

NPS2390, a selective calcium-sensing receptor antagonist controls the phenotypic modulation of hypoxic human pulmonary arterial smooth muscle cells by regulating autophagy

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ABSTRACT

Background and Objectives: Calcium-sensing receptor (CaSR) is known to regulate hypoxia-induced pulmonary hypertension (HPH) and vascular remodeling via the phenotypic modulation of pulmonary arterial smooth muscle cells (PASMCs) in small pulmonary arteries. Moreover, autophagy is an essential modulator of VSMC phenotype. But it is not clear whether CaSR can regulate autophagy involving the phenotypic modulation under hypoxia. **Methods:** The viability of human PASMCs was detected by cell cycle and BrdU. The expressions of proliferation protein, phenotypic marker protein, and autophagy protein in human PASMCs were determined by western blot. **Results:** Our results showed that hypoxia-induced autophagy was considerable at 24 h. The addition of NPS2390 decreased the expression of autophagy protein and synthetic phenotype marker protein osteopontin and increased the expression of contractile phenotype marker protein SMA- α and calponin via suppressing downstream PI3K/Akt/mTOR signal pathways. **Conclusions:** Our study demonstrates that treatment of NPS2390 was conducive to inhibit the proliferation and reverse phenotypic modulation of PASMCs by regulating autophagy levels.

Key words: NPS2390, calcium-sensing receptors, hypoxia, pulmonary arterial smooth muscle cells, phenotypic modulation, autophagy

INTRODUCTION

The hypoxic pulmonary hypertension (HPAH) is a clinical syndrome caused from increasing pulmonary vascular resistance, which can cause serious impairment of heart and lung function. Hypoxic pulmonary vascular remodeling (HPVR) is an important pathologic basis, but the exact mechanisms have not been fully elucidated. The abnormal proliferation of pulmonary arterial smooth muscle cells (PASMCs) play an important role during this process. Our previous study have found that phenotypic modulation of PASMCs is a key step in initiating HPVR, but the molecular mechanisms controlling the phenotypic

modulation of PASMCs are still not fully understood.

Autophagy is a process of phagocytizing its own cytoplasmic proteins or organelles, coating them into vesicles, and fusing with lysosomes to form autophagosomes, which degrade the contents of their entrapment.^[1] LC3 is an widely accepted autophagic marker. When autophagy is formed, cytosolic LC3 (LC3-I) will enzymatically cleave a small segment of the polypeptide and transform it into an autophagosome membrane type (LC3-II).^[2,3] The previous study has shown that autophagic protein LC3B play a protective role during the development of PAH through regulating

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hypoxic-induced cell proliferation.^[4] Moreover, inhibition of autophagy by drugs can inhibit PDGF on synthetic phenotype.^[5,6] Autophagy may participate in the process of phenotypic modulation of VSMCs. Furthermore, the molecular mechanism of autophagy is complicated and related to several distinct signaling pathways. Especially, the PI3K/Akt/mTOR signaling pathways were demonstrated to regulate autophagy under certain conditions.^[7,8] However, the role of autophagy was still not fully understood in hypoxic pulmonary hypertension (HPAH).

Calcium-sensing receptor (CaSR) is a G-protein-coupled receptor; its activation can increase the concentration of intracellular calcium. In the previous study, we have verified that the activation of CaSR is related to hypoxia-induced proliferation of rat PSMCs through MEK1/ERK1,2 and PI3K pathways. Recently, we also discovered that the expression of CaSR was increased and involved in the phenotypic modulation of PSMCs in small pulmonary artery and vascular remodeling in hypoxic rat. Besides, $[Ca^{2+}]_i$ plays an important role in the process of autophagy.^[9,10] However, whether CaSR regulates the phenotypic modulation via mediating autophagy in human PSMCs under hypoxia is still unclear.

In our study, we observed whether CaSR antagonist can regulate proliferation and phenotypic modulation of PSMCs under hypoxia and whether PI3K/Akt/mTOR signaling pathway is related to these processes.

MATERIAL AND METHODS

Cell culture

Human pulmonary artery smooth muscle cells (HPASMCs) and medium SMCM was purchased from American ScienCell Company. HPASMCs cells were cultured in SMCM in complete medium (89% DMEM + 10% fetal calf Serum + 1% growth factor), and 100 U/mL was added to the medium Cyan-streptomycin. The cells were placed at 37°C, 21% O₂, 5% CO₂, 74% subculture in N₂, incubator under saturated humidity, take within 6 generations counting cells in experiments.

Hypoxia treatment of HPASMCs

The original medium of HPASMCs was discarded and 1% fetal bovine serum was added to DMEM. The culture was incubated for 12 h for synchronization and then changed to SMCM culture. The cells were placed in a hypoxic incubator and treated at 1% O₂ concentration.

BrdU incorporation and detection

BrdU was incorporated into PSMCs, and proliferation of different treatment groups was detected using immunofluorescence technique. Cells from different

treatment groups were cultured in 96-well plates for 44 h, and then BrdU was added to these cells and continued culturing for 4 h at 38.5°C. Subsequently, the cells were fixed with 4% paraformaldehyde for 20 min, washed for 3 times with phosphate buffered saline with Tween-20 (PBST) at a concentration of 2 mol/L HCl, 37 °C and denatured for 30 min. After adding 0.1 mol/L of sodium tetraborate, the cells were kept for 5 min and washed with PBST for 3 times; then the cells were blocked with ready-to-use goat serum for 20 min at room temperature. After adding BrdU primary antibody G3G4 (1:200), the cells were incubated overnight at 4 °C and washed for 3 times with PBST. TRIFC-labeled goat anti-mouse secondary antibody (1:500) was added to the cells and incubated for 1 h at 37°C and washed for 3 times with PBST after incubation. Finally, DAPI was added and the cells were kept at room temperature for 30 min, after which the cells were washed for 3 times with PBST. The BrdU incorporation index was calculated by BrdU-labeled nuclei and DAPI-stained nuclei ratio under 1×70 fluorescence microscopy.

Cell cycle assays

For cell cycle analysis, the PSMCs to be tested were washed with a phosphate buffered saline solution, placed in a centrifuge tube, and centrifuged at 1,000 rpm for 5 min. The supernatant was discarded, and the cells were resuspended in 80% ethanol at 4 °C. The cells were centrifuged again for 24 min at 1,000 rpm for 24 min. The fixative was discarded, and the cells were resuspended in propidium iodide. Cell cycle analysis was performed with a Cy-toFLEX S flow cytometer (manufactured by Beckman Coulter) after 30 min at room temperature.

Western blot

The cells were collected and 300 μL of protein lysate was added. The cells were placed on ice for 15 min and then centrifuged at 15 °C/12,000 rpm for 15 min. The supernatant was collected. After adjusting the concentration, the protein was boiled in boiling water for 10 min. SDS-PAGE gels were prepared and filled with 40 μg of sample per well for electrophoresis. After 300-mA wet rotation for 120 min, the PVDF membrane was blocked at room temperature for 1 h and then added to the antibody dilution of CaSR (1:800), calponin (1:500), SMA-α (1:1000), OPN (1:500), PCNA (1:1000), Ki67(1:1000), LC3 (1:1000), caspase-3(1:1000), AKT (1:1000), p-AKT (1:1000), PI3K (1:1000), p-PI3K (1:1000), mTOR (1:1000), p-mTOR (1:1000), and GAPDH (1:1000) at 4°C overnight. The gel was wash with TBST for 3 times, each time for 10 min, and then horseradish-labeled goat anti-rabbit secondary antibody (1: 50,000) was added, The gel was incubate for 1 h at room temperature and washed with TBST for 3 times; each protein expression was detected by ECL chemiluminescence at 10 min. The resulting protein

strip image, using Quality one software to analyze the image to GAPDH light density. The degree value is used as an internal parameter to correct the optical density value of the target protein.

Measurement of intracellular calcium in HPASMCs

Fluo-4 AM calcium ion fluorescent probe was used to detect the intracellular calcium ion density in each group. The cell density was 2×10^5 /mL. The laser confocal small dish was inserted. After treating the cells according to the above group, 10 μ mol/L of Fluo-4 AM was added according to the instructions. The cells were then incubated in the dark for 30 min, after which they were rinsed in PBS with calcium-free buffer repeatedly, centrifuged for 2 times, and observed under laser confocal microscope.

Statistics

Data were analyzed using SPSS21.0 statistical software, all results according to the mean \pm SEM, the comparison between the different groups was tested in One-way ANOVA, $P < 0.05$ was considered statistically significant.

RESULTS

Hypoxia increases the expression of CaSR in cultured HPASMCs

Primary cultured HPASMCs were incubated for different times (12, 24, and 48 h) at 1% oxygen concentration in the hypoxia chamber, the control group was incubated under normoxia. Western blot analysis indicated that the CaSR expression in HPASMCs under hypoxia was increased from 12 h and peaked at 24 h ($P < 0.05$, Figure 1).

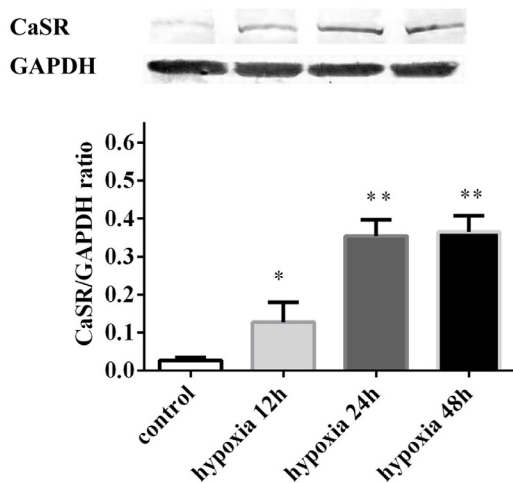


Figure 1: Hypoxia increases the expression of CaSR in human pulmonary arterial smooth muscle cells (HPASMCs). * $P < 0.05$ versus control group, ** $P < 0.01$ versus control group.

Hypoxia increases proliferation and promotes the phenotypic modulation in cultured HPASMCs

Moreover, we detect the proliferation assays and phenotype marker proteins of cultured in HPASMCs. The BrdU incorporation showed that the HPASMCs proliferation were increased demonstrably from 24 h under hypoxia when compared with the normoxic controls ($P < 0.05$, Figure 2A), and western blot analysis revealed that the expression of PCNA and Ki67 were increased from 12 h and peaked at 24 h ($P < 0.05$, Figure 2C-D). Afterwards, the results of cell cycle analysis showed that proportion of cells from G1 phase to S phase was increased under hypoxia ($P < 0.05$, Figure 2B).

Meanwhile, we found that the OPN expression was obviously increased, while the expressions of SMA α and calponin were markedly decreased in HPASMCs from 12 h under hypoxia when compared with the control group ($P < 0.05$; Figure 3). These data showed that the proliferation, cell cycle progression, and phenotypic modulation (from contractile phenotype to synthetic phenotype) of HPASMCs were induced under hypoxic conditions.

The increasing proliferation of HPASMCs and promotion of phenotypic modulation is associated with the activation of autophagy induced by hypoxia

To verify whether autophagy was related to the process that hypoxia induced proliferation of HPASMCs, cells were incubated under hypoxia for different times (12, 24, and 48 h). The expression of autophagic protein was observed by western blot analysis. The expression of LC3-II was increased markedly from 12 h (Figure 4). The results showed that the level of autophagy was increased in the early stage of hypoxia in a time-dependent manner.

To demonstrate the function of autophagy in HPASMCs and phenotypic modulation activated by hypoxia, a selective autophagy inhibitor, 3-MA, was added into hypoxic cell models. The inhibitor has no obvious toxic effects for certain cells including HPASMCs.^[11] When compared with the hypoxia 24 h group, the group in which 3-MA was added showed reduced expression of LC3-II, which indicate that 3-MA control the autophagy activated under hypoxia (Figure 5A). However, there was no variation in the expression of CaSR when 3-MA was given under hypoxia (Figure 5B). In addition, these data showed that the addition of 3-MA reduced the proliferation of HPASMC and reversed the phenotypic modulation of HPASMCs from synthetic phenotype to contractile phenotype at hypoxia for 24 h (Figure 5C–F), which indicate that the activation of autophagy maybe necessary for HPASMCs proliferation and phenotypic modulation under hypoxia, and CaSR is possibly the upstream factor of autophagy.

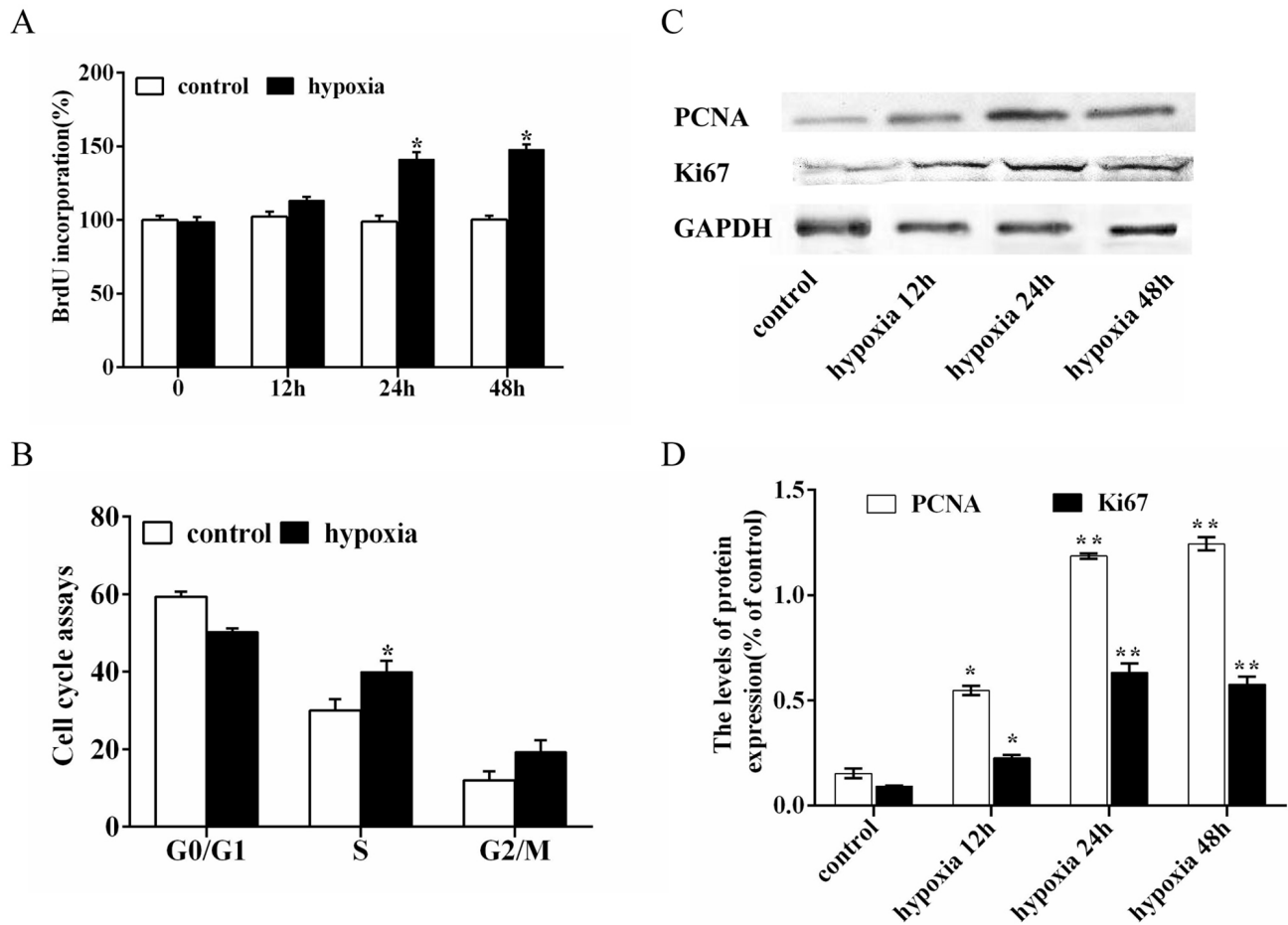


Figure 2: Hypoxia increases the proliferation and cell cycle progression of HPASMCs. (A) HPASMCs proliferation was measured by 5-bromo-2'-deoxyuridine (BrdU) incorporation. Data are presented as mean \pm SEM, $n = 5$. * $P < 0.05$ versus normoxia group. (B) Cell cycle analysis of HPASMCs in hypoxia condition at 24 h by flow cytometry. The results were expressed as relative cell growth in percentage, which was compared with a 21% oxygen control group. The concentration of 21% oxygen was set as control. $n = 5$ for each group. * $P < 0.05$ versus normoxia group. (C) Expression of PCNA and Ki67 proteins in HPASMCs under hypoxia. (D) Densitometry was applied to quantify PCNA and Ki67 protein density. Data were presented as mean \pm SEM, $n = 5$. * $P < 0.05$ versus control group, ** $P < 0.01$ versus control group

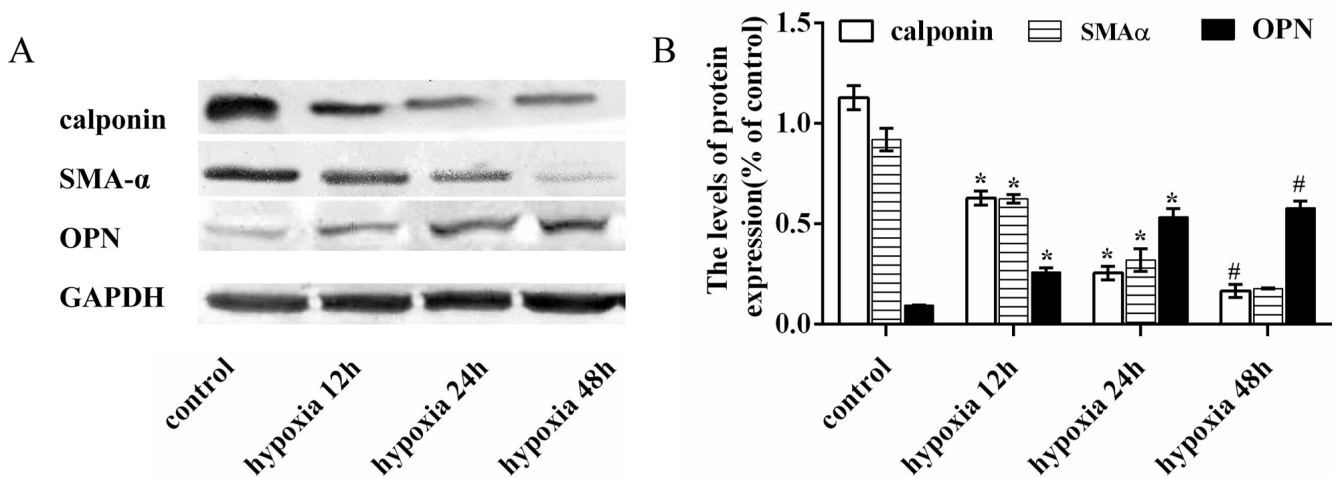


Figure 3: Hypoxia stimulates the phenotypic modulation of HPASMCs. (A) Phenotype marker proteins expression in HPASMCs detected by western blot. (B) Densitometric analysis of (A). Data were presented as mean \pm SEM. * $P < 0.05$ versus control; # $P < 0.05$ versus hypoxia for 24 h.

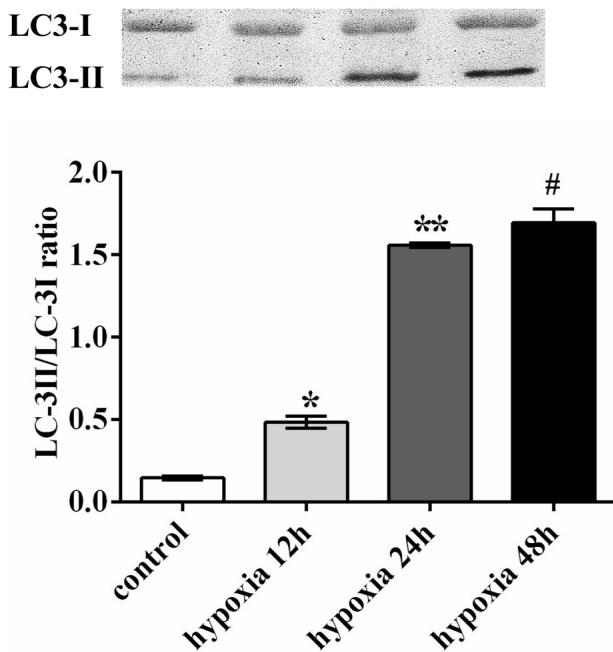


Figure 4: Activation of autophagy in HPASMCs under hypoxia. Data are presented as mean \pm SEM. * $P < 0.05$ versus control; ** $P < 0.01$ versus control group; # $P < 0.05$ versus hypoxia for 24

NPS2390 decreases proliferation and reverses phenotypic modulation via inhibiting autophagy in PSMCs under hypoxia

To identify whether the function of CaSR is involved with the regulation of autophagy in HPASMCs proliferation and phenotypic modulation under hypoxia, HPASMCs were treated with NPS2390 (10 μ M, CaSR inhibitor) for 24 h under hypoxic or normoxic conditions. Our results showed that the addition of NPS2390 reduced the proliferation and reversed phenotypic modulation in HPASMCs under hypoxia (Figure 6A and B). Meanwhile, the expression of LC3-II was also decreased after treating with NPS2390 (Figure 6C). The results indicate that the inhibition of CaSR suppressed the autophagy of HPASMCs under hypoxia (Figure 6D).

Changes in $[Ca^{2+}]_i$ in different groups

We found that hypoxia markedly enhanced $[Ca^{2+}]_i$, and the addition of R568 (30 μ M) raised a cooperativity as the $[Ca^{2+}]_i$, further increases in the hypoxia + R568 group ($P < 0.05$) when compared with the hypoxia group. But NPS2390 can suppress these changes (Figure 7).

NPS2390 inhibits PI3K/Akt/mTOR pathways in HPASMCs under hypoxia

Next, we intend to identify whether the decreasing level of autophagy activated by NPS2390 was attributed to the change in PI3K/Akt/mTOR pathways. After the addition

of NPS2390 for 24 h under hypoxia, the expressions of p-PI3K, p-Akt, and p-mTOR were increased (Figure 8A and B). To further verify whether the function of NPS2390 is dependent on the PI3K/Akt pathways, the PI3K inhibitor LY294002 (10 μ M) was added together with the CaSR agonist R568 into HPASMCs under hypoxia. LY294002 suppressed the activation of Akt and its downstream mTOR, when compared with the R568 treatment hypoxia group. Furthermore, the function of NPS2390 on autophagy-associated protein was verified by western blot. The expression of LC3-II was upregulated by the addition of R568 at hypoxia for 24 h when compared with the hypoxic group without any treatment (Figure 8C and D). The LY294002 treatment obviously controls the expression of LC3-II when compared to the R568 treatment hypoxia group (Figure 8E).

These results demonstrated that the PI3K/Akt signaling pathway targets autophagy activation that is dependent on mTOR inhibition, which may be related to promote the suppression of HPASMC proliferation by NPS2390.

DISCUSSION

Our study identifies a novel function for CaSR in controlling autophagy in PSMCs. We observed that NPS2390, CaSR antagonists, significantly inhibit hypoxia-induced autophagy. Meanwhile, addition of autophagy inhibitor not only markedly suppressed the hyperproliferation of PSMCs under hypoxia but also reversed the phenotypic modulation of PSMCs from the contractile phenotype to the synthetic phenotype stimulate by hypoxia. These findings confirm that NPS2390 can inhibit hypoxia-induced pulmonary vascular remodeling by regulating autophagy to inhibit proliferation and phenotypic modulation of PSMCs.

Pulmonary hypertension is a serious and fatal circulatory disease, mainly caused by excessive proliferation of vascular wall cells, eventually occluding small pulmonary artery.^[12-14] Although several studies have made some progress in the treatment of pulmonary hypertension in the recent years, the current treatment methods are not satisfactory.^[15] Therefore, a better understanding of the pathogenesis of PAH and the selection of more effective treatments are imminent. In 2015, hypoxia-induced pulmonary hypertension (HPH) was listed as one of the most common types in PAH by the European Society of Cardiology and Respiratory Society.^[16] The pathological basis that occurs in HPH contains hypoxic pulmonary vasoconstriction and hypoxic pulmonary vascular remodeling.^[17,18] Moreover, studies have observed that over proliferation of PSMCs plays an important role in the pathology of hypoxic pulmonary vascular remodeling.^[19,20] Meanwhile, PSMCs

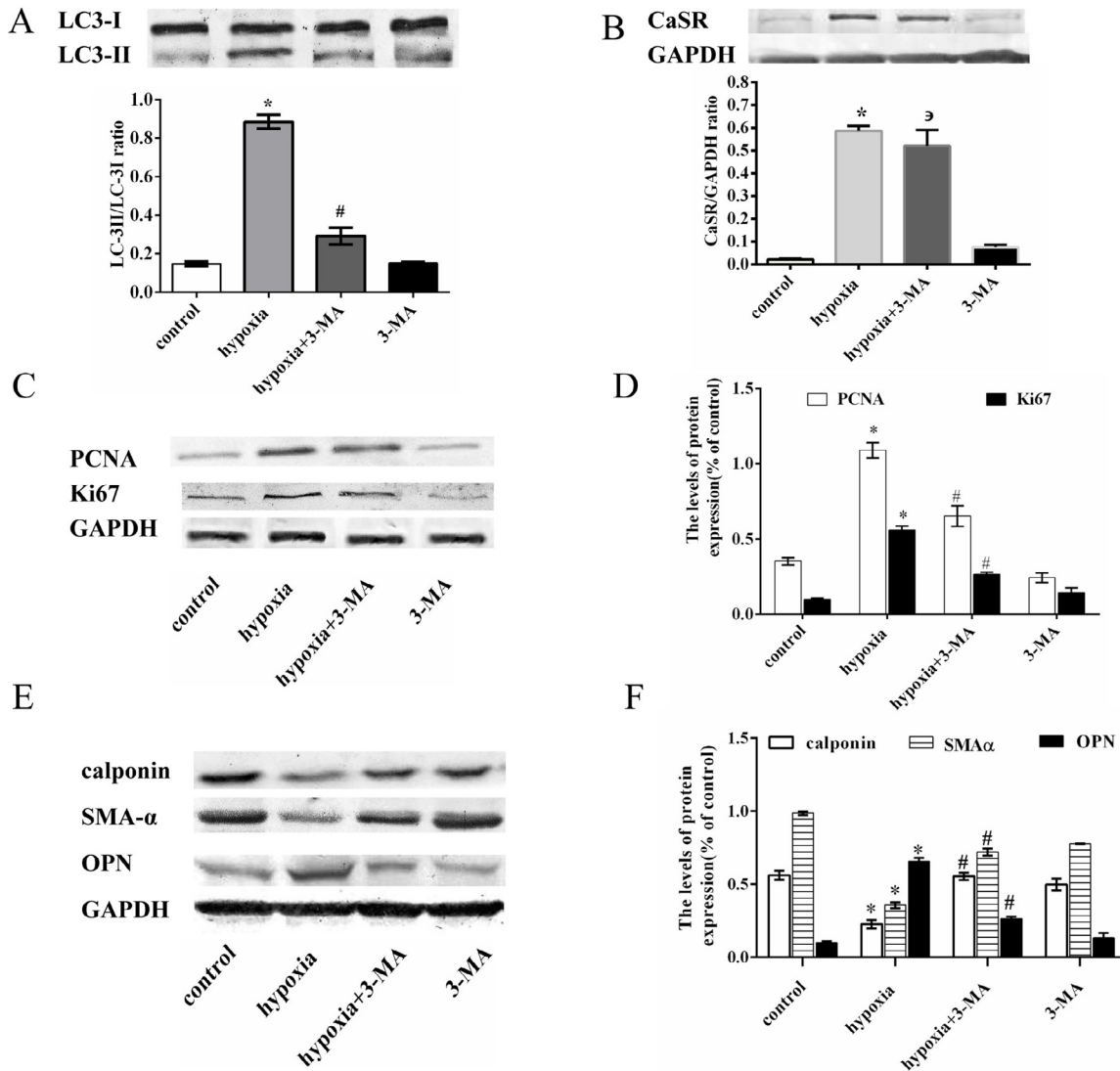


Figure 5: 3-MA inhibits autophagy and CaSR expression, decreases the proliferation, and reverses the phenotypic modulation of HPASMCs induced by hypoxia. (A) The levels of LC3-II and LC3-I were measured in the HPASMCs under hypoxia by western blot analysis. (B) The levels of CaSR were measured in the HPASMCs under hypoxia by western blot analysis. (C) The levels of PCNA and Ki67 were measured in the HPASMCs under hypoxia by western blot analysis. (D) Densitometric analysis of (C). (E) The levels of phenotype marker protein were measured in the HPASMCs under hypoxia by western blot analysis. (F) Densitometric analysis of (E). Data were presented as mean \pm SEM, $n = 5$. * $P < 0.05$ versus control group, # $P < 0.05$ versus hypoxia group, ^ε $P > 0.05$ versus hypoxia group

phenotypic modulation is characterized by an increase in smooth-muscle-cell-specific expression of phenotype-associated proteins that contribute to the proliferation of PSMCs and vascular remodeling of pulmonary hypertension. Although the study of pathological changes in HPH has become clearer, the cellular mechanisms related to excessive and uncontrolled growth of vascular wall cells remain unclear.

An increase in the concentration of cytosolic calcium ($[Ca^{2+}]_{cyt}$) in PSMCs is the main cause for pulmonary vasoconstriction and a critical stimulation for PSMCs

proliferation and migration.^[21] As the major regulator of intracellular calcium concentrations, the CaSR can respond to the changes in extracellular calcium concentrations ($[Ca^{2+}]$) and its activation can induce the release of calcium from the sarcoplasmic reticulum.^[22,23] Our previous study have verified that CaSR activation is related to hypoxia-induced proliferation of rat PSMCs via MEK1/ERK1,2 and PI3K signal pathways. Recently, we also discovered that the expression of CaSR was increased and involved the phenotypic modulation of PSMCs in small pulmonary artery and vascular remodeling in hypoxic rat. In our study, we used human PSMCs to further observe the underlying

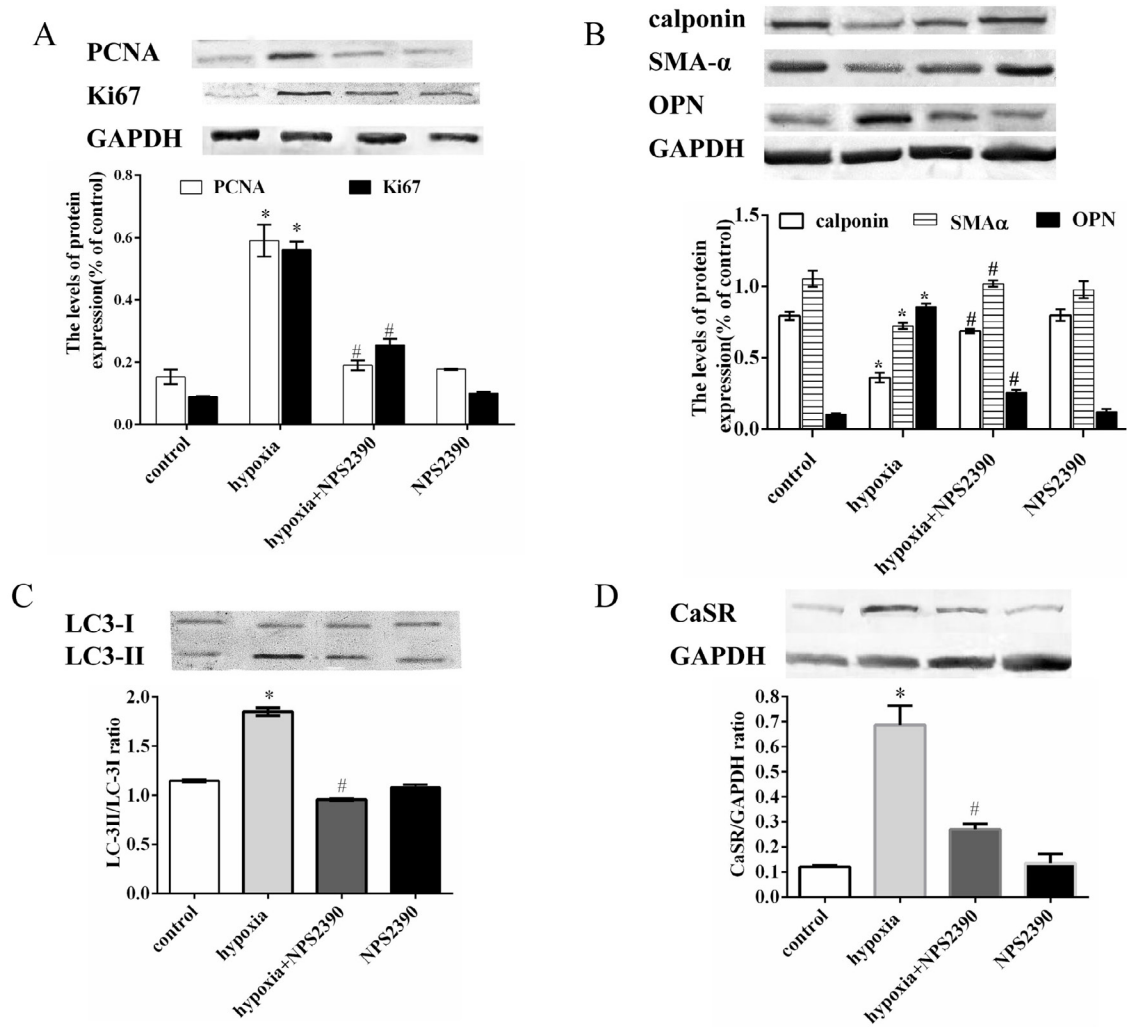


Figure 6: NPS2390 inhibits autophagy and CaSR expression, decreases the proliferation, and reverses the phenotypic modulation of HPASMCs induced by hypoxia. (A) The levels of PCNA and Ki67 were measured in the HPASMCs under hypoxia by western blot analysis. (B) The levels of phenotype marker protein were measured in the HPASMCs under hypoxia by western blot analysis. (C) The levels of LC3-II and LC3-I were measured in the HPASMCs under hypoxia by western blot analysis. (D) The levels of CaSR were measured in the HPASMCs under hypoxia by western blot analysis. Data were presented as mean \pm SEM, $n = 5$. * $P < 0.05$ versus control group, # $P < 0.05$ versus hypoxia group.

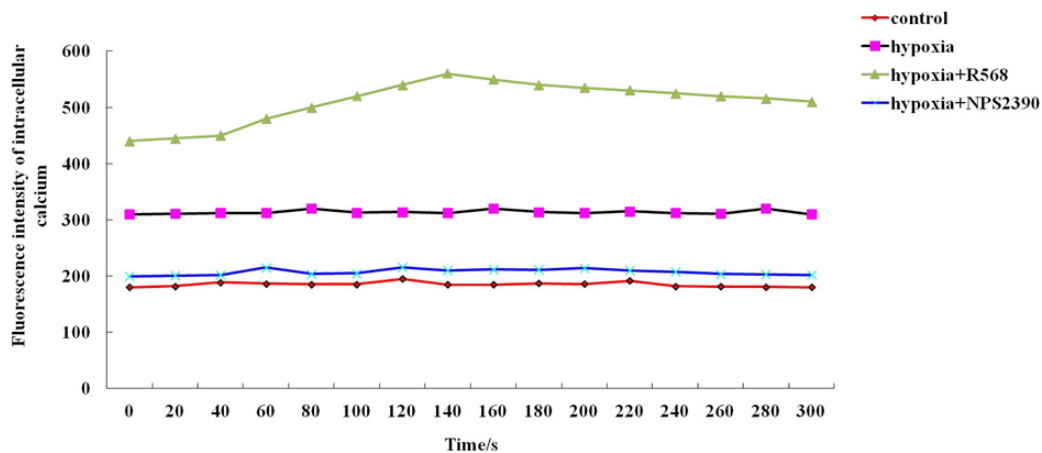


Figure 7: Changes in intracellular calcium concentration ($[Ca^{2+}]_i$) in HPASMCs. Fluorescence intensities in $[Ca^{2+}]_i$ were recorded by confocal laser scanning microscopy in different treatments. Compared with the control group, hypoxia significantly increased $[Ca^{2+}]_i$, and this increase was enhanced by R568 ($P < 0.05$ versus hypoxia group) but was attenuated by the addition of NPS2390 ($P < 0.05$ versus hypoxia group).

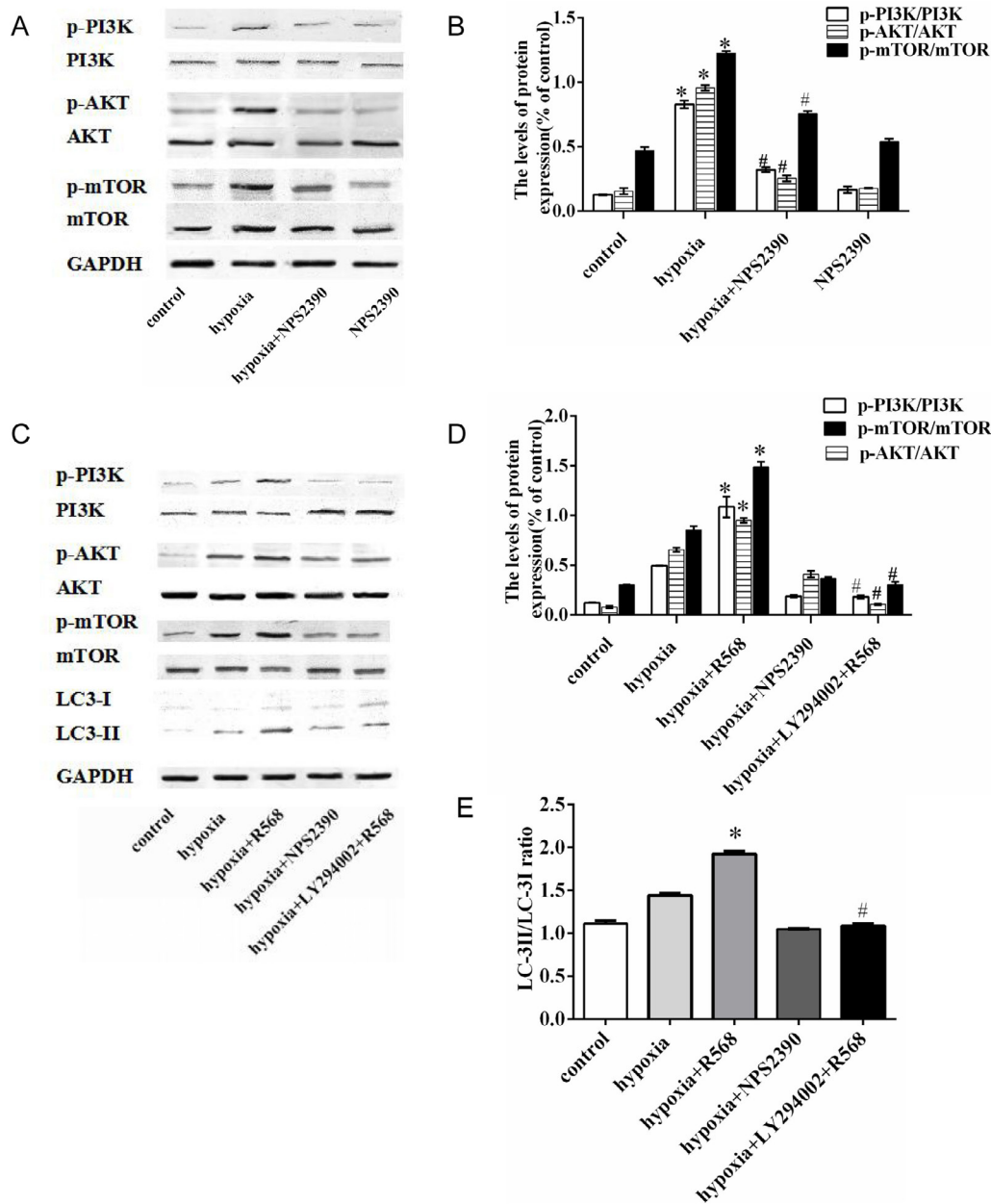


Figure 8: The effect of NPS2390 on autophagy in HPASMCs induced by hypoxia is related to the regulation of PI3K/Akt/mTOR pathways. (A) NPS2390 decreases the phosphorylation of PI3K/Akt/mTOR signals. The protein expressions were measured by western blot analysis. (B) Densitometry was applied to quantify the protein density; data were presented as mean \pm SEM, $n = 5$. * $P < 0.05$ versus control group, # $P < 0.05$ versus hypoxia group. (C) Expression of phosphorylated-PI3K/Akt/mTOR and LC3 proteins in HPASMCs under hypoxia with NPS2390, R568, and Akt inhibitor LY294002. (D) The ratio of normalized LC3-II to LC3-I. (E) Densitometry was applied to quantify the density of phosphorylated-PI3K/Akt/mTOR protein; data were presented as mean \pm SEM, $n = 5$. * $P < 0.05$ versus hypoxia group, # $P < 0.05$ versus R568-treated hypoxia group.

function of the CaSR in pulmonary vascular remodeling induced under hypoxia; we found the same changes in the expression of CaSR and the proliferation factors PCNA and Ki67. Meanwhile, we also observed that hypoxia induced the increased expression of synthetic phenotype marker protein osteopontin and decreased the expression of contractile phenotype marker proteins SMA- α and calponin. CaSR antagonist NPS2390

weakened the effect of hypoxia. The results showed that hypoxia can induce increased expression of CaSR and proliferation and phenotypic modulation of HPASMCs. Therefore, we hypothesized that hypoxia-induced CaSR activation plays an important role in promoting PASMCS proliferation and phenotypic modulation, and this change is due to CaSR-mediated increase in intracellular calcium concentration.

To verify the hypothesis, we detected the level of $[Ca^{2+}]_i$ and observed that lower oxygen conditions obviously increased $[Ca^{2+}]_i$, this increase can be further upregulated by CaSR agonist R568 and downregulated by NPS2390. NPS R568 is an allosteric agonist of CaSR, also known as calcimimetic, which binds specifically to CaSR, thereby altering its spatial conformation and increasing the sensitivity of CaSR to extracellular Ca^{2+} .^[24] Moreover, autophagy, as an important process of cell death and survival, has been shown to be involved in mediating SMC proliferation.^[25,26] However, whether CaSR regulates phenotypic modulation of PSMCs via autophagy remain elusive.

Autophagy is a highly conserved protein or organelle degradation process found in eukaryotes^[27] and is related to the pathogenesis of HPAH.^[4] So far, only a few sporadic studies of autophagy have been published. Seon-Jin Lee have found that autophagic protein LC3B play a protective role during the development of PAH through regulating hypoxic-induced cell proliferation.^[4] Recently, studies have found that chloroquine inhibits autophagy and blocks lysosomal degradation of bone morphogenetic protein type II receptors, thereby inhibiting the proliferation and increased apoptosis of PSMCs in HPH animal and cell models.^[28] The latest study shows that the activation of autophagy is related to hypoxia-induced proliferation and migration of PSMCs and the inhibition of autophagy leads to decreased cell proliferation and cell cycle arrest,^[29] which is consistent with our results.

In this study, we showed that hypoxia-induced activation of autophagy is related to the proliferation and phenotypic modulation of PSMCs, and autophagy-specific inhibitor, 3-MA, can reduce proliferation and reverse the phenotypic modulation of PSMCs, proposing that the activation of autophagy converted the PSMCs from contractile phenotype to synthetic phenotype and promoted PSMCs proliferation under hypoxic condition, which may be an important factor in the progression of HPH disease and vascular remodeling. Concurrently, CaSR antagonist, NPS2390, inhibits the autophagy induced by hypoxia. These results showed that CaSR antagonists treatment may suppress the proliferation and reverse phenotypic modulation of PSMCs through regulating autophagy level.

CaSR has been shown to promote the activation of the PI3K/Akt pathway, which plays a protective function in cell proliferation. Moreover, it is common knowledge that as an upstream pathway, the PI3K/Akt/mTOR signal is critical for the regulation of autophagy.^[30-32] But the function of CaSR and PI3K/Akt/mTOR in HPASMCs of hypoxic cell models has not been discussed. In the current study, we first found that the inhibition of the PI3K/Akt/mTOR

pathway is critical for the inhibition of hypoxia-induced autophagy by NPS2390. In addition, NPS2390 reduces autophagy levels in PSMC by inhibiting the downstream signaling of Akt and mTOR. The data from these in vitro experiments suggest that regulation of autophagy and its downstream signaling may be related to the effects of NPS2390, which is beneficial for inhibiting vascular remodeling in HPH.

However, our findings need further research and confirmation in vivo to confirm the role of NPS2390 in the regulation of autophagy involved in vascular remodeling and HPH formation by hypoxic animal models, further verifying the feasibility of NPS2390 as a therapeutic target for HPH.

In summary, our results demonstrated that CaSR inhibition regulates proliferation, cell cycle progression, and phenotypic modulation in PSMCs caused by hypoxia, which relies on PI3K/Akt/mTOR signal pathway. Inhibition of this pathway prevents PSMC proliferation and reverses HPVR. Our study reveals the mechanism of HPVR and provides more targets for the treatment of PAH.

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Conflict of Interest

The authors declare that they have no competing interests.

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