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Sitagliptin inhibits vascular inflammation via the SIRT6-dependent signaling pathway

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ABSTRACT

Sitagliptin has recently been shown to inhibit inflammatory response in cardiovascular disease. Sirtuin6 (SIRT6), a NAD^+-dependent class III histone deacetylase, participates in the regulation of cellular inflammation. We hypothesized that sitagliptin could attenuate vascular inflammation via modulation of SIRT6 pathway. It was found that sitagliptin decreased the expression of monocyte chemotactic protein-1 (MCP-1), interleukin (IL)-6 and IL-1β, but up-regulated SIRT6 expression, both in mice and in TNF-α-stimulated endothelial cells. Moreover, knockdown of SIRT6 reversed the inhibitory effect of sitagliptin on MCP-1, IL-6 and IL-1β expression. Further study revealed that sitagliptin also decreased the expression of MCP-1, IL-6 and IL-1β partly through suppression of reactive oxygen species (ROS). In vivo, hypercholesterolemia in mice was induced by intraperitoneal administration of poloxamer 407 for 1 month. Hyperlipidemia-induced production of MCP-1, IL-6 and IL-1β was significantly suppressed in the sitagliptin-supplemented animals, but the effect of was abolished by SIRT6 knockout in endothelium. These results indicate that sitagliptin protects against vascular inflammation via the SIRT6/ROS-dependent signaling pathway.

1. Introduction

Sitagliptin, as a highly selective DPP-4 inhibitor, was used for the treatment of type 2 diabetes mellitus (T2DM). It can be used alone or in combination with other drugs, such as metformin [1]. Recently, many researches have turned attention to the vascular effects of DPP-4 inhibitors, and it has been found that DPP-4 inhibitors could ameliorate endothelial function in T2DM patients, rats and normoglycemic apolipoprotein-E-deficient mice [2,3]. Sitagliptin also increased circulating endothelial progenitor cells in patients with type 2 diabetes, which participates in repair of vascular endothelial injury [4]. Another study indicated that sitagliptin treatment could improve the endothelium-dependent relaxation and attenuate the vascular endothelial impairment of Zucker diabetic fatty rats [5]. However, little is known about the effect of sitagliptin on inflammation response in vascular endothelial cells.

Sirtuin 6 (SIRT6) is one of the sirtuin family members, a kind of NAD^+-dependent histone deacetylase and ADP-ribose transferase enzyme. It plays an important role in a variety of physiological and pathological processes in CVDs, including chronic inflammation, diabetes, cardiac hypertrophy, obesity [6–8]. In addition to mediating these processes, SIRT6 expression is decreased in endothelial cells under stimulation with lipopolysaccharide (LPS) [9], hydrogen peroxide [10] and high glucose [11–13], all of that are risk factors associated with endothelial dysfunction and atherogenesis. In our previous studies, we found that SIRT6 exerts potential anti-inflammatory effect in vascular adventitial fibroblasts (VAFs) [14]. Moreover, a recent study demonstrated that DPP-4 inhibitors may promote a more stable plaque phenotype and it could prevent downregulation of SIRT6 expression in diabetic patients [11]. However, whether the regulation of sitagliptin on inflammation is mediated by SIRT6 remains unknown.

In the present study, we investigated the potential role of sitagliptin on inflammation and SIRT6 expression in Human umbilical vein endothelial cells (HUVECs), and examined whether SIRT6 was involved in the inhibition of sitagliptin against proinflammatory factor induction.

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2. Materials and methods

2.1. Materials

TNF-α was purchased from PeproTech (Rocky Hill, NJ, USA). The goat anti-rabbit IgG (H + L) was purchased from Abbkine (USA). 4′, 6-Diamidino-2-phenylindole (DAPI), reactive Oxygen Species Assay Kit and reactive oxygen species (ROS) scavenger *N*-acetyl-L-cysteine (NAC) were obtained from Beyotime (Haimen, China). Lipofectamine 2000 transfection reagent and TRIzol reagent were obtained from Invitrogen (Carlsbad, CA, USA). Protease inhibitor cocktail was obtained from Roche (Roche, Mannheim). All-in-One cDNA Synthesis SuperMix and SYBR Green qPCR Master Mix were purchased from Bimake (Houston, TX, USA). Poloxamer 407 (P-407) was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Sitagliptin (purity > 98%) was purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). Mice TNF-α and IL-1β ELISA assay kit was product of TBhealthcare (Foshan, China).

2.2. Cell culture and treatment

Human umbilical vein endothelial cell line (CRL-1730) was purchased from the American Type Culture Collection (ATCC). HUVECs were grown in DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere with 95% O_2 and 5% CO_2 at 37 °C. When cells were grown to confluence, culture medium was replaced with serum-free medium for 24 h incubation prior to experimental use.

2.3. Mice

$SIRT6^{flox/flox}$ mice and C57BL/6J Tie2-Cre mice were obtained from Jackson Laboratory. The method of generate endothelium specific SIRT6 knockout mice ($SIRT6^{endo-/-}$ mice) as described [15]. The mouse genotype was confirmed by reverse transcription–polymerase chain reaction. The experimental protocol was in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and was approved by the Institutional Animal Care Committee of Xi'an Jiaotong University. All animals were provided standard rodent chow and water ad libitum in a temperature-controlled room. Mice were injected i.p. with 500 mg/kg/d P-407 solution every day for 1 month to establishment hyperlipidemia. To study the effect of sitagliptin on the protection of vascular wall, mice were treated with the sitagliptin (50 mg/kg, p.o.). The aorta was used for protein and RNA isolation.

2.4. Immunofluorescence staining

HUVECs were fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.5% Triton X-100 in PBS and blocked with normal goat serum at room temperature for 1 h. The cells were incubated overnight with rabbit anti-SIRT6 antibody (1:200) at 4 °C, followed by the application of goat anti-rabbit IgG (H&L) antibody (1:1000). The nucleus was stained with DAPI (1 μg/mL). The slides were imaged under an Olympus fluorescent microscope (Olympus, Tokyo, Japan). Fluorescence intensity was quantified using ImageJ software.

2.5. RNA interference

The small interfering RNA (siRNA) and negative control siRNAs were obtained from GenePharma Corporation (Shanghai, China). HUVECs were transfected with either with SIRT6 siRNA or with negative control siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Table 1
Primer sequences used in real-time PCR.

Gene	Primer	Sequence (5′-3′)
Mice GAPDH	F	CATGGCCTTCCGTGTTCCTA
	R	GCGGCACGTCAGATCCA
Mice SIRT6	F	CATGGGCTTCCTCAGCTTCC
	R	CACTTGGGACATTCCTCTACAAACA
Mice MCP-1	F	GTTGGCTCAGCCAGATGCA
	R	AGCCTACTCATTGGGATCATCTTG
Mice IL-6	F	AGAGGAGACTTCACAGAGGATACC
	R	AATCAGAATTGCCATTGCACAAC
Mice IL-1β	F	CTGTGTCTTTCCCGTGGACC
	R	CAGCTCATATGGGTCCGACA
Human GAPDH	F	GGAGCGAGATCCCTCCAAAAT
	R	GGCTGTTGTCATACTTCTCATGG
Human SIRT6	F	CCCACGGAGTCTGGACCAT
	R	CTCTGCCAGTTTGTCCCTG
Human MCP-1	F	CAGCCAGATGCAATCAATGCC
	R	TGGAATCCTGAACCCACTTCT
Human IL-6	F	CCTGAACCTTCCAAAGATGGC
	R	TTCACCAGGCAAGTCTCCTCA
Human IL-1β	F	TTCGACACATGGGATAACGAGG
	R	TTTTTGCTGTGAGTCCCGGAG

2.6. Western blotting

Primary antibody against SIRT6 (1:1000) was purchased from Abcam. Primary antibody against β-actin (1:1000) was product of CMCTAG. Western blotting was performed as previously described [16]. The intensity of protein bands was analyzed by Lane 1D software (Sage Creation Science Co, China).

2.7. Real-time polymerase chain reaction (PCR)

RNA was extracted from cultured HUVECs and aortas of mice with TRIzol reagent. The RNA was reversely transcripted to cDNA using All-in-One cDNA Synthesis SuperMix (Bimake). The mRNA levels of target genes were detected using SYBR Green qPCR Master Mix by StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, USA). Relative changes in mRNA levels were analyzed by the $^{\Delta\Delta}$CT method, using GAPDH served as control. Primer pair sequences are shown in Table 1.

2.8. Cytokine analysis by enzyme-linked immunosorbent assay (ELISA)

The concentration of TNF-α and IL-1β in serum of mice was detected by ELISA assay. The ELISA kit is commercially available and the manufacturers' instructions are followed.

2.9. Measurement of intracellular ROS production

Intracellular ROS levels were measured with DCFH-DA fluorescent probes. HUVECs were pretreated with different concentrations of sitagliptin (0.25, 0.5, and 1 μM) for 1 h in serum-free medium prior to the addition of TNF-α (20 μg/L) for 8 h. Then, the cells were loaded with DCFH-DA (10 μM) for 30 min at 37 °C. The fluorescence was observed with an Olympus fluorescent microscope (Olympus). The relative fluorescence intensity was measured and analyzed with ImageJ software.

2.10. Statistical analysis

Data are expressed as mean ± SEM from three independent experiments. Statistical analyses between two groups were performed by unpaired student's *t*-test, multiple groups were assessed by one-way ANOVA followed by Tukey's test. In all cases, differences were considered statistically significant with $P < 0.05$.

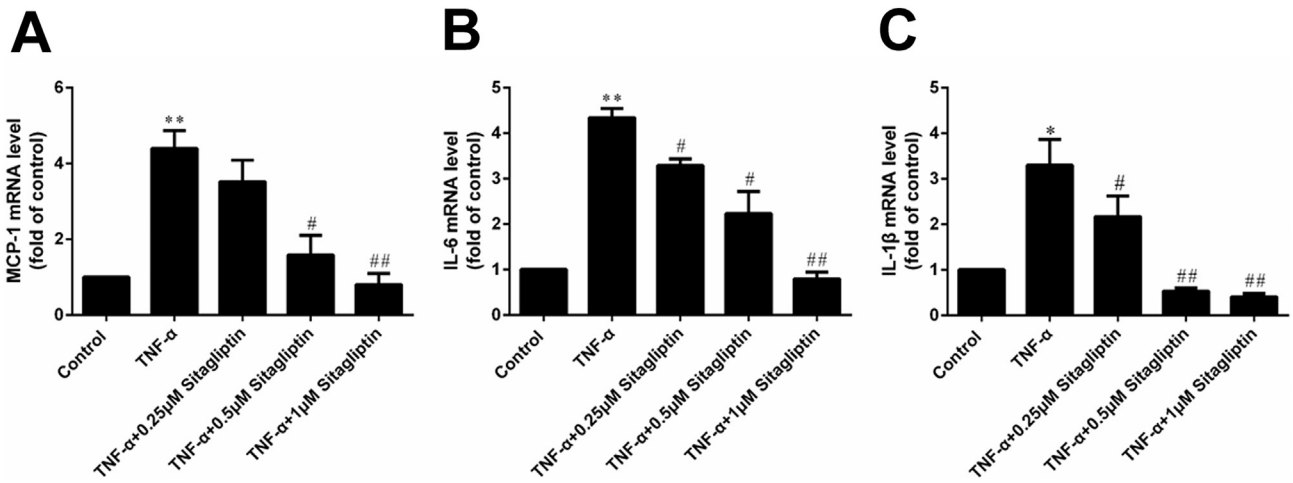

Fig. 1. Sitagliptin inhibits TNF-α-induced inflammation in HUVECs. The cells were pretreated with Sitagliptin (0.25, 0.5, and 1 μM) for 1 h, and then stimulated with TNF-α (20 μg/L) for 8 h. (A–C) The mRNA levels of MCP-1, IL-6 and IL-1β were determined by real-time PCR. Data are expressed as mean ± SEM, n = 3. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. TNF-α group.

3. Results

3.1. Sitagliptin inhibits inflammation in HUVECs stimulated with TNF-α

To determine the effect of sitagliptin on the activation of inflammation in HUVECs, the cells were pretreated with different concentrations of sitagliptin (0.25, 0.5, and 1 μM) for 1 h, and then stimulated with TNF-α (20 μg/mL) for 8 h. As shown in Fig. 1, stimulating the cells with TNF-α increased the mRNA levels of monocyte chemotactic protein-1 (MCP-1), interleukin (IL)-6 and IL-1β, which were obviously inhibited by the pretreatment with sitagliptin. These results imply that sitagliptin has the ability to inhibit TNF-α-induced the activation of inflammation in HUVECs.

3.2. Sitagliptin up-regulates SIRT6 expression in HUVECs stimulated with TNF-α

To investigate the effect of sitagliptin on SIRT6 in HUVECs, the cells were treated with different concentrations of sitagliptin (0.25, 0.5, and 1 μM) for 8 h. As shown in Fig. 2A and B, the protein and mRNA levels of SIRT6 were increased by the treatment with sitagliptin. Based on the above results, we further explored whether SIRT6 was involved in the inhibitory effect of sitagliptin on inflammation activation. HUVECs were pretreated with sitagliptin (0.25, 0.5, and 1 μM) for 1 h, and then stimulated with TNF-α (20 μg/L) for 8 h. The expressions of SIRT6 protein and mRNA were determined by Western blotting and real-time PCR, respectively. As shown in Fig. 2C and D, sitagliptin significantly upregulated the expression of SIRT6 protein and mRNA compared with the TNF-α group in HUVECs. Based on the results with western blotting and real-time PCR, we further examined the effect of sitagliptin (1 μM) on the level of SIRT6 by immunofluorescent analysis. Similarly, the results revealed that SIRT6 expression in HUVECs was also increased after the pretreatment of sitagliptin (Fig. 2E and F).

3.3. Sitagliptin inhibits TNF-α-induced the activation of inflammation via SIRT6

Given our findings that sitagliptin could up-regulate SIRT6 expression and inhibit inflammation activation, we examined the possibility whether the inhibitory effect of sitagliptin on inflammation is related to SIRT6. HUVECs were transfected with SIRT6-specific siRNA or a scrambled siRNA, and then the protein expression of SIRT6 was explored. As expected, SIRT6 expression was significantly declined and knockdown efficiency of SIRT6 was 71% as determined by western blotting (Fig. 3A). The transfected HUVECs were pretreated with sitagliptin (1 μM), and subsequently stimulated with TNF-α (20 μg/L). We found that the inhibitory effect of sitagliptin on the expression of MCP-1, IL-6 and IL-1β was abolished by SIRT6 knockdown (Fig. 3B, C and D), indicating that regulation of MCP-1, IL-6 and IL-1β expression by sitagliptin depends on SIRT6. Together, our findings imply that the modulatory effect of sitagliptin on inflammation in TNF-α-stimulated HUVECs is mediated by SIRT6.

3.4. Sitagliptin suppresses ROS generation in HUVECs stimulated with TNF-α

Oxidative stress is critically considered to be a triggering factor during the activation of inflammation [17]. Therefore, we assessed whether ROS was involved in the inhibition of sitagliptin on inflammation activation. The cells were pretreated with sitagliptin (0.25, 0.5, and 1 μM) before exposure to TNF-α (20 μg/L), and intracellular ROS levels were evaluated with the fluorescence intensity of DCFHDA. As shown in Fig. 4, TNF-α caused an increase in ROS in HUVECs compared with control group. Furthermore, the generation of ROS induced by TNF-α was significantly inhibited by pretreatment with sitagliptin. These results indicate that sitagliptin could suppress TNF-α-induced the generation of ROS in HUVECs.

3.5. Sitagliptin regulates TNF-α-induced inflammation in HUVECs partly via suppression of ROS generation

As mentioned above, sitagliptin is able to inhibit inflammation activation, and reduce ROS generation in TNF-α-stimulated HUVECs. To confirm whether sitagliptin inhibits the activation of inflammation partly via ROS, a known ROS scavenger NAC was used in the study. The cells were subjected to ROS scavenger (10 mM) for 1 h, followed by treatment of sitagliptin (1 μM) for further 1 h, and subsequently stimulated with TNF-α (20 μg/L). As shown in Fig. 5, ROS scavenger significantly reduced the expression of MCP-1, IL-6 and IL-1β in TNF-α-stimulated HUVECs. Treatment with a combination of ROS scavenger and sitagliptin synergistically reversed TNF-α-induced increase of MCP-1, IL-6 and IL-1β expression in comparison with treatment of sitagliptin alone. These preliminary data suggest that the inhibition of sitagliptin on the activation of inflammation may be partly mediated by suppression of ROS generation.

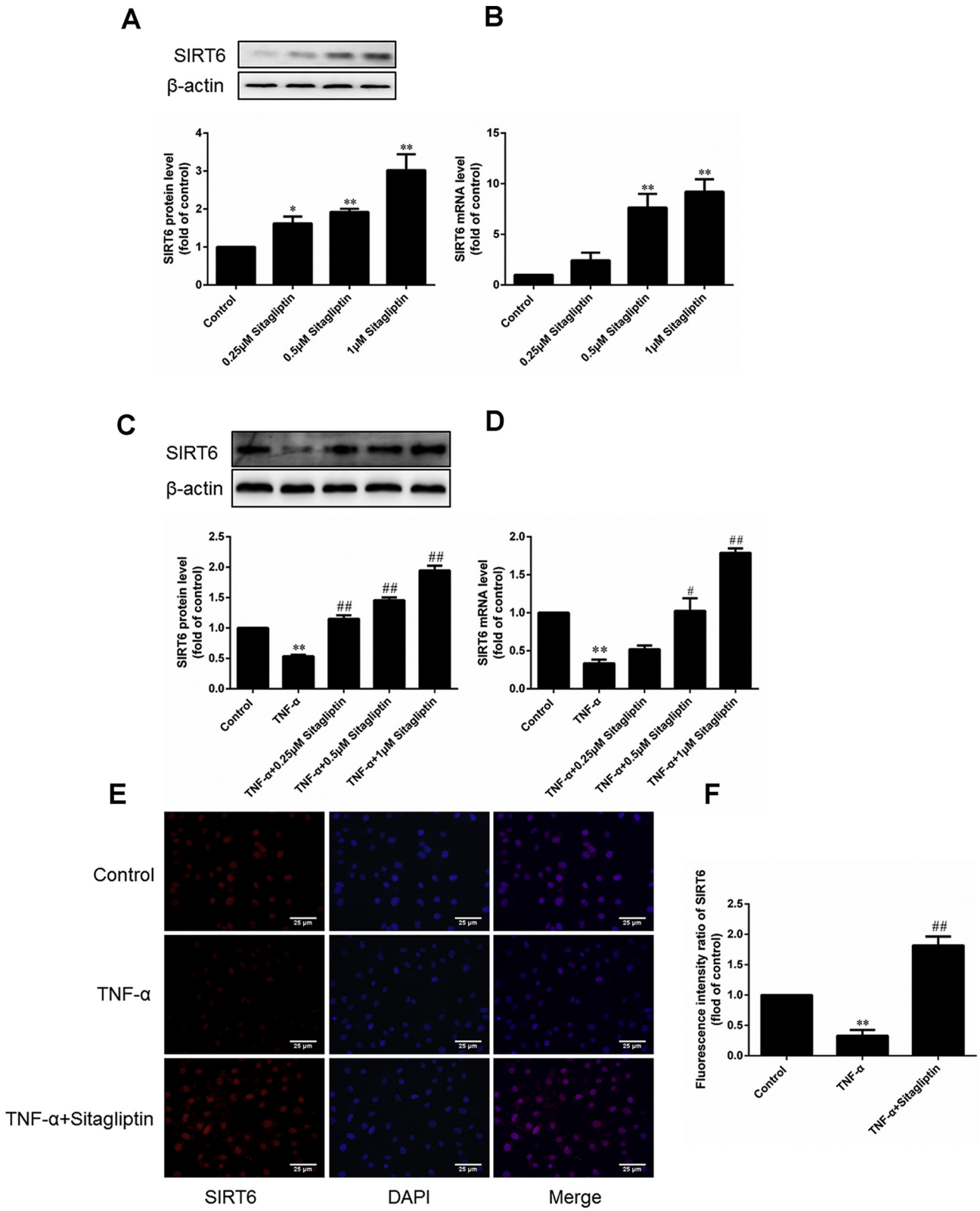

Fig. 2. Sitagliptin up-regulates the expression of SIRT6 in HUVECs stimulated with TNF-α. (A–B) The cells were pretreated with sitagliptin at concentrations of 0.25, 0.5, and 1 μM for 8 h. The expression of SIRT6 protein and mRNA was determined by western blotting and real-time PCR, respectively. (C–D) The cells were pretreated with sitagliptin at concentrations of 0.25, 0.5, and 1 μM for 1 h, and then followed by stimulation with TNF-α (20 μg/L) for 8 h. The expression of SIRT6 protein and mRNA was determined by western blotting and real-time PCR, respectively. (E) HUVECs were pretreated with sitagliptin (1 μM) for 1 h prior to incubation with TNF-α (20 μg/L) for 8 h. SIRT6 was stained with an anti-SIRT6 antibody. The nuclear protein was stained with DAPI. Merge represents the combined image of fluorescence and nucleus staining (bar = 25 μm). (F) Relative fluorescence intensity of SIRT6 staining was quantified from the fluorescence images. Data are expressed as mean ± SEM, n = 3. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. TNF-α group.

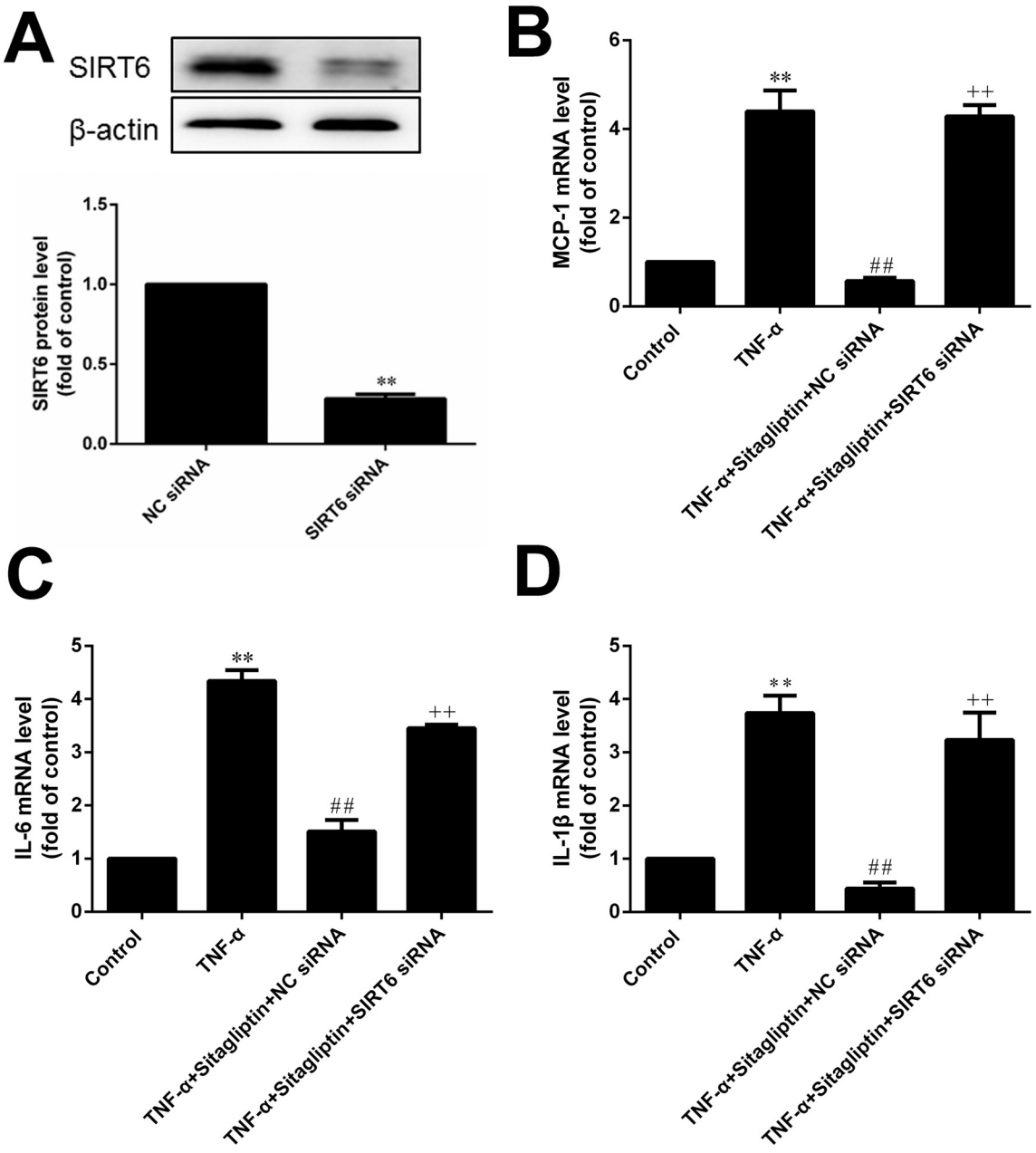

Fig. 3. Sitagliptin inhibits TNF-α-induced inflammation via SIRT6 in HUVECs. (A) HUVECs were transfected with SIRT6-specific siRNA (SIRT6 siRNA) or negative control siRNA (NC siRNA) for 48 h. The expression of SIRT6 was examined by western blotting. (B–D) The SIRT6 transfected HUVECs were pretreated with sitagliptin (1 μM for 1 h), and then stimulated with TNF-α (20 μg/L) for 8 h. The mRNA levels of MCP-1, IL-6 and IL-1β were detected by real-time PCR. Data are expressed as mean ± SEM, n = 3. $^{**}P < 0.01$ vs. control group. $^{\#\#}P < 0.01$ vs. TNF-α group. $^{++}P < 0.01$ vs. NC siRNA + TNF-α + sitagliptin group.

3.6. Sitagliptin increases the expression of SIRT6 in mice with hyperlipidemia

To confirm whether SIRT6 expression could be regulated by sitagliptin in vivo, mice were injected with P-407 (500 mg/kg, i.p.) solution every day for 1 month to establishment hyperlipidemia. And the mice were treated with the sitagliptin (50 mg/kg/d, p.o.). Relative to the model animals, gavage with sitagliptin increased SIRT6 protein and mRNA expression in aortas (Fig. 6A and B). In addition, we also observed the effects of sitagliptin on protein and mRNA levels of SIRT6 were inhibited in SIRT6$^{endo-/-}$ mice.

3.7. Sitagliptin protects against vascular inflammation and reduces serum levels of TNF-α and IL-1β in mice with hyperlipidemia

We further observed the effects of sitagliptin on the expressions of MCP-1, IL-6 and IL-1β in the aortas of mice. Relative to the P-407 group, supplementation with sitagliptin decreased the endothelial expression of MCP-1, IL-6 and IL-1β in aortas. However, the inhibitory effect of sitagliptin on the expression of MCP-1, IL-6 and IL-1β was abolished by SIRT6 knockout in endothelium (Fig. 7A–C). In addition, we also observed a notable reduction of serum levels of TNF-α and IL-1β in mice with sitagliptin supplementation, but SIRT6 knockout in endothelium has no effect on serum inflammatory factor (Fig. 7D and E). These results indicate that sitagliptin has ability to reduce vascular inflammation via SIRT6, and to repress serum TNF-α and IL-1β in the animals.

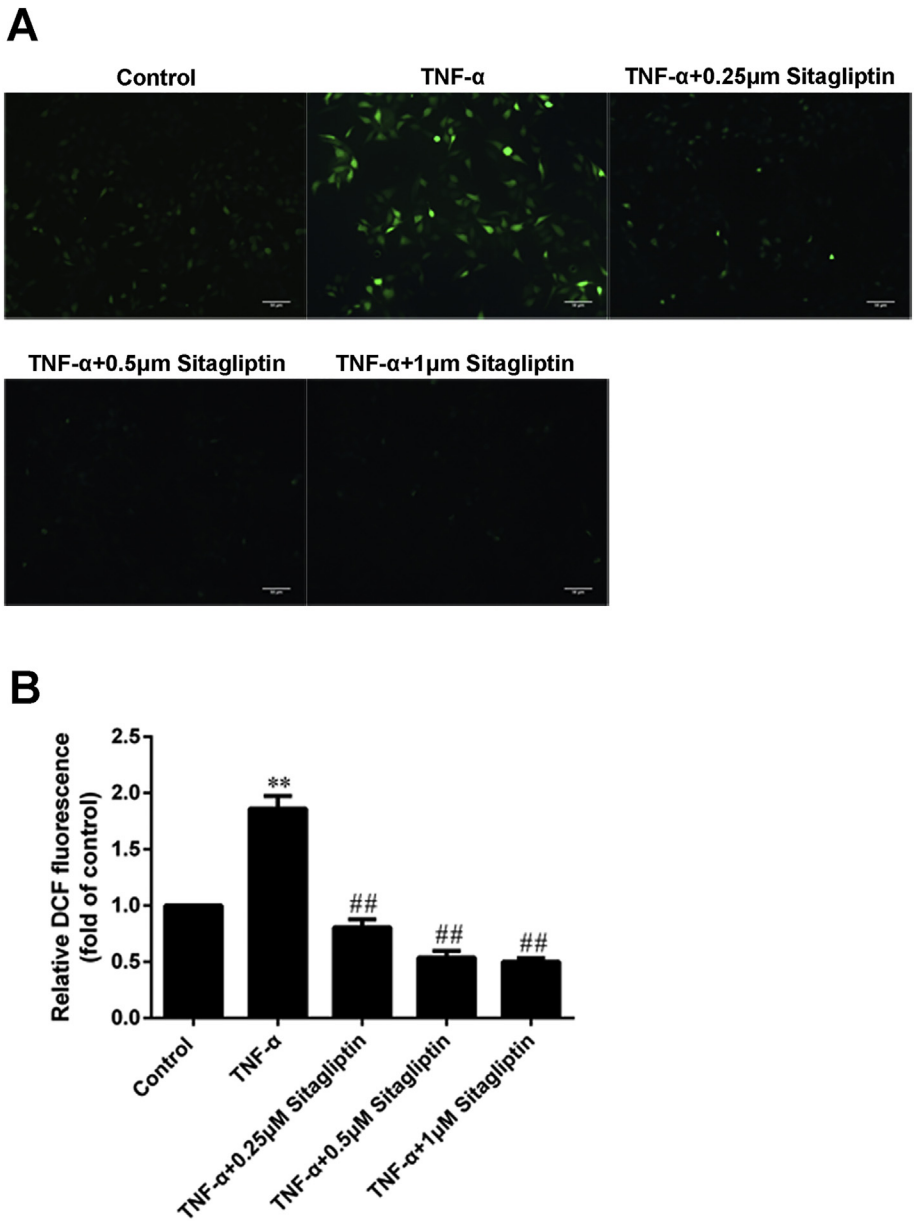

Fig. 4. Effect of sitagliptin on the intracellular ROS generation in HUVECs stimulated with TNF-α. HUVECs were pretreated with sitagliptin (0.25, 0.5, and 1 μM) for 1 h prior to stimulation with TNF-α (20 μg/L) for 8 h, and then incubated with DCFH-DA (10 μM) for 20 min. (A) Representative fluorescence images observed with a fluorescent microscope (bar = 50 μm). (B) Relative fluorescence intensity quantified from fluorescence images. Data are expressed as mean ± SEM, n = 3. $^{**}P < 0.01$ vs. control group. $^{\#\#}P < 0.01$ vs. TNF-α group.

4. Discussion

In the past few years, the beneficial effects of sitagliptin have historically been regarded as a result of its glucose-modifying activity [18]. Recently, the anti-inflammatory potential of sitagliptin has begun to emerge [19,20]. Herein, we demonstrated that sitagliptin inhibited the inflammation in HUVECs. Importantly, our results also showed that sitagliptin exerted the inhibitory effect through up-regulation of SIRT6 expression and suppression of ROS generation.

Emerging studies suggest that the chronic inflammation is closely related to cardiovascular disease [21,22]. It has been showed that sitagliptin has the anti-inflammatory potential in cardiovascular disease [23]. A previous study showed that sitagliptin can reduce the nuclear factor κB1 mRNA in TNF-α-stimulated human vascular endothelial cells [24]. Another report indicated that sitagliptin decreased TNF-α, IL-6, and IL-8 level induced by LPS in the human lung microvascular EC [25]. In the present study, we demonstrated that sitagliptin inhibited MCP-1, IL-6 and IL-1β in TNF-α-stimulated HUVECs. In addition to in vitro experiment, our study also indicated that sitagliptin evidently suppressed the increase of MCP-1, IL-6 and IL-1β mRNA in aortas and IL-1β and TNF-α in serum of the mice with hypercholesterolemia. In light of these findings, sitagliptin may exert an inhibitory effect on the inflammation in HUVECs.

SIRT6 has been generally considered as a key regulator in a wide variety of cellular processes including inflammation [26]. Therefore, we further investigated whether SIRT6 was involved in the inhibitory effect of sitagliptin on inflammation. Balestrieri et al. reported DPP-4 inhibitors could increase the SIRT6 expression in carotid plaques of diabetic patients [11]. The present study showed that the expression of SIRT6 was decreased in the arteries of mice with hyperlipidemia. After the administration of sitagliptin, the protein and mRNA expression of SIRT6 in arteries significantly increased. Our previous study has

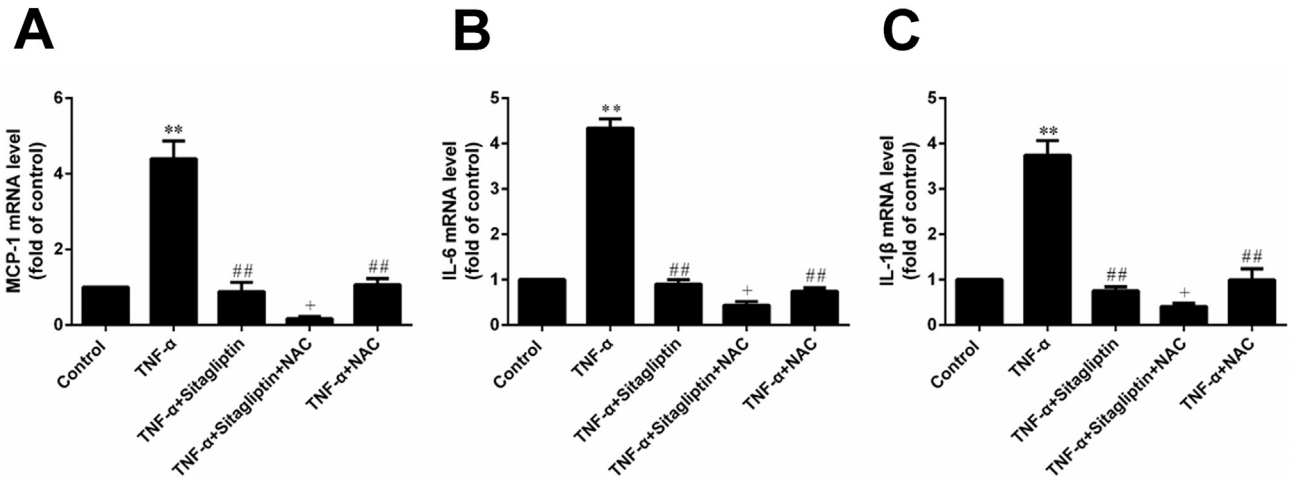

Fig. 5. Sitagliptin regulates TNF-α-induced inflammation in HUVECs partly via suppression of ROS generation. HUVECs were pretreated with ROS scavenger NAC (10 mM) for 1 h prior to stimulation with sitagliptin (1 μM) for 1 h, and then stimulated with TNF-α (20 μg/L) for 8 h. (A–C) The mRNA levels of MCP-1, IL-6 and IL-1β were determined by real-time PCR. Data are expressed as mean ± SEM, n = 3. $^{**}P < 0.01$ vs. control group. $^{\#\#}P < 0.01$ vs. TNF-α group. $^{+}P < 0.05$ vs. TNF-α + sitagliptin group.

showed that SIRT6 expression was significantly reduced in TNF-α-stimulated VAFs [14]. Interestingly, our present study also revealed that stimulation with TNF-α led to a marked decrease of SIRT6 expression in HUVECs. After pretreatment with sitagliptin, the expression of SIRT6 was significantly increased in a concentration-dependent manner. A recent report revealed that icariin upregulated the expression of SIRT6 and had an inhibitory effect on NF-κB inflammatory signaling pathways [27]. And we also found sitagliptin could increase SIRT6 protein and mRNA without stimulation. The results are in concert with our findings that sitagliptin could up-regulate the level of SIRT6 in the arteries of mice with hyperlipidemia. Sitagliptin, as a selective DPP-4 inhibitor, improves glycaemic control by inhibiting DPP-4 inactivation of the endogenous incretin hormones glucagon-like peptide 1 (GLP-1). We also determine the effect of sitagliptin on expression of GLP-1 in HUVECs stimulated by TNF-α. Sitagliptin from 0.25 to 1 μM concentration-dependently increased GLP-1 expression in HUVECs (Fig. S2). But whether sitagliptin affects SIRT6 expression through GLP-1 needs to be further studied. These in vivo and in vitro studies imply that sitagliptin

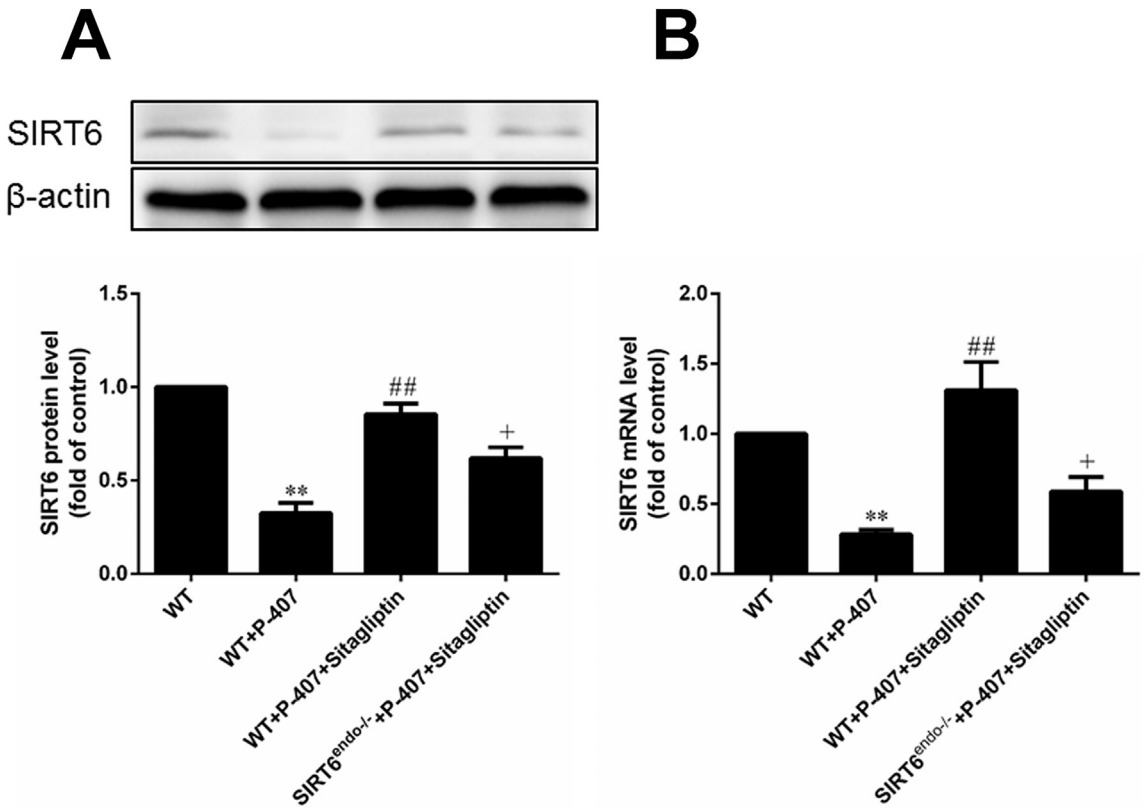

Fig. 6. Sitagliptin up-regulates SIRT6 expression in mice. Mice were injected with P-407 (500 mg/kg/d, i.p.) solution for 1 month to establishment hyperlipidemia. And the mice were treated with the sitagliptin (50 mg/kg/d, p.o.). (A and B) The expression of SIRT6 protein and mRNA in aortas was determined by western blotting and real-time PCR, respectively. Data are expressed as mean ± SEM. $n = 9$. $^{**}P < 0.01$ vs. WT. $^{\#\#}P < 0.01$ vs. WT + P-407. $^{+}P < 0.05$ vs. WT + P-407 + sitagliptin group.

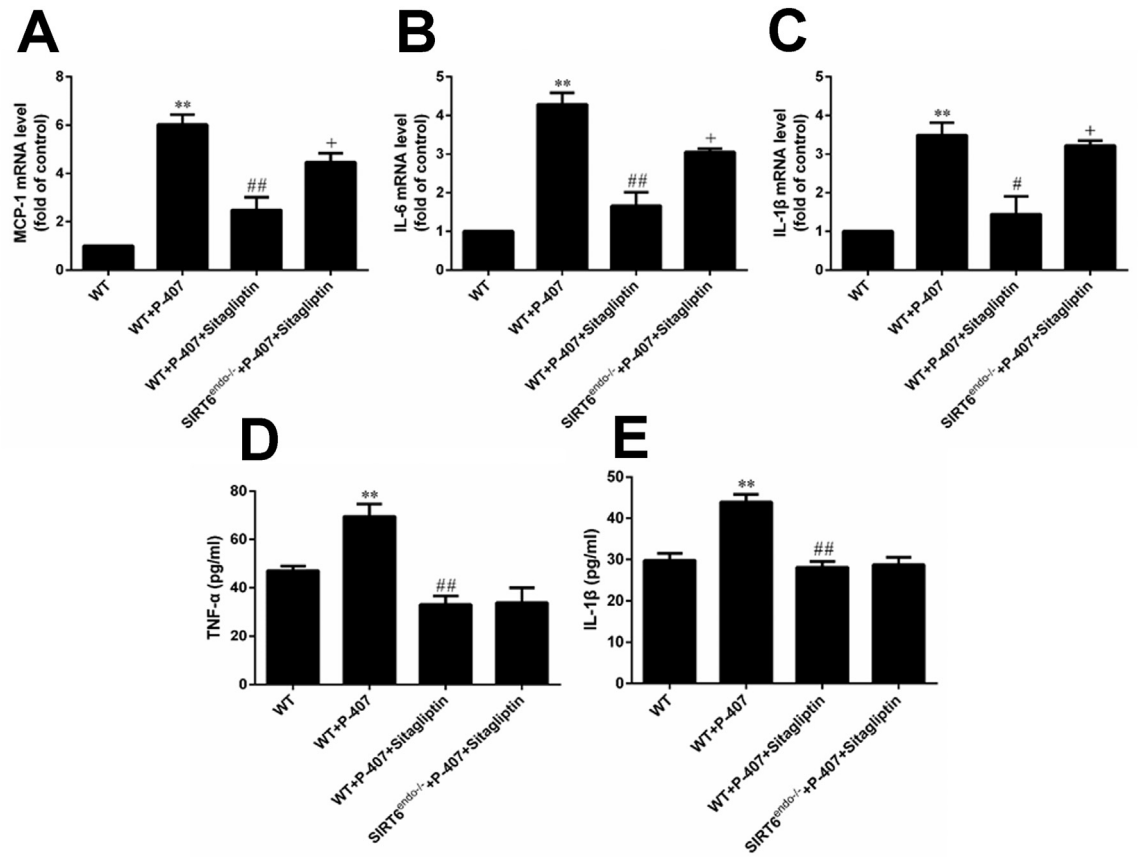

Fig. 7. Sitagliptin inhibits vascular inflammation in aorta and serum. The mice were treated as described in the legend to Fig. 6. (A–C) The expression of MCP-1, IL-6 and IL-1β mRNA in aorta was determined by real-time PCR. (D and E) Serum TNF-α and IL-1β was detected by ELISA analysis. Data are expressed as mean ± SEM. n = 9. $^{**}P < 0.01$ vs. WT. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. WT + P-407. $^{+}P < 0.05$ vs. WT + P-407 + sitagliptin group.

was able to increase SIRT6 expression, which may play an important role in the regulation of the inflammatory process.

On the basis of the results mentioned above, we further investigated whether the anti-inflammation effect of sitagliptin was mediated via SIRT6-dependent pathway. Accordingly, SIRT6 siRNA was applied to HUVECs to clarify whether SIRT6 was involved in the inhibitory effects of sitagliptin. Here, we found that lack of SIRT6 abolished the inhibition of sitagliptin on inflammation in HUVECs treated with TNF-α. In our previous report, we found that SIRT6 exerted anti-inflammatory property by regulating TNF-α-induced expression of MCP-1 and IL-6 in vascular adventitial fibroblasts [14]. In vivo study, we also found that SIRT6 knockout in endothelium could remove the effect of sitagliptin on inflammation. That suggesting that SIRT6 may be associated with the inhibition of sitagliptin on inflammation. The data from the present study suggest that sitagliptin may exert an inhibitory effect on inflammation activation through SIRT6.

Considerable evidences suggest that ROS serves as a triggering factor can induce an inflammatory response, and free radicals are inflammation effectors [28]. Ma et al. reported that aloin suppresses lipopolysaccharide-induced inflammation by inhibiting ROS production in RAW264.7 cells [29]. In addition, our previous study showed that nicotinic acid regulates LPS and ATP-induced NLRP3 inflammasome activation in HUVECs partly via suppression of ROS generation [16]. In the present study, treatment of the cells with sitagliptin markedly decreased the production of ROS in HUVECs stimulated with TNF-α. A recent report revealed that sitagliptin protects fatal arrhythmias by attenuating nerve growth factor-induced sympathetic innervation via inhibiting superoxide production by A1 receptors and xanthine oxidase [30]. Our results showed that the ROS scavenger augmented the inhibition of sitagliptin on TNF-α-induced the expression of inflammation. Meanwhile, the ROS scavenger and sitagliptin synergistically suppressed the inflammation in HUVECs. In combine, these preliminary findings indicate that the inhibition of sitagliptin on inflammation may be partly mediated by ROS. In conclusion, our data provides the evidence that sitagliptin could inhibit the inflammation in HUVECs stimulated with TNF-α. Moreover, the inhibitory effect of sitagliptin on inflammation may be mediated by SIRT6 and ROS in HUVECs. This finding revealed a novel mechanism for the anti-inflammatory effects of sitagliptin for treating cardiovascular diseases.

In conclusion, our data provides the evidence that sitagliptin could inhibit the activation of inflammation in ECs stimulated with TNF-α. Moreover, the inhibitory effect of sitagliptin on inflammation activation may be mediated by SIRT6 and ROS in HUVECs (summarized in Fig. 8). This finding revealed a novel mechanism for the anti-inflammatory effects of sitagliptin for treating cardiovascular diseases. In diabetic patients with the cardiovascular injuries or complications, sitagliptin may be considered as a preferable choice in clinical treatment patients with type 2 diabetes.

Declaration of competing interest

The authors state no conflict of interests.

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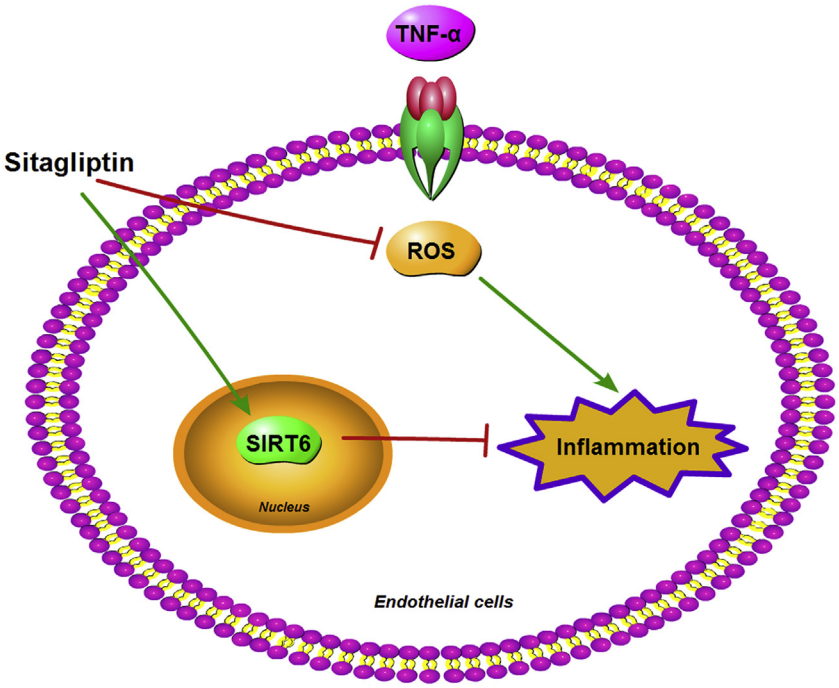

Fig. 8. Schematic illustration of the signaling pathway involved in the effect of sitagliptin on inflammation in HUVECs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105805.

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