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# Effect of Aldosterone on Senescence and Proliferation Inhibition of Endothelial Progenitor Cells Induced by Sirtuin 1 (SIRT1) in Pulmonary Arterial Hypertension

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**Background:** Pulmonary arterial hypertension (PAH) is characterized by a progressive increase in pulmonary circulatory resistance. Pulmonary vascular endothelial dysfunction is one of the main causes of primary PAH. Endothelial progenitor cells (EPCs) can proliferate and differentiate into vascular endothelial cells and play an important role in maintaining normal endothelial function. Mineralocorticoid receptor inhibitor has been reported to be used in the treatment of PAH. However, the role and the underlying mechanism of aldosterone (ALDO) in PAH remains unclear.

**Material/Methods:** Rats were divided to 4 groups (n=10 per group) and treated with 0.9% normal saline, monocrotaline (MCT), spironolactone (SP), or MCT combined with SP. After the rats were sacrificed with an overdose of pentobarbital sodium, hematoxylin and eosin staining was performed to observe the pulmonary artery pathology section. Sirtuin 1 (SIRT1), p53, and p21 protein expression was detected by western blot. Immunofluorescence staining was performed to verify EPCs. EPCs were treated with different concentrations of ALDO. MTT assay and senescence-associated  $\beta$ -galactosidase staining were used to measure cell viability and senescence.

**Results:** MCT increased the vascular arterial wall thickness and wall area, inhibited SIRT1 protein expression and increased p53 and p21 protein expression in the lung tissue of rats, while SP partially reversed this effect. In addition, ALDO inhibited EPCs viability and induced senescence. The expression of p53 and p21 proteins in the EPCs were upregulated and the senescence was accelerated when EPCs were transfected with SIRT1 siRNA.

**Conclusions:** ALDO promoted EPCs senescence and inhibited EPCs proliferation by downregulating SIRT1, which regulates the p53/p21 pathway, thus promoting PAH.

**MeSH Keywords:** **Aldosterone • Hypertension • Sirtuin 1**

**Abbreviations:** **ALDO** – aldosterone; **acLDL** – acetylated low-density lipoprotein; **Dil-acLDL** – 1,1'-octadecyl-3,3,3',3'-tetramethylcyanocarbon labeled acLDL; **EPCs** – endothelial progenitor cells; **H&E** – hematoxylin and eosin; **MCT** – monocrotaline; **MTT** – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **PAH** – pulmonary arterial hypertension; **SA- $\beta$ -gal** – senescence-associated  $\beta$ -galactosidase; **SD** – Sprague-Dawley; **SDS-PAGE** – sodium dodecyl sulfate-polyacrylamide gel electrophoresis; **SIRT1** – Sirtuin 1; **SP** – spironolactone; **UEA-1** – Ulex europaeus agglutinin; **WB** – western blot

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## Background

Pulmonary arterial hypertension (PAH), which is characterized by small pulmonary artery occlusion and remodeling, can result in progressive increases in pulmonary vascular resistance, right ventricular failure, or even death [1–3]. High remodeling of pulmonary vessels in PAH patients is due to gene changes, cell signaling changes, metabolic changes, abnormal pressure, and chronic inflammation [4]. Endothelial progenitor cells (EPCs) are usually defined as cells that can differentiate into endothelial cells and contribute to the formation of new blood vessels [5]. EPCs are a mononuclear cell population derived from bone marrow [6] and play an important role in maintaining vascular integrity through promoting new endothelial growth and endothelial repair mechanisms, which protects tissue perfusion after ischemic injury [7]. It has been reported that the enhanced senescence and dysfunction of circulating EPCs may accelerate endothelial dysfunction and lead to pulmonary vascular remodeling in PAH [8]. Therefore, delaying the senescence of EPCs may help prevent PAH.

Boehm et al. [9] pointed out that at present, the treatment of PAH does not target the process of pulmonary vascular remodeling; however, antagonism of aldosterone (ALDO) with spironolactone (SP) can improve the motor ability and endothelial dysfunction in the process of pulmonary vascular remodeling. It has been reported [10] that there is a positive correlation between plasma ALDO levels and the transpulmonary gradient in PAH patients. Moreover, according to a report by Ogo [11], PAH is associated with accelerated activation of renin-angiotensin-aldosterone system and the sympathetic nervous system. In addition, aldosterone antagonists can reduce morbidity and mortality in patients with severe left heart failure; however, the pathological effects of ALDO on PAH and heart failure have not been fully elucidated [9].

Sirtuin 1 (SIRT1) is a redox-sensitive protein [12] involved in many cellular processes, including metabolism, senescence, circadian rhythm regulation, oxidative stress response, and proliferation [13]. SIRT1 is a NAD<sup>+</sup> dependent deacetylase, which can cause downregulation and deacetylation of target p53, which is an upstream regulator of the transcription of cyclin-dependent kinase inhibitor p21/cip1 [14]. Research [15] found that long noncoding RNA (lncRNA)-mediated SIRT1/p53 and FoxO3a signaling pathways, which regulate type II alveolar epithelial cells senescence in the pathogenesis of chronic obstructive pulmonary disease. Besides, according to a report in 2019 [16], ALDO stimulation may induce metabolic remodeling of beagle dog ventricular myocardial cells via the SIRT1/AMPK signaling pathway. Therefore, in this study, we speculate that the effect of ALDO on PAH is related to stimulating EPCs and regulating the SIRT1/p53/p21 pathway.

## Material and Methods

### Animal models of PAH

Male Sprague-Dawley (SD) rats, aged 12 to 14 weeks, weighing 250±20 g were purchased from Sparford Laboratory Animal Science and Technology Co., Ltd. (Beijing, China). To determine the effect of mineralocorticoid receptors inhibition on monocrotaline (MCT)-induced PAH, the SD rats were randomly divided into 4 groups (n=10 per group): control group, spironolactone (SP) group, MCT group, and MCT+SP group. The SD rats were administered 0.9% normal saline as vehicle control or SP (25 mg/kg/day; Henry Schein, Melville, USA) in the drinking water or MCT (50 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) by intraperitoneal (IP) injection alone or treated with MCT (50 mg/kg) and SP (25 mg/kg/day). After treatment for 23 to 25 days, the rats were sacrificed with an overdose of pentobarbital sodium (100 mg/kg). The lung tissues of rats were removed and washed with phosphate-buffered saline (PBS) and divided into 2 parts. One part was fixed with 4% polyformaldehyde overnight, paraffin-embedded, and sectioned for hematoxylin-eosin staining (H&E) staining; The other part was frozen at -80°C for the detection of SIRT1, p53, and p21 proteins expression levels. Animal experiments were carried out in accordance with the rules for the feeding and use of experimental animals. The study was approved by the ethics review board for animal studies of the Changzhou University.

### H&E staining

H&E staining was performed to observe the pulmonary artery pathology section. The pulmonary artery was fixed with 4% paraformaldehyde, and paraffin was used to embed the pulmonary artery. Then the pulmonary artery was cut into 5 mm segments. Sections were stained with H&E. Each section was randomly selected from 10 microscopic fields and observed under a microscope. To quantify the thickness of pulmonary artery wall, lumen diameter (or basement membrane level area) and total vessel diameter (or adventitia margin area) of 10 muscular arteries with pulmonary segments 50 to 100 mm in diameter were measured. Diameter and area were measured by ImageJ (National Institutes of Health, Bethesda, MD, USA). Then to calculate the vascular arterial wall thickness (%): (total vascular diameter-lumen diameter)/diameter of total vessel×100%, and wall area (%): (total area-lumen area of vessel)/total area of vessel×100%.

### Western blot (WB) analysis

Western blot (WB) analysis was used to detect SIRT1, p53, and p21 proteins in lung tissues. The frozen lung tissues (100 mg) were ground into powder in liquid nitrogen. RIPA pyrolysis solution containing 1% PMSF was added for 1 mL.

The supernatant was centrifuged for 20 minutes at 4°C at 12 000 rpm. The protein content was determined by BCA protein assay kit (Beyotime, China). The protein (30 µg) was subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to a polyvinylidene fluoride membrane (Millipore Billerica, MA, USA). The membrane was blocked with 5% skim milk for 2 hours at room temperature. We added 1: 250 diluted SIRT1 (ab110304, 121kD), p53 (ab131442, 53kD), and p21 (ab109199, 21kD) antibodies and incubate overnight at 4°C. GAPDH (1: 500, 36kD, ab8245) was used as the internal control (antibodies were purchased from Abcam, Cambridge, UK). The samples were incubated with horseradish peroxidase-conjugated secondary antibody (1: 10000; Li-Cor, Inc., USA) for 1 hour at room temperature. ImageJ was performed for the semi-quantitatively analysis.

### Immunofluorescence staining

EPCs from healthy rats were isolated by density gradient centrifugation using Ficoll-Paque™ PREMIUM (GE-Healthcare, Freiburg, Germany). EPCs ( $1 \times 10^7$ ) were seeded on 6-well plates coated with human fibronectin (Millipore, Temecula, USA) in endothelial basal medium-2 (Lonza, Walkersville, MD, USA) containing endothelial growth medium-2 (EGM-2) SingleQuots (Lonza, Walkersville, MD, USA). After culturing for 7 days, EPCs were verified by detecting by immunofluorescence staining, cells that were double positive for Ulex europaeus agglutinin (UEA-1) lectin binding and acetylated LDL (acLDL). Cells were incubated with 1,1'-octadecyl-3,3',3'-tetramethylcyanocarbon labeled acLDL (Dil-acLDL, 2.47 µg/ml) at 37°C for 1 hour, then immobilized in PBS with 2% paraformaldehyde for 10 minutes. Then the cells were stained with fluorescein isothiocyanate-labeled lectin from UEA-1 (10 mg/ml) for 1 hour. DAPI (4'-diamidino-2-phenylindole, dilactate) was used as a control to see the nuclei. The image was enlarged under a fluorescence microscope. Differentiated EPCs were identified as double positive stained with Dil-acLDL and UEA-1 lectin.

### The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

EPCs ( $1 \times 10^4$  cells/well) were inoculated in 96-well plates. EPCs were treated by ALDO at different concentrations (1, 10, 100, and 1000 nmol/L) and MTT was used to detect the cell viability. Control group were set at the same time. After another 24 hours of incubation, MTT was added and cells were incubated for another 4 hours, then dimethyl sulfoxide (DMSO) was added. After crystallization was completely dissolved, the optical density at wavelength of 490 nm was measured by commercial enzyme linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA).

### Cell grouping and transfection

After EPCs were treated with ALDO at different concentrations, and the MTT assay was performed to detect cell viability, resulting in the selection of 100 nmol/L ALDO for further experiments. Then the cells were divided into the 4 groups: control group (untreated EPCs), SP group (EPCs treated with 10 µmol/L SP), ALDO group (EPCs treated with 100 nmol/L ALDO), ALDO+SP group (EPCs treated with 10 µmol/L SP and 100 nmol/L ALDO). In order to explore the underlying mechanism, the cells were transfected with SIRT1 siRNA, and the cells were divided into 3 groups: control group, siNC group (EPCs transfected with siNC), and siSIRT1 group (EPCs transfected with SIRT1 siRNA). EPCs ( $5 \times 10^5$  cells/well) were inoculated in 6-well plates. Then the cells were transfected with control siRNA (siNC) or SIRT1 siRNA (Santa Cruz Biotechnology, Carlsbad, CA USA) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 48 hours of transfection, further experiments were conducted.

### Senescence-associated β-galactosidase (SA-β-gal) staining

The senescence of EPCs was detected by *in situ* staining with SA-β-gal with an acid β-galactosidase staining kit (Beyotime, Nanjing, China). After termination of the reaction by the addition of PBS, the number of β-gal positive cells was examined by counting at least 300 cells per representative field of view under light microscope. The experiments were performed 3 times, and β-gal positive cell numbers was expressed as a percentage of total cells.

### Statistical analysis

GraphPad Prism (Graphpad Software Inc., La Jolla, CA, USA) was used to analyze all data. Data were expressed as mean±standard deviation. The differences between groups were analyzed using one-way ANOVA analysis followed Tukey test. All *P* values <0.05 were considered statistically significant.

## Results

### SP remarkably prevented the development of MCT-induced PAH

After treatment with MCT, the pathology sections of the pulmonary artery were collected. The results of H&E showed that compared to the control group, rats in the MCT group had an increased percentage change in pulmonary artery wall thickness/diameter and ratio of area of pulmonary segmental wall to total vessel area, while SP reversed the development of MCT-induced PAH (Figure 1A–1C). Then, the expression of SIRT1, p53, and p21 proteins were measured by WB. The results revealed that MCT downregulated the expression of SIRT1 and

upregulated the expression of p53 and p21 in the lung tissue of rats, while SP partially reversed the effect of MCT on the lung tissue of rats (Figure 1D, 1E).

### Phenotypic characterization of rats EPCs

We isolated the EPCs from peripheral blood mononuclear cells of healthy rats. EPCs were identified using immunofluorescence staining. Three color fluorescence imaging results showed that uptake of EPCs to DilacLDL (red), bound to FITC-UEA-1 (green). The nuclei were found stained with DAPI (blue). The differentiated endothelial progenitor cells were double-positive, and the covering layer was yellow (Figure 2A).

### ALDO inhibited EPCs activity in a dose-dependent manner in PAH rats

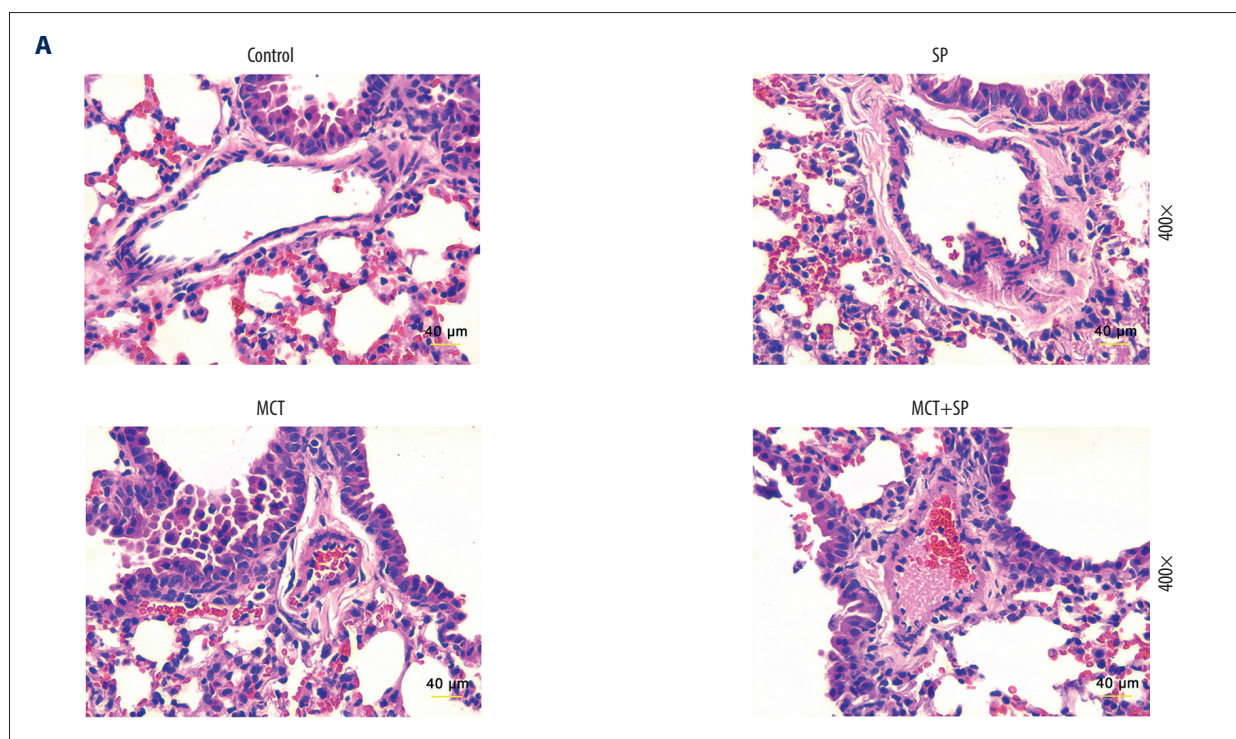
EPCs were treated with different concentrations of ALDO (1, 10, 100, and 1000 nmol/L). Cell viability and expression of SIRT1 protein were measured by MTT and WB. The results found that the inhibitory effect of ALDO on EPCs viability was obvious with the increase of ALDO concentration (Figure 2B). Moreover, ALDO could inhibit the expression of SIRT1 protein in EPCs, and the more the concentration of ALDO increased, the more obvious the inhibitory effect on the expression of protein SIRT1 was (Figure 2C, 2D).

### ALDO inhibited the viability and induced the senescence of EPCs

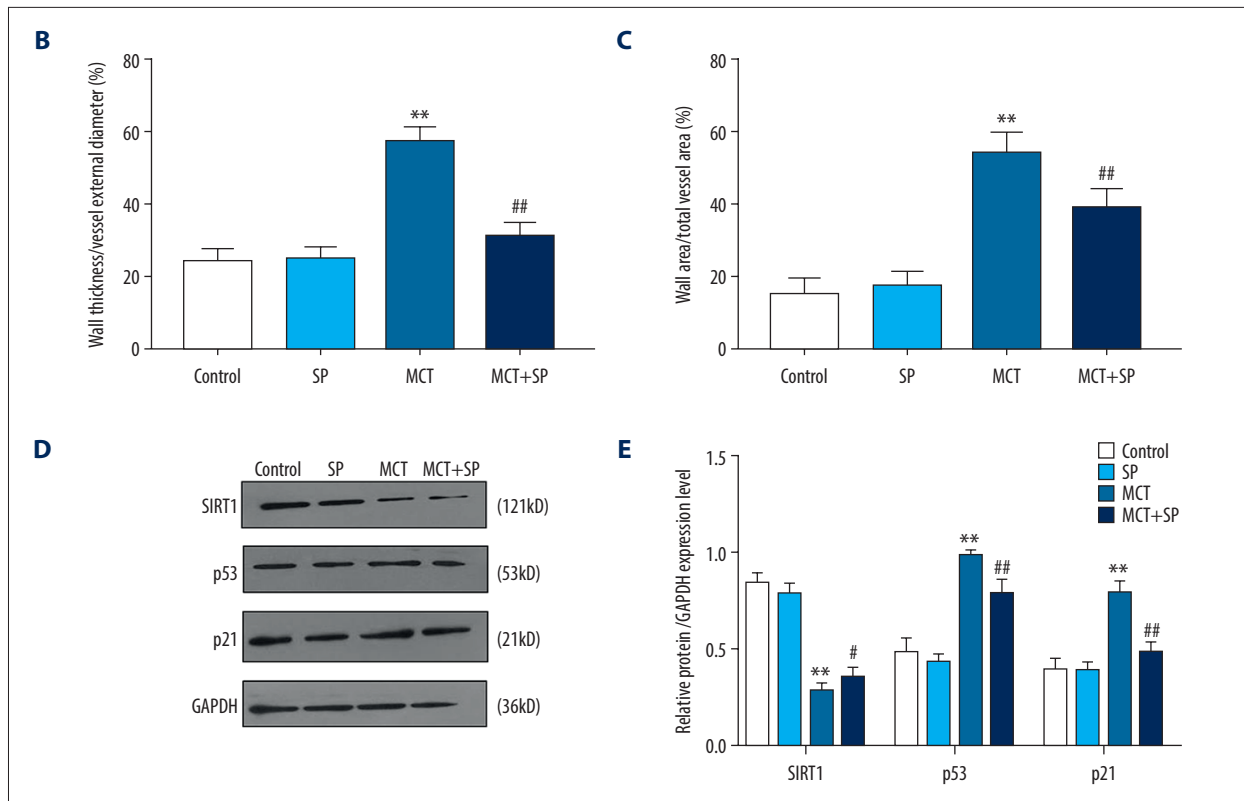
In order to further explore the role of ALDO on EPCs, we measured the cell viability by MTT, and EPCs stained for SA- $\beta$ -gal to detect the senescence. The results revealed that ALDO decreased the optical density at wavelength of 490 nm of EPCs, compared to the control group. And the mineralocorticoid receptor inhibitor SP increased the optical density of EPCs, compared to the ALDO group (Figure 3A). In addition, the percentages of  $\beta$ -galactosidase positive cells in the ALDO group was obviously increased compared to the control group, while SP remarkably reversed the ALDO-induced senescence (Figure 3B, 3C). Moreover, ALDO inhibited the expression of SIRT1 protein in EPCs, while it was upregulated remarkably in EPCs which were treated with ALDO and SP (Figure 3D, 3E).

### ALDO inhibited the viability and induced the senescence of EPCs through downregulating SIRT1

Next, in order to explore the underlying mechanism of the effect of ALDO on EPCs. EPCs were transfected with SIRT1 siRNA. The WB assay was conducted to measure the transfection rate, and the result showed that the SIRT1 protein was remarkably downregulated in EPCs transfected with SIRT1 siRNA (Figure 4A, 4B). In addition, the expression of p53 and p21 proteins were upregulated in EPCs transfected with SIRT1 siRNA (Figure 4C, 4D). Then the SA- $\beta$ -gal staining was conducted to







**Figure 1.** Pathological section of pulmonary artery and expression of sirtuin 1 (SIRT1), p53 and p21 in lung tissue. (A) Representative images of hematoxylin-eosin staining (H&E) for lung sections from each group. (B) The ratio of wall thickness of the lung slices to the total diameter of the total blood vessels was counted. The results showed that compared with the control group, rats treated with monocrotaline (MCT) with increased percentage of pulmonary artery wall thickness/diameter, while spironolactone (SP) reversed this effect of MCT. (C) The ratio of area of pulmonary segmental wall to total vessel area was calculated. The results revealed that rats treated with MCT with increased percentage of pulmonary artery wall thickness/diameter compared with the control group, while SP reversed this effect of MCT. (D, E) The results of western blot (WB) revealed that compared with control group, MCT downregulated the expression of SIRT1 and upregulated the expression of p53 and p21 in pulmonary arterial hypertension (PAH) rats' lung tissue. \*\*  $P < 0.001$  versus control, #  $P < 0.05$ , ##  $P < 0.001$  versus MCT.

detect the senescence of EPCs, and the result showed an increased percentages of  $\beta$ -galactosidase positive cells in the siSIRT1 group compared to the control group and the siNC group (Figure 4E, 4F).

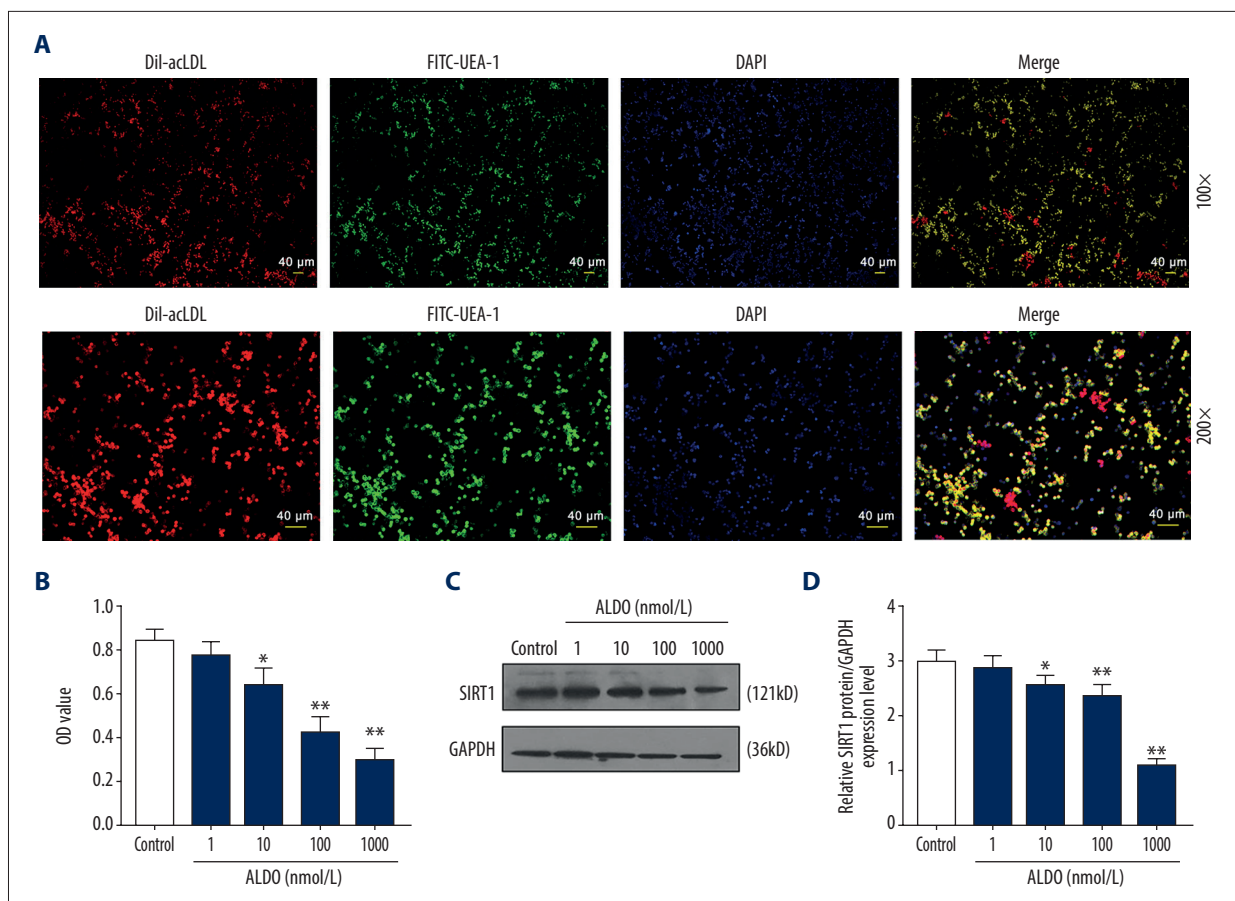
## Discussion

The main finding of this study was that MCT increased the vascular arterial wall thickness and wall area in lung tissue of rats, while SP as antagonism of ALDO partially reversed this MCT effect. MCT inhibited SIRT1 protein expression and increased p53 and p21 protein expression in lung tissue of rats. Cell experiments showed that ALDO inhibited normal EPCs viability and induced senescence, which might occur by regulating the SIRT1/p53/p21 pathway.

At the heart of the pathology of pulmonary hypertension is a plexiform lesion composed of proliferation of vascular smooth

muscle cells, endothelial cells inflammatory cells, and myofibroblasts that block the pulmonary arteries [17]. According to Thompson et al. [18], vascular remodeling is the main pathological feature of PAH, but there are few treatments for this process. According to a review in 2018, EPCs participate in endothelial cell regeneration and are considered to be an important factor in endogenous vascular repair, which can promote the reendothelialization of damaged arteries by replacing dysfunctional EPCs, thus inhibiting the formation of new intima [19]. It has also been reported that the proper homing of EPCs plays a key role in the remodeling of regenerated arteries. However, the mechanism of EPC promoting pulmonary vascular remodeling remains unclear [20].

Animal models are important tools for understanding the pathophysiology of pulmonary hypertension and for discovering and developing new therapies [21]. In this study, PAH rat models were established by MCT. We observed the pulmonary artery pathology section by H&E staining. In addition, the vascular

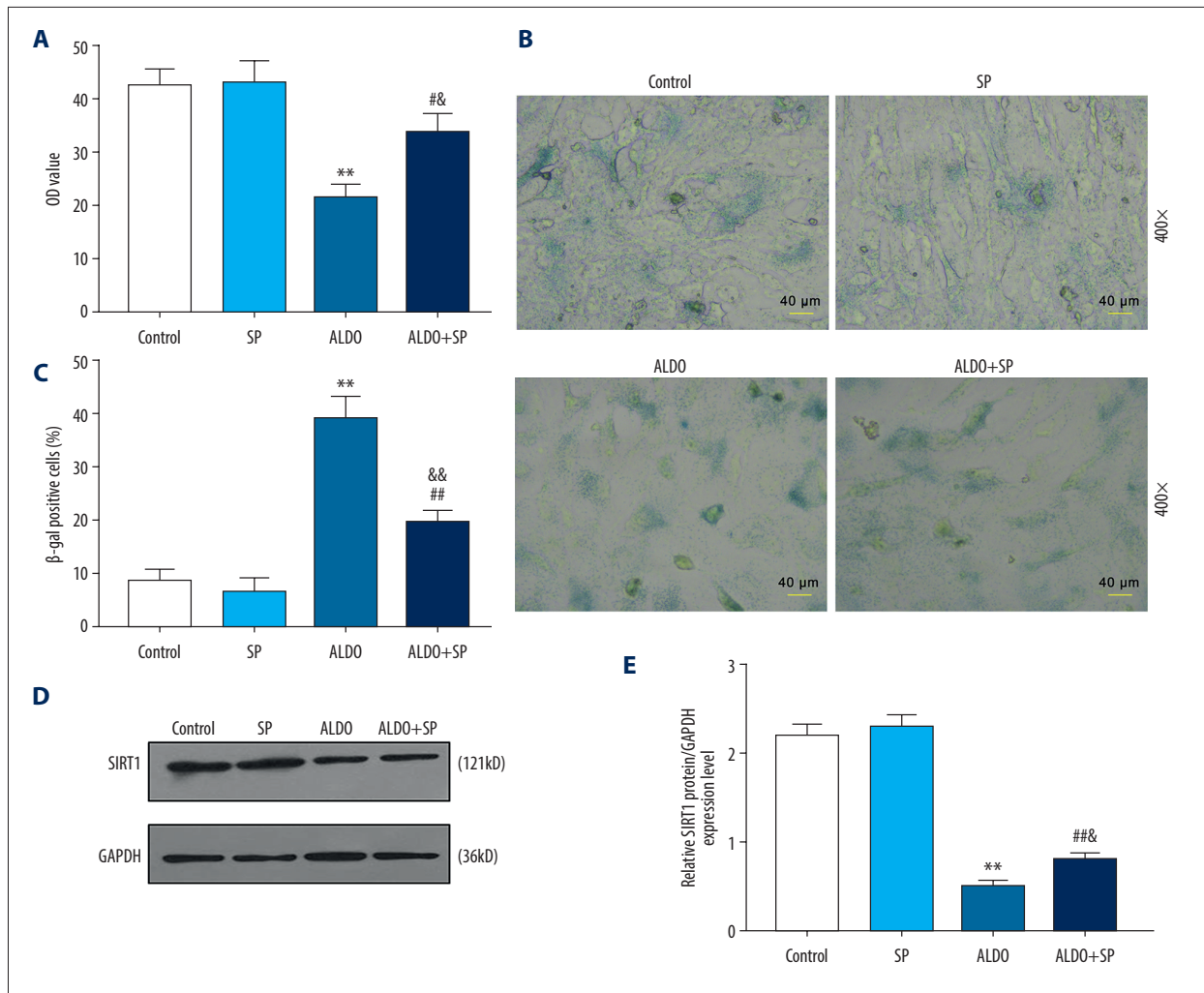


**Figure 2.** Characteristics of endothelial progenitor cells (EPCs) from healthy rat peripheral blood and the effects of different concentrations of aldosterone (ALDO) on EPCs viability and the expression of sirtuin 1 (SIRT1). **(A)** EPCs were identified by immunofluorescence staining. The results showed that EPCs bind Dil-aclDL (red) and FITC-UEA-1 (green). The nuclei were stained with DAPI (blue). The differentiated endothelial progenitor cells were double positive, and the covering layer was yellow. **(B)** The results of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay showed that ALDO inhibited EPCs viability in a dose-dependent manner. **(C, D)** The results of western blot (WB) revealed that ALDO downregulated SIRT1 protein expression in a dose-dependent manner. \*  $P < 0.05$ , \*\*  $P < 0.001$  versus control.

arterial wall thickness and wall area were calculated. The results revealed that the change in vascular arterial wall thickness and wall area in the lung tissue of the model rats. SP was shown to have an inhibitory effect on PAH in rats induced by MCT. SP has a wide range of therapeutic effects in cardiovascular diseases. A previous study [22] reported that SP and rivaroxaban combination therapy partially inhibited cardiovascular remodeling of PAH by inhibiting pal-2 and local aldosterone produced by StAR. A clinical study of 46 patients with PAH by Safdar et al. [23] showed that SP was safe and well tolerated, with no increased side effects in patients with hyperkalemia or liver function tests abnormalities.

We also found in our study that MCT downregulated the expression of SIRT1 and upregulated the expression of p53 and p21 in PAH rat lung tissue. A previous study [24] showed that resveratrol partially improved right ventricular systolic pressure

and reduced right ventricular hypertrophy in PAH rats by enhancing SIRT1 activation, thus producing a beneficial effect. In recent years, many studies have pointed out that SIRT1 plays a key role in the proliferation and apoptosis of cells. For instance, the research of Igarashi et al. [25] showed that in the intestinal tract, SIRT1-activated compounds were predicted to not impair signal transduction in Paneth cells and to activate SIRT1 in intestinal stem cells, which should increase the number of intestinal stem cells, and be beneficial to health. SIRT1 also has been shown to inhibit the proliferation of vascular smooth muscle cells induced by oxidative stress [26]. Similarly, another research study found that creatine provided at least partial protection against nonalcoholic fatty liver disease by inhibiting miR-34a/SIRT1/p66shc pathway [27]. Other researchers [28] found that SIRT1 was gradually inhibited during early intestinal reperfusion, which resulted in intestinal reactive oxygen species accumulation and apoptosis.

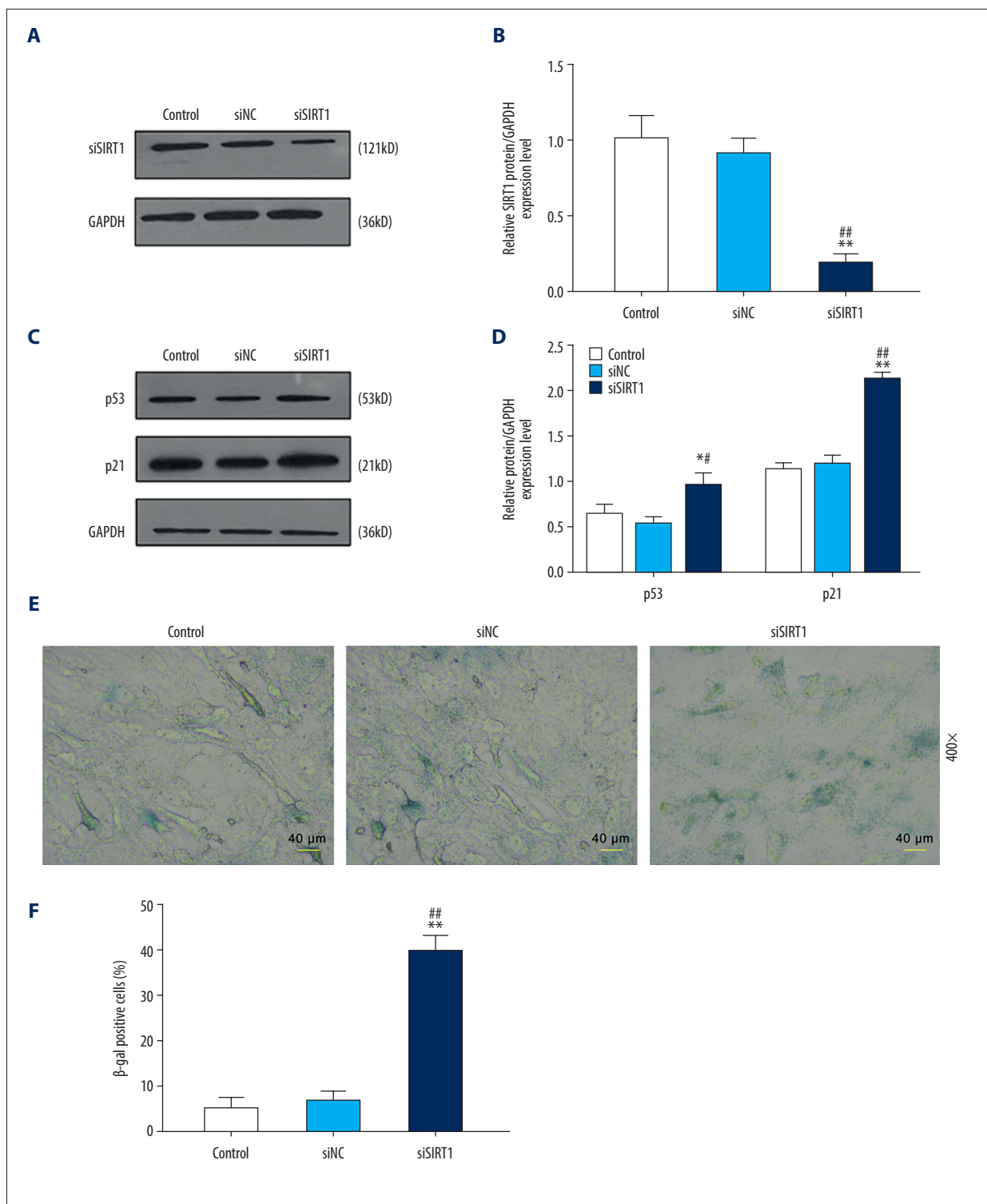


**Figure 3.** The effects of aldosterone (ALDO) and spironolactone (SP) on the proliferation and senescence of endothelial progenitor cells (EPCs). **(A)** EPCs treated with ALDO (100 nmol/L) with lower optical density at wavelength of 490 nm compared with EPCs in control group, while SP (10  $\mu$ mol/L) remarkably reversed the effect of ALDO. **(B, C)** Senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) activity was measured for cell senescence, and the results revealed that increased percentages of  $\beta$ -galactosidase positive cells were found in the ALDO group compared with that in control group, while SP remarkably reversed the ALDO-induced senescence. **(D, E)** The results of western blot (WB) revealed that ALDO downregulated sirtuin 1 (SIRT1) protein expression in EPCs, while SP remarkably reversed the effect of ALDO. \*\*  $P < 0.001$  versus control. #  $P < 0.05$ , ##  $P < 0.001$  versus SP. &  $P < 0.05$ , &&  $P < 0.001$  versus ALDO.

The p53/p21 pathway, which is one of the major pathways to induce cell senescence [29], was one of the focuses of our research. SIRT1 has been found to specifically bind to p53 and deacetylated it, negatively regulating p53-mediated transcriptional activation in oxidative stress, containing p53-mediated p21 transcriptional activation [29]. Based on the aforementioned results, we speculated that the effect of SP on PAH rats might be through the SIRT1/p53/p21 pathways.

We further explored the mechanism of ALDO affecting EPCs function through cell experiments. The results revealed that ALDO inhibited normal EPCs viability and induced senescence.

In addition, ALDO downregulated SIRT1 protein expression and upregulated p53 and p21 protein expression in normal EPCs. We inferred that ALDO stimulated EPCs and promoted PAH by downregulating SIRT1, which promoted EPCs senescence and inhibited EPCs proliferation. Then, we transfected SIRT1 siRNA to EPCs to verify that SIRT1 regulates EPCs senescence by regulating p53/p21. And the results revealed that the expression of p53 and p21 proteins in the EPCs were upregulated and the senescence was accelerated when cells were transfected with SIRT1 siRNA. Our study results were similar to other research [30] that found that MeCP2 inhibited SIRT1 in senescent EPCs, in which telomere activity was low, and the p16



**Figure 4.** The role of sirtuin 1 (SIRT1) in the senescence of endothelial progenitor cells (EPCs) via regulating the p53/p21 pathway. (A, B) The western blot (WB) results showed that the cells were transfected successfully with SIRT1 siRNA. (C, D) The WB results revealed that the expression of p53 and p21 proteins in the EPCs were upregulated when cells transfected with SIRT1 siRNA. (E, F) Measuring for cell senescence by senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) activity, and the results found that the siSIRT1 group with increased percentages of  $\beta$ -galactosidase positive cells compared with control group. \*  $P < 0.05$ , \*\*  $P < 0.001$  versus control. #  $P < 0.05$ , ##  $P < 0.001$  versus siNC.



and p53 mRNAs levels were high, and SIRT1 showed the effect to reverse the effects of MeCP2 on EPCs.

## Conclusions

In summary, the results in our study revealed that ALDO stimulated EPCs. It was found that ALDO downregulated SIRT1,

which regulated the p53/p21 pathway to promote EPCs senescence and inhibit EPCs proliferation, thus promoting PAH. However, there were some limitations to this study. For example, the effect of ALDO on EPCs in PAH rat models needs to be studied directly in future trials, and other pathways that might be involved in cell senescence should be studied.

## References:

1. Weatherald J, Boucly A, Chemla D et al: Prognostic value of follow-up hemodynamic variables after initial management in pulmonary arterial hypertension. *Circulation*, 2018; 137(7): 693–704
2. Maron BA, Galie N: Diagnosis, treatment, and clinical management of pulmonary arterial hypertension in the contemporary era: A review. *JAMA Cardiol*, 2016; 1(9): 1056–65
3. Lau EMT, Giannoulitou E, Celermajer DS, Humbert M: Epidemiology and treatment of pulmonary arterial hypertension. *Nat Rev Cardiol*, 2017; 14(10): 603–14
4. Dummer A, Rol N, Szulcek R et al: Endothelial dysfunction in pulmonary arterial hypertension: loss of cilia length regulation upon cytokine stimulation. *Pulm Circ*, 2018; 8(2): 2045894018764629
5. Medina RJ, Barber CL, Sabatier F et al: Endothelial progenitors: A consensus statement on nomenclature. *Stem Cells Transl Med*, 2017; 6(5): 1316–20
6. D'Avola D, Fernandez-Ruiz V, Carmona-Torre F et al: Phase 1-2 pilot clinical trial in patients with decompensated liver cirrhosis treated with bone marrow-derived endothelial progenitor cells. *Transl Res*, 2017; 188: 80–91.e2
7. D'Apolito M, Colia AL, Lasalvia M et al: Urea-induced ROS accelerate senescence in endothelial progenitor cells. *Atherosclerosis*, 2017; 263: 127–36
8. Liu B, Li T, Peng JJ et al: Non-muscle myosin light chain promotes endothelial progenitor cells senescence and dysfunction in pulmonary hypertensive rats through upregulation of NADPH oxidase. *Eur J Pharmacol*, 2016; 775: 67–77
9. Boehm M, Arnold N, Braithwaite A et al: Eplerenone attenuates pathological pulmonary vascular rather than right ventricular remodeling in pulmonary arterial hypertension. *BMC Pulm Med*, 2018; 18(1): 41
10. Ogihara Y, Yamada N, Dohi K et al: Effects of low-dose tolvaptan on electrolyte abnormality and hemodynamic parameters in a liver cirrhosis-associated portopulmonary hypertension patient: A case report. *Exp Ther Med*, 2017; 13(1): 269–72
11. Ogo T: Transthoracic pulmonary artery denervation: New insight into autonomic nervous system in pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol*, 2019; 39: 979–81
12. Kaszubowska L, Foerster J, Kaczor JJ et al: NK cells of the oldest seniors represent constant and resistant to stimulation high expression of cellular protective proteins SIRT1 and HSP70. *Immun Ageing*, 2018; 15: 12
13. Zha S, Li Z, Cao Q et al: PARP1 inhibitor (PJ34) improves the function of aging-induced endothelial progenitor cells by preserving intracellular NAD(+) levels and increasing SIRT1 activity. *Stem Cell Res Ther*, 2018; 9(1): 224
14. Fujino T, Yokokawa R, Oshima T, Hayakawa M: SIRT1 knockdown up-regulates p53 and p21/Cip1 expression in renal adenocarcinoma cells but not in normal renal-derived cells in a deacetylase-independent manner. *J Toxicol Sci*, 2018; 43(12): 711–15
15. Gu C, Li Y, Liu J et al: LncRNAmiated SIRT1/FoxO3a and SIRT1/p53 signaling pathways regulate type II alveolar epithelial cell senescence in patients with chronic obstructive pulmonary disease. *Mol Med Rep*, 2017; 15(5): 3129–34
16. Liu GZ, Zhang S, Li YY et al: Aldosterone stimulation mediates cardiac metabolism remodeling via Sirt1/AMPK signaling in canine model. *Naunyn Schmiedebergs Arch Pharmacol*, 2019; 392(7): 851–63
17. Batton KA, Austin CO, Bruno KA et al: Sex differences in pulmonary arterial hypertension: role of infection and autoimmunity in the pathogenesis of disease. *Biol Sex Differ*, 2018; 9(1): 15
18. Thompson AAR, Lawrie A: Targeting vascular remodeling to treat pulmonary arterial hypertension. *Trends Mol Med*, 2017; 23(1): 31–45
19. Yang JX, Pan YY, Wang XX et al: Endothelial progenitor cells in age-related vascular remodeling. *Cell Transplant*, 2018; 27(5): 786–95
20. Aliotta JM, Pereira M, Wen S et al: Bone marrow endothelial progenitor cells are the cellular mediators of pulmonary hypertension in the murine monocrotaline injury model. *Stem Cells Transl Med*, 2017; 6(7): 1595–606
21. Bueno-Beti C, Sassi Y, Hajjar RJ, Hadri L: Pulmonary artery hypertension model in rats by monocrotaline administration. *Methods Mol Biol*, 2018; 1816: 233–41
22. Hayashi T, Ogata T, Kondo S et al: A novel therapeutic combination with rivaroxaban and spironolactone attenuates cardiovascular remodeling in PAH through suppression of protease-activated receptor-2 and steroidogenic acute regulatory protein. *Circulation*, 2018; 138(Suppl. 1): A14358-A
23. Safdar Z, Tamez E, Thakur A et al: Effects of spironolactone in pulmonary arterial hypertension: results of Spiro study. *D54 Clinical Pulmonary Hypertension: American Thoracic Society*, 2016; A7380-A
24. Yu L, Tu Y, Jia X et al: Resveratrol protects against pulmonary arterial hypertension in rats via activation of silent information regulator 1. *Cell Physiol Biochem*, 2017; 42(1): 55–67
25. Igarashi M, Guarente L: mTORC1 and SIRT1 cooperate to foster expansion of gut adult stem cells during calorie restriction. *Cell*, 2016; 166(2): 436–50
26. Ma Y, Gong X, Mo Y, Wu S: Polydatin inhibits the oxidative stress-induced proliferation of vascular smooth muscle cells by activating the eNOS/SIRT1 pathway. *Int J Mol Med*, 2016; 37(6): 1652–60
27. Shan W, Gao L, Zeng W et al: Activation of the SIRT1/p66shc anti-apoptosis pathway via carnolic acid-induced inhibition of miR-34a protects rats against nonalcoholic fatty liver disease. *Cell Death Dis*, 2015; 6: e1833
28. Wang G, Yao J, Li Z et al: miR-34a-5p Inhibition alleviates intestinal ischemia/reperfusion-induced reactive oxygen species accumulation and apoptosis via activation of SIRT1 signaling. *Antioxid Redox Signal*, 2016; 24(17): 961–73
29. Zhou N, Lin X, Dong W et al: SIRT1 alleviates senescence of degenerative human intervertebral disc cartilage endo-plate cells via the p53/p21 pathway. *Sci Rep*, 2016; 6: 22628
30. Wang C, Wang F, Li Z et al: MeCP2 mediated dysfunction in senescent EPCs. *Oncotarget*, 2017; 8(45): 78289–99