


Decrease in Tripartite Motif Containing 24 suppresses hypoxia-induced proliferation and migration of pulmonary arterial smooth muscle cells via the AKT/mammalian target of rapamycin complex 1 pathway

Jingwen Xu*, Yujia Zhong*, and Zhang Wang 

Department of Geriatrics, The General Hospital of Western Theater Command, Chengdu, P.R. China

ABSTRACT

Tripartite Motif Containing 24 (TRIM24) is an oncogenic protein and promotes proliferation in several cancer cell lines. Nevertheless, the role of TRIM24 in proliferation and migration of pulmonary artery smooth muscle cells (PASMCs) remains to be clarified. The current study was aimed to reveal the role of TRIM24 in proliferation and migration of PASMCs during the development of pulmonary arterial hypertension (PAH). In pulmonary arteries (PAs) of chronic hypoxia-PAH (CH-PAH) mice and PASMCs challenged with hypoxia, the expression of TRIM24 was increased. Silencing TRIM24 by *Trim24* short hair RNA (shTrim24) suppressed hypoxia-induced increase in Ki-67 positive PASMCs and wound-healing rate. Furthermore, hypoxia caused enhanced phosphorylation of AKT and two major effectors of mammalian target of rapamycin complex 1 (mTORC1), S6 and 4EBP1 in PASMCs. The enhancement was then attenuated after silencing TRIM24. Both restoring mTORC1 activity and AKT reactivation abolished silencing TRIM24-mediated inhibition of proliferation and migration of PASMCs. Additionally, AKT reactivation also reversed the declined phosphorylation of S6 and 4EBP1 induced by shTrim24. In conclusion, TRIM24 is involved in the excessive proliferation and migration of PASMCs after hypoxic stimulus during PAH. The mechanism of TRIM24-mediated regulation of PASMCs is partly illustrated by promoting activity of AKT/mTORC1 signaling pathway.

ARTICLE HISTORY

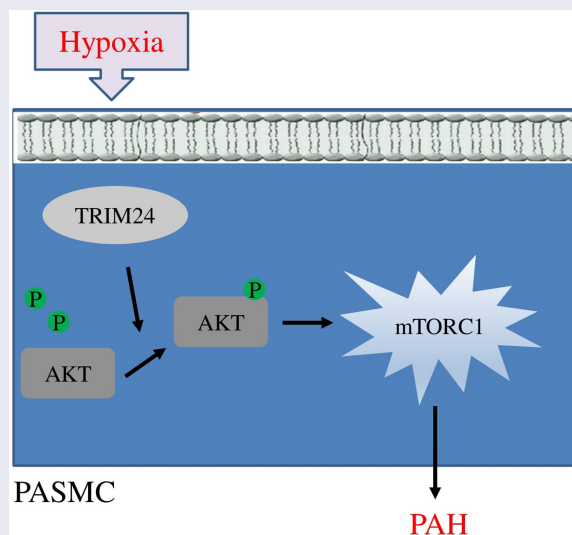
Received 18 April 2022


Revised 17 May 2022

Accepted 17 May 2022


KEYWORDS

TRIM24; pulmonary arterial hypertension; pulmonary artery smooth muscle cells; AKT; mTORC1



CONTACT Zhang Wang  wangzhanggbbf@126.com  Department of Geriatrics, The General Hospital of Western Theater Command, Chengdu, Sichuan 610083, China

*Jingwen Xu and Yujia Zhong are co-first authors.

 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/21655979.2022.2080423>.

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Highlights

- TRIM24 is increased in hypoxia-treated PSMCs and PAs of CH-PAH mice.
- Silencing TRIM24 suppresses hypoxia-induced proliferation and migration of PSMCs.
- The activation of AKT/mTORC1 is responsible for TRIM24-mediated regulation on PSMCs.

Introduction

Chronic pulmonary arterial hypertension (PAH) is a disease characterized by pathological pulmonary vascular remodeling and progressive pathological pulmonary artery (PA) contraction, resulting in high vascular resistance, right ventricular hypertrophy, and failure [1]. PAH is often complicated with chronic hypoxic diseases, such as interstitial lung disease, obstructive sleep apnea and chronic obstructive pulmonary disease [2]. Currently available drugs recovering the vasoconstrictive phenotype of PA mainly target vasoactive factors such as prostacyclin or endothelin-1. However, these drugs do not affect pulmonary vascular remodeling and thus fail to ultimately improve the outcomes of PAH patients [3]. Therefore, it is urgent to search new therapeutic strategies of PAH.

Pulmonary artery smooth muscle cells (PSMCs) are the main component of PA, thereby exerting a crucial role in physiological regulation of pulmonary vessel. Previous research demonstrates that chronic hypoxia-induced abnormal proliferation and migration of PSMCs is involved in the development of PAH and also associated with vascular stenosis and occlusion [4]. A variety of cellular and molecular mechanisms are involved in the modulation of PSMC proliferation and migration, including K^+ channel, Ca^{2+} channel, transforming growth factor- β receptors and membrane receptors, etc [5]. However, the precise events that are involved in the enhanced proliferation and migration of PSMC remain unclear.

Tripartite Motif Containing 24 (TRIM24), also called TIF1 α , belongs to the member of Transcription Intermediary Factor family. It consists of a terminal plant homeodomain (PHD)-bromodomain and a RING (E3 ubiquitin ligase) domain [6]. TRIM24 is abnormally expressed in

multiple tumors and thus functions as oncogenic factors [7,8]. The tumor-promoting effect of TRIM24 is associated with its pro-proliferative role in different cancer cells. Additionally, TRIM24 is also confirmed to exert certain role in cardiovascular events. For example, TRIM24 can promote cardiomyocyte hypertrophy via regulation of dysbindin protein [9]. However, the role of TRIM24 in proliferation, migration of PSMC and PAH remains unknown.

AKT (also known as protein kinase B)/mammalian target of rapamycin (mTOR) is an important intracellular transduction system regulating cell proliferation, differentiation, migration, and protein synthesis [5]. mTOR exerts its role by forming two complexes, mTOR complex (mTORC)1 and mTORC2. AKT inhibits a crucial indirect regulator of mTORC1, the tuberous sclerosis complex 1 (TSC1) to activate mTORC1 [10]. Excessive AKT/mTORC1 activation is implicated in enhanced proliferation and migration of PSMCs [11]. TRIM24 can enhance the phosphorylation of AKT to induce proliferation of glioma cell proliferation [12]. It is unclear whether AKT/mTORC1 acts downstream of TRIM24 in PSMCs.

We hypothesized that TRIM24 might exert a regulatory role in the development of PAH. In the current study, we found TRIM24 was increased in PAs of chronic hypoxia-pulmonary arterial hypertension (CH-PAH) mice and hypoxia-challenged PSMCs. Silencing TRIM24 inhibited proliferation and migration of PSMCs via suppressing AKT/mTORC1 activity. This study aimed to search novel target for preventing PAH.

Materials and methods

Animals

C57BL/6 J mice obtained from Dashuo Animal Science and Technology (Chengdu, Sichuan, China). Mice were housed at room temperature in a 12-h light–dark cycle and have free access to water and food. CH-PAH mice model were established by using SU5416-hypoxia as previously described [13]. Briefly, male mice at the age of 8–10 weeks were subcutaneously injected

with SU5416 (20 mg/kg; Sigma, St Louis, Missouri, USA) once a week and housed in a ventilated chamber with chronic hypoxia (10% O₂) for 3 weeks. Normoxic control mice were subcutaneously injected with equivalent DMSO and kept in room air. All animal experiments in the study were performed in accordance with the Institutional Animal Care and Use Committee and the Ethic Committee of The General Hospital of Western Theater Command (2021EC1-7; Chengdu, Sichuan, China). Mice were finally anesthetized with pentobarbital (100 mg/kg) and sacrificed for other experiments.

Detection of right ventricular systolic pressure (RVSP) and right ventricular hypertrophy index (RVHI)

RVSP and RVHI were measured as previously described [14]. Briefly, mice were anesthetized by intraperitoneal injection with pentobarbital (30 mg/kg). For determination of RVSP, a pressure transducer catheter (Millar Instruments, Houston, TX, USA) was inserted into the right ventricle. For RVHI, cardiac tissues of sacrificed mice were isolated and then the mass ratio of RV to left ventricle + septum was calculated as RVHI.

Immunoblotting

Immunoblotting was performed as previously described [11]. The lysates of PAs and PSMCs were collected after treatment of RIPA buffer (Beyotime Institute of Biotechnology, Shanghai, China). Protein lysates were separated by gel electrophoresis and transferred to nitrocellulose membranes (Beyotime). The membranes were then blocked and subjected to primary antibodies against TRIM24 (cat ab256491, 1: 2000; Abcam; Cambridge, England), p-AKT^{Thr308} (cat 13038S, 1: 1000; Cell Signaling Technology; Danvers, Massachusetts, USA), AKT (cat 9272S, 1: 1000; Cell Signaling Technology), p-S6^{Ser235/236} (cat 4858S, 1: 1000; Cell Signaling Technology), S6 (cat 14467S, 1: 1000; Cell Signaling Technology), p-4EBP1^{Thr37/46} (cat 2855S, 1: 1000; Cell Signaling Technology), 4EBP1 (cat 9644 T, 1: 1000; Cell

Signaling Technology), phosphoinositide 3-kinase (PI3K) p110 α (cat 4255S, 1: 1000; Cell Signaling Technology), cyclin D1 (cat 2978S, 1: 1000; Cell Signaling Technology) and GAPDH (cat 4970S, 1: 8000; Cell Signaling Technology) overnight at 4°C. The membranes were next incubated with secondary antibodies.

Cells

PASMCs were isolated and cultured as previously described [11]. Briefly, PAs were excised and kept in ice-cold PBS to remove their endothelium, adventitia, and connective tissues. The media were dissected, cut into small pieces and then incubated with Dulbecco's modified Eagle's medium (DMEM; HyClone, Carlsbad, California, USA) filled with 10% fetal calf serum (Invitrogen, Carlsbad, California, USA), penicillin (100 units/mL) and streptomycin (100 mg/mL) at 37°C in a humidified 5% CO₂ incubator. PSMCs between passages 3 and 5 was utilized for further experiments. Hypoxic PSMCs were incubated at 37°C under the condition of 3% O₂, 92% N₂ and 5% CO₂ for 6, 12 and 24 hours (h), while normoxic PSMCs were cultured at 37°C under the condition of 21% O₂, 74% N₂ and 5% CO₂ for the same time points. The adenovirus carrying *Trim24* short hairpin RNA (shTrim24) and control viruses (shCon) were constructed according to the manufacturer's instructions (Genechem; Shanghai, China). For transfection in PSMCs, cells were transfected with relative adenovirus (3 pfu/cell) for 72 hours. For siRNA transfection, PSMCs were incubated with the mixture of control siRNA or *Tsc1* siRNA (siTsc1) and 10 μ L X-tremeGENE siRNA transfection reagent for 8 h. Then, PSMCs were cultured for another 48 h before experiments. The sequence of siTsc1: GCUUUGACUCUCCCUUCUA [15]. SC-79 (10 μ M; MCE, Shanghai, China) was used to treat PSMCs for 24 h [16].

Immunofluorescence staining

Immunofluorescence staining of PSMC was performed as previously described [11]. PSMCs were cultured in a 6-well plate, fixed with 4% polyformaldehyde and then incubated with 0.5% triton X-100 T to break the membrane. After being

blocked by a blocking solution, PSMCs were next incubated in dark with a primary antibody against Ki-67 (1:1000; Cell Signaling Technology) at 4°C overnight. PSMCs were then washed by PBS and incubated with a secondary antibody in dark for 1 h. Finally, the nuclei were stained by using DAPI (5 mg/ml; VECTOR Labs, Burlingame, California, USA) at 25°C for 5 s. Images were obtained with an immunofluorescent microscopy (Leica MPS 60, Germany).

Wound-healing assay

Wound-healing assay was performed as a previous study described [15]. PSMCs were cultured on a 6-well plate and grown to 90% confluency. PSMCs were serum-deprived for 12 h and scratches were made by using a 10- μ L sterile pipette in the middle area of cells. PSMCs were then cultured under normoxic or hypoxic condition for 24 h. The images were taken in real time from 0 to 24 hours. The rates of wound closure were directly measured by using microscopic visualization and followed with a reference point at the bottom of the plate to capture the same spot every time.

Statistical analysis

Comparisons between two groups were conducted by Student's *t*-test. Comparisons between multiple groups were conducted by analysis of variance (ANOVA) with an appropriate *post hoc* test. $P < 0.05$ was considered to be statistically significant. All data are expressed as mean \pm standard deviation (SD).

Results

In our current study, we explored the role of TRIM24 in proliferation and migration of PSMCs by utilizing the adenovirus carrying shTrim24. We demonstrated that silencing Trim24 suppressed hypoxia-induced proliferation and migration in PSMCs in an AKT/mTORC1-dependent manner.

The protein expression levels of TRIM24 in CH-PAH mice and hypoxic PSMCs were increased

To investigate whether TRIM24 is implicated in the modulation of PAHs, we first determined the expression of TRIM24 in PAs of CH-PAH mice. The model of CH-PAH mice was confirmed by increased RVSP (Figure 1(a)) and RVHI (Figure 1(b)). We found that PAs in CH-PAH mice displayed significantly higher TRIM24 expression than PAs in control mice (Figure 1(c)). Hypoxia induces excessive proliferation and migration of PSMCs, thus accelerating the development of PAH [4]. We thus determined the expression of TRIM24 in hypoxia-challenged PSMCs. Our data showed that 6, 12, and 24 hours of hypoxia exposure both led to significantly increased expression of TRIM24 (Figure 1(d)). Therefore, upregulation of TRIM24 might play a crucial role in the regulation of abnormal proliferation, migration of PSMCs, and PAH.

Silencing TRIM24 inhibits hypoxia-induced proliferation and migration of PSMCs

To investigate the relationship of TRIM24 upregulation with increased proliferation and migration of PSMCs after hypoxic stimulus, we next used shTrim24 to silence the expression of TRIM24 and analyzed the changes of proliferation and migration of PSMCs. By using Ki-67 immunofluorescent staining, we found that 24 h-treatment with hypoxia significantly increased the number of Ki-67 (a marker of cell proliferation) positive PSMCs compared to normoxic treatment. However, the elevation of Ki-67 positive cell number was obviously reversed after silencing TRIM24 (Figure 2(a)). Subsequent experiments were performed to investigate whether the capacity of migration was also influenced by TRIM24 in PSMCs. In the current study, PSMC migration was analyzed by *in vitro* scratch assay. Hypoxia treatment accelerated the rate of wound-healing compared to normoxic condition. Simultaneously, the increased wound-healing rate of PSMCs was slowed down once TRIM24 was silenced (Figure 2(b)). These data indicate that downregulation of TRIM24 can effectively suppress

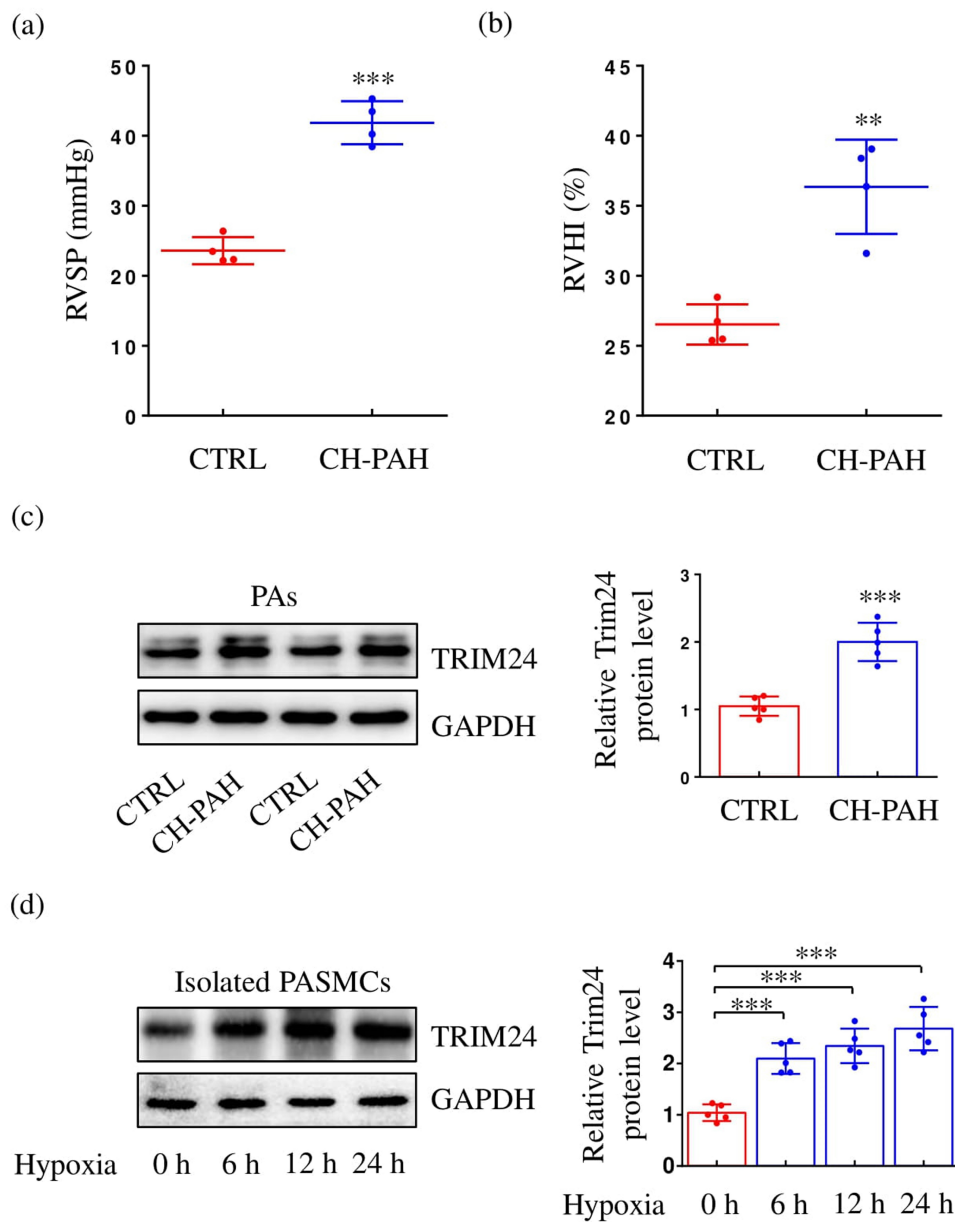


Figure 1. The expression of TRIM24 is increased in pulmonary arteries (PAs) of chronic hypoxia-pulmonary arterial hypertension (CH-PAH) mice and hypoxia-treated PSMCs. RVSP (a) and RVHI (b) of CH-PAH mice were analyzed ($n = 4$). RVSP: right ventricular systolic pressure. RVHI: right ventricular hypertrophy index. (c) The relative protein level of TRIM24 in dissected PAs of CH-PAH mice was measured by immunoblotting ($n = 5$). (d) The relative protein level of TRIM24 in PSMCs exposed to hypoxic conditions for different time courses was measured by immunoblotting ($n = 5$). Data are expressed as mean \pm SD. ** and *** indicate a significant difference of $P < 0.01$ and $P < 0.001$ between the two marked groups, respectively.

hyperproliferation and hypermigration in PSMCs after hypoxic stimulus.

Silencing TRIM24 inhibits proliferation and migration of PSMCs via reducing mTORC1 activity

We next tried to reveal the molecular mechanism by which TRIM24 promoted proliferation and

migration of PSMCs. It was shown that both excessive proliferation and migration of PSMCs and the development of PAH were associated with a significant increase in mTORC1 activity [11]. Whether TRIM24 regulates the activity of mTORC1 in PSMCs is unknown.

S6 and 4EBP1 are two major downstream effectors of mTORC1. Therefore, the phosphorylation levels of the two proteins reflect the activity

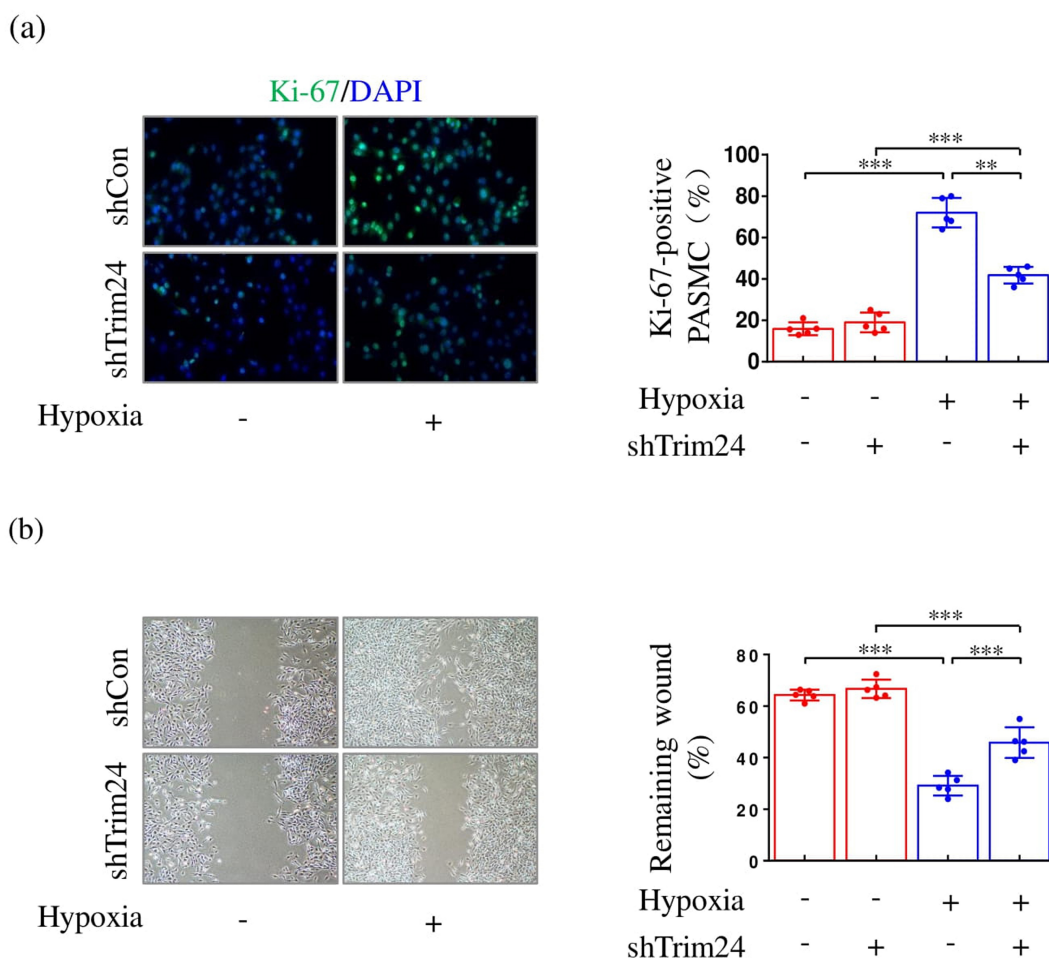


Figure 2. Silencing TRIM24 inhibits hypoxia-induced proliferation and migration of PASCs. PASCs were transfected with the adenovirus carrying *Trim24* shRNA (shTrim24) or control virus (shCon) and incubated under normoxic/hypoxic conditions for 24 h. (a) Ki67 (green) and DAPI (blue) staining was performed by immunofluorescence staining (n = 5). Magnification 400 × . (b) Cell migration was analyzed by wound healing assay (n = 5). Magnification 100 × . Data are expressed as mean ± SD. ** and *** indicate a significant difference of P < 0.01 and P < 0.001 between the two marked groups, respectively.

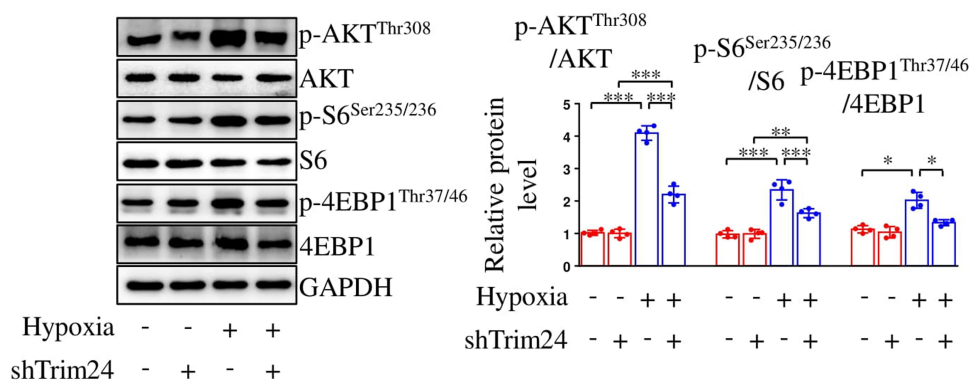


Figure 3. Silencing TRIM24 suppresses hypoxia-induced phosphorylation of AKT, S6 and 4EBP1 in PASCs. PASCs were transfected with shTrim24 or shCon and incubated under normoxic/hypoxic conditions for 24 h. The relative protein levels of p-AKT^{Thr308}, AKT, p-S6^{Ser235/236}, S6, p-4EBP1^{Thr37/46} and 4EBP1 in PASCs were analyzed by using immunoblotting (n = 4). Data are expressed as mean ± SD. *, ** and *** indicate a significant difference of P < 0.05, P < 0.01 and P < 0.001 between the two marked groups, respectively.

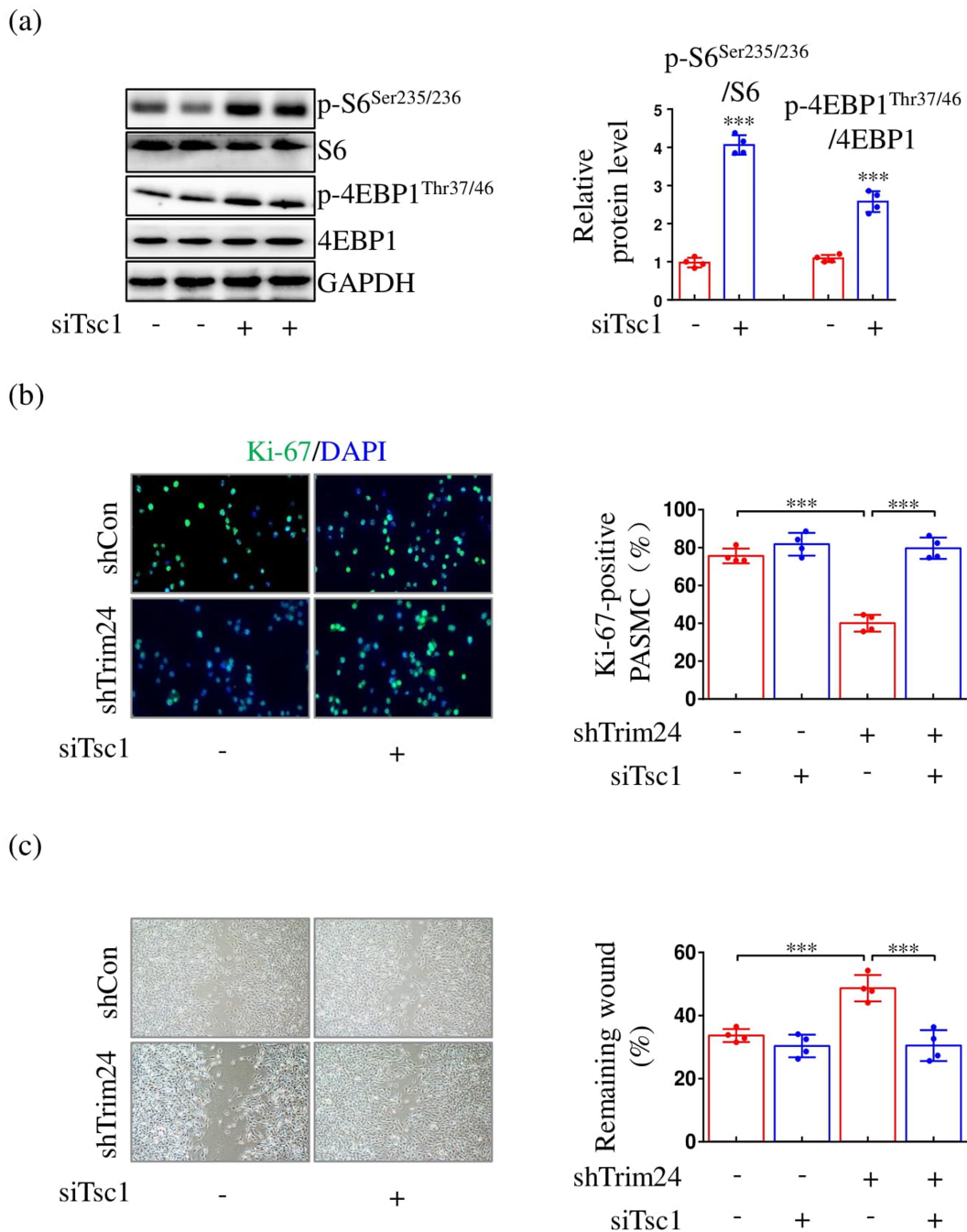


Figure 4. Restoring mTORC1 activity abolishes shTrim24-induced decrease in proliferation and migration of PASCs exposed to hypoxia. PASCs were firstly treated with incubated with siCon or siTsc1 for 8 h. Then, cells were transfected with shTrim24 or shCon and cultured under normoxic/hypoxic conditions for 24 h. (a) The protein expression levels of p-S6^{Ser235/236}, S6, p-4EBP1^{Thr37/46} and 4EBP1 in PASCs transfected with siCon or siTsc1 were evaluated by immunoblotting. Representative bands (left panel) and corresponding quantification (right panel) were shown (n = 4). (b) Ki67 (green) and DAPI (blue) staining was performed by immunofluorescence staining (n = 4). Magnification 400 ×. (c) Cell migration was analyzed by wound healing assay (n = 4). Magnification 100 ×. Data are expressed as mean ± SD. *** indicates a significant difference of P < 0.001 between the two marked groups.

of mTORC1. As shown in our data, the phosphorylation levels of S6 and 4EBP1 in PASCs were both increased after hypoxic stimulus (Figure 3). Moreover, the accelerated

phosphorylation of S6 and 4EBP1 induced by hypoxia was partly rescued after shTrim24 transfection (Figure 3). To investigate whether increased mTORC1 activity was associated with

excessive proliferation and migration of PASCs, we utilized siRNA of a classical gene inhibiting mTORC1 activity, tuberous sclerosis complex (siTsc1) to recover the reduced mTORC1 activity in hypoxia-challenged PASCs (Figure 4(a)). The results demonstrated that the decrease in the proliferation and migration of TRIM24-silenced PASCs reversed when the cells were transfected with siTsc1 (Figure 4(b,c)).

Silencing TRIM24 suppresses mTORC1 activity in PASCs by inhibiting AKT phosphorylation

We next identified the signaling pathways that underlie mTORC1 inactivation in PASCs after shTrim24 transfection. Serving as a sensor for cellular stress and energy metabolism, mTORC1 is affected by various of stress-associated kinases such as PI3K/AKT [17]. TRIM24 is proved to be an activator for AKT activation in several cancer cell lines [12,18]. Whether TRIM24 activates AKT to increase mTORC1 activity in PASCs is unrevealed.

As illustrated in Figure 3, hypoxia enhanced the phosphorylation of AKT at site Thr308 in PASCs, while silence of TRIM24 abolished the increase in AKT phosphorylation (Figure 3). Next, we utilized an agonist of AKT, SC-79 to rescue the declined activity of AKT induced by shTrim24. We found that silencing TRIM24 failed to attenuate the phosphorylation of S6 and 4EBP1 in PASCs after application of SC-79 (Figure 5). However, silencing TRIM24 did not affect the expression of PI3K p110 α in PASCs (Supplementary Figure S1),

indicating PI3K was not involved in TRIM24-mediated regulation of mTORC1. These data identify that TRIM24 silence-induced mTORC1 inactivation in PASCs is mediated by AKT dephosphorylation.

AKT/mTORC1 act downstream of TRIM24 to induce proliferation and migration of PASCs

Published observations confirm the important role of AKT/mTORC1 axis in proliferation and migration of PASCs [5]. Since restoring AKT activity abolished shTrim24-mediated suppression of mTORC1 activity, we investigated whether AKT inhibition also impaired shTrim24-mediated regulation on proliferation and migration of PASCs. The results showed that the proportion of Ki-67 positive PASCs was significantly reduced in hypoxic-challenged PASCs that was transfected with shTrim24 compared to shCon. Simultaneously, AKT reactivation by SC-79 significantly attenuated the decrease in the number of Ki-67 positive PASCs induced by silencing TRIM24 (Figure 6(a)). We also determined the expression of cyclin D1, a cell cycle regulator downstream of AKT in PASCs [19]. We found that silencing TRIM24 inhibited the expression of cyclin D1 in hypoxia-challenged PASCs, which was reversed by SC-79 (Supplementary Figure S2). Decreased migration of TRIM24-silenced PASCs was also demonstrated by wound healing assay. However, the attenuated capacity of cell migration induced by TRIM24 inhibition was also reversed

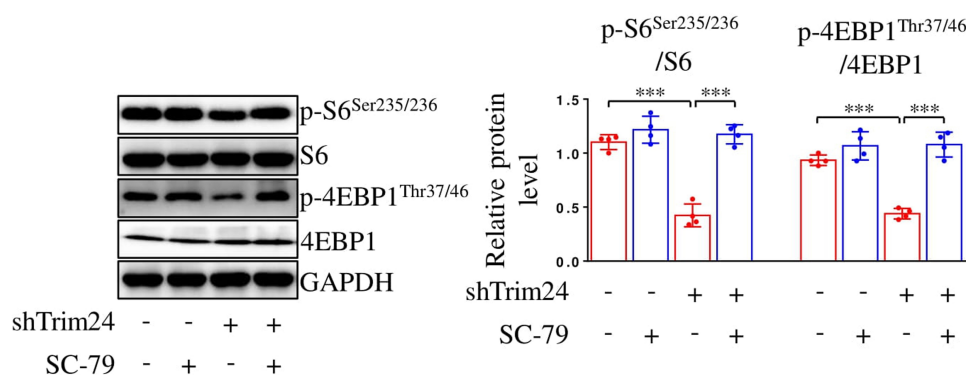


Figure 5. TRIM24-mediated regulation of mTORC1 in PASCs depends on AKT. PASCs were firstly incubated with SC-79 (AKT agonist; 10 μ M) or equivalent vehicle for 24 h. Then, cells were transfected with shTrim24 or shCon and cultured under hypoxic condition for another 24 h. Images of immunoblotting and relative expression levels of p-S6^{Ser235/236}, S6, p-4EBP1^{Thr37/46} and 4EBP1 in PASCs were shown (n = 4). Data are expressed as mean \pm SD. *** indicates a significant difference of P < 0.001 between the two marked groups.

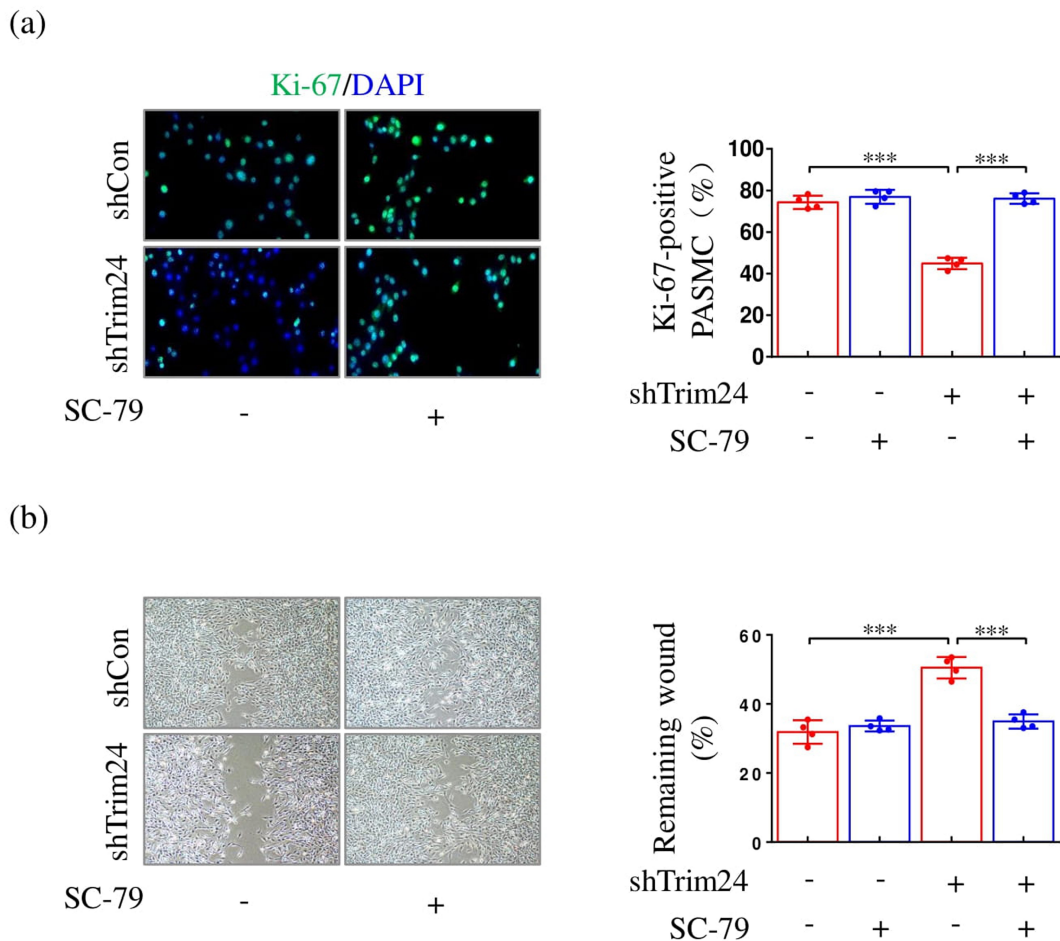


Figure 6. AKT activation reverses shTrim24-induced decrease in proliferation and migration of hypoxia-challenged PSMCs. PSMCs were firstly incubated with SC-79 (AKT agonist; 10 μ M) or equivalent vehicle for 24 h. Then, cells were transfected with shTrim24 or shCon and cultured under hypoxic condition for another 24 h. (a) Ki67 (green) and DAPI (blue) staining was performed by immunofluorescence staining (n = 4). Magnification 400 \times . (b) Cell migration was analyzed by wound healing assay (n = 4). Magnification 100 \times . Data are expressed as mean \pm SD. *** indicates a significant difference of P < 0.001 between the two marked groups.

after SC-79 utilization (Figure 6(b)). Taken together, these findings suggest that AKT dephosphorylation-mediated mTORC1 inactivation by silencing TRIM24 suppresses excessive proliferation and migration of PSMCs.

Discussion

The current study was aimed to reveal the regulatory role of TRIM24 in the proliferation and migration of PSMCs and the underlying mechanism. The protein level of TRIM24 was elevated in both PAs of CH-PAH mice and PSMCs after hypoxic treatment. Silencing TRIM24 *in vitro* reduced proliferation and migration of PSMCs challenged

with hypoxia. In PSMCs, TRIM24 inhibition reduced AKT phosphorylation and mTORC1 activity. AKT reactivation and restoring mTORC1 activity both reversed silencing TRIM24-mediated regulation of PSMCs, which provided novel insight into the regulatory role and mechanism of TRIM24 in proliferation and migration of PSMCs and revealed a potential therapeutic target for PAH.

TRIM24 is a member of the transcription intermediary factor family and plays important roles in T cell differentiation, innate immune regulation, and tumor development [20]. TRIM24 is also reported to be also implicated in cardiovascular regulation, such as cardiomyocyte hypertrophy [9]. We found the expression

of TRIM24 was significantly increased in PAs of CH-PAH mice, reminding us that TRIM24 might play a certain role in the regulation of PAH. Hypoxia-associated abnormal proliferation and migration of PSMCs is an crucial step of pathological vascular remodeling during PAH [1]. Therefore, we also confirmed the expression of TRIM24 in hypoxia-treated PSMCs and observed increased TRIM24 after hypoxic stimulus. In accordance with other cancer cell lines [7,8], silencing TRIM24 by shTrim24 also attenuated hypoxia-induced proliferation and migration of PSMCs. These results showed that TRIM24 might partly illustrate why proliferation and migration of PSMCs was enhanced during the development of PAH. Nevertheless, the exact downstream biological mechanism of TRIM24 remains unclear.

mTORC1 is a crucial serine/threonine kinase and implements regulatory roles in cell growth, proliferation, and differentiation, etc [5]. mTORC1 has also been established as a major mechanism for the development of PAH [5]. For example, mTORC1 over-activation is associated with endothelial dysfunction [21], pathological PSMC proliferation [11], and PSMC-endothelial cell communication [22], thus accelerating the progression of PAH. TRIM24 leads to mTORC1 activation in mouse mammary epithelia [23]. Consistently, we found that silencing TRIM24 also significantly inhibited mTORC1 activity in hypoxic-challenged PSMCs. The inhibitory roles of shTrim24 in proliferation and migration of PSMCs were abolished after restoring mTORC1 activity. Therefore, mTORC1 is essential for the suppressive effects of silencing TRIM24 on proliferation and migration of PSMCs.

The PI3K/AKT is a classical signaling pathway that regulates proliferation, migration of PSMCs and PAH [24]. mTORC1 is a major downstream effector of PI3K/AKT pathway. AKT positively regulates mTORC1 activity via affecting its many kinases, such as suppressing TSC1/2 [10] or promoting phosphorylation of PRAS40 [25]. Our current study showed that AKT phosphorylation at site Thr308 in PSMCs was also obviously decreased by silencing TRIM24. Additionally, shTrim24-induced declined phosphorylation of

S6 and 4EBP1 was also rescued after AKT reactivation. Therefore, we proposed that AKT might also act downstream of TRIM24 to activate mTORC1 in PSMCs. Consistent with our hypothesis, the attenuated proliferation and migration of PSMCs induced by silencing TRIM24 was also reversed after reactivation of AKT. We thus confirmed that TRIM24 promoted hypoxia-induced proliferation and migration of PSMCs via enhancing AKT/mTORC1 activity.

The current study also has some limitations. For example, how TRIM24 affects AKT phosphorylation is still unclear. In addition, though we have confirmed the role of TRIM24/AKT/mTORC1 axis in proliferation and migration of PSMCs, whether this signaling pathway works in humans remains unknown. These doubts need further investigation in our future study.

Conclusion

In summary, our findings identify TRIM24 as a potential target for the prevention and treatment of PAH. We first demonstrated that TRIM24 was increased in hypoxia-challenged PSMCs and PAs of CH-PAH mice. Decrease in TRIM24 abolished hypoxia-associated proliferation and migration of PSMCs, indicating hypoxia-induced upregulation of TRIM24 was responsible for excessive proliferation and migration of PSMCs. Next, we found mTORC1 signaling was activated by TRIM24 and acted downstream of TRIM24 to promote proliferation and migration of PSMCs. Our data also revealed a mechanism by which TRIM24 activated mTORC1 in PSMCs through phosphorylating AKT. The newly elucidated roles of TRIM24 in PSMCs also help us better understand the pathological mechanisms of PAH development.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The work was supported by the grant from the Logistics support research project in China (grant no. 18BJZ16) to Zhang Wang.

ORCIDZhang Wang  <http://orcid.org/0000-0003-1951-7432>**References**

- [1] Morrell NW, Adnot S, Archer SL, et al. Cellular and molecular basis of pulmonary arterial hypertension. *J Am Coll Cardiol*. 2009;54(1 Suppl):S20–S31.
- [2] Simonneau G, Montani D, Celermajer DS, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J*. 2019;53(1):1801913.
- [3] Lajoie AC, Lauziere G, Lega JC, et al. Combination therapy versus monotherapy for pulmonary arterial hypertension: a meta-analysis. *Lancet Respir Med*. 2016;4(4):291–305.
- [4] Humbert M, Sitbon O, Chaouat A, et al. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation*. 2010;122(2):156–163.
- [5] Babicheva A, Makino A, Yuan JX. mTOR signaling in pulmonary vascular disease: pathogenic role and therapeutic target. *Int J Mol Sci*. 2021;22(4):2144.
- [6] Hatakeyama S. TRIM proteins and cancer. *Nat Rev Cancer*. 2011;11(11):792–804.
- [7] Lv D, Li Y, Zhang W, et al. TRIM24 is an oncogenic transcriptional co-activator of STAT3 in glioblastoma. *Nat Commun*. 2017;8(1):1454.
- [8] Groner AC, Cato L, de Tribolet-Hardy J, et al. TRIM24 is an oncogenic transcriptional activator in prostate cancer. *Cancer Cell*. 2016;29(6):846–858.
- [9] Borlepawar A, Rangrez AY, Bernt A, et al. TRIM24 protein promotes and TRIM32 protein inhibits cardiomyocyte hypertrophy via regulation of dysbindin protein levels. *J Biol Chem*. 2017;292(24):10180–10196.
- [10] Zheng M, Zang S, Xie L, et al. Rheb phosphorylation is involved in p38-regulated/activated protein kinase-mediated tumor suppression in liver cancer. *Oncol Lett*. 2015;10(3):1655–1661.
- [11] Hu S, Zhao Y, Qiu C, et al. RAS protein activator like 2 promotes the proliferation and migration of pulmonary artery smooth muscle cell through AKT/mammalian target of rapamycin complex 1 pathway in pulmonary hypertension. *Bioengineered*. 2022;13(2):3516–3526.
- [12] Lv D, Jia F, Hou Y, et al. Histone acetyltransferase KAT6A upregulates PI3K/AKT signaling through TRIM24 binding. *Cancer Res*. 2017;77(22):6190–6201.
- [13] Ciucan L, Bonneau O, Hussey M, et al. A novel murine model of severe pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2011;184(10):1171–1182.
- [14] Wan N, Rong W, Zhu W, et al. Tregs-derived interleukin 35 attenuates endothelial proliferation through STAT1 in pulmonary hypertension. *Ann Transl Med*. 2021;9(11):926.
- [15] Gu M, Wang Z, Feng F, et al. Inhibition of PIKfyve ameliorates the proliferation, migration of vascular smooth muscle cells and vascular intima hyperplasia via reducing mTORC1 activity. *J Cardiovasc Pharmacol*. 2022;79(5):739–748.
- [16] Zhou H, Ning Y, Zeng G, et al. Curcumin promotes cell cycle arrest and apoptosis of acute myeloid leukemia cells by inactivating AKT. *Oncol Rep*. 2021;45(4):11.
- [17] Wang Y, Bai Y, Qin L, et al. Interferon-gamma induces human vascular smooth muscle cell proliferation and intimal expansion by phosphatidylinositol 3-kinase dependent mammalian target of rapamycin raptor complex 1 activation. *Circ Res*. 2007;101(6):560–569.
- [18] Zhang LH, Yin AA, Cheng JX, et al. TRIM24 promotes glioma progression and enhances chemoresistance through activation of the PI3K/Akt signaling pathway. *Oncogene*. 2015;34(5):600–610.
- [19] Wang J, Wu J, Zhu X, et al. Absence of the MFG-E8 gene prevents hypoxia-induced pulmonary hypertension in mice. *J Cell Physiol*. 2021;236(1):587–600.
- [20] Yu T, Gan S, Zhu Q, et al. Modulation of M2 macrophage polarization by the crosstalk between Stat6 and Trim24. *Nat Commun*. 2019;10(1):4353.
- [21] Omura J, Satoh K, Kikuchi N, et al. Protective roles of endothelial AMP-activated protein kinase against hypoxia-induced pulmonary hypertension in mice. *Circ Res*. 2016;119(2):197–209.
- [22] Wang Z, Yang K, Zheng Q, et al. Divergent changes of p53 in pulmonary arterial endothelial and smooth muscle cells involved in the development of pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2019;316(1):L216–L228.
- [23] Shah VV, Duncan AD, Jiang S, et al. Mammary-specific expression of Trim24 establishes a mouse model of human metaplastic breast cancer. *Nat Commun*. 2021;12(1):5389.
- [24] Tang H, Chen J, Fraidenburg DR, et al. Deficiency of Akt1, but not Akt2, attenuates the development of pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2015;308(2):L208–220.
- [25] Wiza C, Nascimento EB, Ouwens DM. Role of PRAS40 in Akt and mTOR signaling in health and disease. *Am J Physiol Endocrinol Metab*. 2012;302(12):E1453–1460.