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

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Abstract

Pulmonary arterial hypertension (PAH) is a chronic disease that is characterized as mean pulmonary artery hypertension (mPAP) > 25 mmHg. PAH is caused by progressive obliteration of small pulmonary arteries due to known or unknown etiologies. The effect of traditional therapy is suboptimal because it can only improve symptoms but cannot cure the disease, and therefore, scientists have turned their attention to stem cell therapy for efficacious treatments. In recent years, accumulating evidences have demonstrated that endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs) are closely related with the occurrence of PAH and have the ability to prevent and reverse this disease. In this review, we turn our attention to a novel therapy for PAH, stem cell therapy, through comparing effect of preclinical research on cells and animals and evaluating the feasibility and potential difficulties of clinical application.

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Abstract

Pulmonary arterial hypertension (PAH) is a chronic disease that is characterized as mean pulmonary artery hypertension (mPAP) > 25 mmHg. PAH is caused by progressive obliteration of small pulmonary arteries due to known or unknown etiologies. The effect of traditional therapy is suboptimal because it can only improve symptoms but cannot cure the disease, and therefore, scientists have turned their attention to stem cell therapy for efficacious treatments. In recent years, accumulating evidences have demonstrated that endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs) are closely related with the occurrence of PAH and have the ability to prevent and reverse this disease. In this review, we turn our attention to a novel therapy for PAH, stem cell therapy, through comparing effect of preclinical research on cells and animals and evaluating the feasibility and potential difficulties of clinical application.

Key words: stem cell, pulmonary arterial hypertension, mesenchymal stem cell, endothelial progenitor cell, therapy

Introduction

Pulmonary arterial hypertension (PAH) is a chronic disease that is characterized as mean pulmonary artery hypertension (mPAP)> 25 mmHg(Southgate, Machado, Graf, & Morrell, 2020). According to an investigation by the World Health Organization (WHO), the morbidity of PAH is approximately 1% for the world population, which equals approximately 100 million deaths(Schermuly, Ghofrani, Wilkins, & Grimminger, 2011). For people over 65 years of age, this number significantly increases to 10%, and a statistic of concern is that more than 80% of patients with PAH originate from developing countries(Mandras, Mehta, & Vaidya, 2020). There are numerous subcategories of PAH, and similar pathological changes occur in almost all of them, including the destruction of endothelial cells (ECs) and proliferation of pulmonary artery mesenchymal stem cells (PASMCs). Over time, the affected blood vessels become stiffer and thicker, which finally leads to PAH. Until now, there has been no existing radical therapy for PAH(Schermuly et al., 2011). Current treatment options include targeted therapies, such as endothelial receptor antagonist, guanylate cyclase antagonist, type 5 phosphodiesterase inhibitor, and prostaglandin drugs. The purpose of these drugs is only to block PAH pathways and delay the progression of disease, but these treatments do not significantly reduce mortality, which still remains at approximately 50% at five years(Schermuly et al., 2011). In this review, we attempt to provide a stem cell therapy that may potentially reverse the occurrence of PAH and effectively reduce its mortality. To analyze the unique advantages and potential challenges, we will discuss five aspects of therapy: (i) classification, (ii) mechanism, (iii) correlation, (iv) preclinical research, and (v) clinical research.

According to the process of differentiation, there are 5 different types of stem cells: (i) totipotent, (ii) pluripotent, (iii) multipotent, (iv) oligopotent, and (v) unipotent precursor cells(Toshner et al., 2009) (Figure 1). Totipotent stem cells have the strongest replication and differentiation capacity, and can differentiate to three germ layers and the trophectoderm (TE, such as the placenta). The most common example of totipotent stem cells is a zygote. Thus, totipotent stem cells are capable of forming every organ, including an entity under the correct support environment. Then, the inner cell mass (ICM), a part of the embryoblast, finally becomes pluripotent stem cells after a session of replication and differentiation(Mitalipov & Wolf, 2009). Pluripotent stem cells also are able to differentiate to three germ layers, but they cannot differentiate to the trophectoderm because the source of pluripotent stem cells is the inner cell mass but not a trophoblast. Therefore, totipotent stem cells can form an entire entity, while pluripotent stem cells can only form mature cells derived from three germ layers. Of course, there are well-known examples of pluripotent stem cells, such as embryonic stem cells (ESCs) and induced-pluripotent stem cells (iPSCs). After differentiation, pluripotent

stem cells become next stage, multipotent stem cells(Ulloa-Montoya, Verfaillie, & Hu, 2005). Multipotent stem cells still possess a strong capacity for replication and differentiation, and these cells can differentiate to a specific germ layer. Therefore, different types of multipotent stem cells have specific names depending on their differentiation orientation, such as hematopoietic stem cells, mesenchymal stem cells, neural stem cells, and skin stem cells.

Along with the process of differentiation, multipotent stem cells gradually lose the capacity to differentiate to a germ layer, and they finally become oligopotent stem cells. Oligopotent stem cells have the capacity to differentiate to a specific category of tissue, but they cannot become other type of cells. For example, myeloid cells can only become granulocytes, and not red blood cells. Unipotent stem cells have the weakest capacity of replication and differentiation. Although this type of cell can only differentiate to specific cells, they possess the ability to self-renewal, which distinguishes them from non-stem cells (e.g., progenitor cells)(M. Xu, He, Zhang, Xu, & Wang, 2019). Some studies found that a small proportion of multipotent stem cells and unipotent stem cells could revert to a trophectoderm or pluripotent stem cells, respectively, and the reason for this phenomenon may be attributed to the redifferentiation process. This discovery may provide a novel method that can be used to broaden the applications of stem cell therapy(Y. Yang et al., 2018).

2. Characterization and Classification

2.1 Endothelial progenitor cells (EPCs)

The controversy surrounding EPC definition are beginning from the first observations of blood vessel development in 1917, but after a long period of discussion, the precise definition of EPC are still unclear(Ferkowicz & Yoder, 2005). The most accessed definition of EPC is that progenitor cells are a heterogeneous population which includes different origins and several residing sites at different maturity stages(Yang, Pan, Wang, Qiu, & Mao, 2018). Due to the different features of cells, it is difficult to produce a worldwide accepted characterization of EPCs(Richardson & Yoder, 2011). Additionally, due to the different characterizations of EPCs, some contrary results have emerged(Harper et al., 2019; Toshner et al., 2009; Zhou et al., 2013). Balistreri et al. argued that EPCs are a type of stem cell(Bianconi et al., 2018; Prokopi & Mayr, 2011). In their opinion, progenitor cells are an intermediate stage between multipotent stem cells and mature cells, and both stem cells and progenitor cells have the ability to replicate and differentiate to mature cells, and even have the same biological markers(Brown & McGuire, 2012). EPCs can adhere to matrix molecules such as fibronectin and demonstrate dual positivity to acetylated low-density lipoprotein (acLDL) and *Ulex europaeus*agglutinin I (UEA-1) lectin(Yang, Pan, Zhao, & Wang, 2013). These types of cells commonly have some common positive protein such as CD34+ and CD133+ (which are two unique markers of hematopoietic stem cells), vascular endothelial growth factor receptor-2 (VEGFR2+, also called kinase insert domain receptor (KDR)/fetal liver kinase-1 (Flk-1), which is an important regulator of development of ECs and vasculature), and von Willebrand factor+ (vWF+), CD31+, CD144+, CD146+, and endothelial nitric oxide synthase+(eNOS+).

EPCs in different classifications are characterized by different abilities and affinities. According to the number of culture days after monocytes are extracted from blood(Hansmann et al., 2011), EPCs can be divided into early-outgrowth EPCs and late-outgrowth EPCs(Pelosi, Castelli, & Testa, 2014; Prokopi & Mayr, 2011). Early-outgrowth EPCs, also called circulatory angiogenic cells (CACs)(Basile & Yoder, 2014; Prater, Case, Ingram, & Yoder, 2007), are a spindle-shaped cell that emerges after culturing for 4-7 days(Murohara, 2010). Its name indicates that this type of EPC possesses little differentiation capacity, and thus, these cells cannot become mature cells(Paneni, Costantino, Kränkel, Cosentino, & Lüscher, 2016). However, early-outgrowth EPCs have a significant capacity to release several cell growth factors and cytokines such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), and interleukin (IL)-8(Guber, Ebrahimian, Heidari, Eliopoulos, & Lehoux, 2018; J. X. Yang et al., 2018). Due to their unique biological behaviors, early-outgrowth EPCs exert their function mainly by secreting growth factors and cytokines to support the growth of adherent cells and surrounding late-outgrowth EPCs. Except for custom EPC markers, early-outgrowth EPCs also express CD45 and CD14(S. J. Zhang et al., 2006).

Other cell groups emerged after 14-21 days of culture, and these cobblestone-shaped cells are named late-outgrowth EPCs, and also are called endothelial outgrowth cells (EOCs)(S. Liu et al., 2018), although they are different from early-outgrowth EPCs. The late-outgrowth EPCs display a significant capacity to replicate and differentiate to mature ECs. Compared with typical EPCs, late-outgrowth EPCs do not express hematopoietic stem cells markers, but instead, express vascular endothelial-cadherin (VE-cadherin) and CD146(Minami et al., 2015).

Given their biological properties, some researchers recognize that early-outgrowth EPCs and late-outgrowth EPCs are not single types of cells in different stages, but they may differentiate from different cells(Medina et al., 2010). There also exist a small proportion of EPCs that remain in the microcirculation of lung vessels before occurrence of injury(Schniedermann et al., 2010). Therefore, we called this type of EPC resident EPCs (in contrast to circulating EPCs). Resident EPCs display a significant replication and differentiation capacity, suggesting that lung blood and lymphatic ECs may be derived from these cells. However, based on our existing research results, we cannot clearly distinguish this cell type, and therefore, further study is required to discern the nature between resident EPCs and circulating EPCs.

2.2 Mesenchymal stem cells (MSCs)

MSCs, also known as mesenchymal stromal cells, are multipotent stem cells that can differentiate into numerous cells types such as osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells), and adipocytes (fat cells)(Ong, Ankrum, Dastidar, Levy, & Karp, 2014; Tonk, Witzler, Schulze, & Tobia Sc H, 2020). The morphology of MSCs is also characteristic, as MSCs have a small cell body with long and thin processes that are widely dispersed and populated in the adjacent extracellular matrix. In label-free live cell imaging, some organelles can be seen, and MSCs are clearly defined due to these distinct morphological features(Richmond, 1992). According to the International Society for Cellular Therapy (ISCT), MSCs are characterized by the following criteria: (1) expression of a specific set of clusters of differentiation CD markers (CD73, CD90, and CD105), (2) lack of expression of hematopoietic lineage CD markers [CD45, CD34, CD14 or CD11b, CD79a or CD19, human leukocyte antigen-antigen D related (HLA-DR)], (3) plastic adherence under standard culture conditions, (4) the ability to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro* (Fukumitsu & Suzuki, 2019).

Due to the clear definition, it is much easier to classify MSCs than EPCs. The most acknowledged classification is cell source, and the most common extraction sources are umbilical cord blood, amniotic fluid, adipose, and bone marrow, and different sources of MSCs possess different biological properties (Figure 2). The abundance and healing capacity of stem cells from umbilical cord blood are much higher as compared to amniotic fluid, and adipose and bone marrow stem cells. However, the replication and differentiation capacity not only correlate with the cell source, but also with the age of donors. Research shows that the doubling time and activity generation significantly decrease when age increases(Fraser, Wulur, Alfonso, & Hedrick, 2006; Pittenger et al., 1999).

The immunogenicity is also different between different cell sources. MSCs from bone marrow possess the highest immunogenicity because they have a higher expression level of HLA-DR under an inflammatory microenvironment (such as a high concentration of tumor necrosis factor-α (TNF-α) and interferon gamma (IFN-γ)), which will cause the immune cells to identify them and more rapidly eliminate them. The differentiation tendency is also different, as MSCs from umbilical cord blood tend to secrete additional hematopoietic factors such as G-CSF, GM-CSF, and HGF. Therefore, this type of MSC is more well-equipped to support the blood system. Additionally, MSCs from bone marrow can secrete a greater amount of VEGF than MSCs from umbilical cord blood, which enables MSCs from bone marrow to more easily form neovessels(Baksh, Yao, & Tuan, 2007; Hass, Kasper, Böhm, & Jacobs, 2011; Peng et al., 2008; Y.-Y. Shi, R. P. Nacamuli, A. Salim, & M. T. Longaker, 2005; Van Harmelen, Röhrig, & Hauner, 2004; Weiss et al., 2006).

2.2 Induced pluripotent stem cells (IPSCs)

IPSCs are a type of pluripotent stem cell that can be directly generated from somatic cells. Due to this characterization, there is great interest in IPSC technology worldwide. IPSC technology was pioneered by Dr.

Shinya Yamanaka in 2006, when he introduced four specific genes named Myc, octamer-binding transcription factor 4 (Oct3/4), sex determining region Y-box 2 (Sox2), and kruppel like factor 4 (Klf4) (which were also named Yamanaka factors), and then, encoding transcription factors could convert somatic cells into pluripotent stem cells. Yamanaka used this technology, and was awarded the 2012 Nobel Prize along with Sir John Gurdon "for the discovery that mature cells can be reprogrammed to become pluripotent(Takahashi & Yamanaka, 2006).

3. Preclinical research on stem cells in PAH

3.1 EPCs

3.11 PAH and EPCs

The usual hypothesis is that a low level of EPCs is one of the conditions for the occurrence of PAH. In research regarding congenital heart disease (CHD) that caused PAH in childhood, severe PAH patients (mean pulmonary arterial pressure $>$ 25 mmHg) show a significantly lower EPC level as compared to non-PAH patients. This phenomenon shows that low level of EPCs is strongly correlated with PAH. However, this relationship was not apparent for patients with mild PAH, indicating that a decreased level of EPC is not the only pathogenesis, or the EPC level will not be significantly decreased in the early stage of PAH(H. X. Sun et al., 2019). The same result was also obtained for adult patients, and in a comparison of COPD and COPD-PAH patients, the latter group showed a significantly lower EPC level as compared to COPD patients(P. Liu et al., 2016). A different conclusion was reached by a group of English scientists, who found that the level of EPCs was increased in idiopathic pulmonary arterial hypertension (iPAH) patients with bone morphogenetic protein type II receptor (BMPR2) mutation (which is a classical mutation that results in the development of iPAH).

In order to explain this aberration, a comparison was made between the EPC screening conditions in different studies (Table 1). We found that almost all scientists use different testing conditions, and the different markers may correspond to different cell groups and different cell functions, finally leading to the chaos of conclusions(Toshner et al., 2009). These different definitions of EPCs cause academic disruption that increases the difficulty and cost of discussions and experiments, and eventually leads to the standstill of progress. Despite the existence of contrary opinions, there is a consensus by most scientists that the low level of EPCs is part of an indispensable process that results in the development of PAH. They suggest that in the initial stage of PAH, high levels of cycling EPCs are able to repair the loss of vessels. However, with the progression of disease, the continuous damage exceeds the compensatory ability of EPCs, which finally leads to the reduction of peripheral EPCs and additional destruction of vessel structure. Ultimately, this process causes stiffer and narrower vessels and a higher pulmonary arterial pressure.

Under this vicious cycle, the symptoms of PAH become increasingly severe and finally lead to death(Toshner et al., 2009). EPCs exist in numerous ways to prevent the development and reverse existing PAH, and mainly include direct incorporation to sites of impaired vessels for repair, playing supportive roles in cellular repair, inhibiting the transfer of ECs, and participating in immunosuppression functions and secretion functions such as secretome, exosome and extracellular vehicles (EVs)(Bayraktutan, 2019; Bianconi et al., 2018; Wei et al., 2013).

3.12 Preclinical research on EPCs in PAH

Because EPCs have numerous capacities such as proliferation, migration, and adhesion to protect the normal structure of ECs and PASMCs, scientists use EPCs to attenuate and reverse PAH. Zhao et al. injected fluorescently labeled EPCs into monocrotaline (MCT)-induced PAH mice, the EPCs integrated into the distal pulmonary artery endothelium and limited the progression of PAH through differentiation to mature ECs and alleviation of neointima formation(Yang et al., 2013). Zhao et al. also detected EPC-derived proangiogenic growth factors (such as IL-8 and adrenomedullin) in the adjacent area of EPCs through autocrine and paracrine methodology. Zheng et al. found that EVs of EPCs transport mRNA to ECs, and express a series of proteins such as chemokines, pathway proteins, and growth factors to enhance EC proliferation

and migration and decrease angiogenesis(Deregibus et al., 2007; Ingram et al., 2004; X. Li et al., 2016). Zhao et al. used EPCs to treat monocrotaline (MCT)-induced PAH mice at two different time points to explore the effect of EPCs on different disease stages. The results seemed inspiring, as compared with the MCT group, the hemodynamic improvement in both groups was significant, but both treatment groups still had a higher mean pulmonary arterial pressure (mPAP) than the control group, indicating that EPCs can prevent but cannot reverse the occurrence of PAH. Zhao et al. also investigated the microstructure of the pulmonary artery under EPC therapy. They stained the pulmonary artery with fluorescent microspheres and alpha-smooth muscle actin (α-sma) to visualize the structure of PASMCs and microvasculature perfusion. The results showed that the 21-day and 35-day groups dramatically increased their microvascular perfusion and microvasculature after EPC treatment(Y. D. Zhao et al., 2005).

As a nonoral therapy, use frequency is also an important indicator of feasibility. Therefore, Harper et al. and Zhou et al. investigated the stay time and stay location after EPC transplantation. Harper et al. selected cells expressing CD34, CD306, CD146, and CD45 by fluorescence-activated cell sorting (FACS) and transfected adenoviral vectors carrying the luciferase and GFP reporter gene. The immunofluorescence results showed that at 1 h and 7 h after EPC transplantation, most of the luminescence was located in the chest region, and there was little or no luminescence in other areas, the luciferase results were same. Compared with the control group, the concentration of EPCs in the lung was two times higher than that in the spleen. This shows that EPCs tend to aggregate in damaged endothelial tissues, but rarely aggregate in integrated tissues. According to the above evidence, EPC therapy seems to be a targeting therapy that has little impact on other organs, at least in concentration relationships(Harper et al., 2019; Zhou et al., 2013). However, the experiment by Zhou et al. produced some opposite results. He found that the retention rate of EPCs was still nearly 50% 25 days after transplantation(Zhou et al., 2013). These contrary results were caused by different screening conditions during FACS.

In order to improve the effect of EPC therapy, scientists use gene editing technology. Wei et al. used Ad.CMV-human endothelialNO synthase enzymes (Ad.CMV-heNOS), Ad.CMV, or Ad.CMV-enhanced green fluorescent protein (Ad.CMV-EGFP) to infect EPCs for 72 h. Two weeks after the injection, the systolic arterial pressure (ASP) of mice did not significantly change, but the systolic pulmonary artery pressure (sPAP) significantly decreased in both the simple EPC group and the heNOS-EPC group. Furthermore, the heNOS-EPC group was significantly lower than the vector-EPC-treated group and the normal EPC-treated group. The histology results also showed the same trend, with a significant reduction in the number of muscular pulmonary arteries and thickness of the muscular coat(Wei et al., 2013).

Cao et al. selected the human hypoxia inducible factor-1 alpha (hHIF-1α) gene and transfected it into EPCs. Compared with the EPC group and blank group, hHIF-1-EPCs significantly reversed the vascular remodeling, and decreased the sPAP, mPAP, and right ventricular/left ventricular + septum (RV/LV+S). Xu et al. chose to disturb the metabolic process of the pulmonary artery to control the progression of PAH. They used lentiviral vectors to inhibit the expression of E2F transcription factor 1 (E2F1) and subsequently increase oxidative metabolism, endothelial differentiation, vascular repair, and decrease the pyruvate dehydrogenase kinase 4/2 (PDK4/2) expression. The metabolic level was measured by the oxygen consumption rate (OCR), extracellular acidification rate (ECAR), and lactate level. The results showed that E2F1-/- mice had a greater OCR, a lower ECAR, and a lower level of lactate (which represents a lower anaerobic respiration level), indicating that E2F1-/-EPC enhanced vascular growth, reduced infarct size, and improved vascular function through increasing the oxygen utilization rate of pulmonary ECs(Cao et al., 2015; S. Xu et al., 2018).

Dysfunctions of the prostacyclin (PGI) pathway are also an important factor in the occurrence of PAH. Therefore, Zhou et al. tried to engineer the cyclooxygenase-prostacyclin (COX-PGI) pathway to improve the effect of EPCs. The engineered EPCs significantly attenuated the RVSP increase, RV hypertrophy, and intimal and medial smooth muscle layer cell proliferation, and enhanced adventitial pulmonary vessel wall apoptosis(Zhou et al., 2013).

3.2 MSCs

3.21 PAH and MSCs

MSCs also have a significant ability to prevent the occurrence of PAH and reverse existing PAH in a manner similar to that of EPCs. Liu et al. found that MSCs can promote the repair of ECs through increasing the proliferation, migration, and tube formation of ECs to inhibit PAH. Inflammatory regulation also plays a role that cannot easily be overlooked, under the treatment of MSCs, the level of anti-inflammatory cytokines (such as TGF-β, IL-1, and prostaglandin E2) were increased(A. Liu et al., 2020; J. Liu et al., 2021), the level of pro-inflammatory cytokines (such as IL-1β, IFN-γ, and TNF-α) and angiogenic factors (such as VEGF, vWF, and fibroblast growth factor) were significantly reduced(Bull, Clark, McFann, & Moss, 2010), what's more, MSCs can attenuate the proliferation of dendritic cells (DCs) and secretion of IL-10 which inhibits the expression of CD80 and CD83 (which are co-stimulation factors of DC), and inhibiting the maturation of DCs(S. Zhang et al., 2018).

What's more, the effect of exosomes and secretomes has also been experimentally verified, both murine MSC extracellular vesicles (mMSC-EXs) and human MSC extracellular vesicles (hMSC-EXs) have a significant healing effect on mouse PAH models (Figure 3)(Jason M. Aliotta et al., 2016; Bian et al., 2014; Hu et al., 2018; Lopatina et al., 2014).

3.22 Preclinical treatment with MSCs in PAH

The research about MSC therapy in PAH is much earlier than EPC therapy, and the effect of MSC therapy is also more recognized. Huang et al. showed that after MSC transplantation, sugen-hypoxia induced-PH (Su-Hx PH) rats and chronic hypoxia-induced pulmonary hypertension (CHPH) rats exhibited a significant decrease in right ventricle pressure and degree of pulmonary artery remodeling. Masson's trichrome staining results showed that MSCs ameliorated the collagen deposition around the pulmonary arterial vasculature. MSCs also attenuated the process of endothelial-to-mesenchymal transition (EndMT) by reducing the expression of HIF-2α(J. Huang et al., 2020). Furthermore, Huang et al. also investigated the secretory function of MSCs by injecting MSC conditional medium (MSC-CM) into mice, and they obtained the same trend as that observed with cell suspension.

These results showed that the both control group and experimental groups exhibited a high level of HIF-2α under hypoxic conditions. However, the group that received MSC-CM treatment exhibited a faster disappearance rate of HIF-2α, indicating that MSC-CM inhibited HIF-2α by promoting its degradation(J. Huang et al., 2020).

Rathinasabapathy et al. found that adipose source MSCs (ASCs) and their conditional medium can modulate numerous types of cytokines, including significantly downregulating pro-inflammatory cytokines such as TNFα, IL-1, and IL-6, markers of immune defense system such as toll-like receptor 4 (TLR-4), cytokine-inducible NOS (iNOS), and markers of tissue remodeling such as TGF-β, and upregulating the markers of anti-inflammatory cytokines such as IL-10(Rathinasabapathy et al., 2016). In the past decade, an increasing number of scientists has concentrated their attention on the exosome of MSCs. Without exception, the exosome of MSCs was also effective, as some scientists found that the expression levels of Wnt5a, Wnt11, BMPR2, BMP4, and BMP9 increased, but β-catenin, cyclin D1, and TGF-β1 decreased in the MSC exosome group in vivo and in vitro. Considering that the immunogenicity of exosomes is lower than that of a cell suspension, if we have an efficient method to obtain sufficient exosomes, then these exosomes may constitute a more effective treatment (Table 2).

Chen et al. transfected MSCs with the eNOS and F92A-Cav1 genes using lentivirus vector to verify the feasibility of gene editing technology in MSCs and increase the effect of MSCs. The results showed that the serum NO concentration significantly increased in eNOS-MSCs, and eNOS/F92A-Cav1-MSCs inhibited proliferation of PASMCs and improved pulmonary hemodynamics, vascular remodeling, and short-term survival(H. Chen et al., 2017). Cheng et al. used adenovirus to transfect MSCs with the lethal-7a (let-7a) microRNA, so that the let-7a-MSCs would overexpress let-7a microRNA, and the results showed that let-7a-MSCs ameliorated MCT-induced ventricular impairment, attenuated pulmonary vascular remodeling, and regulated PASMC proliferation and apoptosis resistance(Cheng, Wang, Li, & He, 2017).

3.3 IPSCs

3.31 Preclinical treatment with IPSCs in PAH

As an rising technology, rely on IPSCs to treat diseases has been applied in many fields including cardiovascular disease, neurodegenerative disease, it also includes respiratory diseases. Huang et al. use IPSCs and IPSCs-CM to treat MCT-induced PAH, the results show that IPSC-based therapy led to decreased accumulation of inflammatory cells and down-regulated the expression of pro-inflammatory factors through inhibit the phosphorylation of NF-ϰB pathway(W. C. Huang et al., 2016).

4. Clinical application of stem cells in PAH

4.1 EPCs

Until now, the clinical application of EPCs in PAH has been rare (Table 3). The search results from clinicaltrials show that only China and Canada have achieved EPC therapy trials using human patients. Of the 31 idiopathic PAH patients in the Chinese trial, 16 patients were randomly selected to receive conventional therapy, and 15 patients were randomly selected to receive EPC infusion plus conventional therapy. This trial lasted for 12 weeks, and the frequency and degree of adverse events were similar between the 2 groups (p >0.05)(X. X. Wang et al., 2007). However, the cell infusion group exhibited a significant improvement in mean distance walked in 6 minutes, mPAP, pulmonary vascular resistance, and cardiac output. The Canadian trial was more radical because they used gene-edited EPCs in patients who were refractory to conventional therapy. They injected seven patients with eNOS-EPCs, but the number of patients was far less than that in the Chinese trial, and there were five female subjects but only two male subjects. Similar to the Chinese trial, the infusion of EPCs was well tolerated in the cell infusion group, and there was a significant increase in the mean distance walked in 6 minutes over all 6 months. However, it is worth noting that some improvements (such as hemodynamics) disappeared after 3 months, which may be due to the short duration of EPCs(Granton et al., 2015).

4.2 MSCs

Until now, the only legal clinical stem-cell therapy was hematopoietic stem-cell therapy for childhood leukemia. Other therapies remain controversial because they are often related to abortion politics and human cloning. However, the research field of stem-cell therapy is constantly expanding, and scientists now have applied stem cells in neurodegeneration, brain and spinal cord injury, frailty syndrome, pancreatic beta cells, orthopedics, and other uses(Gazdic et al., 2018; Golpanian et al., 2017; R. Li et al., 2018; Lyon, 2018; Memon & Abdelalim, 2020). The significant difference between EPCs and MSCs is that a lower immunogenicity is associated with MSCs. However, if low immunogenicity leads to lengthy transplantation time and excessive allogeneic cells in the circulation of patients, this may lead to graft-versus-host disease (GVHD), which is the most severe side effect of stem cell therapy(Malard & Mohty, 2014).

Ethical issues continue to cause problems with allografts because embryonic stem cells are derived from human embryos, but this process will kill the embryo. A key issue is the voluntary actions of donors, and therefore, the choice of donation must be later than the choice of abortion, so as to avoid involuntary activities caused by monetary temptation or other factors. In addition to ethical issues, there are also many criticisms of the experimental procedures. Some critics report that only five basic trials seem to be used in 48 reports, and many trials contradict each other. Therefore, research into clinical stem cell therapy requires further development and stricter standards to limit disordered behavior and prevent damage to the reputation of scientists and the field of stem cell therapy(Heo, Choi, Kim, & Kim, 2016). He identity of patients after allogeneic stem cell transplantation is another inevitable problem. Because allogeneic stem cells can differentiate into allogeneic mature cells, the identity of patients still needs to be further discussed and confirmed. It is advantageous that the new technology of inducing pluripotent stem cells avoids this ethical tissue, and another benefit is that it provides even more convenient access as compared to autologous stem cells(Y. Y. Shi, R. P. Nacamuli, A. Salim, & M. T. Longaker, 2005).

4.3 IPSCs

The appearance of IPSCs has profound implications for the source of pluripotent stem cells. Because the access of embryonic stem cells is controversial, IPSC technology sidesteps a majority of the ethical issues, which results in the acceptability of stem-cell therapy for wide application. However, there is still a lack of understanding regarding IPSCs, which are currently not being used for clinical therapy. Current clinical application of IPSCs is restricted for cell models of diseases, which remain difficult for scientists to simulate in vitro. Because patients' somatic cells can revert back to pluripotent stem cells after utilization of IPSC technology, then under the current steering, normal somatic cells can become model cells. Considering this characterization, IPSC technology is an ideal method for acquiring model cells through patients' somatic cells because it does not cause obvious damage to patients. Sa et al. compared IPSC-derived ECs and native pulmonary ECs of iPAH patients in order to investigate the feasibility of IPSC-derived cells. The results showed that two types of cells manifest a similar phenotype. This result indicates that enormous progress has been made, because whether sugen, hypoxia, or MCT-induced PAH has a certain gap with iPAH, the easier method to derive human-source disease model cells, which is a huge leap for exploring the real mechanisms of iPAH(Sa et al., 2017).

In the past 2 years, IPSC therapy trials in patients have also been established. However, all IPSC clinical therapy trials are still in the recruiting stage. Only after a session of completed research will we be finally able to evaluate the feasibility and effectiveness of IPSC therapy and then consider whether IPSC therapy can finally become a feasible treatment for human diseases.

5. Challenge and future prospects

Both MSC therapy and EPC therapy are promising due to their outstanding effectiveness and low toxicity, and the effects of MSC therapy and EPC therapy also have been verified in many diseases thus far. However, because of our limited understanding of cell differentiation regulation, we poorly predict the further development of transplanted MSCs and EPCs. Except for ethical issues, challenges mainly include the following three aspects (Luo et al., 2013).(1) Low abundance: Human stem cells are derived from human tissue such as bone marrow, adipose, amniotic fluid, and umbilical cord blood, and therefore, due to the sparse volume of tissue, obtaining vast numbers of stem cells will become an insurmountable hurdle. However, the emergence of IPSC technology may solve this problem. There exist many methods to induce somatic cells, including virus, vector, mRNA, protein, small molecules, and miRNA(Ichida et al., 2009; Moradi, Braun, & Baharvand, 2018; Moradi et al., 2017; Zhu et al., 2010). Unfortunately, the most efficient method is accompanied by the highest danger. Therefore, further study is necessary to obtain a method that will balance safety and efficiency(Okita, Nakagawa, Hyenjong, Ichisaka, & Yamanaka, 2008; Stadtfeld, Nagaya, Utikal, Weir, & Hochedlinger, 2008; Woltjen et al., 2009). (2) Genomic insertion and incomplete reprogramming: Gene editing technology and inducing pluripotent stem cell technology require carriers to transfect transcription factors, while under some rare situations, exogenous cDNA can be inserted into the host's chromosomal DNA. However, due to undetectable mutations and unknown genesis positions, nobody knows what will happen if genomic insertion occurs or if incomplete reprogrammed stem cells replicate and differentiate in a patients' body(Selvaraj, Plane, Williams, & Deng, 2010). (3) Tumorigenicity: all stem cells probably develop to tumor cells after replication and differentiation for a long period of time. This phenomenon has been verified by many studies, because all stem cell transplantations finally develop to a tumor. Additionally, the process of inducing pluripotent stem cell technology also increases the tumorigenicity of pluripotent stem cells, because the transcription factor Myc is a protooncogene that will lead to the occurrence of tumors. Fortunately, Yamanaka reported that a new technology can create IPSCs without Myc(Aguilar-Gallardo, Cristóbal, Simón, & Carlos, 2013), and although its efficiency is much lower, this provides us with a satisfactory idea for safety technology(Marión et al., 2009).

The future of stem cell therapy is promising. In the past 2 decades, stem cell therapy has been applied to numerous fields, including interstitial pulmonary fibrosis, limbal stem cell deficiency, progressive multiple sclerosis, diabetes, myocardial infarction, and neurodegenerative disease. Different from other conventional drug therapies for pathogenesis, stem cell therapy has obvious advantages because it can directly involve in cell regeneration. Therefore, stem cells provide a new therapy that conventional drug therapy cannot achieve.

A large number of animal studies and clinical trials show that significant improvement results from combined therapy as compared with traditional drug therapy. However, stem cell therapy is not a mature therapy because we know little about its potential mechanism. Therefore, in the next stage of research, we should first design new experiments to explore the detailed mechanisms of stem cell therapy, and second, we should summarize clinical experiences to provide a suitable therapy course. Last but not least, we should discuss the ethical issues regarding allotransplantation. If we can obtain insight into the specific mechanism of stem cell therapy and achieve a feasible consensus regarding allotransplantation, then the applications for stem cell therapy will greatly expand.

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Authors' contributions

ZRX and XMY collected literatures, prepared figures and tables, and drafted the manuscript. WXH edited the manuscript. YLH, WJ and HXY conceived the idea and reviewed the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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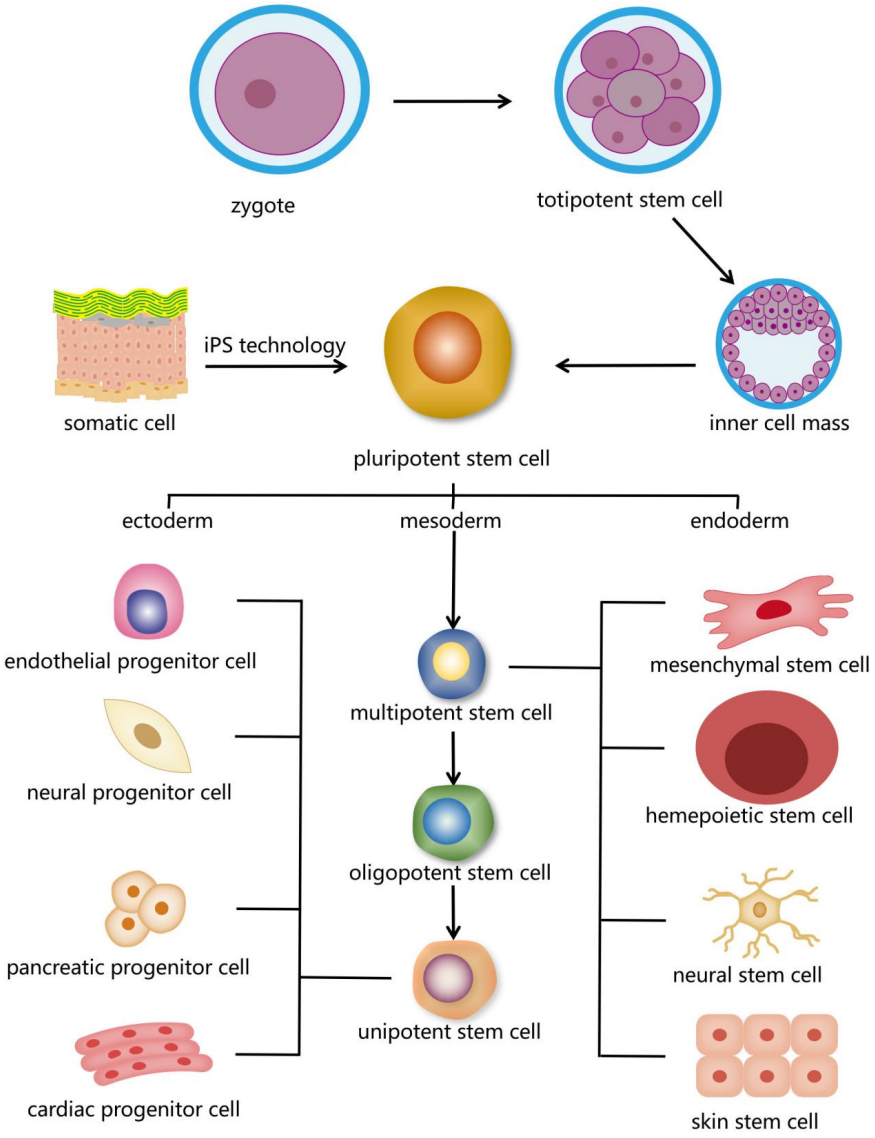

Figure 1. Stem cell differentiation process.

This is the differentiation tree of stem cells from zygote to progenitor cell. Progenitor cells are unipotent stem cells that have the weakest capacity for differentiation and replication. In the mesoderm, endothelial progenitor cells, neural progenitor cells, pancreatic progenitor cells, and cardiac progenitor cells are involved. A multipotent stem cell is a monibus term; in the mesoderm, mesenchymal stem cells, hematopoietic stem cells, neural stem cells, and skin stem cells are involved. iPSCs are manufactured stem cells, and this type of pluripotent stem cell possesses functions similar to those of traditional pluripotent stem cells.

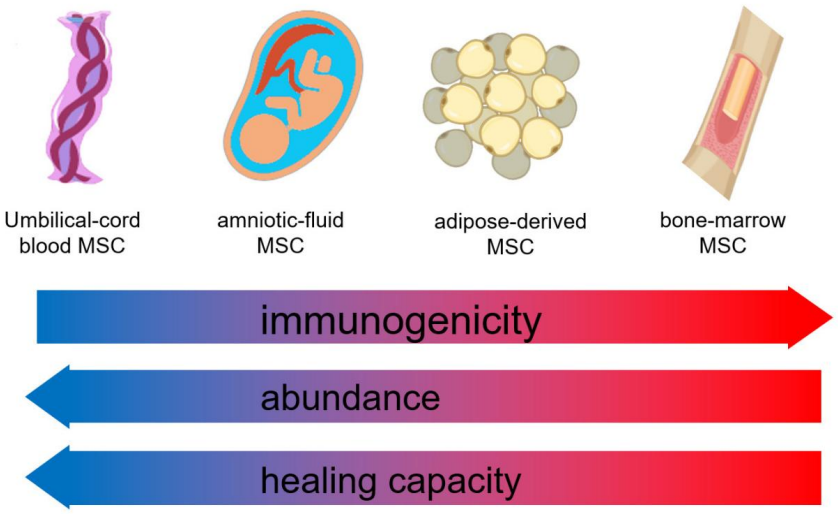

Figure 2. Mechanism of stem cell therapy.

Stem cells can inhibit and reverse vessel pathological changes through numerous methods. Stem cells can inhibit the proliferation of SMCs and promote the differentiation of ECs. Of course, the secretory function also plays a critical role in efficacy and outcomes. Stem cells inhibit the secretion of pro-inflammatory factors and promote the secretion of anti-inflammatory factors. Stem cells also secrete microvesicles and exosomes, and under the comprehensive effect, diseased blood vessel revert to normal blood vessels.

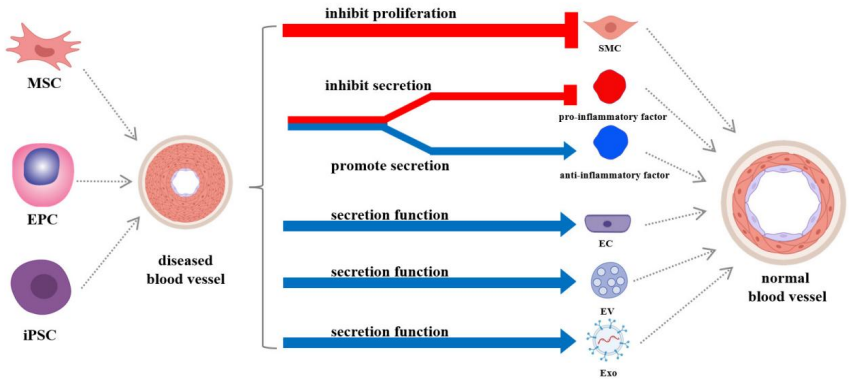

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Table 1. Summary of recent literature regarding EPC immunological markers

Flow cytometry makers	Source	Target	Conclusion	Reference

CD133 (+), VEGFR-2(+)	peripheral blood	Correlation between EPC and PAH	The number of EPC decreased in PAH patients	(H. X. Sun et al., 2019)
CD34(+), CD146(+), CD45(+), CD309(+)	Bone marrow	Gene editing EPC therapy	BMPR2-expressing EPC alleviate PAH	(Harper et al., 2019)
CD34(+), CD133(+), CD309(+)	peripheral blood	Drug effect to EPC and PAH	Riociguat increase the number of EPC to protect CTEPH	(Yamamoto et al., 2020)
CD34(+) CD31(+), CD144(+), VEGFR-2(+), eNOS (+)	peripheral blood	Regulatory Pathway of EPC	Stimulation of NOX2, NOX4 and VPO1 induce EPC's apoptosis and dysfunction	(E. L. Wang et al., 2019)
sca-1(+), CD117(+), VEGFR-2(+)	Bone marrow	extracellular vesicles' different effect under different microenvironment	MSC-EV reduce the number of EPC in increase the expression of EPC genes in MCT-PH mice	(J. M. Aliotta et al., 2017)
CD31(+), VEGFR-2(+), CD144(+), CD34(+), CD14(+)	peripheral blood	Factors influence to distribution of EPC	chemokine and cell Adhesion Molecules attract EPC to lung	(Y. Liu et al., 2020)
CD34(+), CD133(+), CD31(+), CD45(+)	Bone marrow	Drug effect to EPC and PAH	pinocembrin has a supportive effect to EPC in MCT-PH rats	(Ahmed, Rizk, & El-Maraghy, 2017)
CD45(+), CD34(+), CD133(+)	Bone marrow	Correlation between EPC and PAH	COPD patients have a lower level of EPCs	(Pizarro et al., 2014)
CD34(+), VEGFR-2(+)	Peripheral blood and lung tissue	Correlation between EPC and PAH	COPD patients have a lower level of EPCs, but COPD-PH patients have a higher level of EPCs	(Y. Yang et al., 2018)
ZsGreen (+), CD117(+), CD45(+)	Bone marrow	Correlation between EPC and EHT	Sugen-hypoxia mice's EPCs unable to transfer PH pathology to recipient mice	(Liang et al., 2017)
CD34(+), CD45(+), CD14(+)	Peripheral blood	Function of EPCs	different markers EPCs have different function	(Foris et al., 2016)

CD133 (+), VEGFR-2(+)	Peripheral blood	Correlation between EPC and PAH	EPC number and vasculogenesis increase in 48h, but significantly reduce after 6 weeks	(Xia et al., 2009)
CD31(+), VEGFR-2(+)	Bone marrow	Gene editing EPC therapy	E2F1 deficiency EPCs improve the effect of EPC	(S. Xu et al., 2018)
CD34(+), CD14(+), VEGFR-2(+)	Peripheral blood	Correlation between EPC and PAH	number of EPCs is negatively correlated with the PAH	(P. Liu et al., 2016)
CD45(+), CD34(+), CD133(+)	Peripheral blood	Correlation between EPC and PAH	number of EPCs is significantly reduced in PAH patients	(García-Lucio et al., 2017)
CD34(+), AC133(+), VEGFR-2(+)	Peripheral blood	Correlation between EPC and PAH	number of EPCs is significantly reduced in PAH patients	(Diller et al., 2008)
CD34(+), CD133(+)	Peripheral blood	Function of EPC therapy	EPCs can reduce the symptoms of PAH	(Q. Zhao et al., 2007)
CD31(+), VEGFR-2(+), vWF (+)	Bone marrow	Function of EPC therapy	EPCs can alleviate PAH	(C. K. Sun et al., 2012)
CD34(+), CD133(+), VEGFR-2(+)	Bone marrow	Function of EPC therapy	HIF1-EPCs can alleviate PAH	(Cao et al., 2015)
CD31(+), CD144(+), VEGFR-2(+)	Peripheral blood	Function of EPC therapy	EPCs can improve the function of RV	(Loisel et al., 2019)
CD31(+), CD14(+), CD34(+), VEGFR-2(+)	Peripheral blood	Function of EPC therapy	EPCs show a no discernable therapeutic benefic	(Ormiston, Deng, Stewart, & Courtman, 2010)
CD31(+), VEGFR-2(+)	Lung tissue	Correlation between EPC and PAH	number of EPCs is significantly reduced in PAH patients	(Duong et al., 2011)
VEGFR-2(+), Tie2(+), CD14(+), CD31(+), CD34(+)	Bone marrow	Function of EPC therapy	Both COX1-PGIS-EPCs and EPCs can alleviate PAH	(Zhou et al., 2013)
AC133(+), VEGFR-2(+)	Peripheral blood	Correlation between EPC and PAH	number of EPCs is significantly reduced in PAH patients	(Junhui et al., 2008)

CD34(+), CD133(+), VEGFR-2(+)	Peripheral blood	Correlation between EPC and lung disease	Progenitor cell types are present in the neointima of occluded vessels	(Yao et al., 2009)
CD34(+), CD144(+), CD31(+), VEGFR-2(+), eNOS (+)	Peripheral blood	Function of EPC therapy	Inhibition of ROCK reduced EPCs senescence and alleviate PAH	(B. Liu et al., 2016)

VEGFR-2, Vascular Endothelial Growth Factor Receptor-2, also known as KDR (Kinase Insert Domain Receptor)/Flk-1(Fetal Liver Kinase 1); eNOS, endothelial nitric oxide synthase; sca-1, Stem cells antigen-1; ZsGreen, Zoanthus sp. green fluorescent protein; vWF, von Willebrand factor; NOX, NADPH oxidase; ROCK, Rho-kinase;

Table 2 Summary of recent literature regarding MSC therapy in PAH

Cell source	**Application form**	**Route**	**Dosage**	**Modeling**	**Start time and end time**	**Outcome**	**Reference**
Mice UC	Cell suspension	IV	5x10^5 Cells in 50 μL PBS	4 weeks hypoxia and 2 weeks hypoxia/SU5416	Treat at week 6, sacrifice at week 8	Treatment improves hemodynamic variables and histopathological alterations of PAH models	(Alencar et al., 2018)
Rat BM	Cell suspension	IV	1×10^6 cells in 1 ml PBS	3 weeks hypoxia /SU5416	Treat at week 1, Sacrifice at week 5	Treatment improves hemodynamic variables and histopathological alterations of PAH models	(J. Huang et al., 2020)

Mice BM	Exosome	IV	0.1μg or 10μg Exo in PBS once a day	3 weeks hypoxia	Treat at day 4, Sacrifice After week 4 hypoxia	Treatment improves hemodynamic variables and histopathological alterations of PAH models	(Lee et al., 2012)
Rat BM	Microvesicle / Cell suspension	IV	MSCs once a day MSC-MVs once two days	One injection of MCT(50 mg/kg) for 3 weeks	Treat at week 4, Sacrifice at week 6.	Treatment improves hemodynamic variables and histopathological alterations of PAH models	(J. Y. Chen et al., 2014)
Rat AD	Cell suspension	IV	10^5 cells in 50 μl saline	One injection of MCT (60 mg/kg) for 2 weeks	Treat at week 2, Sacrifice at week 4	Treatment improves hemodynamic variables and histopathological alterations of PAH models	(de Mendonça et al., 2017)
Rat BM	Cell suspension	IV	3×10^6 cells in PBS	One injection of MCT (50 mg/kg) for 3 weeks	Treat at week 4, Sacrifice at week 6	Treatment improves hemodynamic variables and histopathological alterations of PAH models .	(Cheng et al., 2017)

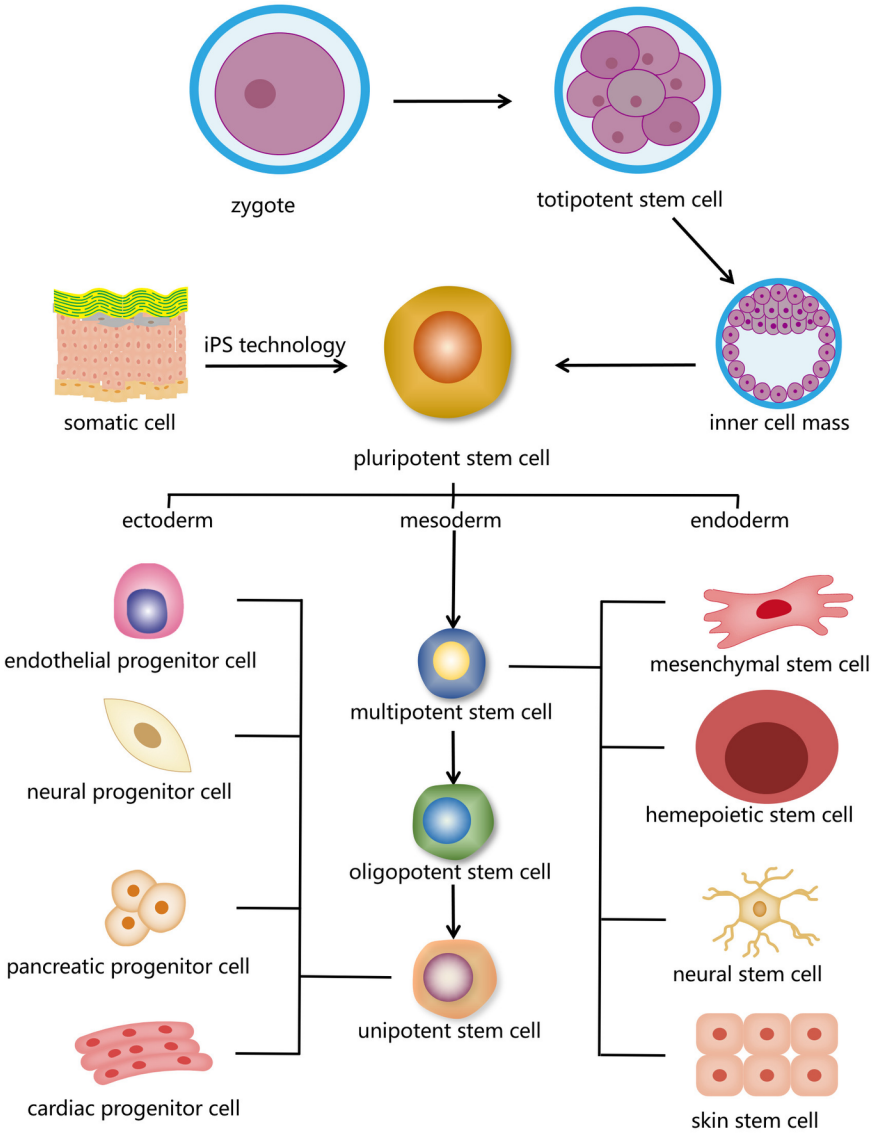
Rat BM	Cell suspension	IT	3×10^6 cells in PBS	One injection of MCT (60mg/kg) for 2 weeks	Treat at week 3, Sacrifice at week 7	Treatment improves hemodynamic variables and histopathological alterations of PAH models	(Baber et al., 2007)
Rat BM	Cell suspension	IV	1×10^5 cells in 0.1 mL LR	Hyperoxia for 15 days	Treat at day 5, Sacrifice at day 15	Treatment improves hemodynamic variables and histopathological alterations of PAH models	(Suzuki et al., 2020)

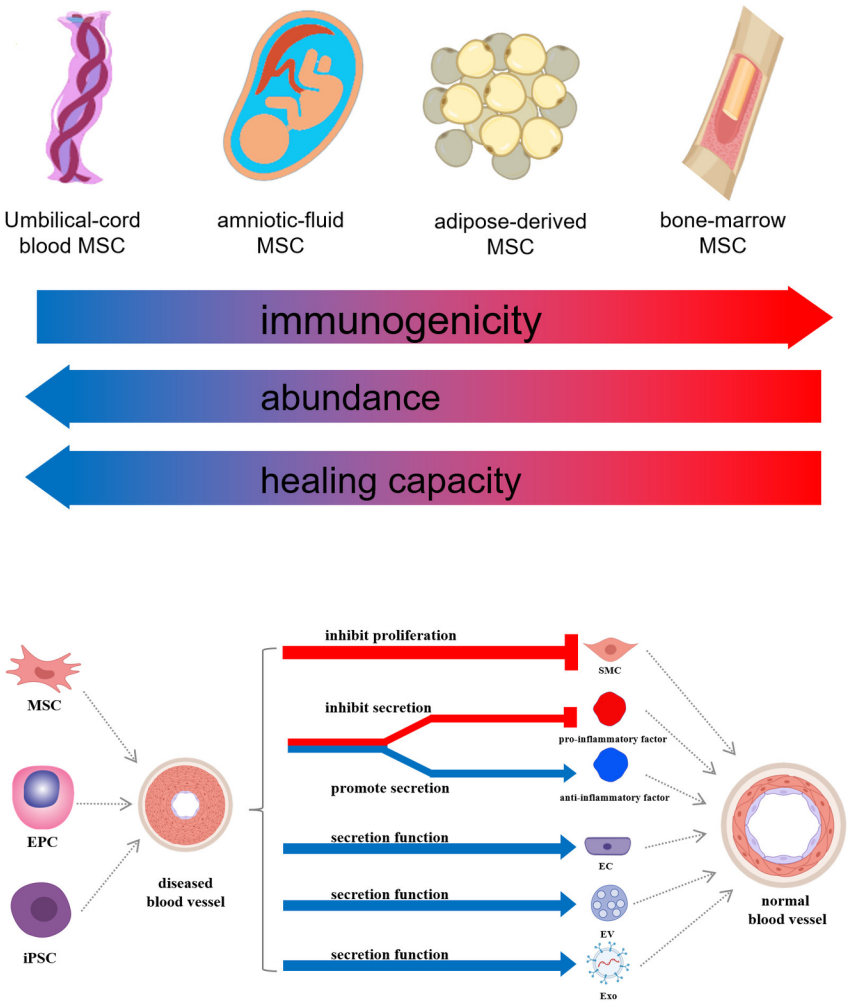
UC, umbilical cord; BM, bone marrow; AD, adipose derived; IV, intravenous; IT, intratracheal; MSC, mesenchymal stem cell; MV, microvesicle; PBS, phosphate buffer saline; MCT, monocrotaline; EV, extracellular vesicle; LR, lactated Ringer's solution

Table 3. Summary of recent stem cell therapy clinical trials

NCT number	**Participants number**	**phase**	**intervention**	**Cell type**	**Study target**	**Status**
NCT00641836	98	Not Applicable	Transplantation	EPC	The ideal quantity, duration and potential toxicity of therapy	completed
NCT03001414	45	2 and 3	Transfection and transplantation	EPC	establish the efficacy and safety of repeated monthly dosage	Recruiting
NCT00491309	45	Not Applicable	Evaluation	EPC	Evaluate the effectivity and safety of low-dose training program	Recruiting

NCT00551408	20	Not Applicable	Evaluation	EPC	Establish the correlation between PAH and EPC	Completed
NCT04055415	60	1 and 2	Transplantation	MSC	establish the efficacy and safety of stem cell therapy	Recruiting
NCT04432545	unknown	Not Applicable	Transplantation	MSC	establish the efficacy and safety of stem cell therapy	Available





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