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Original Research

Dipsacoside B Inhibits the Migration and Proliferation of VSMCs and Blunts Neointimal Formation by Upregulating PTEN Expression

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Abstract

Background: To investigate the effect and potential molecular mechanisms of Dipsacoside B (DB), an herb monomer extracted from *Dipsacusasper* or *Lonicera macranthoides*, on the migration and proliferation of vascular smooth muscle cells (VSMCs) and balloon-induced neointimal formation. **Methods**: *In vivo*, rat abdominal aorta balloon injury model was utilized to investigate the effect of DB on the neointimal formation. *In vitro*, cultured VSMCs were used to investigate the effect of DB on Angiotensin-II (Ang-II)-induced migration and proliferation of VSMCs. Western blot and immunofluorescence were used to measure PTEN expression. **Results**: As compared to vehicle control balloon-injury group, DB treatment significantly inhibited the neointimal formation together up-regulated the expression of phosphatase and tension homolog deleted on chromosome 10 (PTEN). Cell proliferations (MTT and Edu incorporation) assays and wound migration measurement further revealed that treatment with DB significantly blunted Ang-II-induced proliferation and migration potential of VSMCs. Western blot analysis exhibited that DB upregulated the expression of PTEN *in vivo* and *in vitro*. **Conclusions**: DB treatment suppresses the proliferation and migration of VSMCs and reduces neointimal formation by the mechanisms involving regulating the phenotype switch of VSMCs via upregulating PTEN expression.

Keywords: dipsacoside B; vascular smooth muscle cell; phenotype switch; balloon injury; PTEN

1. Introduction

Cardiovascular disease (CVD), which includes atherosclerosis, coronary heart disease, pulmonary hypertension and etc., is the major cause of death worldwide. The morbidity and prevalence remains very high and thus result in great burden on global healthcare [1–3]. Vascular remodeling (VR) is the underlying pathogenesis of CVD. As today, the close relationship between CVD and VR has been confirmed, and more attention has been paid to the strategies for the prevention and treatment of VR in cardiovascular events. However, medications utilized in clinical for VR treatment, such as renin-angiotensin-aldosterone system inhibitors, calcium antagonists, potassium channel openers, and so on, have limited efficacy [4]. Besides, the unpleasant side effects associated with the aforesaid medications, like coughing, rash, angiopaural edema and etc., restrict their applicability. Thus, the search for potential therapeutic agents for VR remains critical.

Intimal hyperplasia and arterial restenosis are two core parts existing in VR. Throughout the progression, vascular smooth muscle cell (VSMC) is the main contributor. Stimulated VSMCs underwent phenotype switch, proliferated and migrated from the media to the intima, thus causing the narrowing or restenosis consequence [5,6]. Therefore, targeting hyper proliferating VSMCs and suppressing VSMCs' phenotype switch can contribute to therapeutic outcome in vascular diseases [7]. Multiple studies [8,9] have demonstrated that phosphatase and tension homolog deleted on chromosome 10 (PTEN) is the key regulator in VSMCs' phenotype switch and sequentially alters behaviors in the migration and proliferation of VSMC. Down-regulation of PTEN promotes the migration and proliferation of VSMCs [10], whereas upregulation of PTEN suppresses the migration and proliferation of VSMCs, blunts neointimal formation, and alleviate stenosis [9,11,12]. All these clues implicate that PTEN may be a pivotal target in VR-related diseases. Herein, searching for medications that can block the development of VR by targeting PTEN becomes an effective strategy for CVD treatment.

In recent decades, natural components from Chinese herbs have exhibited properties of suppressing VR with multiple targets and less negative effects [13–15]. Triterpenoid saponins isolated from Chinese medicine have exhibited attenuation effects in VR. Report has shown that astragaloside IV can inhibit the proliferation and migration of rat VSMC by targeting CDK2 [16]. Besides, panax noto-

ginseng saponins present inhibitory effects in the phenotype transformation of VSMCs and suppress abdominal aortic aneurysm progression [17]. Furthermore, ginsenoside Re inhibits VSMC proliferation induced by PDGF-BB via the eNOS/NO/cGMP pathway [18]. Evidence is mounting that triterpenoid saponins including Notoginsenoside R1, Astragaloside IV exert beneficial effects in oxygen and glucose deprivation-induced injury [19] and myocardial infarction [20] via PTEN expression. Nevertheless, it has not been reported that triterpenoid saponins inhibit the phenotype transformation of VSMC in VR via PTEN. Dipsacoside B (DB), triterpenoid saponins, is an herb monomer extracted from Chinese medicine-*Dipsacusasper* or *Lonicera macranthoides* [21]. However, the pharmacological effects of DB are rarely reported. Recent studies have demonstrated that DB exerts beneficial effects on brain ischemic injury via the inhibition of mitochondrial E3 ubiquitin ligase 1 [21], suggesting the therapeutic potential of DB in VR-related diseases. Our previous study [22] has demonstrated that DB suppresses the proliferation of VSMCs by downregulating TOP2α, but whether DB exert its role by interfering VSMCs' phenotype switch and its interaction with PTEN needs to be further elucidated.

In the present study, we adopted rat abdominal aorta balloon injury model to evaluate the inhibitory effects of DB on neointimal formation *in vivo*. Then, we employed Ang-II to induce VSMCs' proliferation and migration, investigated the effects of DB on the proliferation and migration of VSMCs and further examined the association between DB and PTEN in these effects. Our study may provide a new sight into the protective effect of DB in VR-related diseases.

2. Materials and Methods

2.1 Animals and Study Design

Thirty-two male Sprague-Dawley (SD) rats (280–330 g) were purchased from department of laboratory animal science of university of south China (Hengyang, China). Animals were housed under controlled temperature (25 $\pm$ 1 °C) and humidity (55%), with 12 h light-dark cycles and free access to food and water. All rats were randomly divided into 4 groups: sham operation (sham) group, balloon-injury (BI) group, balloon-injury administrated with 10 mg/kg dipsacoside B (BI + 10 mg/kg DB) group and balloon-injury administrated with 60 mg/kg dipsacoside B (BI + 60 mg/kg DB) group.

DB was obtained from Nanjing spring & autumn biological engineering co., td and dissolved in distilled water.

2.2 Rat Abdominal Aorta Balloon Injury

Balloon injury was administrated as follows: SD rats were anesthetized with isoflurane by aerosol inhalation and fixed on the simple operating plates. The skin around the neck was shaved, disinfected and paved with sterilized sheets. The platysma muscle and left sternocleidomastoid muscle were separated at the left of the midline to find left common carotid artery. The bifurcation between the left external and internal carotid artery was ligated with 1# silk suture, and then the proximal end of the left common carotid artery was ligated with vascular camp to block blood flow. Balloon catheter was inserted along left common carotid artery and put at the end of abdominal aorta, inflated with 8 Kpa and carefully pulled, repeated three times. After the intervention, penicillin was injected intramuscularly with 100,000 U a day for continuous 3 days to prevent infection. After 14 days with indicated administration, rats were sacrificed and tissue samples were obtained for the follow-up detection.

2.3 HE Staining

2 μm sections of abdominal aorta samples were put in xylene twice for de-waxing, each for 5 min and then placed in gradient ethanol gradually (100%–95%–90%–85%–80%–75%), each for 5 min. After the deparaffinization and rehydration, the sample slices were stained with hematoxylin solution for 15 min, rinsed with distilled water followed by 1% hydrochloric acid alcohol for almost 5 s, and again rinsed. The slices were put in gradient ethanol gradually (70%–80%–90%–95% - anhydrous alcohol) for dehydration, each for 3 min, and then stained with eosin solution for 3 min, put in gradient ethanol (90%–95%–100%) for dehydration, each for 3 min, placed in I, II, III for 15 min respectively. The mounted slides were then observed and photographed using fluorescence microscope (IX83, Olympus, Japan). Tcapture software(Shanghai, China)was used to analyze and measure the thickness of intima and media.

2.4 Cell Culture and Transfection

VSMCs were purchased from cell bank of central laboratory of Xiangya hospital central south university (Changsha, Hunan, China). VSMCs were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (12800017, Gibco, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS) (P30 - 3302, PAN-Biotech, Dorset, UK) and 1% penicillin-streptomycin solution (PB180120, Procell, Wuhan, China). All cell lines were incubated in a incubating tank with CO_2 concentration of 5% and temperature of 37 °C.

DB (purity >98%) was obtained from Nanjing spring & autumn biological engineering co., td (Nanjing, China) and dissolved in DMEM with 1% FBS.

2.5 Cell Viability Assay

Briefly, cells were seeded (8×10^3 per well) into 96-well plates. When the cell confluence had reached 80%, cells were pre-incubated with DB of various concentrations (1, 3, 10, 30, 100 μM) for 2 h and then co-incubated with angiotensin II (Ang II 1 μM; A9525, Sigma, St. Louis, MO, USA) for another 24 h. On the second day, 100 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT; M-2128, Sigma, St. Louis, MO, USA) reagent was added into each well and the cells were incubated in cell bank for 4 h. After 4 h, 150 μL of dimethyl sulfoxide (DMSO; D8371, Solarbio, Beijing, China) was added into each well and shaken on the oscillators for 10 min. Absorbance at 570 nm was measured using a microplate reader (Synergy HTX, Biotek, VT, USA).

2.6 EdU Incorporation Assay

5-ethynyl-2'-deoxyuridine (EdU) incorporation assay (C0081S, Beyotime, Shanghai, China) was performed according to the manufacturer's instructions to detect the proliferation of VSMCs. Cells were seeded (1×10^4 per well) into 96-well plates and divided into 6 groups: control group (DMEM with 1% FBS), Ang II group (1 μM Ang II), curcumin + Ang II group (5 μM cur + 1 μM Ang II), DB + Ang II group (3, 10, 30 μM DB + 1 μM Ang II). After 24 h, 50 μM EdU solution (100 μL per well) was added into each well and incubated for 2 h. After EDU staining, cells were fixed with 4% paraformaldehyde (100 μL per well) for 15 min, permeabilized with 0.3% Triton X-100 for 15 min, and incubated with click reaction liquid for 30 min. Nuclei were stained with Hoechst 33342 (100 μL per well) for 10 min. Cells were observed under a fluorescent microscope (IX83, Olympus, Tokyo, Japan).

2.7 Wound Migration Assay

Cells were seeded (5×10^5 per well) into 6-well plates with four parallel lines drawn on the back in advance. When the cell confluence had reached 60–80%, cells were incubated with 1% DMEM for 24 h. The monolayer was scratched using a 1 mL sterilized tip and the detached cells were washed with PBS. Different factors were added into each well respectively according to the corresponding group. VSMCs were photographed at 0, 12 h post-wounding. The average migration distance was calculated using Image-Pro Plus (Media Cybernetics, Maryland, America). The migration rate = $(T_0 - T_{12})/T_0$ (T_0 refers to the width of initial scratch, T_{12} refers to the width of final scratch).

2.8 Western Blot Analysis

Cells were washed with PBS, harvested, and lysed in lysis buffer. Protein samples of 20 μL/well were separated by 8–10% SDS-PAGE (100 V, 100 min), transferred onto polyvinylidene difluoride membranes (PVDF, Millipore, CA) (200 mA, 2 h), blocked in TBST containing 5% fat-free milk at 37 °C for 1 h, incubated with the indicated primary antibodies at 4 °C overnight, and then incubated with the corresponding secondary antibodies at 37 °C for 1 h. Blots were visualized using Image lab 3.0 software (Hercules, CA, USA). GAPDH was used as an internal control. Anti-PTEN (1:1000, ab170941), anti-osteopontin (anti-OPN, 1:1000, ab8448), anti-TAGLN/Transgelin (anti-SM22α, 1:1000, ab14106) and anti-alpha smooth muscle actin (anti-α-SMA, 1:10000, ab124964), anti-PCNA (1:5000, ab29) antibody was obtained from Abcam (Cambridge, UK). Anti-GAPDH (1:2500, 10494-1-AP), goat-anti-rabbit (1:5000, SA00001-2) and goat-anti-mouse (1:5000) were obtained from Proteintech (Wuhan, China).

2.9 Immunofluorescence

Cells were fixed with 4% paraformaldehyde for 15 min, washed with PBS three times, each for 5 min, blocked in the BSA (5%) for 60 min, incubated with the indicated primary antibodies at 4 °C overnight, and then incubated in dark with corresponding secondary antibodies at room temperature for 1 h. Cells were stained with DAPI and washed with PBS three times, each for 5 min. Inverted fluorescence microscope (ZKX53; Olympus, Tokyo, Japan) was used to obtain images. Anti-OPN (1:200, ab8448), anti-SM22α (1:200, ab14106) and anti-α-SMA (1:1000, ab124964) antibody was obtained from Abcam (Cambridge, UK). Dylight 594 red fluorescent secondary antibody (e032410) was purchased from Jakson.

2.10 Immunohistochemistry

2 μm sections of abdominal aorta samples were put in H_2O_2 (3%) at room temperature for 10 min. Then, the slides were soaked in PBS three times, each for 5 min, blocked in BSA (5%) for 30 min. After 30 min, the slides were incubated with primary antibodies against PTEN (1:100, ab170941, abcam) at 4 °C overnight, and then incubated with secondary antibody at 37 °C for 20 min and stained with DAB for 1 min.

2.11 Statistics Analysis

All data were expressed as the mean $\pm$ SD. Graphpad Prism 8.3.0 (San Diego, CA, USA) was used to analyzed the data. The difference among groups was analyzed by one-way ANOVA. Results were regarded as statistically significance at $p < 0.05$.

3. Results

3.1 DB Inhibits Neointimal Formation

In the progression of AS, the proliferation and migration of VSMCs contributes to arterial restenosis. Given the fact, we induced a rat balloon catheter-injured abdominal aorta model to study the role of DB *in vivo*. HE staining showed that DB inhibits the intimal hyperplasia in rats with balloon catheter-injured abdominal artery (Fig. 1A,B).

3.2 DB Inhibits VSMCs Proliferation and Migration

To further elucidate the underlying mechanism of DB in cultured VSMC, MTT, Edu and wound migration assays were used to test the measurements of cell viability, proliferation and migration in VSMCs treated with DB at indicated concentrations for 24 h. MTT assays demonstrated that no cytotoxicity was observed at DB concentrations up to 100 Mm (Fig. 2A). Ang-II significantly increased the

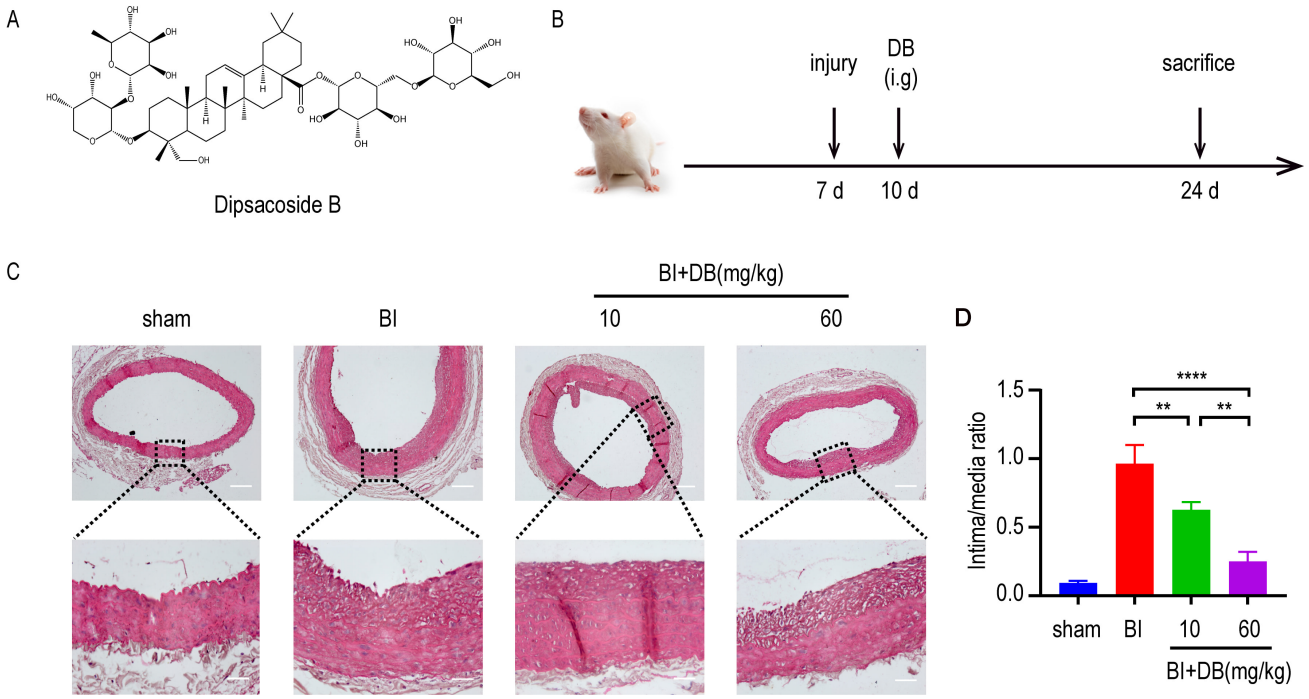

Fig. 1. DB inhibited neointimal formation. (A) The chemical structure of DB. (B) Schematic illustration of animal experimental protocol. (C) H&E staining of neointimal formation in arteries from rats underwent different administration after balloon injury, magnification: upper $\times$200, lower $\times$400. Scare bar: 20 μM. (D) The ratio of intima-to-media in (C). sham, sham operation group; BI, balloon-injury group; BI + 10 mg/kg DB, balloon-injury treated with 10 mg/kg DB group; BI + 60 mg/kg DB, balloon-injury treated with 60 mg/kg DB group. $^{**}p < 0.01$ and $^{****}p < 0.0001$.

cell viability of VSMCs, which was inhibited by DB in a dose-dependent manner (Fig. 2B). Based upon the result, we chose the concentration of 3, 10, 30 μmol/L for further study. Western blot showed that DB decreased PCNA expression (Fig. 2C,D). Edu assay demonstrated that the Ang II-induced proliferation of VSMCs was inhibited by DB (Fig. 2E,F). Furthermore, the wound migration assay demonstrated that DB repressed the migration of VSMCs in a dose-dependent manner (Fig. 2G,H).

3.3 DB Exerts Its Role by Modulating Phenotype Switch of VSMCs

Increasing evidence [5,23] has shown that the phenotype switches of VSMCs from contractile phenotype to synthetic ones secrets growth factors, and promotes the proliferation and migration of VSMCs. To investigate the interaction between DB and phenotype switch, we employed western blot and immunohistochemistry analysis to measure the expression of phenotype switch related protein-alpha smooth muscle actinα (SMA), TAGLN/Transgelin (SM22α) and osteopontin (OPN).

Western blot and immunohistochemistry analysis demonstrated that Ang-II down-regulated the expression of contractile protein-α-SMA and SM22α while it up-regulated the expression of synthetic protein-OPN. DB treatment reversed these alterations in a dose-dependent manner (Fig. 3A–H).

3.4 DB Exerts Its Role via Up-Regulation of PTEN Expression

Increasing evidence [8,11,24] showed that PTEN was a potent regulator in inhibiting the proliferation and migration of VSMCs. To determine whether PTEN was involved in the activity of DB, we employed western blot and immunohistochemistry to measure the expression of PTEN *in vivo*. The results of western blot and immunohistochemistry showed that rat subjected to balloon injury resulted in a significant reduction of PTEN expression. Furthermore, the inhibition of PTEN induced by balloon-catheter injury was dramatically attenuated by DB treatment (Fig. 4A,B). We then measured the expression of PTEN in cultured VSMCs. Interestingly, Ang-II significantly decreased the expression of PTEN while DB treatment significantly increased the expression of PTEN in Ang-II induced VSMCs (Fig. 4B), indicating that DB may exert its effects via up-regulation of PTEN expression.

4. Discussion

The excessive proliferation and migration of VSMCs is the basic pathogenesis in VR-related diseases, such as atherosclerosis, hypertension and etc. [25,26]. Thus, it is urgent to find a promising medicine that can suppress the proliferation and migration in an effective way. Previous studies [27,28] have demonstrated that triterpenoid

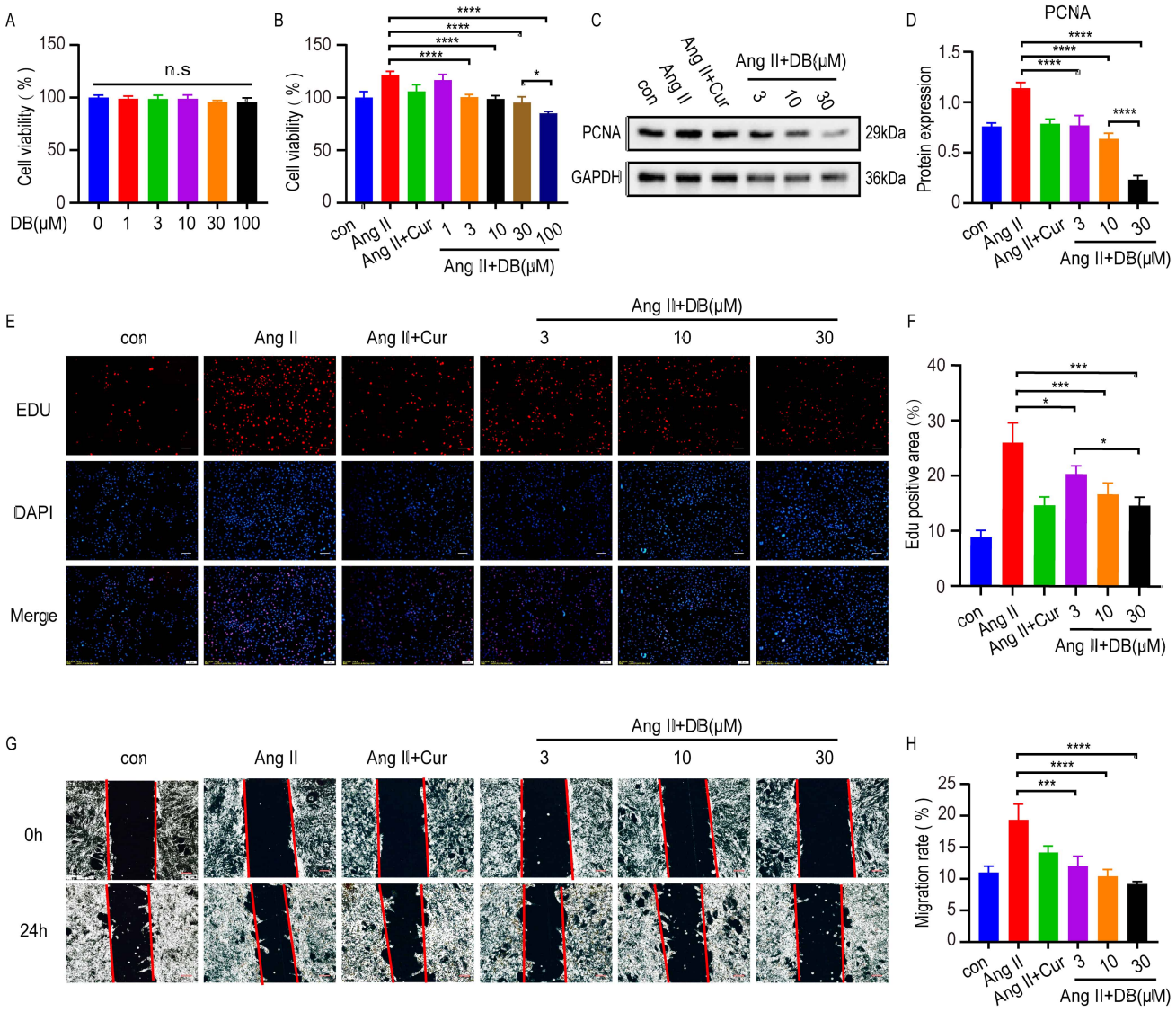

Fig. 2. DB inhibits the viability, proliferation and migration of VSMCs. (A) MTT assay analysis of cell viability rate of VSMCs treated with DB of different concentrations (0, 1, 3, 10, 30, 100 μM) for 24 h. (B) MTT assay analysis of cell viability rate of VSMCs co-incubated with Ang-II (1 μM) and DB of different concentrations (0, 1, 3, 10, 30, 100 μM) for 24 h. (C) Western blot analysis of PCNA expression in VSMCs co-incubated with Ang-II (1 μM) and DB of different concentrations (0, 1, 3, 10, 30, 100 μM) for 24 h. (D) Quantification of PCNA levels in (C). (E) EdU assay analysis of the cell proliferation of VSMCs co-incubated with Ang-II (1 μM) and DB of different concentrations (0, 1, 3, 10, 30, 100 μM) for 24 h, magnification: $\times$200. Scare bar: 50 μM. (F) Quantification of proliferation rate in (E). (G) Wound migration assays analysis of migration potential of VSMCs co-incubated with Ang-II (1 μM) and DB of different concentrations (0, 1, 3, 10, 30, 100 μM) for 24 h. magnification: $\times$100. Scare bar: 50 μM. (H) Quantification of migration rate in (H). DB, Dipsacoside B; Cur, Curcumin; Ang-II, Angiotensin II. GAPDH served as an internal control. *$p < 0.05$, ***$p < 0.001$ and ****$p < 0.0001$.

saponins can inhibit the neointimal formation caused by balloon injury. As a member in triterpenoid saponin family, the effect of DB on neointimal formation remains unknown. Therefore, rat balloon injury animal model was employed to investigate the *in vivo* activity of DB. *In vivo* experiments showed that DB treatment significantly decreased the intima to media ratio, indicating that DB blunted neointimal formation in rats underwent balloon injury.

Ang-II is an important inducer in the proliferation of VSMC. To find out whether DB has an influence on the proliferation and migration of VSMCs, we investigated the role of DB on the Ang-II induced proliferation and migration of VSMCs. CCK-8 assay showed that when the concentration of DB came up to 100 μM, no cytotoxicity was observed on the cell viability of VSMCs. Futhermore, the viability of VSMCs reduced about 20% with 3 μM (100.35 $\pm$ 3.45%),

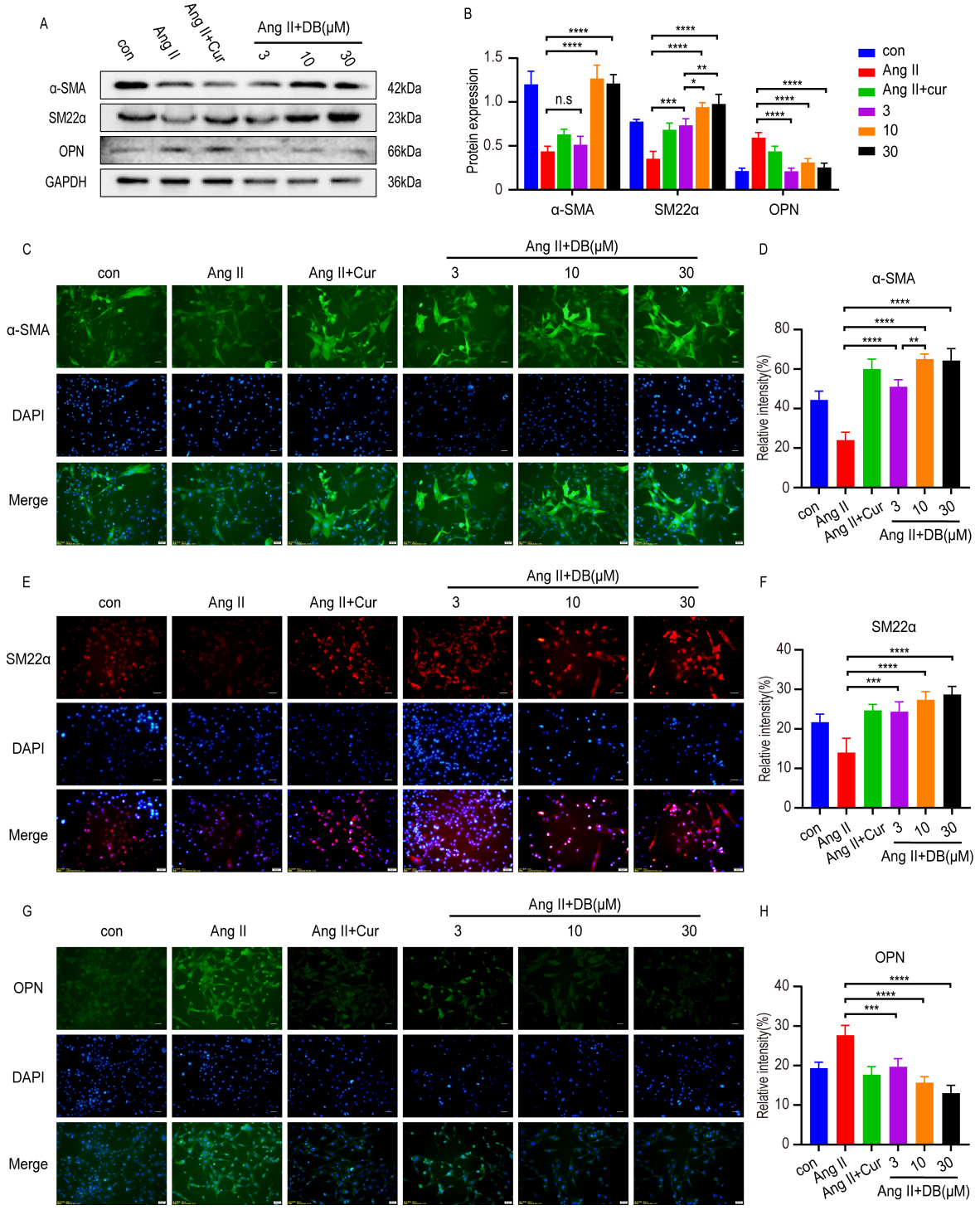

Fig. 3. DB exerts its role by modulating phenotype switch of VSMCs. (A) Western blot analysis of α-SMA, SM22α and OPN expression in VSMCs co-incubated with Ang-II (1 μM) and DB of different concentrations (0, 1, 3, 10, 30, 100 μmol/L) for 24 h. (B) Quantification of α-SMA, SM22α and OPN levels in (A). (C) Immunofluorescence analysis of α-SMA expression in VSMCs co-incubated with Ang-II (1 μM) and DB of different concentrations (0, 1, 3, 10, 30, 100 μmol/L) for 24 h, magnification: $\times$400. Scare bar: 20 μM. (D) Quantification of α-SMA immunofluorescence intensity in (C). (E) Immunofluorescence analysis of SM22α expression in VSMCs co-incubated with Ang-II (1 μM) and DB of different concentrations (0, 1, 3, 10, 30, 100 μmol/L) for 24 h, magnification: $\times$200. Scare bar: 50 μM. (F) Quantification of SM22α immunofluorescence intensity in (E). (G) Immunofluorescence analysis of OPN expression in VSMCs co-incubated with Ang-II (1 μM) and DB of different concentrations (0, 1, 3, 10, 30, 100 μmol/L) for 24 h, magnification: $\times$400. Scare bar: 20 μM. (H) Quantification of OPN immunofluorescence intensity in (G). DB, Dipsacoside B; Cur, Curcumin; Ang-II, Angiotensin II. GAPDH served as an internal control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.

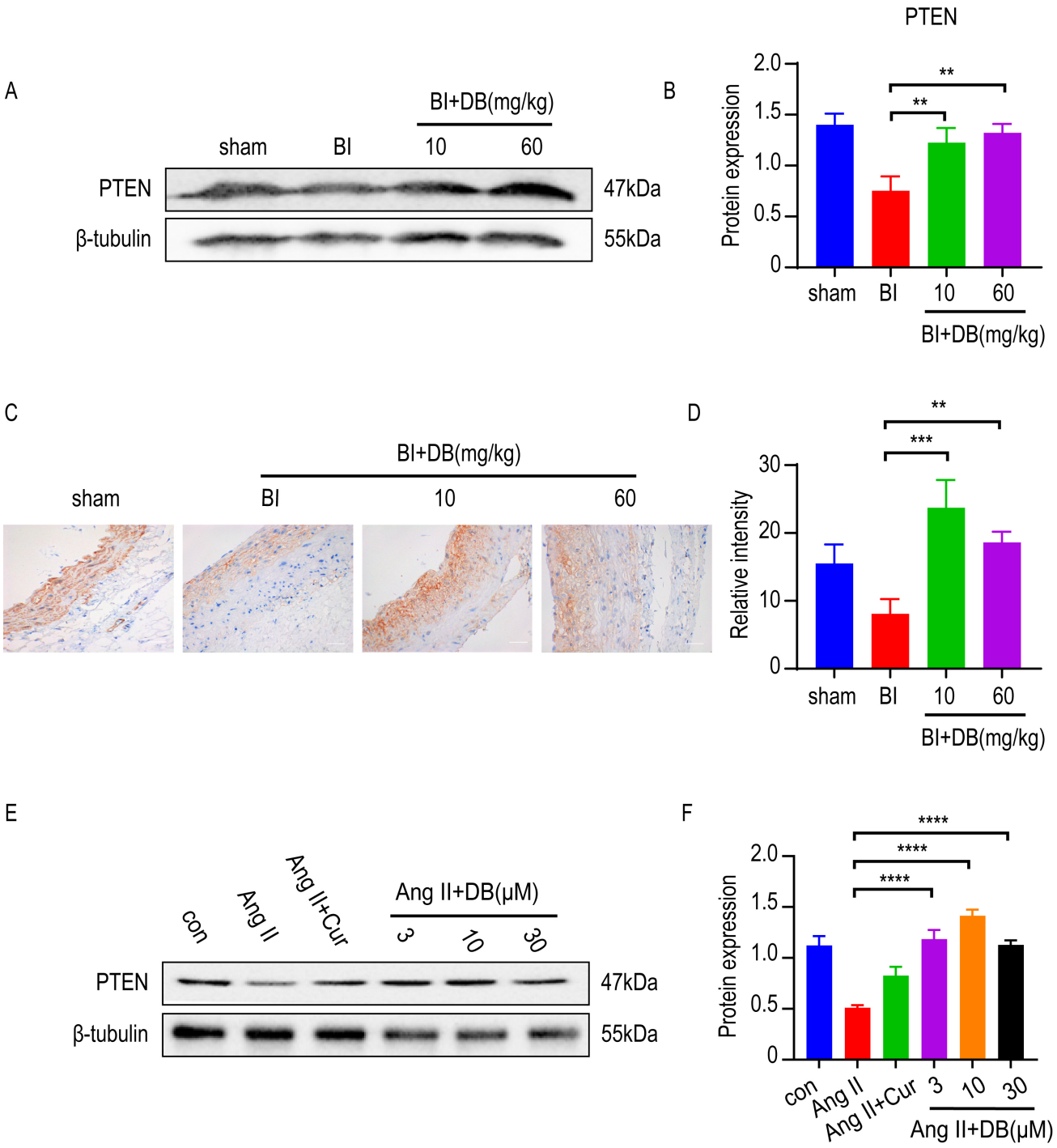

Fig. 4. DB exerts its role via up-regulation of PTEN expression. (A) Western blot analysis of PTEN expression in arteries obtained from rats underwent balloon injury (sham, BI, BI + 10 or 60 mg/kg DB treated groups). (B) Quantification of PTEN levels in (A). (C) Immunohistochemistry analysis of PTEN expression in arteries obtained from rats underwent balloon injury (sham, BI, BI + 10 or 60 mg/kg DB treated groups), magnification: ×400. Scare bar: 20 μM. (D) Quantification of PTEN levels in (C). (E) Western blot analysis of PTEN expression in VSMCs co-incubated with Ang-II (1 μM) and DB of different concentrations (0, 1, 3, 10, 30, 100 μmol/L) for 24 h. (F) Quantification of PTEN levels in (E). sham, sham operation group; BI, balloon-injury group; BI + 10 mg/kg DB, balloon-injury treated with 10 mg/kg dipsacoside B group; BI + 60 mg/kg DB, balloon-injury treated with 60 mg/kg dipsacoside B group; DB, Dipsacoside B; Cur, Curcumin; Ang-II, Angiotensin II. β-tubulin and GAPDH served as internal control. $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$.

10 μM (98.65 $\pm$ 3.13%) or 30 μM (95.15 $\pm$ 5.68%) DB treatment, compared to Ang-II group (121.58 $\pm$ 3.45%). As 3 μM, 10 μM and 30 μM DB showed obvious suppression effect on the Ang-II induced viability of VSMCs, the 3 concentration groups were used for the following experiments. PCNA level and Edu assay are the good methods to exhibit the proliferative states in VSMCs. Although PCNA was obviously expressed in Ang-II group, this effect was reduced by DB treatment. Consistent with the PCNA level in cultured VSMC, Edu assay showed that the addition of DB significantly reduced the increased positive area induced by Ang-II, indicating DB inhibits the proliferation of VSMCs. Wound migration assay showed that DB treatment significantly curb the increased cell migration induced by Ang-II. All these data reveal DB can inhibit the proliferation and migration of VSMCs.

Phenotype switch of VSMCs accompanied by the transition of protein markers is the key process in the proliferation and migration of VSMCs [29]. Among the VSMCs transformative process from contractile type to synthetic type, the expression of synthetic protein marker like OPN increased while the contractile protein marker likeα-SMA and SM22-α decreased, thus contributing to abnormal proliferation and migration [23,30,31]. Previous studies [31] have demonstrated that inhibition of excessive VSMC phenotypic switch are benefits during arterial remodeling. Studies have proven that triterpenoid saponins repressed VSMC proliferation by inhibiting phenotype transformation [17,18]. Therefore, we focus on the biomarkers to investigate whether DB exerts a role by blocking the phenotype switch of VSMC. Here, we used western blot and immunofluorescence to measure the expression of α-SMA, SM22-α and OPN. Compared with Ang II group, DB treatment effectively upregulated the expression of α-SMA and SM22-α, and downregulated the expression of OPN. These results revealed that DB inhibits the proliferation and migration of VSMCs by interfering phenotype switch.

Based on the effect of DB on anti-proliferation and phenotypic regulation *in vivo* and *in vitro*, we then further dissect the underlying mechanism. Increasing evidence [9,32] revealed that PTEN is a powerful target that can regulate VSMCs' proliferation and migration, which shows close association with vascular fibrosis and remodeling [33,34]. Studies have proven that Ang-II decreased PTEN expression in SMC [33]. The low level of PTEN in turn promotes the proinflammatory SMC phenotype [35], and subsequently improves vascular remodeling [36]. The above results demonstrated that DB suppressed neointimal formation, and inhibited Ang-II induced migration and proliferation of VSMC. However, whether DB interfered with PTEN remains to be further elucidated. To investigate whether DB can affect PTEN expression, immunofluorescence and western blot was utilized to measure the effect of DB treatment on PTEN expression. Our data showed that DB significantly upregulated PTEN expression *in vivo* and *in vitro*, indicating DB may exert its role by targeting PTEN expression.

However, there are still a number of issues that need to be further elucidated. Firstly, many investigations regarding the interaction between PTEN stability and proteasome has shown that proteasome has an impact on PTEN protein stability [37]. Through the ubiquitin/proteasome pathway, treatment that modulates PTEN stability can suppress cell proliferation and migration [38]. But the interaction among DB, PTEN and proteasome remains unknown. Then, it is well known [20,24] that PTEN can suppress the proliferation of VSMCs by dephosphorylat-ing phosphatidylinositol (3, 4, 5) triphosphate (PIP3) and preventing the activation of the PI3K/Akt signaling pathway. We hypothesize that DB plays its role through suppressing the PI3K/Akt pathway based on our observations of the elevation effects of DB on PTEN protein levels and the strong link between PTEN and PI3K/Akt pathway, and these effects will be shown in the follow-up research.

In this study, we first demonstrated that DB treatment blunted the intimal formation in balloon injured abdominal aorta. Furthermore, we confirmed that DB treatment suppressed the proliferation and migration of VSMCs. Then, we examined PTEN expression in balloon injured abdominal aorta and cultured VSMCs, and found DB treatment upregulated PTEN expression, revealing that DB may exert its role by upregulating PTEN expression. Therefore, for the first time, we report a novel role of DB in the cardiovascular disease and sheds insights into the application of DB in arterial restenosis.

5. Conclusions

In conclusion, DB can suppress the proliferation and migration of VSMCs and neointimal formation by regulating the phenotype switch of VSMCs and targeting PTEN expression.

Author Contributions

DL and QT designed the research study. JX, DY and ZS advised experimental design. YH and YC performed the research. WQ and YH analyzed the data and wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was approved by the Institute Research Ethics Committee of Hunan University of Chinese Medicine. The animal ethics number is LL2020022001.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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