Activation of autophagy induces monocrotaline-induced pulmonary arterial hypertension by FOXM1-mediated FAK phosphorylation

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Abstract

Background: Activation of autophagy induces the proliferation of pulmonary artery smooth muscle cells (PASMCs) and vascular remodeling in rats, and therefore improves the development of pulmonary arterial hypertension (PAH). However, the possible mechanisms underlying autophagy in PAH have not been completely understood. The aims of this study are to address these issues.

Method: Rat models of PAH were induced by intraperitoneally injection of monocrotaline (MCT). The right ventricle systolic pressure (RVSP), right ventricular hypertrophy index (RVHI), percentage of medial wall thickness (%MT), α -smooth muscle actin (α -SMA) staining and Ki67 staining were performed to evaluate the development of PAH. The protein levels of forkhead box M1 (FOXM1), focal adhesion kinase (FAK), phospho-FAK, and LC3B were determined by immunoblotting and immunohistochemistry.

Results: FOXM1 protein level and the activation of FAK and autophagy were significantly increased in MCT-induced PAH rats. Pharmacological inhibition of FOXM1 and FAK suppressed MCT-induced autophagy activation, and reduced RVSP, RVHI and %MT, and ameliorated the proliferation of pulmonary arterial smooth muscle cells in MCT-induced PAH rats.

Conclusion: FOXM1 induces the development of PAH by activation of FAK and consequent stimulating autophagy in MCT-treated rats.

Introduction

Pulmonary arterial hypertension (PAH) is a severe and life-threatening disease which is associated with increase pulmonary vascular resistance and pulmonary artery pressure [1, 2]. All forms of PAH share a common pathogenesis mechanisms which includes persistent pulmonary vasoconstriction, vascular remodeling, and thrombosis in situ [3]. Pulmonary vascular remodeling, the main pathological feature of PAH, is majorly caused by pulmonary arterial smooth muscle cells (PASMCs) proliferation/migration [4]. Therefore, elucidating the molecular mechanisms underlying the PASMCs proliferation and pursuing appropriate targets to inhibit vascular remodeling are critical in the treatment of PAH.

Autophagy, a conserved and self-degradative process, preserves in eukaryotic cells and has essential roles in cellular homeostasis and energy balance [5–7]. Multiple reports have demonstrated that autophagy regulates diverse biological activities, such as cell proliferation, differentiation, survival and tissue remodeling [8]. A study has suggested that activation of autophagy induces the development of PAH in rats, and inhibition of autophagy decreases PASMCs proliferation in vitro [9]. However, the molecular mechanisms underlying autophagy in PAH are still largely unknown.

Forkhead box M1 (FOXM1), a member of forkhead box family of transcription factors, regulates a variety of cellular processes including cell proliferation, migration, invasion, apoptosis, and angiogenesis by directly binding to target gene enhancers [10, 11]. It has been discovered that FOXM1 is overexpressed in hypoxia-induced PAH in mice, and suppression of FOXM1 inhibits the proliferation and dedifferentiation

of PASMCs [12]. A study has demonstrated that FOXM1 induces the activity/phosphorylation of FAK through directly binding to integrin $\beta 1$, which promotes the proliferation and invasion of triple-negative breast cancer cells [13]. Furthermore, FOXM1 has been reported to promote triple-negative breast cancer cells survival and proliferation through induction of autophagy [14]. However, it is still unclear whether FOXM1 induces the activation of autophagy in MCT-induced PAH. To address the above issues, rat models of monocrotaline (MCT)-induced PAH were used, the effects and mechanisms of FOXM1 on autophagy were further explored.

Materials And Methods

Animals

Ninety male Sprague-Dawley (SD) rats (200 to 250 g) were used in the current study. All animal care and experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of Xi'an Jiaotong University Animal Experiment Centre. All protocols used in this study were approved by the Laboratory Animal Care Committee of Xi'an Jiaotong University. The rats with similar baseline characteristics were randomly divided into six groups: control group (Con group, n = 15), monocrotaline (MCT)-treated group (MCT group, n = 15), MCT and vehicle (dimethylsulfoxide, DMSO)-treated group (MCT + vehicle group, n = 15), MCT and thiostrepton (TRT)-treated group (MCT + TRT group, n = 15), MCT and 1,2,4,5-benzenetetraamine tetrahydrochloride (Y15)-treated group (MCT + Y15 group, n = 15), and MCT and chloroquine phosphate (CQ)-treated group (MCT + CQ group, n = 15). All rats were housed in a 12h light-dark cycle at $22 \pm 2^{\circ}\text{C}$ and free accessed to food pellets and tap water.

Generation of PAH models and drug treatment

MCT (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.1 mol/L HCl, the solution was then titrated to pH 7.4 with 0.1 mol/L NaOH with the final concentration of 30 mg/mL. Thiostrepton (Selleckchem, USA) were dissolved in DMSO, and then diluted with 0.9% NaCl to a final concentration of 10 mg/mL. DMSO was diluted with 0.9% NaCl to a final concentration of 0.5% as a vehicle for thiostrepton. Y15 (Selleckchem, USA) and CQ (Aladdin Bio-Chem Technology Co., Shanghai, China) was dissolved in 0.9% NaCl with the final concentration of 7.5mg/mL and 20 mg/mL.

The PAH model was established by intraperitoneally (IP) injection of MCT (60 mg/kg) on day 1. Once PAH was established 2 weeks post MCT injections, FOXM1 inhibitor thiostrepton (20 mg/kg) or its vehicle was administrated to the rats by IP injection every day for 2 weeks. After the establishment of MCT-induced PAH rat model, FAK inhibitor Y15 (15 mg/kg) or CQ (40 mg/kg) were daily administered to the rats by IP. The healthy control rats were IP injected with the same volume of vehicle solution (DMSO or 0.9% NaCl).

Measurement of RVSP and RVH

At the endpoint of the study, all survived rats were anesthetized by a spontaneous inhalation of isoflurane. After stable anesthesia, we isolated the right internal jugular vein of rats, and then inserted a polyethylene catheter into the right ventricle (RV). A Grass polygraph was used to detect the right ventricle systolic pressure (RVSP). Then hearts and lungs of rats were excised. The RV was divided into the left ventricle (LV) and interventricular septum (S), and then each part was weighed separately. The ratio of the weight of RV to the LV plus S [RV/ (LV+S)] was calculated to assess the index of right ventricular hypertrophy (RVH).

Histologic analysis

Harvested pulmonary tissues from marginal right lower lobes were immersed in 4% paraformaldehyde, and then embedded in paraffin. Tissue blocks were cut in a thickness of 5 mm, and then stained with hematoxylin and eosin (HE). The medial wall thickness (%MT) of vessels (20–70 µm diameters, n = 15 per rat) was observed using a light microscope to assess pulmonary arterioles vascular remodeling. The %MT was calculated as follows: %MT = (2 × medial wall thickness) ×100/external diameter.

Immunohistochemistry

To further assess the pulmonary arterial muscularization and cell proliferation, we used immunofluorescence. Lung sections of rats from each group were dewaxed and dehydrated, followed by antigen retrieval and proteinase digestion. And then, the sections were incubated with anti-α-SMA (BM0002, Boster, CA, USA, 1:200 dilution), anti-Ki67 (YM3064, Immunoway, TX, USA, 1:200 dilution) and anti-LC3B antibodies (18725-1-AP, Proteintech, Chicago, IL, USA, 1:200 dilution). Lung tissue sections were soaked in fluorescence-conjugated secondary antibodies, and then mounted with neutral resin.

Western blot analysis

Harvested lung tissues were immersed in RIPA lysis buffer (HEART, Xi'an, China), and the concentrations of protein were measured by BCA protein assay kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Equal amounts of protein were resolved and separated on 8%–15% SDS-PAGE and then transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad). Membranes were blocked with nonfat dry milk (5%, w/v) for 1h, and then incubated overnight at 4°C with the specific primary antibodies against β-actin (YM3028, Immunoway, TX, USA, 1:1000 dilution), FOXM1 (no 32671, Signalway Antibody, Pearland, TX, 1:1,000 dilution), LC3B (18725-1-AP, Proteintech, Chicago, IL, USA, 1:800 dilution), FAK (12636-1-AP, Proteintech, Chicago, IL, USA, 1:800 dilution) and p-FAK (sc-374668; Santa Cruz, 1:600 dilution). After washing blots three times in PBST, membranes were soaked in horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG secondary antibody (AP156P or AP127P, Sigma-Aldrich, St. Louis, MO, USA, 1:5000) for 1 hours. Blots were visualized by the enhanced chemiluminescence detection system (Amersham Bioscience), and the band densities were measured using Quality One software (Bio-Rad).

Statistical analysis

Experimental data are presented as means $\pm$ standard deviation. Group comparisons were analyzed using one-way analysis of variance followed by a Tukey post hoc test. $P < 0.05$ was considered statistically significant.

Results

Autophagy is activated in MCT-induced PAH model

To examine whether the activation of autophagy are increased in MCT-induced PAH rats, the protein level of LC3B was measured in tissue lysates of rat lung. Fig. 1A shows that LC3B protein level was increased to 1.75-fold over control in MCT-treated PAH rats ($P < 0.05$ versus control). Administration of CQ, an inhibitor of autophagy, further increased the protein level of LC3B to 3.00-fold over control ($P < 0.05$ versus MCT-treated rats; Fig.1A). Furthermore, immunohistochemistry was used to detect the expression of LC3B. As shown in Fig. 1B and C, percentage of LC3B-positive PSMCs was increased from (6.2 ± 1.4) % in control rats to (23.5 ± 3.1) % in MCT-treated PAH rats ($P < 0.05$ versus MCT-treated rats). Meanwhile, the ratio of LC3B-positive PSMCs was increased to (31.5 ± 2.4) % in MCT and CQ treated rats ($P < 0.05$ versus MCT-treated rats, Fig. 1B, C). These results imply that autophagy is activated in MCT-induced PAH.

Inhibition of autophagy prevents the increase of RVSP, RVH, and pulmonary arterial remodeling in MCT-induced PAH model

In MCT-treated PAH rats, RVSP was significantly increased to 43.63 ± 2.33 mmHg versus 22.36 ± 1.69 mmHg in control group ($P < 0.05$ versus control; Fig.2A). This suggests that PAH was successfully induced in MCT-treated rats. The presence of CQ reduced RVSP to 28.66 ± 1.71 mmHg ($P < 0.05$ versus MCT-treated rats; Fig.2A). Similar changes were observed in the index of RVH (RVHI). As shown in Fig. 2B, RVHI was markedly raised from 0.34 ± 0.02 in control rats to 0.59 ± 0.04 in MCT-treated PAH rats ($P < 0.05$ versus control). While treatment of PAH rats with CQ reduced RVSP to 0.36 ± 0.02 ($P < 0.05$ versus MCT-treated rats). These results indicate that inhibition of autophagy effectively prevents the development of PAH.

As shown in Fig. 2C and D, medial wall thickness in small pulmonary arteries in MCT-treated rats [(32.46 ± 2.15) %] was effectively elevated compared with control rats [(21.67 ± 1.48) %] ($P < 0.05$ versus control). However, the increased medial wall thickness was dramatically decreased to (23.31 ± 1.96) % in MCT and CQ-treated PAH rats ($P < 0.05$ versus MCT-treated rats; Fig. 2C, D). In addition, Fig. 2e, f shows that the percentage of muscularization of pulmonary arteries was increased in MCT-treated PAH rats compared with control rats ($P < 0.05$ versus control). We further assessed the proliferation of PSMCs by Ki67 immunostaining. In MCT-induced PAH rats, the percentage of Ki67-positive PSMCs was increased to (19.12 ± 2.2) % over control (6.20 ± 1.0) % ($P < 0.05$ versus control, Fig. 2G, H). However, CQ prevented MCT-induced increase of the pulmonary arteries muscularization and proliferation of PSMCs. The ratio of Ki67-positive PSMCs in MCT-treated PAH rats dropped to (12.23 ± 1.8) % in MCT and CQ-treated rats ($P < 0.05$ versus MCT-treated rats; Fig. 2E–H). These results indicate that inhibition of autophagy suppresses the pulmonary arterial remodeling in MCT-induced PAH.

FAK is activated in MCT-induced PAH model

FAK, a non-receptor tyrosine kinase, has been shown to regulate cell metabolism, survival, proliferation, invasion, migration, and apoptosis [15-17]. Activation of FAK has been shown to promote the proliferation of osteoprotegerin-treated PSMCs[18]. Therefore, we examined the protein levels of FAK and p-FAK using western blotting. Fig. 3 shows that p-FAK protein level was increased to 3.24-fold over control in MCT-treated rats ($P < 0.05$ versus control). After administration of FAK inhibitor Y15 decreased the level of p-FAK to 1.33-fold over control ($P < 0.05$ versus MCT-treated rats; Fig. 3). These results indicate that FAK is activated in MCT-induced PAH.

Inhibition of FAK prevents MCT-induced PAH by suppression of autophagy

In order to determine the effects of FAK inhibition on MCT-induced PAH in rats, we firstly examined RVSP and RVHI. As shown in Fig. 4A and 4B, the increased RVSP and RVHI in MCT-treated rats were significantly decreased from 43.63 ± 2.33 mmHg and 0.59 ± 0.04 to 26.14 ± 1.56 mmHg and 0.35 ± 0.02 in MCT and Y15 co-treated rats, respectively (both $P < 0.05$ versus MCT-treated rats). Similarly, elevated medial wall thickness in small pulmonary arteries was dramatically ameliorated in MCT and Y15 treated rats (Fig. 4C, D). The presence of FAK inhibitor Y15 decreased medial wall thickness from (32.46 ± 2.15) % to (22.54 ± 1.78) % ($P < 0.05$ versus MCT-treated rats; Fig. 4C, D). Moreover, treatment of PAH rats with Y15 also reduced the pulmonary arteries muscularization and PSMCs proliferation, the percentage of Ki67-positive PSMCs dropped from (19.12 ± 2.2) % in MCT-treated rats to (14.53 ± 2.0) % in MCT and Y15-treated PAH rats ($P < 0.05$ versus MCT-treated rats, Fig. 4E–H). The results suggest that inhibition of FAK attenuates pulmonary vascular remodeling and the development of PAH.

We then explored the effect of inhibition of FAK on autophagy in MCT-induced PAH. In MCT-induced PAH rats, LC3B protein level was increased to 4.35-fold over control ($P < 0.05$ versus control), while which was declined to 2.26-fold over control in MCT and Y15-treated rats ($P < 0.05$ versus MCT-treated rats; Fig. 5A). At the same time, the percentage of LC3B-positive PSMCs was decreased from (23.5 ± 3.1) % in MCT-treated rats to (12.3 ± 2.0) % in MCT and Y15 co-treated rats ($P < 0.05$, Fig. 5B, C). These results suggest that inhibition of FAK suppresses autophagy activation in MCT-induced PAH.

The expression of FOXM1 is up-regulated in MCT-induced PAH model

In order to determine whether the expression of FOXM1 is increased in rats with MCT-induced PAH, the protein level of FOXM1 was examined. In MCT and vehicle-treated PAH rats, the protein level of FOXM1 was increased to 2.47-fold over control ($P < 0.05$ versus control; Fig. 6). Administration of thiostrepton, an inhibitor of FOXM1, reduced FOXM1 level in MCT-treated rats to 0.52-fold over control ($P < 0.05$ versus MCT and vehicle-treated rats; Fig. 6). These results suggest that the FOXM1 protein level is up-regulated in MCT-induced PAH.

Inhibition of FOXM1 prevents the increase of RVSP, RVH, and pulmonary arterial remodeling in MCT-induced PAH model

As shown in Fig. 7A and 7B, thioestrepton reduced RVSP and RVHI from 44.55 ± 2.54 mmHg and 0.57 ± 0.04 in MCT and vehicle-treated rats to 31.33 ± 2.00 mmHg and 0.36 ± 0.03 in MCT and thioestrepton co-treated rats ($P < 0.05$ versus MCT and vehicle-treated rats). Quantitative morphometric analysis of the medial wall thickness showed that the increased %MT in MCT and vehicle-induced PAH rats reduced from (33.11 ± 2.35) % to (23.31 ± 1.75) % in MCT and thioestrepton-treated rats ($P < 0.05$ versus MCT and vehicle-treated rats; Fig. 7C, D). Thioestrepton also reduced MCT-induced pulmonary arteries muscularization and PASMCs proliferation, the ratio of Ki67-positive PASMCs dropped from (21.04 ± 2.5) % in MCT and vehicle-treated rats to (15.15 ± 2.1) % in MCT and thioestrepton-treated rats ($P < 0.05$ versus MCT and vehicle-treated rats; Fig. 7E–H). These results indicate that inhibition of FOXM1 suppresses the pulmonary arterial remodeling in MCT-induced PAH.

FOXM1 mediates FAK and autophagy activation in MCT-induced PAH model

To determine whether FOXM1 mediates the activation of FAK and autophagy in rats with MCT-induced PAH, the protein levels of FAK, p-FAK and LC3B were examined. Fig. 8A shows that the protein level of p-FAK was increased to 3.12-fold over control in MCT and vehicle co-treated rats ($P < 0.05$ versus control), while p-FAK was decreased to 1.45-fold over control in MCT and thioestrepton-treated rats ($P < 0.05$ versus MCT and vehicle-treated rats). These results indicate that FOXM1 induces the activation of FAK in MCT-induced PAH rats.

As depicted in Fig. 8B, LC3B protein level was increased to 3.12-fold over control in MCT and vehicle-induced PAH rats ($P < 0.05$ versus control), while treatment of PAH rats with thioestrepton decreased the level of LC3B to 1.49-fold over control ($P < 0.05$ versus MCT and vehicle-treated rats). In addition, the percentage of LC3B-positive PASMCs was decreased from (22.1 ± 2.6) % in MCT and vehicle-treated rats to (15.2 ± 2.2) % in MCT and thioestrepton-treated rats ($P < 0.05$, Fig. 8C, D). These results suggest that FOXM1 up-regulation induces autophagy in MCT-induced PAH rats.

Discussion

This study has indicated that FOXM1 triggers FAK by inducing the phosphorylation of FAK for the activation of autophagy and promotes the development of MCT-induced PAH.

Autophagy maintains cellular homeostasis and protects against genome instability, which plays a crucial role in removing misfolded or aggregated proteins, clearing damaged organelles and eliminating intracellular pathogens [19]. Multiple reports have discovered that inhibition of autophagy alleviates pulmonary artery remodeling in MCT or hypoxic-induced PAH rats [20, 21]. Our previous study has suggested that autophagy is activated in sphingosine-1-phosphate (S1P)-induced proliferation of PASMCs and animal models of PAH [22–24]. In this study, we found that the protein level of LC3B was increased in MCT-induced PAH rats, and inhibition of autophagy by CQ suppressed pulmonary artery remodeling and the development of PAH.

FAK is a cytoplasmic tyrosine kinase and scaffold protein, which is localized at cell adhesion and extracellular matrix [15]. FAK plays essential roles in integrating signals such as growth factors receptors, G-protein coupled receptors and cytokine receptors, which promotes FAK activation by auto-phosphorylation [25, 26]. It has been shown that activation of FAK induces the proliferation and migration of PSMCs from PAH human lungs, and inhibition of FAK alleviates vascular remodeling in MCT-induced PAH rats [27]. Our present study indicated that the phosphorylation of FAK is significantly increased in MCT-induced PAH rats, which was accompanied with increased pulmonary vascular remodeling. In addition, FAK has been reported to be related to autophagy in the development of numerous types of human diseases. Sun et al. have shown that osteopontin enhances autophagy by activating of FAK signaling pathway, and then attenuating early brain injury [28]. However, Zheng et al. have shown that osteoprotegerin inhibits autophagy by activation of FAK in cultured H9C2 cells [29]. In our study, we confirmed inhibition of FAK by administration of Y15 abolished MCT-induced autophagy activation and therefore suppressed pulmonary artery remodeling.

FOXM1, a classic proliferation-associated transcription factor, is widely expressed during the cell cycle, which plays essential roles in regulating G1/S and G2/M transition, and M-phase progression [30, 31]. A previous study has shown that FOXM1 increases the proliferation of PSMCs and contributes to the development of animal model of PAH [32]. Our previous study has also demonstrated that yes-associated protein (YAP) increases the expression of FOXM1 and promotes the proliferation of Galectin-3-induced PSMCs [33]. The results of the present study showed that FOXM1 protein level is increased in rats with MCT-induced PAH. Importantly, FOXM1 has been shown to promote the activity of FAK by binding to integrin $\beta 1$ in triple-negative breast cancer [34]. Moreover, studies have further suggested that inhibition of FOXM1 inhibits starvation and rapamycin-induced autophagy by decreasing the expression of LC3 and Beclin-1 in breast cancer [14]. In this study, we found that inhibition of FOXM1 inhibited the phosphorylation of FAK, and then reduced the protein level of LC3B in MCT-induced PAH. These results indicate that FOXM1 induces autophagy activation by phosphorylating FAK in MCT-induced PAH.

Conclusions

We identify that FOXM1 induces the development of PAH by increasing the phosphorylation of FAK and subsequent activation of autophagy. Our study may contribute to the development of more effective PAH therapeutic strategies

Declarations

Acknowledgements

Not applicable.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Cui Zhai, Nana Zhang, Jian Wang, Meng Cao and Jing Luan. The first draft of the manuscript was written by Cui Zhai, Shaojun Li and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

All animal care and experiments were performed in accordance with Xi'an Jiaotong University Animal Care Policy following the Guide for the Care and Use of Laboratory Animals. Ethics approval for the experimental protocols was received from the Laboratory Animal Care and Use Committee of Xi'an Jiaotong University.

References

1. D. Santos-Ribeiro, P. Mendes-Ferreira, C. Maia-Rocha, R. Adão, A.F. Leite-Moreira, C. Brás-Silva (2016) Pulmonary arterial hypertension: Basic knowledge for clinicians. *Archives of Cardiovascular Diseases* 109(10): 550-561. 10.1016/j.acvd.2016.03.004.
2. S.S. Hahn, M. Makaryus, A. Talwar, M. Narasimhan, G. Zaidi(2017) A review of therapeutic agents for the management of pulmonary arterial hypertension. *Ther Adv Respir Dis* 11(1): 46-63. 10.1177/1753465816665289.
3. Y. Miura, Y. Fukumoto, K. Sugimura, M. Oikawa, M. Nakano, S. Tatebe, S. Miyamichi, K. Satoh, H. Shimokawa(2010) Identification of New Prognostic Factors of Pulmonary Hypertension. *Circulation Journal* 74(9): 1965-1971. 10.1253/circj.CJ-10-0270.
4. T. Tajsic, N.W. Morrell(2011) Smooth muscle cell hypertrophy, proliferation, migration and apoptosis in pulmonary hypertension. *Compr Physiol* 1(1): 295-317. 10.1002/cphy.c100026.

5. Z. Yang, D.J. Klionsky(2010) Eaten alive: a history of macroautophagy. *Nat Cell Biol* 12(9): 814-22. 10.1038/ncb0910-814.
6. N. Mizushima, B. Levine, A.M. Cuervo, D.J. Klionsky(2008) Autophagy fights disease through cellular self-digestion. *Nature* 451(7182): 1069-75. 10.1038/nature06639.
7. S.-M. Zhang, Z.-F. Shang, P.-K. Zhou(2015) Autophagy as the effector and player in DNA damage response of cells to genotoxicants. *Toxicology Research* 4(3): 613-622. 10.1039/c5tx00043b.
8. K. Phadwal, A.S. Watson, A.K. Simon(2013) Tightrope act: autophagy in stem cell renewal, differentiation, proliferation, and aging. *Cell Mol Life Sci* 70(1): 89-103. 10.1007/s00018-012-1032-3.
9. L. Long, X. Yang, M. Southwood, J. Lu, S.J. Marciniak, B.J. Dunmore, N.W. Morrell(2013) Chloroquine prevents progression of experimental pulmonary hypertension via inhibition of autophagy and lysosomal bone morphogenetic protein type II receptor degradation. *Circ Res* 112(8): 1159-70. 10.1161/circresaha.111.300483.
10. D. Nandi, P.S. Cheema, N. Jaiswal, A. Nag(2018) FoxM1: Repurposing an oncogene as a biomarker. *Semin Cancer Biol* 52(Pt 1): 74-84. 10.1016/j.semcancer.2017.08.009.
11. M. Halasi, A.L. Gartel(2013) FOX(M1) news—it is cancer. *Mol Cancer Ther* 12(3): 245-54. 10.1158/1535-7163.mct-12-0712.
12. J. Dai, Q. Zhou, H. Tang, T. Chen, J. Li, P. Raychaudhuri, J.X.J. Yuan, G. Zhou(2018) Smooth muscle cell-specific FoxM1 controls hypoxia-induced pulmonary hypertension. *Cell Signal* 51(119-129). <https://doi.org/10.1016/j.cellsig.2018.08.003>.
13. Z. Hamurcu, N. Kahraman, A. Ashour, B. Ozpolat(2017) FOXM1 transcriptionally regulates expression of integrin β 1 in triple-negative breast cancer. *Breast Cancer Res Treat* 163(3): 485-493. 10.1007/s10549-017-4207-7.
14. Z. Hamurcu, N. Delibaşı, U. Nalbantoglu, E.F. Sener, N. Nurdinov, B. Tascı, S. Taheri, Y. Özkul, H. Donmez-Altuntas, H. Canatan, B. Ozpolat(2019) FOXM1 plays a role in autophagy by transcriptionally regulating Beclin-1 and LC3 genes in human triple-negative breast cancer cells. *J Mol Med (Berl)* 97(4): 491-508. 10.1007/s00109-019-01750-8.
15. P.C. Lv, A.Q. Jiang, W.M. Zhang, H.L. Zhu(2018) FAK inhibitors in Cancer, a patent review. *Expert Opin Ther Pat* 28(2): 139-145. 10.1080/13543776.2018.1414183.
16. X. Zhao, J.-L. Guan(2011) Focal adhesion kinase and its signaling pathways in cell migration and angiogenesis. *Advanced Drug Delivery Reviews* 63(8): 610-615. <https://doi.org/10.1016/j.addr.2010.11.001>.
17. T.M.J. Evers, L.J. Holt, S. Alberti, A. Mashaghi(2021) Reciprocal regulation of cellular mechanics and metabolism. *Nature Metabolism* 3(4): 456-468. 10.1038/s42255-021-00384-w.
18. D. Jia, Q. Zhu, H. Liu, C. Zuo, Y. He, G. Chen, A. Lu(2017) Osteoprotegerin Disruption Attenuates HySu-Induced Pulmonary Hypertension Through Integrin $\alpha\beta$ 3/FAK/AKT Pathway Suppression. *Circulation: Cardiovascular Genetics* 10(1): e001591. doi:10.1161/CIRCGENETICS.116.001591.
19. D. Glick, S. Barth, K.F. Macleod(2010) Autophagy: cellular and molecular mechanisms. *J Pathol* 221(1): 3-12. 10.1002/path.2697.

20. X.-l. Zhou, Z.-b. Liu, R.-r. Zhu, H. Huang, Q.-r. Xu, H. Xu, L. Zeng, Y.-y. Li, C.-h. Huang, Q.-c. Wu, J.-c. Liu(2019) NSD2 silencing alleviates pulmonary arterial hypertension by inhibiting trehalose metabolism and autophagy. *Clinical Science* 133(9): 1085-1096. 10.1042/cs20190142.
21. Y. Liu, Y. Xu, J. Zhu, H. Li, J. Zhang, G. Yang, Z. Sun(2019) Metformin Prevents Progression of Experimental Pulmonary Hypertension via Inhibition of Autophagy and Activation of Adenosine Monophosphate-Activated Protein Kinase. *J Vasc Res* 56(3): 117-128. 10.1159/000498894.
22. C. Zhai, W. Shi, W. Feng, Y. Zhu, J. Wang, S. Li, X. Yan, Q. Wang, Q. Zhang, L. Chai, C. Li, P. Liu, M. Li(2018) Activation of AMPK prevents monocrotaline-induced pulmonary arterial hypertension by suppression of NF-kappaB-mediated autophagy activation. *Life Sci* 208(87-95. 10.1016/j.lfs.2018.07.018.
23. C. Zhai, W. Feng, W. Shi, J. Wang, Q. Zhang, X. Yan, et al.(2020) Sphingosine-1-phosphate promotes pulmonary artery smooth muscle cells proliferation by stimulating autophagy-mediated E-cadherin/CDH1 down-regulation. *Eur J Pharmacol* 884(173302. 10.1016/j.ejphar.2020.173302.
24. W. Feng, J. Wang, X. Yan, C. Zhai, W. Shi, Q. Wang, Q. Zhang, M. Li(2019) Paclitaxel alleviates monocrotaline-induced pulmonary arterial hypertension via inhibition of FoxO1-mediated autophagy. *Naunyn Schmiedeberg's Arch Pharmacol* 392(5): 605-613. 10.1007/s00210-019-01615-4.
25. D.A. Fletcher, R.D. Mullins(2010) Cell mechanics and the cytoskeleton. *Nature* 463(7280): 485-92. 10.1038/nature08908.
26. M.C. Frame, H. Patel, B. Serrels, D. Lietha, M.J. Eck(2010) The FERM domain: organizing the structure and function of FAK. *Nat Rev Mol Cell Biol* 11(11): 802-14. 10.1038/nrm2996.
27. R. Paulin, J. Meloche, A. Courboulain, C. Lambert, A. Haromy, A. Courchesne, P. Bonnet, S. Provencher, E.D. Michelakis, S. Bonnet(2014) Targeting cell motility in pulmonary arterial hypertension. *Eur Respir J* 43(2): 531-44. 10.1183/09031936.00181312.
28. C. Sun, B. Enkhjargal, C. Reis, T. Zhang, Q. Zhu, K. Zhou, Z. Xie, L. Wu, J. Tang, X. Jiang, J.H. Zhang(2019) Osteopontin-Enhanced Autophagy Attenuates Early Brain Injury via FAK-ERK Pathway and Improves Long-Term Outcome after Subarachnoid Hemorrhage in Rats. *Cells* 8(9): 10.3390/cells8090980.
29. D. Zheng, M. Zhang, T. Liu, T. Zhou, A. Shen(2021) Osteoprotegerin prompts cardiomyocyte hypertrophy via autophagy inhibition mediated by FAK/BECLIN1 pathway. *Life Sci* 264(118550. 10.1016/j.lfs.2020.118550.
30. S. Zona, L. Bella, M.J. Burton, G. Nestal de Moraes, E.W. Lam(2014) FOXM1: an emerging master regulator of DNA damage response and genotoxic agent resistance. *Biochim Biophys Acta* 1839(11): 1316-22. 10.1016/j.bbarm.2014.09.016.
31. I. Wierstra(2013) The transcription factor FOXM1 (Forkhead box M1): proliferation-specific expression, transcription factor function, target genes, mouse models, and normal biological roles. *Adv Cancer Res* 118(97-398. 10.1016/b978-0-12-407173-5.00004-2.
32. Z. Dai, M.M. Zhu, Y. Peng, H. Jin, N. Machireddy, Z. Qian, X. Zhang, Y.Y. Zhao(2018) Endothelial and Smooth Muscle Cell Interaction via FoxM1 Signaling Mediates Vascular Remodeling and Pulmonary

Hypertension. Am J Respir Crit Care Med 198(6): 788-802. 10.1164/rccm.201709-1835OC.

33. Q. Zhang, W. Li, Y. Zhu, Q. Wang, C. Zhai, W. Shi, W. Feng, J. Wang, X. Yan, L. Chai, Y. Chen, C. Li, P. Liu, M. Li(2021) Activation of AMPK inhibits Galectin-3-induced pulmonary artery smooth muscle cells proliferation by upregulating hippo signaling effector YAP. Mol Cell Biochem 10.1007/s11010-021-04131-3.
34. Z. Hamurcu, N. Kahraman, A. Ashour, B. Ozpolat(2017) FOXM1 transcriptionally regulates expression of integrin β 1 in triple-negative breast cancer. Breast Cancer Research and Treatment 163(3): 485-493. 10.1007/s10549-017-4207-7.

Figures

Figure 1

Autophagy is activated in MCT-induced PAH model. **A** The protein level of LC3B in lung tissues from different groups was determined using immunoblotting. Representative western blot and quantification of bands are shown. (n = 4 each group). **B** Immunohistochemistry was used to detect the protein level of LC3B in the lung tissues from different groups; cells with brown-stained cytoplasm in the medial layer of pulmonary artery are LC3B-positive PSMCs. **C** The percentage of LC3B-positive PSMCs in different groups (25 arteries each slide, diameter 20-100 μ m). * P < 0.05 versus control group. # P < 0.05 versus MCT group.

Figure 2

Inhibition of autophagy prevents the increase of RVSP, RVH, and pulmonary arterial remodeling in MCT-induced PAH model. **A** Change of right ventricular systolic pressure (RVSP) in different groups of rats (n = 10-15). **B** Change of right ventricle hypertrophy index (RVHI) in different groups of rats (n = 10-15). **C** Hematoxylin and eosin staining of small pulmonary arteries in different groups (n = 10-15) (magnification $\times$ 400). **D** Quantitative analysis of the medial wall thickness of pulmonary arteries (n = 10-15). **E** The degree of muscularization of pulmonary arteries was evaluated by immunostaining of α -SMA. **F** Percentage of muscular, partially muscular and non-muscular arteries in different groups (25 arteries each slide, diameter 20–200 μ m). **G** PSMCs were stained for Ki67 by immunohistochemistry to assess cell proliferation; cells with brown-stained nuclei in the medial layer of pulmonary artery are Ki67-positive PSMCs. **H** The percentage of Ki67-positive PSMCs in different groups (25 arteries each slide, diameter 20–100 μ m). * P < 0.05 versus control group; # P < 0.05 versus MCT group.

Figure 3

FAK is activated in MCT-induced PAH model. The protein levels of p-FAK and FAK in lung tissues from different groups were determined using immunoblotting. Representative western blot and quantification of bands are shown. (n = 4 each group). * $P < 0.05$ versus control group. # $P < 0.05$ versus MCT group.

Figure 4

Inhibition of FAK prevents the increase of RVSP, RVH, and pulmonary arterial remodeling in MCT-induced PAH model. **A** Change of right ventricular systolic pressure (RVSP) in different groups of rats (n = 10-15). **B** Change of right ventricle hypertrophy index (RVHI) in different groups of rats (n = 10-15). **C** Hematoxylin and eosin staining of small pulmonary arteries in different groups (n = 10-15) (magnification $\times 400$). **D** Quantitative analysis of the medial wall thickness of pulmonary arteries (n = 10-15). **E** The degree of muscularization of pulmonary arteries was evaluated by immunostaining of α -SMA. **F** Percentage of muscular, partially muscular and non-muscular arteries in different groups (25 arteries each slide, diameter 20–200 μm). **G** PSMCs were stained for Ki67 by immunohistochemistry to assess cell proliferation; cells with brown-stained nuclei in the medial layer of pulmonary artery are Ki67-positive PSMCs. **H** The percentage of Ki67-positive PSMCs in different groups (25 arteries each slide, diameter 20–100 μm). * $P < 0.05$ versus control group; # $P < 0.05$ versus MCT group.

Figure 5

Inhibition of FAK prevents the activation of autophagy in MCT-induced PAH model. **A** The level of LC3B in lung tissues from different groups was determined using immunoblotting. Representative western blot and quantification of bands are shown. (n = 4 each group). **B** Immunohistochemistry was used to detect the protein level of LC3B in the lung tissues from different groups; cells with brown-stained cytoplasm in the medial layer of pulmonary artery are LC3B-positive PSMCs. **C** The percentage of LC3B-positive PSMCs in different groups (25 arteries each slide, diameter 20-100 μm). * $P < 0.05$ versus control group; # $P < 0.05$ versus MCT group.

Figure 6

The expression of FOXM1 is up-regulated in MCT-induced PAH model. The level of FOXM1 in lung tissues from different groups was determined using immunoblotting. Representative western blot and quantification of bands are shown. (n = 4 each group). * $P < 0.05$ versus control group; # $P < 0.05$ versus MCT group.

Figure 7

Inhibition of FOXM1 prevents the increase of RVSP, RVH, and pulmonary arterial remodeling in MCT-induced PAH model. **A** Change of right ventricular systolic pressure (RVSP) in different groups of rats (n = 10-15). **B** Change of right ventricle hypertrophy index (RVHI) in different groups of rats (n = 10-15). **C** Hematoxylin and eosin staining of small pulmonary arteries in different groups (n = 10-15) (magnification $\times 400$). **D** Quantitative analysis of the medial wall thickness of pulmonary arteries (n = 10-15). **E** The degree of muscularization of pulmonary arteries was evaluated by immunostaining of α -SMA. **F** Percentage of muscular, partially muscular and non-muscular arteries in different groups (25 arteries each slide, diameter 20–200 μm). **G** PASMCs were stained for Ki67 by immunohistochemistry to assess cell proliferation; cells with brown-stained nuclei in the medial layer of pulmonary artery are Ki67-positive PASMCs. **H** The percentage of Ki67-positive PASMCs in different groups (25 arteries each slide, diameter 20–100 μm). * $P < 0.05$ versus control group; # $P < 0.05$ versus MCT group.

Figure 8

FOXM1 mediates FAK and autophagy activation in MCT-induced PAH model. **A** The protein levels of p-FAK and FAK in lung tissues from different groups were determined using immunoblotting. Representative western blot and quantification of bands are shown. (n = 4 each group). **B** The level of LC3B in lung tissues from different groups was determined using immunoblotting. Representative western blot and quantification of bands are shown. (n = 4 each group). **C** Immunohistochemistry was used to detect the protein level of LC3B in the lung tissues from different groups; cells with brown-stained cytoplasm in the medial layer of pulmonary artery are LC3B-positive PASMCs. **D** The percentage of LC3B-positive PASMCs in different groups (25 arteries each slide, diameter 20-100 μm). * $P < 0.05$ versus control group; # $P < 0.05$ versus MCT group.