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Original Article

LncRNA APF suppresses pulmonary vascular remodeling in chronic thromboembolic pulmonary hypertension through the miR-188-3p/ATG7-mediated autophagy pathway

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Abstract: Background: An imbalance in autophagy leads to persistent pulmonary vascular remodeling, resulting from impaired phenotypes of dysfunctional vascular smooth muscle cells in chronic thromboembolic pulmonary hypertension (CTEPH). Long non-coding RNA autophagy-promoting factor (APF) is an essential regulator of the miR-188-3p/autophagy-related gene 7 (ATG7)/autophagy pathway in cardiomyocytes, but its role in CTEPH was uncertain. Methods: In paired vascular specimens from CTEPH patients, APF/miR-188-3p/ATG7 expression changes were explored. Primary human pulmonary artery smooth muscle cells (HPASMCs) freshly isolated from CTEPH lesion sites were used for biological validation We investigated the effect of APF on the growth, migration, invasion ability, and autophagy of HPASMCs. Regulatory interactions along this axis were also verified. Rescue assays a using miR-188-3p inhibitor, ATG7 over-expression plasmids, and the autophagy inducer rapamycin were conducted to establish mechanistic connection. Results: APF and ATG7 were downregulated in CTEPH tissues, while miR-188-3p was upregulated. APF overexpression inhibited HPASMC proliferation, migration, and invasion but increased autophagy; APF knockdown showed the opposite effects. The pro-proliferative and pro-migratory behaviors induced by APF silencing or miR-188-3p upregulation were abrogated by inhibiting miR-188-3p or restoring ATG7, respectively. Reactivation of autophagy with rapamycin reversed the effects of APF silencing. Conclusion: The downregulation of APF in CTEPH promoted excessive proliferation, migration, and invasion in HPASMCs by inhibiting autophagy through the miR-188-3p/ATG7 axis. We highlight the APF/miR-188-3p/ATG7-autophagy pathway as a potential therapeutic target for CTEPH.

Keywords: Chronic thromboembolic pulmonary hypertension, lncRNA APF, miR-188-3p, ATG7, autophagy, human pulmonary artery smooth muscle cells

Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) involves two mechanisms: persistent thrombotic obstruction and pulmonary vascular remodeling [1-3]. Without timely intervention, pulmonary vascular resistance gradually increases over time, eventually affecting right heart function [4]. This disease process leads to sustained elevation of pulmonary vascular resistance and, if untreated, may result in right heart failure [4]. Although CTEPH is a potential long-term outcome of acute pulmonary embolism, its exact incidence remains challenging to ascertain due to diagnostic complexities [5]. The transition from an acute embolic event to this chronic state involves not merely passive clot persistence but active and dysfunctional vascular wall remodeling. This remodeling includes distal small-vessel endo-

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thelial injury, inflammatory-fibrotic responses, and abnormal aggressive vascular smooth muscle cells (VSMCs), collectively leading to sustained elevation of pulmonary vascular resistance [6-8]. However, the molecular triggers linking thrombosis to vascular wall remodeling remain unclear. Therefore, investigating key pathways that regulate these aberrant vascular cell phenotypes is important for better understanding of CTEPH and identifying drug targets.

Aberrant pulmonary vascular remodeling in CTEPH involves hyperproliferation and migration of human pulmonary artery smooth muscle cells (HPASMCs) [9, 10], leading to medial hypertrophy and neointimal formation, which further narrows the vascular lumen. Autophagy is a core process that maintains cellular homeostasis and stress adaptation [11]. Recent research has increasingly highlighted that dysregulation of autophagic activity is correlated with pulmonary vascular remodeling. Previous studies demonstrated downregulation of autophagy markers in a CTEPH rat model, indicating insufficient autophagic activity in CTEPH [12]. Furthermore, autophagy marker levels were negatively associated with the severity of vascular wall thickening [13], suggesting a correlation between autophagy and vascular remodeling. Notably, the expression of tissue factor (TF), a critical initiator of coagulation that is upregulated in CTEPH, was negatively correlated with autophagy marker expression in the CTEPH rat model [13]. TF has been shown to regulate autophagy in pulmonary artery endothelial cells through the p38 mitogen-activated protein kinase (p38 MAPK)/forkhead box O1 (FOXO1) pathway [12], creating a direct molecular link between pro-thrombotic stimulation and autophagic flux. However, current evidence largely remains correlative, indicating that autophagic changes accompany vascular remodeling. Significant knowledge gaps persist regarding the upstream regulatory factors of autophagy and the precise mechanistic cascade through which autophagy specifically drives pro-remodeling phenotypes such as proliferation and migration in vascular cells.

Long non-coding RNAs (lncRNAs) are involved in regulating autophagy in cardiovascular diseases [14, 15]. Among them, lncRNA autophagy-promoting factor (APF) has been established as a direct upstream regulator of a specific autophagic pathway. Earlier research showed that APF suppressed miR-188-3p expression and increased autophagy-related gene 7 (ATG7) levels in cardiomyocytes [16], which is essential for autophagosome formation [16]. Additionally, APF is associated with the development of abnormal myocardial remodeling [17, 18], which involves phenotypic transformation of cardiomyocytes, VSMCs, endothelial cells, and cardiac fibroblasts. This defined APF/miR-188-3p/ATG7 axis has been validated as a regulatory module controlling autophagic cell death in myocardial infarction [16]. However, the function of APF in the pulmonary vasculature and in CTEPH progression has not been explored. Given the established role of APF in orchestrating autophagy through miR-188-3p and ATG7 in other diseases, the APF/miR-188-3p/ATG7 axis may be similarly dysregulated in the CTEPH microenvironment, contributing to pathological autophagic flux and subsequent vascular cell dysfunction.

To test this hypothesis, this study comprehensively analyzed the levels of the APF/miR-188-3p/ATG7 axis in pathologic samples from CTEPH patients. We investigated its effects on proliferation, migration, and autophagy activity of HPASMCs. Our study aimed to reveal a previously uncharacterized molecular mechanism driving pulmonary vascular remodeling in CTEPH, identifying the APF/miR-188-3p/ATG7 axis as a new therapeutic target.

Materials and methods

Clinical samples

The central, organized thrombotic and endothelialized lesional tissues were collected from CTEPH patients (n = 30) undergoing pulmonary endarterectomy (PEA) at the First Affiliated Hospital of Jiamusi University (Jiamusi, China) between March 2024 and January 2025. As paired internal controls, adjacent, relatively normal distal small pulmonary arteries (n = 30) were carefully dissected and collected during the same operation. Inclusion criteria for subjects: (1) Confirmed diagnosis of CTEPH was made by right heart catheterization, chronic organized thrombi/emboli on ventilation/perfu-

sion lung scan, and computed tomography pulmonary angiography (CTPA); (2) Age between 18 and 75 years old; (3) Scheduled for and undergoing PEA. Exclusion criteria: (1) Other significant pulmonary diseases, such as idiopathic pulmonary arterial hypertension, severe chronic obstructive pulmonary disease, or interstitial lung disease; (2) Any serious systemic illness. Approval was obtained from the Ethics Committee of the First Affiliated Hospital of Jiamusi University. All participants gave written informed consent.

Isolation of HPASMCs

Medial tissue segments of the thickened vascular wall were taken from the lesional pulmonary artery of six CTEPH patients. Tissue specimens were minced into 1 mm^3 cubes. HPASMCs were isolated by enzymatic dissociation using 0.1% collagenase II (Sigma-Aldrich, USA) plus 0.05% trypsin (Gibco, USA) at 37°C for 30-45 min, followed by neutralization with complete smooth muscle cell medium to stop digestion. Subsequently, the cell suspension was collected and maintained in smooth muscle cell medium (ScienCell) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C in 5% CO_2; the medium was replenished every other day. Passages 3 to 6 of HPASMCs exhibited a typical elongated, spindle-shaped morphology and were selected for subsequent experiments. Cell homogeneity was confirmed by immunofluorescence labeling of α-smooth muscle actin (α-SMA) and smooth muscle 22α (SM22α); at least 90% labeled cells were considered suitable.

Cell transfection

Overexpression plasmids for APF (oe-APF) and ATG7 (oe-ATG7), miR-188-3p mimic (5'-GUC CCA CAU GCA GGG UUU GCA-3'), miR-188-3p inhibitor (5'-UGC AAA CCC UGC AUG UGG GAG-3'), small interfering RNAs for APF (si-APF; 5'-TTG TGA GAC TAC CTC ACT G-3') [16], and their corresponding negative control (NC) products were provided by GenePharma Company Limited in Shanghai, China. HPASMCs grown in 96-well plates reached approximately 70-80% confluence and were transfected with the aforementioned molecules or oligonucleotides using Lipofectamine 3000 reagent (Invitrogen).

Real-time quantitative polymerase chain reaction (RT-qPCR)

Extraction of total RNA was conducted by using TRIzol reagent (Invitrogen, USA). For mRNA testing (APF and ATG7), total RNA was reverse-transcribed to cDNA using PrimeScript RT Master Mix (Takara, Japan). Quantitative PCR was performed using a QuantStudio 5 real-time PCR system (Applied Biosystems, USA) and SYBR Green-based qPCR master mix (Applied Biosystems, USA). Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method. Primer sequences are as follows: APF, forward: 5'-CTC CTC TAT ATT GTG TTC AAG-3', reverse: 5'-GTG TTC ATA AGG TAA CCT GAA-3'; ATG7, forward: 5'-TCC GGG GAT TTC TTT CAC GG-3', reverse: 5'-GCT TCA TCC AGA GCC GAA GA-3'; GAPDH, forward, 5'-CGG AGT CAA CGG ATT TGG TCG TAT-3', reverse, 5'-AGC CTT CTC CAT GGT GGT GAA GAC-3'; miR-188-3p, forward: 5'-ATT ATT GGC TCC CAC ATG CAG GG-3', reverse: 5'-ATC CAG TGC AGG GTC CGA GG-3'; U6, forward: 5'-GCT TCG GCA GCA CAT ATA CTA A-3', reverse: 5'-AAC GCT TCA CGA ATT TGC GT-3'.

Western blot analysis

Cell lysates were extracted using RIPA lysis buffer (Beyotime) to purify proteins. Proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). The membranes were blocked and then incubated with specific primary antibodies (Cell Signaling Technology) at 4°C overnight: ATG7 (1:1000; #8558), LC3 (1:1000; #3868), p62 (1:1000; #5114), GAPDH (1:1000; #2118). Following incubation with secondary antibodies, signals were visualized using an enhanced chemiluminescence system (Thermo Fisher Scientific), and gray intensity was analyzed using ImageJ software.

Cell Counting Kit-8 (CCK-8) assay

HPASMCs (5×10^3/well) in 96-well plates were cultured for 24 hours, followed by the addition of 10 μL CCK-8 reagent (Sigma-Aldrich). After incubation for 2 hours, the absorbance at 450 nm was measured using a microplate reader (Biotek, USA).

Migration assay

When transfected cells reached 100% confluence in a 24-well plate, a scratch was made

and washed to remove detached cells; then, serum-starved medium (ScienCell) was added. Wound closure was observed and images were taken by microscopy (Olympus, Tokyo, Japan) at 0 h and 24 h. Migration distances were compared using ImageJ.

Invasion assay

Matrigel-coated transwell chambers with 8-μm pore size (Corning, USA) were used. A quantity of 5×10^4 treated cells was added to the upper chamber in serum-free medium, while medium containing 10% FBS was placed in the lower chamber. After 48 hours, invaded cells were stained and counted using a microscope (Olympus, Japan) and ImageJ software.

Luciferase reporter assay

Luciferase reporter constructs containing wild-type or mutant forms of APF/ATG7 were generated. Each constructed plasmid was verified by Sanger sequencing. HPASMCs were co-transfected with the luciferase reporter vector and miR-188-3p mimic. Luciferase activity was measured using a dual-luciferase reporter assay system (Promega, USA).

RNA pull-down assay

For the pull-down reaction, 50 μL of streptavidin-coupled agarose beads (Thermo Fisher Scientific) were incubated with 2 μg of biotin-labeled APF probes (GenePharma, Shanghai, China) at 4°C for 2 h to form probe-bead complexes. The complexes were then incubated with 500 μg of cellular protein extract (containing RNA and protein complexes) at 4°C overnight with gentle rotation. After incubation, the pulled-down RNA complexes were washed, and RNA products were eluted from the beads. RT-qPCR was performed to detect the enrichment of miR-188-3p in the pulled-down complexes.

Statistical analysis

All quantified analyses were performed using SPSS 25.0. Results are presented as mean ± standard deviation (SD). Comparisons between two groups were analyzed using Student's t-test. For comparisons among more than two groups, one-way analysis of variance (ANOVA) with Tukey's post hoc test was applied. $P<0.05$ was considered significant.

Results

Differential expression and correlations of lncRNA APF, miR-188-3p, and ATG7 mRNA in clinical CTEPH samples

To determine the function of the APF/miR-188-3p/ATG7 axis in CTEPH, we first examined whether there were notable differences in expression patterns across vessels from CTEPH patients. Compared to the relatively normal distal pulmonary artery branches, the organized thrombotic lesions showed a different molecular profile. APF was significantly reduced in the pathological tissues (**Figure 1A**). In contrast, miR-188-3p expression was elevated (**Figure 1B**). Accordingly, the mRNA level of its target gene, ATG7, was notably lower than that in normal tissues (**Figure 1C**). Furthermore, APF expression was negatively correlated with miR-188-3p levels across patient samples (**Figure 1D**). miR-188-3p levels were also negatively correlated with ATG7 mRNA expression (**Figure 1E**). Consistent with the proposed axis, there was a stable positive correlation between APF and ATG7 mRNA expression (**Figure 1F**). Collectively, these clinical findings suggest a disordered APF/miR-188-3p/ATG7 pathway in CTEPH vascular lesions, where reduced APF may promote elevation of miR-188-3p levels and subsequently inhibit ATG7 expression.

APF overexpression inhibits proliferation and migration but promotes autophagy in HPASMCs

Primary HPASMCs from CTEPH patients exhibited a clear spindle shape and were confirmed by α-SMA and SM22α immunofluorescence staining; cell purity exceeded 90% in both cases (**Figure 2A**). RT-qPCR confirmed effective upregulation and silencing of APF in cultured HPASMCs (**Figure 2B**). Biological assays demonstrated that APF knockdown significantly stimulated HPASMC proliferation, whereas APF overexpression suppressed it (**Figure 2C**). The migratory (**Figure 2D**, **2E**) and invasive capacities (**Figure 2F**, **2G**) of HPASMCs were significantly enhanced after APF knockdown and reduced after APF overexpression. We then

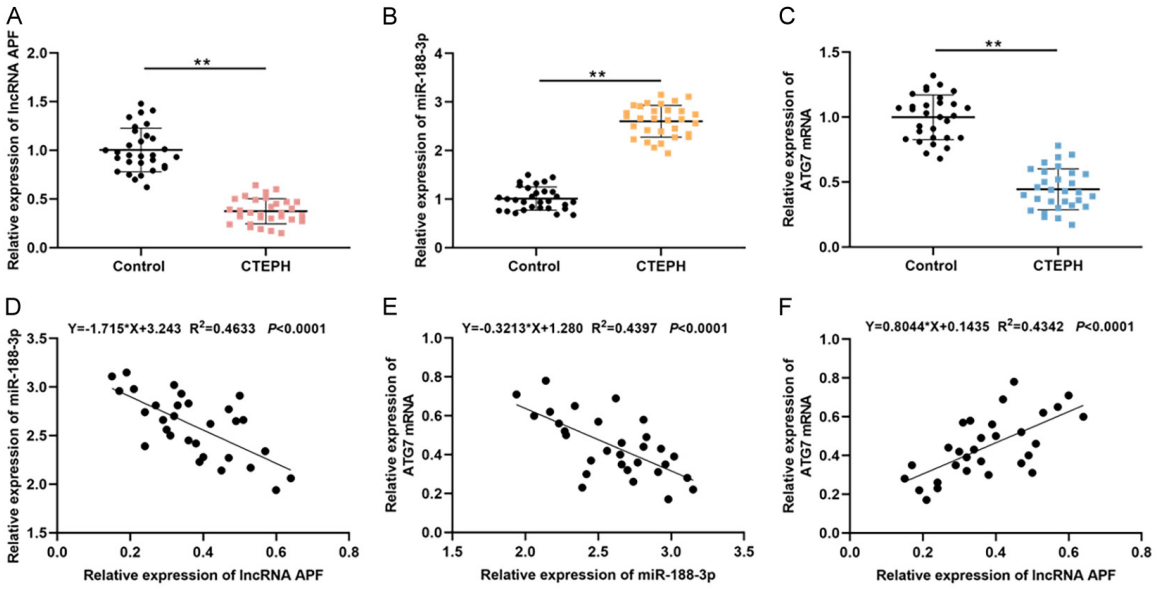

Figure 1. Expression and correlation of the long non-coding RNA autophagy promoting factor (lncRNA APF)/miR-188-3p/autophagy-related gene 7 (ATG7) axis in clinical chronic thromboembolic pulmonary hypertension (CTEPH) tissues. (A-C) Relative expression levels of lncRNA APF (A), miR-188-3p (B), and ATG7 mRNA (C) in paired vascular tissues from CTEPH patients (n = 30), as determined by real-time quantitative polymerase chain reaction (RT-qPCR) (n = 30). (D-F) Pearson correlation analysis showing the relationships between the expression levels of APF and miR-188-3p (D), miR-188-3p and ATG7 mRNA (E), and APF and ATG7 mRNA (F) across all diseased samples. Data are presented as mean ± standard deviation (SD). $^{**}P<0.01$.

examined whether autophagy regulates these effects by assessing key components of autophagosomes. Western blotting revealed that APF knockdown suppressed autophagy, as demonstrated by a reduced LC3-II/I ratio and elevated p62 abundance (**Figure 2H**, **2I**). APF overexpression showed the opposite results. Therefore, APF restricts the abnormal behavior of HPASMCs and enhances autophagy.

LncRNA APF directly binds to and represses miR-188-3p in HPASMCs

The specific binding sites for miR-188-3p in the APF sequence were predicted using the miRcode database (**Figure 3A**). Dual transfection of the APF-wild type vector with the miR-188-3p mimic reduced firefly luciferase activity compared with the negative control. However, this effect was weakened after modification of the binding sites (mutant 1, mutant 2, or mutant 1+2) (**Figure 3B**). The RNA pull-down experiment indicated a significant increase in miR-188-3p bound to the APF probe compared with the control NC probe (**Figure 3C**). In addition, APF overexpression decreased miR-188-3p levels, and APF knockdown elevated them (**Figure 3D**). These results indicate that APF directly represses miR-188-3p in HPASMCs.

APF knockdown promotes proliferation and migration but inhibits autophagy in HPASMCs through upregulation of miR-188-3p expression

We confirmed that miR-188-3p is the main functional downstream target of APF by performing a rescue experiment. APF knockdown increased miR-188-3p expression, which was reduced by co-transfection with the miR-188-3p inhibitor (**Figure 4A**). APF depletion induced HPASMC proliferation (**Figure 4B**), migration (**Figure 4C**, **4D**), and invasion (**Figure 4E**, **4F**). Importantly, these effects were abolished after silencing miR-188-3p. Additionally, APF depletion reduced the LC3-II/I ratio and elevated p62 levels, indicating diminished autophagic flux, whereas miR-188-3p inhibition restored autophagic capacity (**Figure 4G-I**). Collectively, APF knockdown promotes proliferation and migration in HPASMCs by increasing miR-188-3p levels.

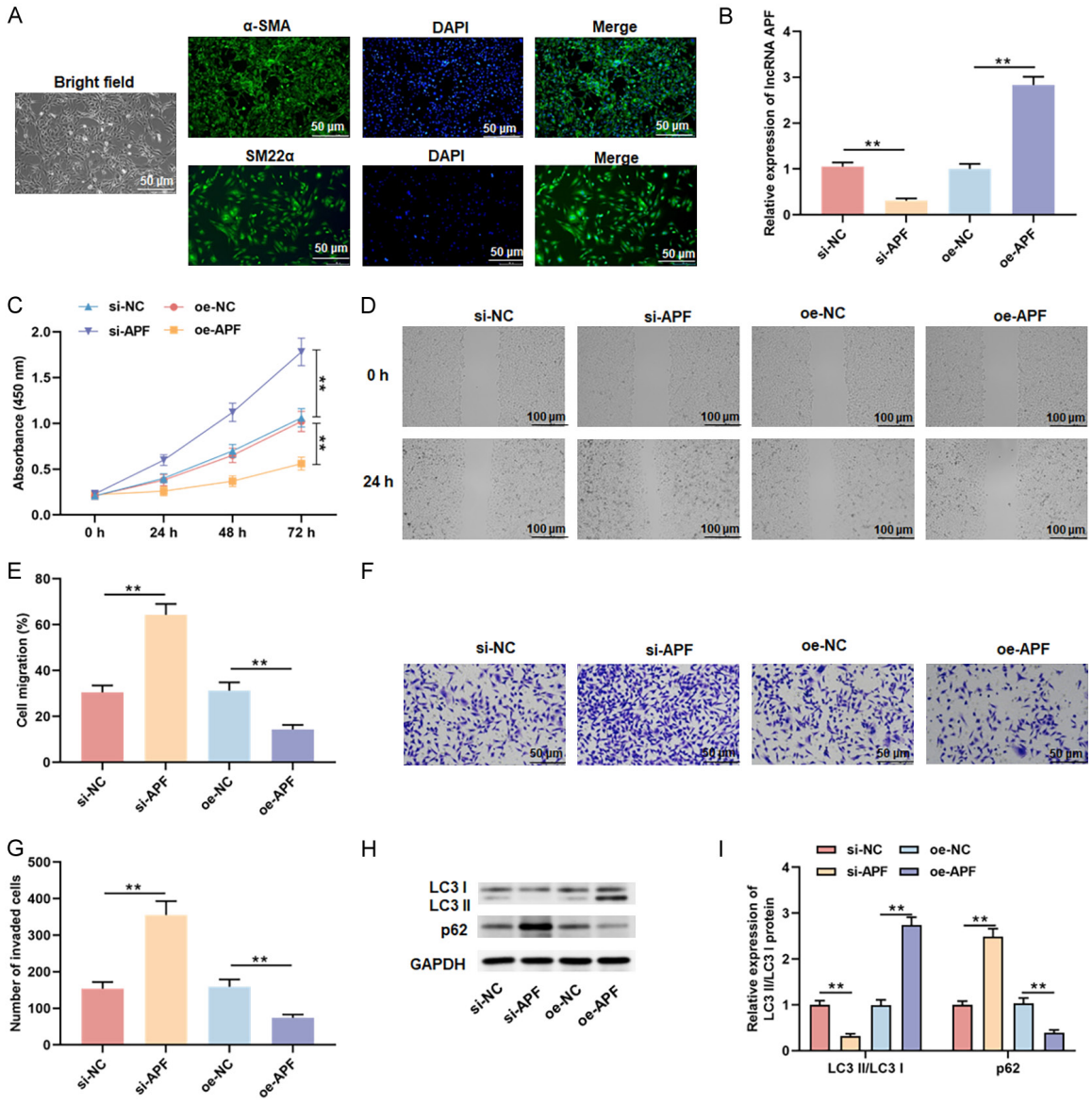

Figure 2. APF overexpression inhibits proliferation and migration and promotes autophagy in human pulmonary artery smooth muscle cells (HPASMCs). A. Representative image showing the characteristic spindle-shaped morphology of primary HPASMCs isolated from CTEPH lesions, and representative immunofluorescence image confirming HPASMC identity by α-smooth muscle actin (α-SMA) and smooth muscle 22α (SM22α) staining. B. RT-qPCR analysis verifying the efficiency of APF overexpression (oe-APF) and knockdown (si-APF) in HPASMCs. C. HPASMC proliferation was assessed using Cell Counting Kit-8 (CCK-8) assay. D, E. HPASMC migration was examined using wound healing assay. F, G. HPASMC invasion was evaluated using Transwell assay. H, I. Western blot analysis of LC3-I/II and p62 protein levels. Data are presented as mean ± SD. $^{**}P<0.01$. APF, autophagy-promoting factor.

miR-188-3p directly targets and downregulates ATG7 in HPASMCs

We next investigated whether ATG7 was a target of miR-188-3p in HPASMCs. A conserved binding motif for miR-188-3p within the 3'-untranslated region (3'-UTR) of ATG7 mRNA was predicted using the TargetScan database (**Figure 5A**). Successful overexpression and inhibition of miR-188-3p in HPASMCs were verified (**Figure 5B**). Thereafter, miR-188-3p upregulation significantly reduced the luciferase activity of the wild-type ATG7 reporter plasmid, whereas no such effect was observed in the mutant group (**Figure 5C**). Furthermore, elevation of miR-188-3p reduced ATG7 mRNA and

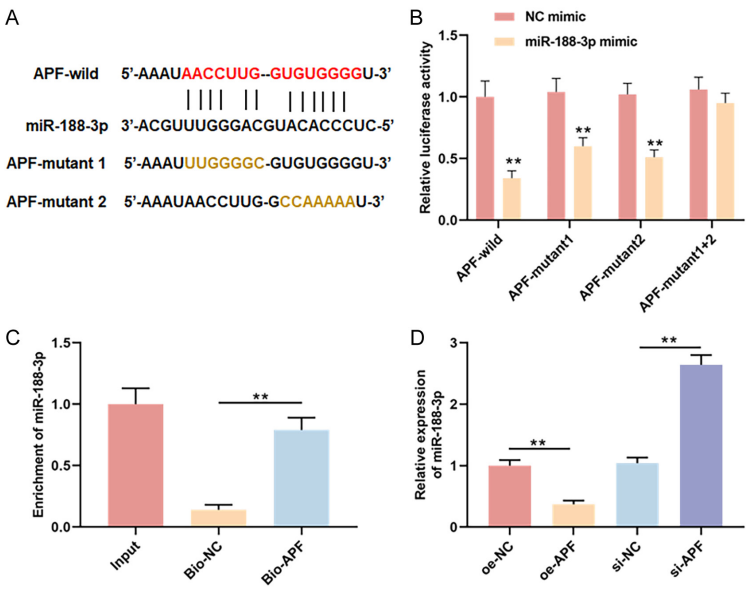

Figure 3. LncRNA APF directly binds to and negatively regulates miR-188-3p in HPASMCs. A. Schematic diagram of the predicted binding site between APF and miR-188-3p using miRcode database, and the designed mutation sites (mutant 1, mutant 2) for the luciferase reporter assay. B. Luciferase activity was measured in HPASMCs co-transfected with either the wild or mutant APF reporter vectors and miR-188-3p/NC mimic using dual-luciferase reporter assay. C. RNA pull-down assay was conducted to confirm the specific binding. D. RT-qPCR analysis showing the expression level of miR-188-3p in HPASMCs. Data are presented as mean ± SD. $^{**}P<0.01$. HPASMCs, human pulmonary artery smooth muscle cells; APF, autophagy-promoting factor.

protein expression (**Figure 5D-F**). In addition, miR-188-3p knockdown significantly increased ATG7 expression. Therefore, miR-188-3p directly targeted and suppressed ATG7 in HPASMCs.

miR-188-3p overexpression promotes proliferation and migration but suppresses autophagy in HPASMCs by inhibiting ATG7 expression

We determined whether miR-188-3p exerted its biological effects through ATG7 by performing rescue assays. ATG7 protein levels decreased after miR-188-3p upregulation and were then restored by transfection with ATG7-overexpressing plasmids (**Figure 6A**, **6B**). Consistently, miR-188-3p overexpression significantly enhanced the proliferation, migration, and invasion of HPASMCs (**Figure 6C-G**). Interestingly, these promoting effects were counteracted by simultaneous ATG7 expression. miR-188-3p overexpression increased LC3-II and decreased p62 levels, indicating impaired autophagic degradation. Critically, this miR-188-3p-mediated autophagy inhibition was reversed by restoring ATG7 expression (**Figure 6H-J**). Collectively, these results indicate that the disease-related characteristics induced by miR-188-3p-mediated inhibition of ATG7 depend on this regulatory mechanism.

Autophagy activation by rapamycin abolishes the effects of APF knockdown on proliferation, migration, and autophagy in HPASMCs

To confirm whether autophagy can regulate the APF/miR-188-3p/ATG7 pathway, we employed the pharmacologic autophagy activator rapamycin. As shown, APF silencing suppressed autophagy. Administration of rapamycin effectively restored autophagic capacity, significantly increasing the LC3-II/LC3-I ratio and decreasing p62 expression (**Figure 7A**, **7B**). Functionally, the increased cell proliferation (**Figure 7C**), motility (**Figure 7D**, **7E**), and invasion (**Figure 7F**, **7G**) induced by APF knockdown were all significantly reduced following rapamycin treatment. These results indicate that suppression of autophagic flux is a necessary downstream event through which the APF/miR-188-3p/ATG7 axis regulates HPASMC proliferation and migration.

Discussion

The development of CTEPH forms a vicious circle through persistent vessel occlusion, incomplete thrombus organization, and progressive vascular remodeling. Although a thrombotic factor is essential to initiate damage, the pathophysiologic changes in the endothelium and HPASMCs are the underlying causes of persistent increases in pulmonary vascular resistance. This study revealed that the lncRNA APF/miR-188-3p/ATG7 axis was dysregulated in CTEPH and served as a critical regulator of HPASMC phenotype, directly linking non-coding RNA-mediated gene regulation to autophagy and vascular remodeling in this disease.

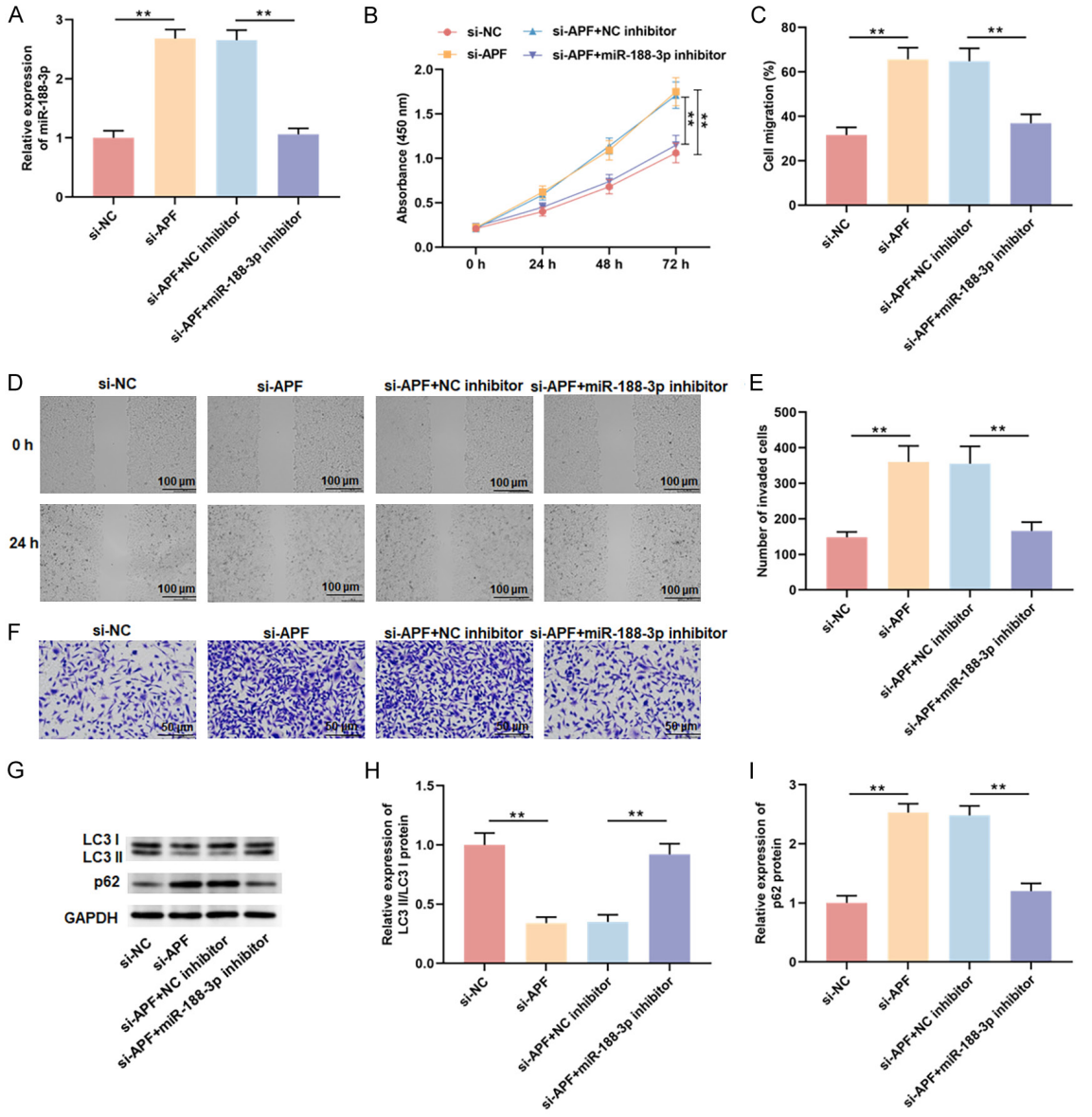

Figure 4. APF knockdown promotes proliferation and migration and inhibits autophagy in HPASMCs by upregulating miR-188-3p expression. A. RT-qPCR analysis showing the expression level of miR-188-3p in HPASMCs. B. HPASMC proliferation was assessed using CCK-8 assay. C, D. HPASMC migration was examined using wound healing assay. E, F. HPASMC invasion was evaluated using a Transwell assay. G-I. Western blot analysis of LC3-I/II and p62 protein levels. Data are presented as mean ± SD. $^{**}P<0.01$. HPASMCs, human pulmonary artery smooth muscle cells; APF, autophagy-promoting factor.

Accelerated proliferation, enhanced migration, and invasiveness are typical features of HPASMCs in pulmonary vessel remodeling, contributing directly to medial hypertrophy and neointima formation [9, 19, 20]. Our findings define lncRNA APF as a novel endogenous regulator of these pathologic processes. We first observed a significant downregulation of APF in the organized thrombotic lesions of CTEPH patients compared to distal normal vessels. This clinical observation was functionally validated *in vitro*, showing that APF knockdown in primary CTEPH-derived HPASMCs significantly stimulated proliferation and migration, consistent with the emerging role of specific lncRNAs as context-dependent regulators of vascular smooth muscle cell phenotype. For instance, downregulation of lncPTSR has been shown to

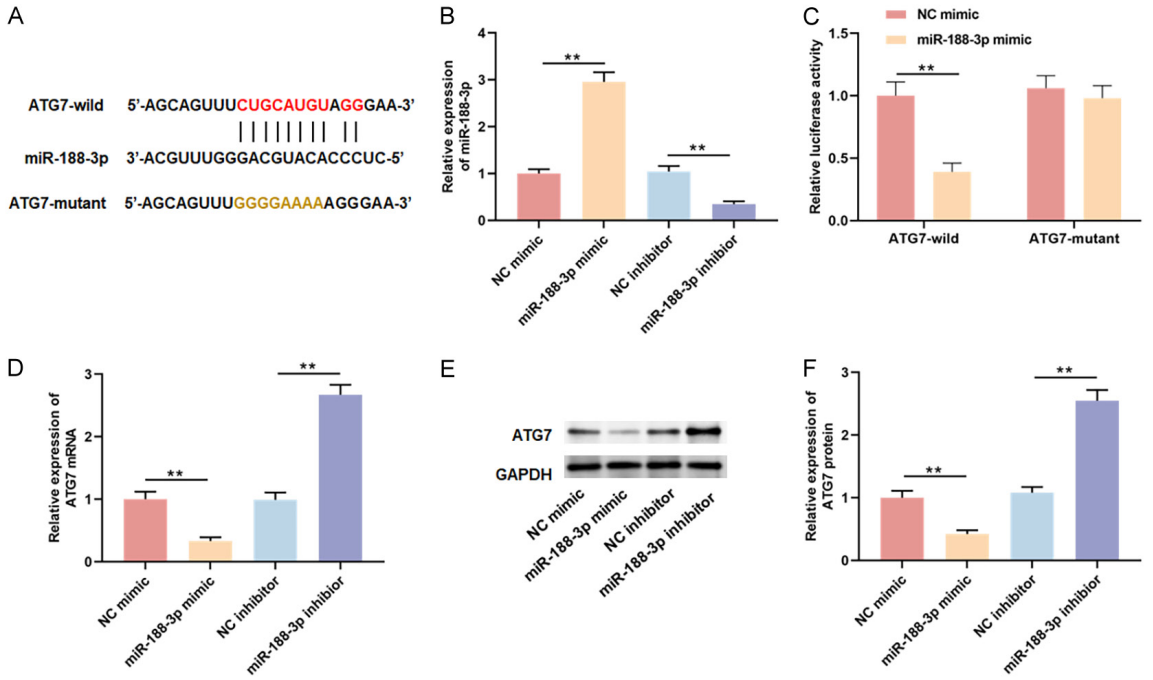

Figure 5. miR-188-3p directly targets and downregulates ATG7 in HPASMCs. A. Schematic diagram of the predicted binding site for miR-188-3p and ATG7 mRNA using TargetScan database, and the designed mutant sequence for the reporter assay. B. RT-qPCR analysis validating the transfection efficiency of the miR-188-3p mimic and inhibitor in HPASMCs. C. Luciferase activity was measured in HPASMCs co-transfected with either the wild or mutant ATG7 reporter vectors and miR-188-3p/NC mimic using dual-luciferase reporter assay. D. RT-qPCR analysis of ATG7 mRNA expression. E, F. Western blot analysis of ATG7 protein expression. Data are presented as mean ± SD. $^{**}P<0.01$. HPASMCs, human pulmonary artery smooth muscle cells; APF, autophagy-promoting factor.

promote PASMC proliferation and migration, resulting in potent pulmonary vascular remodeling [21], while lncRNA ZFAS1 promotes the proliferation and migration of VSMCs in an atherosclerosis model [22]. Our study highlights APF as a central suppressor of pathologic HPASMC phenotypes in the CTEPH microenvironment. The effects of APF knockdown were abrogated by suppression of its downstream target miR-188-3p, establishing a clear functional cascade and revealing the significance of this axis in driving CTEPH-relevant cellular phenotypes.

Autophagy in vascular remodeling plays complex and context-dependent roles [23, 24]. It can serve as a protective mechanism by clearing damaged organelles and proteins, yet excessive or insufficient autophagy can promote cell death or survival, respectively, influencing disease outcomes [25, 26]. In CTEPH, autophagy was reduced in CTEPH rats, as evidenced by downregulated Beclin-1 and LC3-II protein levels [13]. Our results add crucial mechanistic depth to this correlation. We confirmed that APF knockdown led to suppressed autophagic flux (reduced LC3-II/I, increased p62) in HPASMCs. Importantly, this suppression was functionally required for the pro-proliferative and pro-migratory effects, as reactivation of autophagy with rapamycin abolished these phenotypes. This study not only confirms the critical protective role of functional autophagy in restraining abnormal HPASMC proliferation and migration, but more importantly, reveals that in the pathologic context of CTEPH, the APF/miR-188-3p/ATG7 axis serves as a specific upstream molecular switch regulating this autophagic homeostasis. Dysregulation of this axis leads to suppressed autophagic flux, which in turn releases the inherent constraints on the proliferative and migratory phenotypes of HPASMCs, thereby actively driving and accelerating the pathologic progression of pulmonary vascular remodeling. This mechanism reveals that autophagy activation may be a promising therapeutic target in CTEPH. To better contextualize our findings, we first summa-

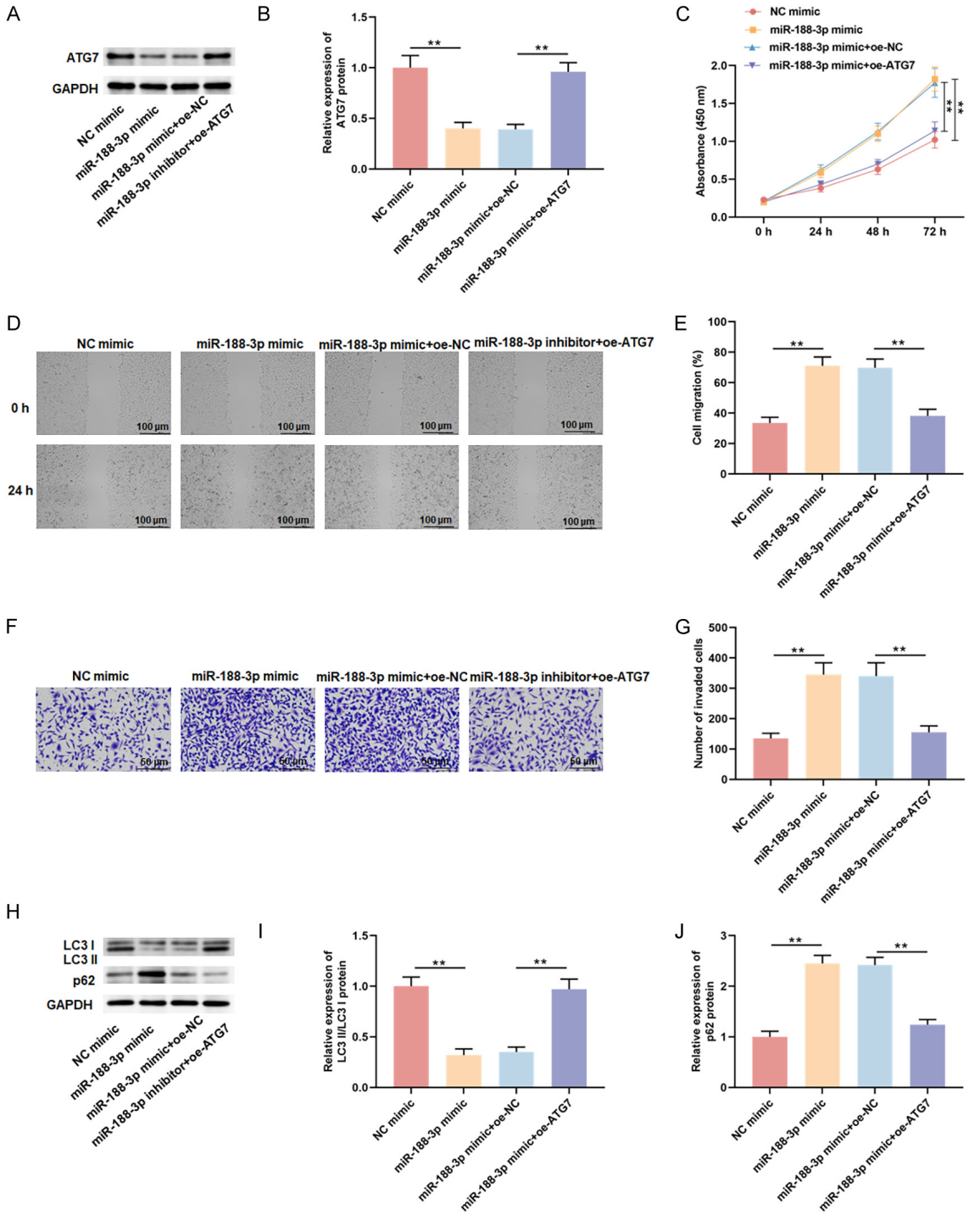

Figure 6. miR-188-3p overexpression promotes proliferation and migration and suppresses autophagy in HPASMCs by inhibiting ATG7 expression. A, B. Western blot analysis of ATG7 protein levels in HPASMCs. C. HPASMC proliferation was assessed using CCK-8 assay. D, E. HPASMC migration was examined using wound healing assay. F, G. HPASMC invasion was evaluated using Transwell assay. H-J. Western blot analysis of LC3-I/II and p62 protein levels. Data are presented as mean ± SD. $^{**}P<0.01$. HPASMCs, human pulmonary artery smooth muscle cells.

rize the established roles of the lncRNA growth arrest-specific transcript 5 (GAS5)/miR-382-3p/ATG7 axis. Specifically, this axis restrains proliferation and promotes autophagy in

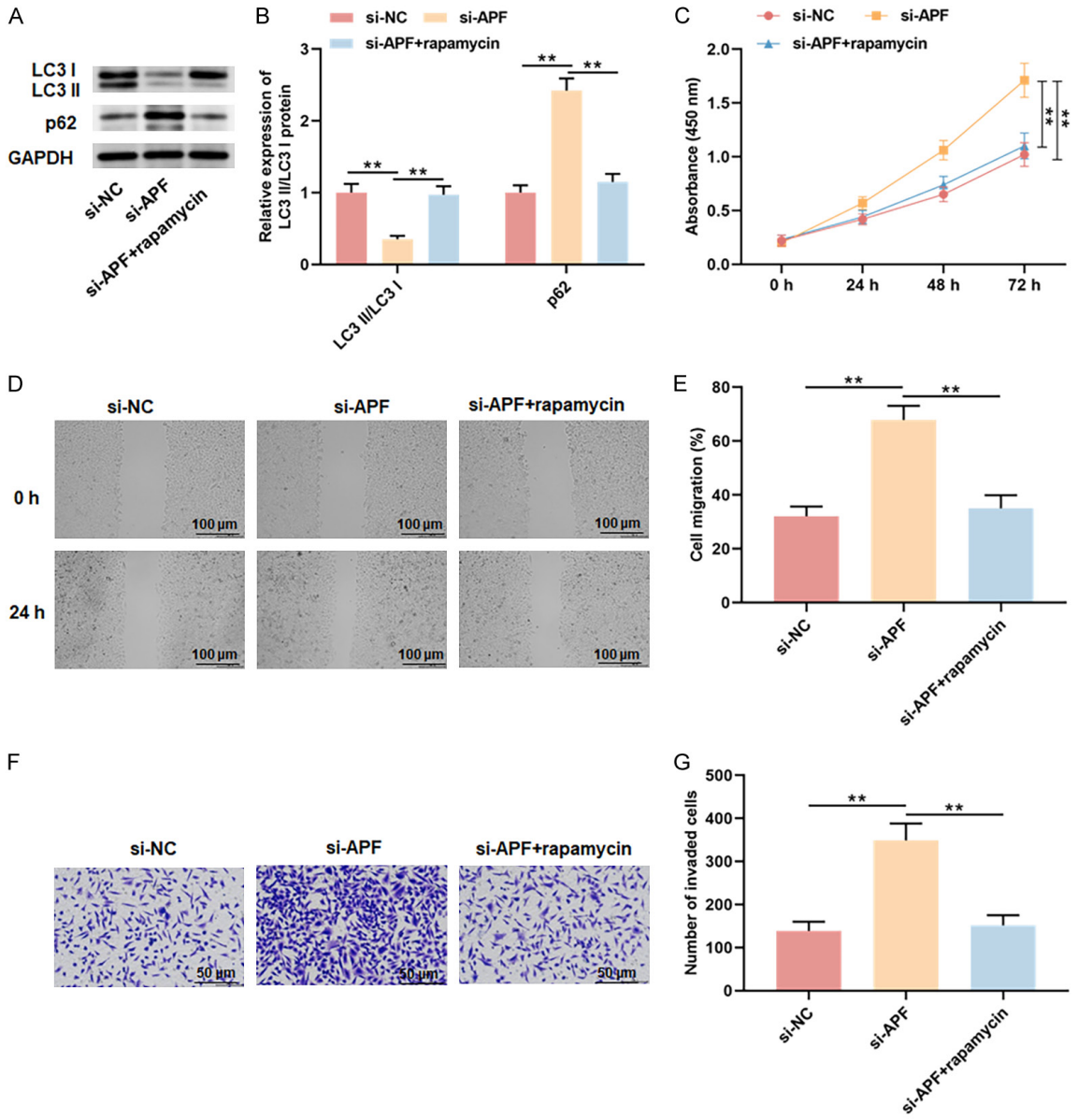

Figure 7. Autophagy activation by rapamycin abolishes the effects of APF knockdown on proliferation, migration, and autophagy in HPASMCs. A, B. Western blot analysis of LC3-I/II and p62 protein levels following treatment with the autophagy activator rapamycin. C. HPASMC proliferation was assessed using CCK-8 assay. D, E. HPASMC migration was examined using wound healing assay. F, G. HPASMC invasion was evaluated using Transwell assay. ***P*<0.01. HPASMCs, human pulmonary artery smooth muscle cells; APF, autophagy-promoting factor.

PASMCs, leading to suppression of pulmonary artery remodeling [27]. In addition, GAS5 promotes spermidine-induced autophagy in pulmonary artery endothelial cells from CTEPH patients through the miRNA-31-5p/N-acetyl aspartate transferase 8 like (NAT8L) axis [28]. Meanwhile, a previous study also demonstrated that miR-382-3p overexpression promoted HPASMC proliferation and migration by inhibiting ATG7, thereby driving pulmonary vascular remodeling in CTEPH [29]. Notably, our study identified ATG7 as a key downstream target of the APF/miR-188-3p pathway regulating autophagy in HPASMCs. Overexpression of ATG7 effectively reversed the pro-proliferative and pro-migratory effects of miR-188-3p. Based on our study, we found that the APF/miR-188-3p/ATG7 axis was disrupted in this disease and led

to impaired basal autophagy, promoting abnormal pulmonary vascular remodeling. When comparing the GAS5/miR-382-3p/ATG7 axis to our APF/miR-188-3p/ATG7 axis, both followed a similar ceRNA regulatory logic: a lncRNA (GAS5 or APF) sponges a specific miRNA to modulate ATG7-dependent autophagy. However, distinct differences exist. The GAS5 axis has been implicated in both PASMCs and endothelial cells, whereas our APF axis focused on HPASMCs. Moreover, our study specifically demonstrated that the rescue of autophagy using rapamycin reversed the pro-proliferative and pro-migratory effects of APF knockdown in HPASMCs, providing direct functional evidence that suppression of autophagic flux is a necessary downstream event through which APF regulates HPASMC proliferation and migration.

The basic molecular relationship of the APF/miR-188-3p/ATG7 network in cardiomyocytes after myocardial infarction has been confirmed and shown to affect autophagic cell death regulation [16]. This is the first time such a well-established mechanism has been reported for pulmonary vascular disease. We systematically validated each interaction of this regulatory axis in HPASMCs. APF sponges miR-188-3p to competitively alleviate its post-transcriptional suppression of ATG7. Crucially, the expression pattern observed in clinical CTEPH tissues, concomitant downregulation of APF and ATG7 with upregulation of miR-188-3p, aligns with and provides *in vivo* corroboration for this mechanistic model. The strong negative correlations between miR-188-3p and APF/ATG7, as well as the positive correlation between APF and ATG7, strongly suggested that this axis is operatively disrupted in the diseased vascular microenvironment. In the hypoxic, inflammatory, and thrombotic microenvironment of CTEPH, the downregulation of APF may represent a maladaptive response, tipping the balance of the axis towards miR-188-3p dominance, ATG7 suppression, and consequently, impaired autophagy and unchecked HPASMC activation.

This study had several limitations. First, although a large number of cases were available, more samples need to be collected in the future to verify whether this axis is related to prognosis. Second, our functional analyses were conducted primarily in HPASMCs. Given the importance of endothelial dysfunction and endothelial-smooth muscle cell crosstalk in CTEPH, it is necessary to investigate the role of this axis in pulmonary artery endothelial cells. Third, genetic methods in animal subjects are needed to verify the *in vivo* functions of the APF axis during CTEPH progression. An ideal mouse model of CTEPH is lacking as a barrier; however, new approaches such as combined embolization and vascular injury models may be pursued [30]. In addition, although this study focused on the ceRNA pathway, it has not explored how APF may regulate genes through other pathways in vascular cells.

In summary, we demonstrated that lncRNA APF sponges miR-188-3p to alleviate its suppression of ATG7 in HPASMCs. The downregulation of APF in CTEPH promotes excessive proliferation, migration, and invasion in HPASMCs by inhibiting autophagy through the miR-188-3p/ATG7 axis. This dysregulated APF/miR-188-3p/ATG7/autophagy axis creates a permissive environment for the pathological vascular remodeling characteristic of CTEPH. This work identifies the APF/miR-188-3p/ATG7/autophagy axis as a promising target for future diagnostic and therapeutic development.

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Disclosure of conflict of interest

None.

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References

[1] Weatherald J, Hemnes AR, Maron BA, Mielniczuk LM, Gerges C, Price LC, Hoeper MM and Humbert M. Phenotypes in pulmonary hypertension. Eur Respir J 2024; 64: 2301633.

[2] Estrada RA, Auger WR and Sahay S. Chronic thromboembolic pulmonary hypertension. JAMA 2024; 331: 972-973.
[3] Hosokawa K and Abe K. Milestones in therapeutic and antithrombotic strategies for chronic thromboembolic pulmonary hypertension. J Cardiol 2025; 86: 127-133.
[4] Mocumbi A, Humbert M, Saxena A, Jing ZC, Sliwa K, Thienemann F, Archer SL and Stewart S. Pulmonary hypertension. Nat Rev Dis Primers 2024; 10: 1.
[5] Gonzalez-Hermosillo LM, Cueto-Robledo G, Navarro-Vergara DI, Torres-Rojas MB, García-Cesar M, Pérez-Méndez O and Escobedo G. Molecular pathophysiology of chronic thromboembolic pulmonary hypertension: a clinical update from a basic research perspective. Adv Respir Med 2024; 92: 485-503.
[6] Liu C, Chen J, Huang X, Xia Q, Yang L, Guo J, Tian J, Wang J, Niu Y, Li L and Gou D. lncRNA VELRP modulates pulmonary arterial smooth muscle cell proliferation and promotes vascular remodeling in pulmonary hypertension. Arterioscler Thromb Vasc Biol 2024; 44: 2560-2576.
[7] Shen H, Gao Y, Ge D, Tan M, Yin Q, Wei TW, He F, Lee TY, Li Z, Chen Y, Yang Q, Liu Z, Li X, Chen Z, Yang Y, Zhang Z, Thistlethwaite PA, Wang J, Malhotra A, Yuan JX, Shyy JY and Gong K. BRCC3 regulation of ALK2 in vascular smooth muscle cells: implication in pulmonary hypertension. Circulation 2024; 150: 132-150.
[8] Kim H, Liu Y, Kim J, Kim Y, Klouda T, Fisch S, Baek SH, Liu T, Dahlberg S, Hu CJ, Tian W, Jiang X, Kosmas K, Christou HA, Korman BD, Vargas SO, Wu JC, Stenmark KR, Perez VJ, Nicolls MR, Raby BA and Yuan K. Pericytes contribute to pulmonary vascular remodeling via HIF2α signaling. EMBO Rep 2024; 25: 616-645.
[9] Viswanathan G, Kirshner HF, Nazo N, Ali S, Ganapathi A, Cumming I, Zhuang Y, Choi I, Warman A, Jassal C, Almeida-Peters S, Haney J, Corcoran D, Yu YR and Rajagopal S. Single-cell analysis reveals distinct immune and smooth muscle cell populations that contribute to chronic thromboembolic pulmonary hypertension. Am J Respir Crit Care Med 2023; 207: 1358-1375.
[10] Aimaier S, Mahemuti A, Abuduhalike R, Lu WK, Guo YJ and Zhao L. Notch signaling enhances PASMC proliferation and vascular remodeling in CTEPH. Open Life Sci 2026; 21: 20251251.
[11] Yang Y, Pan Z, Sun J, Welch J and Klionsky DJ. Autophagy and machine learning: unanswered questions. Biochim Biophys Acta Mol Basis Dis 2024; 1870: 167263.
[12] Wu D, Lin Y, Yang M, Li H, Wang W, Wu Q, Chen M, Shao N and Deng C. Tissue factor regulates autophagy in pulmonary artery endothelial cells from chronic thromboembolic pulmonary hypertension rats via the p38 MAPK-Fox01 pathway. Respir Res 2024; 25: 261.
[13] Deng C, Wu D, Yang M, Chen Y, Ding H, Zhong Z, Lian N, Zhang Q, Wu S and Liu K. The role of tissue factor and autophagy in pulmonary vascular remodeling in a rat model for chronic thromboembolic pulmonary hypertension. Respir Res 2016; 17: 65.
[14] Wang E, Zhang B, Huang L, Li P, Han R, Zhou S, Zeng D and Wang R. LncRNA MIR210HG promotes phenotype switching of pulmonary arterial smooth muscle cells through autophagy-dependent ferroptosis pathway. Apoptosis 2024; 29: 1648-1662.
[15] Wang Y, Zhang X, Ren M, He S, Bie H, Duan M, Chen Z, Jia Q, Chi B, Gan X, Li C, Fu Y, Zhou H, Zhang S, Zhang Q, An F, Chen X and Jia E. LncRNA LUCAT1 offers protection against human coronary artery endothelial cellular oxidative stress injury through modulating hsa-miR-6776-5p/LRRC25 axis and activating autophagy flux. J Transl Med 2024; 22: 1171.
[16] Wang K, Liu CY, Zhou LY, Wang JX, Wang M, Zhao B, Zhao WK, Xu SJ, Fan LH, Zhang XJ, Feng C, Wang CQ, Zhao YF and Li PF. APF lncRNA regulates autophagy and myocardial infarction by targeting miR-188-3p. Nat Commun 2015; 6: 6779.
[17] Zhou H, Wang B, Yang YX, Jia QJ, Zhang A, Qi ZW and Zhang JP. Long noncoding RNAs in pathological cardiac remodeling: a review of the update literature. Biomed Res Int 2019; 2019: 7159592.
[18] Shen S, Jiang H, Bei Y, Xiao J and Li X. Long non-coding RNAs in cardiac remodeling. Cell Physiol Biochem 2017; 41: 1830-1837.
[19] Gao RJ, Aikeremu N, Cao N, Chen C, Ma KT, Li L, Zhang AM and Si JQ. Quercetin regulates pulmonary vascular remodeling in pulmonary hypertension by downregulating TGF-β1-Smad2/3 pathway. BMC Cardiovasc Disord 2024; 24: 535.
[20] Zhou JJ, Jing L, Luo CL, He YH and Qian YL. NEK2 inhibition reverses vascular remodeling in pulmonary arterial hypertension associated with congenital heart disease. Cell Signal 2025; 131: 111720.
[21] Deng L, Chen J, Chen B, Wang T, Yang L, Liao J, Yi J, Chen Y, Wang J, Linneman J, Niu Y and Gou D. LncPTSR triggers vascular remodeling in pulmonary hypertension by regulating [Ca(2+)](i) in pulmonary arterial smooth muscle cells. Am J Respir Cell Mol Biol 2022; 66: 524-538.
[22] Wang H, Hu H, Ma J, Jiang Y and Cheng R. lncRNA ZFAS1 promotes the ox-LDL induced proliferation, invasion and migration of vascular

smooth muscle cells. Exp Ther Med 2021; 22: 835.
[23] Jiang B, Bai F, Hu Y, Ren Y, Su Y, Song W, Xie K, Wang D, Pan J, Liu Y, Feng Y, Li X, Zhang H, Zhu X, Bai H, Yang Q, Ben J and Chen Q. Endothelial major vault protein alleviates vascular remodeling via promoting Parkin-mediated mitophagy. Nat Commun 2025; 16: 4365.
[24] Mu SQ, Lin JJ, Wang Y, Yang LY, Wang S, Wang ZY, Zhao AQ, Luo WJ, Dong ZQ, Cao YG, Jiang ZA, Wang SF, Cao SH, Meng L, Li Y, Yang SY and Sun SG. Hsa_circ_0001304 promotes vascular neointimal hyperplasia accompanied by autophagy activation. Commun Biol 2025; 8: 146.
[25] Feng H, Wang N, Zhang N and Liao HH. Alternative autophagy: mechanisms and roles in different diseases. Cell Commun Signal 2022; 20: 43.
[26] Cao W, Li J, Yang K and Cao D. An overview of autophagy: mechanism, regulation and research progress. Bull Cancer 2021; 108: 304-322.
[27] Feng X, Wang K, Yang T, Liu Y and Wang X. LncRNA-GAS5/miR-382-3p axis inhibits pulmonary artery remodeling and promotes autophagy in chronic thromboembolic pulmonary hypertension. Genes Genomics 2022; 44: 395-404.
[28] Wu Q, Zhou X, Wang Y and Hu Y. LncRNA GAS5 promotes spermidine-induced autophagy through the miRNA-31-5p/NAT8L axis in pulmonary artery endothelial cells of patients with CTEPH. Mol Med Rep 2022; 26: 297.
[29] Feng X, Wang K, Yang T, Dang B and Wang X. Overexpressed microRNA (miR)-382-3p promoted vascular remodeling via suppressing autophagy-related protein 7 (ATG7) in chronic thromboembolic pulmonary hypertension. Curr Mol Med 2023; [Epub ahead of print].
[30] Zhang H, Lu X, Guo Z, Jiang X, Zhang W, Wang S, Liu Q, Dong X, Li Y, Guo L, Zhang Y, Liu J, Zhang Z, Xie W, Song W, Zhang H, Zhai Z and Yang P. Development of a clinically relevant rat model of chronic thromboembolic pulmonary hypertension by combining splenectomy with pulmonary thromboembolism. Thromb Res 2025; 249: 109310.