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Mesenchymal Stem Cell Therapy and Lung Diseases

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Abstract Mesenchymal stem cells (MSCs), a distinct population of adult stem cells, have amassed significant interest from both medical and scientific communities. An inherent multipotent differentiation potential offers a cell therapy option for various diseases, including those of the musculoskeletal, neuronal, cardiovascular and pulmonary systems. MSCs also secrete an array of paracrine factors implicated in the mitigation of pathological conditions through anti-inflammatory, anti-apoptotic and immunomodulatory mechanisms. The safety and efficacy of MSCs in human application have been confirmed through small- and large-scale clinical trials. However, achieving the optimal clinical benefit from MSC-mediated regenerative therapy approaches is entirely dependent upon adequate understanding of their healing/regeneration mechanisms and selection of appropriate clinical conditions.

Keywords Mesenchymal stem cells · Multipotent · Lung · Acute lung injury · Chronic lung disease · Chronic obstructive pulmonary disease · Cystic fibrosis · Idiopathic pulmonary fibrosis

Abbreviations

MSC	Mesenchymal stem cell
CFU-F	Colony-forming unit fibroblast
AEC	Alveolar epithelial cell
AECI	Type I alveolar epithelial cells
AECII	Type II alveolar epithelial cells
VEGF	Vascular endothelial growth factor
eNOS	Endothelial nitric oxide synthase
ALI	Acute lung injury
ARDS	Acute respiratory distress syndrome
LPS	Lipopolysaccharide
KGF	Keratinocyte growth factor

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COPD	Chronic obstructive pulmonary disease
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
IPF	Idiopathic pulmonary fibrosis
SP-C	Surfactant Protein-C
HSCs	Haematopoietic stem cells
PH	Pulmonary hypertension

Contents

1 Introduction

Mesenchymal stem cells (MSCs) are a population of adult stem cells that have amassed significant interest from the medical and scientific community since their initial discovery [44]. The interest in MSCs arises from their potential applications in regenerative medicine, consequent to their proposed ability to aid in the regeneration and repair of otherwise incurable diseases and physiological damage, including articular cartilage damage, neurological disorders, immunological diseases, and the development of irreversible lung fibrosis (a hallmark of idiopathic pulmonary fibrosis). Through continuing research, many new insights have been gained in our understanding of MSCs; however, there are still many unanswered questions regarding the functionality of MSCs and how best to use their clinical potential. Due to the scope of this chapter, we limit our discussion to the general properties of MSCs and their potential applications in the treatment of selected pulmonary diseases.

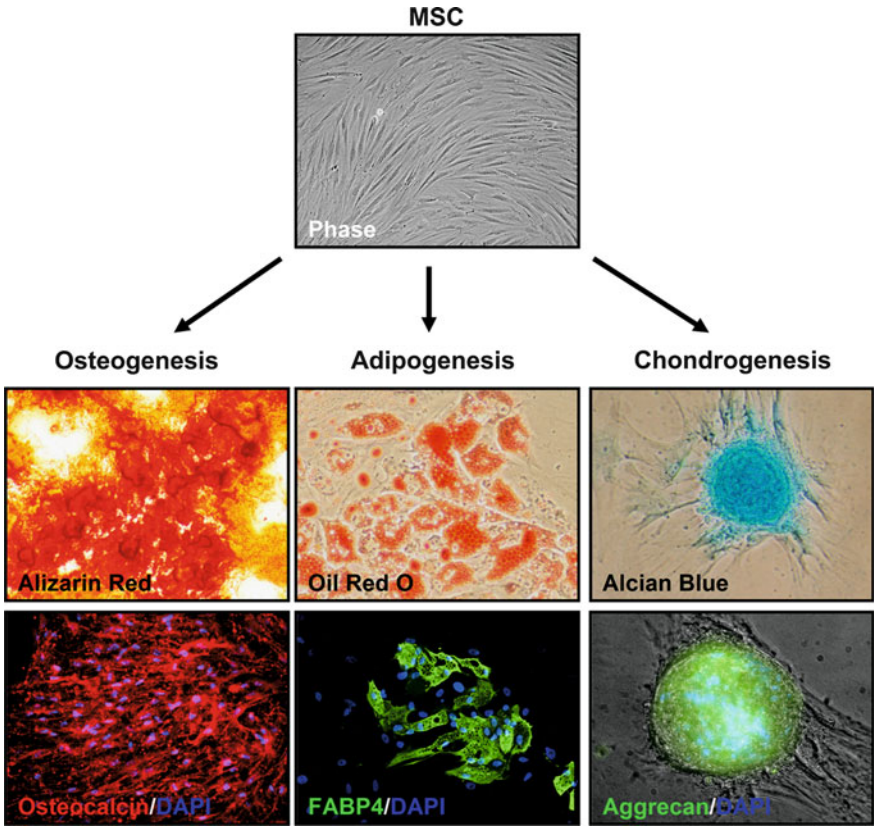

Fig. 1 Morphology of human MSCs and their classical tri-lineage differentiation. Phase images show the typical spindle-shaped morphology of adherent human MSCs. Osteogenesis: deposited calcium by differentiated osteoblasts was stained with Alizarin Red and osteocalcin was labelled by anti-osteocalcin antibody. Adipogenesis: differentiated adipocytes produce triglyceride which was stained with Oil Red O and adipocytes were stained with anti-FABP4 antibody. Chondrogenesis: chondrogenic nodules were stained with Alcian Blue and anti-aggrecan antibody. N.B. Histological images and immunofluorescence images are taken from representative and not identical fields of view

2 History, Definition and Properties of MSC

The first descriptions of MSCs took shape with the work of Friedenstein and colleagues with the discovery of multi-potential precursor cells that were spindle-shaped in nature within bone marrow samples. Further in vitro experiments demonstrated a colony-forming capacity associated with these cells, defined as colony-forming unit fibroblast (CFU-F) [44]. The cells within the CFU-F had the potential to differentiate into chondrocytes, adipocytes, osteoblasts (Fig. 1) and were also postulated to form a stromal layer that is essential in maintaining haematopoiesis. However, it was the capacity for differentiation that accrued most interest [95].

Since their initial discovery, extensive research has attempted to understand and harness the enormous medicinal potential of MSCs. Although well studied and documented, no agreement on the true definition of the MSC has yet been reached.

To define the MSC, the individual components of the mesenchyme and the stem cell should first be considered. Mesenchyme or stroma describes the tissue that provides structural and functional support for the growth and development of numerous organ systems. The bone marrow mesenchyme is a layer of cells that delivers the essential support that haematopoietic stem cells require for self-renewal and differentiation [115].

The definition of a stem cell has evolved largely through increased understanding of haematopoietic stem cell biology. The cell line must demonstrate self-renewal with the production of a clone daughter cell, the ability to differentiate into multi-lineage cell lines, and also in vivo reconstruction of a functional tissue [129]. Functional classification focuses on the capabilities of the stem cell and begins with a description of their nature as pluripotent or multipotent. Pluripotent describes a group of stem cells that are capable of self-renewal and differentiation into all three germ layers; a classical example is the embryonic stem cell [16]. Multipotent stem cells describe a group with the capability for self-renewal but their ability to differentiate is limited to lineages contained within a specific germ layer; an example is, the haematopoietic stem cell, which can differentiate into cells of the immune and haematological cell lines [66].

A difficulty in defining the MSC is the variation in nomenclature that is used within scientific literature. Terminology used to refer to MSCs includes multipotent stromal cells, multipotent progenitor cells, non-haematopoietic stem cells, and stromal progenitor cells [19]. All of these terms are essentially synonymous with the term MSC. This variation within the literature may stem from the lack of evidence for in vivo self-renewal and reconstruction of functional tissue [28]. The International Society for Cellular Therapy has categorized MSCs with a broad definition: "firstly MSCs must be adherent to plastic when maintained in culture, secondly they must express surface antigens CD105, CD73 and CD90 and lack the surface markers for monocytes, macrophages, and B cells in addition to lacking markers of the haematopoietic antigens CD45 and CD34. Finally the MSC must have the potential to differentiate into osteoblast, chondrocytes and adipocytes" [56].

By definition, MSCs under the influence of appropriate growth factors can differentiate into multiple cell lines, in particular to osteoblasts, chondrocytes and adipocytes. Therefore, through demonstration of the presence of these three cell lineages after directed differentiation of a colony of cells in vitro, one can retrospectively deduce that the original cells are MSCs [103]. However, this technique in itself contains numerous pitfalls as it is often very difficult to isolate and culture MSCs without altering and manipulating their original phenotype. A further difficulty in the definition of the MSC is that no single marker has been described that is specific to the MSC, thus making them extremely difficult to identify in vitro and in vivo [136]. Current practice is to define MSCs based on a combination of their differentiation potential, phenotype features, and morphological features—often in a retrospective manner (Figs. 1, 2).

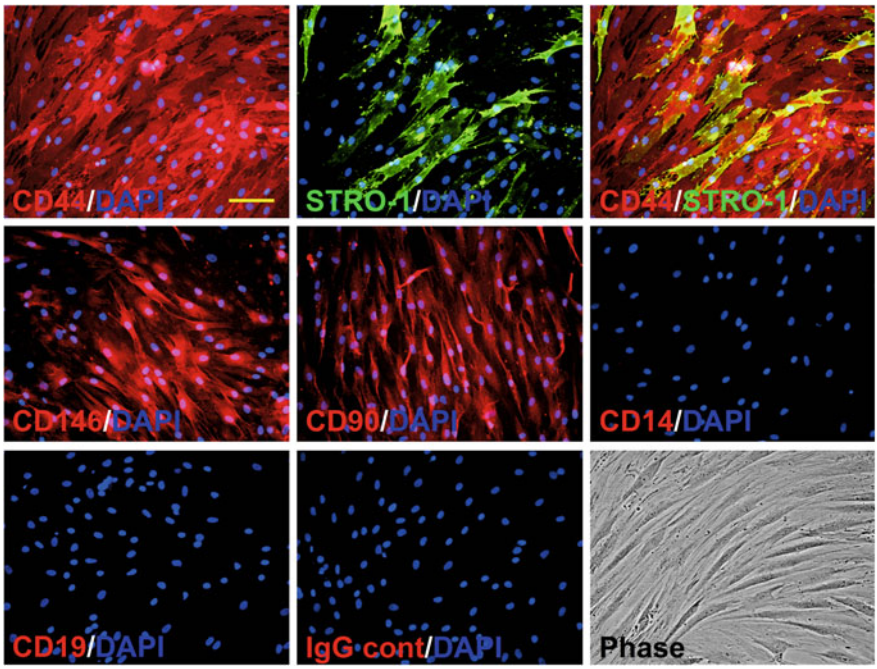

Fig. 2 Phenotypic antigenic markers of MSCs. Human MSCs demonstrate positive expression of CD44, STRO-1, CD90, CD146 and negative expression to haematopoietic markers CD14 and CD19. Nuclei are stained with DAPI. Phase images show typical morphology of MSC. Scale bar = 100 μm

A number of recent reports suggest an additional differentiation capacity of MSCs into a wide range of mesodermal and non-mesodermal adult phenotypes, including cardiomyocytes [34, 121], neurons [37, 135], hepatocytes [105] and lung epithelial cells [65, 72]. The benchmark definitions of MSCs may evolve over the coming years to reflect these descriptions.

3 Sources of MSCs

Friedenstein and others used cells that were isolated from collected bone marrow. Further locations and sites for MSC isolation have emerged, but bone marrow-derived MSCs are still the most frequently used MSCs in experimental research and are considered to be the criterion standard against which the newer sources of MSCs are compared [96].

There are numerous drawbacks and disadvantages associated with MSC isolation from bone marrow. Firstly, the procedure required for collecting bone marrow is through needle aspiration; this is accompanied by a mild discomfort that can be painful and can cause patient distress. Furthermore, there is a risk of infection as a

result of the procedure, with osteomyelitis posing a particular threat [24]. Because of the risk of the aforementioned difficulties and other potential complications, there has been extensive research into searching for other potential sources of MSCs.

Adipose tissue, peripheral blood, the lung, deciduous teeth, and the myocardium are all documented as potential sources of adult MSCs, while the placenta, amnion, umbilical cord and cord blood have been studied as potential birth-associated sources of MSCs. However, there does appear to be differences within the phenotypes, quality, and quantity of the MSCs collected at the various sites [15].

Adipose tissue is a potential source of adult MSCs. One of the main advantages of using adipose tissue is the relative ease with which it can be collected and the quantity of adipose tissue available [68]. Adipose tissue is collected through liposuction, which is a commonly performed and safe procedure with minimal patient distress or risk [106]. Furthermore, the frequency of MSCs in the adipose tissue is 1–10 in 100 stromal vascular fractions [50, 86], whereas, in bone marrow it is 1–10 in 1,00,000 mononuclear cells [13, 74]. In addition, adipose tissue-derived MSCs have a greater proliferative potential than bone marrow-derived MSCs, particularly in long-term cultures [62]. However, there are variations within the markers of the bone marrow and adipose tissue MSCs [12, 74].

Peripheral blood is another potential site for MSC collection. Collection of peripheral blood is performed through venipuncture and is thus a minimally invasive procedure with a low risk of complications [107]. However, studies have determined that although MSCs can be isolated from peripheral blood with subsequent differentiation, the frequency of peripheral blood MSC is much lower than that of adipose tissue and bone marrow, thus meaning a much larger sample of peripheral blood is required to evaluate MSC quality [127].

To negate the requirement for bone marrow-derived MSCs, numerous studies have investigated the effectiveness of using MSCs derived from birth-associated tissue with some promising results. Much interest has developed in isolating MSCs in this manner as it negates the use of invasive procedures such as bone marrow aspiration and is also more readily available. Furthermore, the cells collected from birth-associated tissues have been documented to demonstrate an improved capacity for self-renewal, differentiation, and an increased rate of proliferation when compared to their adult bone marrow-derived counterparts [15].

Human placental tissue is a potential source of birth-associated MSCs. Placental tissue has been characterised from four different locations: amniotic epithelial, amniotic mesenchymal stromal cells, chorionic mesenchymal stromal cells, and chorionic trophoblastic tissue [94]. There are four potential sources of placental tissue MSCs, but only the chorionic and amniotic mesenchymal stromal cells have been shown to demonstrate MSC properties [119]. Placental MSCs are reported as having a limited proliferative lifespan and as lacking adipogenic differentiation potential; further research is required to achieve a comprehensive conclusion [94].

Umbilical cord blood can be subdivided into whole umbilical cord, umbilical cord blood, and Wharton's jelly [40]. Umbilical cord MSCs demonstrate distinct features in comparison to bone marrow-derived MSCs. Umbilical MSCs and cord blood MSCs display an initially higher proliferative capacity when compared to

bone marrow MSCs, but similar to placental MSCs they appear to lack an adipogenic differentiation capacity [13, 29].

In conclusion, although bone marrow was the original site for isolation of MSCs, recent advances in our understanding of MSC biology have determined that there are other locations that may also yield MSCs. However, despite these recent advances, bone marrow remains the standard location for MSC isolation; further research will evaluate alternative locations and determine their value in practical and functional applications.

4 Bio-markers of MSC

Although no specific marker for MSCs has yet been identified, there are an abundance of non-specific surface antigens on MSCs. To provide clarification, the International Society for Cellular Therapy has provided guidance on MSC markers; MSCs must express CD73, CD90, CD105 and lack the expression of CD34, CD45, CD14, CD11b, CD19 or MHC class II antigens [39]. However, there are MSC marker variations readily located within the literature; STRO-1 provides a good example [25, 56, 57, 112] (Fig. 2).

5 Reparative Mechanistic Properties of MSC

Preclinical studies and clinical trials demonstrate that the application of MSCs stimulates wound repair and regeneration with efficient amelioration of a number of clinical conditions [18, 65, 80, 100], (www.clinicaltrials.gov). However, the precise mechanism of MSC-mediated wound repair and regeneration is not clear. One of the unique properties of MSCs is their site-specific migration and engraftment to injured tissues and differentiation into specific cell types. A variety of experimental animal models suggest active participation in wound repair and tissue regeneration [65, 80, 100]. On the other hand, some studies postulate that MSC-secreted paracrine factors play a vital role for wound repair, most likely through their anti-inflammatory, anti-apoptotic, angiogenic and immunomodulatory properties [9, 24, 83, 90, 137]. Additional reports suggest that MSC secretory products are capable of stimulating tissue-specific regional progenitor cells propagating tissue regeneration [47, 118].

5.1 Functional Contribution of MSCs in Tissue Repair

In 2002, Toma and colleagues injected human bone marrow MSCs isolated from healthy donors into the myocardium of healthy mice. They observed that MSC had differentiated into cardiomyocyte-like cells after a week [121]. Berry and

colleagues injected MSCs into the infarct region of the cardiac wall of myocardial infarction rat models and demonstrated that MSC treatment improved cardiac function; it reduced cardiomyocyte apoptosis and fibrosis scars in comparison to non-MSC treated control groups [18]. They also showed that transplanted MSCs expressed the cardiomyocyte-specific protein 'troponin T' while lacking a cardiomyocyte morphology, suggestive of a putative paracrine role that underpinned the reparative process.

MSC differentiation into type I and type II alveolar epithelial cells (AECI and AECII respectively) in vivo has been reported [65, 100]. Studies on bleomycin-induced animal lung fibrosis models demonstrated that following intratracheal and intravenous administration of MSCs, a small proportion of transplanted cells were engrafted to the affected lung and differentiated into AECI and AECII cells with an accompanying amelioration of pulmonary fibrosis [65, 100]. Human MSCs are capable of in vitro differentiation into Surfactant Protein-C (SP-C; a bio-marker of AECII)—expressing AECII-like cells when co-cultured with fetal lung mesenchymal cells [72]. In addition, the systemic application of murine MSCs in a cisplatin-induced acute renal failure mouse model resulted in migration and engraftment to the affected kidneys. This migration and engraftment was associated with differentiation into renal tubular epithelial cells and amelioration of renal dysfunction with augmentation of renal tubular regeneration. This is suggestive of the MSC as a potential candidate cell for a regenerative medicine-based therapy for the treatment of acute renal failure [80].

The differentiation of MSCs into hepatocytes was demonstrated when Sato and colleagues injected human MSCs directly into an alcohol-induced injury in the rat liver and assessed for expression of hepatocyte-specific bio-markers over an ensuing time-course [105]. From 7 days post-transplant, MSCs displayed expression of hepatocyte-specific and linked proteins including human-specific alpha-fetoprotein (AFP), albumin (Alb), cytokeratin-19 (CK-19), cytokeratin-18 (CK-18), and asialoglycoprotein receptor (AGPR) [105]. In addition, MSCs have been shown to differentiate into functional neuronal phenotypes [37, 135], retinal pigment epithelial cells [7] and skin epithelial cells [81].

Increased reports describe differentiation of MSCs into a variety of adult cell phenotypes. In many of these instances, differentiation into the desired cell-type was confirmed based on their cell-type specific biomarkers. Although some markers are specific for certain cells, this is not the case in every instance. Empirical analysis on both human and rodent MSCs demonstrated that the MSC is, by nature, primed for osteogenic, chondrogenic, adipogenic, and vascular smooth muscle differentiation and can undergo active differentiation under appropriate culture condition via activation of either transforming growth factor-beta, hedgehog, peroxisome proliferation-activated receptor-mediated interaction, and mitogen-activated protein kinase pathways, respectively [36]. Thereby, precaution must be taken in the application of MSCs in vivo to avoid any unwanted ectopic differentiation as a consequence of their relatively non-specific responsiveness to external cues.

5.2 Tissue Repair by MSC-Mediated Paracrine Mechanism

A growing body of evidence supports the hypothesis that paracrine mechanisms may underpin the role that the MSCs play in tissue repair and the regenerative process. MSCs possess an immunomodulatory function that has been demonstrated through their therapeutic efficacy in alleviation of graft-versus-host disease and animal models of bronchial asthma through putative roles in modulating Type-1 (Th1) and Type-2 (Th2) immune responses [84]. MSC-secreted factors are cyto-protective as demonstrated in the cardiac injury animal model driven by anti-apoptotic and inotropic effects [47]. The MSC-mediated anti-apoptotic effect can be driven by up-regulation of the anti-apoptotic gene Bcl-2, which was demonstrated in an animal model of emphysema [137]. Animal models of myocardial infarction and pulmonary hypertension have demonstrated that transplanted MSCs improve cardiac function and pulmonary vasculature by stimulating neovascularisation possibly via their secretory VEGF (vascular endothelial growth factor) and eNOS (endothelial nitric oxide synthase) [9, 24, 61]. The anti-inflammatory function of MSCs has been documented in many animal model studies, in which the mechanism is paracrine in nature and occurs via blocking of anti-inflammatory cytokines such as TNF-α and IL-1 [52, 90].

6 MSC Therapy in Pulmonary Disease

6.1 Acute Lung Injury

Acute lung injury (ALI) represents a continuum of clinical and radiological changes that affect the lungs. ALI can occur at any age and is characterised by a rapid onset of severe hypoxemia that is not secondary to left atrial hypertension [17]. Acute respiratory distress syndrome represents the most severe form of ALI. The definition of ALI has evolved through time as our understanding of the condition has improved. ALI was first described by Ashbaugh in 1967 with the description of a group of 12 patients who had refractory hypoxemia with abnormal changes on radiographic and pulmonary function tests [8].

6.1.1 Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS) is a common and devastating clinical syndrome of ALI caused by various direct and indirect insults including infection, trauma, and major surgery. It can result in respiratory failure and ultimately death [128]. The pathological hallmarks of ARDS include diffuse alveolar damage with presence of neutrophils, macrophages, erythrocytes, formation of hyaline membranes, accumulation of protein rich oedema fluid in the alveolar spaces, capillary

injury and disruption of the alveolar epithelium [4, 10, 11]. ARDS is a leading cause of death and disability in critically ill adults and children [101]. In the United States, there are 2,00,000 new cases of ARDS diagnosed each year, with a high mortality rate of 40 % (comparable to that seen in breast cancer; [102]). To date, there is no curative treatment for this devastating disease and the management is widely supportive [55].

A growing number of animal model studies demonstrate compelling data on the beneficial effects of MSCs in resolving acute lung injuries induced by endotoxin [32, 52, 70, 78], hyperoxia [26], pneumonia [67] and systemic sepsis [83]. In a recent description, endotoxin-induced lung injury in explanted human lungs was ameliorated with the infusion of MSCs [70]. The accumulation of this pre-clinical data offers considerable hope that MSCs could be a potential candidate for the effective therapy of ARDS. However, MSCs have not yet been evaluated for the therapeutic efficacy for ARDS in clinical trials.

In the ALI model, injury is induced by administration of bacterial endotoxin lipopolysaccharide (LPS) either via the intraperitoneal or intratracheal route, which drives the development of acute pulmonary inflammation within 24–48 h of LPS challenge in mice [100]. Evaluation of the LPS-induced mouse ALI model demonstrated that intravenous or intratracheal administration of MSCs within 1–4 h of LPS challenge significantly attenuated pulmonary inflammation, alveolar injuries, improved alveolar fluid clearance, and reduced mortality [52]. This improvement of the pulmonary condition was observed in the absence of significant engraftment of MSCs in the lung, suggesting a paracrine role of MSCs in the alleviation of ALI. This alleviation could be through down-regulation of pro-inflammatory responses via repression of TNF-α and increased anti-inflammatory cytokine IL-10 [52]. In support of MSC-paracrine mediated anti-inflammatory effects, Ortiz and colleagues demonstrated that MSCs and/or acellular conditioned media collected from cultured MSCs attenuated acute pulmonary inflammation. This attenuation was via suppression of both IL-1α-dependent T-lymphocyte proliferation and inhibition of TNF-α secretion by activated macrophages via MSC-secreted IL-1 receptor antagonist in vitro and in the bleomycin-induced murine lung injury model [90].

Nemeth and colleagues demonstrated that MSCs were stimulated by pro-inflammatory cytokines and endotoxins such as TNF-α and LPS. MSC endotoxin-based activation occurred via toll-like receptor-4, resulting in increased production of cyclooxygenase-2 and increased prostaglandin-E2 release. MSC-secreted prostaglandin-E2 drove increased macrophage IL-10 secretion and attenuated sepsis and sepsis-associated lung injury [83]. The explanted human lung model provided the demonstration that MSC enhanced LPS-induced ALI repair had likely occurred in a keratinocyte growth factor (KGF)-dependent manner [70]. Preclinical data are promising; however, clinical trials will decide the ultimate fate of MSCs as a therapeutic modality for ARDS in the near future.

6.2 Chronic Lung Disease

Chronic lung disease refers to any condition resulting in the long-term impairment of the lung that affects an individual's daily functioning [134]. The conditions that result in chronic lung disease are varied in their etiology, progression, clinical features and management [117]. For example, cystic fibrosis occurs due to a genetic defect, chronic obstructive pulmonary disease may occur as a result of an environmental irritant such as cigarette smoke [92], and finally some chronic diseases, such as idiopathic pulmonary fibrosis, may occur due an unknown cause. Although some chronic lung diseases such as asthma can be controlled and treated, many eventually result in respiratory failure.

6.2.1 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide and has been projected to be the third leading cause in 2020 [27]. No curative therapy is available for COPD at this time. COPD is characterised by an ongoing cycle of repeated destruction and repair of bronchilo-alveolar regions with subsequent tissue remodelling and sustained irreversible airway obstruction [2]. Approximately 20 % of patients with COPD present with emphysema, which is characterised by destruction of terminal bronchioles and alveolar walls resulting in an irreversible enlargement of alveolar spaces. The pathogenesis of COPD is not well understood. However, a significant reduction of circulating CD34+ progenitor cells has been observed in patients with end-stage COPD [92] and an elastase-induced experimental lung emphysema model [1]. Circulating bone marrow-derived CD34+ cells are haemopoietic progenitors thought to play a role in tissue repair [92]. The causes of progenitor cell destruction in COPD are not clear; however, it is assumed that the products of smoking create oxidative stress that may cause or contribute to progenitor cell destruction and apoptosis [63].

Systemic administration of bone marrow-derived MSCs was reported to ameliorate the emphysematous changes in the irradiation and papain-induced experimental mouse models [137]. Here Zhen and colleagues demonstrated that transplanted MSCs were localised to the emphysematous lung parenchyma and had differentiated into AECIIs. This was accompanied by reduced alveolar epithelial cell apoptosis, via Bcl-2 expression, and reduced enlargement of alveolar spaces [137]. Autologous intratracheal transplantation of bone marrow stem cells significantly mitigated elastase-induced pulmonary emphysema in the rabbit model [133]. The transplantation of bone marrow stem cells was associated with improved lung function, an attenuation of inflammation, an inhibition of epithelial apoptosis, a decrease in matrix metalloproteinase-2 expression, and the stimulation of alveolar and bronchiolar cell proliferation where engraftment and differentiation of the transplanted stem was negligible [133].

A Phase II, multicenter, randomized, double-blind, placebo-controlled clinical trial for the evaluation of safety and efficacy of MSCs for the treatment of moderate to severe COPD has recently been completed (www.clinicaltrial.gov). The trial enrolled 62 patients with COPD in six different centers in the United States. MSCs were administrated through an intravenous route. The complete report has yet to be published; however, preliminary reports are indicative of an improvement of quality of life with reduction of serum C-reactive protein, suggestive of a mitigation of inflammation (http://copsonlinenews.blogspot.com/2011/04/osiris-therapeutics-reports-interim.html).

6.2.2 Cystic Fibrosis

Cystic fibrosis (CF) of the lung is an autosomal recessive disorder caused by a mutation in the gene encoding the CF transmembrane conductance regulator (CFTR). CFTR is expressed in airway epithelial cells and the protein located on the luminal side of the plasma membrane, where it serves as a regulator of the Cl^- channel to maintain fluid and ions transport [75, 111, 116]. Activation of CFTR negatively regulates the epithelial Na^+ channel, which is why mutation of CFTR causes dysfunction of both Na^+ and Cl^- channels [75, 116]. According to current hypotheses on CF lung disease, the loss of Cl^- ion secretion and increased Na^+ ion absorption by airway epithelia reduce the thickness of the airway surface liquid layer overlying the epithelia, resulting in impaired mucociliary clearance [77]. Loss of CFTR function also suppresses mucous and antimicrobial factors secretion by airway submucosal glands [130]. Therefore, dysfunction of CFTR causes formation of thick and dehydrated mucous membranes that provides an ideal environment for persistent bacterial infection, triggering chronic inflammation and ultimately resulting in organ failure. At present, there is no curative treatment for CF. Because a genetic mutation underpins the pathogenesis of this disease, gene therapy is thought to be a valid option for the cure of CF [30]. Stem cell therapy has also been proposed to restore CFTR defective airway epithelia and to alleviate the concomitant inflammation [113, 117].

The main hurdle for stem cell therapy in the restoration of CFTR-defective epithelial cells is their low engraftment efficiency in the lung. Animal models demonstrate that transplantation of wild-type CFTR-expressing engineered bone marrow-derived MSCs in the CFTR knock-out transgenic mice results in a lung engraftment rate of about 0.025 % [71]. Moreover, the CFTR-expressing airway epithelial cells represented less than 0.01 % of the total airway epithelial cells, which was insufficient to replenish the lung with CFTR-expressing epithelial cells [71]. A low engraftment efficacy of CFTR-expressing MSCs in the intestinal epithelia of CFTR knock-out mice was reported by Bruscia and colleagues, where the engraftment was less than 0.01 % [22, 23]. These two studies indicate that complete restoration of CFTR-defective lung epithelial cells by transplanted CFTR-expressing MSCs, at least in the current animal models of CF, is virtually impossible.

An in vitro study has suggested that to restore epithelial ion and fluid clearance, it is not necessary to replace 100 % of CFTR-defective cells. The restoration of 6–20 % of CFTR-expressing epithelial cells was sufficient for effective Cl^- secretion by airway epithelial cells [42, 60]. Conversely, earlier reports suggested that all cells must express CFTR to re-establish the negative regulatory effects on airway Na^+ channel for effective Na^+ ion absorption homeostasis [48, 59].

Patients with CF frequently suffer from severe repeated pulmonary infections and chronic inflammation. This is often the main cause of sickness, disability, and mortality due to failure of lung function. It has been suggested that the anti-inflammatory and immunomodulatory functions of MSCs (discussed above) could serve a role in the ablation of the inflammatory conditions of CF lungs with potential therapeutic benefits.

6.2.3 Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown etiology, occurring primarily in older adults, limited to the lungs, and associated with the histopathological and/or radiological pattern of usual interstitial pneumonias [98]. This disease was also referred as cryptogenic fibrosing alveolitis before being displaced by the term IPF [110]. IPF is characterised by repeated microinjuries to the alveolar epithelium and consequent abnormal wound repair. This is accompanied by the accumulation of fibroblasts and myofibroblasts, with the deposition of excessive extracellular matrix resulting in the replacement of normal lung tissue with fibrotic scars. Accompanying the alteration of normal lung architecture is the clinical manifestation of progressive dyspnea worsening and reduced lung function resulting in respiratory failure [46, 98]. Unlike other inflammatory and fibrotic lung diseases, IPF does not respond to steroids and other potent immunosuppressive agents largely fail to reduce death rates in patients with IPF; the only potential curative treatment option at the moment is lung transplantation [108].

Patients with IPF are generally more than 50 years of age and two-thirds are older than 60 years at disease presentation. The median survival of patients with IPF is 2.8 years [20]. In the United States, the incidence and prevalence of IPF are 16.3 and 42.7 per 1,00,000 people, respectively [99]. Higher incidences are noted for the 75 years or older age group, in which it is 76.4 per 1,00,000 people, as compared to age group 18–34 years, with 1.2 per 1,00,000 people. An estimated 48,000 new IPF cases are diagnosed annually in the United States alone [98, 99]. In the United Kingdom, the overall incidence rate of IPF is 4.6 per 1,00,000 cases per annum [49]. More than 4,000 new IPF cases are currently diagnosed each year in the United Kingdom. The mortality rate from IPF has also increased over the last two decades [87]; death rates from IPF have reported to be higher than death rates from some cancers [58].

Unlike CF, to date, no specific genetic or acquired cause has been identified for IPF; however, mutation in the genes encoding for hTERT [6, 38, 123] and SP-C [120]

have been reported in the familial form of IPF. The pathophysiological process of IPF is widely unknown. Previously, IPF was thought to be a consequence of chronic pulmonary inflammation. However, non-responsiveness to anti-inflammatory or anti-fibrotic drugs and lack of histopathological evidence of inflammation in IPF lungs suggest that inflammation may not be an initiating trigger in the pathogenesis of this disease [109]. An evolving hypothesis describes IPF as a consequence of aberrant alveolar wound repair and regeneration, most likely due to a combination of repeated AEC injury [31, 109], increased AEC apoptosis [14, 69, 125], dysregulated epithelial-mesenchymal cross-talk [110], polarised immune response [114, 124] and altered coagulation cascade [27, 64].

Stem cell-mediated regenerative therapeutic approaches have been proposed for the treatment of IPF. To assist in these studies, several animal models of pulmonary fibrosis have been developed [79], including the bleomycin-induced pulmonary fibrosis model [53, 79], radiation-induced fibrosis [54], silica-induced fibrosis [33] and asbestos-induced lung fibrosis models [21].

The bleomycin-induced pulmonary fibrosis mouse model provided a demonstration of migration and engraftment of endotracheal or systematically transplanted MSCs towards the site of injuries of the lung and subsequent attenuation of pulmonary fibrosis [91, 100]. Systemic administration of bone marrow-derived MSCs after 4 h of bleomycin administration attenuated pulmonary inflammation, reduced fibrosis, and decreased mortality after 14 days of injury. Transplanted MSCs had engrafted into the injured alveoli with accompanying differentiation into type II AEC-like phenotype [91]. However, when MSCs were administered after 7 days of injury, the MSC-mediated protective function was abrogated [91]. Complementary results were noted in independent studies [100]. In 2007, Ortiz and colleagues showed that MSCs protected against bleomycin-induced lung injury and reduced fibrosis by blocking pro-inflammatory cytokines such as TNF-α and IL-1 by MSC-associated IL-1 receptor antagonist [90].

The administration of KGF-expressing MSCs or HSCs (haematopoietic stem cells) in the bleomycin-induced mouse lung fibrosis model was associated with reduced fibrosis via suppression of collagen accumulation [3]. KGF has an established role in the repair of alveolar epithelium through stimulation of type II AEC proliferation, migration and spreading [51, 93, 132]. This proof-of-concept experiment demonstrated that genetically modified MSCs or HSCs with suitable cytokine/growth factor have potential as a therapeutic strategy for pulmonary fibrosis [3].

The pre-clinical studies described previously suggest a role for MSCs as a potential candidate for regenerative therapy for IPF. There are remaining concerns that MSC have pro-fibrotic effects and could deteriorate the pathological condition if they are applied in chronic lung fibrosis [131]. Yan and colleagues demonstrated that after systemic application of MSCs at 4 h of irradiation-induced lung injury, transplanted cells engrafted in the alveolar and bronchiolar epithelium and differentiated into epithelial phenotype; however, MSCs administered at 60 and 120 days post-injury localised in interstitial spaces and differentiated into myofibroblasts, a fibrotic cell that plays major role in fibrogenesis [131]. These authors

concluded that fate of MSC differentiation is controlled by the microenvironment milieu and warned that MSC therapy might be ideal for ALI but may augment fibrosis in chronic lung fibrosis, such as IPF.

Supporting the putative profibrotic nature of MSCs, an in vitro study demonstrated that human and mouse MSCs secrete TGF-β1 and Wnt proteins that stimulate both human/mouse lung fibroblast proliferation and collagen production—two major hallmarks of lung fibrosis [104]. Prostaglandin E2 treatment significantly inhibited resident MSC proliferation and collagen secretion and abrogated fibrotic differentiation into myofibroblasts [126]. If this is true for MSCs from common sources such as bone marrow and cord blood, prostaglandin E2 could be administered concomitantly with MSCs to reduce putative fibrotic effects.

Conversely, no TGF-β1 expression was detected in MSCs isolated from the bone marrow of normal healthy individuals or patients with IPF; the expression of fibroblast growth factor and VEGF was not significantly different in either case [5]. However, CXCR4, a potent chemokine receptor, was significantly over-expressed in patients with IPF. The increased CXCR4 expression by IPF MSCs suggests that the bone marrow is probably implicated in the pathophysiology of IPF by mobilising resident MSCs in response to or preceding lung injury [5]. Further study will confirm that whether this MSC mobilisation is a mere attempt to repair lung injury or solely aggravates fibrosis in IPF.

6.2.4 Bronchial Asthma

Bronchial asthma, one of the most common chronic inflammatory lung diseases, affects over 300 million people world-wide [76]. Asthma is characterised by reversible airway obstruction, hyper-responsiveness of airway smooth muscle, and airway inflammation. There is no permanent curative treatment for asthma; most of the patients remain symptomatically controlled by combined mediation of bronchodilator and steroids. However, approximately 5 % of patients with asthma are resistant to conventional therapy and suffer from substantial morbidity and mortality [117]. The ability of MSCs to modulate the immune system encouraged researchers to explore the potential of MSCs as an anti-asthmatic therapy.

The ragweed-induced mouse asthma model was used to demonstrate that administration of bone marrow-derived MSCs ameliorated allergic and inflammatory responses in the airway [84]. After transplantation, animals were protected from the majority of asthma-specific pathological changes, including inhibition of eosinophil infiltration and excess mucus production in the lung, decreased levels of Th2 cytokines (IL-4, IL-5, and IL-13) in bronchial lavage, and lowered serum levels of Th2 immunoglobulins (IgG1 and IgE) [84].

6.3 Pulmonary Vascular Disease

Pulmonary vascular disease is an umbrella term used to describe a group of conditions associated with damage or alterations to the lung vasculature [35]. Diseases within this realm include pulmonary hypertension, pulmonary embolism, pulmonary veno-occlusive diseases, arterio-venous malformation and pulmonary edema [41]. Pulmonary hypertension is frequently associated with lung parenchymal damage and can present as a secondary complication of chronic lung disease [35, 41]. The remaining conditions within the group are frequently associated with vascular and cardiac pathologies.

6.3.1 Pulmonary Hypertension

Pulmonary hypertension (PH) is rapidly progressive and often fatal disease characterised by increased pulmonary arterial pressure, right heart dysfunction, and lung vasculature remodelling leading to loss of alveolar vasculature [97]. MSC-based therapy has been explored for application in the regeneration of pulmonary vasculature because they secrete VEGF a potent stimulator of neovascularisation. Intratracheal administration of bone marrow-derived MSCs in the monocrotaline-induced rat PH model attenuated PH [9]. Transplantation of MSCs reduced monocrotaline-induced pulmonary arterial pressure and improved pulmonary vasculature through paracrine mediator(s). Immunohistochemistry showed no evidence of endothelial differentiation of MSCs [9].

Intravenous administration of MSCs and eNOS-overexpressing MSCs in the monocrotaline-induced rat PH model also resulted in attenuation of PH and improved right ventricular hypertrophy in comparison to un-treated control groups [61]. Interestingly, the reduction of right ventricular hypertrophy was significantly higher in the eNOS-overexpressing MSC treated group in comparison to the MSC groups, suggesting that MSC-mediated improvement of pulmonary vasculature in PH could be driven by modulation of nitric oxide secretion by the vascular endothelium [61].

7 Tissue Engineered Lung Tissue

Current research in the tissue engineering field is focused on exploration of 3-dimensional tissue culture systems for use in development of functional lung tissue. The ultimate ambition of these studies is to reduce donor-dependent lung transplantation [85, 122]. Because of the unique architecture of the lung and its anatomical and physiological complexity, this presents a major challenge. Tissue-engineered tracheas (wind pipe) have been developed using MSCs isolated from various sources before being cultured on biodegradable and biosynthetic scaffolds to generate tracheal cartilage for the repair of congenital tracheal defects in both animal

and human clinical trial models [88, 89]. Very recently, a group of tissue engineers, stem cell researchers, and medical professionals developed a functional human airway by culturing MSC-derived chondrocytes on an acellular tracheal scaffold, which was subsequently transplanted in a female patient who had suffered airway damage from tuberculosis [73]. Macchiarini and colleagues first decellularised a 7-cm long segment of human trachea taken from a 51-year-old white female donor who had died of cerebral hemorrhage. The recipient's bone marrow-derived MSCs were differentiated into chondrocytes and airway epithelial cells cultured using *in vitro* tissue culture system. The MSC-derived chondrocytes were seeded on the external surface of the acellular trachea and epithelial cells seeded on the luminal surface, in an equal ratio, and cultured in an air–liquid interface rotating bioreactor for 96 h. After *in vitro* preparation, the tracheal construct was transplanted to the left bronchus of the recipient, which improved breathing difficulties without graft rejection [73]. This achievement should encourage the development of more complicated parts of the lung, such the alveoli and pulmonary vasculature, in the near future.

8 Challenges for MSC Therapy in Pulmonary Disease

Although pre-clinical data provide evidence of promising therapeutic benefits of MSCs in various pulmonary diseases, many hurdles remain. Some important parameters such as MSC choice, dose, timing, route of administration, and selection of suitable clinical conditions for cell therapy need to be established before clinical application [2]. As a route of administration, intravenous, intra-arterial, and intra-tracheal routes have all been implemented in animal models for MSC delivery. MSC engraftment was higher when administered into injured lungs through the intravenous route [43], whereas administration through the intra-arterial route was accompanied by complications associated with microvasculature occlusion [45]. The intratracheal route was also demonstrated to be suitable for efficient engraftment [52, 70]. Clinical trials of MSCs in pulmonary diseases, such as COPD, although safe, have not yet evidenced an appropriate efficacy of repair. The prospects of MSC-based regenerative cell therapy for the treatment of pulmonary diseases will be determined by the outcome of future large-scale clinical trials.

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