Statins protect diabetic myocardial microvascular endothelial cells from injury

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Abstract

To study the protective effect and mechanism of statins on myocardial microvascular endothelial cells injury in diabetic rats. The rats were administrated with atorvastatin (10 mg/kg/day), rosuvastatin (5 mg/kg/day), or placebo for 1 week. The expression of small GTP-binding protein dissociation stimulator (SmgGDS) was analyzed using western blot. The expression of SmgGDS was also analyzed in cultured rat cardiac microvascular endothelial cells using western blot. The cells were divided into normal glucose group, hyper glucose group, and atorvastatin-treated group. The superoxide dismutase (DHE) staining was used to detect the oxidative stress in the rat cardiac microvascular endothelial cells. The expression of SmgGDS was detected using TUNEL staining after knockout of Akt1 and β 1-integrin in cardiac microvascular endothelial cells. The followings were the significant findings of this study: (1) SmgGDS was expressed in aorta endothelium of diabetic rats and cardiac microvascular endothelial cells cultured with high glucose, (2) high glucose increased the production of ROS, the activity of NADPH, and the rate of apoptosis in cardiac microvascular endothelial cells, but after atorvastatin pretreatment, the production of ROS, the activity of NADPH, and the rate of apoptosis in cardiac microvascular endothelial cells decreased, (3) the expression of SmgGDS decreased and the oxidative stress increased after knockdown of Akt1 or β 1-integrin. Statins can protect diabetic microvascular endothelial cells from oxidative stress and apoptosis by upregulating SmgGDS partly through β 1-integrin/Akt1 pathway.

Keywords Statins · Cardiac microvascular endothelial cells (CMECs) · β 1-integrin, Akt (protein kinase B, PKB) · SmgGDS (small GTP-binding protein dissociation stimulator)

Introduction

Diabetes, as a disease of metabolic disorder, is a serious threat to human health. Increased levels of chronic blood glucose caused by diabetes can lead to a series of complications [1], among which, cardiovascular complications are the leading cause of death. There are evidences that microvascular damage in cardiovascular disease caused by diabetes plays a very important role [2].

Myocardial microvascular located in the terminal circulation, to some extent determines the level of myocardial perfusion and coronary reserve. Microvascular injury may also lead to complications such as microvascular angina, diabetic cardiomyopathy, no-reflow phenomenon after coronary intervention, and myocardial ischemia-reperfusion injury [3]. Clinical studies have shown that microvascular damage occurs much earlier than that of large vessels and myocardial cells. HMG-CoA reductase inhibitors (statins) are effective cholesterol-lowering drugs that are widely used for primary prevention and secondary prevention of coronary artery disease. In addition to their cholesterol-lowering effects, the pleiotropic effect of statins, showing the benefit effect on heart and vascular, has attracted people's attention [4].

The pleiotropic effects of statins are currently thought to be mediated by a reduction in the synthesis of isoprenoids, the intracellular proteins, responsible for posttranslational regulation. Because the activity of the GTPase of membrane localization and small GTP-binding proteins (such as Rho, Rac,

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and Ras) depends on isoprenylation, the pleiotropic properties of statins are thought to be mediated by the inhibition of small GTP-binding proteins [5]. Recent studies have shown that the cardiovascular protective effect of statins is mediated in part by small GTP-binding protein dissociation stimulator (SmgGDS) [6], which leads to Rac1 degradation and oxidative stress reduction, but it is unknown whether statins in high glucose conditions have such effect on myocardial microvascular through SmgGDS. The SmgGDS-mediated effect of statins represents a third mechanism of action for statins, the other two mechanisms being the inhibition of cholesterol synthesis in the liver and the inhibition of small GTP-binding proteins. The aim of this study was to investigate whether statins have cardioprotective effects and what is the possible molecular mechanisms through SmgGDS in the presence of high glucose.

Materials and methods

Materials

Reagents High glucose and low glucose DMEM medium, trypsin (Sigma, USA), fetal bovine serum (USA Sigma), DII-Ac-LDL (US Invitrogen), atorvastatin (US Pfizer), rosuvastatin (Shandong Qilu), superoxide detection kit (Jiangsu Biyo times Biotechnology Research Institute), NADPH TUNEL Apoptosis Detection Kit (Roche, USA), BCA protein detection assay kit (Shanghai Biotech Pharmaceutical Technology Co., Ltd.), superoxide anion detection kit (Jiangsu Biyo time Biotechnology Research Institute), HRP-labeled goat anti-rabbit IgG (Beyotime), EC chemiluminescence detection kit (USA Millipore), Akt1-siRNA (Qiagen, Germany), β 1-integri-siRNA (Qiagen, Germany), mocksiRNA anti-Smg GDS antibody (US BD transduction Lab), anti- β 1-integri antibody (US Cell Signaling), anti-Akt1 antibody (US Cell Signaling), horseradish peroxidase-labeled (Sigma), PVDF membrane (US GE) rabbit anti-mouse, goat anti-rabbit antibody, and β -actin mouse anti-rat monoclonal antibody (Abcam, USA), etc. were utilized in this study.

Apparatus CO₂ incubator (Japan Ikebukuro Co., Ltd.), western blotting system (Bio-Rad Company, USA), and confocal microscope (Olympus, Japan), etc. were utilized in this study.

Animal experiments

SD rats, male, 6–8 weeks of age, weighing 180–220 g, were provided by the Experimental Animal Center of Soochow University, with the consent of the Experimental Animal Ethics Committee of Suchow University. Firstly, the rat model of type 2 diabetes mellitus was established according to

reference [7]. Streptozotocin (STZ) 65 mg/kg was injected into the abdominal cavity of the rats, and the blood glucose of the tail vein blood was measured after 1 week, with two times of random blood glucose ≥ 16.7 mmol/L as the standard of success of diabetes model. Secondly, the rats were divided into three groups ($n = 6$ each group). And then the animals were respectively treated with atorvastatin (10 mg/kg/day, Pfizer, USA), rosuvastatin (5 mg/kg/day, Qilu, Shandong), or placebo by gavage once a day for 1 week. And then after 1 week of treatment, the animals were anesthetized with ether, and the aorta and heart were frozen by liquid nitrogen after dissection. The frozen tissue was lysed in a cell lysis reagent and homogenized by a homogenizer. Finally, the homogenate was centrifuged and the supernatant was used for western blot analysis to detect SmgGDS protein expression.

Cardiac microvascular endothelial cells (CMECs) culture and identification

The cardiac microvascular endothelial cells were isolated from Sprague-Dawley rat heart by the enzyme dissociation method. Briefly, 4-week-old SD rats were anesthetized by pentobarbital injection. The heart was removed from the rat thoracic cavity under aseptic conditions, the left ventricle was then isolated, washed with PBS, the epicardium was removed, and the myocardial tissue was cut into 1-mm³ tissue pieces, and the tissue pieces were immersed in 0.2% II collagenase at 37 °C for 10 min, and then digested by addition of 0.02% trypsin for 10 min to digest the tissue. DMEM with 10% FBS was added to the tissue to stop digestion, and the solution was filtered through a 100- μ m nylon mesh to remove undigested tissue. The solution was centrifuged, resuspended in DMEM/low glucose, 15% FBS was added, and then grown on petri dishes. After incubation for 6 h at 37 °C in a humidified atmosphere of 5% CO₂, unadherent cells were removed, and the medium was changed again after 24 h. The medium was changed every 3 days. After the cells reached about 80% confluency, they were passaged with 0.25% trypsin-0.02% EDTA.

The microvascular endothelial cells were cultured for 7 days. Cells were digested, centrifuged and inoculated on coverslips, and cultured in 5% CO₂ incubator at 37 °C. Dil-Ac-LDL (dil-acetylated-low density lipoprotein) was added at a final concentration of 15 μ g/mg. After cultured together for 8–10 h, the coverslips were removed, rinsed gently with PBS three times for 3 min, fixed with 4% paraformaldehyde for 20 min, and washed three times with PBS for 3 min. The cells were incubated with DAPI at a 1: 500 concentration drops in the cell lobes, incubated at room temperature for 5 min, and washed three times with PBS, each time for 3 min. The coverslips were placed in the dark and ventilated place, dried and then followed by mounting with neutral glycerine, and microscopic examination with a confocal microscopy.

Detection of SmgGDS expression in cardiac microvascular endothelial cells and aorta

CMECs were seeded in 6-well plates (1.5×10^5 cells/well). They were divided into four groups, namely control group (the final concentration of glucose in the cell culture medium was 5.5 mmol/L), high glucose group (the final concentration of glucose in the cell culture medium was 25 mmol/L), atorvastatin group (10 μ mol/L), atorvastatin plus high glucose group. Western blot was used to detect the expression level of SmgGDS. Briefly, the cells were washed with PBS and the plate was left on ice for 30 min to lyse the cell. Cells were collected in a centrifuge tube with a cell scraper, and then lysed with ultrasonic irradiation for 3 min two times. Cell debris was removed by centrifugation of the lysates at 12000 r/min for 2 min at 4 °C, and protein concentration was determined by using the BCA protein assay kit. The working liquid was prepared according to the instruction, and the standard curve was made, the plate was incubated in 37 °C for half an hour, and then the optical density (570) was tested by a microplate reader. Finally, the protein concentration was computed according to the standard curve. All the specimens were adjusted to the same concentration, after the protein supernate was mixed with the buffer, the liquid was boiled for 10 min, followed by cooling, centrifugation, and 12% SDS-PAGE. Following the same method, the protein in the aorta was separated by SDS-PAGE and transferred to PVDF membranes. The proteins were transferred to PVDF membranes. The membranes were then washed and incubated with anti-SmgGDS overnight at 4 °C. After adding horseradish peroxidase-labeled goat anti-rabbit IgG (1:5000), the membranes were incubated at room temperature for 1 h, and then the chemiluminescent reagents A and B were mixed in a ratio of 1:1 for 3 min; finally, the blots were visualized by the enhanced chemiluminescence system.

Cell groups and index detection

CMECs were seeded in a 6-well plate (1.5×10^5 cells/well) and divided into four groups: the control group (the final concentration of glucose in the cell culture medium was 5.5 mmol/L); the high glucose group (the final concentration of glucose in the cell culture medium was 25 mmol/L); the atorvastatin group (the concentration of atorvastatin was 10 μ mol/L and the final concentration of glucose in the cell culture medium was 5.5 mmol/L); and the atorvastatin + high glucose group (the final concentration of glucose in the cell culture medium was 25 mmol/L, and the concentration of atorvastatin was 10 μ mol/L).

Detection of reactive oxygen species

CMECs were seeded in 96-well plates (5×10^5 cells/well), and the medium was discarded. After washed with PBS, the cells

were incubated with superoxide detection working solution for 3 min, and then the optical density (450) was tested by a microplate reader.

Detection of superoxide anion

CMECs were seeded in the coverslips and dealt as we described in the grouping part. Dihydroethidium (DHE8 μ mol/L, in 0.1% DMSO) was added in the medium, and then incubated for 1 h. DAPI (1:500) was added and followed by incubation at room temperature for 5 min. The coverslip was washed with PBS again, dried in the air, and then followed by mounting with neutral glycerine and microscopic examination with a confocal microscopy. If the red fluorescence was enhanced, it suggested the level of superoxide anion was elevated.

Detection of NADPH oxidase activity

CMECs were seeded in 6-well plates (1.5×10^5 cells/well) and dealt as we described in the grouping part. TRIzol was added to lyse the cells, and placed at -70 °C for further use. NADPH peroxidase activity was detected using a chemiluminescence apparatus according to the instruction of the kit. The activity of the NADPH peroxidase was computed by the formulation:

$$RLU_{\text{the real activity of the sample}} = RLU_{\text{total activity of the sample}} - RLU_{\text{specific activity of the sample}} - RLU_{\text{control group}}$$

Detection of apoptosis

CMECs were seeded in the coverslips and dealt as we described in the grouping part. The coverslips was fetched out, washed with PBS, and then fixed in 4% paraformaldehyde. After washed with PBS, 3% hydrogen peroxide was added and incubated for 20 min to remove the endogenous peroxidase. Triton 100 was added and then put on the ice. Before and after the Triton 100 was added, the coverslips should be washed with PBS carefully. The apoptosis rate of the CMECs was detected by using the TUNEL apoptosis detection kit, and the working liquid was prepared according to the instruction. Briefly, the working liquid was added onto the coverslips, after incubated in 37 °C for 1 h. Then, it was washed with PBS, and the cells were incubated with DAPI (1:500) for 5 min at room temperature. It was washed with PBS again. The cells were incubated in a dark place, air-dried, sealed with glycerol, and observed under a fluorescence microscope. The number of apoptotic cells was represented by nuclear blue staining, and ten visual fields were selected randomly to count.

Molecular mechanism of statins on CMECs

CMECs were transfected with siRNA and the protein expression was detected by western blot

Multiple siRNA duplexes for Akt1 and β 1-integrin were purchased from Qiagen (Germany). A functional non-targeting siRNA that was bioinformatically designed by Qiagen was used as a mock control. CMECs were transfected with HiPer-Fect Transfection Reagent (Qiagen) with either 10 nmol/L mock control siRNA or 10 nmol/L siRNA specific for target proteins as described previously. After 72-h post-transfection, the cells were analyzed using western blot. In the case that CMECs transfected siRNA was treated with atorvastatin, atorvastatin was treated for 24 h. To quantify the expression levels of several proteins in cultured cells and mouse tissues, the same amount of protein sample was separated by SDS–PAGE and transferred to PVDF membranes. The membranes were immunoblotted with the primary antibodies, including anti-SmgGDS, anti-phospho-Akt, anti-Akt (pan), anti-Akt1, anti- β 1-integrin, and anti-b-actin (Abcam). After incubating with horseradish peroxidase-conjugated rabbit anti-mouse, goat anti-rabbit antibody, blots were visualized by the enhanced chemiluminescence system.

Fig. 1 **a** Cobblestone-like CMECs isolated from rats. **b** Identification of rat CMECs, $\times 400$, DAPI: blue, AcLDL: red

Transfection of CMECs with siRNA, detection of the reactive oxygen species (ROS)

The cells were divided into six groups, control group (concentration of glucose was 5.5 mmol/L); high glucose group (concentration of glucose was 25 mmol/L); atorvastatin + high glucose group; SC79(Akt agonist) + high glucose group; atorvastatin + high glucose + siRNA-Akt1 group; atorvastatin + high glucose + siRNA- β 1-integrin groups, and reactive oxygen species were detected according to the previous experimental method.

Statistical analysis

SPSS19.0 software was used for statistical analysis in this study. All data are expressed as mean $\pm$ SD, and *t* test was applied to perform statistical analysis. *p* < 0.05 was considered significantly different.

Results

Culture and identification of CMECs

CMECs were spindle or polygonal shapes that showed a typical pebble-like morphology under a microscope

(Fig. 1a). DiL-Ac-LDL phagocytosis test showed that cells can phagocytize DiL-Ac-LDL, which showed that our experimental cultured cells were CMECs (Fig. 1b).

Statins upregulate SmgGDS expression in diabetic microvascular endothelial cells

Statins upregulate SmgGDS expression in the aorta of diabetic rats

To examine the upregulation of SmgGDS in diabetic rats, we first examined the effect of oral atorvastatin (10 mg/kg per day) and rosuvastatin (5 mg/kg per day) therapy in diabetic rats for 1 week. Statins significantly increased SmgGDS protein expression in aortic (Fig. 2a).

Fig. 2 **a** SmgGDS expression in aorta, vehicle: placebo, ROSU: rosuvastatin, ATOR: atorvastatin (10 mg/kg per day) and rosuvastatin (5 mg/kg per day) significantly increased SmgGDS expression in the aorta. There was significant difference compared with placebo ($p < 0.05$). **b** Atorvastatin upregulated SmgGDS expression in cardiac microvascular endothelial cells cultured in high glucose. Atorvastatin (1 $\mu\text{mol/L}$) began to upregulate the expression of SmgGDS. The effect of atorvastatin on SmgGDS was enhanced with the increase of concentration, and there was significant difference compared with the control group ($p < 0.05$). Vehicle: placebo; ROSU, rosuvastatin; ATOR, atorvastatin

Atorvastatin upregulated the expression of SmgGDS in cultured CMECs

We examined the effects of atorvastatin on the expression of SmgGDS in rat cardiac microvascular endothelial cells (CMECs) cultured in high glucose. Atorvastatin significantly increased SmgGDS expression in CMECs in a concentration-dependent manner.

Inhibition effect of atorvastatin on generation of ROS in CMECs

We first examined the effects of atorvastatin on hyperglycemia in CMECs induced by high glucose. ROS production was significantly increased ($p < 0.05$) in the CMECs

Fig. 3 **a** Effect of atorvastatin on ROS production in cardiac microvascular endothelial cells. **b** Effect of atorvastatin on DHE in cardiac microvascular endothelial cells. con, control group; ATOR, atorvastatin group; HG, high glucose group

induced by high glucose and decreased significantly in the atorvastatin pretreatment group ($p < 0.05$) (Fig. 3a). Compared with the normal control group, the fluorescence intensity of CMECs increased significantly ($p < 0.05$), and

the fluorescence intensity of CMECs was significantly attenuated after atorvastatin treatment ($p < 0.05$), indicating that atorvastatin inhibited the production of ROS in CMECs induced by high glucose (Fig. 3b).

Fig. 4 Influence of atorvastatin on NADPH oxidase activity in CMECs. It showed the activity of NADPH peroxidase in CMECs was much higher in the high glucose group than the control group, the difference was statistically significant ($P < 0.05$). After treated with atorvastatin, the activity of NADPH peroxidase was declined significantly ($p < 0.05$)

Fig. 5 **a** CMECs apoptosis (TUNEL) immunostaining images. **b** Inhibition effect of atorvastatin on apoptosis of CMECs, comparison between control group and high glucose group, $p < 0.05$ and comparison between high glucose group and high glucose + atorvastatin group, $p < 0.05$

Figure 3 showed that CMECs in high glucose group produced more ROS than control group. Figure 3b showed

that compared with the control group, the fluorescence intensity of CMECs in the high glucose group was greatly

improved, while after added with atorvastatin, the fluorescence intensity was declined clearly, and the differences were statistically significant ($p < 0.05$).

Inhibition effect of atorvastatin on NADPH oxidase activity in CMECs

Current basic and clinical studies have shown that NADPH oxidase is the main source of ROS produced by endothelial cells. In the hyperglycemic state, the activity of NADPH oxidase in vascular endothelial cells increased and ROS production increased, which induced oxidative stress, causing vascular injury. Therefore, we further detected the activity of NADPH oxidase in CMECs after various kinds of intervention and found that the activity of NADPH oxidase in the high glucose treatment group was higher than that in the normal control group ($p < 0.05$). After pretreatment with atorvastatin, NADPH oxidase activity was significantly lower ($p < 0.05$) (Fig. 4).

Inhibition effect of atorvastatin on apoptosis of CMECs

TUNEL staining showed that the apoptosis rate of CMECs in the high glucose group was significantly higher than that in the control group [(40.06 ± 2.81%) vs. (11.20 ± 2.64%)] ($p < 0.05$), the apoptosis rate of CMECs treated with atorvastatin significantly declined [(27.11 ± 2.62%) vs. (40.06 ± 2.81%)], compared with group with normal glucose level ($p < 0.05$) (Fig. 5).

Atorvastatin mediates the phosphorylation of Akt in CMECs under high glucose

Next, we examined the molecular mechanism of atorvastatin upregulating SmgGDS, and we examined whether atorvastatin

enhanced Akt phosphorylation in CMECs cultured under high glucose. The results showed that Akt phosphorylation was significantly increased by atorvastatin at 15 min in high glucose-cultured CMECs ($p < 0.05$, Fig. 6).

Akt1 mediates atorvastatin-induced SmgGDS expression

Previous studies have shown that only Akt1 in three subtypes of Akt contributes to atorvastatin up-regulation of SmgGDS expression in normal CMECs [8]. We next examined whether Akt1 was responsible for the expression of SmgGDS in CMECs under high glucose condition induced by atorvastatin. Compared with CMECs under high glucose, the effect of Akt1 on the expression of SmgGDS influences induced by atorvastatin was observed. The results showed that atorvastatin upregulated the expression of SmgGDS mediated by Akt1 in CMECs under high glucose condition, which was significantly different from that of control group ($p < 0.05$, Fig. 7a). We also found that SC79 (Akt activator) significantly increased the expression of SmgGDS in CMECs under high glucose conditions ($p < 0.05$, Fig. 7c).

β1-integrin mediated upregulation of atorvastatin SmgGDS expression and Akt phosphorylation

We examined the upstream mediator of Akt1 for upregulation of SmgGDS by atorvastatin. We found that upregulation of SmgGDS and the phosphorylation of Akt by atorvastatin was reduced after knockout β1-integrin ($p < 0.05$, Fig. 8). The results showed that atorvastatin increased the expression of SmgGDS in the CMECs under high glucose in vitro partly through β1-integrin/Akt1 signaling pathway.

Fig. 6 Atorvastatin increased the phosphorylation of Akt in high glucose-cultured CMECs. Akt phosphorylation was significantly increased by atorvastatin at 15 min in high glucose-cultured CMECs. HG, high glucose group; ATOR, atorvastatin group, ($p < 0.05$)

Fig. 7 a–c Akt1 induced atorvastatin to upregulate the expression of SmgGDS in CMECs under high glucose condition. The effect of atorvastatin on the expression of SmgGDS in CMECs was abolished after Akt1 knockout, which was statistically different from that of atorvastatin group ($P < 0.05$). HG: high glucose group, ATOR: atorvastatin group, 1:mocksiRNA+HG;2: mocksiRNA + ATOR;3:Akt1siRNA + HG; 4:Akt1siRNA + ATOR. Figure 7d, SC79 (Akt activator) significantly increased the expression of SmgGDS in CMECs under high glucose conditions

Atorvastatin inhibits ROS production in CMECs via β 1 integrin/Akt1 signaling pathway

To further test the role of β 1-integrin/Akt1 signaling pathway in the protection of atorvastatin against high glucose-induced

oxidative stress in myocardial microvascular endothelial cells, we examined ROS production again. The ROS production of myocardial microvascular endothelial cells under high glucose was increased ($p < 0.05$), and the ROS production was decreased after atorvastatin pretreatment ($p < 0.05$). At the same

Fig. 8 **a–b** Atorvastatin upregulated SmgGDS expression through β 1-integrin in CMVCs under high glucose. The effect of atorvastatin on the expression of SmgGDS in atorvastatin group disappeared after knockout β 1-integrin. There was statistically significant difference ($p < 0.05$). **c** The phosphorylation of Akt reduced after knockout β 1-integrin ($p < 0.05$). HG: high glucose group, ATOR: atorvastatin group. 1: mocksiRNA + HG; 2: mocksiRNA + ATOR; 3: β 1-integrin siRNA + HG; 4: β 1-integrin siRNA + ATOR

time, Akt agonist SC79 also inhibited the production of ROS induced by high glucose. After we knocked out β 1-integrin or Akt1, and then treated CMECs under high glucose with atorvastatin, we found that the ROS production increased compared with that treated with atorvastatin alone ($p < 0.05$, Fig. 9).

Discussion

Diabetes mellitus is a disease that has the same danger with coronary heart disease. The incidence of the two diseases in

the world increases year by year, and both of them become the most common disease endangering human health, with high morbidity and mortality.[9] Statins are one of the commonly used drugs for the treatment of diabetes mellitus and coronary heart disease. In addition to the lipid-modifying effect of statins, there are many aspects of cardio protection that are independent of lipid-modifying effects, which is called pleiotropic effects. The pleiotropic effects of statins have been recognized as various biological actions, including improvement of endothelial dysfunction, reduction of inflammatory responses, stabilization of atherosclerotic plaques, and

Fig. 9 Comparison of ROS levels among different groups, HG vs. control, $p < 0.05$; HG vs. HG + ATOR, $p < 0.05$; HG + ATOR vs. HG + ATOR + siRNA-Akt1, $p < 0.05$; HG + ATOR vs. HG + ATOR + siRNA- β 1-integrin; HG, high glucose group; ATOR, atorvastatin group

attenuation of procoagulant activity and platelet function. The current guidelines recommend patients with acute coronary syndromes to start statin therapy early, considering the pleiotropic effect of statin, in addition to its function of lower blood lipids [10]. The pleiotropic mechanism of statins is currently a hot topic. It has been demonstrated that statins activate the phosphatidylinositol 3-kinase/serine/threonine-specific protein kinase (Akt) pathway and enhance the expression and activity of eNOS [11]. It is now thought to be mediated by a reduction in the synthesis of isoprenoids, which are responsible for posttranslational regulation within the protein. Because membrane localization and GTPase activity of small GTP-binding proteins (such as Rho, Rac, and Ras) are dependent on isoprenylation, it has been considered that statins exert anti-oxidation by inhibiting those small GTP-binding proteins [12]. Recent studies have shown that the new mechanism of cardiovascular protection of statins is partly mediated by SmgGDS, which leads to Rac1 degradation and oxidative stress reduction [6]. However, the study was under normal physiological conditions, and there are a few research studies about this in the pathological states, such as diabetes and so on.

We made diabetic rat models, fed them with statins, and verified that SmgGDS was expressed in the aorta of diabetic rats. We isolated and cultured CMECs in vitro from rats and observed the protective effect of atorvastatin on CMECs under high glucose conditions. We found that atorvastatin reduced the expression of ROS and NADPH peroxidase in CMECs under high glucose conditions and inhibited oxidative stress and apoptosis. We also found that statins increased the expression of SmgGDS in CMECs under high glucose and the phosphorylation of Akt. We observed the expression of SmgGDS was increased by Akt agonists. We also observed that knockout of β 1-integrin attenuated the phosphorylation of Akt and decreased the SmgGDS expression, suggesting that the upregulation of SmgGDS by statins on CMECs under

high glucose conditions was achieved partly through the β 1-integrin/Akt1 signaling pathway, showing the novel mechanism of statin pleiotropic effects.

The organism will produce a large amount of free radicals under the stimulation of high glucose, and then start the oxidative stress reaction [13, 14]. We found that high glucose induced the overexpression of ROS in CMECs, and the decrease of ROS after atorvastatin treatment was decreased. The results suggested that atorvastatin can inhibit the oxidative stress induced by high glucose to a certain extent. NADPH peroxidase is the main source of ROS produced by endothelial cells; therefore, we tested our results by detecting the level of NADPH peroxidase, and we also found that atorvastatin significantly inhibited NADPH peroxidase activation induced by high glucose. Oxidative stress is the initial factor of diabetic vascular complications. Cells are exposed to high blood sugar to produce large amounts of ROS, activation of a series of biological reactions, such as cell proliferation, apoptosis, migration, and inflammatory response. These biological responses will damage the vascular endothelial cells, and increase vascular permeability, leading to pathological angiogenesis, and vasomotor disorders, which ultimately can lead to diabetic vascular complications [15].

β 1-integrin protein, one of the integrin family members, is a transmembrane receptor that mediates the connection between cells and their external environment (such as extracellular matrix, ECM). β 1-integrin plays an important role in cell adhesion, migration, survival, angiogenesis, endothelial cell polarity, and lumen formation [16]. Studies have shown that down-regulation of β 1-integrin by miR-223 reduces VEGF-R2 expression and Akt phosphorylation in VEGF-induced endothelial cells [17], and enhances Akt phosphorylation by β 1-integrin activation [18]. VEGF-R2 is one of the main receptors of VEGF in endothelial cells, and VEGF has multiple biological activities, such as enhancing endothelial cell viability in endothelial cells. β 1-integrin may be the

pharmacological target of SmgGDS upregulation. Integrins play a mechanical role in the endothelial cells transducer function, and play an important role in activation through the mechanical stress of VEGF-R2 [19]. Clinical application of non-invasive shock wave and ultrasound angiogenesis therapy are related to β 1-integrin mechanical stimulation of VEGF expression upregulated [20]. Thus, it is envisaged that the pharmacological and mechanical stimulation of β 1-integrin by means of SmgGDS may be a novel therapeutic strategy for cardiovascular disease.

Akt, also known as protein kinase B (PKB), is a serine/threonine-specific protein kinase that plays an important role in various cellular processes, such as glucose metabolism, apoptosis, cell proliferation transcription, and cell migration. Akt1, Akt2, and Akt3 are widely expressed in all mammalian tissues. Akt1 is involved in cell survival through inhibition of apoptosis, and also induces protein synthesis [21]. Studies have shown that only Akt1 is involved in SmgGDS expression [8]. Akt activator (SC79) treatment significantly upregulated SmgGDS expression, indicating that Akt played an important role in SmgGDS expression. In addition, Akt is a known activator of eNOS [22] and has been shown potentially interact with nitric oxide (NO) production, which may be a key beneficial pleiotropic effect of statins. In this study, β 1-integrin/Akt1 signal transduction was regarded as one of new therapeutic targets of statin in the cardiovascular protection. More experiments are needed in the future to demonstrate the feasibility of SmgGDS upregulation as a potential therapeutic target for cardiovascular disease.

Our research has many limitations. Firstly, in our study, we only carried out cell experiments, animal experiments still need to be further confirmed. Secondly, in the human body whether there are the same results need to be tested in the future. Lastly, the clinical application of statin dose and experimental dose have some differences, and we need further research to invest.

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Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

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