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Invited Review

Potential of the TRPM7 channel as a novel therapeutic target for pulmonary arterial hypertension

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Abstract

Pulmonary arterial hypertension (PAH) is an intractable vascular disease characterized by a progressive increase in pulmonary vascular resistance caused by pulmonary vascular remodeling, which ultimately leads to right-sided heart failure. PAH remains incurable, despite the development of PAH-targeted therapeutics centered on pulmonary artery relaxants. It is necessary to identify the target molecules that contribute to pulmonary artery remodeling. Transient receptor potential (TRP) channels have been suggested to modulate pulmonary artery remodeling. Our study focused on the transient receptor potential ion channel subfamily M, member 7, or the TRPM7 channel, which modulates endothelial-to-mesenchymal transition and smooth muscle proliferation in the pulmonary artery. In this review, we summarize the role and expression profile of TRPM7 channels in PAH progression and discuss TRPM7 channels as possible therapeutic targets. In addition, we discuss the therapeutic effect of a Chinese herbal medicine, *Ophiocordyceps sinensis* (OCS), on PAH progression, which partly involves TRPM7 inhibition.

Key words: TRPM7, pulmonary arterial hypertension, cardiovascular remodeling

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Overview of Pulmonary Arterial Hypertension (PAH)

Pulmonary hypertension is an intractable vascular disease characterized by a progressive increase in pulmonary vascular resistance (PVR). Pulmonary hypertension was divided into five groups according to clinical, hemodynamic, and etiological characteristics and treatment strategy, including idiopathic and heritable pulmonary arterial hypertension (PAH) (group 1), PAH due to left heart disease (group 2), PAH due to lung diseases and/or hypoxia (group 3), PAH associated with chronic thromboembolism (group 4), and PAH forms with unclear or multifaceted origins (group 5). The estimated incidence of primary PAH ranges from 1 to 2 cases per million people in the general population.

Under physiological conditions, blood flows from the right ventricle to the lungs via the pulmonary artery and contributes to the gas exchange. Endothelial cells generate constriction factors, such as endothelin (1), and relaxing factors, such as nitric oxide (NO) and prostacyclin (2). Under physiological conditions, endothelium-derived relaxing factors play an important role in maintaining tissue homeostasis, preventing thrombosis, and protecting vessel walls from remodeling. In contrast, PAH is characterized by vasoconstriction and vascular wall remodeling, which are associated with intimal and medial thickening and thrombosis, resulting in an increase in PVR and pulmonary arterial pressure (3).

The resultant thickening of the pulmonary arterial wall and narrowing of the vessel diameter increases the PVR and pulmonary arterial pressure to over 25 mmHg at rest (4–6). At the 6th World Symposium on Pulmonary Hypertension (Nice, France, 2018), it was recognized that the original hemodynamic definition of PAH was arbitrary, and it was recommended that the mean pulmonary artery pressure (MPAP) threshold should be lowered to 20 mmHg. Pulmonary arterial wedge pressure (PAWP) and PVR thresholds were ≤15 mmHg and >3 Wood units, respectively, as measured using right heart catheterization (7).

Increased cardiac afterload due to increased PVR causes right heart hypertrophy, which eventually results in fatal right heart failure. Delaying the progression of right ventricular insufficiency by targeting vasoconstriction and vascular remodeling is anticipated to alleviate symptoms and improve the survival of patients with PAH (8). Before the development of current therapeutic options, idiopathic PAH progressed rapidly, leading to right-sided heart failure and death. In a recent study, the survival rates of patients with PAH at 1-, 2, and 3 years of follow-up were 85.7%, 69.6%, and 54.9%, respectively (9). In Japan, the survival of patients with PAH has been significantly improved by more specific treatments; the 3- and 10 year survival showed 88.2–92.1% and 69.5%, respectively (10, 11).

Several causative conditions have been suggested in the pathogenesis of certain types of PAH. Portal hypertension (12), human immunodeficiency virus infection (13, 14) and appetite-suppressant drugs such as fenfluramine (15, 16) have been proposed as the causative conditions for a type of associated PAH. More than 20 years ago, a genetic predisposition to a heterozygous mutation in the bone morphogenetic protein receptor type II (BMPR2), a member of the transforming growth factor β (TGF-β) superfamily receptor, was discovered in heritable PAH. At least 16 other genes have been identified in patients with hereditary PAH, including activin A receptor type II-like 1 (ACVRL1), SMAD1, SMAD4, SMAD9, and caveolin 1 (CAV1) (17). However, in most cases of PAH, the precise mechanism of pathogenesis is poorly understood and may vary among cases. Endothelial-to-mesenchymal transition (EndoMT) of pulmonary artery endothelial cells (PAECs) and increased proliferative activity of pulmonary arterial smooth muscle cells (PASMCs) are the main mechanisms underlying pulmonary vascular remodeling (18–21). The transcription factors hypoxia-inducible factor (HIF), CCAAT-enhancer-binding protein (CEBP), runt-related transcription factor (RUNX), activator protein-1 (AP-1), C-terminal binding protein-1 (CtBP1), forkhead box M1 (FoxM1), pyruvate kinase muscle-2 (PKM2), nu-

clear factor-kappa B (NF-κB), β-catenin, twist family basic helix–loop–helix transcription factor 1 (TWIST1), and SLUG have been suggested to contribute to pulmonary vascular remodeling in PAH development and progression (7).

Pulmonary Artery Remodeling in PAH

The major remedies for the current treatment of PAH are prostacyclin analogs, endothelin receptor antagonists, and phosphodiesterase type 5 inhibitors (8). These vasodilators target pulmonary vasoconstriction in PAH. Vascular remodeling is another critical condition that contributes to the pathogenesis and progression of PAH. Therefore, our study focused on vascular remodeling, which may serve as a therapeutic target for the development of novel PAH treatment strategies.

EndoMT is a biological process in which endothelial cells progressively change their endothelial phenotype to a mesenchymal or myofibroblastic phenotype (22). It contributes to vascular fibrosis and angiogenesis, which are often closely associated with cardiac neovascularization, atherosclerosis, arteriosclerosis, and PAH (19, 23). In the pulmonary artery in cases of PAH, most cells within various intimal lesions show a phenotype of myofibroblasts that are positive for smooth muscle-type α actin (α-SMA) but negative for endothelial markers, such as nitric oxide synthase (eNOS), vascular endothelial cadherin, and cluster of differentiation (CD) 31 (24–26). Among the various pathological insults to endothelial cells, including oxidative stress (15, 27), mechanical stress (28) and inflammatory cytokines (29), TGF-β is one of the most relevant inducers of EndoMT in PAH as it augments myofibroblast transformation in numerous cell types and contributes to tissue fibrosis. Several TGF isotypes, such as TGF-β1, TGF-β2, and TGF-β3 (30), contribute to angiogenesis (31, 32). The canonical TGF-β signaling pathway commences with the binding of TGF-β to the TGF-β type 2 receptor (TGF-βR2), which phosphorylates and activates the type 1 receptor (TGF-βRl) via its constitutive kinase activity. Activated TGF-βRl phosphorylates the transcription factors SMAD-2 and SMAD-3, which in turn promotes collagen synthesis (33, 34). Fibrotic diseases are characterized by EndoMT. In particular, upregulation of mesenchymal markers, such as α-SMA and type 1 collagen, and downregulation of endothelial markers, such as vascular endothelial (VE)-cadherin, caused by the TGF-β/Smad signaling pathway, have been shown (35).

The proliferation of PASMCs is another key element in vascular remodeling during PAH progression (36). PASMCs derived from patients with PAH show enhanced proliferation compared with those derived from patients without PAH (37). Increased proliferative activity is associated with the increased activity of cyclin-dependent kinases. Moreover, the calcineurin/nuclear factor of activated T-cell signaling pathway has been reported to contribute to increased proliferation and decreased apoptosis in PASMCs derived from PAH model rats (38).

The aforementioned signaling pathways that contribute to EndoMT and the proliferation of PASMCs are assumed to be good targets for the treatment of PAH, especially when focusing on vascular remodeling.

Role of Transient Receptor Potential (TRP) Channels in PAH Pathophysiology

Intracellular Ca^{2+} signaling contributes not only to vasoconstriction but also to many events of vascular remodeling, such as EndoMT, migration, proliferation, and production of extracellular matrix. An influx of extracellular Ca^{2+} and release of intracellularly stored Ca^{2+} are the main mechanisms underlying the increase in intracellular calcium concentration ($[Ca^{2+}]_i$). Calcium entry involves various plasmalemmal Ca^{2+} channels,

including the superfamily of stretch-activated channels. Stretch-activated channels are nonselective Ca^{2+}-permeable cation channels. Some transient receptor potential (TRP) channels and members of the Piezo channel superfamily serve as stretch-activated channels.

The TRP channel superfamily is a group of non-selective cation channels that are permeable to mono- and divalent cations such as Na^+, K^+, Cs^+, Li^+, Ca^{2+}, and Mg^{2+} (39). TRP channels were initially discovered during a study on *Drosophila* phototransduction (40, 41). Twenty-eight distinct members of the TRP channel superfamily have been identified, 20 of which are expressed in mammals. All members of the TRP channel superfamily share a six-transmembrane structure, comprising 553–2022 amino acid residues. The development of X-ray diffraction analysis and electron microscopy is helpful in elucidating the structure of TRP channels (42, 43). Based on their structural differences, TRP channels can be categorized into six types: ankyrin (TRPA), canonical (TRPC), melastatin (TRPM), polycystin (TRPP), vanilloid (TRPV), and mucolipin (TRPML).

The expression of TRPC1, TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM3, TRPM4, TRPM7, and TRPM8 has been detected in the pulmonary arteries of rats, mice, and humans. More precisely, TRPC1, TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, TRPV1, TRPV2, TRPV4, TRPM1, TRPM3, TRPM4, TRPM6, TRPM7, and TRPM8 have been detected in PAEC (39).

The expression of TRPC channels was reported to be upregulated in PASMCs of PAH model mice, and these channels contribute to an increase in $[Ca^{2+}]_i$ (44). Table 1 summarizes the influence of various PAH-inducing conditions on the expression of TRP channels, with the exception of TRPM7. The enhanced expression and function of TRP channels in PAH highlight their importance as potential targets for pharmacological interventions.

The involvement of TRPM7 channels in cardiovascular remodeling has been reported (45–52). TRPM7 channels are activated by oxidative, mechanical, and osmotic stress, thereby contributing to various physiolog-

Table 1. Altered expression of TRPCs, TRPMs (except for M7), and TRPVs in PAH-related conditions

Cell or tissue (animal species)	PAH-related stimulation	TRP channel	Expression	Ref.
PASMC (rat)	Hypoxia	C1, C6	Upregulation	(44)
PASMC (rat)	Hypoxia	C1, C6	Upregulation	(81)
Pulmonary artery (rat)	MCT	C1, C4 C3	Upregulation (C1, C4) Downregulation (C3)	(82)
PASMC (mouse)	Hypoxia	C1	Upregulation	(83)
Lung (rat)	Chronic intermittent hypoxia	C1, C4, C6	Upregulation	(84)
PASMC (human)	Hypoxia	C6	Upregulation	(85)
PASMC (human)	Hypoxia	C6	Upregulation	(86)
PASMC (rat)	Hypoxia	C1, C6	Upregulation	(87)
Pulmonary artery (rat)	Hypoxia MCT	M8	Downregulation	(88)
PASMC (rat)	Hypoxia	M8	Downregulation	(89)
PASMC (rat)	Hypoxia	M2	Upregulation	(90)
Pulmonary artery (rat)	Hypoxia	V4	Upregulation	(91)
Lung (rat)	Hypoxia	V3	Upregulation	(92)
Lung microvascular endothelial cell (rat)	SuHx	V4	No changing, but regulate migration and proliferation	(93)
Adventitia (rat)	Hypoxia MCT	V4	Upregulation	(94)

PASMC: pulmonary arterial smooth muscle cell; MCT: monocrotaline; SuHx: Sugen5416 + hypoxia.

ical events, such as inflammation, fibrosis, cell migration, contraction, and cell growth (53). The TRPM7 channel is a non-selective cation channel with unique features of divalent (Ca^{2+}, Mg^{2+}) conductance and a structure involving the alpha-kinase domain in its C-terminal region (54, 55). Mg^{2+} permeability in TRPM7-transfected cell lines is reportedly slightly higher than that of Ca^{2+}, while the relative permeability of Na:Ca:Mg is estimated to be 1:1.23:1.29 (56, 57). Moreover, the difference between intracellular and extracellular Ca^{2+} concentrations ($[Ca^{2+}]_o$ / $[Ca^{2+}]_i \geq 10^4$) was much larger than that of Mg^{2+}($[Mg^{2+}]_o$ / $[Mg^{2+}]_i \geq 2$). Endogenous TRPM7 currents are typically small; this is particularly true for Mg^{2+} currents because the driving force (Nernst potential) for Mg^{2+} across the plasma membrane is much smaller than that for Ca^{2+} (58, 59).

Ca^{2+} permeation via TRPM7 channels is associated with several important events such as those related to tissue remodeling. Ca^{2+} influx via TRPM7 contributes to angiotensin II-induced cardiac fibrosis (60) and epithelial-to-mesenchymal transition and migration in breast cancer cells (61). TRPM7-mediated activation of extracellular signal-regulated kinase (ERK) 1/2 and signal transducer and activator of transcription 3 (STAT3) essentially plays important roles in these processes. Similarly, TRPM7 may contribute to endothelial remodeling. Indeed, TRPM7 channel activation has been shown to induce EndoMT by increasing $[Ca^{2+}]_i$ in sepsis (62). TRPM7 also plays a critical role in EndoMT of PAECs. In addition, TRPM7-mediated Ca^{2+} influx enhances endothelial cell migration (63).

The TRPM7 channel, which is associated with shear stress, regulates physiological and pathophysiological events such as cell migration, differentiation, and fibrogenesis (64–66). Shear stress-induced by fluid flow upregulates TRPM7 channel expression and activates it in vascular smooth muscle cells derived from the aorta (67). This indicates that vessel damage caused by shear stress triggers TRPM7 channel upregulation and activation. In the pulmonary artery, TRPM7 and TRPV4 are required for shear stress-induced Ca^{2+} increases in $[Ca^{2+}]_i$ in PASMCs (51). TRPM7 is also responsible for the shear stress-induced Mg^{2+} influx. Moreover, the shear stress-induced Mg^{2+} increase observed in PASMCs derived from patients with PAH was greater than that observed in normal PASMCs (51). Accordingly, TRPM7 expression in PASMC was remarkably higher in PAH lungs than in non-PAH lungs. The TRPM7 channel is involved in platelet-derived growth factor BB (PDGF-BB)-induced proliferation of hepatic stellate cells (68). PDGF-BB is known to promote proliferation and migration of human PASMCs (69). The miR-1181/STAT3 axis has been suggested to contribute to the PDGF-BB-induced proliferation and migration of PAMSCs. TRPM7 activation induces STAT3 phosphorylation in glioma cells (70). In the lungs of patients with PAH, increased STAT3 phosphorylation has been implicated in the proliferative and survival phenotypes of cells that comprise plexogenic lesions (71). STAT3 is a cytoplasmic transcription factor. Upon phosphorylation of its tyrosine 705 residue in response to cytokines, such as interleukin-6 (IL-6) and PDGF (69, 72), STAT3 translocates to the nucleus and binds to DNA after dimerization, thereby regulating gene expression (73). In contrast, the IL-6/STAT3 axis modulates TRPM7 function (74).

Extracts of Medicinal Mushroom *Ophiocordyceps sinensis* Ameliorate PAH Partially via TRPM7 Inhibition

In addition to the current therapeutic agents for PAH, it is necessary to develop highly safe agents that target vascular remodeling. Therefore, we explored these agents as ingredients in natural herbal medicines. FTY-720, which is synthesized from a component derived from the medicinal mushroom *Isaria sinclairii*, is now known to be a TRPM7 inhibitor (Supplementary Fig. 1) (75, 76). Another medicinal mushroom, *Ophiocordyceps sinensis* (OCS), known as *Yarsagumba*, has long been used as a folk remedy for altitude sickness, which is usually encountered above 4,000 m sea level (77). OCS enhances cardiopulmonary function and

exerts therapeutic effects on fibrotic lesions and malignant tumors (78, 79). Therefore, we examined the therapeutic effects of OCS and FTY-720 on the pathophysiology of PAH (45). We observed that the OCS extract ameliorated PAH and suppressed TRPM7 channel activity. However, the specific ingredients of OCS targeting the TRPM7 channel are undefined. Figure 1 summarizes the proposed mechanism underlying the therapeutic effects of OCS in PAH, based on our own investigation.

In vitro experiments showed that FTY-720 and OCS inhibited TGF-β-induced Smad2 phosphorylation and EndoMT in human PAECs and the proliferation of PASMCs derived from patients with PAH. Furthermore, inhibition of TRPM7 by the OCS extract and FTY-720, as well as the knockdown of TRPM7 channel expres-

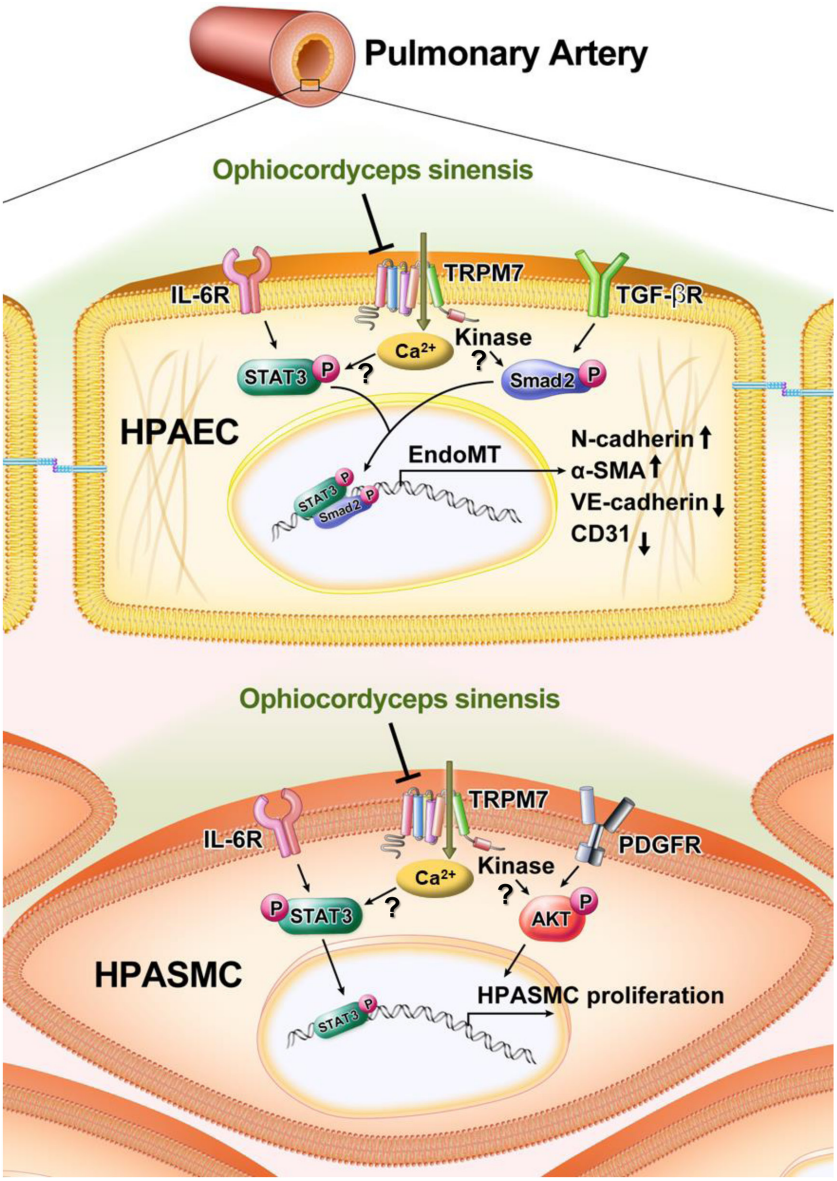

Fig. 1. Proposed mechanism for the therapeutic effects of Ophiocordyceps sinensis (OCS) on vascular remodeling in pulmonary hypertension.
In human pulmonary artery endothelial cells (HPAEC), inhibition of TRPM7 channels by OCS inhibits the phosphorylation of STAT3 and Smad2, and endothelial-to-mesenchymal transition (EndoMT). In human pulmonary artery smooth muscle cells (HPASMC), inhibition of the TRPM7 channel by OCS inhibits the phosphorylation of STAT3 and Akt and cell proliferation.

sion, suppressed the phosphorylation of STAT3 and Akt induced by IL-6 in PAECs, which are intracellular signaling pathways relevant to the EndoMT. Upregulation of TRPM7 channel expression, colocalized with αSMA in the medial wall and occlusive lesions, was also observed in the pulmonary arteries of patients with PAH. Furthermore, OCS extracts and FTY-720 induced relaxation in the human pulmonary artery during contraction induced by the thromboxane A_2 analog, U46619.

In vivo experiments using a rat model of PAH induced by monocrotaline showed that treatment with the OCS extract improved survival, right ventricular hypertrophy and dysfunction, and vascular remodeling. In PAH rats, TRPM7 channel expression is upregulated in α-SMA-positive cells. Administration of OCS extracts also ameliorated pathological progression in a mouse model generated by monocrotaline pyrrole. Furthermore, in TRPM7 channel knockout mice, monocrotaline pyrrole-induced increases in the right ventricular pressure and muscularization of the pulmonary artery were ameliorated compared with those in wild-type mice.

Our results suggest that targeting TRPM7 may be a novel strategy for PAH treatment, with the aim of ameliorating pulmonary artery remodeling. However, other studies have suggested that TRPM7 inhibition leads to PAH progression (80). One study showed that PASMCs derived from hypoxia-induced PAH model rats exhibited decreased expression of TRPM7 protein, and that TRPM7 knockdown and treatment with waixenicin A, a TRPM7 inhibitor, enhanced proliferation and apoptosis in PASMCs, suggesting that the TRPM7 channel is a negative regulator of PAH progression. Mechanistically, TRPM7 channel-mediated Mg^{2+} transport underlies attenuation of PAH progression. Furthermore, Mg^{2+} supplementation was shown to improve pulmonary arterial pressure, right heart hypertrophy, and medial wall thickening in a rat model of severe PAH (52). Mg^{2+} supplementation also inhibited PASMC proliferation and migration and enhanced the apoptosis of PASMCs. These reports contradict our findings and should thus be considered. The contribution of the TRPM7 channel to the pathogenesis of PAH may vary depending on the experimental PAH model. In other words, the therapeutic effects of targeting TRPM7 may depend on the type of PAH.

Summary

Despite significant advancements in therapeutic strategies over the last two decades, PAH remains an incurable disease. The present study proposes OCS, a traditional Chinese medicine, as a new treatment option for PAH. OCS ameliorates pathological changes in a rodent model of PAH. At least part of the observed anti-remodeling effects associated with EndoMT and STAT3 signaling involve the TRPM7 channel. TRPM7 has been suggested to be a therapeutic target for OCS. In addition, OCS improved PAH by inhibiting the proliferation and contraction of pulmonary artery smooth muscle. Based on these results, OCS may be a novel therapeutic agent for PAH.

Conflict of Interest

No conflict of interest needs to be disclosed.

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