REVIEW ARTICLE

Research Progress on Pulmonary Arterial Hypertension and the Role of the Angiotensin Converting Enzyme 2-Angiotensin-(1–7)-Mas Axis in Pulmonary Arterial Hypertension

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Abstract

Pulmonary arterial hypertension (PAH) is a progressive disease with a complex aetiology and high mortality. Functional and structural changes in the small pulmonary arteries lead to elevated pulmonary arterial pressure, resulting in right heart failure. The pathobiology of PAH is not fully understood, and novel treatment targets in PAH are desperately needed. The renin-angiotensin system is critical for maintaining homeostasis of the cardiovascular system. The system consists of the angiotensin converting enzyme (ACE)-angiotensin (Ang) II-angiotensin type 1 receptor (AT_1R) axis and the ACE2-Ang-(1–7)-Mas receptor axis. The former, the ACE-Ang II-AT_1R axis, is involved in vasoconstrictive and hypertensive actions along with cardiac and vascular remodelling. The latter, the ACE2-Ang-(1–7)-Mas axis, generally mediates counterbalancing effects against those mediated by the ACE-Ang II-AT_1R axis. Based on established functions, the ACE2-Ang-(1–7)-Mas axis may represent a novel target for the treatment of PAH. This review focuses on recent advances in pulmonary circulation science and the role of the ACE2-Ang-(1–7)-Mas axis in PAH.

Keywords Pulmonary arterial hypertension · Renin-angiotensin system · ACE-Ang II-AT_1R axis · ACE2-Ang-(1–7)-Mas axis

Abbreviations

ACE	angiotensin converting enzyme
ACE2	angiotensin converting enzyme 2
Ang-(1–7)	angiotensin-(1–7)
Ang II	angiotensin II
AT_1R	angiotensin II type 1 receptor
BMPR-II	bone morphogenetic protein receptor II
cGMP	3′,5′-cyclic guanosine monophosphate
ECs	endothelial cells
eNOS	endothelial nitric oxide synthase
ET-1	endothelin-1
EYA3	eyes absent 3
IL-6	interleukin-6
IL-8	interleukin-8
MCT	monocrotaline
NMDAR	N-methyl-D-aspartate receptor
NO	nitric oxide
PAH	pulmonary arterial hypertension
PASMCs	pulmonary artery smooth muscle cells
PH	pulmonary hypertension
PKG	protein kinase G
RAS	renin-angiotensin system
ROS	reactive oxygen species
sGC	soluble guanylate cyclase
SOD2	superoxide dismutase 2

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Pulmonary Arterial Hypertension (PAH) Introduction

PAH is a chronic disorder characterized by a progressive elevation in pulmonary vascular resistance, ultimately leading to right heart failure and death. PAH is defined as a pulmonary artery wedge pressure ≤ 15 mmHg, pulmonary vascular resistance >3 Wood units and an increase in mean pulmonary arterial pressure ≥ 20 mmHg at rest [1, 2]. PAH is classified as group 1 pulmonary hypertension (PH) and has different forms [1]. These different aetiologies share common pathological features in PAH. Hallmark pathophysiologic changes of PAH include enhanced sustained vasoconstriction, obstructive vascular remodelling, in situ thrombosis and inflammation. Pharmacological agents currently approved for the treatment of PAH range from endothelin receptor antagonists, soluble guanylate cyclase stimulators, nitric oxide donors and phosphodiesterase inhibitors to prostacyclin analogues and prostacyclin receptor agonists. Although these treatments augment pulmonary vasodilation and improve symptoms, they do not significantly reverse vascular remodelling [3, 4]. This gap emphasizes the need to better understand the molecular mechanisms involved in the pathophysiology of PAH and the urgency to identify novel therapeutic strategies.

Pathophysiology of PAH

Endothelial Dysfunction and Pulmonary Vasoconstriction

The role of sustained vasoconstriction in the pathogenesis of PAH is well established. Endothelial dysfunctions are characterized by both reduced secretion of potent vasodilators, such as nitric oxide (NO) and prostacyclin, along with increased production of vasoconstrictors, such as endothelin-1 (ET-1), thromboxane and serotonin [4–7].

Vasodilatory effects of NO are mediated by soluble guanylate cyclase (sGC)/3′,5′-cyclic guanosine monophosphate (cGMP) [8]. Research in patients [9] and animal experiments [10] have confirmed that NO deficiency is involved in the pathogenesis of PAH. Endothelial RhoA/Rho kinase signalling is responsible for the inhibition of both endothelial NO synthase (eNOS) expression and its activity in cultured endothelial cells (ECs) [11].

Prostacyclin stimulates the formation of cyclic adenosine monophosphate after binding to its receptors on smooth muscle cells (SMCs), leading to SMC relaxation. A deficiency of prostacyclin and an excess of thromboxane are found in patients with PAH and are associated with abnormal vasoconstriction [12].

ET-1 causes pulmonary artery SMC (PASMC) contraction by activating the Gq-protein-inositol triphosphate-Ca^{2+}

pathway after binding to two receptors, ET_A and ET_B, on SMCs [4].

Pulmonary Vascular Remodelling

Pulmonary vascular remodelling is manifested by the thickening of all three layers of the blood vessel wall—the intima, the media and the adventitia. Hypertrophy of the media and hyperproliferation of ECs and SMCs significantly drive the thickening of the vessels [13].

In PAH, dysfunction of ECs is manifested by increased secretion of vasoconstrictors, reduced secretion of vasodilators, increased proliferative capacity, a pro-inflammatory landscape and a smooth muscle-like mesenchymal phenotype transition [14]. Plexiform lesions are pathognomonic for PAH and are characterized by dysregulated proliferation and reduced apoptosis of ECs, leading to vascular obliteration. In fact, additional studies indicate that hypoxia-inducible factor-1α and hypoxia-inducible factor-1β are highly expressed in ECs derived from plexiform lesions during hypoxia and may contribute to the overproduction of mitogenic factors and their receptors, such as vascular endothelial growth factor [15, 16]. More recently, increased hypoxia-inducible factor-2α has also been reported to contribute to severe PAH by causing endothelial-to-mesenchymal transition [17].

Several lines of evidence suggest that increased activities of platelet-derived growth factor, epidermal growth factor, fibroblast growth factor and c-kit-receptor signalling pathways contribute to the excessive proliferation and migration of SMCs and ECs [18–21].

In addition to the role of ECs in promoting proliferation, ECs in PAH secrete increased levels of procoagulant factors and antifibrinolytic mediators. Failure of the thrombus in situ in the pulmonary circulation to dissolve over long periods is thought to contribute to vascular remodelling and fibrosis and eventually to increase pulmonary artery resistance [22].

Under pathological conditions, PASMCs can undergo phenotypic changes, including proliferation, migration and secretion, in response to bioactive agents [23]. Hyperplasia of PASMCs in the media layer of the pulmonary artery also leads to muscularization of previously non-muscularized arteries and an increase in the degree of muscularization of already muscularized pulmonary arteries [24]. Numerous studies have investigated the molecular mechanisms underlying the hyperproliferative phenotype of PASMCs. Imbalance between vasodilators and vasoconstrictors not only contributes to increased vasoconstriction, as mentioned before but also leads to PASMC proliferation. Prostacyclin inhibits PASMC proliferation and decreases platelet aggregation [25]. ET-1 may contribute to the abnormal proliferation of PASMCs in PAH by stimulating the mitogen-activated protein kinase family signalling pathway after binding to its receptors [26]. Recently, epigenetic factors were suggested to play a role in

PAH. Several studies have shown evidence to support the role of microRNAs in the hyperproliferation of PASMCs. The expression of microRNA-21 was increased in human PASMCs exposed to hypoxia. Inhibition of microRNA-21 reduced hypoxia-induced cell proliferation and migration [27]. In contrast, the expression of microRNA-204 was decreased in human PAH and in rats with hypoxia- and mono-crotaline (MCT)-induced PAH. Treatment with synthetic microRNA-204 in animals with PAH ameliorated pulmonary haemodynamics and vascular remodelling [28]. The DNA damage/poly(ADP-ribose) polymerase-1 signalling pathway may further mediate the role of microRNA-204 [29]. More recently, several studies have reported that ZIP12 [30], the glutamate-N-methyl-D-aspartate receptor (NMDAR) axis [31] and the Eyes Absent 3 (EYA3) tyrosine phosphatase may underlie hyperplasia of PASMCs [32]. Despite significant progress, the mechanisms underlying vascular remodelling in PAH remain unclear.

Inflammation in PAH

A significant number of studies have highlighted the contribution of inflammatory responses to the development and progression of PAH [33]. A study reported that patients with PAH have increased inflammatory cytokines and lipid peroxidation with decreased antioxidant capacity [34]. Experimental PAH models show that inflammation precedes pulmonary vascular remodelling and PAH, and furthermore, suppression of the immune response prevents PAH development [35]. Additional animal work has revealed that high mobility group box 1 protein induces the release of pro-inflammatory cytokines, the maturation of dendritic cells and the proliferation as well as aggregation of PASMCs, all leading to the formation of PAH [36]. Previous studies have revealed that germline mutations in bone morphogenetic protein receptor II (BMPR-II) in the pulmonary endothelium contribute to enhanced vasoconstriction and vascular remodelling [37, 38]. In fact, reductions of BMPR-II also lead to the elevated secretion of pro-inflammatory cytokines, increased vascular permeability and increased infiltration of leukocytes in response to inflammatory stimuli. These data suggest that BMPR-II may contribute to endothelial barrier function and act as an anti-inflammatory molecule; its loss may be a key mechanism responsible for the initiation of PAH [39].

In view of the above pathogenesis, the currently approved specific drugs for PAH target the NO pathway, prostacyclin pathway and endothelin pathway (Table 1) to restore endothelial-dependent vasodilation function [28]. While there have been many studies that have evaluated a myriad of molecular targets to restore endothelial-dependent vasodilation, the prognosis for PAH remains poor. The field has an urgent need to develop new therapeutic targets to reverse the progressive narrowing of the pulmonary vasculature, perivascular

Table 1 Summary of specific drugs approved for pulmonary arterial hypertension

Category	Drugs
Guanylate cyclase stimulators	Riociguat
Phosphodiesterase type 5 inhibitors	Sildenafil Tadalafil Vardenafil
Prostacyclin analogues	Epoprostenol Iloprost Treprostinil
Prostacyclin receptor agonists	Selexipag
Endothelin receptor antagonists	Ambrisentan Bosentan Macitentan

inflammation and pathological cardiac remodelling with the goal of improving patient outcomes.

The Role of the ACE2-Ang-(1–7)-Mas Axis in PAH

ACE2-Ang-(1–7)-Mas Axis

The renin-angiotensin system (RAS) plays an important role in maintaining cardiovascular homeostasis and the balance of water and electrolytes in mammals. Classically, renin secreted by juxtaglomerular cells catalyses angiotensinogen to Ang I, which is then cleaved by ACE into Ang II. It is believed that AT_1R mediates most functions of Ang II in the system. Systemic RAS functions include regulating blood pressure, excreting sodium and diuresis, and regulating blood volume [40]. In addition to the endocrine RAS, all components of the RAS have been found in tissues such as the heart, blood vessels and kidneys. Local functions of RAS span regulating local vascular-bed blood flow, controlling stimulation responses locally, and participating in cell proliferation, differentiation and apoptosis [41]. More importantly, it is documented that the system consists of two antagonistic (i.e. against each other) and coordinated axes, the ACE-Ang II-AT_1R axis and the ACE2-Ang-(1–7)-Mas axis [42, 43].

The ACE2 protein was found by cloning technology and is a widely distributed enzyme found in the heart, lungs, kidneys, intestines, uterus and testis, among other tissues [44]. The ACE2 enzyme is a specific monocarboxypeptidase that differs from ACE in substrate specificity and function. ACE2 can hydrolyze one amino acid residue to transform Ang I into Ang-(1–9), which is further degraded to Ang-(1–7) under the action of ACE. ACE2 can also directly hydrolyze the eighth phenylalanine of Ang II to produce Ang-(1–7). Because ACE2 has the ability to hydrolyze Ang II at 400 times that of Ang I, Ang-(1–7) is considered its main by-product [45,

46]. Ang-(1–7) is an active heptapeptide composed of aspartic acid, arginine, valine, tyrosine, isoleucine, histidine and proline, which is mainly distributed in blood vessels, the heart, kidneys and ovaries, among other organs. Mas is a specific G protein-coupled receptor encoded by the Mas proto-oncogene, and Ang-(1–7) is its endogenous ligand [47].

The ACE2-Ang-(1–7)-Mas axis has complex biological effects. It can promote the release of prostaglandin and NO by ECs through an Akt-dependent signalling pathway after Mas receptor binding, resulting in vasodilation as well as anti-proliferative, anti-inflammatory and anti-thrombotic effects [46, 48–51]. Our recent study on spontaneous hypertensive rats has also shown that Ang-(1–7)-Mas relaxes isolated pulmonary arteries through the NO-cGMP-protein kinase G (PKG) pathway [52]. Studies on hypertensive rats with thrombosis have found that decreased ACE2 activity is involved in increased thrombus formation in hypertensive rats [53], and treatment with Ang-(1–7) exerts an anti-thrombotic action by inducing the release of NO and prostacyclin from ECs [54]. In addition, it can elicit protective effects on infarcted hearts [55]. These data together suggest that the ACE2-Ang-(1–7)-Mas axis can negatively regulate blood pressure, inhibit myocardial remodelling, decrease the production of inflammatory factors and resist adverse effects of the ACE-Ang II-AT_1R axis, which are all functions that may benefit PAH. In the following section, we will review recent research progress on the ACE2-Ang-(1–7)-Mas axis in PAH.

Beneficial Role of the ACE2-Ang-(1–7)-Mas Axis in PAH

The ACE2-Ang-(1–7)-Mas Axis Improves Endothelial-Dependent Vasodilation

A number of studies have consistently reported an imbalance of ACE-Ang II-AT_1R axis and the ACE2-Ang-(1–7)-Mas axis in PAH. Several clinical studies confirmed reduced levels of ACE2 [56] or the activity of ACE2 [57] in serum in PAH. Furthermore, enhancement of ACE2 activity decreases the Ang II/Ang-(1–7) ratio and ameliorates abnormal pulmonary haemodynamics by reducing reactive oxygen species (ROS) via superoxide dismutase 2 (SOD2) and inhibiting inflammation in patients with PAH [57]. A recent clinical study reported that ACE2 activity and the concentration of Ang-(1–7) are reduced, while Ang II levels are increased in patients with PAH [58]. Studies on animal models with PAH suggest that reduced activity of the ACE2-Ang-(1–7)-Mas axis may be attributed to an unstable ACE2, which is due to decreased phosphorylation of Ser680 by AMP-activated protein kinase [59]. Inhibition of the ACE-Ang II-AT_1R axis and stimulation of the ACE2-Ang-(1–7)-Mas axis have been shown to decrease pulmonary arterial pressure and to improve vascular remodelling by increasing the production of NO [60].

A study on rat models of MCT-induced PAH found that microvesicles derived from mesenchymal stem cells improve haemodynamics, inflammation scores, and pulmonary vascular and cardiac remodelling by upregulating the ACE2-Ang-(1–7)-Mas axis and downregulating the ACE-Ang-II-AT_1R axis [61]. A further study showed that ACE2 activation ameliorates PAH by inducing phosphorylation of eNOS, leading to increased release of NO [62]. The above results support that the ACE2-Ang-(1–7)-Mas axis exerts beneficial effects on PAH by potentially improving endothelial function. A schematic diagram of the proposed mechanisms of the ACE2-Ang-(1–7)-Mas axis in ECs and PASMCs in PAH is shown in Fig. 1.

ACE2-Ang-(1–7)-Mas Axis Inhibits Vascular and Cardiac Remodelling

The ACE-Ang II-AT_1R axis also promotes vascular and cardiac remodelling. A previous study in a hypoxia-induced rat model of PH found that ACE activity is significantly increased in the right ventricle [63]. Furthermore, Ang II-induced cardiac fibroblast proliferation and collagen deposition through protein kinase Cδ, and β II-dependent inactivation of p38 may be an underlying mechanism of right ventricular fibrosis in PAH [64]. ACE-Ang II-AT_1R axis-induced vascular and cardiac remodelling may involve multiple processes, including stimulating cardiomyocyte hypertrophy and fibrocyte proliferation, increasing the release of catecholamine from sympathetic nerve endings and secretion of endothelin. Other studies have shown that Ang II can lead to pathological remodelling characterized by calcium signalling dysfunction, overexpression of primitive genes, inactivation of glycogen synthetase kinase-3 β and aggregation of activated nuclear transcription factors in T cells, resulting in cardiac hypertrophy. Ang-(1–7), in contrast, prevents Ang II-mediated pathological remodelling via the NO/cGMP signalling pathway [65].

Ang-(1–7) also inhibits platelet-derived growth factor- and Ang II-mediated proliferation of PASMCs. Ang-(1–7) also exhibits anti-angiogenic features and improves endothelial function after stent implantation [66]. Administration of Ang-(1–7) into wild-type mice reduces neointimal area and PASMC proliferation by reducing oxidative stress and the inflammatory response. However, the effect was less obvious in Mas-deficient mice than in wild-type mice [67]. In addition, recent animal work in PH models found that activation of ACE2 improves pulmonary arterial remodelling mediated by the Hippo signalling pathway-induced apoptosis [68], while a Mas inhibitor blocks the protective effects of ACE2 on pulmonary arteries. In other animal work, an adeno-associated virus vector containing both a Tie2 promoter and hypoxia response elements was designed to target the Ang-(1–7) gene [called HTSFcAng(1–7)]. The expression and release of Ang-(1–7) in HTSFcAng(1–7)-transfected ECs
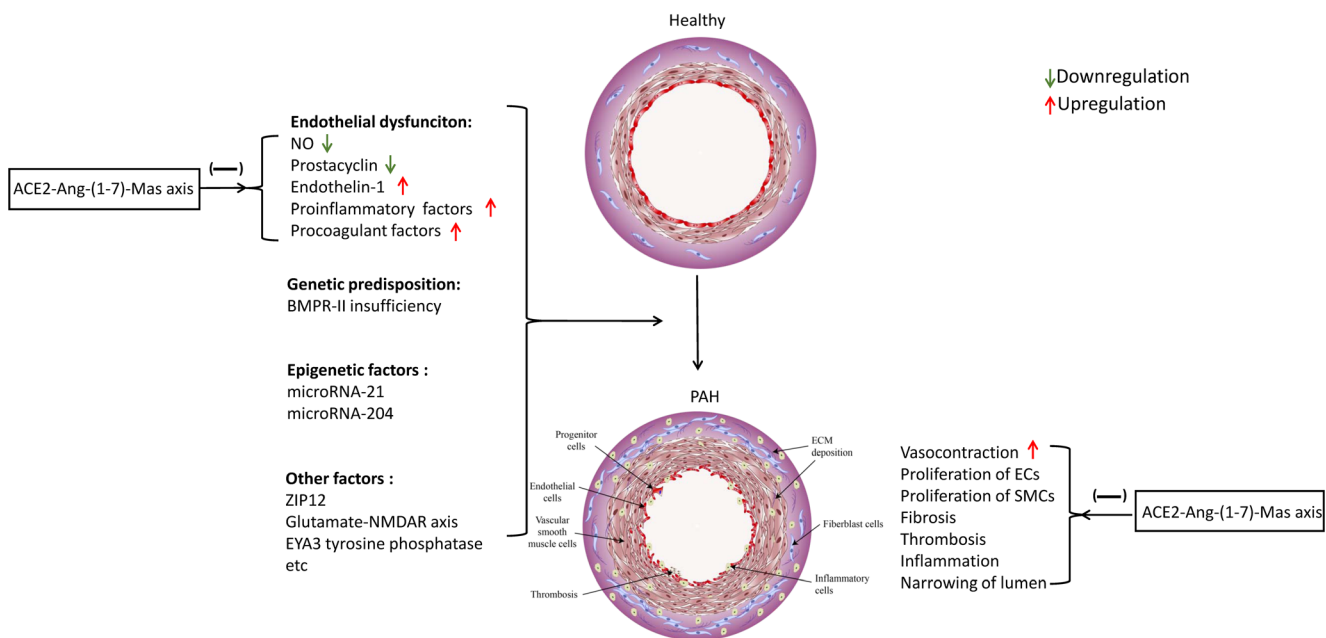
Fig. 1 A schematic diagram of the main mechanisms of the ACE2-Ang-(1–7)-Mas axis in ECs and PASMCs in PAH

were increased under both normoxic and hypoxic conditions, resulting in reduced hypoxia-induced proliferation of pulmonary arterial PASMCs with increased relaxation of pulmonary artery rings. In rats with hypoxia-induced PH, HTSFcAng(1–7) relieved pulmonary haemodynamics and pulmonary arterial remodelling [69]. In addition, treatment of MCT-induced PH rats with orally delivered ACE2 and Ang-(1–7) bioencapsulated within plant cells ameliorated right ventricular and pulmonary artery remodelling and attenuated total pulmonary resistance.

These beneficial effects of ACE2 and Ang-(1–7) in rodents involved alterations in Mas receptors, AT$_1$ receptors, IL-1β and TGF-β1 [70] (in Fig. 1).

The pathophysiologic mechanisms of right ventricular remodelling and dysfunction include calcium overload [71], oxidative stress injury and cardiomyocyte apoptosis [72, 73]. Ang-(1–7) supplementation can slow down the development of right ventricular dysfunction and improve the maximum rate of right ventricular pressure rise during the isovolumic systolic period [74,

Fig. 2 The general effects of the ACE2-Ang-(1–7)-Mas axis on vascular remodelling of the pulmonary artery in PAH
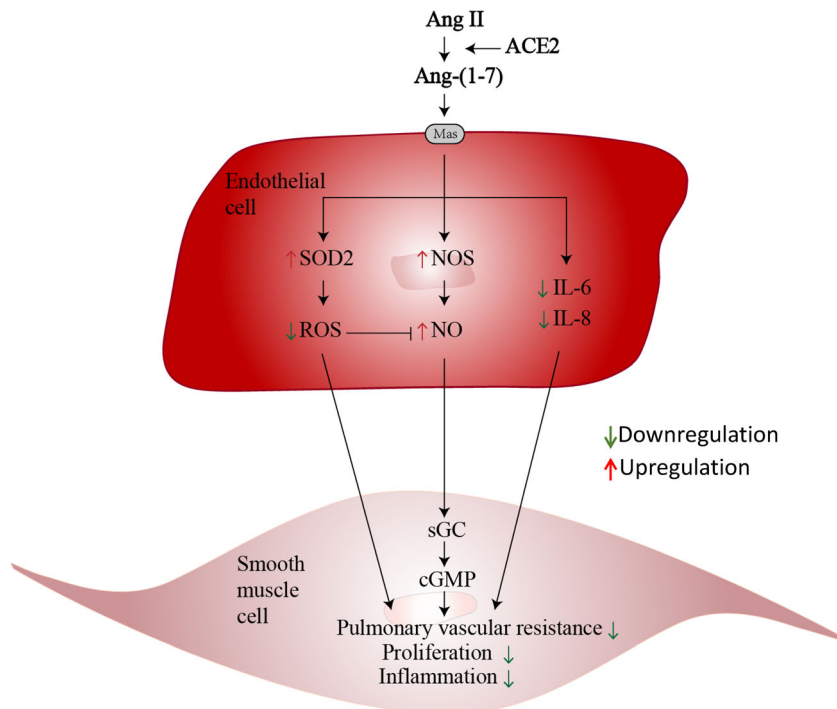
75]. Evidence was reported that Ang-(1–7) has protective effects on rat neonatal cardiomyocytes with hypoxia exposure in vitro, which is related to decreased tumour necrosis factor-α and interleukin (IL)-6 gene expression as well as increases in ACE2, bradykinin B2 receptor and IL-10 gene expression [76]. Overexpression of Ang-(1–7) can also improve cardiac function and alleviate left ventricular remodelling after myocardial infarction [76]. Further studies indicate that the beneficial effect of Ang-(1–7) on myocardial remodelling mediated by Ang II is related to the upregulation of the activity of dual-specificity phosphatase-1-mediated inhibition of the activity of mitogen-activated protein kinases [77].

The effects of the ACE2-Ang-(1–7)-Mas axis on vascular remodelling in PAH are shown in Fig. 2. Given that the currently approved treatments for PAH have little effect on improving cardiac function, the ACE2-Ang-(1–7)-Mas axis may hold significant promise.

The ACE2-Ang-(1–7)-Mas Axis Inhibits the Inflammatory Response

It has been reported that MCT increases the expression of inflammatory factors, such as IL-6 and IL-1β [78, 79]. Another study revealed that tumour necrosis factor α contributes to MCT-induced PH by suppressing pyruvate dehydrogenase [80]. The above studies suggest that overproduction of inflammatory factors is involved in the development of MCT-induced PH. In contrast, Ang-(1–7) overexpression reduces the expression of IL-1β, IL-6, tumour necrosis factor-α and other pro-inflammatory factors involved in inflammation and increases the production of IL-10, an anti-inflammatory factor [75]. These data indicate that Ang-(1–7) can also improve PAH by inhibiting the pulmonary vascular inflammatory response.

Conclusion

PAH can be considered a chronic progressive disease with a complex aetiology and high morbidity and mortality. The ACE2-Ang-(1–7)-Mas axis represents a novel therapeutic pathway in PAH. Ang-(1–7) and ACE2 overexpression have both shown protective effects in PAH and right heart dysfunction. Therefore, further studies on the mechanisms of the ACE2-Ang-(1–7)-Mas axis can provide new potential drug targets in PAH.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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