

The key role of PGC-1 α in mitochondrial biogenesis and the proliferation of pulmonary artery vascular smooth muscle cells at an early stage of hypoxic exposure

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Abstract Peroxisome proliferator activated receptor gamma coactivator 1 α (PGC-1 α) induced by hypoxia regulates mitochondrial biogenesis and oxidative stress. However, the potential role of PGC-1 α in hypoxia-promoted proliferation of pulmonary arterial vascular smooth muscle cells (PASMCs) is completely unknown. In this study, we found that hypoxia significantly induced the expression of PGC-1 α in cultured PASMCs and activated mitochondrial biogenesis through upregulation of nuclear respiratory factor-1 and mitochondria transcription factor A in a time-dependent manner. Knockdown of PGC-1 α by siRNA abrogated hypoxia-induced PASMCs proliferation via the downregulation of PCNA, cyclinA, and cyclinE. Furthermore, we observed that PI3K/Akt signaling pathway was involved in hypoxia induced PGC-1 α expression and PASMCs proliferation. Taken together, these data reveal PGC-1 α as the key regulator to mediate mitochondrial biogenesis and the proliferation of PASMCs at an early stage of hypoxic exposure. This process might bring to light a potential adaptive mechanism for PASMCs to minimize hypoxic damage and our novel findings provide new insight into the development of hypoxic pulmonary hypertension.

Keywords PGC-1 α · Mitochondrial biogenesis · Hypoxia · Pulmonary vascular remodeling · Hypoxic pulmonary hypertension

Introduction

Pulmonary arterial hypertension (PAH) is a severe disease of the pulmonary vasculature characterized by elevated pulmonary vascular resistance and progressive right ventricular hypertrophy causing right heart failure and eventually death [1, 2]. Persistent hypoxia is considered as one of the most predominant factors of PAH [3, 4]. The initial event of hypoxic pulmonary hypertension is acute pulmonary vasoconstriction and followed by pulmonary arteries vascular remodeling [5, 6]. In previous study, we have reported that hypoxia-stimulated excessive proliferation and inadequate apoptosis of pulmonary arterial vascular smooth muscle cell (PASMC) are important component of pulmonary vascular remodeling [7, 8]. However, the mechanisms of hypoxia-induced PASMCs proliferation remain largely unknown.

Mitochondria play a critical role in energy metabolism, cell proliferation, and apoptosis activity. An aberrant increase of mitochondrial biogenesis has been implicated in many diseases such as asthma and cancer [9, 10]. In addition, mitochondria biogenesis is stimulated by hypoxia as a response to the hypoxia damage [11]. Recently a variety of evidence obtained on human PAH and experimental PAH (including hypoxia models) clearly demonstrate that PASMCs and endothelial cells in pulmonary hypertensive conditions have impaired mitochondrial function, decreased mitochondrial oxidation, and metabolic shift to glycolysis, which is critical for increased proliferation, survival and

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pulmonary vascular remodeling [12–15]. However, a mitochondrial biogenic response is mounted. Especially at an early stage of hypoxic exposure the molecular mechanism by which hypoxia-induced mitochondrial biogenesis and dysfunction regulates the proliferation and function of PSMCs remains elusive.

Peroxisome proliferator activated receptor gamma coactivator 1 α (PGC-1 α) is a 795-amino acid protein, that mediates many metabolic functions through the interaction and activation of a large number of transcription factors including Peroxisome proliferator activated receptor gamma (PPAR γ) [16], estrogen receptor-related α [17], myocyte enhancer factor-2 [18], and nuclear respiratory factor (NRFs) [19]. PGC-1 α coordinates nuclear and mitochondrial gene expression into a program of mitochondrial biogenesis through regulating the NRF-1 and NRF-2, which transactivate genes for oxidative phosphorylation and mediate mtDNA transcription and replication via mitochondrial transcription factor A (TFAM) [20, 21]. Lack of oxygen can significantly induce expression of PGC-1 α which then regulates mitochondrial biogenesis and oxidative stress [22–24]. Interestingly it was shown recently that PGC-1 α dependent PPAR γ signaling was down-regulated in human and experimental pulmonary hypertension. Furthermore, activation of PPAR γ signaling suppressed smooth muscle cell excessive proliferation and migration, thus preventing pulmonary vascular remodeling [25].

Based on these previous studies, we hypothesized that hypoxia induces PGC-1 α expression in PSMCs, which then regulates mitochondrial biogenesis and PSMCs proliferation. In this study, we examined the expression of PGC-1 α and mitochondrial biogenesis in PSMCs subjected to hypoxia and observed the phenotypes of PSMCs. In addition, we explored the potential signaling pathways involved in the regulation of PGC-1 α expression in hypoxic PSMCs. Our results showed for the first time that PGC-1 α expression, mitochondrial biogenesis, and proliferation of PSMCs were significantly increased in PSMCs at an early stage of hypoxic exposure, which was markedly reversed by knockdown of PGC-1 α . Furthermore, PI3K/Akt signaling pathway was identified to contribute to hypoxia induced PGC-1 α expression and PSMCs proliferation.

Materials and methods

Materials

Antibodies against PGC-1 α , TFAM, β -actin, PCNA, cyclinA, and cyclinE were purchased from Santa Cruz Biotechnology (CA, USA). Polyclonal antibody against NRF-1 was obtained from cayman (Ann Arbor, MI, USA).

LY294002 were provided by Beyotime Institute of Biotechnology (Haimen, China). Enhanced chemiluminescence reagents were purchased from Amersham (Amersham, UK). Superscript First-Strand cDNA Synthesis Kit was obtained from Invitrogen (CA, USA). Bromodeoxyuridine (BrdU) Cell Proliferation Assay Test kit was purchased from Millipore (Billerica, MA, USA). Other regular reagents were from common commercial sources.

Animals

Adult male Wistar rats (180–250 g) obtained from the Experimental Animal Center of Harbin Medical University, which is fully accredited by the Institutional Animal Care and Use Committee (IACUC). The animals were maintained on a 12-h artificial light–dark cycle and housed in separate cages with standard rodent chow and water ad libitum. During our experiments, the rats were anesthetized with 4 % halothane, then chests were opened to take heart and lungs, pulmonary arteries were dissected under a stereo-microscope.

Cell culture and exposure hypoxia

PASMCs were separated according to our previously published protocol [8]. Cells were cultured using DMEM medium containing 20 % fetal bovine serum (FBS). Determined by Trypan Blue exclusion, cell viability was consistently >98 %. PASMCs purity of the primary cultures was confirmed by specific monoclonal antibodies raised against smooth muscle α -actin (Boehringer Mannheim Germany). Cells were cultured in a humidified incubator with 5 % CO₂ at 37 °C as normoxic control. After appropriate treatment, hypoxic group of cells were grown in a chambers equilibrated (3 % O₂) for 24, 48, or 72 h, respectively. Passage 2–4 cells were used for all experiments.

RNA isolation and real-time quantitative RT-PCR

Total RNA was extracted from cultured PSMCs using Trizol reagent according to the manufacturer's instructions. The concentration of RNA was quantified from the optical density measured at 260 nm by ultraviolet spectrophotometry. Total RNA from each sample was reverse transcribed with the Superscript First-Strand cDNA Synthesis Kit (Invitrogen CA, USA). The Quantitative RT-PCR was performed with SYBR Green I on an ABI Prism 7300 sequence detection system (Applied Biosystem, Foster City, CA). β -Actin was used as a control. The specific primers sequences for Real-time Quantitative RT-PCR were designed and synthesized by Shinegene Co, Shanghai shown in Table 1.

Table 1 Real time PCR primers

	Accession no.	Primers (5′–3′)	Expected size
PGC-1 α	NM_031347.1	(forward) GTGCAGCCAAGACTCTGTATGG (reverse) GTCCAGGTCATTCACATCAAGTTC	121 bp
NRF-1	NM_001100708.1	(forward) ACATTGGCTGATGCTTCAGAA (reverse) TGGTCGTCTGGATGGTCAT	134 bp
TFAM	NM_031326.1	(forward) GAAAGCACAAATCAAGAGGAG (reverse) CTGCTTTTCATCATGAGACAG	175 bp
β -Actin	NM_031144.2	(forward) AGGCCCTCTGAACCCTAAG (reverse) CCAGAGGCATACAGGGACAAC	118 bp

Western blot analysis

After specific treatment, cells were washed twice with cold PBS and lysed on ice for 30 min in 0.3 ml lysis buffer (Tris 50 mM, pH7.4, NaCl 150 mM, TritonX-100 1 %, EDTA 1 mM and PMSF 2 mM), centrifugation at 13,500 rpm for 15 min at 4 °C, then the supernatants were collected as total protein, the protein concentrations were determined using the BCA method (Pierce, Rockford, IL, USA). The protocol for Western blot was used as previously described [7].

Electron microscopic study

At a confluence of 70–80 %, PSMCs were cultured in normoxic or hypoxic condition for 24 h after growth arrest. Then, the cells were detached with trypsin and centrifuged at 2,000 rpm 5 min. The cell pellets were fixed in 2 % glutaraldehyde/osmium tetroxide, after dehydration in ethanol and embedded with spurr resin. Thin sections were obtained from a microtome and viewed with a Philips transmission electron microscope. 20 randomly selected areas were photographed at 20,000 \times magnification.

siRNA transfection

To knockdown PGC-1 α expression, PSMCs were transfected using small interfering RNA (accession numbers NM_031347.1), siPGC sequence: 5′-GCUCUUGAGAAU GGAUAUATT-3′ and non-targeted control siRNA (siNC) sequence: 5′-UUCUCCGAACGUGUCACGUTT-3′ which were designed and synthesized by Gene Pharma. Non-targeted control siRNA (siNC) was used to exam and optimize the efficiency of transfection and served as a negative control. siRNA was transfected into PSMCs using X-tremeGene siRNA transfection reagent (Roche, Mannheim, Germany) according to the manufacturer's instructions. In brief, 2 μ g siRNA and 10 μ l X-tremeGene siRNA transfection reagent were incubated with serum-free medium for 5 min respectively, and mixed them together. After

incubator for 20 min at room temperature, the mixture was added to cells. Following another culture for 6–8 h, cells were washed and subsequently cultured in DMEM containing 5 % FBS for 24 h. The efficiency of silencing was detected by western blot.

MTT assay

PASMCs were cultured in 96 well culture plates and starved for 24 h. Then, each group of cells received appropriate treatment. After 24 h, the cells were incubated with a medium containing the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), with a final concentration at 0.5 mg/ml. Finally, the MTT reaction was terminated via adding 150 μ l DMSO into each well and incubation for 10 min at room temperature. The absorbance value was read at 570 nm using a spectrophotometer.

Cell proliferation assay

Cell proliferation was assayed with a commercially available 5-bromo-2′-deoxy-uridine (BrdU) labeling kit (Millipore Billerica, MA, USA) according to the manufacturer's instruction. In brief, PSMCs were seeded in 96-well plates (about 1×10^5) 24 h, the cells were stopped growth for another 24 h. Then cells were transfected with non-targeted control siRNA (siNC) or targeted small interfering RNA (siPGC), respectively. After a 6–8 h culture, the medium was changed to DMEM containing 5 % FBS and 10 ng/ml BrdU. Then cells were exposed to normoxia or hypoxia for 24 h. After removal of the culture medium, the cells were fixed by the fixation reagent provided in the kit, then the cells were washed and incubated with mouse anti-BrdU monoclonal antibody, washed and added peroxidase goat anti-mouse IgG at room temperature, washed three times, and 100 μ l substrates were added to each well and incubated in the dark. Finally, the reaction product was quantified by measurement of the absorbance at 450 nm using a spectrophotometer.

Statistics

The composite data were expressed as means \pm SEM. Statistical analysis was performed with Student's *t* test or one-way ANOVA followed by Dunnett's test where appropriate. Differences were considered to be significant at $p \leq 0.05$.

Result

Hypoxia induced PGC-1 α expression in cultured PSMCs

To identify whether hypoxia activates PGC-1 α in cultured PSMCs, the mRNA and protein expression levels of PGC-1 α were evaluated by real-time Quantitative RT-PCR and Western blot. The results showed that both PGC-1 α mRNA and protein levels increased after exposed to hypoxia for 24 h, which continued to elevate until 48 h but then returned to control level at 72 h (Fig. 1). These results suggested that hypoxia induced expression of PGC-1 α in cultured PSMCs in a time-dependent manner.

Hypoxia activated mitochondrial biogenesis

To determine whether hypoxia promotes mitochondrial biogenesis in cultured PSMCs, the mitochondrial ultra-structure was examined by transmission electron microscope. The results indicated some damaged mitochondria surrounding the healthy ones, including medullary degeneration and vacuolar degeneration (Fig. 2a). By quantitative analysis, an apparently increased number of mitochondria were observed in PSMCs under hypoxia compared with normoxia (Fig. 2b).

To confirm the morphological changes we observed, next we detected the expression of nuclear respiratory factor-1 (NRF-1) and mitochondrial transcription factor A (TFAM), which are biomarkers of mitochondrial biogenesis. Real-time Quantitative RT-PCR and Western blot analysis showed that NRF-1 mRNA and protein expression levels increased in a time-dependent manner in PSMCs after exposed to hypoxia, beginning at 24 h and remaining significantly elevated at 48 h then returning to baseline after 72 h (Fig. 2c, d). Strikingly, hypoxia induced up-regulation of TFAM at both mRNA and protein levels after 24 h, and reached to maximum by 72 h (Fig. 2e, f). These data suggested that hypoxia activated mitochondrial biogenesis in PSMCs through upregulation of NRF-1 and TFAM in a time-dependent manner.

PGC-1 α was required for hypoxia induced mitochondrial biogenesis in PSMCs

To further test whether PGC-1 α is involved in hypoxia-mediated mitochondrial biogenesis and mitochondrial related gene expression in PSMCs, we performed RNA interference technology to knock down PGC-1 α . The efficiency of the siRNA was measured by western blot analysis of PGC-1 α protein. Our results showed that there were no differences between cells treated with siNC and untreated cells (Blank) or cells treated only with the transfection reagent (Mock). In contrast, PGC-1 α small interfering RNA (siPGC) reduced PGC-1 α protein expression remarkably compared with siNC (Fig. 3a). The mRNA and protein expression of NRF-1 and TFAM were detected in PSMCs with PGC-1 α knocked down. The results showed that hypoxia-induced increase on mRNA and protein expression of NRF-1 and TFAM was significantly blocked by PGC-1 α siRNA (Fig. 3b–e), suggesting

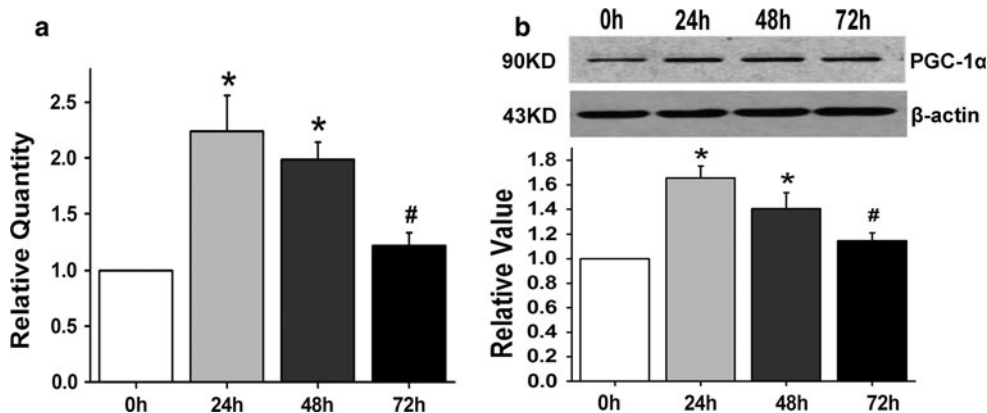


Fig. 1 Hypoxia induced PGC-1 α expression in cultured rat PSMCs. PGC-1 α mRNA expression from PSMCs was measured by real-time Quantitative RT-PCR and at different time points under hypoxia, using β -actin as internal standard (a). Western blots of PGC-1 α expression in PSMCs was analyzed after exposing to hypoxia for

different time points (b). All of the values are denoted as means \pm SEM (* $P < 0.05$ vs. hypoxia 0 h; # $P < 0.05$ vs. hypoxia 24 h $n = 5$). Data shown are representative of at least three independent experiments

Fig. 2 Hypoxia activated mitochondrial biogenesis. The number of mitochondria and expressions of mitochondrial biogenesis-related genes were increased in cultured PSMCs exposed to hypoxia. Transmission electron microscope image of sections from cultured PSMCs after normoxia and hypoxia condition. Randomly selected areas were photographed at $\times 20,000$ magnification (a). Quantitative analysis of the number of PSMCs mitochondria was tested in per photomicrograph (b). (* $P < 0.05$ vs. Normoxia) *Nor* Normoxia, *Hyp* hypoxia. *Nu* nucleus, *arrows* indicates mitochondria. NRF-1, TFAM mRNA were measured at the indicated times after hypoxia with real-time Quantitative RT-PCR (c and e). Protein expression of NRF1 and TFAM in PSMCs were measured using Western bolt at various time points after hypoxia (d and f). All of the values are denoted as means \pm SEM from three or more separate experiments (* $P < 0.05$ vs. hypoxia 0 h; ** $P < 0.01$ vs. hypoxia 0 h; # $P < 0.05$ and ## $P < 0.01$ vs. hypoxia 24 h $n = 5$)

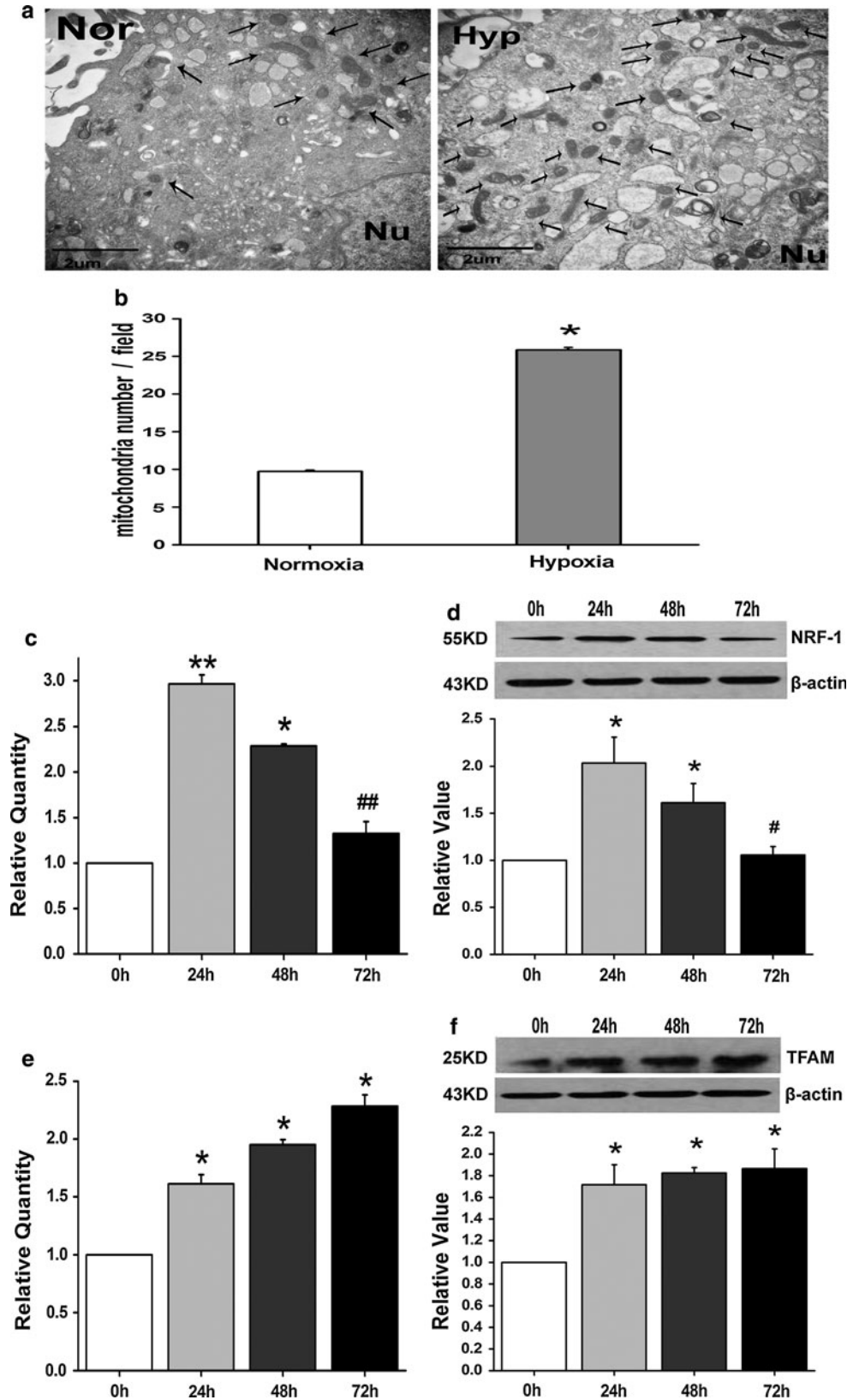
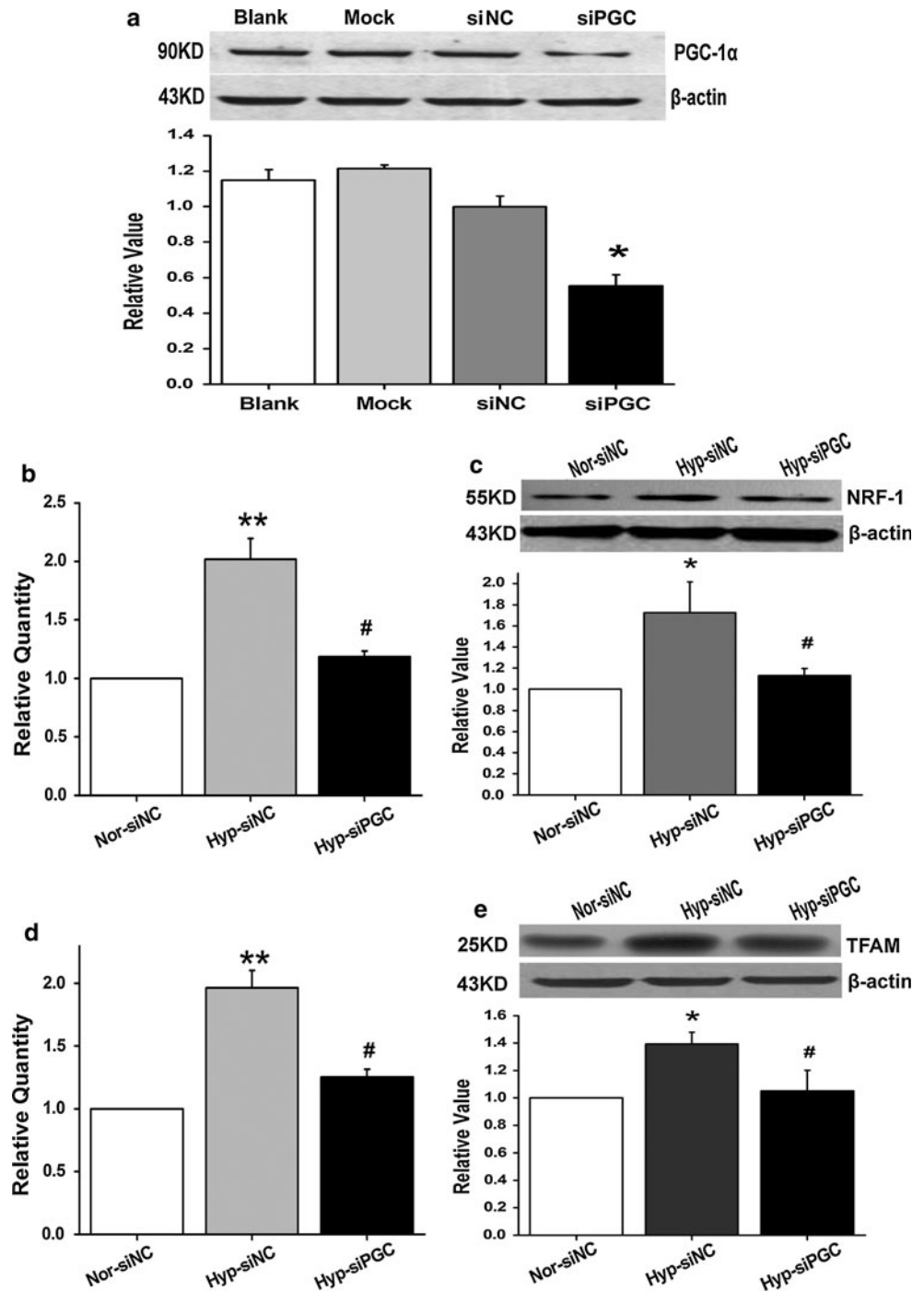


Fig. 3 PGC-1 α was required for hypoxia induced mitochondrial biogenesis in PASCs. Western blots analyzed PGC-1 α expression in untreated PASCs (Blank), PASCs treated with transfection vehicle alone (Mock), non-targeted small interfering RNA control (siNC), and siRNA targeted to PGC-1 α (siPGC) (a). (* $P < 0.05$ vs. siNC $n = 6$). Knockdown of PGC-1 α interfered with NRF1 and TFAM expression induced by hypoxia. NRF1 mRNA (b) and protein (c) expression were analyzed from normoxia with siNC, hypoxia with siNC, and hypoxia with siPGC. *Nor* Normoxia, *Hyp* hypoxia. In the same way, the mRNA and protein expressions of TFAM were shown (d, e). All values are denoted as mean \pm SEM from three or more independent experiments (* $P < 0.05$ vs. normoxia with siNC, ** $P < 0.01$ vs. normoxia with siNC, # $P < 0.05$ vs. hypoxia with siNC $n = 6$)



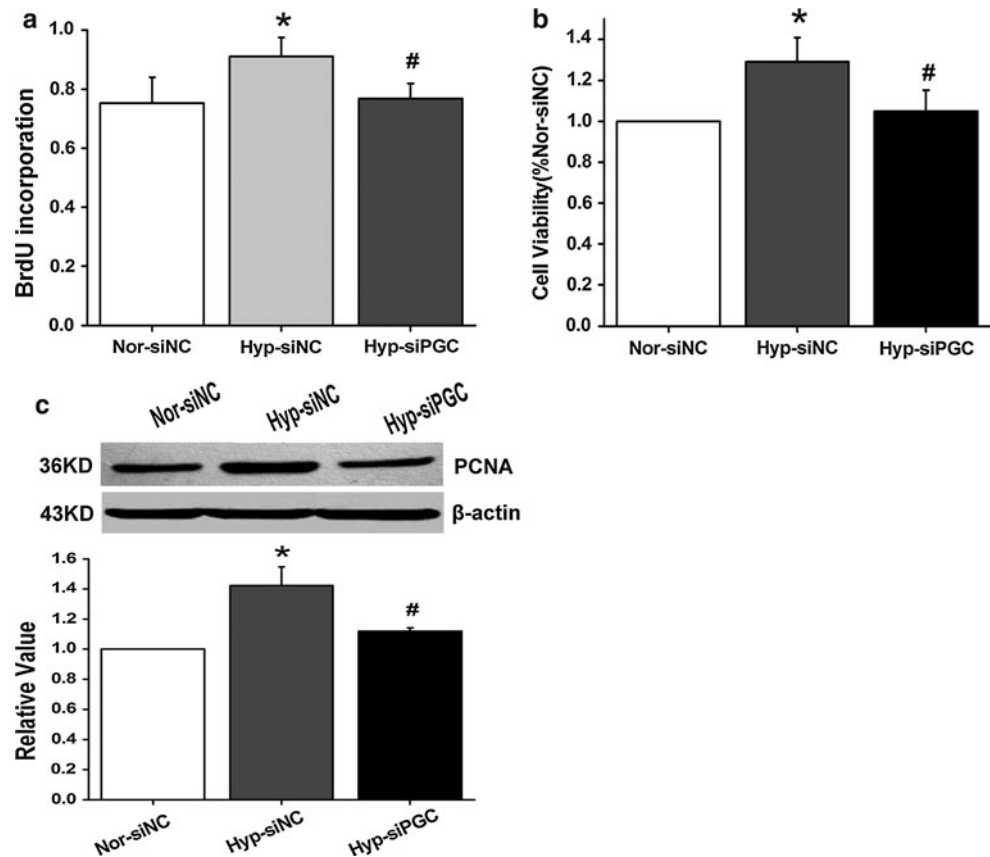
that hypoxia-induced expression of NRF-1 and TFAM was mediated by PGC-1 α .

Knockdown of PGC-1 α inhibited hypoxia induced proliferation of PASCs

To investigate the role of PGC-1 α in PASCs proliferation at an early stage of hypoxia exposure, we employed loss of function approach to knock down PGC-1 α in PASCs.

MTT assay demonstrated that cell viability was significantly increased by hypoxia compared with normoxic condition. However, knockdown of PGC-1 α abrogated the cell viability induced by hypoxia (Fig. 4a). Similarly, to assess the population of PASCs which are actively synthesizing DNA, a BrdU Cell proliferation assay was performed, which showed that hypoxia-induced DNA synthesis was also abolished by PGC-1 α siRNA (Fig. 4b). Moreover, we analyzed the expression of proliferating cell

Fig. 4 Effect of siPGC on PASCs proliferation. After 24 h growth arrest, transfected with siRNA, PASC cell viability was determined by MTT assay. Knockdown of PGC-1 α inhibited the increase of cell viability by 24 h hypoxia exposure (a). 5-Bromodeoxyuridine (BrdU) incorporation was measured in a similar experimental protocol, which demonstrated that hypoxia-induced cell DNA synthesis was inhibited by transfecting with siPGC (b). The protein expression of PCNA (c) was analyzed by western blot using PASCs under normoxia with siNC, hypoxia with siNC and hypoxia with siPGC. *Nor* normoxia, *Hyp* hypoxia. All of the values are denoted as mean \pm SEM from at least three separate experiments (* $P < 0.05$ vs. normoxia with siNC, # $P < 0.05$ vs. hypoxia with siNC $n = 6$)



nuclear antigen (PCNA) in PASCs. Western blot analysis showed that hypoxia induced the up-regulation of PCNA, which was abolished by knockdown of PGC-1 α (Fig. 4c). These results suggested that hypoxia induced cell proliferation of PASCs was mediated partly by PGC-1 α .

Knockdown of PGC-1 α inhibited hypoxia promoted cyclin expression

To further investigate the effect of PGC-1 α in PASCs proliferation at an early stage of hypoxia exposure, we sought to determine whether knockdown of PGC-1 α in PASCs affect the cell cycle progression. We checked cell cycle regulators cyclinA and cyclinE expression via western blot. The results showed that hypoxia induced the upregulation of cyclinA and cyclinE in control siRNA transfected PASCs (siNC) but not in PGC-1 α siRNA transfected PASCs (siPGC) (Fig. 5). These results suggested that PGC-1 α contributed to hypoxia-induced proliferation of PASCs through promoting the cell cycle progression.

PI3K/Akt signaling pathway was involved in hypoxia-induced PGC-1 α expression and PASC proliferation

PI3K/Akt signaling play an important role in hypoxia-induced cell survival and proliferation [26]. To clarify

whether PI3K/Akt signaling pathway is upstream of PGC-1 α , we analyzed the effect of LY294002 (20 μ M), an inhibitor of PI3K/Akt on hypoxia induced PGC-1 α expression in PASCs. The results showed that hypoxia-induced upregulation of PGC-1 α expression at both mRNA and protein levels was abolished in the presence of LY294002 (Fig. 6a, b). Furthermore, hypoxia-induced upregulation of PCNA, cyclinA and cyclinE were all blocked by LY294002 (Fig. 6c, d).

Discussion

PGC-1 α as a key regulator of energy metabolism, mitochondrial biogenesis, and oxidative stress, is correlated with oxygen content. In this study we novel findings demonstrate that PGC-1 α is significantly up-regulated in a time-dependent manner, which promotes mitochondrial biogenesis and cell proliferation during the early stage of hypoxia exposure. Furthermore, potential signaling pathway PI3K/Akt is found to be associated with the process.

Emerging reports show high expression of PGC-1 α in cardiac myocytes and myocardium of TOF patients is hypoxia dependent [24]. Hypoxia stimulates PGC-1 α expression, leading to angiogenesis in cultured skeletal muscle cells [23]. And PGC-1 α expression is rapidly

Fig. 5 PGC-1 α knockdown inhibited hypoxia promoted cyclin expression. The protein expression of cyclinA (a) and cyclinE (b) was analyzed by western blot using PASMCS under normoxia with siNC, hypoxia with siNC and hypoxia with siPGC. *Nor* normoxia, *Hyp* hypoxia. (* $P < 0.05$ vs. normoxia with siNC, # $P < 0.05$ vs. hypoxia with siNC; $n = 6$)

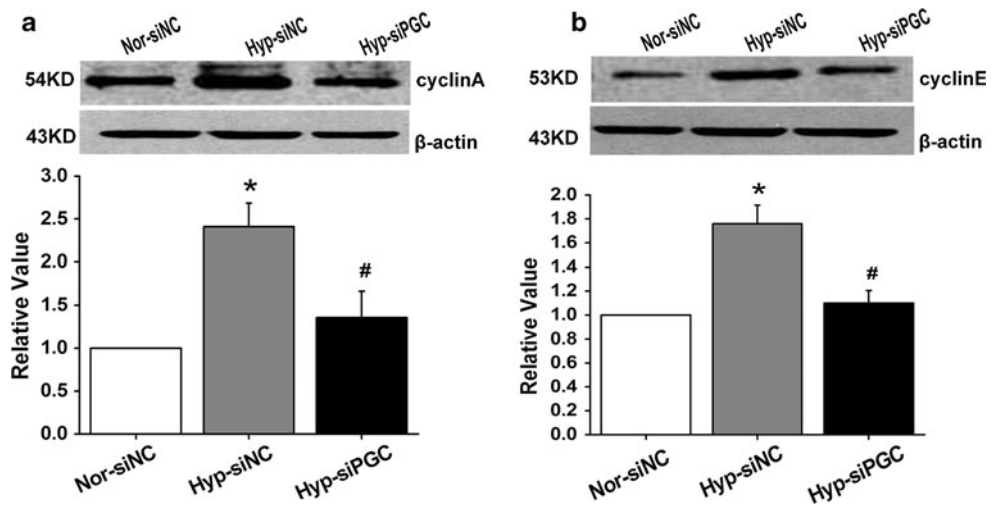
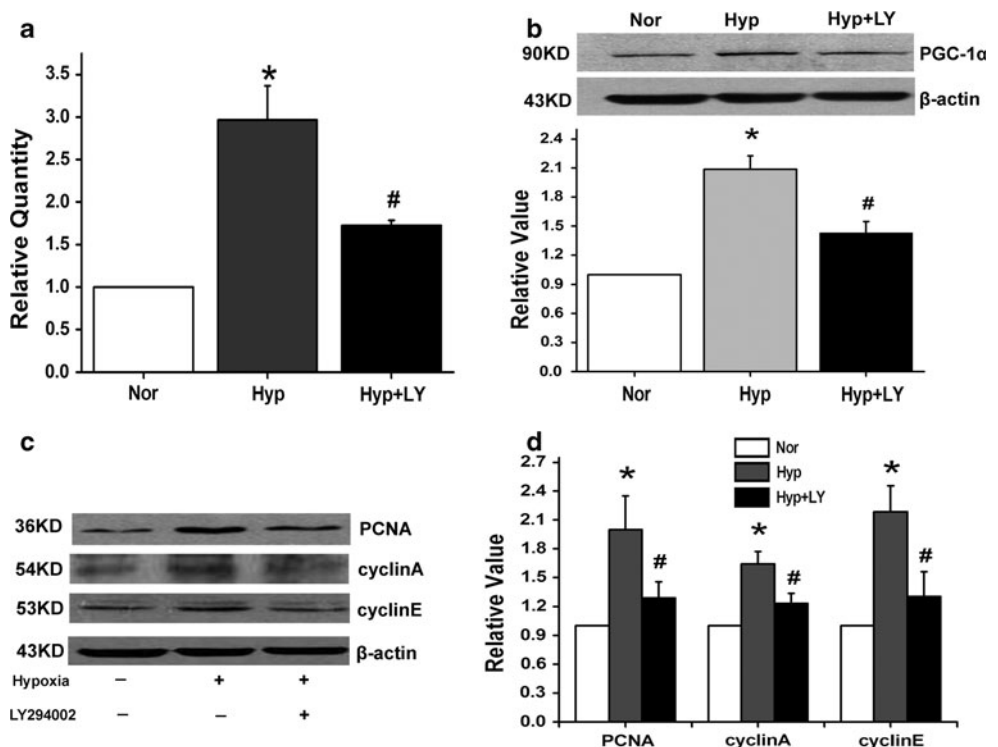


Fig. 6 PI3K/Akt signaling pathway was involved in hypoxia-induced PGC-1 α expression and PASM proliferation. The mRNA and protein expression of PGC-1 α in cultured PASMCS from normoxia, hypoxia and hypoxia with LY294002 (20 μ M) (a, b) was detected by real-time Quantitative RT-PCR and Western blots. The expressions of PCNA, cyclinA and cyclinE in cultured PASMCS by western blot (c, d) were shown. *Nor* normoxia, *Hyp* hypoxia, *LY* LY294002, an inhibitor of PI3K/Akt. (* $P < 0.05$ vs. normoxia, # $P < 0.05$ vs. hypoxia; $n = 6$)



elevated in brain subcortex by transient hypoxia stimulates and then returned to baseline [27]. However, PGC-1 α in hypoxic PASMCS is poorly known, although it was reported recently that PGC-1 α dependent PPAR γ signaling was implicated in pulmonary vascular remodeling [25]. In the current study, clearly proof is provided to demonstrate a time-dependent enhancement of PGC-1 α expression in PASMCS by hypoxia exposure, which peaks after 24 h and decreases to control level at 72 h. However, the reason for prolong hypoxia results in loss of the adaptive increase of PGC-1 α is unclear and may be related to oxidative stress or the development of a chronic inflammation.

It has been reported that transgenic over-expression of PGC-1 α directly improves mitochondrial biogenesis mediating muscle fiber type determination [28]. In addition, PGC-1 expression is induced in the mouse heart resulting in increased cellular mitochondrial number and stimulated coupled respiration [20]. Given the fundamental role of PGC-1 in mitochondrial biogenesis, we wonder whether hypoxia-induced PGC-1 α expression promotes mitochondrial biogenesis in cultured PASMCS. Transmission electron microscopy analysis showed that mitochondrial number was significantly higher in cultured PASMCS under hypoxia condition. As basic mitochondrial transcription factor,

TFAM binds to the D loop of mitochondrial DNA and activates mitochondria DNA replication and transcription, which has been realized to be regulated by PGC-1 α through coactivation with NRF-1 [21]. Our findings reveal TFAM and NRF-1 are rapidly increased in PSMCs after hypoxia stimulation, while PGC-1 α knockdown abolishes hypoxia-induced NRF-1 and TFAM expression. Our results provide evidence that PGC-1 α is essential for the induction of mitochondrial biogenesis at early stage of hypoxia in cultured PSMCs.

The acute and chronic responses to hypoxia include the angiogenesis and cell proliferation. These responses may sustain oxygen supply to tissues, whereas excessive proliferation of PSMCs contributes to the pathogenesis of pulmonary vascular remodeling [7]. In the present study, our result demonstrates that PGC-1 α knockdown strikingly inhibited hypoxia-induced DNA synthesizing, cell viability, and PCNA expression of PSMCs. Moreover, similar result shows that blockage of PGC-1 α inhibits both hypoxia-induced cyclinA and cyclinE expression. Based on these data, we speculate that the up-regulated PGC-1 α expression evokes mitochondrial biogenesis and PSMCs proliferation during the early period of hypoxia exposure, which process could be an important adaptive mechanism in attempt to optimize oxygen utilization, meet the energy demand and minimize hypoxic damage. This is a very early event, whereas prolonged challenges to the PSMCs might cause different changes. Some data on human PAH samples and chronic hypoxia models show impairment of mitochondrial and PPAR γ signaling. Therefore, it is worthy to investigate the role of PGC-1 α in chronic hypoxic pulmonary artery remodeling in future studies.

PI3K/Akt signaling is crucial in many cellular adaptive processes. Hypoxia activated PI3K/Akt pathway is required for the proliferation of human pulmonary vascular smooth muscle cells [26]. Blockade of Akt activity is reported to inhibit PGC-1 α activity but had no effect on expression levels of PGC-1 α [29]. However, it is suggested that PI3K/Akt is a downstream effector of PGC-1 α [30]. To clarify these conflicting results, we employ Akt inhibitor LY294002 to examine its effect on PGC-1 α expression in PSMCs. The results show that it inhibits PGC-1 α upregulation at both mRNA and protein levels. We also confirm that blockade of PI3K/Akt pathway inhibits the upregulation of cyclin and PCNA by hypoxia in cultured PSMCs. These results suggest that PI3K/Akt pathway positively regulates PGC-1 α expression, which then mediates downstream effectors.

In conclusion, our results indicate for the first time that hypoxia induces early PGC-1 α expression and mitochondrial biogenesis in cultured PSMCs in a time-dependent manner, and PGC-1 α promotes proliferation of PSMCs during the early stage of hypoxia exposure. We also find

that PI3 K/AKT pathway is involved in PGC-1 α up-regulation and cell proliferation by hypoxia. These findings reveal PGC-1 α as the key regulator for mitochondrial biogenesis and the proliferation of PSMCs during the early stage of hypoxia. It might bring to light a potential adaptive mechanism for PSMCs to minimize hypoxic damage and provide new insight into the development of hypoxic pulmonary hypertension.

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