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Atorvastatin enhances endothelial adherens junctions through promoting *VE-PTP* gene transcription and reducing VE-cadherin-Y731 phosphorylation

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ABSTRACT

Vascular endothelial protein tyrosine phosphatase (VE-PTP) is essential for endothelial cells (ECs) adherens junction and vascular homeostasis; however, the regulatory mechanism of *VE-PTP* transcription is unknown, and a drug able to promote VE-PTP expression in ECs has not yet been reported in the literature. In this study, we used human ECs as a model to explore small molecule compounds able to promote VE-PTP expression, and found that atorvastatin, a HMG-CoA reductase inhibitor widely used in the clinic to treat hypercholesterolemia-related cardiovascular diseases, strongly promoted *VE-PTP* transcription in ECs through activating the *VE-PTP* promoter and upregulating the expression of the transcription factor, specificity protein 1 (SP1). Additionally, atorvastatin markedly reduced VE-cadherin-Y731 phosphorylation induced by cigarette smoke extract and significantly enhanced stability of endothelial adherens junctions. Together, our findings reveal that atorvastatin up-regulates VE-PTP expression, increases VE-cadherin protein levels, and decreases VE-cadherin-Y731 phosphorylation to strengthen EC adherens junctions and maintain vascular cell monolayer integrity, offering a new mechanism of atorvastatin against CSE-induced disruption of vascular integrity and relevant cardio-cerebrovascular disease.

1. Introduction

The maintenance of vascular wall integrity and homeostasis is essential for proper blood circulation, the health, and the life of human beings. Various pathological factors, including hyperlipidemia, hypertension, inflammation, and toxic components from smoking and air pollution can reduce endothelial cell (EC) adherens junctions, break down the integrity of blood vessels, and disrupt vascular homeostasis. These factors often result in cardiovascular and cerebrovascular diseases [1–3]. Accumulated evidence shows that the EC plasma membrane proteins, vascular endothelial protein tyrosine phosphatase (VE-PTP) and VE-cadherin, play pivotal roles in EC adherens junctions and vascular homeostasis [4–13]. However, how to maintain EC adherens junctions and vascular homeostasis under pathological conditions is still a challenge to the blood and cardiovascular research fields.

Vascular ECs have a unique cell membrane protein known as VE-PTP. This protein consists of 2251 amino acids with 18 domains, comprised of 16 extracellular domains, a short transmembrane domain, and an intracellular domain with a tyrosine phosphatase [4–7]. VE-PTP hydrolyzes the phosphate group at the tyrosine 731 site of VE-cadherin, a master EC surface adherent protein essential to vascular cell adherens junctions. Dephosphorylated VE-cadherin binds to β-catenin and F-actin to form a VE-cadherin/β-catenin/F-actin complex that anchors to cell skeleton proteins. This complex plays important roles in maintenance of cell surface VE-cadherin stability, EC adherens junctions, and vascular integrity [9–13]. *VE-PTP*-knockout mice die on embryonic day 10 from defects in embryonic angiogenesis and vascular remodeling, therefore, *VE-PTP* is essential for vascular remodeling, homeostasis, and angiogenesis [4]. Gong H et al. [14] and Aguirre Palma et al. [15] recently reported that HIF-2alpha and Angiopoietin-2 increased VE-PTP mRNA levels; however, the regulatory mechanism of *VE-PTP* transcription is unknown, and a drug able to promote VE-PTP expression has not yet been reported in the literature.

It is well established that smoking causes vascular cell dysfunctions and various diseases, including disruption of endothelial cell adherens junctions, loss of barrier function, inflammation, and apoptosis, resulting in development of diseases, such as pulmonary hypertension, atherosclerosis, cardio-cerebrovascular disease, emphysema, and acute respiratory distress syndrome [16, 17]. Smoking causes five million people to die worldwide every year [16–19]; hence, it is highly desired to find a way to effectively prevent and treat smoking-induced vascular diseases.

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In the current investigation, we used human ECs as a model to explore small molecule compounds for the ability to promote VE-PTP expression and strengthen EC adherens junctions. We report that atorvastatin, a HMG-CoA reductase inhibitor widely used in the clinic to treat hypercholesterolemia-related cardiovascular diseases, strongly promoted *VE-PTP* transcription in ECs through upregulation of the transcription factor, specificity protein 1 (SP1). Atorvastatin also effectively abolished cigarette smoke extract (CSE)-induced phosphorylation of VE-cadherin-Y731. This resulted in marked enhancement of EC adherens junctions and reduction of CSE-induced endothelial cell layer damage. For the first time, we conceptually demonstrate that overexpression of VE-PTP by atorvastatin strengthens EC adherens junctions and protects the endothelial cell monolayer from damage by CSE.

2. Materials and methods

2.1. Materials

Atorvastatin was from Pfizer (City of New York, New York, USA). VE-PTP antibody was purchased from BD Biosciences (Franklin Lakes, New Jersey, USA). VE-cadherin antibody was from Alexis (Lausanne, Vaud, Switzerland). Phosphorylated VE-cadherin (Y731) antibody was from Millipore (Bill Rika, Massachusetts, USA). SP1 antibody was from Santa Cruz (Dallas, Texas, USA). SP1 inhibitor was purchased from Selleck (Houston, Texas). Lipofectamine® 2000 Reagent Kit was obtained from Invitrogen (Carlsbad, CA, USA). Dual-luciferase Reporter Assay Kit was from Promega (Madison, WI, USA). Human lung microvascular endothelial cells (HLMVEC) and Eahy926 cells (a cell line from an endothelial hybridoma with endothelial characteristics and function [20–23]), but without tumorigenic capability [24] were obtained from Applied Biological Materials Inc. (Richmond, BC, Canada). Cigarette smoke extract (CSE) was prepared in our lab according to the protocol as we previously described [25].

2.2. Cell culture

Human ECs HLMVEC and Eahy926 cells were cultured in DMEM (high glucose) supplemented with 10% fetal bovine serum (complete medium). The cells were maintained at 37 °C in a humidified atmosphere of 5% CO2 as previously described [26]. By the way, HLMVECs display all major endothelial phenotypic markers, including platelet endothelial cell adhesion molecule (PECAM-1, CD31), V*E*-cadherin, intercellular adhesion molecule (ICAM-1), von Willebrand factor (vWF), and E-selectin [27].

2.3. Treatment of cells with atorvastatin and cigarette smoke extracts

HLMVEC and Eahy926 cells were cultured in a 10 cm dish to confluence and treated with either atorvastatin at the doses of 0–20 μM, or 0–6% CSE solution diluted in the DMEM complete medium, or the complete medium as a control, respectively. After 48 h treatment, the cells were harvested for measurement of various parameters as stated below.

2.4. Endothelial monolayer permeability assay

The endothelial monolayer layer permeability assay was performed in a transwell system as described before [25, 28–31]. In brief, Eahy926 cells were seeded in the upper chamber of transwell inserts (8 μm pore size, BD Falcon) for 24 h to reach cell confluence and treated with 4% CSE along or 4% CSE together with 5 μM atorvastatin. FITC-Dextran (MW 3000–5000 kDa) was added to the upper chamber and incubated for 0.5 h. The amount of FITC-Dextran infiltrated into the lower chamber well was determined using a fluorescence spectrophotometer (Thermo, America) at the excitation wavelength of 488 nm and the emission wavelength of 520 nm, representing the permeability of endothelial monolayer.

2.5. RT-PCR and quantitative real time-PCR

Total RNA was extracted from the ECs. cDNA was generated by reverse transcription using First Strand cDNA Synthesis Kit and oligo (dT) primers (Takara, Dalian, China). For RT-PCR, PCR was performed with 25 μL reactions containing 1 μL cDNA, 1 μL Taq DNA polymerase, and 1 μL primers listed in Supplementary Table 1. The PCR products were analyzed on 1.0% agarose gels. The quantitative real time-PCR (QT-PCR) was performed as we previously described [11] by Applied Biosystems ABI7500 (ABI, Carlsbad, CA, USA) using SYBR Green PCR Master Mix(ABI) and the primers listed in the Supplementary Table 2. The QT-PCR products were analyzed by ABI 7500 QT-PCR data processing software.

2.6. Western blotting

Proteins in ECs were extracted using the Mammalian Protein Extraction Kit as we reported before [25]. Equal amounts of proteins were loaded into each lane and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with Tris-glycine running buffer and transferred to nitrocellulose membranes. Then the membrane was blocked with 5% nonfat milk for 2 h at room temperature and incubated with primary antibodies at 4 °C overnight, followed by incubation with HRP-conjugated secondary antibody for 1 h at room temperature. The blots were visualized using enhanced chemiluminescence detection reagents and exposed to X-ray film.

2.7. Cell immunostaining and confocal microscopy imaging

ECs were seeded on small round slides pre-coated with gelatin and treated by atorvastatin at the doses of 0–10 μM. The cells were fixed by 4% PFA for 10 min, permeabilized with 0.1% Triton X-100 for 15 min, and blocked with 5% BSA for 1 h at room temperature, then incubated with primary antibody diluted by 5% BSA overnight at 4 °C and fluorescence-labeled secondary antibody diluted by 5% BSA for 1 h at room temperature. Cell nucleus was stained with DAPI for 15 min at room temperature. The slides were imaged by confocal microscopy (Olympus Optical Co., Ltd).

2.8. Luciferase activity analysis

The genomic DNA fragments in the 5′flanking promoter region of *VE-PTP* gene were cloned into a luciferase reporter vector pGL4.17. HLMVEC cells were transfected with the pGL4.17-VE-PTP promoter constructs or the control pGL4.17 vector by Lipofectamine® 2000 Reagent Kit. The stable transfected cells were selected using G418 for 3 weeks and treated with atorvastatin at the dose of 10 μM. The cells were lyzed and the luciferase activity in the cell lysates was measured using Dual-luciferase Reporter Assay Kit and detected by Chemiluminescence reading instrument.

2.9. Statistical analysis

The results of the experiments are expressed as means ± SD. Statistical evaluation of the data was performed by the unpaired Student-*t*-test for the comparison between two groups and by one-way ANOVA in Graphpad Prism. $P < .05$ and $P < .01$ were deemed as significant and very significant, respectively.

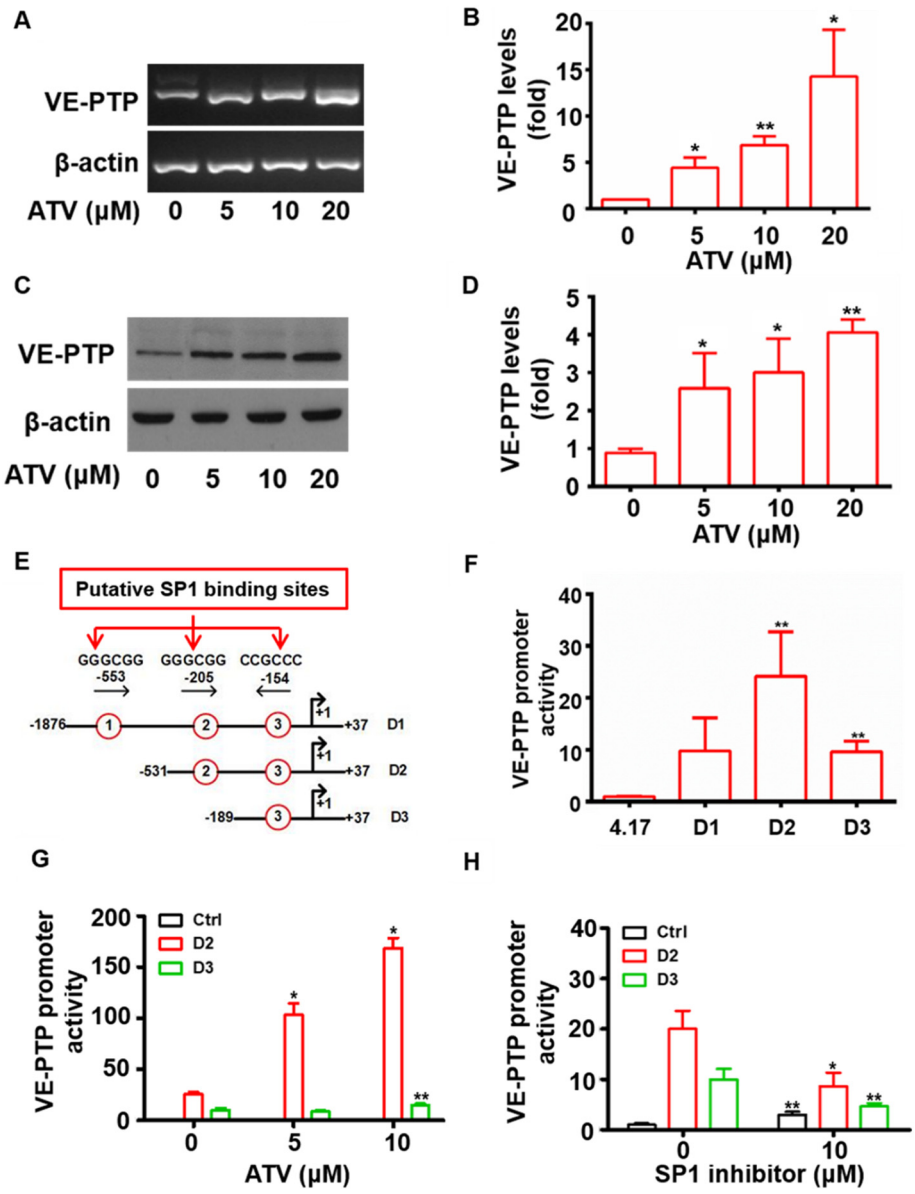

Fig. 1. Atorvastatin notably promotes *VE-PTP* gene transcription in endothelial cells.
After the human Eahy926 cells were treated with atorvastatin (ATV) at the doses of 0–20 μM, VE-PTP expression levels were measured by RT-PCR (A), quantitative real time PCR (qRT-PCR) (B), and Western blotting (C, D). In addition, three SP1-binding sites and the core consensus DNA sequences in the 5′flanking promoter region of the *VE-PTP* gene were analyzed by bioinformatics and shown by circled numbers 1, 2, and 3, respectively (E).The human lung microvascular endothelial cells (HLMVECs) were transfected with either pGL4.17 vector containing different VE-PTP promoter DNA fragments D1, D2, and D3 (E), or pGL4.17 vector as a control, respectively. The luciferase activity of the D1, D2, and D3 in the cells without (F) or with 0–10 μM ATV was measured (G). In addition, treatment of ECs transfected with D2-pGL4 concurrent with 10 μM azithromycin, a SP1-selective inhibitor, suppressed VE-PTP gene promoter activity (H). The data represent three repeats, means ± SD, $^{*}P < .05$, $^{**}P < .01$.

3. Results

3.1. Atorvastatin promotes VE-PTP transcription in human ECs

VE-PTP plays critical roles in vascular remodeling and homeostasis [4–7]; however, the agent that regulates *VE-PTP* expression has not yet been reported. In the current study, we screened various small molecule compounds to identify potential agents to promote VE-PTP expression in human ECs. Among the various compounds tested (data not shown), we found that atorvastatin notably promoted *VE-PTP* expression in ECs (Fig. 1A). Quantitative real time polymerase chain reaction (qRT-PCR) showed that *VE-PTP* mRNA levels in ECs were increased 9-fold by atorvastatin in a dose-dependent manner compared to the control (Fig. 1B). Western blotting demonstrated that atorvastatin significantly increased the amount of VE-PTP protein in ECs (Fig. 1C, D).

Because the mechanism controlling *VE-PTP* transcription is unclear, we studied the mechanism of atorvastatin-dependent *VE-PTP* expression. Bioinformatics analysis identified three putative promoters located at the 5′flanking region of the *VE-PTP* gene, which contained transcription factor SP1-binding consensus core sequences. These three sequences were (1) GGGCGG, (2) GGGCGG, and (3) CCCGCC, respectively (Fig. 1E). Accordingly, we cloned the three genomic DNA fragments referred to as D1 (−1876 to +37), D2 (−531 to +37), and D3 (−189 to +37), respectively, into a luciferase gene reporter system vector, pGL4.17 (Fig. 1E). The luciferase assay [26] indicated that all three genomic DNA fragments had promoter activity, but the D2 fragment had the highest promoter activity of the three (Fig. 1F). Interestingly, the dual-luciferase assay showed that atorvastatin remarkably activated D2 promoter activity in a dose-dependent way, while D3 promoter activity was only slightly elevated by atorvastatin (Fig. 1G). This suggests that the genomic DNA fragment, D2, plays a major role in atorvastatin-induced *VE-PTP* transcription. To further confirm the effect of atorvastatin on SP1-promoted *VE-PTP* transcription, we treated ECs transfected with D2-pGL4.17 concurrent with 10 μM azithromycin, a SP1-selective inhibitor. The dual-luciferase assay showed that *VE-PTP* promoter activity was significantly reduced by the SP1 inhibitor (Fig. 1H), suggesting that SP1 plays an important role in atorvastatin-mediated *VE-PTP* transcription.

Next, we investigated whether atorvastatin promoted VE-PTP overexpression was related to increased SP1 expression in ECs. Western blotting showed that atorvastatin significantly increased SP1 levels in ECs in a dose-dependent manner (Fig. 2A, B). Additionally,

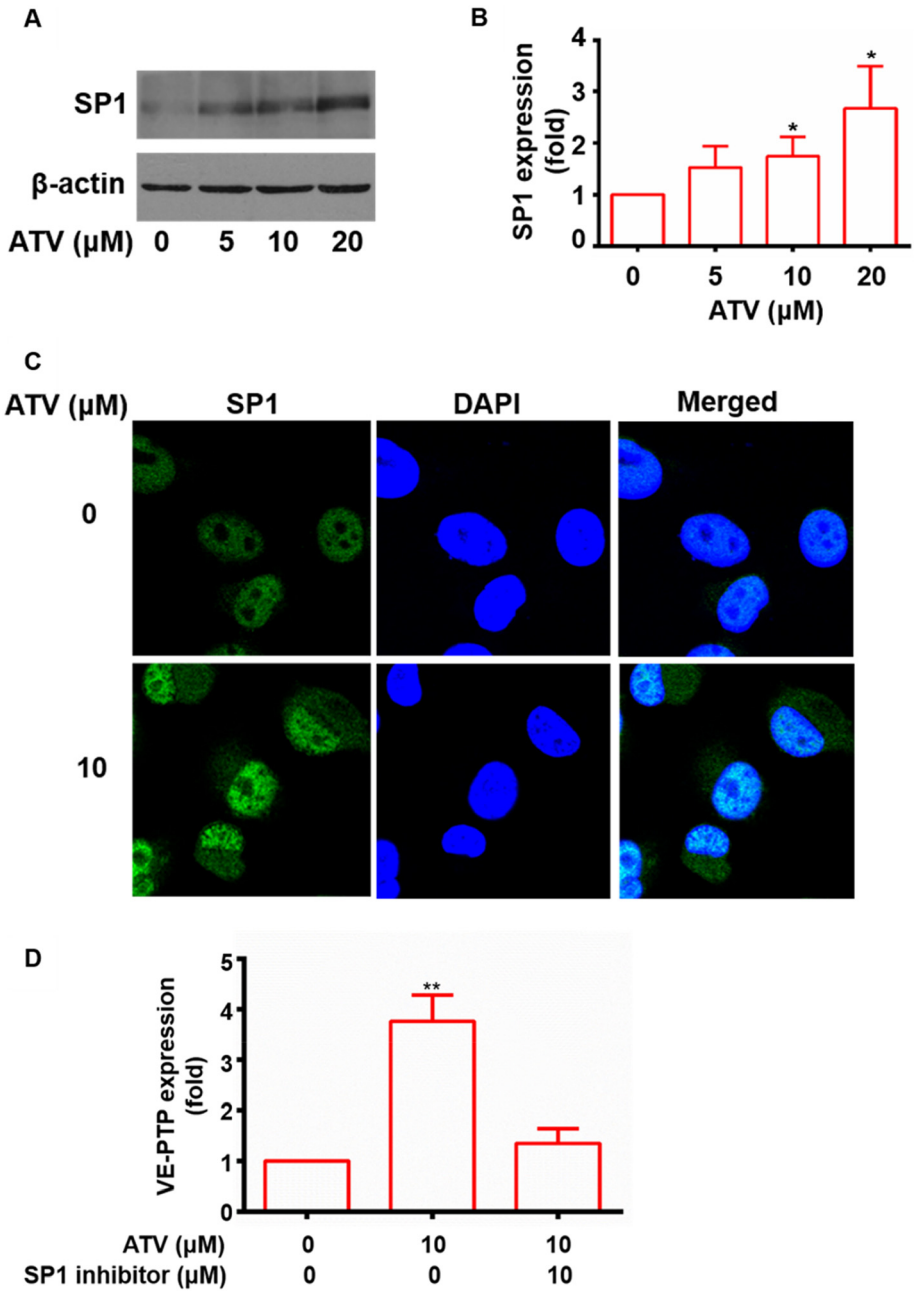

Fig. 2. Atorvastatin raises transcription factor SP1 levels to promote VE-PTP gene expression in endothelial cells.

Specificity protein 1 (SP1) levels were elevated in Eahy926 cells after the cells were treated with atorvastatin (ATV) at the doses of 0–20 μM (A), and the data in A were statistically analyzed (B). The intracellular SP1 was also be detected by immunofluorescent staining (400×), SP1 (C left panel, green) and DAPI (C middle panel, blue) in the cell nucleus were shown in the presence or absence of 10 μM ATV. qRT-PCR showed that ATV notably induced SP1 overexpression, which was effectively reduced by SP1 inhibitor azithromycin (D). The data represent three repeats, means ± SD, $*P < .05$, $**P < .01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

immunofluorescent staining demonstrated that the amount of SP1 in the cell nucleus was notably elevated after 10 μM atorvastatin treatment (Fig. 2C). Furthermore, qRT-PCR indicated that atorvastatin significantly promoted *VE-PTP* expression in ECs, but this was abolished by the SP1-selective inhibitor, azithromycin (Fig. 2D). Collectively, these data imply that atorvastatin promotes *VE-PTP* transcription in ECs primarily through activation of the gene promoter containing the SP1-binding consensus DNA sequence and the elevation of SP1 levels in ECs.

3.2. *VE-PTP overexpression enhances endothelial cell VE-cadherin stability and adherens junctions via reduction of VE-cadherin phosphorylation*

Given that VE-cadherin plays a pivotal role in EC adherens junctions and vascular integrity, we studied whether VE-PTP overexpression could increase VE-cadherin protein stability and enhance endothelial adherens junction. To do this, we applied two models, *VE-PTP* overexpression and atorvastatin-induced VE-PTP overexpression, respectively. The human lung microvascular cell line, HMVEC, was first transfected with a *VE-PTP* cDNA-VENUS vector construct, and then VE-PTP and VE-cadherin levels in ECs were detected. The results showed that overexpression of *VE-PTP* (Fig. 3A) not only significantly increased VE-cadherin protein levels in ECs (Fig. 3B), but also increased VE-cadherin protein distribution among ECs (Fig. 3C). This suggests that overexpression of *VE-PTP* enhances endothelial adherens conjunctions. Intriguingly, atorvastatin significantly elevated VE-cadherin protein levels in a dose-dependent manner (Fig. 3D, E), implying that atorvastatin increases VE-cadherin protein levels in ECs.

Next, we investigated the effect of VE-PTP and atorvastatin on VE-cadherin stability in ECs. It is well established that the dephosphorylated form of VE-cadherin functions as a bridge to form EC adherens junctions. However, phosphorylated VE-cadherin rapidly dissociates from β-catenin and enters the cytoplasm to be degraded, resulting in loss of EC adherens junctions. Thus, the phosphorylation state of VE-cadherin is critical to VE-cadherin stability and EC adherens junctions [9–13]. Accordingly, we examined whether overexpression of *VE-PTP* or treatment of ECs with atorvastatin could reduce VE-cadherin

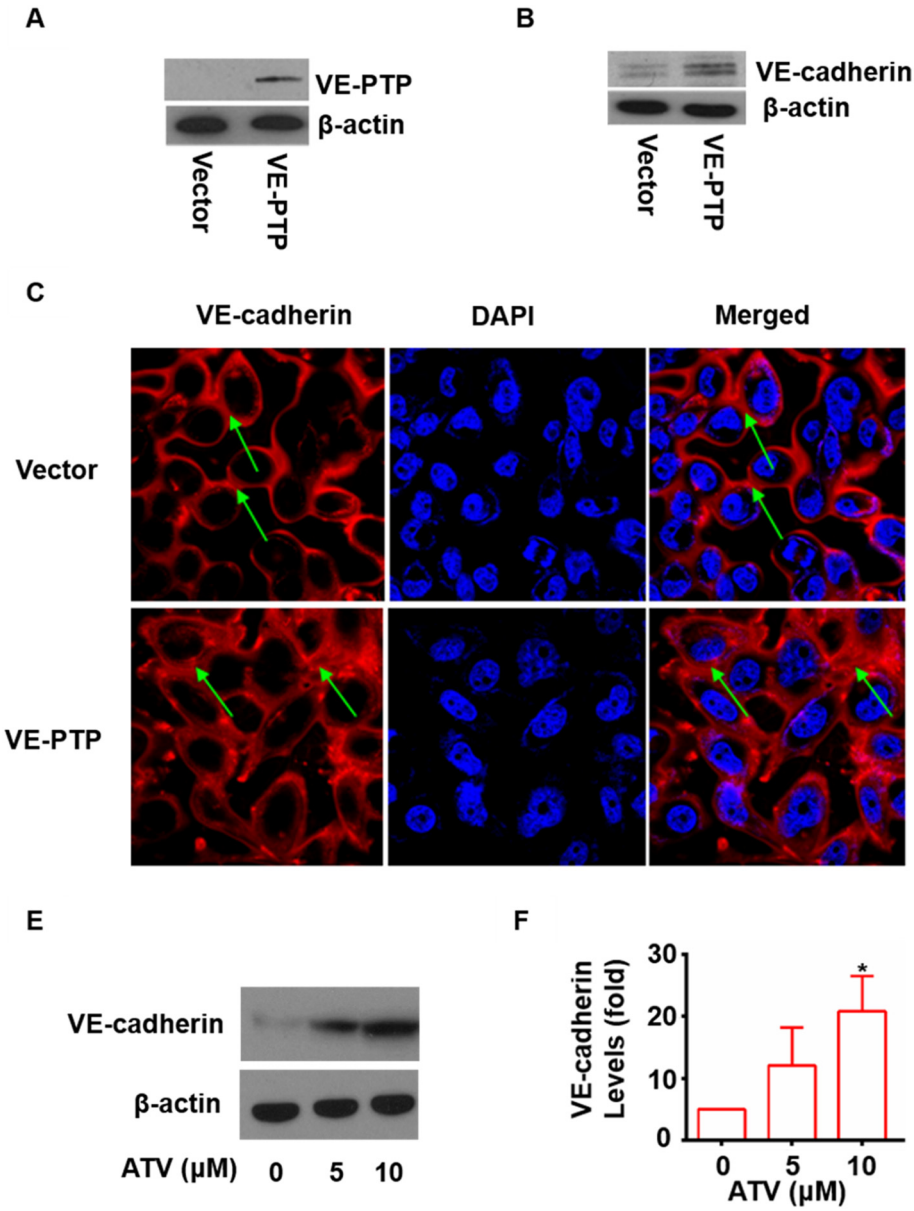

Fig. 3. Either VE-PTP overexpression or atorvastatin increases VE-cadherin distribution on the cell surface and enhances endothelial adherens junction.
After HLMVECs were transfected by either VE-PTP cDNA-vector or vector as a control, VE-PTP protein was detected by Western blotting (A). Overexpression of VE-PTP caused an increase in VE-cadherin amount in ECs (B). The VE-cadherin protein distribution on the cell surface were shown by immunefluorescent staining (C left panel, red: VE-cadherin; C middle panel, blue: DAPI), and VE-cadherin protein located at the endothelial adherens junction between ECs was indicated with green arrows (C). In addition, Western blotting indicated that atorvastatin significantly increased VE-cadherin protein levels in ECs in a dose-dependent manner (D, E). The data are shown as mean ± SD of three replicates, $^{*}P < .05$, $^{**}P < .01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

phosphorylation and enhance EC adherens junctions. The results showed that EC surface VE-cadherin formed a clear lining in the absence of CSE, while the lining was notably disrupted by 4% CSE treatment (Fig. 4A). Endothelial VE-cadherin protein has at least three forms with molecular weight 97 kDa, 115 kDa, and 130 kDa, respectively dependent on the glycosylation of the protein. The 97 kDa band represents unglycosylated form, the 115 kDa and 130 kDa bands represent partially and highly glycosylated forms of VE-cadherin proteins. Western blotting showed that 2–4% CSE markedly increased VE-cadherin-Y731 phosphorylation, but did not obviously alter the levels of VE-cadherin-Y685 phosphorylation (Fig. 4B). Overexpression of *VE-PTP* effectively prevented VE-cadherin-Y731 phosphorylation, but not significantly affected VE-cadherin-Y685 phosphorylation (Fig. 4C), implying that VE-PTP selectively reduces VE-cadherin-Y731 phosphorylation. In contrast, atorvastatin significantly diminished the amount of phosphorylated VE-cadherin-Y731, but increased the total VE-cadherin levels in ECs (Fig. 4D, E); of note, atorvastatin at the concentrations of 10 and 20 μM significantly increased the levels of both 115 kDa and 97 kDa VE-cadherin bands, which may be related to the with or without glycosylation of the protein. More interestingly, atorvastatin completely abolished 6% CSE-induced VE-cadherin-Y731 phosphorylation and concurrently elevated total VE-cadherin levels (Fig. 4F, G). Collectively, these data suggest that induction of VE-PTP expression by atorvastatin reduces VE-cadherin-Y731 phosphorylation and increases VE-cadherin levels in ECs, providing a new method to strengthen endothelial adherens junctions.

3.3. Atorvastatin effectively reduced cigarette smoke extract–induced EC monolayer permeability

Finally, we investigated whether upregulation of VE-PTP by atorvastatin could protect the endothelial monolayer from damage by CSE. Using the widely used FITC-Dextran dye leakage assay in a transwell system [25–27, 32–34], we observed that after Eahy926 cells were treated with 6% CSE for 24 h, most cells detached from bottom of the top compartment (Fig. 5A and B middle panels). Specifically, CSE induced EC apoptosis (data not shown), and the FITC-Dextran dye easily leaked from the top compartment to the bottom (Fig. 5C). In contrast, when ECs were incubated with 6% CSE combined with 5 μM atorvastatin for 24 h, the EC monolayer was well-maintained (Fig. 5A and B right panels), and the leakage of the dye was almost completely prevented (Fig. 5C). This implies that atorvastatin effectively protects the

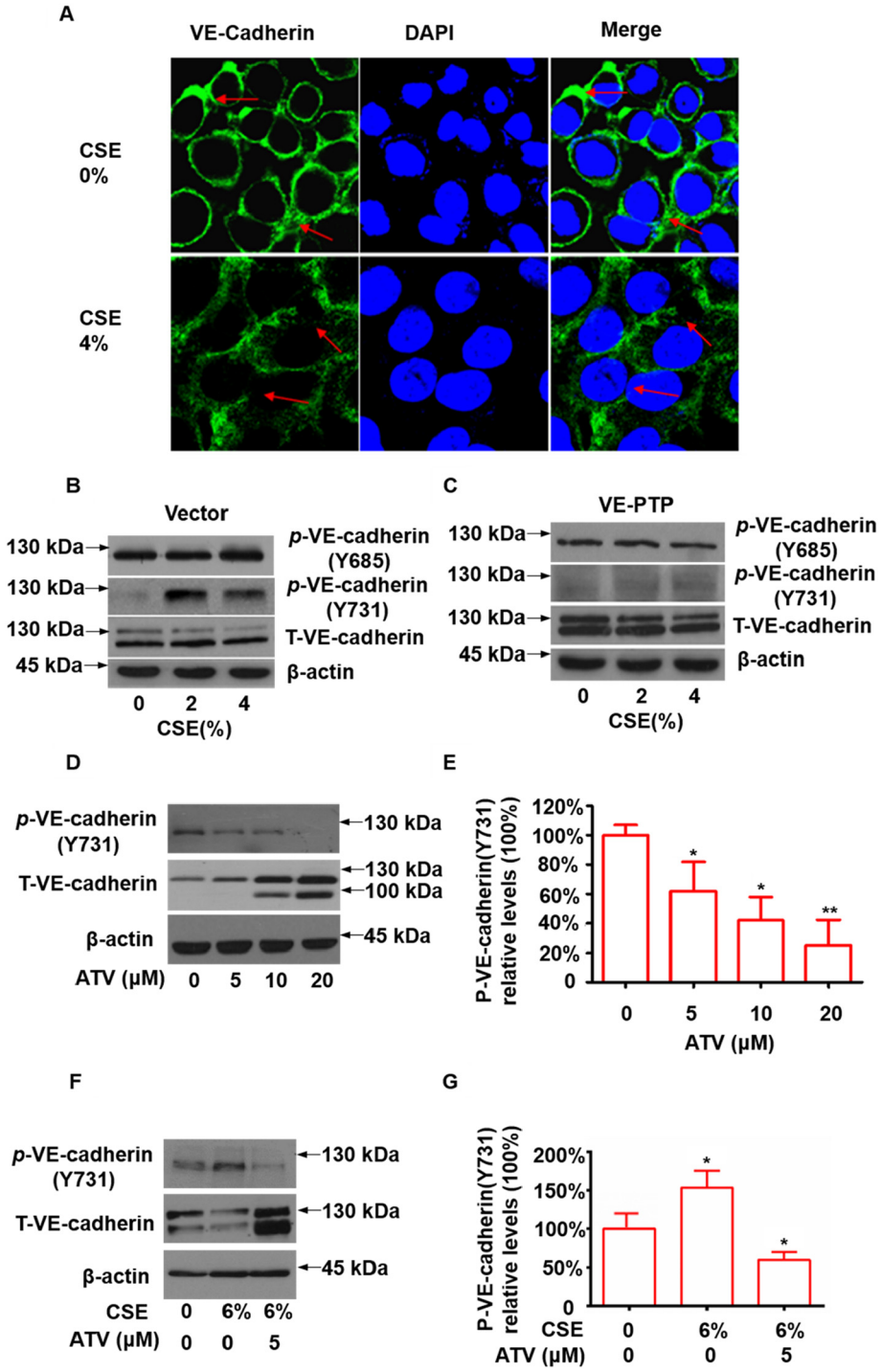

Fig. 4. Atorvastatin remarkably inhibits cigarette smoke extraction-induced VE-cadherin phosphorylation in endothelial cells.
Eahy926 cells were treated with 4% cigarette smoke extracts (CSE) for 48 h, stained by VE-cadherin specific antibody, and imaged using confocal microscope (1000×) (4A). The green represents VE-cadherin protein, the red arrows indicate the adherens junction (4A up panels) or disruption of the adherens junction (4A low panels). CSE at the concentrations of 0–4% markedly induced VE-cadherin-Y731 phosphorylation, but not VE-cadherin-Y685 the phosphorylation as shown by Western blotting (B). Overexpression of VE-PTP almost completely inhibited CSE-induced VE-cadherin-Y731 phosphorylation (C). On the other hand, atorvastatin (ATV) reduced VE-cadherin-Y731 phosphorylation in a concentration-dependent way (D, E); additionally, 5 μM ATV effectually abolished 6% CSE-induced VE-cadherin-Y731 phosphorylation (F, G). Of note, endothelial VE-cadherin protein has at least three forms with molecular weight 97 kDa, 115 kDa, and 130 kDa, respectively. The 97 kDa band (D) represents unglycosylated form, the 115 kDa and 130 kDa bands represent partially and highly glycosylated two forms of VE-cadherin proteins (B, C, D, and E). The data represent three repeats, means ± SD, $^{*}P < .05$, $^{**}P < .01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

EC monolayer from damage by CSE. Taken together, these data indicate that upregulation of VE-PTP with atorvastatin enhances adherens junctions and protects the EC monolayer from damage by CSE, indicating atorvastatin treatment as a new technique to strengthen endothelial adherens junctions and promote vascular homeostasis (Fig. 5).

4. Discussion

Atorvastatin is one of the widely marketed statins that selectively inhibits a key cholesterol synthesis-limiting enzyme, HMG-CoA reductase. It has also been used clinically to treat hypercholesterolemia and various cardiovascular diseases. Another statin family member, simvastatin, enhances human lung EC contact by increasing cell surface localization of the VE-cadherin protein, but does not increase total VE-cadherin protein levels in ECs [27]. Atorvastatin promotes the transcription of thrombomodulin, an endothelial thrombin receptor that can act as an anticoagulant and antithrombotic agent [33]. Additionally, atorvastatin modulates VE-cadherin expression [14, 34, 35] and reduces VE-cadherin tyrosine phosphorylation in ECs [36]. However, the mechanism by which atorvastatin decreases VE-cadherin phosphorylation is unknown. In the current study, we found that atorvastatin promoted expression of VE-PTP and dephosphorylated VE-cadherin, resulting in enhanced EC adherens junctions and protection of the EC monolayer from damage by CSE. Our findings provide new insight into atorvastatin-mediated dephosphorylation of VE-cadherin and offer a novel approach to strengthen vascular EC adherens

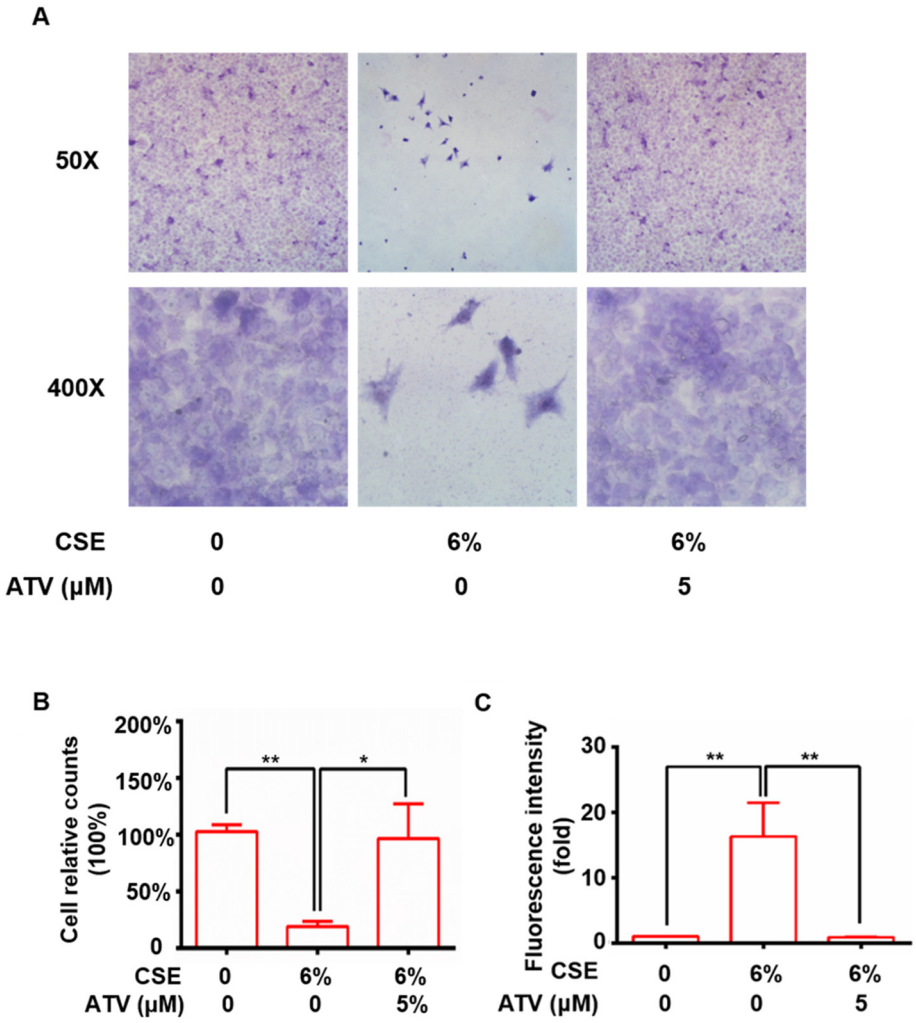

Fig. 5. Atorvastatin protects endothelial cell monolayer from damage by cigarette smoke extraction.
The confluent Eahy926 cells were treated for 48 h with 6% cigarette smoke extraction (CSE) along, or CSE together with 5 μM atorvastatin (ATV), stained by Giemsa-staining solution (A), and the numbers of ECs attached on the bottom of transwell were counted (B). Meanwhile, the endothelial cell monolayer permeability was measured in a transwell system using FITC-Dextran (MW 3000–5000 kDa) and fluorescence spectrophotometer, and the results were statistically analyzed (C).The data represent three repeats, means ± SD, $*P < .05$, $**P < .01$.

junctions and maintain EC monolayer integrity (Graphical abstract).

Smoking is one of the most serious health problems in the world [37]. Tobacco use induces pathogenesis in the lungs and cardiovascular system [38,39] and results in the death of > 5 million people worldwide each year [40]. Statins have been previously reported to protect lung function in patients that smoke [41–44]; however, the mechanism responsible for the protective effect of statins is not fully understood. In the current study, we reveal that atorvastatin can effectively reverse CSE-induced VE-cadherin phosphorylation, disruption of endothelial adherens junctions, and damage of the EC monolayer. Specifically, these effects occur through upregulation of *VE-PTP* expression and removal of the phosphate group at Tyr731 of VE-cadherin. Thus, our study reveals a new mechanism of atorvastatin-mediated protection of the lung and vascular system against the hazards of smoking.

Together, we have conceptually demonstrated that upregulation of *VE-PTP* expression by atorvastatin effectively enhances EC adherens junctions and protects the EC monolayer from damage by CSE. This provides a new approach to strengthen vascular EC adherens junctions and maintain EC monolayer integrity. Furthermore, our data indicate that VE-PTP is a viable target for novel anti-cardiovascular and cerebrovascular drug discovery. Finally, our findings support the notion that atorvastatin and perhaps other statin family members may potentially protect vascular integrity in the mass smokers and second hand smokers, providing a new mechanism of atorvastatin against cardiovascular and cerebrovascular diseases.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contribution

Zihe Huo: Conduct various molecular and cellular biology experiments, collect the original experimental data, and write a part of first draft of the manuscript.

Ying Kong: Carry out molecular biology-related experiments and prepare Figures.

Mei Meng: Prepare CSE, perform CSE-related experiments and confocal microscopy.

Zhifei Cao: Perform atorvastatin-related experiments and provide technical support.

Quansheng Zhou: Design whole research and experiments, interpret the data, and write and modify the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vph.2018.06.003.

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