Stem cell therapy in pulmonary hypertension: current practice and future opportunities

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A review of stem cell therapy in pulmonary hypertension discussing preclinical achievements, therapeutic effects in clinical trials and the challenges and future perspectives for large-scale applications https://bit.ly/3DgF4ht

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Abstract

Pulmonary hypertension (PH) is a progressive disease characterised by elevated pulmonary arterial pressure and right-sided heart failure. While conventional drug therapies, including prostacyclin analogues, endothelin receptor antagonists and phosphodiesterase type 5 inhibitors, have been shown to improve the haemodynamic abnormalities of patients with PH, the 5-year mortality rate remains high. Thus, novel therapies are urgently required to prolong the survival of patients with PH. Stem cell therapies, including mesenchymal stem cells, endothelial progenitor cells and induced pluripotent stem cells, have shown therapeutic potential for the treatment of PH and clinical trials on stem cell therapies for PH are ongoing. This review aims to present the latest preclinical achievements of stem cell therapies, focusing on the therapeutic effects of clinical trials and discussing the challenges and future perspectives of large-scale applications.

Introduction

Pulmonary hypertension (PH) is a complex pathophysiological disorder that frequently co-occurs with cardiovascular and respiratory diseases [1]. According to various known or unknown causes, PH can be categorised into five distinct groups: 1) PH caused by arterial disease, 2) PH caused by left heart disease, 3) PH induced by chronic hypoxia, 4) PH resulting from chronic thromboembolic events and 5) PH associated with multifactorial mechanisms [2]. PH is characterised by the progressive remodelling process of the pulmonary blood vessels, including pulmonary intima hyperplasia, inflammatory cell infiltration and endothelial-to-mesenchymal transition (EndMT), and ultimately leads to the pulmonary vascular load increasing and life-threatening right-sided heart failure. 1- and 3-year survival rates for patients with PH range from 68 to 93% and from 39 to 77%, respectively [3]. Although conventional PH target therapies, such as calcium channel blockers, prostacyclin analogues (PCAs), endothelin receptor antagonists, phosphodiesterase type 5 inhibitors (PDE5is) and soluble guanylate cyclase agonist, effectively improve the quality of life of patients, the 5-year mortality rate remains as high as 50% [4, 5]. Therefore, an efficient, accurate, convenient therapy is urgently required to treat PH and improve patient survival.

Stem cells are undifferentiated cells that have the ability to self-renew, proliferate and differentiate into various specific cells [6]. Endothelial progenitor cells (EPCs) [7, 8], mesenchymal stem cells (MSCs) [9] and induced pluripotent stem cells (iPSCs) [10] are the most common stem cell types used to treat PH. Recent studies have demonstrated the treatment effects of cell infusion therapy [8, 9] and its detailed

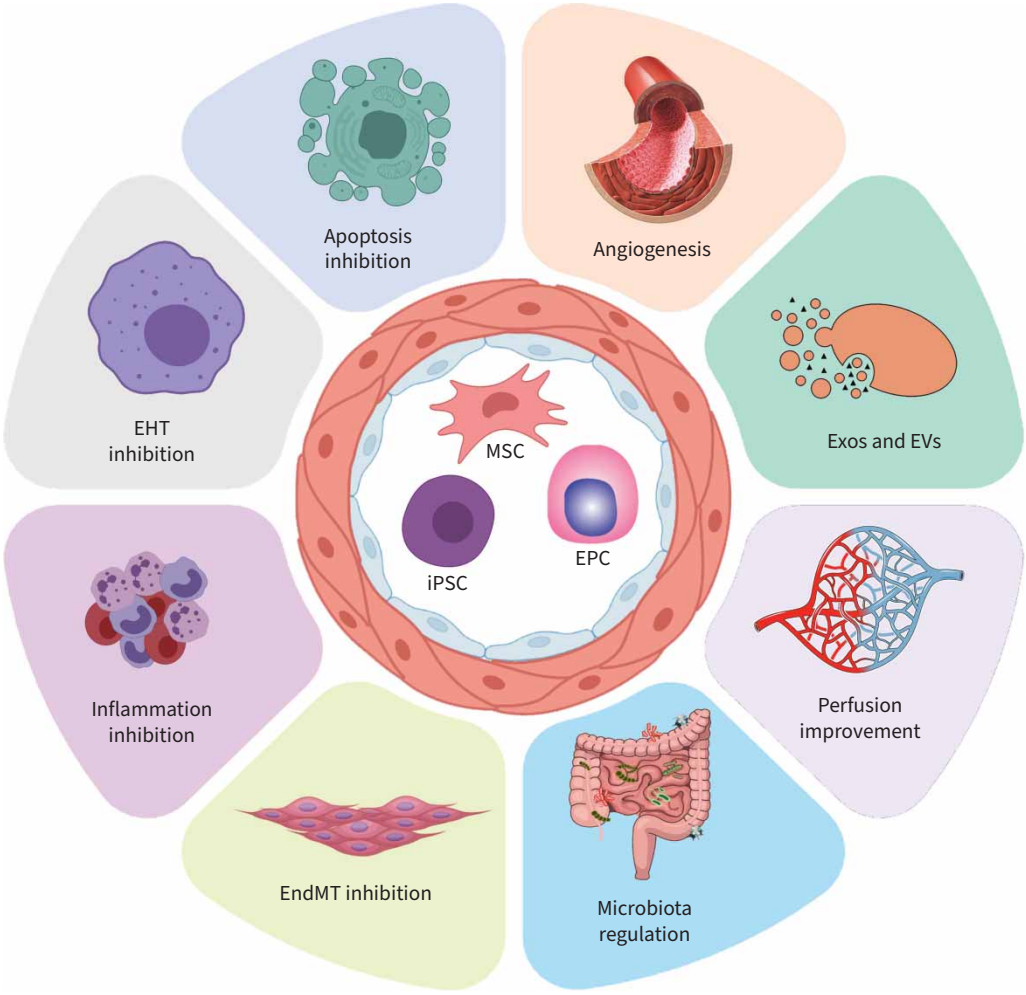

FIGURE 1 Overview of the treatment mechanisms of stem cell therapies in pulmonary hypertension. EHT: endothelial-to-haematopoietic transition; EndMT: endothelial-to-mesenchymal transition; EPC: endothelial progenitor cell; EV: extracellular vesicle; Exo: exosome; iPSC: induced pluripotent stem cell; MSC: mesenchymal stem cell. Image partially created using FigDraw.com (reproduction permission ID OWYSR2fb74.

mechanisms are gradually being elucidated [11–15]. Additionally, due to their distinct advantages, cell appendage therapy, such as extracellular vesicle (EV) [16], exosome (Exo) [17] and cell regulation therapy [18], have also garnered attention from researchers (figure 1).

This review summarises the latest advances associated with the use of stem cell therapy for PH. The first section provides a definition and classification of stem cells. The subsequent section highlights the remarkable outcomes of the preclinical research and analyses the treatment mechanisms. The third section lists the status of relevant clinical trials and discusses their results. The final section considers the future prospects and challenges in stem cell therapy and provides valuable recommendations to promote clinical applications.

In brief, stem cell therapy, encompassing EPCs, MSCs and iPSCs, along with cell appendage therapy, has shown remarkable treatment effects and elucidated detailed mechanisms in PH. Consequently, it holds immense promise as a treatment option for PH patients. However, to establish its long-term safety and efficacy, additional studies are necessary. We are optimistic about the future of these studies.

Definition and classification of stem cells

Stem cells are undifferentiated cells that have the ability to self-renew, proliferate and differentiate [19]. Stem cells both exist in embryos and adults. Under the complex regulation of the human body, stem cells can differentiate into every kind of cell and fulfil their metabolism requirements [6, 20]. According to their stemness and differentiation process, stem cells are classified into five classes: 1) totipotent, 2) pluripotent, 3) multipotent, 4) oligopotent and 5) unipotent [21] (figure 2). Based on the cellular composition of

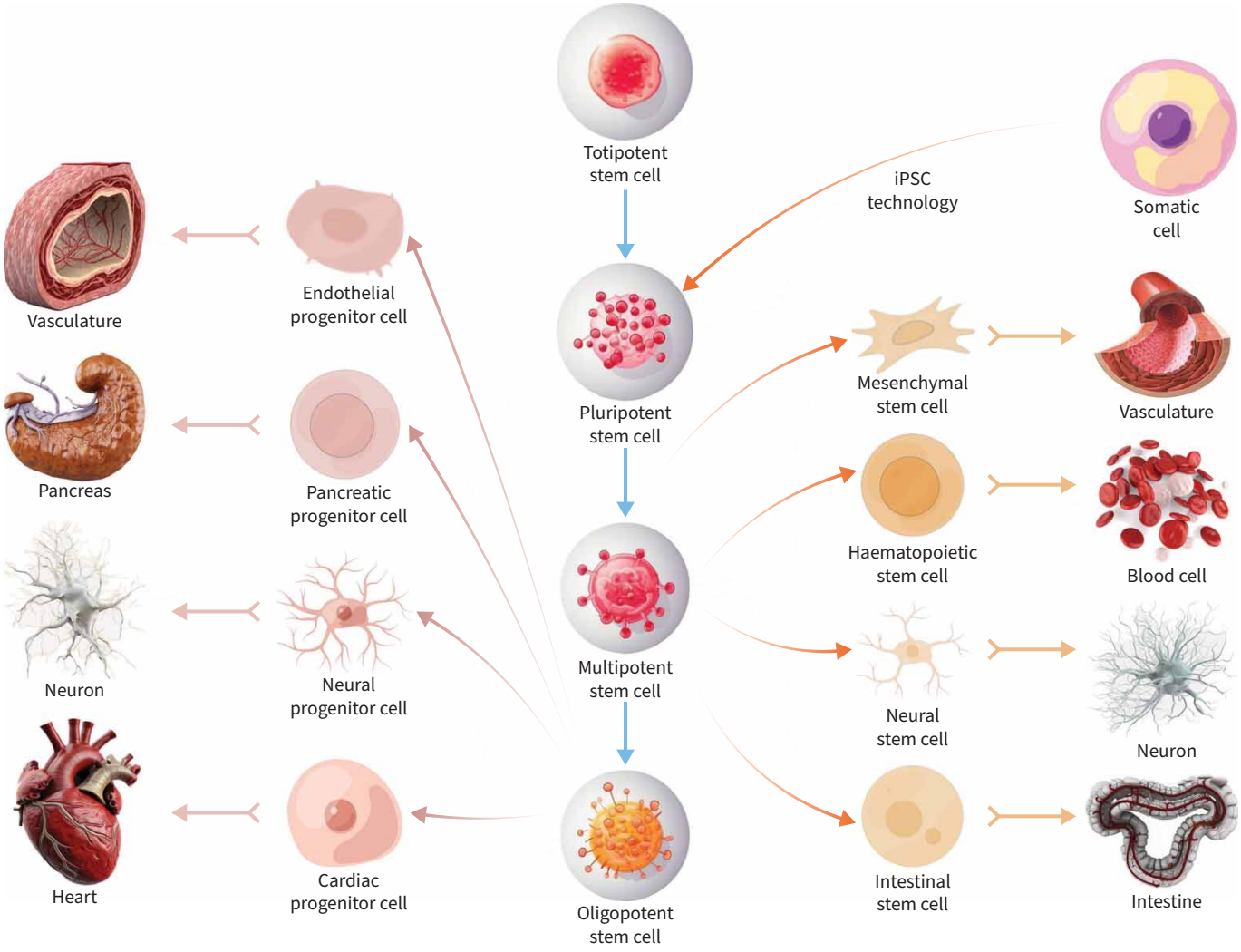

FIGURE 2 Differentiation tree of stem cells from zygote to progenitor cell. iPSC: induced pluripotent stem cell. Image partially created using FigDraw.com (reproduction permission ID OWYSR2fb74.

pulmonary vessels and the right ventricle, as well as previous studies that revealed their pathogenic mechanisms, pluripotent [22], multipotent [23] and oligopotent stem cells [24] were identified as the most promising stem cell types in terms of therapeutic potential, and iPSCs, MSCs and EPCs are representative cells [25] (figure 1).

EPCs

EPCs are a type of oligopotent stem cell that have a propensity for endothelial cell (EC) differentiation [26, 27]. Normally, the majority of EPCs in the human body are typically stored in the bone marrow (BM), while a smaller fraction is found within specialised niches within the vascular wall system [27]. According to the culture time of peripheral blood mononuclear cells, EPCs can be classified into two categories: early EPCs and late EPCs. The adherent cells collected on day 10 are referred to as early EPCs and their distinct morphology when collected on day 14 are referred to as late EPCs [28]. Given the marked discrepancies in angiogenic potential observed between early and late EPCs, the majority of researchers opt to utilise late EPCs as a therapeutic intervention for vascular injury diseases [29]. Classic EPC surface markers include CD31, CD34, CD144 (also known as vascular endothelial–cadherin or cadherin 5), CD146, vascular endothelial growth factor receptor-2 (VEGFR2, also known as CD309, kinase insert domain receptor or fetal liver kinase-1). To distinguish EPCs from haematopoietic stem cells, it is crucial for CD14 and CD45 and to be negative in the EPC group [30–32]. Nowadays, researchers use markers, such as vWF (von Willebrand factor) [33], CD133 (also known as AC133) [34–36], *Ulex europaeus* agglutinin-1 and acetylated low-density lipoprotein [37], to identify EPCs.

MSCs

MSCs are a type of multipotent stem cell that have a mesenchymal differentiation tendency and can differentiate into mesenchymal cells such as osteoblasts, adipocytes, chondrocytes and myocytes [38, 39]. The International Society for Cellular Therapy proposed that human MSCs must meet the following criteria: 1) positive for CD73, CD90 and CD105, 2) negative for CD14, CD19, CD34, CD45 and major histocompatibility complex II DR, 3) plastic adherence under standard culture conditions, and 4) the ability to differentiate into osteoblasts, adipocytes and chondroblasts *in vitro* [40]. The classification of MSCs is primarily based on their extraction source, which includes BM [41] and neonatal tissue, such as umbilical cord [42], adipose tissue [43] and amniotic fluid [44]. Each source exhibits distinct characteristics that make them suitable for different treatment situations.

iPSCs

iPSCs are a type of artificially pluripotent stem cell generated from somatic cells [45]. Takahashi *et al.* [46] first reprogrammed human fibroblasts by using four kinds of transcription factors, including myelocytomatosis oncogene, octamer-binding transcription factor 3/4, sex-determining region Y-box 2 and Krüppel-like factor 4. Nowadays, a wide range of somatic cells, including blood cells, fat cells and even urine cells, can be reprogrammed by various programming factors. For instance, undifferentiated embryonic cell transcription factor 1 [47], Sal-like protein 4 [48] and many transcription factors [49] have been identified as effective factors for cellular reprogramming.

Preclinical research of stem cell therapies for PH

Preclinical research of EPC therapies for PH

Previous hypothesis supposed that under the stimulation of adverse factors, the number of peripheral EPCs would increase and exert repair functions [50]. However, the opposite is true [51, 52]. Patients with PH and other diseases such as congenital heart disease [35] and COPD [53] had lower levels of EPCs. Furthermore, the pathological status of EPCs could also promote the development of PH. EPCs extracted from a hypoxic neonatal calf exhibited higher migratory and tube formation capacities [54]. Patients with proliferative EPCs also often experience more severe clinical courses [55]. Therefore, it is crucial to correct EPC deficiencies and regulate the EPC abnormal repair process in patients with PH (table 1).

EPC infusion therapy for PH

The simplest and most straightforward way to restore EPC deficiency is *via* cell infusion. As early as 2005, Zhao *et al.* [8] and Nagaya *et al.* [7] revealed the excellent treatment effect of EPC infusion therapy in monocrotaline (MCT) rats. After EPC transfusion, various haemodynamic parameters, including mean pulmonary arterial pressure (mPAP) [7], right ventricular systolic pressure [8], right ventricle to left ventricle plus septum [8] and pulmonary vascular resistance (PVR) [7], were improved and survival in an animal model was also ultimately prolonged [7].

EPC distribution tendency and retention time in infusion therapy

The distribution tendency and retention time of stem cells are two important indicators that researchers use to decide the most suitable therapy strategy. Relying on live imaging technology, Harper *et al.* [63] revealed that administered labelled EPCs mainly aggregated in the lung region, rather than the liver, heart or spleen. The fluorescence intensity of MCT rats was found to be 14.3 times higher than that of healthy rats at both 1 and 6 h and dissipated at 24 h [63]. Positron emission tomography technology also showed that most of the radioactivity aggregated in the lung region at 1 h and the intensity in MCT rats was higher from 24 h until 254 h [62]. Furthermore, evidence of EPC aggregation could even be detected by PCR after 25 days [72]. Studies revealed that the EPCs tended to aggregate in the injured pulmonary vasculature. However, there are contradictory results regarding the retention time of EPCs among the different studies. These conflicts may be due to the different experimental methods employed. The limit of detection of the different analysis methods significantly influenced the minimum positive results, which subsequently affected the final results.

Mechanisms of EPC infusion therapy

Vascular repair function

Zhao *et al.* [8] revealed that injected EPCs clustered around the distal arterioles, integrated into arterial endothelium and significantly improved capillary perfusion in MCT rats. Yip *et al.* [11] verified that EPCs were engrafted and trapped in the pulmonary arterioles of MCT rats at day 7. In addition, the disruption of the connexin43 cellular gap junctions was also reversed by EPCs at day 90 after transplantation.

Apoptosis inhibition

Excessive apoptosis of ECs can induce severe disruption of the vascular architecture and haemodynamic abnormalities [12]. In the MCT rat model, the administration of EPCs significantly decreased the

TABLE 1 Summary of recent literature regarding endothelial progenitor cell (EPC) markers

Year	EPC markers	Cell source	Cell donor	Conclusion	Reference
2022	$CD45^+$, $CD144^+$	BM	VE-cadherin lineage tracing mice	Inhibition of RUNX1 could be a novel treatment for pulmonary arterial hypertension	[56]
2022	$CD31^+$, $CD117^+$, $CD45^-$	Lung tissue	$Foxf1^{WT/S52F}$ mice	EPCs promoted neonatal lung angiogenesis and alveolarisation through FOXF1-mediated activation of BMP9/ACVRL1 signalling	[57]
2022	$CD133^+$, VEGFR-2^+	BM	Broiler chickens	Chronic TNF-α challenge directed early-EPCs to macrophage differentiation and significant increased plexiform lesion density	[34]
2021	$CD31^+$, $CD117^+$, $CD45^-$	Lung tissue	$Foxf1^{WT/S52F}$ and $Foxf1^{+/-}$ mice	Single-cell sequencing revealed the heterogeneity of pulmonary EPCs and constructed an ACDMPV disease model in gene-edited mice	[58]
2021	UEA-1^+, acLDL$^+$	PB	Healthy and MCT rats (50 mg·kg^{-1})	Aldosterone could inhibit the senescence and proliferation of EPCs and alleviate PH	[37]
2021	$CD31^+$, $CD34^+$, vWF^+	PB	Healthy human volunteers	Microgravity environment could enhance the angiogenic properties of EPCs	[59]
2021	$CD34^+$, $CD133^+$, $VEGFR2^+$	PB	Female SSc patients	EPCs might be a protentional disease status biomarker of SSc-related PH	[60]
2020	$CD34^+$, $CD133^+$, VEGFR-2^+	PB	CTEPH patients	Riociguat improved the number of EPCs and reverses the symptoms of CTEPH	[61]
2020	$CD31^+$, VEGFR-2^+, $CD144^+$, $CD34^+$, $CD14^-$	PB	Healthy human volunteers	The administrated EPCs spontaneously aggregated in the lung region	[62]
2019	$CD34^+$, $CD146^+$, $CD45^-$, VEGFR-2^+	BM	Healthy and MCT rats (60 mg·kg^{-1})	BMPR2-EPCs showed better treatment effects than unedited EPCs	[63]
2019	$CD133^+$, VEGFR-2^+	PB	0–6 years old CHD and non-CHD patients	Number of EPCs was decreased in patients with CHD-related PH than in patients with only CHD	[35]
2019	$CD34^+$ $CD31^+$, $CD144^+$, VEGFR-2^+, $eNOS^+$	PB	Healthy rats and week 4 hypoxia rats	Hypoxia disturbed the function and enhanced the apoptosis of EPCs through the NOX1/VPO1 pathway	[64]
2019	$CD31^+$, $CD144^+$, VEGFR-2^+	PB	Healthy large white piglets	EPCs significantly reversed the abnormal haemodynamics in the piglet CTEPH model	[65]
2018	$CD34^+$, VEGFR-2^+	Lung tissue	COPD and non-COPD patients	EPC numbers were decreased in patients with COPD-related PH than in patients with only COPD	[66]
2017	$CD34^+$, $CD133^+$, $CD31^+$, $CD45^-$	BM	Healthy rats	Pinocembrin significantly reversed the MCT-induced RV dysfunction	[67]
2017	$CD144^+$, $CD117^+$, $CD45^-$	BM	Healthy CD45.1 and CD45.2 mice	Haematopoietic transformation of EPCs played a critical role in the development of PH	[68]
2017	sca-1^+, $CD117^+$, VEGFR-2^+	BM	Healthy and MCT rats (60 mg·kg^{-1})	Administration of MCT-treated EPCs and EVs promoted the occurrence of PH	[69]
2017	$CD45^-$, $CD34^+$, $CD133^+$	PB	Treatment-naïve and PH-targeted therapy PH patients	Number of EPCs in patients with CTEPH was decreased and associated with exercise tolerance	[36]
2016	$CD34^+$, $CD14^-$, VEGFR-2^+	PB	COPD and PH-COPD patients	Number of EPCs was decreased in COPD-related PH patients than simple-COPD patients and showed multiple dysfunctions	[53]
2015	$CD34^+$, $CD133^+$, VEGFR-2^+	BM	Healthy rabbits	HIF-1α-EPCs showed better treatment effect than unedited EPCs	[70]
2014	$CD45^-$, $CD34^+$, $CD133^+$	BM	COPD and non-COPD patients	Number of EPCs was decreased in patients with COPD-related PH than in patients with only COPD and showed multiple dysfunctions	[71]
2013	VEGFR-2^+, $Tie2^+$, $CD14^-$, $CD31^+$, $CD34^+$	BM	Healthy rats	COX1-PGIS-EPCs showed better treatment effect than unedited EPCs	[72]
2012	$CD31^+$, VEGFR-2^+, vWF^+	BM	Healthy rats	Combined therapy had a better treatment effect than either EPCs or sildenafil alone	[33]
2009	$CD34^+$, $CD133^+$, VEGFR-2^+	PB	CTEPH patients	Microenvironment of the occluded vessel could promote the aggregation and differentiation of EPCs	[73]
2009	$CD133^+$, VEGFR-2^+	PB	Healthy dogs and dehydro-MCT dogs	EPCs from DHMC-treated dogs suffered from exhaustion and senescence	[74]

Continued

TABLE 1 Continued

Year	EPC markers	Cell source	Cell donor	Conclusion	Reference
2008	$AC133^+$, $VEGFR\text{-}2^+$	PB	Healthy volunteers and idiopathic PH patients	Number of EPCs is significantly reduced in patients with idiopathic PH	[75]
2008	$CD34^+$, $AC133^+$, $VEGFR\text{-}2^+$	PB	Healthy volunteers, idiopathic PH patients and Eisenmenger syndrome patients	Number of EPCs is significantly reduced in patients with PH	[52]

ACDMPV: alveolar capillary dysplasia with misalignment of pulmonary veins; acLDL: acetylated low-density lipoprotein; ACVRL1: activin A receptor like type 1; BM: bone marrow; BMP9: bone morphogenetic protein; BMPR2: bone morphogenetic protein receptor type II; CHD: congenital heart disease; COX1: cyclooxygenase 1; CTEPH: chronic thromboembolic pulmonary hypertension; DHMC: dehydromonocrotaline; eNOS: endothelial nitric oxide synthase; FOXF1: forkhead box F1; HIF-1α: hypoxia inducible factor-1α; MCT: monocrotaline; NOX1: NADPH oxidase 1; PB: peripheral blood; PH: pulmonary hypertension; PTGIS: prostaglandin I2 synthase; RUNX1: runt-related transcription factor 1; RV: right ventricular; sca-1: stem cell antigen-1; SSc: systemic sclerosis; Tie2: tyrosine kinase receptor 2; TNF-α: tumour necrosis factor-α; UEA-1: *Ulex europaeus* agglutinin 1; VE: vascular endothelial; VEGFR: vascular endothelial growth factor receptor; VPO1: vascular peroxidase 1; vWF: von Willebrand factor.

expression of caspase-3, which is a hallmark of apoptosis. This effect of apoptosis inhibition was also verified in a cell model. The treatment with EPC conditional medium successfully reversed the high shear stress and serum starvation induced apoptosis, decreased the apoptosis rate, and increased the percentage of proliferation [76]. The findings of these studies provide important insights into the considerable vascular repair and cytoprotective capacity of EPCs, including their ability to integrate into damaged blood vessels and impede the development of damage. Numerous studies have also shown that EPC infusion therapy can reverse PH in multiple models, such as in an MCT rat model [8] and a chronic thromboembolic PH model [65]. However, some researchers have reported contradictory results. For instance, Ikutomi *et al.* [77] found that treatment with any type of EPC could not prevent the progression of MCT-injured PH. Therefore, additional evidence is required to verify the feasibility of EPC infusion therapy and reveal further therapeutic mechanisms.

Gene-edited EPC infusion therapy

The use of viruses or plasmids to regulate genes is an established method to enhance treatment effectiveness [78]. Therefore, bone morphogenetic protein receptor type II (BMPR2)-overexpressing EPCs were constructed. Compared to unedited EPCs, BMPR2-EPCs successfully increased the expression of BMPR2 and its downstream pathways and phosphorylated-small precursors of decapentaplegic-1/5/8, and more desirable improvement effects were achieved in an MCT rat model [63]. The systemic administration of PCAs induce substantial side-effects which hinder their large-scale application [79]. To address this issue, EPCs fused with cyclooxygenase isoform 1 and prostacyclin synthase (PGI) were generated to release PGI at the damaged vessel site. After 4 weeks of treatment, the edited EPCs had exerted a considerable improvement in haemodynamic disorders, inhibited intimal and medial smooth muscle layer proliferation, and prolonged the survival rate in an MCT rat model [72]. Other gene-edited EPCs, such as hypoxia inducible factor-1α EPCs and endothelial nitric oxide synthase (eNOS) EPCs, have also been successfully constructed and have achieved substantial efficacy improvements in rabbit and rat PH models [70, 80, 81].

EPC regulation therapy for PH

Pharmacological interventions that aim to correct the pathological status and increase the number of EPCs hold promise as potential therapeutic approaches for the treatment of PH [18].

Endothelial-to-haematopoietic transition (EHT) inhibition

Liang *et al.* [68] revealed that EHT may be an important cause of decreased levels of EPCs. Under PH conditions, the EPCs expressed abnormally high levels of haematopoietic transcription factors, which reactivated their haematopoietic tendency and led to the excessive infiltration of immune cells. To address this issue, Liang *et al.* [68] administered an EHT inhibitor, runt-related transcription factor 1 (Runx1), which successfully blocked the EHT process, reversed the decreasing levels of EPCs and prevented the progression of SU5416 combined with hypoxia (SuHx) in an MCT mice PH model.

Cell mobilisation

Erythropoietin (EPO) has been reported to have a vascular protective effect in various studies [82]. In the aortocaval shunt surgery model, the administration of EPO increased the expression of heme oxygenase-1, mobilised the dormant BM-EPCs, restored the number of circulating EPCs, and finally attenuated vascular remodelling in a mice PH model [83].

In conclusion, the above research results provide valuable insights into correcting the abnormal activities of endogenous EPCs and restoring vascular homeostasis. However, the potential side-effects of drugs need to be carefully considered. For instance, as a regulator of the critical haematopoietic transcription process, further systematic investigations are required to reveal the potentially fatal consequences of Runx1 induction, such as myeloid cell or platelet deficiency [84]. Overall, intravenous administration of drugs appears to be a more attractive option for patients when compared to the complex BM extraction, time-consuming cell culture and dangerous cell transplantation processes.

Preclinical research of MSC therapies for PH

MSC infusion therapy for PH

As early as 2006, Horimoto *et al*. [9] had demonstrated that MSCs could improve haemodynamic disorders induced by MCT and its underlying mechanisms are gradually being revealed (table 2).

MSC distribution tendency and retention time in infusion therapy

Jiang *et al*. [101] demonstrated that in MCT rats, injected MSCs stably adhered to the arterioles of the lung and the fluorescence in the lung was significantly higher than in the spleen from day 7 to day 21. Luan *et al*. [99] extended this observation period to 6 months to verify the long-term retention effect of MSCs in an MCT rat model. After 6 months, the injected MSCs were still present in the pulmonary arterioles and had differentiated into mature cells. This evidence suggests that MSC infusion therapy has good targeting properties and the injected MSCs could spontaneously gather at the damaged region and exert their functions.

Mechanisms of MSC infusion therapy

Vascular repair function

In the Yorkshire pigs pulmonary vein banding model, injected MSCs were found to aggregate in the ischaemic area of the right ventricle. Furthermore, these aggregated cells exhibited robust proliferation, as evidenced by the high expression levels of Ki67 and vascular cell adhesion molecule-1 [102], and differentiation ability, as determined by the widespread expression of vWF and α-smooth muscle actin (α-SMA, mesenchymal marker) [87, 103].

EndMT inhibition

The EndMT of ECs is a notable characteristic of late-stage PH [104]. In chronic hypoxia and SuHx rat models, the administration of MSCs reversed collagen deposition and reduced the overlap rate of vWF and α-SMA around the pulmonary artery. Western blot analysis also demonstrated the downregulation of EndMT-related proteins such as fibronectin 1, Snail and twist family BHLH transcription factor 1 [13].

Inflammation and apoptosis inhibition

Elevated levels of inflammatory and apoptosis factors are a prominent feature of PH. In an MCT rat study conducted by Kim *et al*. [14], the levels of lung tissue pro-inflammatory and pro-apoptotic factors, including interleukin 6, tumour necrosis factor-α and B-cell leukaemia/lymphoma-2 were inhibited by MSC infusion. This ultimately led to an improvement in pulmonary artery and right ventricle structures.

Microbiota regulation

The gut microbiota is important for the maintenance of homeostasis in the human body [105]. RNA sequencing analysis demonstrated that both SuHx and MCT can disrupt the homeostasis of the mice gut microbiota by increasing the Firmicutes-to-Bacteroidetes ratio, promoting the growth of harmful microbiota and reducing the abundance of beneficial microbiota. However, treatment with MSCs partially reversed these changes. In the MSC-treated group, the levels of anti-inflammatory microbiota recovered and immunomodulatory functional microbiota improved [15].

Gene-edited MSC infusion therapy

The potential protective role of let-7a has been exhibited in numerous pulmonary diseases [106]. In comparison to unedited rat MSCs, let-7a-rat MSCs activated the downstream signal transducer and activator of transcription 3–BMPR2 pathway and demonstrated a superior ability to resist vascular remodelling and proliferation of pulmonary artery smooth muscle cells [97]. Thus, promoting the expression of nitric oxide could alleviate abnormal pulmonary vasoconstriction. Based on this, the eNOS-rat MSCs possess a stronger ability to counteract MCT-induced haemodynamic disorder and vascular remodelling and prolong the survival rate in a rat model [107]. Additionally, the enhancement effect of PGI-MSCs were also verified in an MCT rat model [108].

Overall, the results of these studies indicated that MSC infusion therapy has potential for the treatment of PH. However, there are concerns regarding the clinical application of MSC infusion therapy, particularly regarding

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TABLE 2 Summary of recent literature regarding mesenchymal stem cells (MSC) therapy for pulmonary hypertension (PH)

Year	Cell source	Application form	Animal model	Route and dosage	Treatment course	Major findings	Reference
2022	BM and WJ	EVs	Rats and 2 weeks hyperoxia	*i.v.*/*i.t.* and 2/12/60×10^8·g^{-1} EVs in 50 μL saline	Treat at day 3, sacrifice at day 14 or week 3	EVs from MSCs has a potential therapy to treat BPD-PH, and WJ-MSCs, and BM-MSCs have similar treatment effect on BPD-PH	[85]
2022	BM	Hypoxic cells	Rats and 2 weeks hyperoxia	*i.t.* and 5×105 cells in 40 μL saline	Treat at day 0, sacrifice at day 14	Hypoxic MSCs successfully improved BPD-PH through restoring alveolar structure and lung function	[86]
2022	BM	PGE1-MSCs	Rats and 4 weeks MCT (60 mg·kg^{-1})	*i.v.* and 2 ×10^7·mL^{-1} in 0.2 μL PBS	Treat at day 3, sacrifice at week 4	PGE1-treated BM-MSCs could regulate the HIF-1 pathway and enhance the treatment effect of MSCs in MCT rats	[87]
2022	UC	NVs and EVs	Rat and 4 weeks MCT (60 mg·kg^{-1})	*i.v.* and 500 μg·kg^{-1} in PBS	Treat at day 15, 19 and 23, sacrifice at day 30	Both MSC EVs and NVs could significantly improve the symptoms of PH and the miRNA may be the therapeutic mechanism	[88]
2021	AD, BM and UC	Cells	Rats and 4 weeks MCT (60 mg·kg^{-1})	*i.v.* and 1.0×10^6 cells	Treat at week 2, sacrifice at week 4	UC-MSCs showed better treatment effect than BM-MSCs	[89]
2021	UC	Exos	Rats and 3 weeks MCT (50 mg·kg^{-1})	*i.v.* and 50 μg·day^{-1} Exos	Treat at day 0, 1 and 2, sacrifice at week 3	UC-MSCs-Exos successfully reversed the development of PH and suppressed vascular modelling and EndMT	[90]
2021	BM	Cells	Mice and 3 weeks hypoxia	*i.v.* and 1.0×10^6 cells	Treat at week 0, sacrifice at week 3	BM-MSCs could reverse hypoxia-induced gut microbiota disturbance and PH	[15]
2020	UC	Cells	Rats and 3 weeks SuHx (20 mg·kg^{-1})	*i.v.* and 5×10^5 cells	Treat at day 14, sacrifice at week 3	UC-MSCs successfully reversed the development of PH and lodenafil combination therapy could improve the therapeutic effect of UC-MSCs	[91]
2020	BM	EVs	Rats and 3 weeks SuHx (25 mg·kg^{-1})	*i.v.* and 20 or 100 mg·kg^{-1} EVs in 500 μL PBS	Group I treat at day 0, 1 and 2, group II treat at day 5, 10, 15 and 20, sacrifice at week 3	MSC-EVs successfully reversed the SuHx-induced PH and the different protocol showed similar treatment effects	[92]
2020	BM	EVs	Rats and 3 weeks SuHx	*i.v.* and 100 mg·kg^{-1} EVs in 500 μL PBS	Treat at day 1, 2 and 3, sacrifice at week 4	MSC-EVs could regulate the inhibited macrophage activation and recruitment in the lung region to inhibit the development of PH	[92]
2020	UC	Exos	Rats and 3 weeks MCT (50 mg·kg^{-1})	*i.v.* and 25 μg·day^{-1} in 100 μL PBS	Treat at day −3, −2 and −1, sacrifice at week 4	Administration of MSC-Exos could reverse the development of PH and increase the expression level of Wnt5a	[93]
2019	AD	Cells	Rats and 4 weeks MCT (60 mg·kg^{-1})	*i.v.* and 3×10^6 MSCs in 200 μL PBS	Treat at week 2, sacrifice at week 4	MSCs could regulate a series of miRNAs to reverse the development of PH	[94]
2019	AD	bFGF-MSCs	Rats and 4 weeks MCT (60 mg·kg^{-1})	*i.v.* and 3×10^6 MSCs in 200 μL PBS	Treat at week 2, sacrifice at week 4	bFGF-MSCs improved the PH symptoms of MCT rats and increased the expression of the PI3K–Akt pathway	[95]
2018	UC	Cells	Mice and 3 weeks SuHx (20 mg·kg^{-1})	*i.v.* and 5×10^5 cells in 50 μL PBS	Treat at week 2, sacrifice at week 4	MSCs significantly regulated the abnormal apoptosis and inflammation and reversed the development of PH in the SuHx mice model	[96]
2017	BM	Let7a- MSCs	Rats and 6 weeks MCT (60 mg·kg^{-1})	*i.v.* and 3×10^6 cells in PBS	Treat at week 3, sacrifice at week 6	Let7a-MSCs showed a better treatment effect than unedited MSCs by increasing the expression of the STAT3–BMPR2 pathway	[97]
2016	UC	Cells	Rats and 4 weeks MCT (60 mg·kg^{-1})	*i.v.* and 3×10^6 cells	Treat at week 1, sacrifice at week 2 and week 4	Administration of MSCs significantly inhibited the apoptosis, proliferation and inflammation of the MCT-PH model	[14]
2015	UC	Cells	Rats and 4 weeks MCT (60 mg·kg^{-1})	Subcutaneous injection and 500 μL CM	Treat at day 5 and day 9, Sacrifice at week 4	CM from MSCs could inhibit the over-proliferation of PASMC and potential therapy to treat MCT-PH	[98]
2014	BM	Cells	Rats and 3 weeks MCT (50 mg·kg^{-1})	*i.v.* and 1×10^7 cells	Treat at week 1, sacrifice at week 3	MSCs significantly inhibited the MCT-induced vascular remodelling and treatment effect lasted until month 6	[99]
2012	BM	Cells	Rats and 3 W MCT (50 mg·kg^{-1})	*i.v.* and 5×10^5 cells in 100 μL saline	Treat at week 1, sacrifice at week 3	Administration of MSCs significantly reversed the abnormal haemodynamics of the MCT-PH model	[100]

AD: adipose tissue derived; eNOS; bFGF: basic fibroblast growth factors; BM: bone marrow; BMPR2: bone morphogenetic protein receptor type II; BPD: bronchopulmonary dysplasia; CM: conditional medium; EndMT: endothelial-to-mesenchymal transition; EV: extracellular vesicle; Exo: exosome; HIF-1α: hypoxia inducible factor-1α; *i.v.*: intravenous; *i.t.*: intrathecal; MCT: monocrotaline; miRNA: microRNA; NV: nanovesicle; PGE1: prostaglandin E1; STAT3: signal transducer and activator of transcription 3; SuHx: SU5416 combined with hypoxia; UC: umbilical cord; WJ: Wharton's jelly.

the cell source of autologous MSCs. Patients with PH often carry PH-related mutant genes that may attenuate the treatment effects of the MSCs and a poor cell status can substantially hinder the culture process of MSC infusion therapy. However, the disadvantages of allogeneic stem cell transplantation, such as graft-*versus*-host disease (GVHD) and ethical issues also concern researchers. To overcome these disadvantages, further research is required to identify new ways to optimise the clinical application of MSC infusion therapy.

MSC appendage therapy for PH

Although MSC infusion therapy has shown therapeutic benefits for PH, researchers remain concerned about adverse reactions, including autoimmune reactions [109], genetic instability [110] and long-term tumour formation [111]. Therefore, cell appendages, such as Exos and EVs, naturally attracted the attention of researchers. Since Exos and EVs do not include cell membrane surface receptors or cell components, the risk of immune reactions or tumour development are reduced [112]. Willis *et al*. [113] injected Exos from BM-MSCs into hyperoxia bronchopulmonary dysplasia (BPD) mice models. The BM-MSC-Exos significantly improved the hyperoxia-induced lung function and structural abnormalities. Furthermore, the BM-MSC-Exos also regulated the differentiation tendency of immune cells and promoted a macrophage shift from M1 to M2 phenotype [113]. Other therapeutic mechanisms such as cytoprotective effects [114], mitochondrial health [115] and vascular protection functions [93] have also been verified in mouse and rat PH models. In recent studies, the functional contents of EVs and Exos, including miRNAs (miR-125-5p, miR-00-5p [88], miR-34a, miR-122, miR-124, miR-127 [17], miR-17 and miR-204 [114]) and proteins (Wnt family member 5a (Wnt5a), Wnt11, BMPR2, bone morphogenetic protein (BMP) 4 and BMP9 [116]) have been revealed, suggesting that they may represent a promising alternative to stem cell therapy in the treatment of PH.

Preclinical research of iPSC therapies for PH

The advent of iPSC technology offers a new solution for researchers and doctors to resolve the limitation of stem cell sources [117]. Huang *et al*. [10] administrated iPSCs at prevention (with MCT) and reversal (14 days after MCT) time points in MCT rats. Both the prevention and the reversal therapy considerably improved MCT-induced haemodynamic abnormalities, pulmonary artery hypertrophy and downregulation of inflammatory factors. However, the limitations of iPSCs are also obvious, such as tumorigenicity, genetic and epigenetic abnormalities, and long-term safety and efficacy [118]. Therefore, iPSCs are commonly used as cell models to investigate different phenotypes under various interventions, such as the different differentiation fates [119], cell models for drug screening [120], influence of gene mutations [120, 121] and RNA sequencing [122, 123]. For example, Gu *et al*. [120] reprogrammed fibroblasts from six patients with PH as iPSCs and induced them to differentiate into ECs and then exposed them to 4500 compounds and performed assays to determine improved cell survival. Finally, the lead compound AG1296 was selected and verified in animal model. With the support of iPSCs, additional potential therapeutic drugs, pathogenicity genes and target cells are being explored and further in-depth studies are planned.

Clinical application of stem cell therapy for PH

EPC therapy clinical trials

According to research results on clinicaltrials.gov, six clinical trials have been associated with EPCs and PH, of which three have been completed, two are currently active and one is unknown (table 3). 31 adult patients with idiopathic PH were randomised to receive conventional therapy or conventional plus EPC infusion therapy. The cell infusion plus group received an average intravenous infusion of 1.1×10^7 EPCs. After a 12-week follow-up, the mean distance walked in 6 min (6-MWD) of the cell infusion plus group increased by 48.2 m, compared to the 5.7 m increase of the conventional therapy group. Other hemodynamic parameters, such as mPAP, PVR and cardiac output, were also substantially improved. Notably, there were no observed severe life-threatening adverse events [124]. Similar improvements and adverse events were observed in children with idiopathic PH [125]. A Canadian group further demonstrated the feasibility of gene-edited EPCs, where a total of 50 million eNOS-EPCs were injected into seven patients across three dosages. All patients showed an improvement in total pulmonary resistance during the delivery period and 6-MWD also improved at 1, 3 and 6 months [126]. However, one of the seven patients experienced a severe adverse event (death) after discharge. These findings suggest the potential of EPCs as a therapeutic option for PH, although further studies are warranted to evaluate their safety and efficacy. In EPC regulation therapy, riociguat has been proposed as a potential therapeutic intervention for PH [127]. A study conducted by Yamamoto *et al*. [61] investigated the effects of riociguat on PH-related parameters and its potential mechanism of action. The results showed that the riociguat group did not exhibit significant improvements in terms of demographic and haemodynamic parameters, oxygenation or the 6-MWD compared to the naïve group. However, a notable finding was that the riociguat group exhibited a significantly higher number of peripheral EPCs compared to the naïve group. Subsequent PCR analysis revealed the upregulation of several angiogenesis-related genes in riociguat-treated EPCs. In addition, *in*

TABLE 3 Summary of recent clinical trials regarding the use of stem cells to treat pulmonary hypertension (PH)

www.clinicaltrials.gov identifier number	Number of participants	Phase	Disease	Cell type	Trial targets	Last update posted	Current Status
NCT00641836	98	NA	Idiopathic PH	EPCs	To verify the clinical feasibility of autologous EPC transplantation therapy in patients with idiopathic PH	24 March 2008	Completed
NCT03001414	45	2/3	Symptomatic severe PH	eNOS-EPCs	To verify the clinical feasibility of eNOS-EPC transplantation therapy in patients with progressive PH	11 January 2023	Recruiting
NCT00372346	40	NA	Idiopathic PH	EPCs	To verify the clinical feasibility of autologous EPC transplantation therapy in patients with idiopathic PH	6 September 2006	Recruiting
NCT00257413	/	NA	Idiopathic PH	EPCs	To verify the clinical feasibility of autologous EPC transplantation therapy in patients with idiopathic PH	22 November 2005	Completed
NCT00491309	45	NA	Symptomatic PH	EPCs	To investigate the correlation between EPCs and PH	14 July 2022	Recruiting
NCT00551408	20	NA	Idiopathic PH	EPCs	To investigate the correlation between EPCs and PH	16 October 2018	Completed
NCT04055415	60	1/2	COPD-PH	MSCs	To verify the clinical feasibility of autologous AD-MSCs transplantation therapy in patients with COPD-PH	13 August 2019	Recruiting
NCT04432545	/	/	SSc-related PH	MSCs	To verify the clinical feasibility of autologous MSC transplantation therapy in patients with SSc-related PH	16 June 2020	Available
NCT02443961	10	1	BPD	MSCs	To verify the clinical feasibility of autologous MSC transplantation therapy in preterm babies with BPD	30 March 2023	Completed

AD: adipose tissue derived; BPD: bronchopulmonary dysplasia; eNOS: endothelial nitric oxide synthase; EPC: endothelial progenitor cell; MSC: mesenchymal stem cell; NA: not applicable; PH: pulmonary hypertension; SSc: systemic sclerosis; /: information not available from www.clinicaltrials.gov.

vitro cell experiments demonstrated that ECs co-cultured with riociguat-treated EPCs displayed enhanced tube formation, migration and proliferation capacity. These findings suggest that riociguat may exert potentially beneficial effects in PH by promoting angiogenesis through the modulation of EPCs.

MSC and iPSC therapy clinical trials

To date, only three clinical trials have attempted to utilise MSC therapy to treat idiopathic PH, diffuse cutaneous systemic sclerosis with PH and BPD-PH. However, the status of these trials was unknown, available and active and not recruiting, respectively, and there were no available results to analyse the therapeutic effects and adverse events. Furthermore, to date there are no iPSC therapy trials for PH.

In conclusion, clinical trials have shown promising results for stem cell therapy, including both simple stem cell infusion therapy and gene-edited stem cell infusion therapy. However, a severe adverse event was reported, which may have been caused by thrombosis-induced acute ischaemia, such as cell embolus, fat embolus or hypercoagulable state-induced thrombosis. As such, the establishment of a well-defined cell pre-processing and cell infusion workflow is essential for safe and effective treatments. Additionally, further evidence is required to evaluate the long-term therapeutic effects and potential side-effects of stem cell therapy, as most trials have only reported up to 6 months. Therefore, further research is required to provide convincing evidence to support the widespread use of stem cell therapy for the treatment of PH.

Challenges of the PH stem cell therapy in clinical applications

Autologous GVHD (auto-GVHD)

GVHD is a serious immune disease that occurs in patients who have received a transplant. The grafted immune cells attack the recipient's healthy tissues and organs, resulting in the disturbance of multiple organs and even death [128]. Although GVHD primarily occurs in allogeneic transplantation, auto-GVHD should not be disregarded. Zenz *et al*. [129] reported that the incidence of auto-GVHD substantially increased after the use of short-course cyclosporine during autologous stem cell transplantation, with

symptoms that included conjunctivitis, sicca syndrome and cholestasis. Despite the growing interest in autologous stem cell transplantation, there remains a lack of systematic research on the mechanism of auto-GVHD occurrence.

Culture process

Autologous cell transplantation requires complex extraction processes and a waiting period for cell number replication. It is important to carefully monitor and optimise the culture conditions of stem cells to ensure their viability and activity for successful treatment outcomes in patients with PH. In patients with severe PH, the number and viability of stem cells are likely reduced, which could significantly prolong the required culture time, thereby increasing the likelihood of failure and ultimately decreasing treatment efficacy. To address this issue, universal allogeneic cell products may be a direction for future research.

Tumour formation

As previously mentioned, concerns regarding potential tumour formation should not be overlooked. While stem cells have demonstrated a strong capacity of self-renewal and proliferation, studies have also highlighted their potential oncogenicity. This may be attributed to the differences in culture conditions between laboratory settings and the human body, such as the use of multiple growth factors in the stem cell culture medium [130], harder culture substrates [129] and altered metabolic environments [131]. Animal models have shown that the risk of cancer in recipient animals is higher than in normal animals over a long-term period [132]. Therefore, it is crucial to thoroughly assess the potential risks of stem cell therapy before clinical implementation. Further research is required to better understand the mechanisms underlying the oncogenic potential of stem cells and the development of safer and more effective methods for their use in clinical settings.

Ethical issues and political controversies

The promising outcomes of advanced stem cell therapy research offer hope to patients suffering from various refractory diseases, including pulmonary diseases [133], diabetes [134], spinal cord injury [135], Parkinson's disease [136] and so on. However, the ethical concerns and political controversies surrounding stem cell therapy have also raised concerns among researchers [137]. It is important to note that current clinical trials primarily focus on patients with severe PH [124–126] and there is currently no evidence supporting the efficacy of stem cell therapy in treating mild PH. Patients with mild PH should use conventional drugs to control disease progression before considering stem cell therapy. Hence, a systematic evaluation of indications for stem cell therapy is necessary. Moreover, it should be acknowledged that autologous stem cell transplantation therapy is a costly treatment and not all patients may have the financial means to afford it. Therefore, physicians must carefully assess the indications for PH and consider the patient's financial situation before recommending stem cell therapy [138]. EPCs, MSCs and iPSCs can be derived from the patient's own tissue, avoiding the need for blastocyst derivation. However, if researchers try to apply more stem cell types in PH treatments, such as embryonic stem cells, the blastocyst derivation source also needs further consideration. With increasing restrictions on abortion and embryo donation, the availability of cells for research and treatment is limited. Although embryonic stem cell lines, such as H1 and H9 cell lines, could meet academic research requirements, their long culture time has been shown to lead to the accumulation of mutations and potentially induce uncontrollable severe side-effects. The development of universal cell lines for clinical stem cell therapy applications still requires significant progress [137, 139]. Despite these difficulties, we remain optimistic about the future of these studies.

Points for clinical practice

Preclinical studies and clinical trials have demonstrated the excellent treatment effects of stem cell and cell appendage therapies and revealed their detailed treatment mechanisms. However, there still remains a gap in their large-scale clinical application. There is an urgent need for an explicit clinical guideline. In the diagnostic phase, it is crucial to clearly define which phase of PH patients should be treated. For example, mild PH patients may benefit from traditional target therapy as the first-line choice. On the other hand, stem cell therapy should be considered for patients in the progressive phase or those with refractory PH to potentially prolong their life. Furthermore, a standard treatment protocol needs to be established. This should include defining the cell donor, cell source, cell culture time, infusion cell number, delivery route, usage and dosage, and treatment course. Having a standardised protocol will help ensure consistent and effective treatment approaches. Lastly, it is essential to investigate the long-term treatment effects and side-effects of stem cell infusion through a large cohort study. Multiple factors should be recorded, including the effects of symptom remission, stem cell infusion-associated side-effects and any unrelated side-effects that may arise from stem cell infusion. Understanding the long-term effects and potential risks of stem cell therapy will provide valuable information for clinical decision-making and patient safety.

Conclusion and perspectives

The demand for a radical therapy for PH has driven the rapid advancement of stem cell therapy research, resulting in promising outcomes. Stem cell therapy operates through a complex and synergistic mechanism, which includes differentiation, angiogenesis, apoptosis inhibition, secretion, EndMT inhibition, microbiota regulation and other functions. Additionally, stem cell-related studies, such as those investigating Exos, EVs and drug regulation therapies, have greatly expanded the application range of stem cell therapy. These exciting findings consistently reinforce efforts toward clinical translation, highlighting the potential of stem cell therapy as a viable treatment option for PH. Further research is required to fully elucidate the mechanisms and optimise the clinical application of stem cell therapy in PH treatment.

Although stem cell therapy has shown promise for the treatment of PH, there are several challenges that must be addressed before its large-scale application can be considered. In preclinical research, a general definition of EPCs is still lacking. Researchers subsequently choose CD31, CD34, VEGFR-2, CD133, CD144, *etc*. as markers to filter EPCs. Different markers present different cell clusters and lead to different treatment effects. Therefore, a clear definition of EPCs is necessary. In clinical research, while the treatment effect has been shown to be excellent, the potential side-effects of stem cell therapy remain unclear and are the most important obstacle to clinical translation. The potential risk of tumour formation is of particular concern, where one cell out of a million with harmful mutations could have disastrous consequences due to the robust self-renewal and differentiation capacity of stem cells. Additionally, the complex culture process and its induced issues, such as infection, thromboembolism and low vitality, also pose challenges for researchers. Furthermore, the interaction between EPCs and conventional drugs, such as PCA and PDE5is, which are often long-term PH treatments, should be investigated in more systemic clinical trials. Therefore, multidimensional studies are necessary to investigate the long-term side effects of stem cell therapy and address these challenges before its widespread clinical application can be considered.

In conclusion, stem cell therapy has demonstrated remarkable treatment efficacy for PH and has revolutionised the treatment approach for both patients and healthcare professionals. However, concerns from experts regarding the long-term safety and efficacy of this therapy must be addressed through further research and detailed reporting. Despite these challenges, we remain optimistic and positive about the future of stem cell therapy for PH treatment. These findings highlight the potential of stem cell therapy as a promising treatment option for PH and further studies are required to determine its long-term safety and efficacy.

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