Caveolin-1 modulates hypertensive vascular remodeling via regulation of the Notch pathway

QIAN WANG[1], MINXI LAO[1], ZHEN XU[2], MEILIN DING[1], SHAOLEI GUO[3*] and LING LI[4*]

[1]Special Medical Service Center, The First Affiliated Hospital of Sun Yat-Sen University; [2]Department of Rehabilitation, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University; Departments of [3]Neurosurgery and [4]Neurology, National Key Clinical Department and Key Discipline of Neurology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong 510080, P.R. China

Received February 19, 2020; Accepted July 24, 2020

DOI: 10.3892/mmr.2020.11508

Abstract. Hypertension is one of the critical risk factors of cerebrovascular disease. Caveolin-1 (Cav-1) has been suggested to be involved in the development of hypertension; however, the underlying mechanism remains largely unknown. Therefore, the present study aimed to investigate the mechanism underlying Cav-1 in hypertension. In the present study, the hypertension model was induced by infusion of angiotensin II (Ang-II) in rats. Cell Counting Kit-8 assay was used to detect the viability of human umbilical vein endothelial cells (HUVECs). Flow cytometry was used to determine the apoptosis of HUVECs. Transmission electron microscopy was utilized to address the thickness of the vessel walls. Reverse transcription-quantitative PCR, western blotting and immunofluorescence staining were used to assess the mechanism of cav-1/Notch1 involved in hypertensive vascular remodeling. In the present study, an Ang-II-induced hypertension model was successfully established in rats. With this model, it was found that the expression levels of cav-1 and Notch1 were significantly increased in brain tissues in the hypertension group compared with the sham-operated group. In cultured HUVECs, knockdown of cav-1 regulated Ang-II-induced HUVEC viability and apoptosis, and modulated hypertensive vascular remodeling, which was mediated by the Notch pathway. The data of the present study demonstrated that the cav-1/Notch signaling plays an important role in the regulation of Ang-II-induced hypertension and vascular remodeling.

Correspondence to: Professor Ling Li, Department of Neurology, National Key Clinical Department and Key Discipline of Neurology, The First Affiliated Hospital of Sun Yat-Sen University, 58 Zhongshan Er Road, Guangzhou, Guangdong 510080, P.R. China
E-mail: wangqianmaomao@163.com

*Contributed equally

Abbreviations: Ang-II, angiotensin II; DAPT, N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester; VSMCs, vascular smooth muscle cells; VEGFR, vascular endothelial growth factor receptors

Key words: hypertension, caveolin-1, Notch pathway, vascular remodeling, apoptosis

Introduction

Hypertension, which promotes the occurrence of cerebral venous dysfunction-mediated brain tissue damage, such as venous sinus thrombosis, venous cerebral infarction, venous cerebral hemorrhage, periventricular venous disease, venous neurocytotoxicity, neurodegeneration and demyelination, is one of the primary risk factors of cerebrovascular disease (1-4). Vascular remodeling has been shown to be important for the progression of hypertension-mediated cerebrovascular disease (5). Under hypertensive conditions, blood vessels undergo a series of structural and functional changes to develop and maintain high blood pressure, which leads to hemiplegia, epilepsy and dementia, thus seriously affecting the health of the patient (6). Therefore, understanding the underlying mechanism of vascular remodeling will aid in the prevention of hypertension-mediated cerebral venous disease.

Caveolin-1 (cav-1) is a primary structural protein and a regulatory component of caveolae. It participates in various physiological processes, such as vesicle transport, membrane phospholipid metabolism, cholesterol transport and cell signaling (7). Aberrant expression of cav-1 is closely associated with the occurrence of various diseases, including cardiovascular diseases, lung injury, tumorigenesis and infectious diseases (8-11). Cav-1 regulates its activity through complementary binding with other signal molecules and thus, modulates physiological processes, such as cell proliferation and differentiation, via different signaling pathways. Increasing evidence has indicated that cav-1 may be implicated in the development of hypertension. It has been reported that, in mice, cav-1 acted on inflammation and vascular remodeling, independent of the regulation of blood pressure or cardiac hypertrophy (12). Another study demonstrated that the upregulation of cav-1 and caveolae increased agonist-induced production of pulmonary arteries via the modulation of store-operated $Ca(2^+)$ entry and receptor-operated calcium entry, contributing to the progression of pulmonary hypertension in rats (13). However, the

detailed mechanism of cav-1 in Ang-II-induced hypertension remains largely unknown.

The Notch pathway has been shown to play a crucial role in vascular development (14). The activation of the Notch signaling pathway is mediated by the binding of cellular surface receptors (Notch1-4) to their ligands, which results in the cleavage of the intracellular domain of the receptors. Subsequently, the intracellular domain enters the nucleus where it promotes the transcription of downstream effectors (15). Previous studies reported that Notch1 signaling is implicated in the progression of pulmonary hypertension, but the function and mechanism of Notch1 in Angiotensin II (Ang-II)-induced hypertension remains unclear (16-18).

The Ang-II-induced hypertension model is commonly used in studies on hypertension (12,19). In the present study, the Ang-II-induced hypertension model was successfully established in rats and it was found that the expression of cav-1 and Notch1 were significantly upregulated in the brain tissues of hypertensive rats. Moreover, the present data demonstrated that cav-1 modulated hypertensive vascular remodeling by regulating the Notch signaling pathway. These findings may provide novel therapeutic candidate targets for the treatment of hypertension.

Materials and methods

Cell culture. Human umbilical vein endothelial cells (HUVECs) were purchased from the American Type Culture Collection and were cultured in endothelial cell medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2. HUVECs were treated with 5 ng/ml Ang-II (Sigma-Aldrich; Merck KGaA). A total of 24 h later, cells were harvested and used for further experiments.

Cell treatment. Cells were treated with 20 μM DAPT (N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester) (Sigma-Aldrich; Merck KGaA), a Notch pathway inhibitor, for 24 h at 37˚C.

Cell Counting Kit-8 (CCK-8) assay. Cells ($5x10^3$) were seeded in 96-well plates. After treatment with Ang-II (5 ng/ml) for 24 h, cell viability was determined with a CCK-8 assay (Beyotime Institute of Biotechnology), according to the manufacturer's instructions.

Apoptosis detection. Apoptosis analysis was performed using a FITC-Annexin V apoptosis detection kit (BD Biosciences). Cells ($1x10^6$) were harvested and washed once with PBS. Then, cells were incubated with propidium iodide and FITC-Annexin V for 15 min at 4˚C. After washing with PBS three times, cells were suspended in binding buffer and analyzed using a CytoFLEX flow cytometer (Beckman Coulter, Inc.) and FlowJo software (version 10; FlowJo LLC). Early + late apoptosis was assessed.

Establishment of hypertension model. A total of 12 female Sprague-Dawley (SD) rats (age, 6 weeks; weight, 153±12.4 g) were randomly divided into two groups: Hypertension model group (n=6) and sham operation group (sham group, n=6). One rat in the hypertension model group died, the cause of death was cerebral infarction, it was found with symptoms of hemiparalysis and the dissection was performed after death. The duration of the animal experiment was 1 month. Animal health and behavior were monitored once a day post-surgery. All the animals were kept in the specific-pathogen-free animal laboratory at 24±1˚C with 50±10% humidity, 12-h light/dark cycles, and free access to food and water. The weight of the SD rats was measured before surgery. Rats were anesthetized with pentobarbital sodium (30 mg/kg). Under sterile conditions, an incision was made in the mid scapular region and an osmotic pump containing angiotensin II (infusion rate, 0.7 mg/kg per day) was implanted. Blood pressure was measured once before surgery, and 1 week and 3 weeks after surgery. Sham-operated rats underwent the same surgical procedure after anesthesia, except that no osmotic pump was implanted. Rats were euthanized by pentobarbital sodium (150 mg/kg), and the heart rates were detected to verify death. All experimental protocols were approved by the Committee of Animal Care and Use at Sun Yat-sen University (approval no. SYSU-IACUC-2019PGY260K; Guangdong, China).

Western blotting. Protein from cells or tissues were isolated and lysed in RIPA lysis buffer (Sigma-Aldrich; Merck KGaA), containing protease inhibitors (Sigma-Aldrich; Merck KGaA). Protein concentration was determined by the Braford method (BioRad Laboratories, Inc.). Proteins (50 μg) were separated via SDS-PAGE on a 10% gel, and subsequently transferred onto PVDF membranes. The membrane was blocked with 5% non-fat milk for 1 h at room temperature, and then incubated overnight at 4˚C with anti-collagen (COL)-I (cat. no. ab260043), anti-matrix metalloprotease (MMP)2 (cat. no. ab92536), anti-MMP9 (cat. no. ab38898), anti-cav-1 (cat. no. ab18199), anti-Notch1 (cat. no. ab167441) and GAPDH (cat. no. ab9485) antibodies (all 1:1,000; all purchased from Abcam). Subsequently, the membrane was incubated with a HRP-conjugated goat anti-rabbit IgG H&L secondary antibody (1:5,000; cat. no. ab6721; Abcam) for 2 h at room temperature. The proteins were detected by ECL chemiluminescence (EMD Millipore) and analyzed using ImagePro Plus software (version 6.0; Media Cybernetics, Inc.).

Small interfering (si)RNA transfection. siRNAs (si-1, si-2, and si-3) targeting cav-1 and control siRNA were synthesized by Shanghai GenePharma Co., Ltd. siRNA transfection (50 nmol) was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Then, 48 h post-transfection, cells were collected and used for further experiments.

Reverse transcription-quantitative (RT-q)PCR. Total RNA from cells and tissues was isolated using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RNA was reverse-transcribed into cDNA using a RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.). The following temperature protocol was used for reverse transcription: 37˚C for 2 min, 23˚C for 10 min, 55˚C for 10 min and 85˚C for 10 min. RT-qPCR was conducted on a PRISM 7500 System (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR-Green (Tiangen Biotech Co., Ltd.). The following thermocycling conditions were used for qPCR: 95˚C for 5 min,

followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. The expression was calculated using the $2^{-\Delta\Delta Cq}$ method (20). GAPDH was used as a control. Primers used are shown in Table I.

Immunofluorescence staining. Tissues were fixed with 4% paraformaldehyde overnight at room temperature. Paraffin-embedded tissue slices (thickness, 4 μm) were dewaxed and rehydrated. Slices were blocked with 10% goat serum (Thermo Fisher Scientific, Inc.) for 30 min at room temperature, and then incubated with anti-cav-1 (1:1,000; cat. no. ab17052; Abcam) at 4˚C overnight. After washing with PBS, slices were incubated with Alexa 555-conjugated secondary antibody (1:5,000, cat. no. A28180; Thermo Fisher Scientific, Inc.) for 2 h at room temperature. Diamidino-phenyl-indole (DAPI) was utilized to stain the nuclei. Images were obtained using a fluorescence microscope (Nikon Corporation) and analysed using ImagePro Plus software (version 6; Media Cybernetics, Inc.).

Transmission electron microscopy. To observe the alteration of the cerebral vein following the induction of hypertension, rat brain tissues were sliced into ~1 mm^3 and the white-matter region with small veins were incubated in 2.5% glutaraldehyde for 12 h at 4˚C. After washing with PBS, tissues were fixed with 1% osmic acid for 3 h at 4˚C. Tissues were dehydrated and embedded in epoxy resin for 4 h at room temperature. Then, the tissue was cut into 70-nm sections. After staining with uranyl acetate at room temperature for 2 h and lead citrate at room temperature for 15 min, tissues were observed using a transmission electron microscope (Leica Microsystems, Inc.).

Statistical analysis. Statistical analysis was carried out using SPSS 19.0 software (IBM Corp.). Comparisons were analyzed by one-way ANOVA and followed by Tukey's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Ang-II infusion induces hypertension in rats. In order to understand the molecular mechanism of cav-1 in the pathogenesis of hypertension, an Ang-II-induced hypertension model was established in rats. As shown in Fig. 1A, rats infused with Ang-II (the hypertension group) showed a significantly lower venous flow velocity compared with the sham-operated rats. In contrast, the blood pressure in the hypertension group was significantly higher compared with the sham-operated group (Fig. 1B). Moreover, the thickness of the vessel walls was significantly higher compared with the sham-operated animals (Fig. 1C). Together, these results suggested that the rat hypertension model induced by Ang-II was successfully established.

Cav-1 and Notch1 are upregulated after Ang-II infusion. The expression levels of cav-1 and Notch1 were examined in rat brain tissues. The protein levels of cav-1 and Notch1 were significantly increased in the brain tissue of the hypertension group compared with the sham-operated group, as characterized by immunofluorescence staining and western blotting, respectively (Fig. 2A and B). Consistent with these results, RT-qPCR analysis showed that the mRNA levels of cav-1 and Notch1 in the brain tissues of hypertensive rats were significantly higher compared with the sham-operated animals (Fig. 2C). Also, the mRNA expression of Notch3 was examined in the two groups, but there was no significant difference between them (Fig. S1).

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Genes	Primer sequences (5'→3')
Caveolin-1	F: GCTGAGCGAGAAGCAAGTGT
	R: GGTGAAGCTGGCCTTCCAAA
Notch1	F: AATGTGGATGCCGCAGTTGT
	R: TGATGTCCCGGTTGGCAAAG
MMP2	F: CCCATGAAGCCCTGTTCACC
	R: CGGTCGTAGTCCTCAGTGGT
MMP9	F: GACGGCAATGCTGATGGGAA
	R:GCAGAAGCCGAAGAGCTTGT
COL-1	F: CCAGTGTGGCCCAGAAGAAC
	R: GCAGGAAGGTCAGCTGGATG
β-actin	F: CGTAAAGACCTCTATGCCAACA
	R: CGGACTCATCGTACTCCTGCT

F, forward; R, reverse; COL, collagen; MMP, matrix metalloprotease.

Ang-II regulates HUVEC viability and extracellular matrix-related genes. To provide further support for the *in vivo* results, cultured HUVECs were utilized. A CCK-8 assay was carried out to determine the effect of Ang-II on HUVEC viability. The results showed that the viability of Ang-II-treated HUVECs was significantly decreased compared with the control cells (Fig. 3A). A flow cytometry assay revealed that Ang-II exposure significantly increased the apoptotic rate of HUVECs (Fig. 3B). In addition, RT-qPCR and western blotting demonstrated that Ang-II treatment significantly increased the mRNA and protein levels of collagen-1, MMP2 and MMP9, which are important regulators of the extracellular matrix (Fig. 3C and D). Therefore, these results indicated that Ang-II regulated HUVEC viability and altered extracellular matrix-associated protein expression.

Cav-1 modulates HUVEC viability and the extracellular matrix. Increased expression of cav-1 was also observed in HUVECs, following the administration of Ang-II (Fig. 4A). Thus, it was investigated whether cav-1 might contribute to Ang-II-mediated HUVEC cell viability and extracellular matrix alteration. To assess this, cav-1 expression was knocked down by siRNAs. As shown in Fig. 4B, the expression levels of cav-1 were significantly downregulated after transfection with cav-1 siRNAs (si-1, si-2, and si-3), as si-2 was shown to be the most effective it was selected for further experiments. It was found that the silencing of cav-1 restored the Ang-II-inhibited viability of HUVECs (Fig. 4C) Additionally, the enhancement of apoptosis in HUVECs induced by Ang-II was also

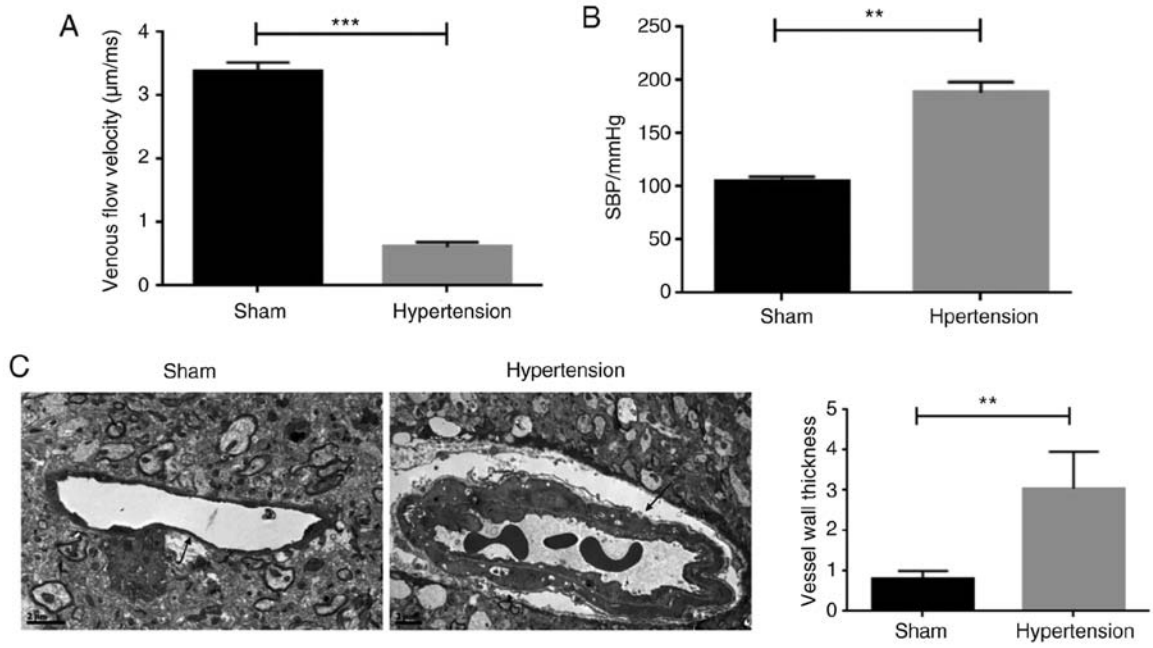

Figure 1. Establishment of Ang-II-induced hypertension model in rats. (A) Determination of venous flow velocity. (B) Blood pressure. (C) Determination of vessel wall thickness by transmission electron microscopy in hypertensive rats (hypertension) and sham-operated rats (sham). Scale bar, 2 μm. Arrows indicate the vessel. **P<0.01, ***P<0.001. Ang, angiotensin.

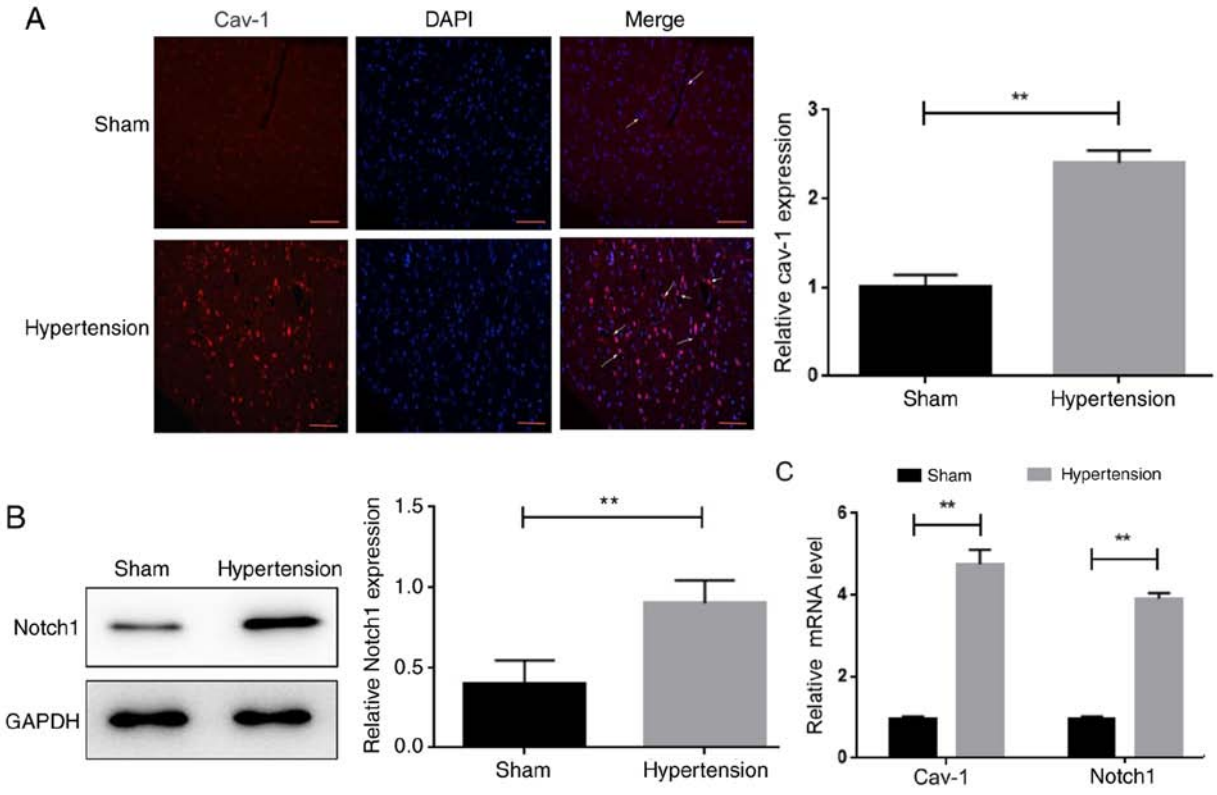

Figure 2. Expression of cav-1 and Notch1 in the brain tissues of hypertensive rats. (A) Immunofluorescence staining of cav-1 in brain tissues of hypertensive rats (hypertension) and control rats (sham). Scale bar, 100 μm. Arrows indicate the expression of cav-1. (B) Western blotting analysis of Notch1 expression in brain tissues of hypertensive rats (hypertension) and sham-operated rats (sham). (C) Reverse transcription-quantitative PCR analysis of cav-1 and Notch1 mRNA expression in the brain tissues of hypertensive rats (hypertension) and sham-operated rats (sham). **P<0.01. Cav-1, caveolin-1.

reversed by cav-1 knockdown (Fig. 4D). Moreover, the depletion of cav-1 downregulated the mRNA and protein levels of collagen-1, MMP2 and MMP9, which were increased by Ang-II (Fig. 4E and F). Together, these results suggested that cav-1 reversed Ang-II-induced viability and extracellular matrix alteration in HUVECs.

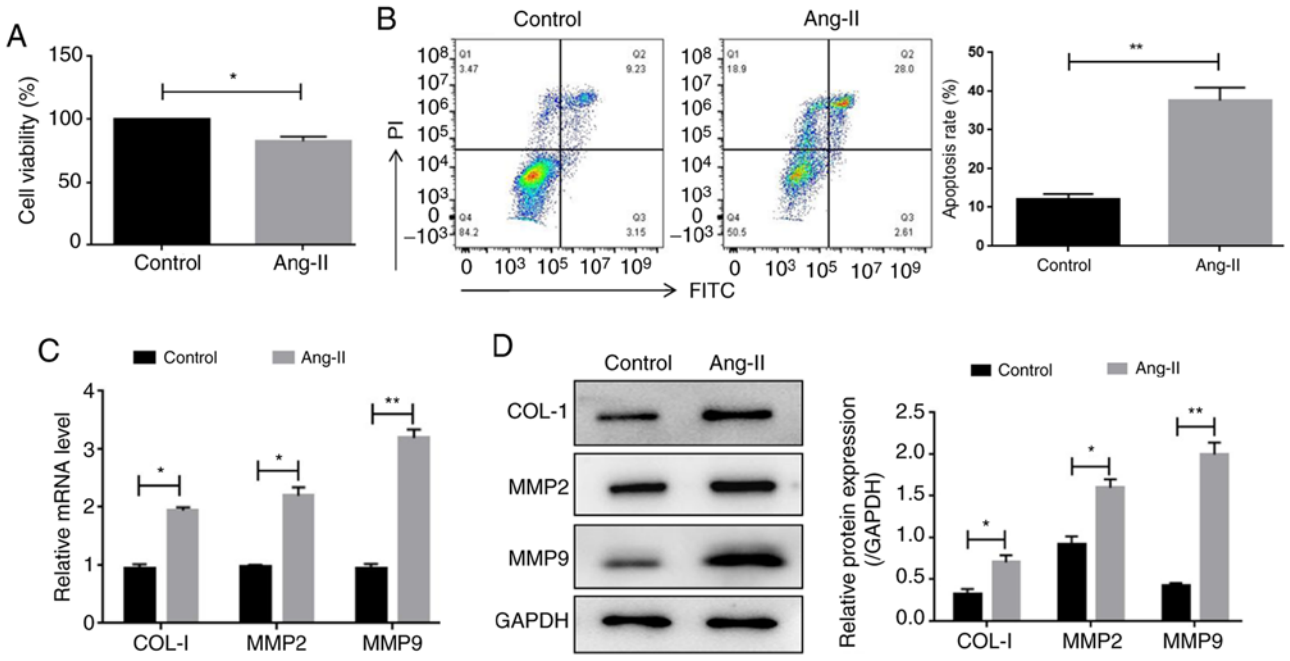

Figure 3. Ang-II induces hypertension model in HUVECs. (A) Cell Cycle Kit-8 assay was used to assess the viability of HUVECs after Ang-II treatment. (B) Flow cytometry was used to assess apoptosis of HUVECs after Ang-II exposure. (C) Reverse transcription-quantitative PCR was used to measure mRNA expression and (D) western blotting was used to determine protein expression levels of vascular remodeling-associated genes, COL-1, MMP2 and MMP9. *P<0.05, **P<0.01. HUVECs, human umbilical vein endothelial cells; Ang, angiotensin; COL, collagen; MMP, matrix metalloprotease.

Cav-1 modulates HUVEC viability and the extracellular matrix via Notch signaling. The upregulation of Notch1 in Ang-II-infused mice allowed for the investigation of whether cav-1 may regulate vascular remodeling via Notch signaling. Indeed, consistent with the *in vivo* results, treatment with Ang-II increased the expression of Notch1, whereas knockdown of cav-1 decreased the protein expression of Notch1 (Fig. 5A). To confirm the involvement of Notch signaling activation in vascular remodeling, the Notch pathway inhibitor, DAPT (N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester), was applied, which was demonstrated to decrease the expression of Notch1 (Fig. 5B). It was observed that while Ang-II treatment decreased the viability of HUVECs, DAPT treatment reversed this effect (Fig. 5C). Consistently, the increased apoptosis observed in Ang-II-treated HUVECs was decreased following DAPT administration (Fig. 5D). Moreover, treatment with DAPT reversed the Ang-II-mediated upregulation of Notch1, collagen-1, MMP2 and MMP9 (Fig. 5E and F). Overall, these results implied that cav-1/Notch functions in vascular remodeling.

Discussion

In the present study, a hypertension model induced by infusion of Ang-II was constructed. Using this model, it was found that cav-1 and Notch1 expression levels were significantly increased in the brain tissues of hypertensive rats compared with control animals. In cultured HUVECs, it was found that knockdown of cav-1 promoted Ang-II-induced HUVEC viability and altered hypertensive vascular remodeling. Furthermore, the data demonstrated that cav-1 exerted its function via regulation of the Notch pathway. Therefore, the present findings revealed a novel mechanism of cav-1/Notch in hypertension.

Multiple studies have suggested that cav-1 is implicated in different models of hypertension. For example, it has been reported that the depletion of C-C motif chemokine 5 rescued pulmonary vascular dysfunction and reversed pulmonary hypertension via activation of bone morphogenetic protein receptor type 2 by cav-1 (21). Fluvastatin protected against monocrotaline-induced pulmonary arterial hypertension via the downregulation of cav-1 in rats (22). In addition, cav-1 participated in the regulation of vascular remodeling. The upregulation of cav-1 decreased cavin-1 expression and promoted the viability and migration of vascular smooth muscle cells (VSMCs) in balloon injury-induced neointimal hyperplasia (23). It was also found that cav-1 deficiency prevented Ang II-mediated hypertrophy and inward remodeling of pial arterioles, which indicated that cav-1 promoted Ang-II-mediated hypertrophy and vascular remodeling (24). Furthermore, another study reported that the overexpression of cav-1 in mice enhanced medial hypertrophy and perivascular fibrosis of the aorta, and its coronary and renal arteries, and that cav-1 promoted hypertensive vascular remodeling (12). The results of the present study were consistent with previous studies. It was observed that cav-1 was increased in the brain tissue of Ang-II-infused hypertensive rats. Moreover, cav-1 depletion reversed the effects of Ang-II on HUVEC viability and apoptosis. Additionally, the knockdown of cav-1 decreased the expression levels of collagen-1, MMP2 and MMP9, which are critical factors involved in promoting vascular remodeling. Therefore, the present data indicated a crucial role for cav-1 in vascular remodeling, which may further contribute to hypertension.

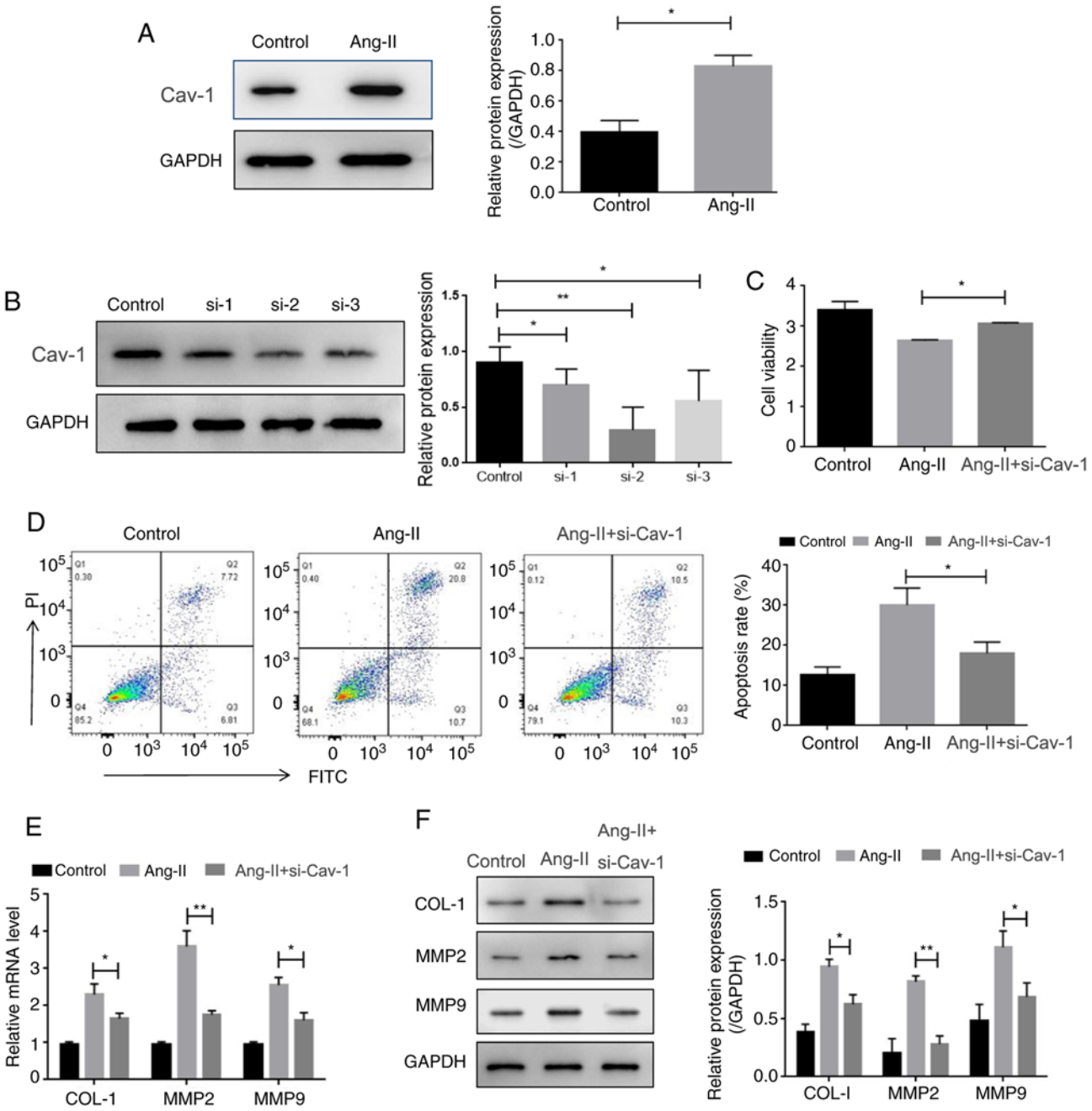

Figure 4. Effect of cav-1 knockdown on vascular remodeling. (A) Western blotting was used to determine expression of cav-1 in Ang-II-treated HUVECs. (B) Verification of the knockdown efficiency of cav-1 siRNAs via western blotting. (C) Depletion of cav-1 restored Ang-II-induced inhibition of HUVEC viability. (D) Depletion of cav-1 reversed the Ang-II-induced increase of HUVEC apoptosis. (E) Reverse transcription-quantitative PCR was performed to measure mRNA expression of vascular remodeling-associated genes in HUVECs treated with Ang-II alone or together with si-cav-1. (F) Western blotting was used to determine the expression of vascular remodeling-associated proteins in HUVECs treated with Ang-II alone or together with si-cav-1. *P<0.05, **P<0.01. HUVECs, human umbilical vein endothelial cells; Ang, angiotensin; siRNA, small interfering RNA; cav-1, caveolin-1; COL, collagen; MMP, matrix metalloprotease.

Previous studies have reported that, in coronary microvascular remodeling, the administration of Ang-II antagonists in rats increased caveolin-1 protein expression, and nitric oxide synthase (NOS) 3 gene and protein expression, as well as NOS activity, thereby reducing collagen expression in the vessel walls and suppressing vascular remodeling (25). The difference in the results of Ang-II-mediated cav-1 protein expression and the effects on vascular remodeling among different studies may be the result of studying different tissues, and the involvement of additional signaling modules. Thus, further research is required.

The Notch signaling pathway is a critical regulator in the occurrence and development of the vascular system (26). Notch belongs to the membrane protein receptor family. Notch1 and Notch4 are expressed in endothelial cells, whereas Notch1 and Notch3 are expressed in VSMCs (27,28). Notch activation regulates angiogenesis by inhibiting vascular endothelial growth factor receptors (VEGFR) and limiting the amount of vascular sprouting (28). It has been reported that treatment with DAPT attenuated intimal hyperplasia by inhibiting Notch1 signaling (29). Notch receptors 1 and 3 have

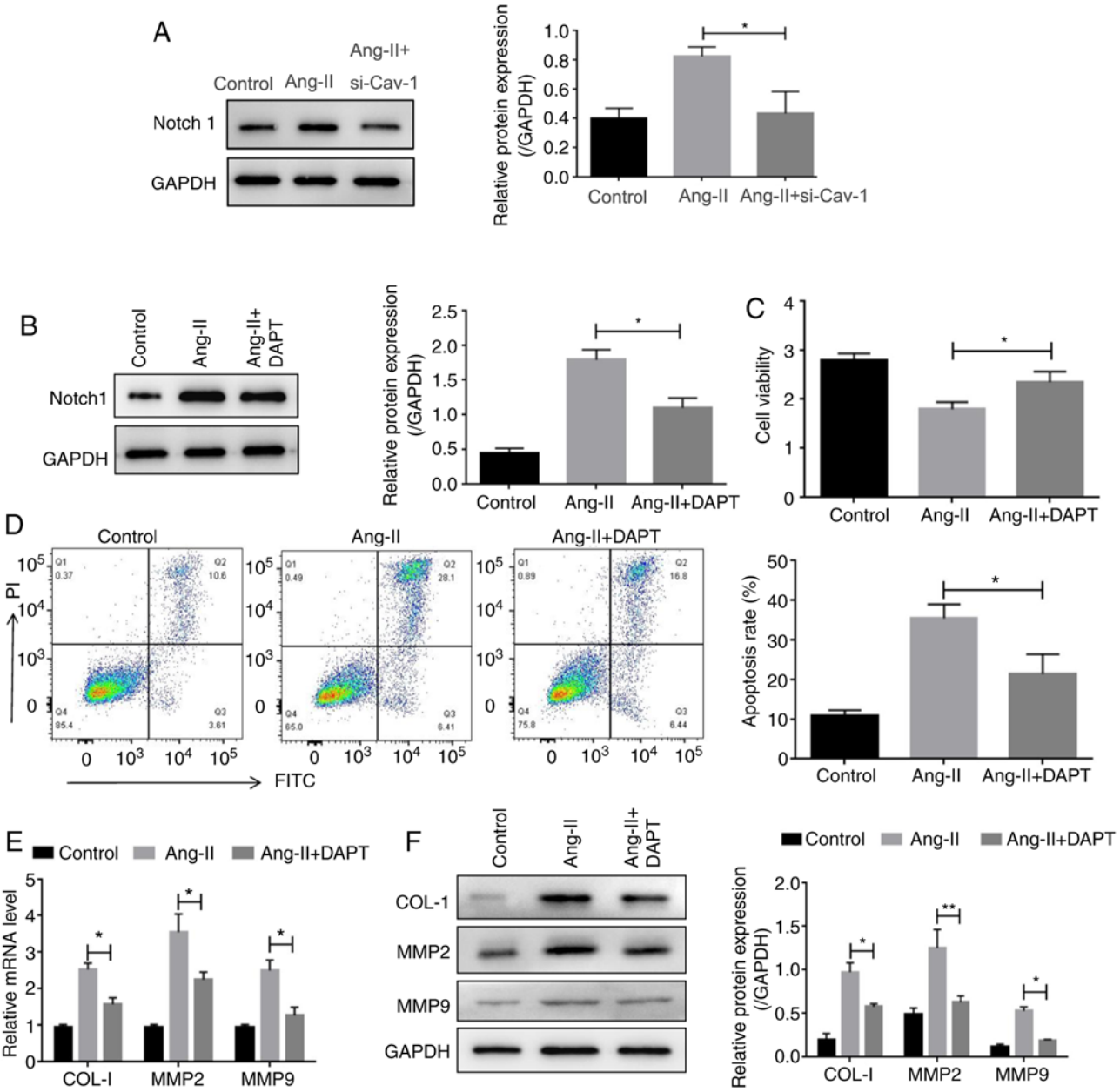

Figure 5. Cav-1 regulates vascular remodeling via Notch. (A) Western blotting was used to measure Notch1 expression in HUVECs treated with Ang-II alone or together with si-cav-1. (B) Western blotting was used to determine Notch1 expression in HUVECs treated with Ang-II alone or together with DAPT. (C) Cell Counting Kit-8 assay was used to measure viability of HUVECs treated with Ang-II alone or together with DAPT. (D) Apoptosis analysis of HUVECs treated with Ang-II alone or together with DAPT. (E) Reverse transcription-quantitative PCR was used to measure mRNA expression of vascular remodeling-associated genes in HUVECs treated with Ang-II or together with DAPT. (F) Western blotting was used to determine expression of vascular remodeling-associated proteins in HUVECs treated with Ang-II or together with DAPT. *P<0.05, **P<0.01. HUVECs, human umbilical vein endothelial cells; Ang, angiotensin; siRNA, small interfering RNA; DAPT, N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester; cav-1, caveolin-1; COL, collagen; MMP, matrix metalloprotease.

been demonstrated to play important roles in the proliferation and migration of VSMCs, and the secretion of MMP2 and MMP9 (30,31), which have roles in the modulation of the degradation of the extracellular matrix and the destruction of vascular wall matrix. Previous studies also suggested that the Notch pathway is involved in the development of hypertension. It has been reported that TNF-α triggered pulmonary arterial hypertension by repressing the bone morphogenetic type-II receptor and modulating the Notch pathway (32). Notch was revealed to regulate the Ca^{2+}-sensing receptor, and thus promoted hypoxia-induced pulmonary hypertension (33). In the present study, it was found that Notch1 expression was elevated in the brain tissues of Ang-II-induced hypertensive rats compared with sham-operated rats. The silencing of cav-1 decreased the expression of Notch1 induced by Ang-II. Moreover, treatment with DAPT reversed the effects of Ang-II on cell viability and apoptosis. Furthermore, DAPT decreased the Ang-II-induced upregulation of collagen-1, MMP2 and MMP9 expression. These results indicated that cav-1/Notch signaling contributed to Ang-II-induced vascular remodeling.

Of note, a limitation of the present study was that the alteration of cav-1 in hypertensive rats was determined

using brain tissue instead of cerebrovascular tissue. This was because after the rats were sacrificed, the cerebral veins started shrinking and thus it was difficult to separate them from the brain tissue.

In summary, the present data demonstrated an important role for cav-1/Notch1 signaling in the regulation of Ang-II-induced hypertension and vascular remodeling. Targeting the cav-1 or Notch signaling pathways may present promising strategies for the treatment of hypertension. The process of vascular remodeling includes degradation of the extracellular matrix, cell proliferation, apoptosis, vascular inflammation and fibrosis. Whether additional signaling molecules, such as EPH receptor 4, VEGF and epidermal growth factor receptors, are involved in the process, and how important the signaling pathway is *in vivo* in animals, will be investigated further in future research.

Acknowledgements

Not applicable.

Funding

The study was supported by grants from: National Natural Science Foundation of China (NSFC) (grant no. 81671153); the Natural Science Foundation of Guangdong Province, China (grant no. 2016A030313203); Guangdong Provincial Key Laboratory for Diagnosis and Treatment of Major Neurological Diseases (grant no. 2017B030314103); The Southern China International Cooperation Base for Early Intervention and Functional Rehabilitation of Neurological Diseases (grant no. 2015B050501003); Guangdong Provincial Engineering Center for Major Neurological Disease Treatment; and Guangdong Provincial Translational Medicine Innovation Platform for Diagnosis and Treatment of Major Neurological Disease.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

QW designed and performed the experiments, analyzed the data and wrote the manuscript. ML conducted the experiments. ZX and MD performed some of the experiments. SG and LL participated in the experimental design, provided financial support and supervised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experimental protocols were approved by the Committee of Animal Care and Use at Sun Yat-Sen University (Guangdong, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Matsubara T, Ayuzawa S, Aoki T, Ikeda G, Shiigai M and Matsumura A: Cerebral venous thrombosis after ventriculoperitoneal shunting: A case report. Neurol Med Chir (Tokyo) 54: 554-557, 2014.
2. Binnani P, Bahadur MM and Dalal K: Dural sinus thrombosis-a rare manifestation of internal jugular venous occlusion. Saudi J Kidney Dis Transpl 23: 799-803, 2012.
3. Albano B, Gandolfo C and Del Sette M: Post-coital intra-cerebral venous hemorrhage in a 78-year-old man with jugular valve incompetence: A case report. J Med Case Rep 4: 225, 2010.
4. Chung SW, Hwang SN, Min BK, Kwon JT, Nam TK and Lee BH: Unilateral thrombosis of a deep cerebral vein associated with transient unilateral thalamic edema. J Cerebrovasc Endovasc Neurosurg 14: 233-236, 2012.
5. Gao M, Sun L, Liu YL, Xie JW, Qin L, Xue J, Wang YT, Guo KM, Ma MM and Li XY: Reduction of glyoxalase 1 (GLO1) aggravates cerebrovascular remodeling via promoting the proliferation of basilar smooth muscle cells in hypertension. Biochem Biophys Res Commun 518: 278-285, 2019.
6. Mills KT, Stefanescu A and He J: The global epidemiology of hypertension. Nat Rev Nephrol 16: 223-237, 2020.
7. Fernandez-Rojo MA and Ramm GA: Caveolin-1 function in liver physiology and disease. Trends Mol Med 22: 889-904, 2016.
8. Haines P, Samuel GH, Cohen H, Trojanowska M and Bujor AM: Caveolin-1 is a negative regulator of MMP-1 gene expression in human dermal fibroblasts via inhibition of Erk1/2/Ets1 signaling pathway. J Dermatol Sci 64: 210-216, 2011.
9. Ma X, Liu L, Nie W, Li Y, Zhang B, Zhang J and Zhou R: Prognostic role of caveolin in breast cancer: A meta-analysis. Breast 22: 462-469, 2013.
10. Santibanez JF, Blanco FJ, Garrido-Martin EM, Sanz-Rodriguez F, del Pozo MA and Bernabeu C: Caveolin-1 interacts and cooperates with the transforming growth factor-beta type I receptor ALK1 in endothelial caveolae. Cardiovasc Res 77: 791-799, 2008.
11. Huang JH, Lu L, Lu H, Chen X, Jiang S and Chen YH: Identification of the HIV-1 gp41 core-binding motif in the scaffolding domain of caveolin-1. J Biol Chem 282: 6143-6152, 2007.
12. Forrester SJ, Elliott KJ, Kawai T, Obama T, Boyer MJ, Preston KJ, Yan Z, Eguchi S and Rizzo V: Caveolin-1 deletion prevents hypertensive vascular remodeling induced by angiotensin II. Hypertension 69: 79-86, 2017.
13. Jiao HX, Mu YP, Gui LX, Yan FR, Lin DC, Sham JS and Lin MJ: Increase in caveolae and caveolin-1 expression modulates agonist-induced contraction and store- and receptor-operated Ca(2+) entry in pulmonary arteries of pulmonary hypertensive rats. Vascul Pharmacol 84: 55-66, 2016.
14. Watson O, Novodvorsky P, Gray C, Rothman AM, Lawrie A, Crossman DC, Haase A, McMahon K, Gering M, Van Eeden FJ and Chico TJ: Blood flow suppresses vascular Notch signalling via dll4 and is required for angiogenesis in response to hypoxic signalling. Cardiovasc Res 100: 252-261, 2013.
15. D'Souza B, Meloty-Kapella L and Weinmaster G: Canonical and non-canonical notch ligands. Curr Top Dev Biol 92: 73-129, 2010.
16. Qiao L, Xie L, Shi K, Zhou T, Hua Y and Liu H: Notch signaling change in pulmonary vascular remodeling in rats with pulmonary hypertension and its implication for therapeutic intervention. PLoS One 7: e51514, 2012.
17. Dabral S, Tian X, Kojonazarov B, Savai R, Ghofrani HA, Weissmann N, Florio M, Sun J, Jonigk D, Maegel L, *et al*: Notch1 signalling regulates endothelial proliferation and apoptosis in pulmonary arterial hypertension. Eur Respir J 48: 1137-1149, 2016.
18. Qiao LN, Xu HB, Shi K, Zhou TF, Hua YM and Liu HM: Role of notch signal in angiotensin II induced pulmonary vascular remodeling. Transl Pediatr 2: 5-13, 2013.
19. Atış M, Akcan U, Uğur Yılmaz C, Orhan N, Düzgün P, Deniz Ceylan U, Arıcan N, Karahüseyinoğlu S, Nur Şahin G, Ahıshalı B and Kaya M: Effects of methyl-beta-cyclodextrin on blood-brain barrier permeability in angiotensin II-induced hypertensive rats. Brain Res 1715: 148-155, 2019.
20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.

21. Nie X, Tan J, Dai Y, Liu Y, Zou J, Sun J, Ye S, Shen C, Fan L, Chen J and Bian JS: CCL5 deficiency rescues pulmonary vascular dysfunction, and reverses pulmonary hypertension via caveolin-1-dependent BMPR2 activation. J Mol Cell Cardiol 116: 41-56, 2018.
22. Zhu S, Wang J, Wang X and Zhao J: Protection against monocrotaline-induced pulmonary arterial hypertension and caveolin-1 downregulation by fluvastatin in rats. Mol Med Rep 17: 3944-3950, 2018.
23. Zhou LJ, Chen XY, Liu SP, Zhang LL, Xu YN, Mu PW, Geng DF and Tan Z: Downregulation of Cavin-1 expression via increasing Caveolin-1 degradation prompts the proliferation and migration of vascular smooth muscle cells in balloon injury-induced neointimal hyperplasia. J Am Heart Assoc 6: e005754, 2017.
24. Umesalma S, Houwen FK, Baumbach GL and Chan SL: Roles of caveolin-1 in angiotensin ii-induced hypertrophy and inward remodeling of cerebral pial arterioles. Hypertension 67: 623-629, 2016.
25. Kobayashi N, Mori Y, Nakano S, Tsubokou Y, Kobayashi T, Shirataki H and Matsuoka H: TCV-116 stimulates eNOS and caveolin-1cav-1 expression and improves coronary microvascular remodeling in normotensive and angiotensin II-induced hypertensive rats. Atherosclerosis 158: 359-368, 2001.
26. Rizzo PL, Miele L and Ferrari R: The Notch pathway: A crossroad between the life and death of the endothelium. Eur Heart J 34: 2504-2509, 2013.
27. Hofmann JJ and Iruela-Arispe ML: Notch signaling in blood vessels: Who is talking to whom about what. Circ Res 100: 1556-1568, 2007.
28. Kume T: Novel insights into the differential functions of Notch ligands in vascular formation. J Angiogenes Res 1: 8, 2009.
29. Aoyama T, Takeshita K, Kikuchi R, Yamamoto K, Cheng XW, Liao JK and Murohara T: Gamma-Secretase inhibitor reduces diet-induced atherosclerosis in apolipoprotein E-deficient mice. Biochem Biophys Res Commun 383: 216-221, 2009.
30. Zhou X, Xiao Y, Mao Z, Huang J, Geng Q, Wang W and Dong P: Soluble Jagged-1 inhibits restenosis of vein graft by attenuating Notch signaling. Microvasc Res 100: 9-16, 2015.
31. Delbosc S, Glorian M, Le Port AS, Béréziat G, Andréani M and Limon I: The benefit of docosahexanoic acid on the migration of vascular smooth muscle cells is partially dependent on Notch regulation of MMP-2/-9. Am J Pathol 172: 1430-1440, 2008.
32. Hurst LA, Dunmore BJ, Long L, Crosby A, Al-Lamki R, Deighton J, Southwood M, Yang X, Nikolic MZ, Herrera B, *et al*: TNFα drives pulmonary arterial hypertension by suppressing the BMP type-II receptor and altering NOTCH signalling. Nat Commun 8: 14079, 2017.
33. Guo Q, Xu H, Yang X, Zhao D, Liu S, Sun X and Huang JA: Notch activation of Ca2+-sensing receptor mediates hypoxia-induced pulmonary hypertension. Hypertens Res 40: 117-129, 2017.