The proliferation of pulmonary artery smooth muscle cells (PASMCs) is an important cause of pulmonary vascular remodeling in pulmonary hypertension (PH). It has been reported that miR-137 inhibits the proliferation of tumor cells. However, whether miR-137 is involved in PH remains unclear. In this study, male Sprague-Dawley rats were subjected to 10% O₂ for 3 weeks to establish PH, and rat primary PASMCs were treated with hypoxia (3% O₂) for 48 h to induce cell proliferation. The effect of miR-137 on PASMC proliferation and calpain-2 expression was assessed by transfecting miR-137 mimic and inhibitor. The effect of calpain-2 on PASMC proliferation was assessed by transfecting calpain-2 siRNA. The present study found for the first time that miR-137 was downregulated in pulmonary arteries of hypoxic PH rats and in hypoxia-treated PASMCs. miR-137 mimic inhibited hypoxia-induced PASMC proliferation and upregulation of calpain-2 expression in PASMCs. Furthermore, miR-137 inhibitor induced the proliferation of PASMCs under normoxia, and knockdown of calpain-2 mRNA by siRNA significantly inhibited hypoxia-induced proliferation of PASMCs. Our study demonstrated that hypoxia-induced downregulation of miR-137 expression promoted the proliferation of PASMCs by targeting calpain-2, thereby potentially resulting in pulmonary vascular remodeling in hypoxic PH.

1. Introduction

Pulmonary hypertension (PH) is a rare vascular disorder, now defined clinically as a mean pulmonary artery pressure (mPAP) over 25 mmHg at rest or over 30 mmHg during activity. Pulmonary vascular remodeling plays an important role in PH pathology, which is mainly characterized by endothelial cell injury, smooth muscle cell proliferation, fibroblast muscularization, extracellular matrix increase, in situ thrombosis, varying degree inflammation, and plexiform arterial changes [1, 2]. In these pathological changes, the proliferation of pulmonary arterial smooth muscle cells (PASMCs) is the most important cause of pulmonary vascular remodeling in PH. Therefore, inhibition of PASMC proliferation is expected to be a crucial pathway for PH treatment.

Calpain is a Ca²⁺-dependent cysteine protease that has been found to contain at least 15 subtypes, calpain-1 (μ-calpain) and calpain-2 (m-calpain), which are the two best-characterized members of the calpain family and are ubiquitously expressed in mammals [3]. Calpain-1 and calpain-2 constitute a distinct larger catalytic subunit, and calpain-4 as a common smaller subunit is responsible for maintaining calpain activity [4]. Recent studies have linked calpain with a variety of diseases,
such as Alzheimer’s and Parkinson’s diseases, cancer, diabetes, atherosclerosis, and PH [5]. In hypoxia and monocrotaline-induced PH of mice and rats, the expression of calpain-1/2/4 in the lung tissues and pulmonary arteries was significantly increased [6–8]. Research focusing on the role of calpain-2 in hypoxia-induced PH becomes a meaningful work.

It has been reported that a variety of miRNAs participate in the pathogenesis of PH. For example, miR-223 [9] and miR-let-7g [10] have been found to regulate the proliferation of PASMCs participating in pulmonary vascular remodeling of PH. To fully reveal the role of miRNAs in hypoxic PH, we did the pilot microarray assay in pulmonary arteries of hypoxic PH rats and found that the expression of miR-137 was significantly downregulated. It has been reported that miR-137 inhibits the proliferation and migration of a variety of tumor cells [11, 12]. Over 1000 genes have been predicted to be targets of miR-137 by using a bioinformatic approach, and highlighted target genes are involved in a large number of pathways including neural development, cell cycle, differentiation, and proliferation [13]. However, whether miR-137 is involved in PH remains unclear. Bioinformatic analysis suggests that the 3′-UTR of calpain-2 contains a potential binding element for miR-137 with a 7-nt match to the miR-137 seed region, and miR-137 has been found to directly target calpain-2 in motoneurons [14]. We therefore hypothesize that miR-137 contributes to hypoxic PH by targeting calpain-2 and designed this study to explore the regulatory role of miR-137 in hypoxia-induced PASMC proliferation and pulmonary arterial remodeling in rat hypoxic PH, and the regulating effect of miR-137 on calpain-2 expression was also certified.

2. Materials and Methods

2.1. Animal Experiments. About 180-220 g, male Sprague-Dawley (SD) rats were purchased from the Laboratory Animal Center of Xiangya School of Medicine, Central South University, Changsha, China (SCXK (XIANG) 2019-0014). All protocols of animal experiments (No. CSU2017009) were approved by the Central South University Veterinary Medicine Animal Care and Use Committee. Regarding the methodology, we followed the PH preclinical guidelines as previously described [15].

SD rats were randomly divided into hypoxia group and control group. Rats were exposed to continuity hypoxia (10% O2) for up to 21 days in the hypoxia group while maintained in normal oxygen condition (21% O2) in the control group. At the 21 days after subjected to hypoxia, the rats were weighed and anesthetized by intraperitoneal injection of 2% sodium pentobarbital (60 mg/kg). A Vevo 2100 (VisualSonics, Canada) ultrasound system equipped with 21 MHz probe was used for echocardiographic assessment of pulmonary arterial acceleration/ejection time ratio (PAAT/PAET). Right-sided heart catheterization was conducted to detect right ventricular systolic pressure (RVSP) and mPAP. The right ventricle (RV) was separated from left ventricle and septum (LV+S) and weighed. The ratio of RV to (LV+S) was calculated to assess the extent of right ventricle hypertrophy. The pulmonary arterial samples were collected for mRNA and protein expression analysis. The right lower lung was fixed in 4% paraformaldehyde for hematoxylin-eosin (HE) staining and in situ hybridization analysis of miR-137.

2.2. HE Staining. For HE staining, the fixed lungs were embedded in paraffin and then cut into approximately 5 μm thick sections by microtome. HE staining of right lung was conducted in accordance to the same method used in our previous study [6].

2.3. In Situ Hybridization. In situ hybridization kit (Boster, Wuhan, China) was used to detect the expression of miR-137 in lung tissues according to the manufacturer’s instructions. In brief, 5μm sections were used for sodium citrate antigen retrieval and then incubated with blocking buffer overnight with miR-137 detection probe which was labeled with 3′ and 5′ digoxigenin. After washed with phosphate-buffered saline (PBS) and SSC buffer, immunodetection was performed with a biotinylated anti-DIG antibody at 37°C for 60 min and the avidin-biotin-peroxidase complex (ABC kit, Vector Laboratories, Burlingame, CA) at 37°C for 20 min. After washed with PBS, the slides were detected by 3,3-diamino benzidine (DAB) staining.

2.4. Preparation of Primary Rat PASMCs. As our previous study described, primary rat PASMCs were extracted from the pulmonary arteries using tissue block anchorage method [10]. Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 20% (v/v) fetal bovine serum was used to culture primary rat PASMCs at 37°C in a humidified atmosphere of 5% CO2. Smooth muscle α-actin (α-SMA) immunohistochemistry and immunofluorescence using anti-rat α-SMA antibody (1 : 50, ab7817, Abcam) were used to identify PASMCs. The three to five passages of PASMCs were used for all experiments.

2.5. Cell Transfection. PASMCs reached 60% to 70% of confluence were starved with low serum sputum (2% FBS) for 24h. To validate the effects of miR-137 and calpain-2 on hypoxia-induced PASMC proliferation and gene expression, the mimic and inhibitor of miR-137 and calpain-2 siRNA (Ribobio Co. Ltd., Guangzhou, China) were transiently transfected by ribo FECT™ CP transfection kit (Ribobio Co. Ltd., Guangzhou, China) according to the manufacturer’s instructions. Then, the cells were maintained in hypoxia (3% O2) or normoxia chamber for up to 48h according to grouping. Quantitative real-time polymerase chain reaction was used to detect the transfection efficiency of miR-137 mimic. Real-time PCR and Western blot were used to test the expression of calpain-2 mRNA and protein to detect the transfection efficiency of calpain-2 siRNA. The target sequences of calpain-2 siRNAs were CCAATT TGTTCGAAGATCAT.

2.6. Assay of Cell Proliferation. For the MTS assay as described previously [10], PASMCs were seeded in 96-well culture plates (6 × 103 cells/well) and then starved with low serum sputum (2% FBS) for 24h. After treatment, the cells were washed with PBS. According to the manufacturer’s
2.9. Western Blot Analysis. Proteins were extracted from cultured PASMCs and pulmonary arteries with RIPA buffer (contain 1% PMSF) for 30 min on ice and quantified by BCA kit (P0010, Beyotime, China). About 20–60 μg protein of each sample was separated by 10% SDS-polyacrylamide gels and transferred onto PVDF membranes. Membranes were blocked with 5% skim milk for 1 h and then incubated with primary antibodies for calpain-2 (ab39165, Abcam, 1:1000), PCNA (A0264, Abclonal, 1:1000), and β-actin (AF0003, Beyotime, 1:1000) and subsequently incubated with horseradish peroxidase- (HRP-) coupled goat anti-rabbit (A0208, Beyotime, 1:1000) and HRP-coupled goat anti-mouse (A0216, Beyotime, 1:1000). The chemiluminescence signals were visualized with the LuminaTM Crescendo substrate (WBLUR0100, Millipore). The densitometric analysis was conducted with ChemiDoc XR® system (Bio-Rad Co. Ltd., USA).

2.10. Luciferase Assay. The 3′-UTR of calpain-2 mRNA with putative/mutant miR-137 binding site was cloned into the firefly luciferase reporter construct pmiR-RB-ReportTM Vector (Ribobio, Guangzhou, China). Firefly luciferase (Luc) acts as a control, and renilla luciferase (Rluc) acts as a reporter. For the reporter assay, PASMCs grown in 96-well plates were cotransfected with calpain-2-3′-UTR-Luc (2μg) and miR-137 mimic (50nM) by ribo FECT™ transfection kit. Dual-Luciferase® Reporter Assay System (E1910, Promega) was used to detect the renilla and firefly luciferase activities after incubation for 48h.

2.11. Statistics. Data were shown as mean ± S.E.M. (standard errors). Statistical analysis was performed by the permutation test when the sample size is only 3 and by Student’s t-test for two groups or by one-way ANOVA followed by Student-Newman-Keuls test for multiple groups when the sample size is greater than 3. A value of p less than 0.05 was considered to be statistically significant. All statistical analyses were performed by the SPSS18.0 software, and GraphPad Prism 7 was used for drawing figures.

3. Results

3.1. miR-137 Was Downregulated in Remodeled Pulmonary Arteries and Hypoxia-Treated PASMCs in Hypoxic PH. To induce hypoxic PH, the rats were exposed to hypoxia (10% O₂) for 21 days. As keeping with our previous study [10], PAAT/PAET (Figure 1(a)) was markedly decreased in the hypoxia group; meanwhile, mPAP (Figure 1(b)), RVSP (Figure 1(c)), and the right heart remodeling index RV/(LV+S) (Figure 1(d)) were significantly increased in the hypoxia group. The body weight of hypoxic PH rats was decreased compared with the control group (Figure 1(e)). HE staining demonstrated that hypoxia induced obvious thickening of the pulmonary vascular wall and the stenosis of the lumen (Figure 1(f)).

According to our pilot study based on the microarray assay (mentioned in Introduction), the expression of miR-137 was measured in pulmonary arteries and PASMCs. As shown in Figures 1(g) and 1(h), hypoxia significantly
FIGURE 1: Continued.
downregulated the expression of miR-137 in pulmonary arteries of hypoxic PH rats. As expected, PASMCs exposed to 3% O₂ for different times (6 h, 12 h, 24 h, 48 h, and 72 h) showed significant proliferation in a time-dependent manner (Figures 1(i) and 1(j)). With the proliferation of hypoxia-induced PASMCs, hypoxia also significantly downregulated the expression of miR-137 in PASMCs (Figure 1(k)).

3.2. miR-137 Inhibited Hypoxia-Induced Proliferation of PASMCs. As mentioned above, miR-137 regulates the proliferation of a variety of tumor cells [11, 12]. We therefore explored the regulatory effect of miR-137 on hypoxia-induced proliferation of PASMCs by transfecting the mimic of miR-137. The results demonstrated that the transfection of miR-137 mimic significantly increased the expression of miR-137 (Figure 2(a)) and remarkably relieved hypoxia-induced proliferation of PASMCs (Figures 2(b)–2(f)).

3.3. miR-137 Inhibitor Induced the Proliferation of PASMCs. To further confirm the role of miR-137 in the proliferation of PASMCs, we transfected the inhibitor of miR-137 (100 nM) to PASMCs under normoxia. As Figure 3 shown, miR-137 inhibitor decreased the expression of miR-137 (Figure 3(a)) and induced the proliferation of PASMCs (Figures 3(b)–3(f)).

3.4. Hypoxia Induced the Expression of Calpain-2. It has well been documented that calpain-2 is mediated in promoting the proliferation of PASMCs, thereby resulting to pulmonary arterial remodeling in hypoxic PH [6–8]. In our setting, we therefore measured the expression of calpain-2 and found that exposure of rats to continuity hypoxia (10% O₂) for 21 days significantly upregulated the protein expression of calpain-2 in pulmonary arteries (Figure 4(b)) but not the expression of calpain-2 mRNA meanwhile (Figure 4(a)). Accordantly, treatment of PASMCs with 3% O₂ for 6 h, 12 h, 24 h, and 48 h also upregulated the mRNA and protein expression of calpain-2 in a time-dependent manner (Figures 4(c) and 4(d)).

3.5. miR-137 Inhibited Hypoxia-Induced Upregulation of Calpain-2 Expression. It has been documented that miR-137 inhibits the mRNA of calpain-2 by directly targeting at 3′-UTR of calpain-2 [14, 16]. To explore whether miR-137 targets 3′-UTR of calpain-2 mRNA in PASMCs, we mutated the putative binding site (Figure 5(a)). As shown in Figure 5(b), miR-137 mimic significantly downregulated the fluorescence values of wild-type vectors, whereas luciferase activity was unchanged using 3′-UTR binding site-mutated construct. These results indicated that miR-137 repressed the translation of calpain-2 mRNA by binding to its 3′-UTR. We then observed the effect of the transfection of miR-137 mimic on the expression of calpain-2 in PASMCs and found that miR-137 mimic downregulated the expression of calpain-2 mRNA and protein expression under normoxic condition (Figures 5(c) and 5(d)). It is of note that miR-137 mimic (25 nM) reversed the upregulated expression of calpain-2 (both mRNA and protein) induced by hypoxia (Figures 5(e) and 5(f)).

3.6. Knockdown of Calpain-2 Inhibited Hypoxia-Induced PASMC Proliferation. Inhibition of calpain-2 has been shown to attenuate proliferation of PASMCs induced by PH mediators (platelet-derived growth factor [PDGF], serotonin [5-HT], and interleukin 6 [IL-6]) [17, 18]. In this study, we therefore used the calpain-2 small interfering RNA (siRNA) to knock down the expression of calpain-2 mRNA to explore whether calpain-2 mediates hypoxia-induced PASMC proliferation. Different fragments and different concentrations of calpain-2 siRNA were transfected into PASMCs, resulting in the decrease of calpain-2 mRNA and protein expression in PASMCs, especially the effect of
Figure 2: Continued.
fragment 2 of calpain-2 siRNAs in a concentration-dependent manner (Figures 6(a) and 6(b)). Then, we used the fragment 2 of calpain-2 siRNAs at the concentration of 40 nM for the subsequent experiments. The MTS and EDU assay showed that knockdown of calpain-2 inhibited hypoxia-induced proliferation of PASMCs (Figures 6(c)–6(e)).

4. Discussion

This study represents the first evidence of the role of miR-137 in mediating hypoxia-induced proliferation of PASMCs, thereby potentially contributing to pulmonary arterial remodeling in PH. The main findings of the present study are as follows: (1) miR-137 was downregulated in pulmonary arteries of hypoxic PH rats and hypoxia-treated PASMCs; (2) miR-137 mimic inhibited hypoxia-induced proliferation of PASMCs by targeting calpain-2, and miR-137 inhibitor induced the proliferation of PASMCs under normoxia; (3) knockdown of calpain-2 by siRNA suppressed hypoxia-induced proliferation of PASMCs.

Hypoxia is one of the commonest causes of PH [19]. Hypoxia not only causes vasoconstriction by activating voltage-gated calcium channels resulting to increased cytosolic calcium of PASMCs, but also leads to pulmonary vascular remodeling by activating rho kinase and hypoxia-inducible factor-(HIF-1α) [20]. Hypoxia also compels the differential expression of miRNAs through response elements in their promoters of HIF-1 or through indirect hypoxia-associated stimulus [21]. The role of several miRNAs including miR-206 [22], miR-130/301 [23], miR-103/107 [24], miR-150 [25], miR-let-7g [6, 10], miR-17/92 [26], miR-92b-3p [27], miR-204 [28], and miR-27a [29] in hypoxic pulmonary arterial remodeling has been reported. The present study found for the first time that miR-137 was downregulated in pulmonary arteries of hypoxic PH rats and hypoxia-treated PASMCs. Studies have reported that the downregulation of miR-137 expression is caused by the ubiquitous in hypoxic-microenvironment [30], and that miR-137 is silenced by methylation and reduction of hypermethylation of the miR-137 promoter by inhibiting DNA methyltransferase which promotes its reexpression in hypoxia condition [31, 32]. In our setting, whether these potential mechanisms are involved in hypoxia-induced, the downregulation of miR-137 expression needs further investigation.

In a variety of cancer cells, miR-137 is significantly downregulated, and transfection of miR-137 mimic to restore miR-137 expression results in significant inhibition of cell proliferation, migration, and epithelial-mesenchymal transition [11, 12, 33]. miR-137 also regulates nervous system development and synaptic plasticity [13, 34]. In high glucose-induced human umbilical vein endothelial cell injury, miR-137 is significantly upregulated and inhibition of miR-137 inhibits oxidative stress and cell apoptosis [35]. In PDGF-induced proliferation of vascular smooth muscle cells, miR-137 is significantly downregulated and overexpression of miR-137 suppresses the cell proliferation and migration by suppressing the activity of mTOR/Stat3 signaling [36]. As we described above, excessive proliferation of PASMCs is the most important cause of pulmonary vascular remodeling in PH [37]. In this study, we for the first time found that miR-137 mediated the pathogenesis of hypoxic PH by inhibiting the proliferation of PASMCs. However, the destruction of vascular intima after vascular endothelial cell injury is usually the starting point of cardiovascular
Figure 3: miR-137 inhibitor induced the proliferation of PASMCs. (a) PASMCs were transfected with miR-137 inhibitor (100 nM), and the expression of miR-137 was detected by real-time PCR \((n = 4)\). (b) PASMCs were transfected with miR-137 inhibitor (100 nM), and the proliferation of PASMCs was detected by MTS assay \((n = 4)\). (c) Statistic diagram of EDU staining \((n = 3)\). (d) PASMCs were transfected with miR-137 inhibitor (100 nM), and the proliferation of PASMCs was detected by EDU staining. (e) PASMCs were transfected with miR-137 inhibitor (100 nM), and RT-PCR was used to detect the mRNA expression of PCNA \((n = 3)\). (f) PASMCs were transfected with miR-137 inhibitor (100 nM), and Western blot detected the protein expression of PCNA \((n = 3)\). The data are presented as means ± S.E.M.; \(^* p < 0.05\) and \(^{**} p < 0.01\) vs. control.
diseases. In the process of PH, apoptosis, necrosis, and endothelial to mesenchymal transition occur in pulmonary arterial endothelial cells [38]. Therefore, the role of miR-137 in pulmonary arterial endothelial functions also deserves to be further studied.

miRNAs bind to the 3′-UTR of target genes, resulting in inhibition of the target genes, to participate in physiological process and the pathogenesis of diseases. Bioinformatic analysis suggests that a potential binding element for miR-137 is contained in the 3′-UTR of calpain-2. Studies have demonstrated that miR-137 binds to 3′-UTR of calpain-2 to inhibit the expression of calpain-2 [14, 16, 39]. In this study, miR-137 also suppressed the translation of calpain-2 mRNA by binding to its 3′-UTR, suggesting that the calpain-2 is a direct target of miR-137 in hypoxia which induced the proliferation of PASMCs. Moreover, as we described above, miR-137 mediates the PDGF which induced the proliferation of VSMCs by regulating the activity of mTOR/Stat3 signaling. Stat3 has been demonstrated as a key mediator of PH pathology, and the inappropriate Stat3 activation in PH has been linked to miRNA expression, such as miR-204 and miR-17/92 [40]. Therefore, whether not only calpain-2 but also Stat3 participates in the proliferation of PASMCs mediated by miR-137 in hypoxic PH or other category of PH needs further investigation.

Calpain-2 (m-calpain) belongs to calpain family, which is activated by hypoxia-induced intracellular calcium fluxes. Our previous study found that calpain-1/2/4 expression was increased in pulmonary arteries of hypoxic PH rats, and the specific calpain inhibitor MDL28170 inhibited hypoxia-induced PASMC proliferation [7]. Others have also reported that global knockout or smooth muscle specific knockout of calpain-4 and MDL28170 prevent pulmonary vascular remodeling of MCT- or hypoxia-induced PH and EGF- and PDGF-BB-induced cell proliferation of PASMCs [8, 17, 18]. In this study, knockdown of calpain-2 by siRNA inhibited hypoxia-induced proliferation of PASMCs. Bioinformatic analysis showed that calpain-1/4 may be not targets of miR-137 (data not shown). Notably, calpain-1 has been implicated strongly in cell motility and adhesion, while calpain-2 has been implicated strongly in cell proliferation [41]. Emerging evidence has suggested an important role of calpain-2 in proliferation of PASMCs. In hyperproliferated
Figure 5: miR-137 inhibited hypoxia-induced upregulation of calpain-2 expression. (a) The putative binding site of miR-137 in 3' UTR of calpain-2 mRNA. (b) Luciferase analysis for examining whether miR-137 targets 3' UTR of calpain-2 mRNA (n = 3). (c) The mRNA expression of calpain-2 in PASMCs after transfecting miR-137 mimic under normoxic condition (n = 5). (d) The protein expression of calpain-2 in PASMCs after transfecting miR-137 mimic (25 nM) under normoxic condition (n = 3). (e) The mRNA expression of calpain-2 in PASMCs after transfecting miR-137 mimic (25 nM) under hypoxic condition (n = 4). (f) The protein expression of calpain-2 in PASMCs after transfecting miR-137 mimic (25 nM) under hypoxic condition (n = 4). WT: wild type; Mut: mutant; NC: negative control. The data are presented as means ± S.E.M.; *p < 0.05 and **p < 0.01 vs. control or WT+NC control and ##p < 0.01 vs. hypoxia.
Figure 6: Continued.
Figure 6: Knockdown of calpain-2 inhibited hypoxia-induced proliferation of PASMCs. (a) PASMCs were transfected with calpain-2 siRNA, and the mRNA expression of calpain-2 was detected by real-time PCR ($n = 3$). (b) PASMCs were transfected with calpain-2 siRNA, and the protein expression of calpain-2 was detected by Western blot ($n = 3$). (c) PASMCs were transfected with calpain-2 siRNA (40 nM), and the proliferation of PASMCs was detected by MTS assay ($n = 4$). (d) PASMCs were transfected with calpain-2 siRNA (40 nM), and the proliferation of PASMCs was detected by EDU staining ($n = 3$). (e) Statistic diagram of EDU staining ($n = 3$). The data are presented as means ± S.E.M.; **$p < 0.01$ vs. control and ##$p < 0.01$ vs. hypoxia.

Figure 7: Schematic diagram of the role of miR-137 in the proliferation of PASMCs during hypoxia-induced pulmonary hypertension. Our study for the first time demonstrated that miR-137 is a novel regulator of proliferation of PASMCs in hypoxia-induced pulmonary hypertension by targeting calpain-2 pathway. DHPR: dihydropyridine receptor; RyR: ryanodine receptor; cADPR: CD38-cyclic ADP-ribose; IP3R: inositol 1,4,5-trisphosphate receptor; SERCA: sarco (endo) plasmic reticulum calcium ATPase.
PASMCs treated with PH mediators (PDGF, 5-HT, and IL-6), the extracellular signal-regulated kinase (ERK) 1/2 activated calpain-2 through phosphorylation of calpain-2 at Ser50 and ERK-1/2 inhibitor PD98059 or knockdown of calpain-2 prevented calpain activation, resulting in inhibition of proliferation of PASMCs [21, 42]. In this study, we demonstrated that miR-137 mimic reduced the expressions of calpain-2, but not measured the activity of calpain-2. However, there is a study showing that miR-137 mimic pretreatment effectively prevented the oxygen-glucose deprivation and reperfusion-induced \([\text{Ca}^{2+}]\) increase, whereas the miR-137 inhibitor aggravated the \([\text{Ca}^{2+}]\) increase [39]. Given that increased intracellular \([\text{Ca}^{2+}]\) levels can activate calpain-2, we speculate that miR-137 mediates the activation of calpain-2 by regulating calpain-2 in pulmonary vascular remodeling and further study has indeed some limitations. Transgenic or gene knockdown animals of miRNA-137 and calpain-2 need to be introduced to further prove the in vivo functions of miRNA-137 and calpain-2 in pulmonary vascular remodeling and further confirm the inhibitory effect of miR-137 in hypoxia-induced proliferation of PASMCs by targeting calpain-2.

In conclusion, the present study for the first time demonstrated that hypoxia-induced downregulation of miR-137 promoted PASMC proliferation by targeting calpain-2. miR-137, a new miRNA involved in proliferation of PASMCs, further in pulmonary vascular remodeling of PH, would be a novel potential therapeutic target for PH.

**Data Availability**

The research data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

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