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Original Article

Atorvastatin inhibits PDGF-BB induced vascular smooth muscle cells proliferation and migration in cerebrovascular diseases

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Abstract: Backgrounds: Vascular smooth muscle cells (VSMCs) dedifferentiation is known to be critical in the genesis of lots of cerebrovascular diseases like atherosclerosis, intimal hyperplasia, and restenosis. This study aims to verify whether atorvastatin (ATV) inhibits rat brain arterial VSMCs dedifferentiation through down-regulation of krüppel-like transcription factor 4 (KLF4). Material and methods: The primary culture and identification of rat brain arterial VSMCs was conducted and VSMCs in passage 2-8 were used for the following experiments. Platelet derived growth factor-BB (PDGF-BB) was used to induce VSMCs dedifferentiation. KLF4-siRNA or pcDNA3.1+HA-KLF4 were used to suppress or increased the expression of KLF4 in VSMCs respectively. After incubation in different concentrations of ATV, the proliferation and migration ability of the VSMCs were tested by MTT assay, Transwell chambers, and wound healing assay. Immunocytochemistry (ICC) was used to evaluate VSMCs morphology and SM-actin rearrangement. Western blotting was employed to investigate the expressions of SM-actin, calponin, SM-MHC, OPN, KLF4 in VSMCs. Results: PDGF-BB improved the proliferation and migration ability of VSMCs and increased the expressions of calponin, SM-MHC, KLF4. ATV significantly inhibited the induction of these phenotypic changes induced by PDGF-BB. Meanwhile ATV suppressed the expression of KLF4 in the VSMCs. Identical to ATV, down-regulation of KLF4 by KLF-siRNA could also inhibit VSMC dedifferentiation. In addition, up-regulation of KLF4 by pcDNA3.1+HA-KLF4 reversed the ATV-induced inhibition of VSMC dedifferentiation. Conclusion: ATV inhibits PDGF-BB induced VSMCs dedifferentiation by down-regulation of KLF4.

Keywords: Vascular smooth muscle cells, atorvastatin, dedifferentiation

Introduction

Vascular smooth muscle cells (VSMCs) dedifferentiation is known to be critical in the genesis of lots of cerebrovascular diseases like atherosclerosis, intimal hyperplasia, and restenosis [1]. Unlike skeletal or cardiac muscle cells that have undergone terminal differentiation, VSMCs of adult animals retain plasticity. The mature, quiescent, "contractile" phenotype VSMCs could dedifferentiate to the proliferative, "synthetic" phenotype in response to various physiological and pathological factors [2]. Differentiation status of VSMCs in vitro can be measured by testing the proliferation, migration ability and investigating the expression of smooth muscle-specific phenotype marker proteins, including smooth muscle actin (SM-acitn), calponin, smooth muscle myosin heavy chain (SM-MHC) and osteopontin (OPN) [3].

Statins, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, including atorvastatin (ATV), rosuvastatin and simvastatin, exerted a variety of cerebrovascular protective effects including anti-oxidant effects, anti-inflammatory effect, and inhibiting ERS, apoptosis and autophagy in VSMCs which are independent of their lowering cholesterol effect [4-8]. Studies in both basic experiment and clinical trial shown that ATV exhibited anti-atherosclerosis effect by improving endothelial function, resisting oxidation and inhibiting VSMCs proliferation and migration [5, 9, 10]. Our previous study found that ATV attenuated homocysteine-induced VSMCs migration by down-regulation of mevalonate pathway [9]. In addition, Liu et al. demonstrated that by inducing autophagy via suppression of the beta-catenin pathway, ATV protectd VSMCs from TGF-beta1-stimulated calcification [10]. However, further

studies are still needed to elucidate the underlying mechanism how ATV involves in the regulation of VSMCs functions.

Platelet-derived growth factor-BB (PDGF-BB) is a primary regulator of VSMCs proliferation and has been shown to be one of the most robust of VSMCs differentiation-modulating agents [11]. Antibodies against PDGF or PDGF receptors, PDGF aptamers, or antisense oligonucleotides to PDGF-BB receptors inhibited VSMCs accumulation in the intima after balloon injury [12, 13]. In addition, VSMCs lacking PDGF receptor β showed strikingly diminished neointimal accumulation after carotid artery ligation [14]. Moreover, pharmacological inhibition of PDGF signaling decreased VSMC proliferation, migration and dedifferentiation [15]. So, in this experiment, PDGF-BB was chose to be used as the inducer of VSMCs dedifferentiation.

Studies have showed that krüppel-like transcription factor 4 (KLF4) palyed an important role in PDGF-BB induced VSMCs migration and proliferation [16, 17]. Besides, researches in these years demonstrated that up-regulation of KLF4 was essential for VSMCs dedifferentiation [12, 18, 19].

In this study, we aimed to explore whether ATV could attenuate PDGF-BB induced rat brain arterial VSMCs dedifferentiation. Furthermore, we planned to exam whether KLF4 participated in this progress.

Materials and methods

Animals used in this study were in compliance with the Guide for Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of The Second People's Hospital of Jingmen.

Materials

Animals: SPF degree SD rat (50 d, body weight 150-180 g, bought form Nanjing animal center), regardless of gender, were obtained from the animal center laboratory of Shandong Province Institute of Medicine.

Reagents: Dulbecco's modified eagle medium (DMEM)-High Glucose, PBS, 0.25% trypsase-EDTA, Penicillin and streptomycin were purchased from Jinuo Biotech Company (Hangzhou, China). Atorvastatin and PDGF-BB were purchased from Sigma (USA), dimethyl sulfoxide (DMSO) from MP Biomedicals (USA), fetal calf serum (FBS) from GIBCO (USA), MTT from Emresco (USA), and DAPI from Rcohe (USA). Antibodies against SM-actin, calponin, OPN, SM-MHC and β-actin were purchased from Abcam (Cambridge, MA, USA), and horseradish peroxidase-conjugated goat anti-mouse and goat anti-rabbit IgG antibody were purchased from Jackson Immuno Research Laboratories (USA). Gelatin was purchased from Abcam (Cambridge, MA, USA). The other reagents for immunoblot assay were purchased from Beyotime (Jiangsu, China). NC-siRNA and KLF4-siRNA were synthesized chemically in Suzhou GenePharma Co. Ltd. (Suzhou, China). Empty vector and pcDNA3.1+HA vector constructed with KLF4 gene was purchased from Life Technologies (Invitrogen, CA, USA).

Cell culture

VSMCs were primarily cultured using enzyme digestion method from rat brain aorta. The VSMCs were identified through immunofluorescence detection of SM-actin and ensured their purity through multiple fluorescent staining with DAPI and SM-actin antibody. Cells between 3 to 5 generations were used in the following experiments.

For transfection, after the cells were cultured to 70-80% confluence, NC-siRNA or KLF4-siRNA were transfected by using Lipofectamine 2000 (Invitrogen, CA, USA) according to the manufacturer's instructions for inhibition the expression of KLF4 in the cells. Meanwhile, pcDNA3.1+HA-KLF4 or pcDNA3.1+HA empty vector were transfected by using Lipofectamine 2000 for overexpression of KLF4 in the cells. Then, PDGF-BB (20 ng/ml) was added to induce VSMCs dedifferentiation. After incubation in PDGF-BB for 12 h, VSMC were treated with vehicle (ethanol) or different concentrations of ATV (1 μM, 10 μM, 100 μM).

MTT assay

Normal VSMCs or VSMCs transfected with KLF4-siRNA or DNA3.1+HA-KLF4 were used in this experiment. About 6×10^3 cells were seeded into 96-well plates per well. After 24 h incubation with non-serum DMEM and 12 h with PDGF-BB, cells were treated with different concentrations of atorvastatin and incubated at

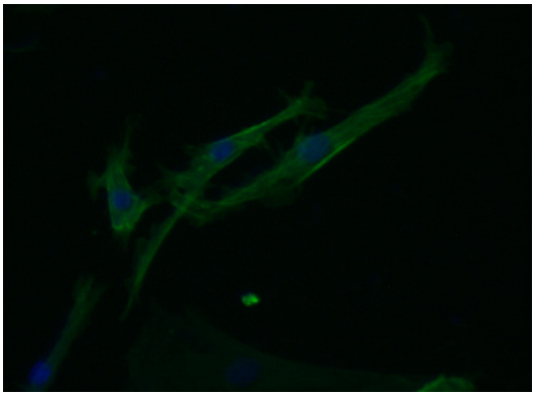

Figure 1. Identification of the VSMCs by immunocytochemistry. VSMCs were SMα-actin positive showed by immunofluorescent staining.

37°C under 5% CO_2. 12 h, 24 h, and 48 h later, 20 ul MTT solution was added to each well, followed by 4-6 h incubation at 37°C. The supernatant was removed with a pipette, and 150 μl dimethyl sulfoxide (DMSO) was added to each well. After 10 min of incubation at room temperature, plates were read on a microplate reader (Anthos, Austria) at 490 nm. Values were normalized using the control value.

Wound-Healing Assay

Normal VSMCs or VSMCs transfected with KLF4-siRNA or DNA3.1+HA-KLF4 were used in this experiment. When cells grew to 80% confluence, they were cultured in serum-free medium containing hydroxyurea for 24 h to synchronize cells and suppress cells proliferation. After 12 h incubation with PDGF-BB, the wound were created by manually scraping the cell monolayer with a 100 ul pipet tip. After being washed to remove the isolated cells, different concentrations of atorvastatin were added to each group. Multiple photographs of the wounds were then taken at 0 and 18 h post-wounding under an inverted Nikon microscope (Nikon Corporation, Tokyo, Japan) at a ×200 magnification. The migration area was analyzed with Image-pro plus 6.0 (Media Cybernetics Inc., Bethesda, MD, USA) and the ratio of the cellular re-coverage area to the whole wound area was used to evaluate cells migration.

Transwell assay

Normal VSMCs or VSMCs transfected with KLF4-siRNA or DNA3.1+HA-KLF4 were used in this experiment. After being synchronized by serum free medium, cells were seeded into the upper chamber of the Transwell inserts (2×10^4 cells per well) (Corning, St. Lowell, MA). Cells were subsequently allowed to migrate for 18 h at 37°C. The cells on the upper side of the inserts were softly scraped off. Cells that migrated to the lower side of the inserts were fixed with 4% paraformaldehyde and stained with crystal violet (1 ug/ml), and then the cells from nine independent, randomly chosen visual fields were counted under an microscope (×100 magnification) for quantification of cells.

Immunohistochemistry

Normal VSMCs or VSMCs transfected with KLF4-siRNA or DNA3.1+HA-KLF4 were used in this experiment. VSMC were cultured on glass coverslips. After 24 h incubation with non-serum DMEM and 12 h with PDGF-BB, cells were treated with different concentrations of atorvastatin and incubated at 37°C under 5% CO_2 for 24 h. Cells were washed with PBS and fixed in 4% paraformaldehyde. Cells were permeabilized by 0.1% Triton. Then, VSMCs were blocked with 10% goat serum in PBST and incubated with SMα-actin antibodies in PBST for 1 h. After washing, anti-mouse fluorescein isothiocyanate (FITC) conjugated second antibody was incubated for 1 h and washed. Coverslips were then processed for immunofluorescent microscopy.

Quantitative real-time PCR

After transfected with KLF4-siRNA, NC-siRNA or DNA3.1+HA-KLF4, empty vector. Total RNA was extracted from VSMCs by using trizol reagent (Invitrogen, USA) and then miRNA were reverse transcribed to cDNA by using reverse transcription kit (Takara, Japan). Quantitative real-time PCR (qRT-PCR) were performed by using SYBR Green PCR Kit on ABI 7500 Fast Real-Time PCR system according to the manufacturer's recommendation. The expression of KLF4 mRNA was normalized to U6. All experiments were done triplicate. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of KLF4 mRNA.

Western blot analysis

Normal VSMCs or VSMCs transfected with KLF4-siRNA or DNA3.1+HA-KLF4 were used in this experiment. After 24 h incubation with non-

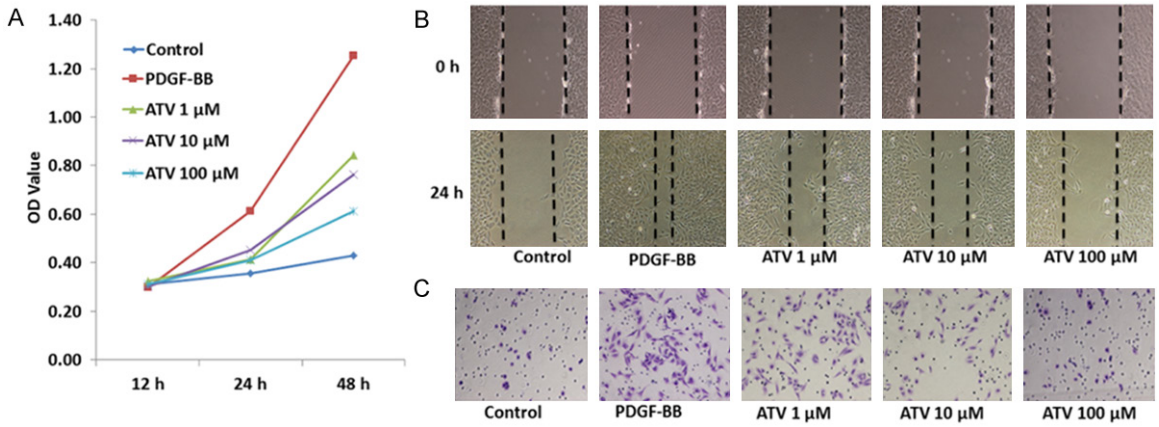

Figure 2. ATV inhibited PDGF-BB induced VSMCs proliferation and migration. A: MTT assay showed that ATV inhibited VSMCs proliferation in a dose-dependent manner. B: Wound healing assay demonstrated that ATV inhibited VSMCs migration in a dose-dependent manner. C: Transwell chambers revealed that ATV inhibited VSMCs invasion in a dose-dependent manner. ATV: atorvastatin.

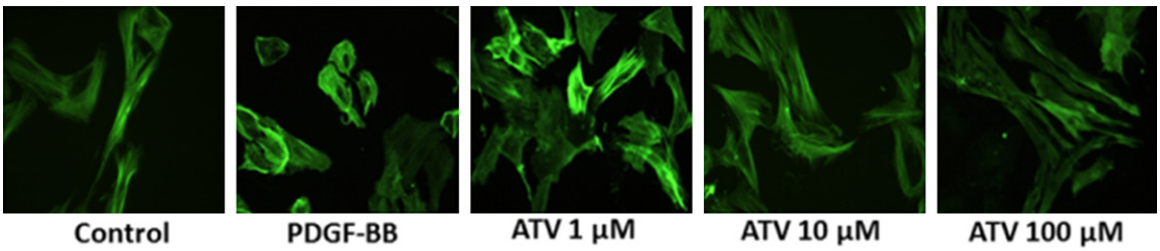

Figure 3. ATV restored the regular distribution of SMα-actin in VSMCs. ATV: atorvastatin.

serum DMEM and 12 h with PDGF-BB, cells were treated with different concentrations of atorvastatin and incubated at 37°C under 5% CO_2 for 24 h. Cellular protein was obtained with the radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Haimen, China) for Western blot analysis. The supernatants were then separated on SDS-PAGE (10%) and transferred to polyvinylidene fluoride membranes. After that, the membranes were blocked with blocking buffer for 30 mins at room temperature and then incubated with the rabbit anti-SMα-actin, calponin, OPN, SM-MHC, KLF4 monoclonal antibody (1:1,000 dilution), and mouse anti-β-actin monoclonal antibody (1:10,000 dilution) overnight at 4°C. TBS-T was used to wash the membranes (3 times for 10 min), and the membranes were incubated with goat anti-rabbit IgG-HRP (1:10,000 dilution), or goat anti-mouse IgG-HRP (1:10,000 dilution) for 1 h at room temperature. The standard chemical luminescence method (Beyotime Company, China) was used to detect the antigen by exposing the membranes to Kodak X-Omat AR film. The resultant films were scanned on a gel imaging and analysis system and analyzed by Quantity One 4.4 (Bio-Rad, Hercules, CA, USA).

Statistical analysis

All experiments were repeated three times. SPSS 20.0 was used for all statistical analyses. Student's t-test was performed for the comparisons between two different groups. One-way analysis of variance (ANOVA), followed by Tukey's post-hoc analysis was used to compare between multiple experimental groups. *P* value of <0.05 was considered as statistically significant.

Results

ATV inhibited VSMCs proliferation and migration

The primary culture of rat brain arterial VSMCs exhibited a typical spindle-shaped appearance

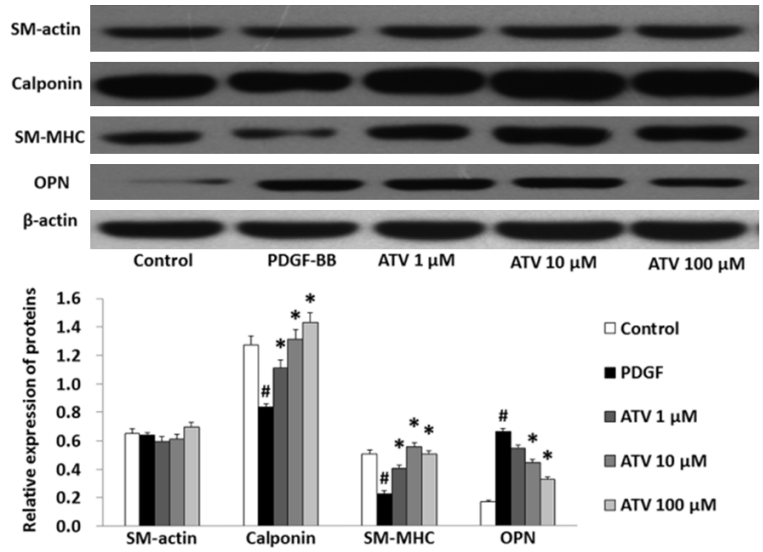

Figure 4. ATV decreased the expression of OPN and increased the expression of SM-MHC and Calponin. ATV: atorvastatin, #P<0.01 compared with control, *P<0.01 compared with PDGF-BB.

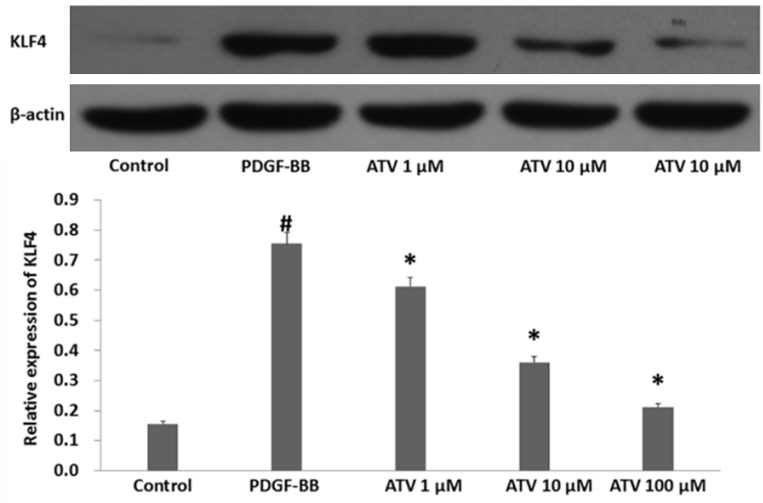

Figure 5. ATV decreased PDGF-BB induced expression of KLF4. ATV: atorvastatin, #P<0.01 compared with control, *P<0.01 compared with PDGF-BB.

with a characteristic "hill-and-valley" pattern, and immunofluorescence showed 98% positive SMα-actin staining (**Figure 1**).

MTT assay were performed to explore the function of ATV on VSMCs proliferation. As shown in the **Figure 2A**, PDGF-BB significantly increased the proliferation ability of VSMCs and ATV could dramaticlly inhibit the proliferation ability of VSMCs in a dose-dependent manner, P<0.01.

Images of the scratches were captured at 0 and 24 h after ATV were added. We found that ATV markedly inhibited PDGF-BB induced VSMCs migration after 24 h in a dose-dependent manner (**Figure 2B**, P< 0.01). In agreement, transwell chamber assay revealed that ATV could also decrease cell invasion ability after 18 h in a dose-dependent manner (**Figure 2C**).

ATV suppressed PDGF-BB induced VSMCs morphology and cytoskeleton rearrangement

Immunocytochemistry was used to assess VSMCs morphology and cytoskeleton. VSMCs in the control group exhibited elongation and spindle morphology with an organized cytoskeleton network and aligned arrangement of actin filaments. In contrast, VSMCs in the PDGF-BB group showed orderless distribution of smooth muscle actin filaments and aggregation around the perinuclear region without clear organized cytoskeleton network. Meanwhile, VSMCs treated by ATV restored the spindle morphology and the regular distribution of actin filaments (**Figure 3**).

AVT inhibited VSMCs dedifferentiation

VSMCs are able to dedifferentiate to a synthetic phenotype in response to vascular injury. The dedifferentiated VSMCs demonstrate low expression of SM-specific contractile markers, such as calponin and SM-MHC and high expression of OPN. Therefore, we used western blot to test whether ATV can affect the expression of VSMCs phenotype marker genes. As shown in **Figure 4**, compared with the control group, PDGF-BB decreased the expression of SM-MHC and calponin, meanwhile, increased the expression of OPN, P<0.01. In contrast, compared with the PDGF-BB group, ATV significantly suppressed the expression of OPN and increased

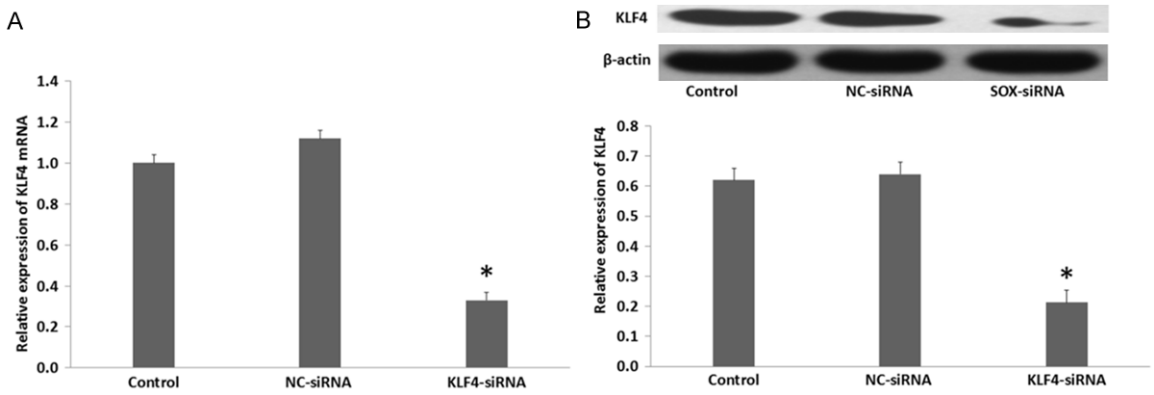

Figure 6. KLF-4 siRNA decreased the expression of KLF4 in VSMCs. A: PCR showed that KLF4-siRNA decreased the expression of KLF4 in VSMCs. B: Western blot analysis showed that KLF4-siRNA decreased the expression of KLF4 in VSMCs.

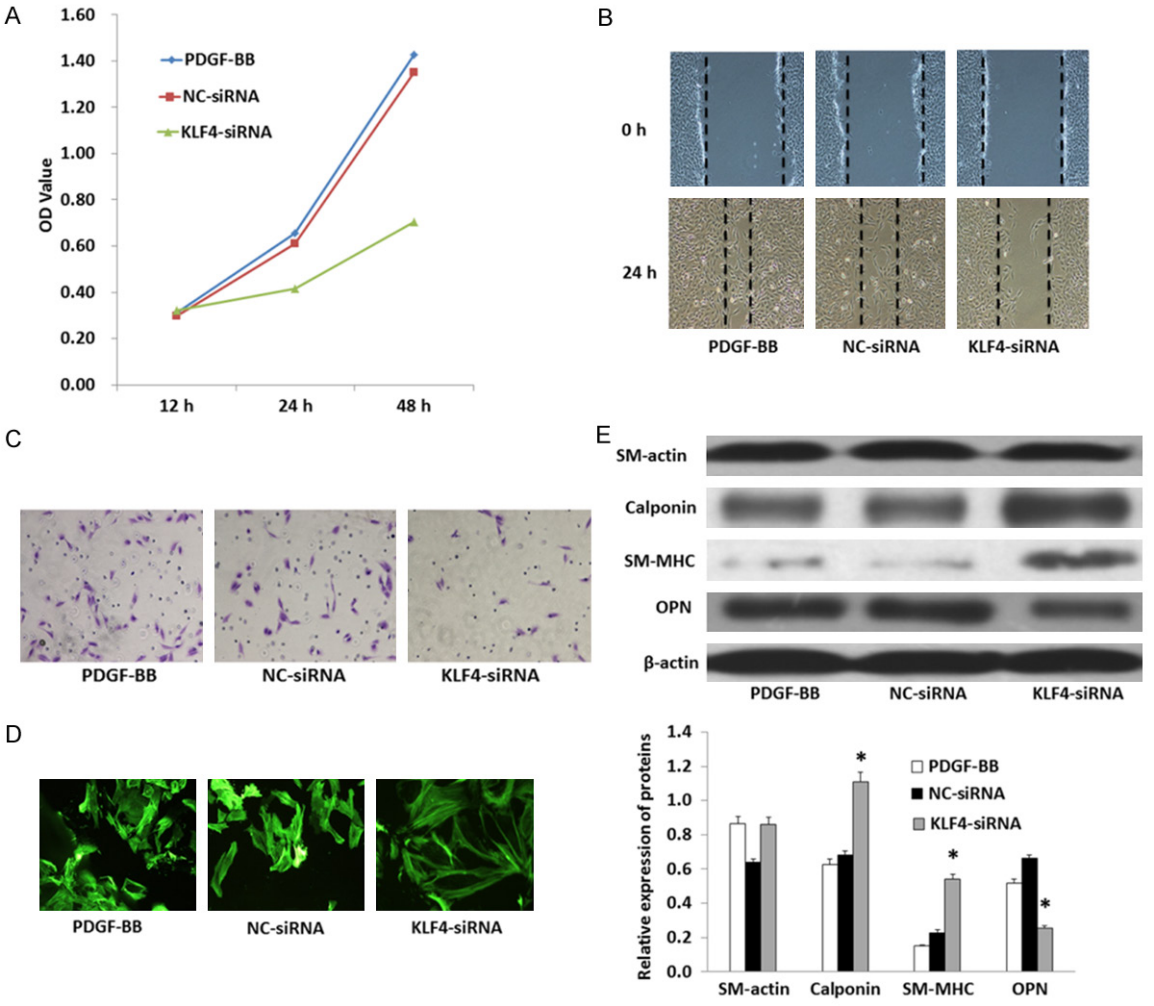

Figure 7. Down-regulation of KLF4 inhibited PDGF-BB induced VSMCs dedifferentiation. A: MTT assay showed that down-regulation of KLF4 inhibited VSMCs proliferation. B: Wound healing assay demonstrated that down-regulation of KLF4 inhibited VSMCs migration. C: Transwell chambers revealed that down-regulation of KLF4 inhibited VSMCs invasion. D: Down-regulation of KLF4 restored the regular distribution of SMα-actin in VSMCs. E: Down-regulation of KLF4 decreased the expression of OPN and increased the expression of SM-MHC and Calponin. *P<0.01 compared with PDGF-BB.

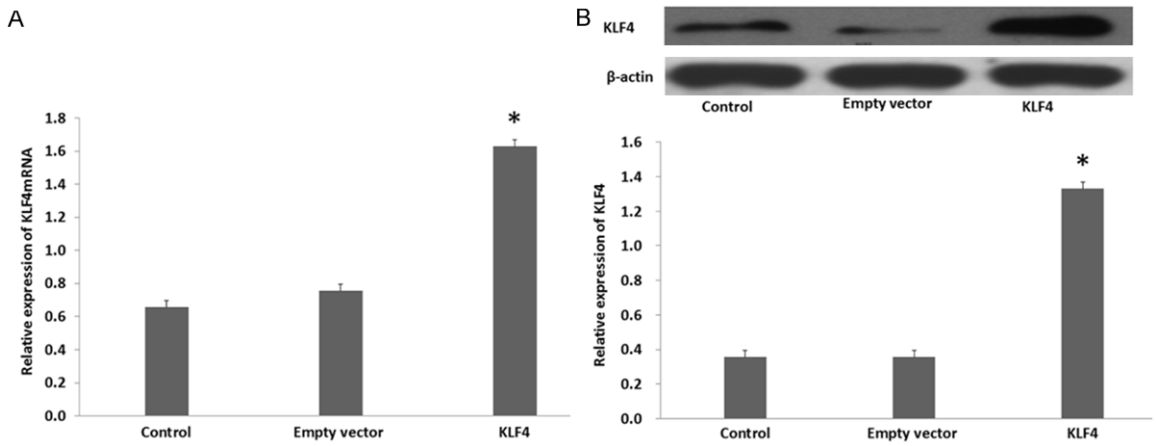

Figure 8. pcDNA3.1+HA KLF4 increased the expression of SOX4 in VSMCs. A: PCR showed that pcDNA3.1+HA KLF4 increased the expression of KLF4 in VSMCs. B: Western blot analysis showed that pcDNA3.1+HA KLF4 increased the expression of KLF4 in VSMCs. *P<0.01 compared with Empty vector.

the expression of SM-MHC, calponin, P<0.05. Taken together, the above results indicated that ATV inhibited PDGF-BB induced VSMCs dedifferentiation.

ATV inhibited the expression of KLF4 in VSMCs

After incubated with increasing concentrations of ATV, Western blot analysis showed that the expression of KLF4 was increased in the PDGF-BB group, P<0.01. Meanwhile, we found that ATV decreased PDGF-BB induced expression of KLF4 in a dose-dependent manner (**Figure 5**, P<0.01). Considering the critical role of KLF4 in the dedifferentiation of VSMCs, these results prompted us to focus on KLF4 in the following study.

Down-regulation of KLF4 suppressed VSMCs dedifferentiation

To examine the role of the KLF4 in dedifferentiation of VSMCs, KLF4-siRNA was used to knock the expression of KLF4 in VSMCs. After transfected with KLF4-siRNA or NC-siRNA, Quantitative real-time PCR showed that KLF4-siRNA decreased the expression of KLF4 in VSMCs, P<0.01 (**Figure 6A**). In addition, western blot analysis showed that KLF4-siRNA decreased the expression of KLF4 in VSMCs, P<0.01 (**Figure 6B**).

After transfected with KLF4-siRNA or NC-siRNA, all the VSMCs were incubated with PDGF-BB to induce dedifferentiation. We found that VSMCs pre-transfected with KLF4-siRNA showed a notable decrease in VSMCs proliferation and migration (**Figure 7A-C**), P<0.05). In addition, the same as ATV, down regulation of KLF4 by KLF4-siRNA could also suppressed PDGF-BB induced VSMCs morphology and cytoskeleton rearrangement (**Figure 7D**). What's more, western blot analysis revealed that, down-regulation of KLF4 decreased the expression of OPN and increased the expression of SM-MHC, calponin (**Figure 7E**, P<0.05). Taken together, these data indicated that, the same as ATV, down-regulation of KLF4 could also suppress VSMC dedifferentiation.

Over-expression of KLF4 reversed the ATV-induced inhibition of VSMC dedifferentiation

To further verify the role of the KLF4 in ATV-induced inhibition of VSMC dedifferentiation. pcDNA3.1+HA-KLF4 were transfected into VSMCs to increase the expression of KLF4. Quantitative real-time PCR showed that the mRNA of KLF4 was increased in the cells transfected with pcDNA3.1+HA-KLF4 compared with the pcDNA3.1+HA-empty plasmid (**Figure 8A**, P<0.01). Besides, western blot analysis demonstrated the same results according to RT-PCR (**Figure 8B**, P<0.01).

After transfected with pcDNA3.1+HA-KLF4 or pcDNA3.1+HA-empty plasmid, all the VSMCs were incubated with PDGF-BB to induce dedifferentiation. After that, the cells were treated with ATV or vehicle. We found that VSMCs transfected with pcDNA3.1+HA-KLF4 showed a notable increase in proliferation and migration abil-

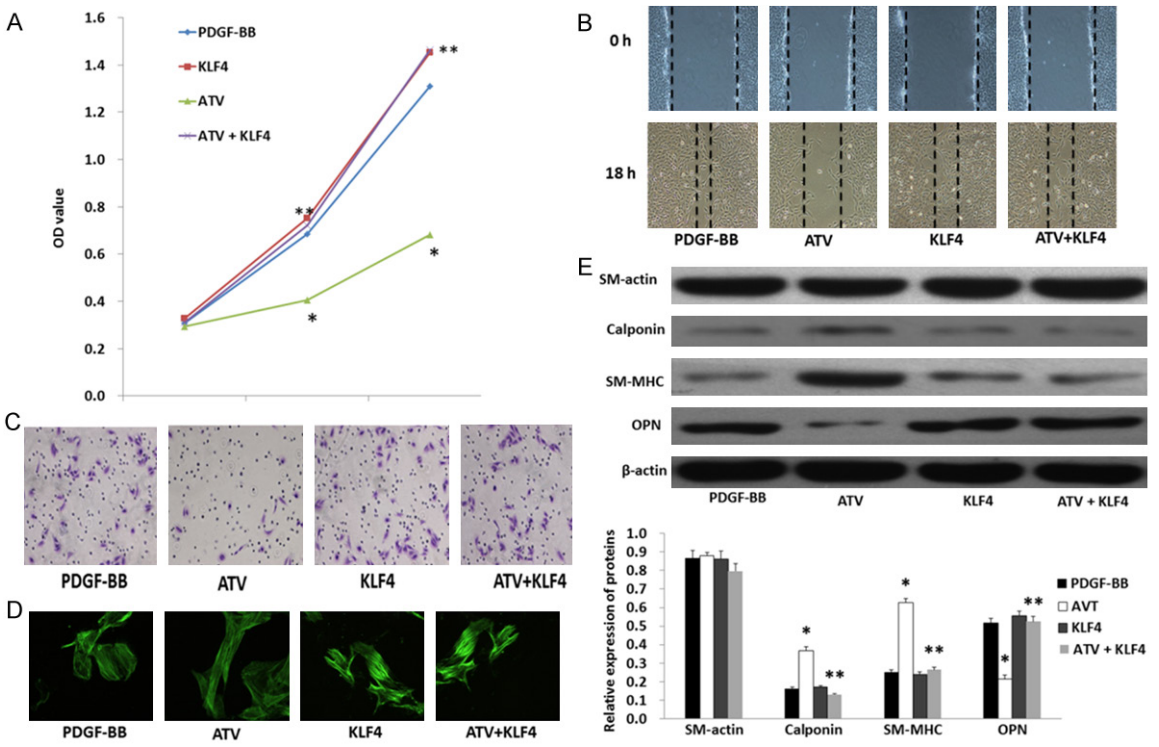

Figure 9. Over-expression of KLF4 reversed ATV-induced inhibition of VSMCs dedifferentiation. A: MTT assay showed that over-expression of KLF4 reversed ATV-induced inhibition of VSMCs proliferation. B: Wound healing assay demonstrated that over-expression of KLF4 reversed ATV-induced inhibition of VSMCs migration. C: Transwell chambers revealed that over-expression of KLF4 reversed ATV-induced inhibition of VSMCs invasion. D: Over-expression of KLF4 reversed the effect of ATV on the regular distribution of SMα-actin in VSMCs. E: Over-expression of KLF4 increased the expression of OPN and decreased the expression of SM-MHC and Calponin. ATV: atorvastatin, *P<0.01 compared with PDGF-BB; **P<0.01 compared with ATV.

ity compared with the ATV group (**Figure 9A-C**). What's more, over-expression of KLF4 suppressed the ATV-induced inhibition of VSMCs morphology and cytoskeleton rearrangement (**Figure 9D**). In addition over-expression of KLF4 could also reversed the ATV-induced increased expression of calponin, SM-MHC and decreased expression of OPN, P<0.01 (**Figure 9E**). These results further verified that KLF4 was involved in ATV-induced inhibition of VSMCs dedifferentiation.

Discussion

VSMCs are essential regulators of vascular function. VSMCs are located in the medial layer of the healthy arteries where they express contractile proteins which help to regulate the vessel tone and blood flow [20]. During atherogenesis and arterial restenosis, VSMCs dedifferentiate from a "contractile" phenotype to a "synthetic" phenotype. Although there are likely many alternative phenotypic states of VSMCs, in general VSMC phenotypic switching is characterized by markedly reduced expression of VSMC-selective differentiation marker genes and increased VSMC proliferation, migration, and synthesis of extracellular matrix components required for vascular repair [21]. These phenotype changes in VSMCs appear to be common and necessary in the development in a large number of major cerebrovascular disease, including atherosclerosis, arterial aneurysm, and restenosis [1, 22]. Zhu found that by regulating vascular smooth muscle cell phenotype, mindin prevented neointima formation in cerebral artery [23]. In addition, more recent genetic lineage tracing studies have shown that VSMC phenotypic switching results in less-differentiated forms that lack VSMC markers including macrophage-like cells, and this switching directly promotes cerebral artery atherosclerosis [24].

Inhibition of the VSMCs dedifferentiation is the mechanism by which lots of drugs paly their

cerebrovascular protective role [25]. Wagner et al. demonstrated that in addition to its lipid-lowering effect, lovastatin suppressed VSMC dedifferentiation through inhibition of Rheb [26]. Meanwhile, Kaimoto et al. revealed that nifedipine, a calcium antagonist, inhibited VSMC dedifferentiation in injured arteries and suppressed neointimal thickening after balloon injury by modulation of the akt signaling [27]. In agreement, researchers found that resveratrol, a component of the red wine polyphenols, inhibited phenotypic switching of neointimal VSMCs after balloon injury through blockade of Notch pathway or by stimulation of SirT1 and AMPK [28, 29]. What's more, Lee et al. found that resveratrol inhibited VSMCs dedifferentiation and proliferation rate by interruption of the balance of Akt, 42/44MAPK, and p38MAPK pathway activation which were induced by PDGF-BB [11]. Consistent with these experimental findings, we demonstrated that ATV decreased the proliferation and migration ability of VSMCs and made the SMα-actin distributed regular. Meanwhile, ATV could also increase the expression of VSMC-selective differentiation marker genes and decrease the expression of OPN. All these data suggested that ATV could inhibit VSMCs dedifferentiation, and this maybe another mechanism, besides lowing lipid level, by which ATV palyed its cerebrovascular protective role.

KLF4 is involved with many cellular processes, including tumor development, stem cell biology, and inflammatory endothelial activation [30-32]. In VSMCs, KLF4 both promotes proliferation and induced VSMCs dedifferentiation [19]. What's more, Yoshida et al. showed conditional deletion of murine KLF4 delayed down-regulation of VSMC marker genes following vascular injury in vivo experiments [33]. Consistent with these experimental findings, our study found PDGF-BB treatment caused up-regulation of KLF4 and down-regulation of VSMC marker genes which could be prevented by down-regulation of KLF4. These results indicated that KLF4 participated in PDGF-BB induced VSMCs dedifferentiation. In addition, we found that AVT decreased the expression the KLF4 in a dose dependent manner which suggested that AVT might inhibit PDGF-BB induced VSMCs dedifferentiation by down-regulation of KLF4. We further used KLF4-siRNA to knock off the expression of KLF4 in VSMCs and found that the same as ATV, KLF4-siRNA inhibit PDGF-induced VSMCs dedifferentiation. What's more, we found that over-expression of KLF4 by pcDNA3.1+HA-KLF4 reversed the ATV-induced inhibition of VSMC dedifferentiation. These findings suggested that ATV inhibits PDGF-induced VSMCs dedifferentiation by down-regulation of KLF4.

In this study, we demonstrated that atorvastatin (ATV) inhibited PDGF-BB induced VSMCs differentiation in a dose-dependent manner. Besides, we found ATV decreased the expression of KLF4 at the same time, and further testified the critical role of KLF4 in ATV's effects on VSMCs dedifferentiation. All these results indicated that ATV inhibited PDGF-BB induced VSMCs dedifferentiation by down-regulation of KLF4. This maybe another mechanism, beside lowing lipid level, by which ATV plays its cerebrovascular protective role.

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Disclosure of conflict of interest

None.

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