Chapter

Stem Cells in Hypertension

Harmandeep Kaur Randhawa, Madhu Khullar and Anupam Mittal

Abstract

Endothelial dysfunction and vascular remodeling are the hallmarks of pulmonary arterial hypertension (PAH). For PAH treatment, there is a rising demand of Stem cell therapy. Interestingly, research reveals that stem/progenitor cells may have an impact in disease progression and therapy in PAH patients. Clinical trials for stem cell therapy in cardiac cell regeneration for heart repair in PAH patients are now underway. The clinical potential of stem/progenitor cell treatment that offers to PAH patients helps in lesion formation which occurs through regaining of vascular cell activities. Majorly the stem cells which are specifically derived from bone marrow such as mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs) and induced pluripotent cells (iPSCs), adipose-derived stem cells (ADSCs), and cardiac stromal cells (CSCs) are among the subtypes that are proved to play a pivotal role in the repair of the heart. But with only MSCs and EPCs, have shown positive outcomes and act as therapeutically efficient in regaining cure for PAH in clinical trials. This chapter also seeks to explain the potential limitations and challenges with most recent achievements in stem/progenitor cell research in PAH.

Keywords: pulmonary arterial hypertension, mesechymal stem cells and induced pluripotent stem cells

1. Introduction

Stem cells have the potential to regenerate tissues and organ systems owing to their capability of self regeneration and multilineage differentiation [1]. Increased pulmonary artery pressure (PAP) causes right ventricular heart failure, which is one of the main reasons why PAH is thought to be incurable [2]. Two essential cell types in the pulmonary arteries that have been significantly impacted by PAH: endothelial cell loss of function and pulmonary artery smooth muscle cells (PASMCs) expansion [3]. Additionally, the pathologic characteristics that were introduced, such as endovascular diameter constriction into the endothelial cells, excessive proliferation of fibroblast and smooth muscle cells (SMC), and a lack of communication between pericytes, contributed to the dysfunction process [4]. The pathobiology of disease can be characterized in terms of changes in RV vascular capacity, and it is shown that in order to preserve coordination between normal cardiac output and RV ventricular-arterial (VA) coupling, there is a distinct transition from adaptive to

maladaptive state [5]. Because contractility and thickness of artery wall are increasing which result in RV dilatation, decreased contractility and cardiac output, and VA uncoupling [6, 7]. Patients who have been diagnosed with long-term RV failure will eventually die. Maladaptive RV remodeling has been associated to decreased angiogenesis, increased metabolic alterations, fibrosis, and disruption of the autonomic nervous system at numerous cellular levels. As a result, PAH is still a fatal disorder with no corrective treatment [8]. Stem cells (SC), on the other hand, are seen as a new cell based therapy approach for those suffering with PAH, as they successfully address symptoms associated to mitochondrial and pulmonary vascular endothelial failure, as well as controlling pulmonary artery expansion in smooth muscle cell [9]. The ultimate goal of SC therapy is to restore cardiopulmonary function while avoiding serious side effects. Another compelling feature is genetic alterations which ultimately boost the effectiveness of stem cells in treating PAH. Adult stem cells are attributed to multipotency with an exception of pluripotent nature found in umbilical cord blood [10]. The right ventricle can be treated using stem cells for instance, MSCs, EPCs, iPSCs, ADSCs, and CPCs. Clinical investigations have shown that the two cell types of differentiating cells which are MSCs and EPCs have been regarded as a putative therapy for PAH as illustrated in **Figure 1** [9, 11]. This pulmonary vascular remodeling process includes endothelial injury and repair, development of smooth muscle cells, and the participation of resident and circulating stem/progenitor cells [10, 12–14]. Stem/progenitor cells have the ability to develop into vascular cell lineages, which may aid in the regeneration process and be effective in the treatment of this condition [15, 16].

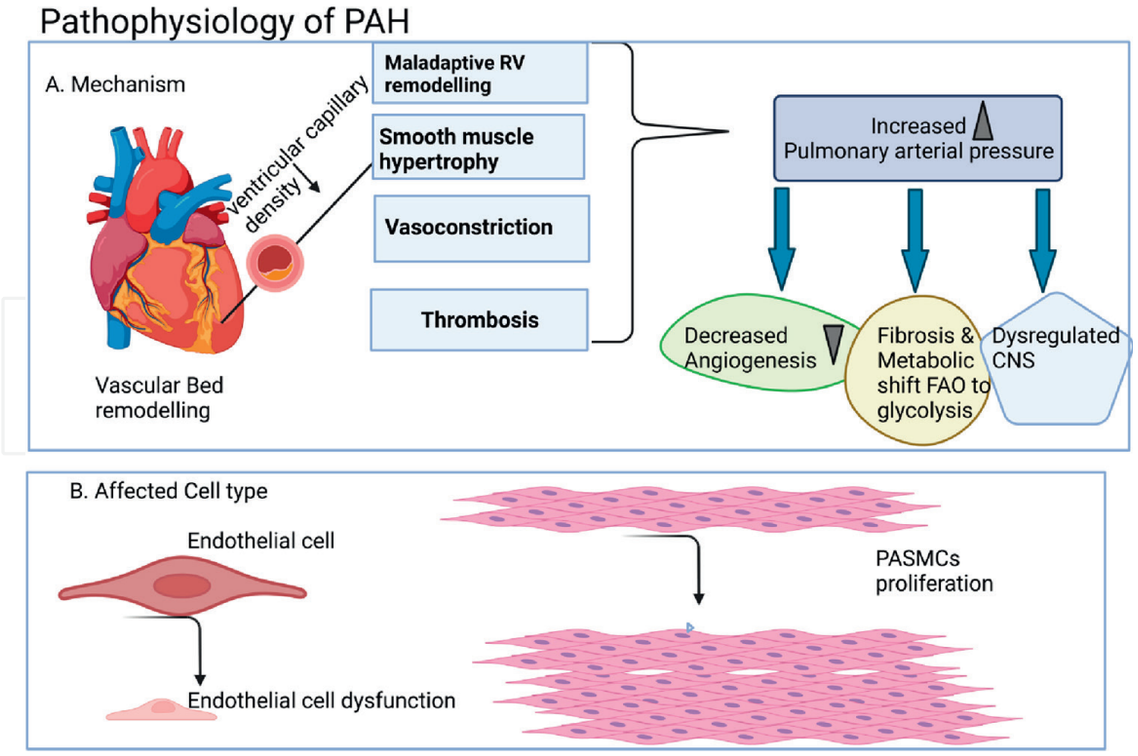

Figure 1.
Mechanism of progression of pulmonary arterial hypertension. (a) defects in right ventricle takes place through decrease in ventricular capillary density. (B) cardiac endothelial cells dysfunction and over proliferation of PASMCs.

2. Regaining of PA endothelial dysfunction by stem cells

The following sections will specifically discuss the different types of stem/progenitor cells involved in PAH.

2.1 Mesenchymal stem cells (MSCs)

MSCs, often referred to as mesenchymal stromal cells, are non-hematopoietic cells that are present in bone marrow stroma. They are a diverse cell population with a built-in capacity for self-regeneration and cell differentiation into a variety of different cell types. MSC-based stem cell treatment has gotten a lot of attention in healing wounded tissue since MSCs are unique in that they can differentiate into multiple cell types and release paracrine substances [17–20]. Such cells can be obtained from different origins such as from bone Marrow, peripheral blood, amniotic fluid, placenta and umbilical cord blood etc. as shown in **Figure 2**. Apparently, the differentiation of stromal cells into adipocytes, osteoblasts and chondroblasts, is due to its remarkable multipotent feature among other cell types. They display specific markers on their

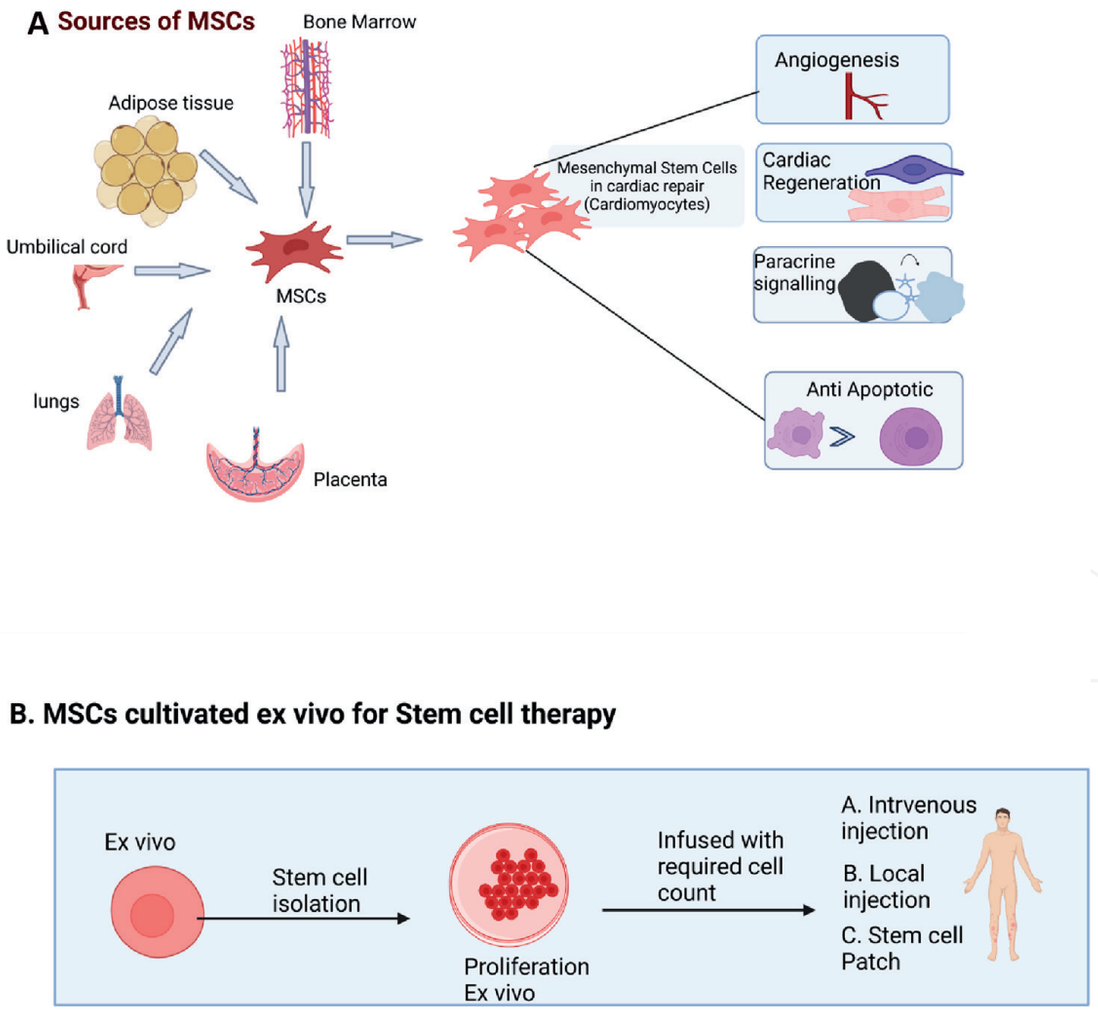

Figure 2.
A. the origin of MSCs and their lineage into cardiomyocytes cells with their characteristics role in regeneration. B MSCs ex vivo preparation for transplantation.

cell specific surface markers: CD90, CD73, and CD105, but not CD14, CD24, CD31, or CD45 [21]. MSCs shows the competence to move to wounded lung tissue and secrete anti-apoptotic (Bcl-2), angiogenic Vascular endothelial growth factor (VEGF), and anti-inflammatory interferon (IFN), Interleukins (IL-10), and Hepatocyte Growth Factor (HGF) proteins. MSC's immunological tolerance is a crucial trait that makes them ideal for clinical usage [22]. In PAH, MSCs are highly suitable for right ventricular (RV) cell therapy because of their tendency to release paracrine chemicals which has proangiogenic and protect cells against harmful agents by secreting various compounds (cytoprotective effects). They may protect cardiomyocytes against hypertrophy and fibrosis by increasing capillary density. Finally, their immunostimulatory attribute make them very appealing for stem cell treatment [23].

As research suggest the Prostacyclin synthase (PCS) gene in MSC's have been demonstrated to reduce right ventricular hypertrophy (RVH) and inhibit monocrotaline induced pulmonary arteriolar remodeling [19, 23]. More significantly, this research found that if PCS-MSCs injected once can potentially improve the life expectancy of PAH induced rats to seven weeks after the injection. When it came to reducing RVH and right ventricular systolic pressure (RVSP) in monocrotaline-induced PAH rat model, MSCs produced from human embryonic stem cells outperformed MSCs derived from adult bone marrow in preclinical studies [24]. Clinical trials done so far are currently centered mainly on increasing pulmonary function, and MSCs have clearly proved their efficacy in treating PAH. MSCs are stable enough to remain at the site of tissue injury and inflammation, and they are also simple to genetically manipulate, isolate, and cultivate ex vivo. The systemic infusion of MSC-conditioned media was shown to reduce lung inflammation and stimulate vascular development in wounded tissue. The release of chemicals that perform tissue healing is most likely the underlying process that promotes vascular growth and heals wounded vascular endothelium. Another effect known as paracrine signaling has been seen, which leads to MSCs engraftment and differentiation into particular lung cell types. Because paracrine signaling is present in modest quantities at wounded tissue, it has a favorable impact on damage responses such as PAH [6, 7, 25].

2.2 Epigenetic alterations of stem cells in treating PAH: role of microRNA (miRNAs) in PAH

miRNAs are non-coding RNAs that are found in the human body and are important regulators in a variety of pathophysiologic processes. Recent research suggests that by influencing gene expression of multiple mRNAs, transfection of stem cells with particular microRNA could alleviate the related inflammatory pathways in PAH [26]. Moreover, miRNA abundance and their activation are tissue specific in both healthy and pathological situations, hence they are of critical importance. To exemplify, some clinical and preclinical studies have already found that the differential expression of number of miRNAs that plays crucial role in prolonged hypoxia condition in the lungs of PAH monocrotaline rat model [26, 27]. DNA methylation, histone acetylation, and microRNA dysregulation all contribute to PAH production. Histone acetylation is important in pulmonary arterial hypertension. MiR-17 promotes the STAT3-BMPR pathway, whereas miR-145 inhibits BMPR activity MiR-30, MiR-22, and let-7f were down regulated in both hypoxic and monocrotaline models, however miR-322 and miR-451 were significantly up regulated throughout the progression of PAH. In PASMCs from people with PAH, miR-204 was consistently down regulated [26, 28]. Absence of regulation of miR-17 in PASMCs has been linked to PAH and is likely to

be related to cell proliferation. Overexpressed miR-21 appears to proliferate human PASMCs and their interconnected proteins, such as cyclin D1 and Bcl-xl, in vitro genetic alteration, providing strong evidence for its role in cell proliferation [29].

The survival and widespread mortality of the transplanted MSCs in the injured tissue made MSC its efficacy unacceptably low. Cell differentiation, neovascularization, cell death, and other processes all involve miRNAs. Therefore, epigenetic pathways must be explored when taken into consideration for transplantation therapy for PAH disease, and a detailed analysis of how miRNAs regulation might reverse PAH will be crucial for further study into its pharmacological properties. As a nutshell, another promising strategy for treating PAH is epigenetic alteration of stem cells using miRNAs [29, 30].

2.3 Endothelial progenitor cells (EPCs)

The first changes in PAH takes place through apoptosis of endothelial cells and further loss of endothelium integrity that contributes to pathophysiology, creation of occlusive vascular lesions produced by subsequent uncontrolled expansion in vascular adventitia and smooth muscle media [4, 31]. ESPCs are thought to develop into mature endothelial cells at locations of vascular injury and provide a vital part in regenerating tissues for endothelial function recovery during PAH. CD34 and VEGFR-2 were the first cell surface markers screened out to determine the EPCs, according to Asahara. Currently, cells must display a series of distinct markers to be classified as EPCs, although however there is a closed resemblance with surface markers that are present on circulating endothelial cells (CEC) and hematopoietic stem cells (HSC). The CD34, CD45-, CD133+, KDR+, CD14-, CD146+ are phenotypic markers for EPCs [32, 33]. The limitations of EPCs extracted from adult peripheral blood ranging from 0.002 to 0.01% is that they are in small proportion which is required for stem cell treatment. For accomplishing the required number of cells, culturing of cells in vitro for several weeks is required. It is the long culture period necessary to generate a viable therapeutic dosage. The isolation of EPCs from 0.2 to 1% in umbilical cord blood (UCB) produces far more EPCs in comparison to adult blood with greater number of active cells as well [32]. Despite the fact that EPCs are taken from different individuals of the same species thus allogenic in nature and the underlying capability for immunological refusal must be considered. Additionally, the absence of identification of the kind of EPCs makes it difficult to compare and adapt study findings to clinical practice. EPCs has been shown to be helpful to the right RV in PAH animal models. The study reveals that no direct impact was evaluated on EPCs in heart as whole concern was on improving lung circulation or if they were caused by the transplanting stem cells for improving the pulmonary vascular disease. Therefore, EPCs likely to be potential candidates for treating PAH with RV-targeted cells [16, 23, 32].

The present limitation facing are due to its low frequency in peripheral and cord blood, which is one of the key constraints of EPCs therapy. Firstly its inherent immunogenicity, it can only be delivered autologously. Secondly, the transplanted SPCs have a low survival rate [32]. Henceforth the obstacles to their isolation and identification, as well as concerns with expansion efficiency and immunogenicity, must be overcome before EPCs may be used clinically in the PAH area. Because of their clonal proliferation potential and ability to create blood vessels, EPCs also have vascular reparative effects in PAH, making them a feasible method for pulmonary vascular regeneration [34].

2.4 Induced pluripotent stem cells (iPSCs)

In 2006, Takahashi and Yamanaka discovered that reprogramming somatic cells from human through overexpression of transcription factors (OCT4, SOX2, KLF4, c-MYC, LIN28, and NANOG) has developed iPSc, a novel pathway for different disorders through regenerative therapy as shown in **Figure 3**. Mouse embryonic or adult fibroblast cells have successfully employed to create induced pluripotent stem cells [35].

Human iPSCs share many properties with human ESCs, such as morphological similarities, ability to proliferate and pluripotency markers for their differentiation potential, but their traits related to epigenetics are significantly differ [36]. The production of endothelium cells, cardiomyocytes, or SMCs from human iPSCs can be achieved after reprogramming of human fibroblasts to differentiate into appropriate cell types in PAH disease. Although iPSCs can also be produced patient specific through patient's own fibroblast from skin.

In preclinical settings, the iPSCs derived cardiomyocytes are infused into the right ventricle of PAH animal model and their beneficial effects on RV performance were recorded through pulmonary unit, however although these stem cell therapies still aren't injected directly to the RV in humans. In autologous stem cell therapies, disease-causing mutations can be easily restored using gene editing advances, resulting in the formation of iPSCs originated to produce functional cells which can replace non functional tissues and organs with healthy cells, such as those affected by neurological, cardiovascular, hepatic, and retinal disease [1, 35, 37]. The use of iPSCs in a rat monocrotaline model produced therapeutic outcomes in one investigation [38]. When treated PAH model with iPSCs, there is a reduction in right heart dysfunction, as a result there is a downfall in hemodynamic parameters which are responsible to maintain right ventricular systolic pressure. Furthermore, by limiting inflammation, such therapy has prevented pulmonary arteriole vascular remodeling deterioration and

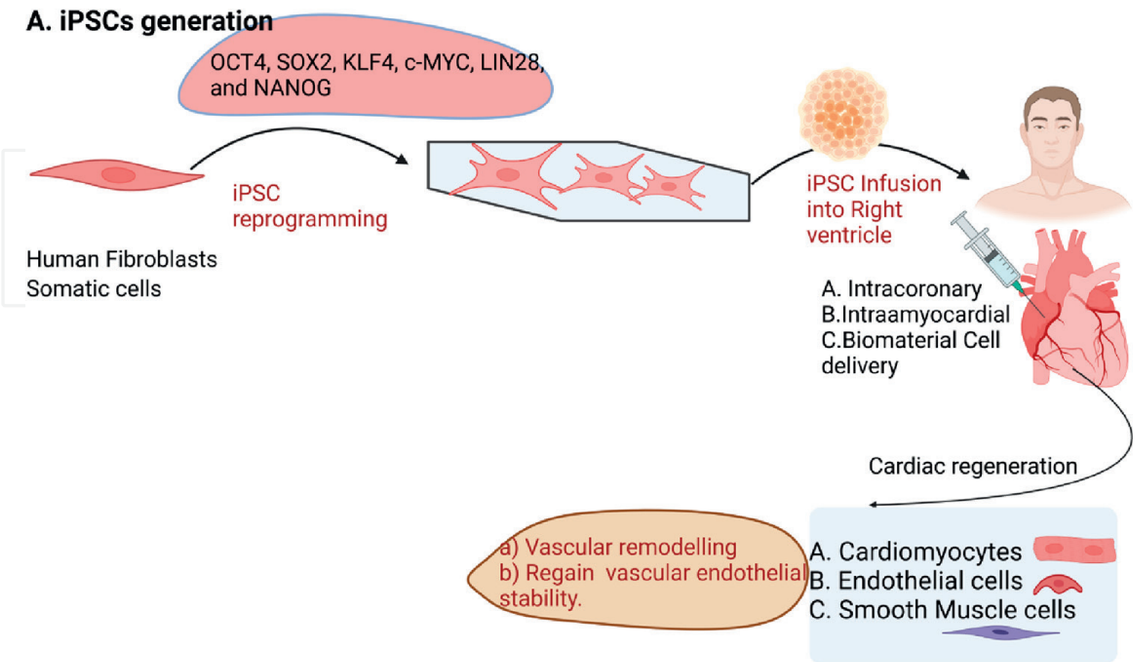

Figure 3.
Development of cell derived iPSCs for cardiac regenerative therapy for treating PAH. The resetting of differentiation of adult fibroblasts with specific factors give rise to generation of iPSCs that can be differentiated into the desired cell type for cardiac regenerative therapy.

reduced media layer proliferation. As nothing more than a nutshell, there that iPSC-based therapy can improve vascular remodeling and making repairs in PAH, as well as restore vascular endothelial stability. The limiting use of iPSCs are due to its tumorigenic nature because of its similarity with embryonic stem cell-like features [38, 39].

2.5 Adipose-derived stem cells (ADSCs)

ADSCs are unique type of adult stem cells for the treatment of cardiovascular disorders that can be easily extracted and grown from adipose tissue. For transplantation, through liposuction technique the ADSCs can be extracted from white adipose tissue [40, 41]. After differentiation, they have a remarkable potential to change into vascular SMCS, endothelial cells and cardiomyocytes [42] for PAH treatment. Most significantly ADSCs are regaining prominence in cardiac research because of its regeneration potential by secreting a number of paracrine substances that promote neovascularization and decrease apoptosis while also preventing fibrosis.

Adipose tissue has a far higher density of stem cells than bone marrow stem cells (5 percent versus 0.01 percent). Miranville et al. [43] used in vitro experiments to identify the subset of ADSCs (CD34+/CD31–) that may differentiate into ECs when cultivated in endothelial growth media supplemented with IGF and VEGF. The cells had a spindle-shaped morphology and strong expression of EC markers like CD31. This group has features that were similar to human umbilical vein endothelial cells.

ADSCs have tissue regenerating potential during injury because it produce angiogenic and anti- growth factors for apoptosis such as colony-stimulating factor (VEGF), transforming growth factor alpha (TGFα), granulocyte–macrophage basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF).

ADSCs can induce angiogenesis in models of hind limb ischemia by undergoing differentiation into endothelial cells that integrate into the walls of newly formed arteries and secreting paracrine substances [44, 45]. Research reveals that ADSCs separated from heart adipose tissue propagated better, and possess the strongest cardiac functional recovery, and the highest rate of recruitment to ischemic myocardium in contrast to cells isolated from heart, visceral, and subcutaneous sub scapular adipose tissues.

Overall, ADSC is considered as better option for treating the right ventricle in PAH because of it can give rise to angiogenesis without undergoing apoptosis, and in addition to being autologous, they are numerous, and easily accessible [42, 46].

2.6 Cardiac progenitor cells (CPCs)

The CPCs are the cells which are endogenous and possess capability of a cell for self-regeneration and differentiation that distinguishes CPCs from other cells in the adult heart. They have attributes of c-Kit, Sca-1, and SSEA-1 expression in contrast to others cells subtype. In pre clinical settings the myocardial infarction (MI) was therapeutically treated using CPCs [47]. They're supposed to boost cardiomyocyte and transdifferentiation of vascular cells, along with generation of paracrine chemicals that promote CPCs activity and neovascularization [48]. These features may help the RV in PAH by increasing capillary density and promoting the development of healthy cardiomyocytes. Adult CPCs isolation, on the other hand, is an invasive procedure that involves extracting cells from the patient's cardiac cells. While employing embryo-derived CPCs raises ethical difficulties, autologous iPS cells can alleviate these concerns. Finally, CPCs contain a significant number of various forms of cell

with varied surface markers, making it difficult to conclude which will provide most efficient treatment. Additionally CPC, can be considered as an option if another alternative cardiosphere derived cells (CDC) are available, which can be derived from adult human biopsies [23, 48, 49]. The use of cardiosphere derived cells (CDCs) or CPC have potential to inhibit cardiomyocyte fibrosis and apoptosis in stem cell therapies.

2.7 Pericytes in PAH

Pericytes in the lungs play a significant role in PAH. Pericyte multiplication has recently been investigated as an early manifestation of PAH, and clinical specimens with PAH have shown aberrant pericyte covering of the pulmonary vasculature. Pericytes have been found to exhibit MSCs like progenitor capacities in recent investigations, and as a result, they are anticipated to play a variety of roles in PAH-related pathological changes to lung structure and function [50]. Pericytes or MSCs are vascular progenitor cells capable of regenerating a variety of cell types while preserving lineage-allegiance responsive to tissue demands, as seen by their extensive distribution in the vasculature [51]. Pericytes have a significant pathogenic role in the development of PAH because they regulate angiogenesis, inflammation, and have progenitor capacity [52]. For the integrity and preservation of the vessel wall's basement membrane, pericytes and endothelial cells must communicate [53].

Despite its importance in developing new blood vessels, vessels permeability, and contractility, pericytes that just lately been researched in relation to PAH. Pericytes that operate as progenitor cells have recently been discovered, specific tissue-localized pericytes which can grow as the normal MSCs trio of chondrocytes, adipocytes and osteocytes has been confirmed [54]. Pericytes and MSCs from the vasculogenic zone, it has been proposed, may be closely related. Pericytes have been discovered to contain MSCs like progenitor capacities in recent investigations, and are more likely to have a role in PAH-related pathological in modifying structural and functional aspect of liver [53, 55]. A better understanding of the regulatory mechanisms that increase pericyte progenitor proficiency, particularly in the context of cardiac remodeling, could usher in a new era of PAH treatment.

3. Current limitations and challenges of stem cell

Despite the tremendous development in MSCs therapies, there is still a dearth of data on MSCs bio distribution, their target cells' cellular and molecular structures, and the methods used by MSCs to achieve these targets [56]. In addition, even though MSCs are successful in clinical trials but it certainly pose various challenges that must be resolved prior to the use of particular kinds of MSCs in tissue engineering. As a result, improving the bioprocess for producing MSCs from humans and their products will increase stem cell treatments' effectiveness and safety [57]. Additionally, they are important in enhancing the outcome of MSCs-based tissue engineering is increasing the cultural context of MSCs and identifying appropriate target and inducing factors [58]. Due to its low frequency in peripheral and cord blood, one of the key constraints of ESPCs therapy is the long culture period necessary to generate a viable therapeutic dosage. Furthermore, due to its inherent immunogenicity, it can

only be delivered autologously. Finally, the transplanted ESPCs have a low survival rate. Firstly, Obstacles in their isolation and identification, as well as concerns with expansion efficiency and immunogenicity, must be overcome before ESPCs may be used clinically in the PAH sector. Secondly, unregulated biodistribution: a significant factor for the limited competence of few cell therapy experiments is poor implantation of rejuvenating stem cells at the site of damage or diseased tissue portion [59, 60]. In numerous clinical trials, the successful incorporation of autologous cells of stem cells in cardiac repair has been injected intravenously or intracoronary in patients. Afterward 24 to 48 hours following transplantation, just a small percentage of transplanted cells (approximately 5%) usually remain at the transplanted area. Almost 99% of the employed cells do not live for even four to six weeks following transplantation [61]. Few undiscovered unfavorable factors in the heart environment or in other organs might inhibits the cell growth, speeds up programmed cells death, and leads to migration to other organs, is thought to be one of the causes of decreased cell viability. The danger of tumorigenicity and immunogenicity is the third and most significant factor to consider [59, 62].

4. Conclusion

Stem cells show remarkably progress in modulating biomolecular pathways, restoring normal mitochondrial function, reversing pulmonary artery remodeling and PAH via improving lung vascular endothelial malfunctioning, lowering cell escalation, and ameliorate other PAH conditions. But on the other hand it is important to note that RV cell therapy should be performed as part of already established treatment targeting the RV and the pulmonary circulation. Patients with PAH who have dysregulation of specific genes may benefit from stem cells modified with therapeutic genes. Autologous stem cells would reduce transplant rejection. Combining stem cell therapy with other types of treatment may be used in some circumstances to provide PAH patients with synergistic therapeutic advantages [63]. Generally, a substantial body of evidence shows that PAH stem cell therapy should be studied further. Because it's hard to know if stem cells are incorporated into the organ and paracrine impact may be the primary route of action of stem cells. miRNAs can also help MSCs with cell differentiation and anti-apoptosis. As exosomes secreted by MSCs can provide microRNAs, it's possible that overexpression of particular miRNAs in MSCs, such as miR-204/206/328, will boost their PAH therapeutic efficiency [64]. To prevent immunological reactions while achieving the same results, exosomes or cell-derived products could be used. One positive outcome till today is that in the preclinical research, through established diseased models, iPSCs have shown practically successful application in regaining viability as curing agents for lung illnesses reflecting stem cell therapy pharmaceutical stability through screening tests [35, 65]. Exosomes clinical trials are now underway or have been completed in over 200 countries, and regenerative medicine requires more research, because of their diverse character, low repeatability, and risk of immunologic responses in recipients, exosomes must have long-term stability before being used as therapeutic agents. The transcriptional and epigenetic similarities between iPSCs and embryonic stem cells are also available as a functional similarity. iPSCs provide an extremely significant resource for determining epigenetic alterations during development.

Abbreviation

bFGf	basic fibroblast growth factor
BMPR2	bone morphogenetic protein receptor type-2
ESPC	endothelial stem/progenitor cell
iPSC	induced pluripotent stem cell
MSC	mesenchymal stem cell
PAH	pulmonary arterial hypertension
TGF-β	transforming growth factor-β
SMCs	smooth muscle cell
CEC	circulating endothelial cells
HSC	hematopoietic stem cells
ADSCs	adipose derived stem cells
CPCs	cardiac progenitor cells
RVH	right ventricular hypertrophy
RVSP	right ventricular systolic pressure
PASMCs	pulmonary artery smooth muscle cells
UCB	umbilical cord blood

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