

Expression of key enzymes in the mevalonate pathway are altered in monocrotaline-induced pulmonary arterial hypertension in rats

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Abstract. Pulmonary arterial hypertension (PAH) is a serious pulmonary vascular disease. The changes in the structure, function and metabolism of endothelial cells are some of the important features of PAH. Previous studies have demonstrated that the mevalonate pathway is important in cardiovascular remodeling. However, whether the mevalonate pathway is involved in the development of PAH remains to be elucidated. The present study aimed to investigate the expression pattern of mevalonate pathway-related enzymes in monocrotaline (MCT)-induced PAH. F344 rats were randomly divided into two groups (n=6/group): Control group rats were injected with a single dose of saline, and MCT group rats were injected with a single dose of MCT (60 mg/kg). After 4 weeks, the right ventricular systolic pressure (RVSP) was measured, and lung and pulmonary artery tissue samples were collected. It was demonstrated that the RVSP increased and pulmonary vascular remodeling was detected in the PAH group. The expression levels of the enzymes farnesyltransferase α and geranylgeranyltransferase type I increased in the PAH group, which suggested that the mevalonate pathway may be involved in the pathological development of PAH.

Introduction

Pulmonary arterial hypertension (PAH) is a serious pulmonary vascular disease, which is defined by a mean pulmonary arterial pressure ≥ 25 mmHg (1). The pathological features of PAH include distal pulmonary artery intimal hyperplasia, plexiform lesions, medial hypertrophy, muscle infarction and thrombosis, gradually leading to occlusion of the lumen and high pulmonary arterial pressure, eventually leading to right heart failure (2,3). In previous years, several studies have demonstrated that the structure, function and metabolic changes of endothelial cells are important features in the development of PAH, which has a marked effect on vessel homeostasis (4-6).

A typical feature of endothelial injury is an imbalance of nitric oxide (NO)-reactive oxygen species (ROS) levels, which is caused by reductions in endothelium-derived NO synthesis, release and activity, and is increased by ROS generation and release (7,8), which is involved in the excessive contraction and remodeling of pulmonary vessels. It has been demonstrated that the small G proteins, Ras homolog family, member A (RhoA) and Ras-related C3 botulinum toxin substrate 1 (Rac1), are involved in changes of endothelial function through the Rho kinase-endothelial NO synthase (eNOS) pathway or regular NADPH oxidase, respectively (9). Therefore, repairing endothelial function and reversing excessive oxidative stress represent the pathophysiological foundation of PAH therapy (7).

Previous studies have demonstrated that the mevalonate pathway is involved in small G-protein activation (10,11). The mevalonate pathway is an important pathway of cholesterol synthesis at the cellular level. Mevalonate is a precursor of cholesterol, in addition to several non-steroid isoprenoid complexes, including farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) (12). These non-steroid isoprenoids are important in small G protein post-translational modification (10). Studies have indirectly demonstrated that the mevalonate pathway maybe involved in the development of PAH. It has been suggested that mevalonate and other mid products produced in the mevalonate pathway can regulate signaling proteins, including those of Ras family (13), which are essential for cell proliferation and other important features. The inhibitor of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCR) enzyme, a key enzyme of the mevalonate pathway, decreases blood lipids, inhibits the proliferation of smooth muscle cells, ameliorates endothelial cell function and

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increases the expression of eNOS (14,15). Animal experiments have shown that statins were able to mitigate pulmonary pressure and right ventricular hypertrophy in monocrotaline (MCT)-induced PAH rats, and this was associated with an increased expression of eNOS (16,17).

At present, the effects of key enzymes, including farnesyl-diphosphate synthase (FDPS), farnesyltransferase α (FNTA), farnesyltransferase β (FNTB) and geranylgeranyltransferase type I (GGTase-I) on endothelial dysfunction have not been reported, with the exception of the initial enzyme, HMGR. The pathway downstream of FPP produced in the mevalonate pathway has three branches: Asterol branch, which primarily contributes to cholesterol synthesis, and two non-sterol branches, which regulate signal transduction proteins, including those of the Ras and Rho families (13,14). Therefore, the mevalonate pathway may affect protein prenylation, vasoactive substance generation and endothelial function, and regulate excessive vascular contraction and remodeling in the pulmonary vascular tissues of PAH rats.

Based on previous studies, the present study aimed to determine whether the expression of key enzymes, including HMGR, FDPS, FNTA, FNTB and GGTase-I, in the mevalonate pathway are altered in MCT-induced PAH rats, which may potentially serve as novel therapeutic targets for PAH.

Materials and methods

Animal model. The present study was performed in adherence with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the ethics committee of Zhejiang University (approval no. ZJU20170874; Hangzhou, China). A total of 100 F344 male rats (6-weeks old; weighing 200 ± 10 g) were obtained from Shanghai Laboratory Animal Center (Chinese Academy of Sciences, Shanghai, China). A total of 5 rats/cage, temperature of 22°C, light and dark cycle 12-h, free access to drinking water and normal feed. The rat PAH model was induced by injecting a single dose of MCT (60 mg/kg, dissolved in 1N HCl, neutralized with 1N NaOH, diluted with saline), purchased from Sigma-Aldrich; Merck Millipore (Darmstadt, Germany) and fed for 4 weeks.

The rats were randomly divided into two groups ($n=6$); in the control group, each rat was injected with a single dose of saline; in the PAH group, each rat was injected with a single dose of MCT (60 mg/kg). On day 28, all rats were sacrificed.

Hemodynamic parameters. The rats were injected with 8% chloral hydrate following weighing (1 ml/200 g), and fixed on an autopsy table. Right ventricular systolic pressure (RVSP) was measured by insertion of a PE50 pipe through the jugular vein to the right ventricle; the RVSP was transferred into an electric signal and recorded using MedLab software (version 6.3; Nanjing Medease Science and Technology, Nanjing, China).

Measurement of right ventricular hypertrophy (RVH). All rats were sacrificed following measurement of pulmonary arterial pressure, and the hearts, lungs and pulmonary arteries were harvested. The blood was removed in cold PBS. The right ventricle (RV) was isolated from the left ventricle (LV)

and septum (S), and these two components were weighted separately. RVH was determined as the ratio of RV weight to (LV+S) weight.

Histological analysis. A sample of lung tissue was fixed in prepared 4% paraformaldehyde for 24 h, made into paraffinized sections (4- μ M thick), and then stained with hematoxylin and eosin. A fluorescence microscope (Nikon Eclipse 80i; Nikon, Tokyo, Japan) was used to observe the pulmonary arteries in the stained sections.

Western blot analysis. The pulmonary arteries were cleared in cold PBS and frozen in liquid nitrogen prior to being stored at -80°C. The pulmonary artery tissues were homogenized in lysis buffer (radioimmunoprecipitation assay buffer, PMSF; 100:1) and then centrifuged at $13,800 \times g$ for 15 min at 4°C. The protein concentrations were determined using a bicinchoninic protein assay kit, and 30 μ g were separated on a 10% SDS-PAGE gel, followed transferal onto a polyvinylidene difluoride membrane. The membrane was blocked in 5% skim milk (5 g skim milk dissolved in 100 ml Tris-buffered saline Tween solution) at room temperature for 1 h. The membrane was incubated with the following antibodies: HMGR (cat. no. ab174830, 1:2,000), FDPS (cat. no. ab189874, 1:1,000), FNTA (cat. no. ab109738, 1:1,000), and FNTB (cat. no. ab109748, 1:1,000) (all from Abcam, Cambridge, UK), GGT-I (cat. no. sc18996, 1:200; Santa Cruz Biotechnology Co., Ltd., Dallas, TX, USA), phosphorylated (p)-eNOS (cat. no. 95719, 1:1,000), eNOS (cat. no. 9586, 1:1,000), and RhoA (cat. no. ARH04, 1:1,000) (all from CST Biological Reagents Company Limited, Shanghai, China), Rac1 (cat. no. ARC03, 1:1,000; BD Biosciences, Franklin Lakes, NJ, USA) at 4°C for 16 h. The membranes were then incubated with the appropriate secondary antibody: Goat-anti-rabbit immunoglobulin G (IgG) (cat. no. 1268, 1:2,500), goat-anti-mouse IgG (cat. no. 1265, 1:2,500), and rabbit-anti-goat IgG (cat. no. M1102, 1:2,500) (all from Biovision, Inc., Milpitas, CA, USA) for 2 h at room temperature. The target protein bands were visualized using chemiluminescence and quantified using ImageJ software (version 1.47; National Institutes of Health, Bethesda, MD, USA). GAPDH was used as an endogenous control (cat. no. 377R, 1:5,000; Biovision, Inc.). All antibodies were diluted in 5% BSA (HuaBio, China).

NADPH oxidase activation assay. The activation of NADPH oxidase was detected using a Tissue NADPH Oxidase Activation Assay kit (Genmed Scientifics, Inc., Arlington, MA, USA). The pulmonary arteries were homogenized in lysis buffer and protein concentrations were determined using a BCA protein assay kit. According to the manufacturer's instructions, this was finally detected at 550 nm using a microplate reader (Thermo Fisher Scientific, Inc., Waltham, MA, USA.).

ROS kinase activation assay. A Tissue ROS Kinase Activation Assay kit (Genmed Scientifics, Inc.) was used to measure the level of ROS in the pulmonary artery. The pulmonary arteries were homogenized in lysis buffer and protein concentrations were determined using a BCA protein assay kit. The results were detected at 340 nm using a microplate reader (Thermo Fisher Scientific, Inc.).

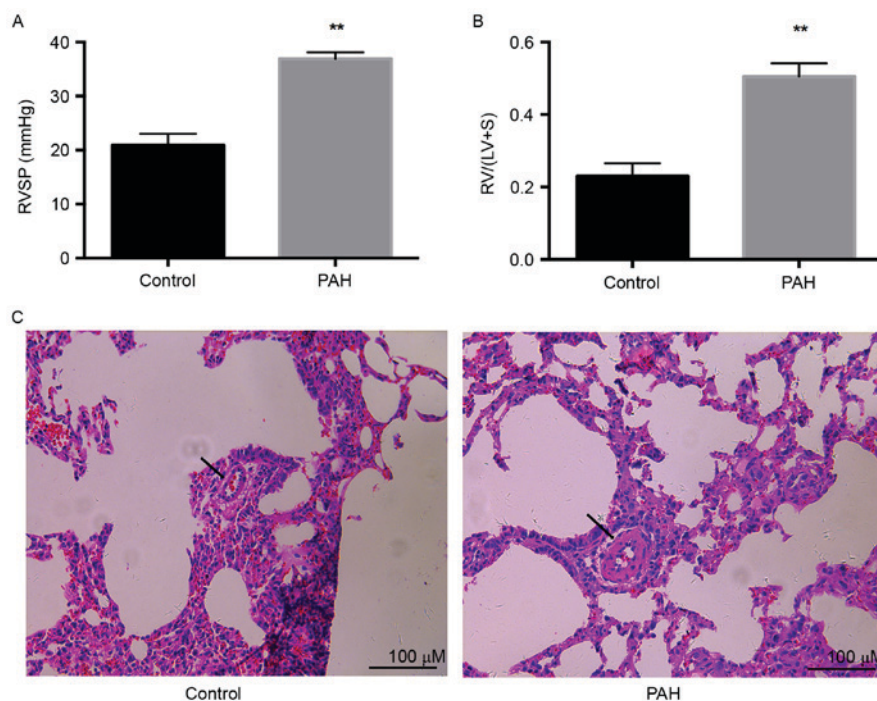


Figure 1. Development of PAH 4 weeks following a single injection of monocrotaline (60 mg/kg). (A) RVSP in control and PAH groups. Data are expressed as the mean \pm standard deviation. ** $P < 0.01$ compared with the control group. (B) RV/(LV+S) in each group. (C) Hematoxylin and eosin staining of lung tissues. The arrows indicate pulmonary micro-arteries (original magnification, $\times 200$). RVSP, right ventricular systolic pressure; PAH, pulmonary arterial hypertension; RV, right ventricle; LV, left ventricle; S, septum.

Measurement of serum NO levels. The blood samples were collected and centrifuged at $13,8000 \times g$, 4°C for 15 min. The serum was then removed into a new EP tube, and serum NO levels were determined using a Nitric Oxide Fluorometric Assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The results were finally detected using a microplate reader (Thermo Fisher Scientific, Inc.).

Statistical analysis. All values are expressed as the mean \pm standard error of the mean. Statistical significance was measured using one-way analysis of variance. Software used for analysis was GraphPad Prism (version 6.0c; GraphPad Software, Inc., La Jolla, CA, USA.). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

MCT-induced PAH. The PAH model was induced by injection of a single dose of MCT (60 mg/kg). After 4 weeks, RVSP was measured by insertion of a PE50 pipe through the jugular vein to the right ventricle. The pressures were transferred into electric signals and collected using MedLab software. The RVSP of the PAH group was significantly increased, compared with that in the control (36.9 ± 1.1 vs. 20.9 ± 1.1 ; $P < 0.01$) as exhibited in Fig. 1A. RVH, calculated as the ratio of RV weight to (LV+S) weight, was significantly increased by ~ 2 -fold in the PAH group compared with that in the control (0.50 ± 0.02 vs. 0.23 ± 0.02 ; $P < 0.01$), as exhibited in Fig. 1B. Pulmonary vascular remodeling was a notable characteristic of PAH. Significant thickening of micro-pulmonary arteries was demonstrated in the PAH group, compared with the control group (Fig. 1C). These results

indicated that injection with a single dose of MCT (60 mg/kg) successfully induced PAH 4 weeks later.

Expression of key enzymes in the mevalonate pathway are altered in MCT-induced PAH. The present study aimed to determine whether the expression levels of key enzymes in the mevalonate pathway are altered in PAH. This involved comparing the expression levels of key enzymes in the pulmonary artery between the PAH and control groups.

HMGR is an initial enzyme in the mevalonate pathway, and the present study found no significant difference in its expression between the PAH and control group (Fig. 2A and B). A significant increase in the expression of FDPS was detected in the PAH group, compared with that in the control group ($P < 0.05$), as exhibited in Fig. 2C. As FTase and GGTase-I catalyze the farnesylation for several small G-proteins, including the Ras and Rho family respectively (18,19), the levels of FNTA and GGTase-I were significantly increased in the PAH group, compared with those in the control group ($P < 0.01$), whereas no significant difference in FNTB was demonstrated (Fig. 2D-F). Overall, the expression levels of enzymes FDPS, FNTA and GGTase-I were elevated in the PAH rats.

Expression of small G-protein RhoA and Rac1 in MCT-induced PAH. Small G-proteins are important in regular specific cell function and gene expression (11), and evidence indicates that the prenylation and activation of small G-proteins are regulated by the enzymes GGTase-I and FTase (18). The expression levels of small G-protein RhoA and Rac1 in the present study were determined using western blot analysis (Fig. 3A). The level of RhoA was significantly elevated in the PAH group, compared with that in the control group (Fig. 3A

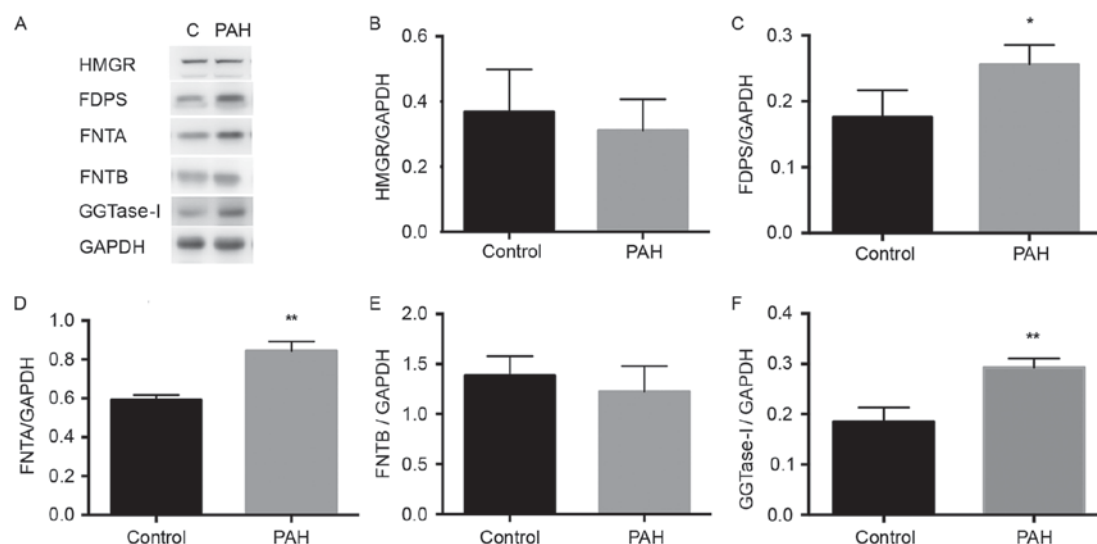


Figure 2. Expression levels of HMGR, FDPS, FNTA, FNTB and GGase-I in the MCT-induced PAH rat pulmonary artery. The proteins were extracted from pulmonary arteries of each group. (A) Western blot analyses demonstrate the expression of HMGR, FDPS, FNTA, FNTB and GGase-I in the control and MCT-induced PAH rats. GAPDH was used as the endogenous loading control. Graphs demonstrate the relative changes in (B) HMGR, (C) FDPS, (D) FNTA, (E) FNTB and (F) GGase-I in the control and MCT groups. Data are expressed as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$ compared with the control group. PAH, pulmonary arterial hypertension; MCT, monocrotaline; HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A; FDPS, farnesyl diphosphate synthase; FNTA, farnesyltransferase α ; FNTB, farnesyltransferase β ; GGase-I, geranylgeranyltransferase type I.

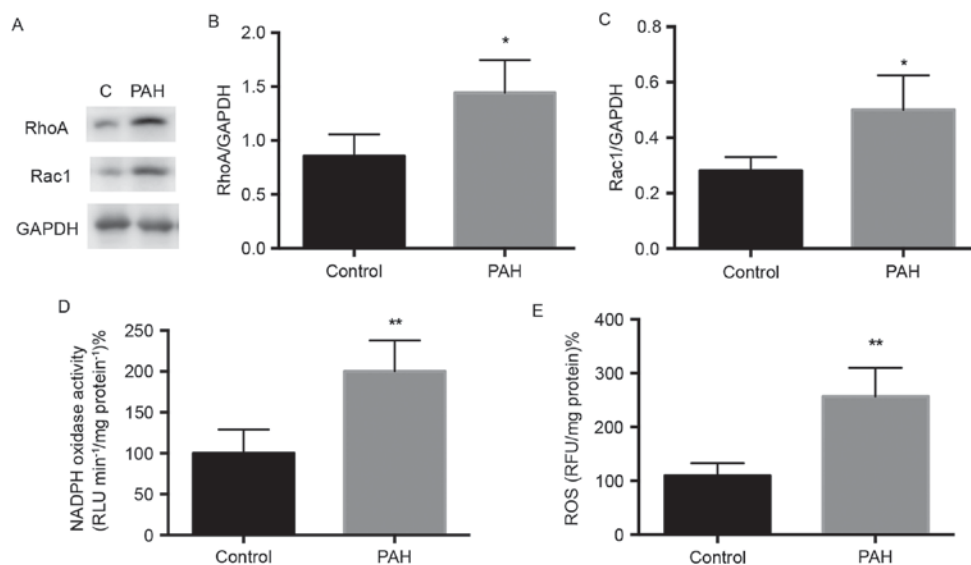


Figure 3. Oxidative stress and small-G protein expression in control and PAH rats. Proteins were extracted from the pulmonary artery of control and PAH rats. (A) Western blot analyses demonstrate the expression of RhoA and Rac1 in the control and PAH rats. GAPDH was used as an endogenous loading control. Graphs demonstrate the relative changes of (B) RhoA and (C) Rac1 in each group. (D) Changes in NADPH oxidase activity in each group were detected using a tissue NADPH oxidase activation assay kit. (E) ROS changes in each group were detected using a tissue ROS kinase activation assay kit. Data are expressed as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$ compared with the control group. PAH, pulmonary arterial hypertension; ROS, reactive oxygen species; RhoA, Ras homolog family, member A; Rac1, Ras-related C3 botulinum toxin substrate 1; C, control.

and B; $P < 0.05$), and Rac1 was also significantly increased in the PAH group (Fig. 3A and C; $P < 0.05$). These results indicated that the expression of small G-proteins were increased in MCT-induced PAH rats.

NADPH activity is increased in MCT-induced PAH. NADPH oxidase is the downstream effector of small G-protein, and its activation depends on the Rac protein (20). The present study detected whether the expression of NADPH oxidase, the downstream effector, was altered in PAH. Following the

treatment of protein lysates according to the manufacturer's protocol of the NADPH Oxidase Activation Assay kit, it was found that NADPH oxidase activity was increased by ~2-fold in the PAH group, compared with that in the control (Fig. 3D).

ROS kinase activity is increased in MCT-induced PAH. NADPH oxidase is a resource for generating ROS (21). The overexpression of ROS is involved in endothelial injury and vessel remodeling (8). The present study measured ROS kinase activity and found a significant increase in the PAH group,

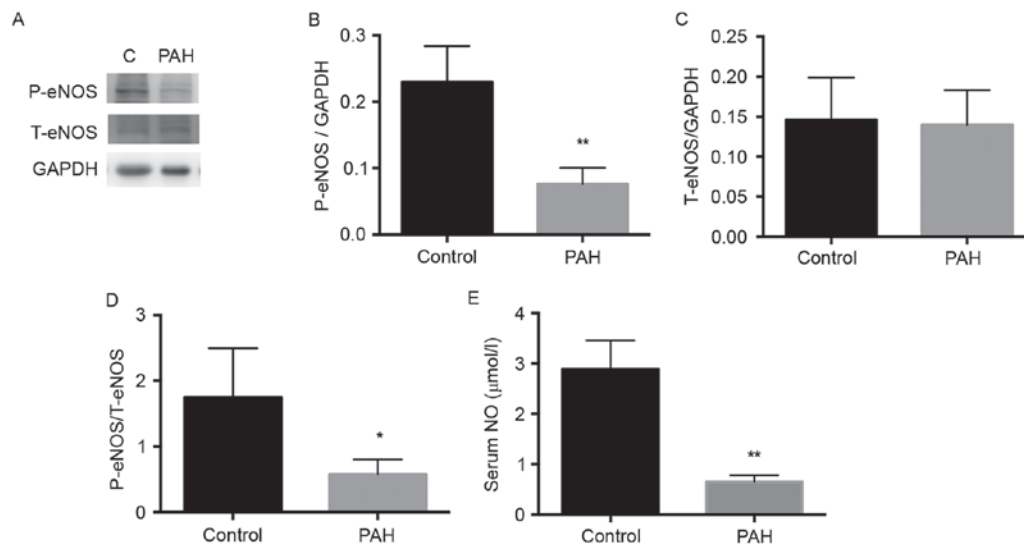


Figure 4. Alterations in eNOS activity and serum NO release in control and PAH rats. Proteins were extracted from the pulmonary artery of animals each group. (A) Western blot analyses demonstrate the expression of P-eNOS and T-eNOS in the control and PAH groups. GAPDH was used as an endogenous loading control. Graphs demonstrate the relative changes of (B) P-eNOS and (C) T-eNOS in each group. (D) Graph demonstrating the ratio of P-eNOS to T-eNOS. (E) Graph demonstrating the changes in serum NO release in the control and PAH groups. Data are expressed as the mean \pm standard deviation. * $P<0.05$ and ** $P<0.01$ compared with the control group. PAH, pulmonary arterial hypertension; NO, nitric oxide; eNOS, endothelial NO synthase; P, phosphorylated; T, total.

compared with that in the control (Fig. 3E; $P<0.01$). This indicated that oxidative stress was elevated in the pulmonary artery of rats with MCT-induced PAH.

Activation of eNOS is decreased in MCT-induced PAH. The eNOS enzyme catalyzes the biosynthesis of NO, and NO in its molecular form has been reported to be important for the development of PAH (22). Therefore, the present study detected the expression of eNOS (Fig. 4A). The level of p-eNOS was significantly decreased in the PAH group, compared with that in the control group (Fig. 4B; $P<0.01$), whereas no significant difference in total NOS was observed between the PAH and the control group (Fig. 4C). Therefore, the activity of eNOS, represented by the ratio of p-eNOS to total eNOS, was significantly decreased in the PAH group, compared with the control (Fig. 4C; $P<0.05$).

Serum levels of NO are decreased in MCT-induced PAH. NO is an important molecule for cardiovascular health. It is released as an endothelium-derived relaxing factor (23). The present study detected the serum levels of NO using a Nitric Oxide Fluorometric Assay kit. The serum level of NO in the PAH group was significantly lower, compared with that in the control (Fig. 4D; $P<0.01$). This may be due to the decreased activity of eNOS in the pulmonary artery of MCT-induced rats.

Discussion

PAH is a serious pulmonary vascular disease, which can lead to right heart failure following qualitative changes in the artery and lumen, blood flow and pressure, and cardiac muscle (2,24). In the present study, changes in the expression of key enzymes of the mevalonate pathway were detected in PAH rats. The MCT-induced PAH model is an established method generally

used in rat experiments (25). In the present study, initial measurements of RVSP revealed a significantly increase in the PAH group 4 weeks following MCT injection. This result was accompanied by a high ratio of RV/(LV+S) in the PAH group. These results indicated that the PAH model and right ventricular remodeling had been successfully established.

In the present study, it was demonstrated that the expression of key enzymes in the mevalonate pathway, including FDPS, FNTA and GGTase-I, were significantly increased in the pulmonary artery of MCT-induced PAH rats. The expression of small G-protein Rac1 and RhoA were also increased, which was consistent with the results of GGTase-I and FTase. Small G-proteins downstream effectors, including NADPH and ROS, were also examined; significant increases in ROS and NADPH oxidase activity were demonstrated, whereas the protein expression of eNOS and release of serum NO decreased.

HMGR is the initial enzyme in cholesterol synthesis. The therapeutic effect of statins on pulmonary hypertension is controversial in clinical trials. In the present study, no significant change was demonstrated in HMGR in the PAH rats, consistent with a previous meta-analysis, which reported that HMGR inhibitor statins had no benefit in patients with PAH (26). Isoprenoid is the intermediate of the mevalonate pathway, and is important for diverse cellular functions (12). The three known end-products of isoprenoid are cholesterol, dolichol and the polyisoprene side chain of ubiquinone (27). Steroidal and non-steroidal isoprenoids partially regulate the expression of enzymes in the mevalonate pathway (28). FDPS is an important enzyme in the mevalonate pathway, which catalyzes the synthesis of FPP and GPP from the substrate mevalonate (28). In the present study, an increase in the enzyme FDPS was found in the pulmonary artery tissue of the PAH rats. This increased expression of FDPS may be responsible for pulmonary vessel remodeling and right ventricular hypertrophy. Therefore, FDPS

inhibitors are being investigated as a treatment option for certain diseases in which FDPS is overexpressed. A previous study demonstrated inhibiting FDPS by N6-isopentenyladenosine, an adenosine and isoprenoid derivative, was demonstrated to inhibit the proliferation of tumor cells (29). In addition, bisphosphonates are widely used FDPS inhibitor in the treatment of osteoporosis (30). The chronic inhibition of FDPS can also attenuate cardiac hypertrophy and fibrosis (31), although the specific signaling mechanism remains to be elucidated. It was hypothesized that the abnormal expression of FDPS may be a potential therapeutic target for the treatment of PAH.

The higher level of FDPS induced the accumulation of downstream products, including GPP and FPP. As an intermediate product, FPP is an important precursor in the synthesis of sterols, dolichols and ubiquinones (32). It is well known that the activation of proteins, including Ras and Rab, require farnesylation by FTase with FPP as substrate in the transmembrane, which responds to cellular signaling. Therefore, Ras can activate genes involved in cell growth, proliferation, and differentiation, and lead to abnormal vessel growth and remodeling (33). The present study demonstrated that the expression levels of FTase and GGTase-I were increased in the PAH group. The enzyme GGTase-I catalyzes the geranylgeranylation of proteins, including the Rho family and Rac. The elevated expression level of GGTase-I has been reported in several human diseases, including renal fibrosis, spontaneous hypertension and cancer (34). Previous studies have attempted to use GGTase-I inhibitor to treat diseases, including renal fibrosis and cancer, and have achieved promising results (35). In order to localize in correct subcellular membranes, small G-proteins of the Rac and RhoA family require post-translational prenylation by FTase and GGTase-I, and then transducing signals to downstream effectors (36). Unlike certain cardiovascular diseases, including pressure overload-induced cardiac hypertrophy and spontaneous hypertension, in which the expression of FNTB is elevated (37), the present study found no significant change in the expression of FNTB in the PAH model. This may be due to differences in tissue expression and disease models, indicating that FNTA is more important in the development of PAH than FNTB.

The Rho family, including Rac and Rho, can regulate the function of the cytoskeleton (38). Rho proteins are involved in the activation of extracellular signal-regulated kinase (ERK) in response to angiotensin II or stretch-induced hypertrophy (39). Certain studies have demonstrated that Rac can provoke the c-Jun N-terminal kinase/p38 subgroup of nuclear mitogen-activated protein kinases (MAPKs) (40). Activated Rac can stimulate the activity of p21-activated kinase (PAK) (41), and activated PAK can affect the activation of ERK via the phosphorylation of the serine/threonine protein kinase Raf (42). The transcription of nuclear genes associated with cell proliferation and growth can be regulated following the activation of Raf, MAPK and ERK proteins (43,44).

NADPH oxidase is a membrane-bound enzyme complex. Several studies have shown that the activation of NADPH oxidase depends on the Rac protein and two other cytosolic proteins, p47phox and p67phox (10). Rac-GDP is converted into Rac-GTP and translocated to the correct membrane, and NADPH oxidase is activated (45). The present study measured the activity of NADPH oxidase and found it was markedly

increased in the PAH rats, consistent with the increase of Rac1. This result was supported by a previous study, which demonstrated the same increased activity of NADPH oxidase in an MCT-induced PAH model (46).

NADPH oxidase is a source of superoxides, and superoxides undergo further reactions to generate ROS (47). According to the present study, the PAH rats generated more NADPH-derived ROS, compared with the control rats, which was consistent with previous studies (46,48). ROS is an essential regulator of normal cell physiology. The overproduction of ROS is associated with cardiovascular disorders, metabolic dysfunction and other diseases (47,49), therefore, it was hypothesized that generated ROS contributes to the development of PAH. Accumulated ROS may function through reacting with cellular components, affecting the function of endothelial cells, inducing the proliferation of smooth muscle cells, and vascular remodeling (50,51).

In several heart diseases, NO is an important molecule in vasodilation. The present study found decreased activity of eNOS and serum levels of NO in the PAH group. Although the bio-activity of eNOS was weaker in PAH, no differences in the expression of eNOS were demonstrated between PAH and the control group. This contradicted the results of a previous study, which reported that the expression level of eNOS decreased in the PAH model (52). However, previous studies commonly used endothelial cells, including human umbilical vein endothelial cells, whereas the experiments in the present study used whole pulmonary artery tissue. This may be the reason for the difference in results. Reductions in NO have not been observed in previous studies (53). The superoxide-like ROS can react with NO to reduce NO bioavailability (9), and the imbalance of ROS-NO levels may lead to endothelial dysfunction, consequently stimulating the development of PAH.

The present study detected changes in the expression of key enzymes of the mevalonate pathway in an MCT-induced PAH model, which may serve as drug targets for PAH treatment. The inhibitors of certain enzymes, including FDPS and GGTase-I, have been used in the treatment of certain human diseases. However, whether treatment with the inhibitors of altered enzymes in PAH can attenuate persistent pulmonary artery remodeling and high pressure remains to be elucidated, for which further investigations are required.

Acknowledgements

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