ECV-304 and U937 monocytes intervened with different concentrations of lycopene under H_2O_2 stimulation. b: Western blotting detection of protein levels of E-selectin, VCAM-1, and ICAM-1 in cells intervened with lycopene under H_2O_2 stimulation. c: EIA detection of protein contents of E-selectin in cells intervened with lycopene under H_2O_2 stimulation. d: EIA detection of protein contents of VCAM-1 in cells intervened with lycopene under H_2O_2 stimulation. e: EIA detection of protein contents of ICAM-1 in cells intervened with lycopene under H_2O_2 stimulation. $^{***}P<0.001$ versus the control group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ versus H_2O_2 group. $n=3$.

immunofluorescence dihydroethidium (DHE) fluorescence probes to detect the content of ROS expression in the HMEC-1 and ECV-304 cells of each group. The results presented in Figure 4a,b show that the intracellular green fluorescence intensity was significantly enhanced in HMEC-1 and ECV-304 cells under H_2O_2 stimulation, suggesting an increase of ROS content. However, the content of ROS gradually decreased after different doses of lycopene intervention. In addition, ELISA results (Figure 4c,d) revealed that MDA content was obviously increased in HMEC-1 and ECV-304 cells under H_2O_2 stimulation, while glutathione (GSH) production was prevented, and the above results were all reversed by lycopene treatment in a dose-dependent manner. It is well known that glutamate cysteine ligase (GCL), a key enzyme in the synthesis of GSH, is composed of a glutamyl-cysteine synthetase catalytic (GCLC) subunit and a glutamate-cysteine ligase modified (GCLM) subunit. The mRNA and protein levels of GCLC and GCLM in HMEC-1 and ECV-304 cells were obviously increased under the stimulation of

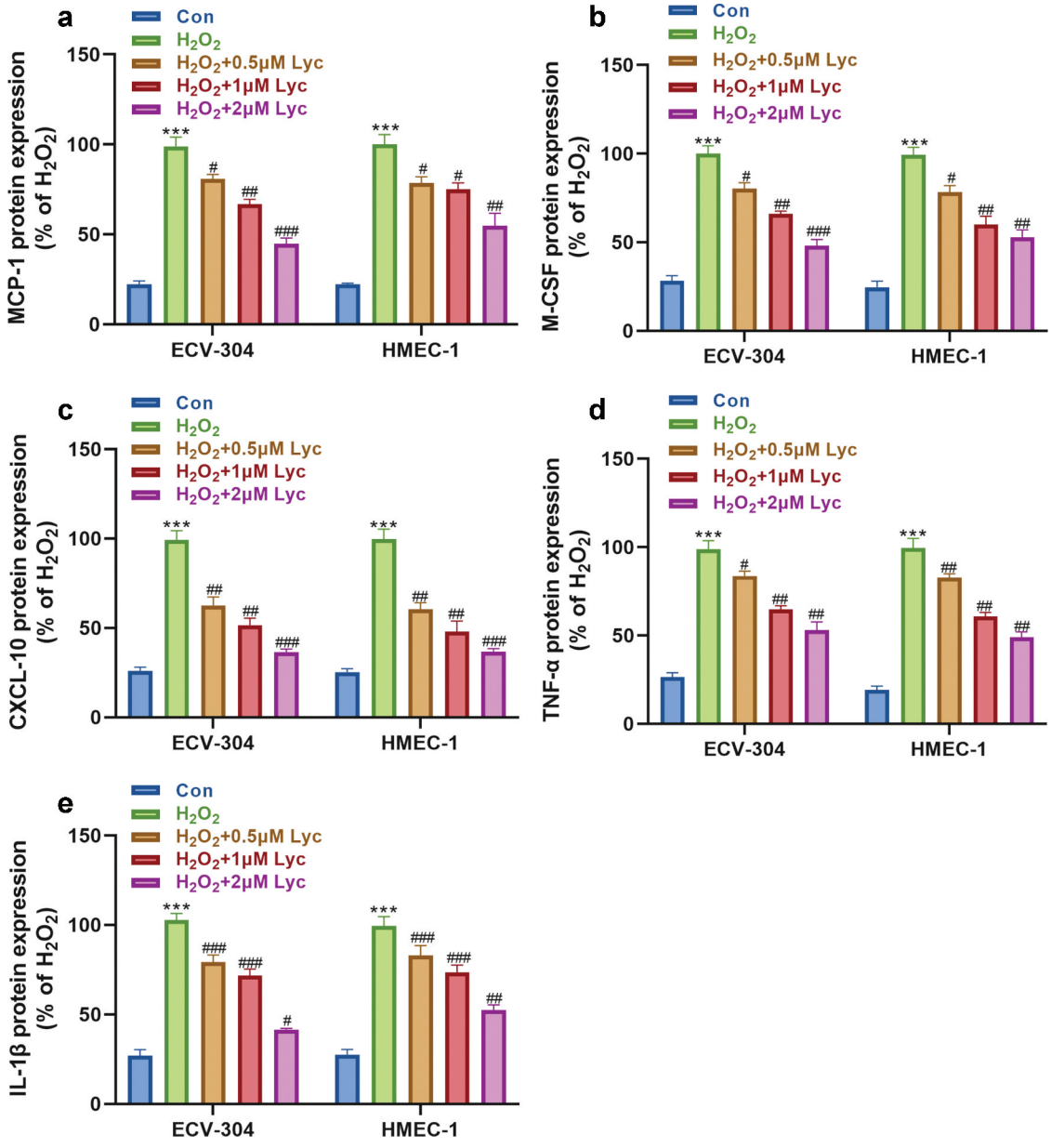

Figure 3. Lycopene can lighten H_2O_2 induced inflammatory responses in endothelial cells. a: ELISA detection of contents of chemokines MCP-1 in HMEC-1 or ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. b: ELISA detection of production contents of chemokines M-CSF in HMEC-1 or ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. c: ELISA detection of production contents of chemokines CXCL-10 in HMEC-1 or ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. d: ELISA detection of production contents of proinflammatory factors TNF-α in HMEC-1 or ECV-304 cells intervened with of lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. e: ELISA detection of production contents of proinflammatory factors IL-1β in HMEC-1 or ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. ***P<0.001 versus the control group. $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 versus H_2O_2 group. n=3.

H_2O_2, which resulted in the low production of GSH (Figure 4e-h). However, GCLC and GCLM levels were gradually recovered after lycopene intervention in a dose-dependent manner (Figure 4e-h).

Lycopene encourages the SIRT1/Nrf2/HO-1 pathway in endothelial cells

Previous studies have found that lycopene increases intracellular glutathione levels synthesized by the nuclear factor erythroid 2-related factor 2 (Nrf2)-related glutamylcysteine synthase. Western blotting results (Figure 5a) displayed that the protein levels of SIRT1, HO-1, and nuclear Nrf2 were noticeably downregulated after H_2O_2 stimulation in HMEC-1 and ECV-304, whereas total Nrf2 protein levels remained unchanged. Subsequently, SIRT1, HO-1, and nuclear Nrf2 protein levels (but not total Nrf2 protein) were gradually upregulated after lycopene intervention at different doses. It is reported that Nrf2 can regulate the level of driving specific gene ARE. Further findings revealed that lycopene indeed leads to an increase in Nrf2 transcriptional activity by transfecting HMEC-1 or ECV-304 with luciferase reporter constructs (Figure 5b,c).

Discussion

In the past few years, oxidative stress has been widely involved in many disease processes, including myocardial infarction,

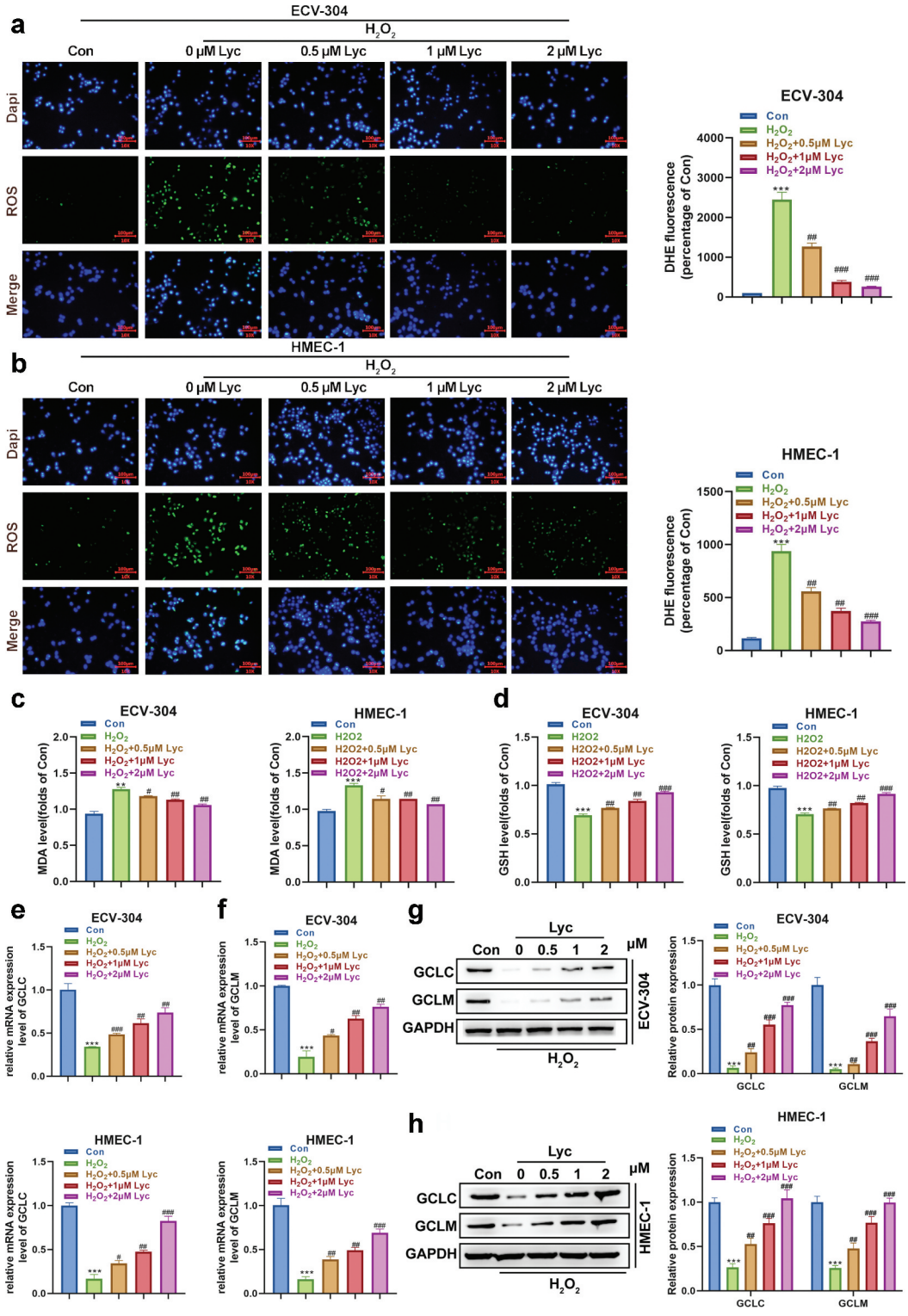

Figure 4. Lycopene ameliorates H_2O_2-induced oxidative stress in HMEC-1 and ECV-304 cells. a: Immunofluorescence labeling was applied to examine the ROS content in ECV-304 cells intervened with lycopene under H_2O_2 stimulation. The DHE labels ROS and DAPI labels nuclei. b: Immunofluorescence labeling was applied to examine the ROS content in HMEC-1 cells intervened with lycopene under H_2O_2 stimulation. c: ELISA detection of production contents of MDA in HMEC-1 or ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. d: ELISA detection of the production content of GSH in HMEC-1 or ECV-304 cells intervened with of lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. e: RT-qPCR detection of GCLC mRNA levels in HMEC-1 or ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. f: RT-qPCR detection of GCLM mRNA levels in HMEC-1 or ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. g: Western blotting detection of GCLC and GCLM protein levels in ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. h: Western blotting detection of GCLC and GCLM protein levels in HMEC-1 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. ***P<0.001 versus control group. $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 versus H_2O_2 group. n=3.

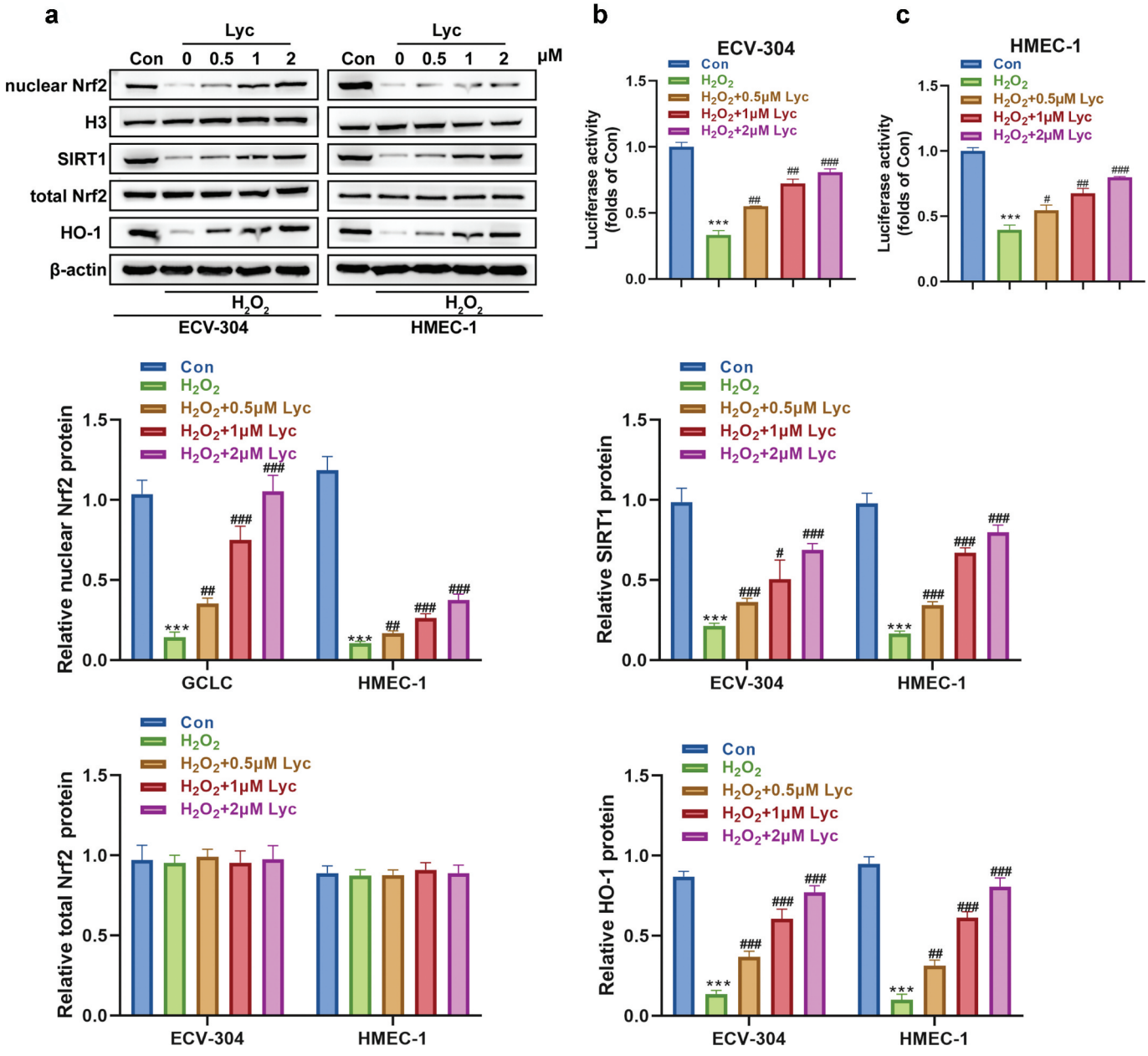

Figure 5. Lycopene activates the SIRT1/Nrf2/HO-1 pathway in endothelial cells. a: Western blotting detection of protein levels in nuclear Nrf2, SIRT1, total Nrf2, and HO-1 in HMEC-1 and ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. b: Luciferase reporter experiments detection of transcriptional activity protein of Nrf2 in ECV-304 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. c: Luciferase reporter experiments detection of transcriptional activity protein of Nrf2 in HMEC-1 cells intervened with lycopene (0.5, 1, or 2μM) under H_2O_2 stimulation. ***P<0.001 versus the control group. $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 versus H_2O_2 group. n=3.

atherosclerosis, myocardial ischemia-reperfusion injury, and other cardiovascular diseases (30,31). H_2O_2, as a major redox metabolite, is essential in redox sensing, signaling, and redox regulation. Supraphysiological concentrations of H_2O_2 can cause damage to cellular functions, resulting in oxidative stress damage (32). Vascular endothelial cells are an important part of the heart and vascular system, and the nutrition and proper biochemical function of vascular endothelial cells are related to the proper functioning of cardiovascular function (33). The accumulation of ROS in endothelial cells under oxidative stress stimuli induces endothelial dysfunction, which has a major impact on the cardiovascular system and the health of living organisms (34). In the present study, to mimic vascular endothelial cell oxidative stress injury in cardiovascular disease, we used a high concentration of H_2O_2 to stimulate HMEC-1 and ECV-304 cells. The results showed an increase in ROS and MDA production and a decrease in GSH, GCLC, and GCLM expression in the cells under H_2O_2 stimulation. Among them, MDA can be used as a marker of oxidative stress damage. In addition, there are antioxidant stress injury substances in the cell, such as GSH as well as other enzyme class and non-enzyme class substances, which can alleviate the oxidative stress injury (35).

Adhesion of blood-borne monocytes to the vascular endothelium and entry into the intima is an event in the development and progression of AS (36). Endothelial cell

injury could induce monocytes to adhere to the endothelium by elevating the ICAM-1 and VCAM-1 levels (37). A study found that atherosclerosis models exhibit a high aortic wall ICAM-1/VCAM-1 expression (38). Zheng *et al.* (39) demonstrated that resveratrol down-regulated E-selectin VCAM-1 and ICAM-1 by partially blocking NF-κB activation in endothelial progenitor cells, which in turn exerted anti-atherosclerotic effects. Our results similarly found that H_2O_2 intervention significantly increased monocyte adhesion in HMEC-1 and ECV-304 cells, as well as elevated the protein levels of E-selectin, VCAM-1, and ICAM-1. Further, ROS-induced oxidative stress production not only damages the body's inherent antioxidant defense system but also is essential in the inflammatory response, apoptosis, and cell growth. Research discovered that treatment of cultured HUVECs with different concentrations of nickel sulfate (0, 62.5, 250, and 1000 μM) revealed that nickel sulfate exerted dose- and time-dependent inhibitory effects on cell growth and was able to trigger inflammation, increase the protein and IL-6 levels, and decrease the protein and mRNA levels of TGF-β. In addition, nickel sulfate led to increased apoptosis in human VECs by activating oxidative stress, manifested by elevated protein levels of cleaved caspase 3 and Bax, and reduced Bcl-2 and Bcl-XL protein levels (40). Similarly, our results displayed that the proliferation of HUVECs and human microvascular endothelial cells was significantly decreased under H_2O_2 stimulation, and cell apoptosis was evidently enhanced, and the content of pro-inflammatory factors was distinctly increased.

Lycopene has a strong ability to scavenge free radicals and can enhance cell antioxidant capacity (41). Li *et al.* (28) discovered that lycopene protected neuronal injury by repressed oxidative stress. Ni *et al.* (42). demonstrated that lycopene prevented and reversed lipotoxicity-induced inflammation and fibrosis in nonalcoholic steatohepatitis mice by reducing oxidative stress. Epidemiological studies have shown that there are many favorable characteristics between dietary lycopene consumption and the reduction of cardiovascular disease risk (43). It is reported that lycopene can protect acutely infarcted myocardium by inhibiting lipid peroxidation injury, improving endogenous antioxidant enzyme activity, and attenuating the damage of oxygen-free radicals to the myocardium (44). Zeng *et al.* (45) showed that lycopene inhibits cardiac hypertrophy by inhibiting ROS-dependent mechanisms. Further, Tang *et al.* (26) confirmed that lycopene attenuates H_2O_2-induced oxidative damage to endothelial cells, repressed p53 and caspase-3 mRNA expressions in injured cells reduces apoptosis of injured cells, and in turn plays a preventive role against AS. In our study, we found that lycopene intervention could reverse the inhibitory effects of H_2O_2 stimulation on the proliferation of HMEC-1 and ECV-304 cells, as well as its facilitative effect on oxidative stress-induced apoptosis, cell adhesion, and inflammation in a dose-dependent manner.

Recently, Nrf2 has gained much attention and attention in the search for therapeutic agents that attenuate cardiovascular diseases by inhibiting oxidative stress (46). Nrf2, a key neuroprotective transcription factor belonging to the cap'n'collar family that is an essential component of the antioxidant stress pathway (47). Nrf2 could bind to antioxidant response elements and promote the transcription of downstream antioxidant enzymes, mainly including HO-1, SOD, CAT, *etc.* (48–50). In addition, Nrf2 also could regulate the biosynthesis of GSH and then enhance the body's antioxidant capacity (51). A large number of basic experimental studies confirmed that enhancement of the Nrf2/HO-1 pathway has a significant protective effect on cardiovascular diseases. A study revealed that theaflavin may exert anti-atherosclerotic effects by strengthening the Nrf2/HO-1 pathway (52). Poria cocos polysaccharides exert their inhibitory effects on oxidative stress to treat AS by enhancing the Nrf2/HO-1 pathway (53). Further, previous studies have discovered that lycopene encourages nuclear ectopy of Nrf2 and activates the Nrf2/HO-1 pathway, which in turn protects hepatocytes from hypoxia/reoxygenation injury (54). Our results similarly showed that lycopene treatment could partially counteract the inhibition of H_2O_2 stimulation on SIRT1/Nrf2/HO-1 pathway proteins in ECV-304 and HMEC-1 cells. Nrf2 could be activated by multiple regulators and pathways among them SIRT1, a member of the sirtuin family of proteins, which is widely expressed in various tissues and organs, and is able to be involved in a variety of pathological processes (55,56). Multiple studies have confirmed that activation of the SIRT1/Nrf2 signaling pathway can alleviate cardiovascular diseases. Zhang *et al.* (57) demonstrated that hydrogen sulfide could restore the cardioprotective effects of sevoflurane postconditioning-induced diabetic rats by activating oxidative stress mediated by the SIRT1/Nrf2 signaling pathway. Besides, yang *et al.* (58) have found that quercetin significantly reduced cerebral infarct volume, neurological deficits, blood–brain barrier permeability, and reactive oxygen species production through the SIRT1/Nrf2/HO-1 pathway. This further confirms the findings of the present study.

In conclusion, lycopene could alleviate H_2O_2-induced oxidative damage to human vascular endothelial cells by reducing intracellular reactive oxygen species levels, inflammatory factor production, cell adhesiveness, as well as apoptosis rate under oxidative stress conditions in cells through enhancement of the SIRT1/Nrf2/HO-1 pathway. The above results contribute to a more comprehensive and in-depth understanding of the relationship between lycopene and oxidative damage in cardiovascular diseases, providing theoretical support for further treatment of the biological mechanisms underlying cardiovascular diseases. Nevertheless, this research content is mainly based on *in vitro* cell experiments and is missing to do further validation on animal experiments, which will be the focus of our subsequent research.

Disclosure statement

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Data availability statement

The data that support the findings of this study are available from the corresponding author, SDL, upon reasonable request.

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