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Review Article

Stem cell therapy in chronic obstructive pulmonary disease. How far is it to the clinic?

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Abstract: Chronic obstructive pulmonary disease (COPD) is a respiratory disease that has a major impact worldwide. The currently-available drugs mainly focus on relieving the symptoms of COPD patients. However, in the latter stages of the disease, the airways become largely obstructed and lung parenchyma becomes destructed due to underlying inflammation. The inappropriate repair of lung tissue after injury may contribute to the development of disease. Novel regenerative therapeutic approaches have been investigated with the aim of repairing or replacing the injured functional structures of the respiratory system. Endogenous and exogenous sources of stem cells are available for the treatment of many diseases. Stem cell therapy is newly introduced to the field of COPD. Currently the research is in its infancy; however, the field is profoundly growing. Previous studies suggest that cell-based therapies and novel bioengineering approaches may be potential therapeutic strategies for lung repair and remodelling. In this paper, we review the current evidence of stem cell therapy in COPD.

Keywords: Stem cell, chronic obstructive pulmonary disease

Cellular and structural inflammation in COPD

In order to understand how stem cell therapy and regenerative medicine would act in COPD, one should understand the current knowledge about pathological changes in airways and lung parenchyma.

In COPD, airflow limitation is resulted from two mechanisms: 1) The loss of elastic recoil and the obstruction of small airways. The loss of elastic recoil is an outcome of the destruction of the airways distal to the terminal bronchioles. This is called as emphysema, which results in hyperinflation. 2) The thickening of small airways increases the airway resistance and results in air trapping as well. Those processes are progressive and irreversible changes leading to dyspnoea, exercise intolerance, impairment of quality of life, disability and death [1-4].

Those pathological changes are profoundly the result of smoking-induced inflammatory changes and structural abnormalities. In a background of genetic susceptibility, smoke exposure causes an exaggerated immune response resulting in activation of adaptive and native immune response. During this cellular and structural immune response, several mechanisms are being proposed. Protease-antiprotease imbalance, oxidative stress, proteolytic mechanisms, apoptosis and excessive aging are among them. Those mechanisms were initiated by smoke fume or inflammatory cells. Interestingly, if the smoke exposure is ceased, the inflammatory process persists [5].

Cellular aging in COPD

Senescence results in serious of pertubation in cell morphology and cell cycle arrest. Cellular senescence is associated with DNA damage, abnormal DNA repair, impairment of epigenetic modifications of DNA, telemore shortening, free radical formation and protein damage. It has been shown that lung fibroblasts and type II

Table 1. Definitions of terms used in the stem cell biology [12]

Dedicated stem cell	A relatively undifferentiated cell usually in localized niches. Divides infrequently; is capable of both long-term ('lifetime') self-renewal and of giving rise to daughter cells that differentiate into one or more specialized cell type; and it functions in both tissue homeostasis and repair.
Facultative stem cell	Differentiated cell that is normally quiescent but responds to injury by dividing and self-renewing, and giving rise to progeny that differentiate into one or more cell types.
Metaplasia	Strictly, the process by which a stem or progenitor cell of one tissue switches to become a progenitor of cells of another tissue type.
Post-mitotic differentiated cell	A cell that can no longer divide and must be replenished during normal turnover or injury.
Progenitor cell	Either a cell in the developing organ, usually multipotent, that is the source of an initial population of adult cells before turnover begins, or, more loosely, a cell that gives rise to another cell. Cell lineage relationships during development may not necessarily reflect those that occur during repair.
Self-renewing differentiated cell	Differentiated cell that divides and self-renews over the long term. Functions in both normal tissue homeostasis and in response to injury.
Transit amplifying (TA) cell	An intermediate between a dedicated stem cell and its final differentiated progeny. Can proliferate, self-renew over the short term and give rise to one or more differentiated cell type.
Transdifferentiation	This refers to the transformation of one well-defined type of fully differentiated cell into another well-defined type. 'Transdifferentiation with proliferation' requires at least one intervening round of cell proliferation. The direct mechanism is more likely to involve the transient existence of a cell that co-expresses differentiation markers of both old and new phenotypes.

alveolar and endothelial cells have increased expression of senescense associated molecules. Sirtuin, which act on histone residues to mediate DNA silencing, has been shown to be reduced in COPD compared to healthy smokers. Telemores, as an indicator of accelerated aging, are much more shortened in COPD when compared with healthy smokers and nonsmokers. The senescence of alveolar epithelial and endothelial cells is accelerated in patients with emphysema. Cellular senescence may explain the abnormal cell turnover that promotes the loss of alveolar cells in emphysematous lungs [6, 7].

Aging causes an increase in collagen and a decrease in elastin in the lung parenchyma. In young individuals stem cells are able to migrate towards injured lung tissues and repair those damages. But with aging, stem cells' repair capacity is diminished. Aged stem cells have decreased telomere length and shortening of telomeres leads to rapid cell turn over and decreased capacity of tissue to cope with inflammatory insult [8-10].

Lung repair

Throughout adult life, multicellular organisms must generate new cells (regeneration) to maintain the structure and function of their tissue. In normal homeostasis, the reparative and regenerative process can keep up with the excess destruction and inflammation. However, multiple injuries in addition to aging can hamper these magnificent mechanisms. What is known classically is that organs may follow a hierarchical algorithm in response to injury. But emerging data have shown, in reality, different organs use different strategies to renew themselves and it should not be necessarily a certain hierarchical order in each organ [11].

Some organs have very fast turnover rate such as hair follicles, blood and gut. They have unspecialized "dedicated" stem cells that possess self-renewal properties for long term and low rate of division. Dedicated stem cells produce transit amplifying (TA) daughter cells. They have a very high proliferative ability, can self-renew for short period of time and give rise to many differentiated precursors. However, recent data shown that the hierarchical order is not too strict in every situation. For example, TA cells could differentiate into another tissue cell type when exposed certain signals. This process is called transdifferentiation. In contrast to the rapid turnovering organs (skin, gut), some organs do not need undifferentiated stem cells. For example, in liver, after hepatectomy, regeneration involves the differentiated hepatocytes. If hepatocyte population is inhibited, interlobuler bile duct can replenish the hepatocyte population. Those cells are called by facultative stem cells. **Table 1** shows definitions of terms and **Figure 1** shows various stem cell, cell therapy, and exvivo bioengineering approaches for lung disease [11, 12].

The adult lung is a complex organ with relatively slow turnover time. However, the epithelial cells lining the airways are continuously exposed to air which may bring toxic and noxious particles. Therefore, after cellular damage, the epitheli-

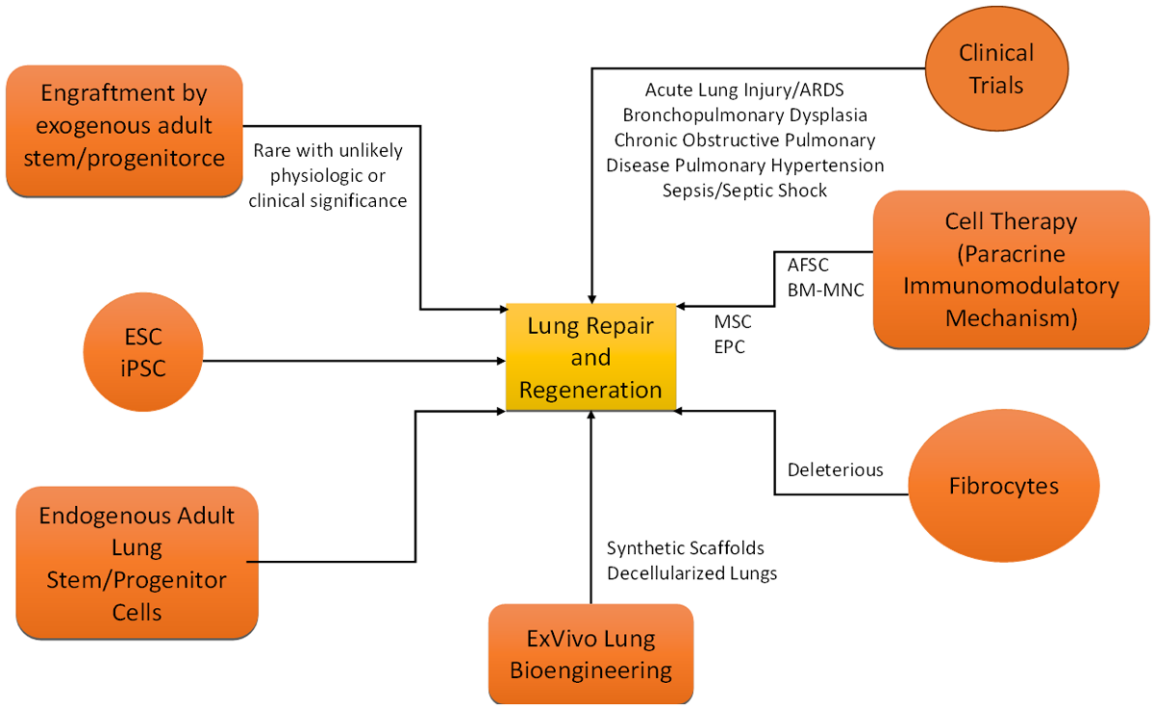

Figure 1. Transamplifying Progenitors: Schematic illustrating various stem cell, cell therapy, and exvivo bioengineering approaches for lung disease. Abbreviations: AFSC, amniotic fluid stem cell; BM-MNC, bone marrow-derived mononuclear cells; EPC, endothelial progenitor cell; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; MSC, mesenchymal stem (stromal) cell [11].

um must respond quickly and constantly to those challenges. The lung epithelium undergoes a slow but constant renewal under normal healthy conditions, with a turnover to occur every 30 to 50 days. To date, most studies indicate that the damaged lung epithelium is repaired by resident lung progenitor cells, with only a possible minor contribution of circulating or bone marrow-derived stem/progenitor cells. Moreover, current evidence suggests that these adult resident lung progenitor cells are not identical to the embryonic cells that form lung tissue during embryonic development [12, 13].

However, the mechanisms under lung repair stimulated with different challenges in chronic conditions have not been elucidated yet. There is evidence that lung repair is defective in COPD. It could be either inappropriate, delayed or impaired [11, 12].

Resident stem/progenitor cells within the lung

Epithelial progenitor cells

The lung is composed of functionally and anatomically different units and, accordingly, more than 40 differentiated cell types [14]. Despite recent advances, little is known about the lung stem cells, their characteristics, and their functional abilities related with regeneration. However, several resident regenerative cells have been identified in lung. They are located in different niches and express distinct levels of genes within different regions of the lungs. In trachea and airways, basal cells express transcription factor p63+ and cytokeratin 5/14. Secretory (Clara) cells produce secretoglobulins (Scgb1a1) also known as (CCSP, CC10 or CCA). Cells residing in submucosal glands and neuroepithelial bodies (NEB) are identified as progenitor cells. In more distal airways, variant clara cells (Clarav) and bronchioalveolar stem cells (BASCs) and in alveola, alveolar type II epithelial cells (AECs) have the properties of progenitor cells [15, 16]. **Figure 2** showed regenerative cell locations both in proximal and distal airways. The functional definition of those cells is rather complex. In the proximal airway, undifferentiated basal cells can function as classical stem cells. They can be self-renewing and can give a rise to ciliated cells and secretory cells. Clara cells can also give rise to ciliated cells. In more distal lung, basal cells are absent. The

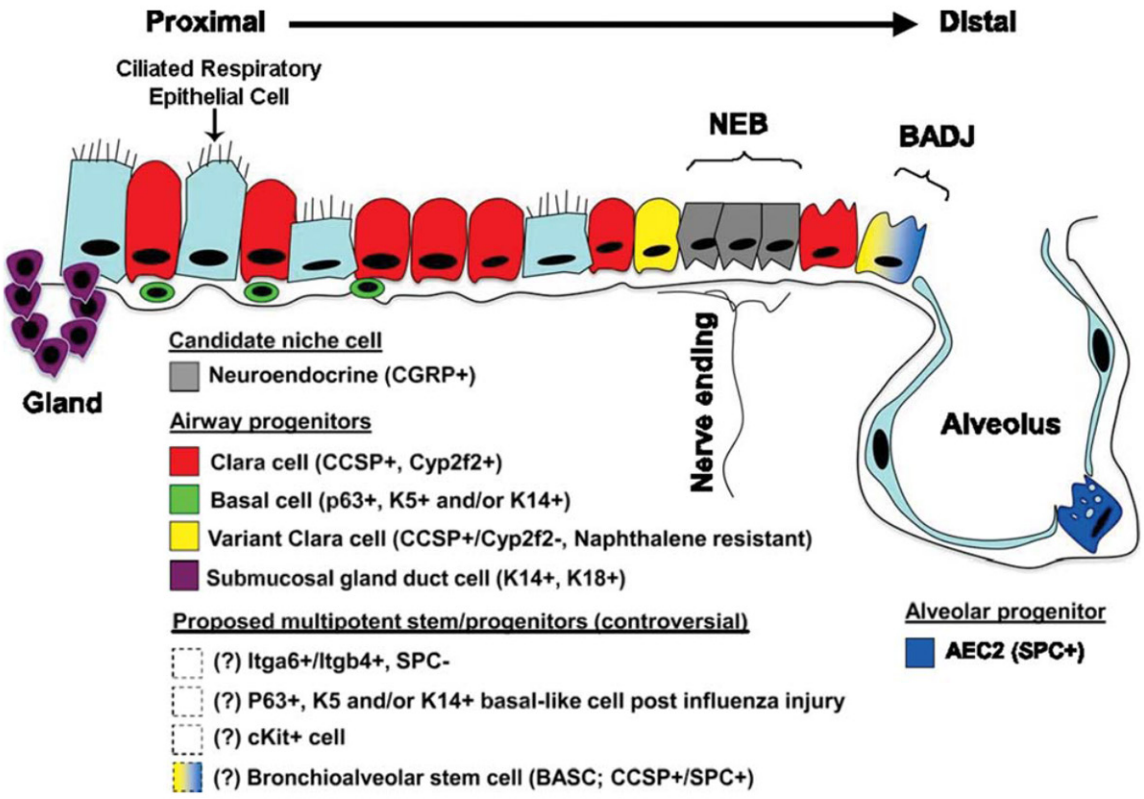

Figure 2. Lung epithelial stem and progenitor cell candidates. Shown is a schematic of proposed lung epithelial candidate stem or progenitor cells and their niches in the proximal conducting airways and distal alveoli. Cells whose localization or existence is not yet clear or accepted are indicated with dashed boxes. Modified with permission from Weiss DJ. Concise review: current status of stem cells and regenerative medicine in lung biology and diseases. Stem Cells. 2014; 32(1): 16-25. Abbreviations: AEC2, type 2 alveolar epithelial cell; BADJ, bronchoalveolar duct junction; CCSP, Clara cell secretory protein; CGFP, calcitonin gene-related peptide; Itg, integrin; K, cytokeratin; NEB, neuroepithelial body; SPC, surfactant protein C [17].

variant Clara and BASCs under specific conditions can self-renew and give rise to different cell types. We do not know if ciliated cells can proliferate and transdifferentiate. There is a need for more specific markers and better techniques for better characterization of those cells. Recently, lineage negative c-kit positive cells were identified from lung tissue possessing the fundamental properties of stem cells -self-renewal, clonogenicity, and multipotency. The delivery of clonal human lung stem cells to cryo-injured mice results in the formation of human bronchioles, alveoli, and pulmonary vessels providing evidence that, human lung stem cells may have role in lung homeostasis and tissue regeneration following damage. The authors suggest that basal epithelial cells, Clara cells, BASCs, and AECs may derive from lineage specification of c-kit positive human lung stem cells [17]. This observation should be confirmed before this information is generalized.

Resident mesenchymal stem/progenitor cells

Lung MSCs have been identified and characterized by using flow cytometry to detect Hoechst 33342 vital dye efflux by lung cells in combination with absence of the hematopoietic marker, CD45. They express the characteristic mesenchymal cell surface markers CD73, CD44, STRO-1, CD73, CD90, CD105, CD166, CCR2b, CD13, prolyl 4-hydroxylase, vimentin and, ScaI. In addition, they lack the hematopoietic markers c-kit, CD11b, CD31, CD14 and CD34. Lung MSCs also exhibit high telomerase activity which indicates the capacity for self-renewal [18, 19].

One of the proposed mechanisms behind COPD is abnormal myofibroblast proliferation. There is a possibility that in COPD, myofibroblast differentiated from resident stem cells can contribute remodelling as opposed to repair. Therefore, resident mesenchymal stem cells could

be a good candidate to target for treatment COPD. However, we do not know how COPD affects the differentiation of those cells. The functionality of stem cells is highly influenced by the local microenvironment or niche. Disease process may alter the niche that influences the resident stem cells' proliferation and differentiation process. Depending on their microenvironment, the lung MSCs have the ability to differentiate different cells including myofibroblasts. They have anti-inflammatory properties and suppression of T cell proliferation. Tissue resident mesenchymal stem cells are important regulators of tissue repair or regeneration, fibrosis, inflammation, angiogenesis and tumor formation. Jun et al [19] documented that replacement of resident stem cells by administration of isolated lung MSCs attenuated the bleomycin-associated pathology and inhibited the development of pulmonary arterial hypertension suggesting that lung MSC's function is to protect lung integrity following injury. Global gene expression analysis indicated that the lung resident MSC are a unique stromal population differing from lung fibroblasts in terms of proinflammatory mediators and pro-fibrotic pathways. Jun et al demonstrated that lung MSCs function to protect lung integrity following injury. However, MSCs' functioning is depending on their microenvironment. Normally MSC should undergo apoptosis after completing lung repair however under the certain conditions, MSCs may survive longer leading pathogenic changes within the lung. As an example, it was shown that under profibrotic factors such as transforming growth factor beta (TGF-β) and interleukine 13 (IL-13) stimulation, MSCs differentiate into myofibroblasts in several settings indicating their critical role in lung remodelling [20, 21].

Platelet derived growth factor (PDGF) and Wnt signalling are two key pathways involved in the "programming" of MSC. PDGF-BB which is expressed from adult lung parenchyma, endothelium, platelets and macrophages interacts with its receptors (PDGFRβ) located in lung mesenchymal cells leading them profibrotic phenotype that promoting proliferation and collagen production. Elevated levels of PDGF are shown in lung fibrosis, bronchiolitis obliterans and pulmonary vasculature in PAH and the inhibition of PDGFB reverses these fibrotic processes [16, 22].

The Wnt family is an important group of proteins in development and repair process. β-catenin protein is a central mediator of canonical Wnt signalling and its activation can result in hypercellularity of tissue and deregulated self-renewal. The genes induced by β-catenin regulate MSC proliferation, differentiation, migration, and survival. Studies have shown that the Wnt/β-catenin pathway also have roles in the development of experimental emphysema [23, 24].

Regenerative approach in COPD

In the mouse models with H1N1, despite the loss of 50-60% of lung parenchyma due to severe inflammatory response, mice can survive and after three to five months of the incident, they regain the normal lung histology. That may indicate that adult stem cell may responsible for this rapid lung regeneration and, therefore, lung has regenerative capacity that may lead a new era for the treatment of severely limiting and mortal lung disease such as COPD [25]. Although it is not well understood, not only temporary injury but also severe destruction may be responsive to correct regenerative approach. Alveolar repair mechanism has been presented in a mice model. Ovariectomized adult mice developed larger alveoli and less alveolar surface compared to controls. Three weeks of oestrogen treatment resulted in alveolar regeneration [26]. Now, there has been a major interest on to understand how stem cell initiates the repair process in conjunction with airway epithelial remodelling and defecting repair processing.

However, we do not know how COPD affects the differentiation of tissue resident stem cells and the bone marrow derived stem cells [27]. There is a possibility that resident stem cells differentiate into myofibroblasts to participate in remodelling in COPD. COPD may alter niche that highly affect stem cell functionality [28]. In COPD, it is yet to be determined whether bone marrow (BM) derived stem cells participate in lung repair, whether the reparative capacity of those cells impaired or whether self mobilization of those cells to the lungs is deranged [27]. In a recent study it was shown that cigarette smoke exposure caused the depression of bone marrow hematopoietic stem cell function [29].

What is regenerative medicine?

Regenerative medicine is a branch of translational research in tissue engineering and molecular biology which deals with the "process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal function". This field holds the promise of engineering damaged tissues and organs via stimulating the body's own repair mechanisms to functionally heal previously irreparable tissues or organs. Regenerative medicine also includes the possibility of growing tissues and organs in the laboratory and safely implant them when the body cannot heal itself [30].

Three different mechanisms are represented for regenerative approach. First is administration of small molecules to activate resident stem cells to stimulate endogenous repair process. Second is exogenous stem cell administration. Third, exogenous cells or tissue administration to replace lost tissue via ex vivo tissue engineering.

Although, this research field is growing fast, lung regeneration models are very challenging to develop because the lung 3D complex architecture and different types of progenitor cells are located in different regions. Expanding these cells ex vivo and adequate administration of them to the injured area for appropriate homing are all challenging issues to overcome.

Stimulating endogenous stem cells

Resident stem cells have the capability of regenerate tissue after injury. Angiogenic factors such as hepatocyte growth factor (HGF), Granulocyte colony-stimulating factor (GCSF), fibroblast growth factor-2, -7 (FGF-2), adrenomedullin and all-trans-retinoic acid (atRA) have been evaluated and are shown to stimulate stem cells in animal models [16].

HGF is a pleotropic growth factor. It has been shown to be a potent mitogen for type II cells. In elastase-induced emphysema models, HGF induces alveolar regeneration and reverses elastase-induced emphysema by induction of both BM derived and resident stem cells. There is no published data for the use of exogenous HGF in patients with COPD [16].

GCSF, induces angiogenesis in cardiac, brain and limb ischemia models by mobilization of BM-derived stem cell populations. GCSF has been reported to improve morphometric parameters of emphysema model [16].

Fibroblast growth factor-7 (FGF-7) or keratinocyte growth factor (KGF) is an important signalling molecule released by fibroblasts and has been shown to promote survival, proliferation, migration and differentiation. In the lung the main target of KGF is alveolar type 2 cells. In an elastase-induced emphysema model, KGF protected against elastase induced pulmonary inflammation but did not reverse morphometric parameters in established disease [31].

The 3-hydroxy-3 methyl glutaryl coenzyme A reductase inhibitors (statins) have pleiotrophic effects such as anti-inflammatory effect, reducing reactive oxygen species, improving endothelial functions, supression of proinflammatory cytokines, adhesion molecules, matrix metalloproteinases. They promote endothelial progenitor cells from BM, increase the secretion of vascular endothelial growth factor (VEGF). In an elastase induced emphysema model, authors showed that simvastatin inhibited the development emphysema in mice. They concluded that this therapeutic effect was due not only to anti-inflammation but also to the promotion of alveolar epithelial cell regeneration and restoring endothelial cell functions [32].

Adrenomedulin (AM) is a vasoactive regulatory peptide originally isolated from human phaeochromocytoma. It is found in many tissues including the lung. It promotes vascular regeneration and has been found in airway basal cells and type II cells of the lung [16].

Retinoids including the biologically active molecule atRA are essential for correct development of a number of organs including the lung. atRA is generated from vitamin A (retinol). atRA acts via nuclear retinoic acid receptors (RARs), which are members of the glucocorticoid/thyroid hormone receptor superfamily. Dexamethasone and retinoids have antagonistic effects. atRA supplementation during alveolar septation increases the number of alveoli in rats treated with dexamethasone. Those data provided the first experimental evidence to suggest that exogenous pharmacological therapy

might be a potential approach for human diseases characterized by alveolar destruction. There are also some evidence that retinol also affects lung development in human. In a study carried out in a chronically undernourished population, maternal repletion with vitamin A at recommended dietary levels before, during, and after pregnancy improved lung function in offsprings. In animal models some studies show that retinoids effect genes involved in lung development and to promote alveolar regeneration. Oestrogens have also been demonstrated to have a regulatory role in alveolar formation. In a study performed in adult ovariectomized mice showed that oestrogen is required for maintenance of already formed alveoli and induces alveolar regeneration after their loss. Human studies also confirmed that there is accelerated loss of lung function in women after the menopause. Hormone replacement therapy has been shown to be related with higher forced expiratory flow rate (FEV1) in postmenopausal women [33, 34].

Bioengineered models

In vitro, embryonic and adult stem cells can be induced to differentiate into lung epithelial cells. However, engraftment following systemic administration rarely occurrs. Thus, scientists have focused on bioengineered three-dimensional matrices or artificial scaffold to generate functional lung tissue. These models have been used to regenerate skin, blood vessels, bone, and cartilage. Stem cells have also been used to repair congenital tracheal defects in animal models and human clinical trials. Omori et al [35]. developed a tissue scaffold made from a Marlex mesh tube covered by collagen sponge. That scaffold implant was successfully applied to repair the larynx and trachea in 4 patients In June 2008, a history making surgery was performed by Dr Paolo Macchiarini and his team. Macchiarini et al [36], for the first time in the world, generated a bioengineered trachea using a donated tracheal matrix colonized by patient's own BM derived stem cells. The engraftment provided a functional airway without immunosuppressive treatment. Recently, the 5-year follow-up results are presented and showed that although the naive trachea developed cicatricial stenosis that required repeated endoluminal stenting, the tissue engineered trachea completely recellularized and was functioning well [37]. Despite the fact that the research in that field is growing quite fast, the technical challenges have to be overcome for complex 3D organs such as heart or lung [38]. Developing distal airways and lung parenchyma are more challenging than developing airways. Peterson et al [39] developed a rat model of acellular scaffold cultured with pulmonary epithelium and vascular endothelium and then implanted the bioengineered lung into rat. The model participated gas exchange in-vivo for a short period of time (45 to 120 minutes). That researchers providing inspiring results for the ultimate goal of functioning bioengineered whole lung.

Exogenous stem cells or progenitor cells

Adult lung progenitor cells

In respiratory disease, it is reasonable to propose enhancing or restoring endogenous resident stem cells by introducing lung epithelial progenitor cells. Although it seems to be a promising way to perform in theory, it is very challenging to obtain sufficient numbers of primary lung epithelial progenitor cells. There are only few animal studies of investigating transplanted lung progenitor cells in lung regeneration. Serrano Molar et al [40] successfully showed that intratracheal transplantation of alveolar type II cells in a rat model of bleomycin induced lung fibrosis could halt and reversed the fibrotic process. More recently alveolar type II cells were transplanted into pneumonectomized mice to investigate the effect of those cells in compensatory lung growth after pneumonectomy. The investigators showed the engraftment of stem cells that stimulated lung regeneration in remnant lung [41].

Embryonic stem cells

Due to the fact that lung progenitor stem cells are difficult to obtain or repopulate in vitro, investigators have focused on obtaining progenitor stem cells from embryonic stem cells. Embryonic stem cells (ESC) are undifferentiated, pluripotent cells isolated from the inner cell mass of preimplantation blastocyst stage embryos. Under well-defined culture conditions, ES cells can be maintained indefinitely and the ability to give rise to cells of all three embryonic germ layers (ectodermal, mesodermal, and endodermal) [42, 43].

Although there is tremendous amount of research examining the potential use of ESC for other organs, the number of investigations for pulmonary regenerative medicine have remained scarce. Lung has a complex structure and lung progenitor cells are difficult to identify and differentiate in vitro, therefore the respiratory regenerative research has been slow comparing to other organs. However, there are few positive examples that showed successful engraftment and beneficial results. Wang et al [44] used embryonic stem cells to generate alveolar type II epithelial cells and transplanted those cells to the mice subjected to bleomycin induced acute lung injury. Cells were engrafted and the clinical results were promising showing increased survival and recovered oxygenization. They also demonstrated reversal of fibrosis in some areas of alveolar epithelium that did not harbor engrafted cells considering paracrine effects [42, 44].

Embryonic stem cells may lead the ethical and rejection problems. Hence, if embryonic stem cells are transplanted before differentiation, there could a possibility to develop teratomas in vivo. Therefore, differentiation should be exerted ex vivo prior to the transplantation. However, when prior differentiation occurs, they may lose their ability to develop complex lung tissue [16].

Induced pluripotent stem cells (IPS)

Recently, it was shown that ectopic expression of defined transcription factors can reprogram human somatic cells to a pluripotent state [45]. These cells are called induced pluripotent stem cells (IPS). Although the revolutionary cells can be created from several cells including dermal skin fibroblasts, neural stem cells and keratinocytes, the research is still in infancy and possibilities are searched to generate lung progenitor cells from them [40]. To develop autologous iPS cells, retroviral transfection of somatic cells with specific transcription factors is required. The cells are very alike to ESC that can maintain undifferentiated status and differentiate into any desired somatic cells. A recent report just showed that human fibroblasts can be reprogrammed as IPS. However, there is a number of obstacles that need to be cleared before IPS is introduced to specific human diseases. Retroviral transfection, potential threat of tumour formation and technical problems of differentiation are main concerns about that method. The final objective of that field would be generating safe, virus-free, and transgene-free autologous iPS cells [45, 46].

Bone marrow derived stem cells

There are many animal studies showing the usefulness of external sources of stem cells which are recruited mainly from bone marrow. A subpopulation of adherent bone marrow cells, when cultured in special condition can express markers of lung epithelial cells. Hence myeloid progenitor cell harvested from the bone marrow has been shown to restore lung structure in neonatal mice model. Bone marrow derived mesenchymal cells (MSC) are the other most studied cells of bone marrow. The other cell type studied extensively is the endothelial progenitor cells (EPC). Those are cells that can differentiate into endothelial cells in vitro. A bone marrow-derived circulating cells with fibroblast-like features termed fibrocyte was recently described and shown to home in lung and differentiate lung fibroblasts and myofibroblasts. Besides endogenous epithelial progenitor cells, there are bone marrow derived epithelial stem cells that are shown to incorporate into the airway epithelium that contribute tissue repair. Different levels of engraftment of bone marrow stem cells, including hematopoetic stem cells, MSCs, multipotent adult progenitor cells, and other populations, into alveolar and airway epithelial tissue, has been shown in several animal studies [47, 48]. In 2001, Krause et al [49] showed multipotency of bone marrow derived cells. They transplanted allogeneic bone marrow CD34 + lin neg cells into irradiated female mice. They were able to have varies engraftments including several bone marrow cells and other organs. That observation was expanded by others showing that bone marrow-derived cells can home injured lung, engraft and adopt the phenotype of one or many of the cells [49].

Mesenchymal stem cells

Multipotent mesenchymal stem cells (MSCs) are one of the most applied form of bone marrow derived stem cells. They were first described in 1976 by Friedenstein et al [42]. MSCs self-renewing multipotent, nonhematopoietic stem cells, could be isolated from main-

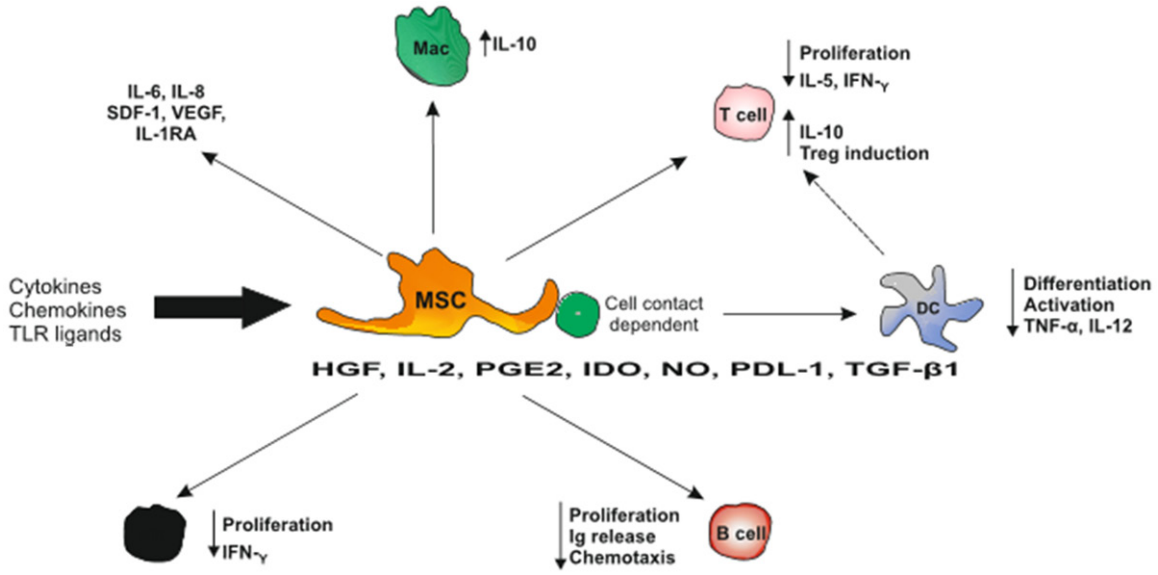

Figure 3. Schematic illustrating the range of in vitro immune-modulating effects described for mesenchymal stem cells (MSCs). DC = dendritic cell; HGF = hepatocyte growth factor; IDO = indoleamine 2,3-dioxygenase; IFN-γ = interferon γ; Ig = Immunoglobulin; IL = Interleukin; IL-1RA = Interleukin-1 receptor antagonist; Mac = Macrophage; NK = natural killer; PGE2 = prostaglandin E-2; SDF-1 = stem-cell derived factor 1; TNF-α = tumor necrosis factor-α; TGF-β1 = transforming growth factor-β1; TLR = Toll-like receptor; VEGF = vascular endothelial growth factor. Modified with permission from Viranuj Sueblinvong and Daniel J. Weiss. Stem Cells and Cell Therapy Approaches in Lung Biology and Diseases. Transl Res. 2010; 156(3): 188-205 [52].

ly from bone marrow but also other mesodermal sources such as, adipose tissue, liver, synovial membrane, teeth and tendons. Those cells have the capacity to differentiate into both mesenchymal and nonmesenchymal lineages. Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed minimal criteria to define human MSCs. MSCs have three important properties: 1) Plastic-adherence, 2) Expression of surface markers CD73, CD90, and CD105, lack of expression of hematopoietic markers CD14, CD34, CD45 or CD11b, CD79alpha or CD19, and human leukocyte antigen-DR (HLA-DR) therefore low immunogenity, 3) They have capacity to self-renew and differentiate into various lineages, including bone, adipose and cartilage in vitro [50, 51].

MSCs also have the ability to differentiate into alveolar cells, lung epithelial cells, vascular and endothelial cells. However, that ability depends on dosage, timing and route of their introduction and their role in lung regeneration in diseased lung is inconclusive [10]. **Figure 3** summarized in vitro immune-modulating effects of mesenchymal stem cells.

Origin of mesenchymal stem cells

The sources of MSCs are included bone marrow (BMMSC), umbilical cord tissue (Wharton's Jelly derived-WJMSC), adipose tissue (ATMSC) umbilical cord blood (UCMSCS), and menstrual blood (MBMSC). The BMMSC product Prochymal R (Osiris R Technologies) has been approved in Canada for treatment of acute Graft Versus Host Disease (GVHD) refractory to corticosteroids in pediatric patients. The most investigated source has been the BMMSC. Human MSCs can be obtained directly from patient, thus the subsequent use of these cells in the clinical setting would have the advantage of being autologous transplantation providing no rejection [53].

WJMSCs are very promising cells because the source is quite available and collection of the cells is noninvasive. WJMSCs have compatible surface markers and cellular characteristics with BMMSCs and ATMSCs. They have better proliferation capacity and there has been no tumorigenicity reported up to date. WJMSCs can show adipogenic, osteogenic and chondrogenic differentiation under certain cultural conditions [50-53].

Homing and mechanism of action

MSCs have potential beneficial effects in some respiratory models such as bleomycin-induced lung injury, abdominal sepsis and ventilator induced lung injury. When injected intravenously, MSCs can accumulate in the injured regions. The damaged lung released some cytokines to attract circulating or bone marrow derived MSCs and promote lung regeneration. However, in most of the cases, MSC infusion provides a very low engraftment rate. Therefore, the way to explain those effects are called as paracrine effects. MSCs can release several mediators in response to injury, downregulate proinflammatory mediators and upregulate the antiinflammatory ones. Despite their low capacity for homing into the lung and differentiate into lung cells, they demonstrate beneficial effects with paracrine actions. Hence, those actions could stimulate the expansion, homing and differentiation of endogenous stem cells on one hand and the differentiation of MSCs toward alveolar epithelial cells, endothelial cells, fibroblasts and bronchial epithelial cells on the other [54-56].

Homing and regeneration

MSCs specifically localize to the injured lung tissue by virtue of the chemokine receptor type 4 (CXCR4). Homing molecule expression increases in the presence of inflammatory cytokines. In mouse experiments, MSCs showed better engraftment when the lungs were challenged with bleomycin. Bleomycin led to induction of hyaluran and osteopontin which augment the engraftment of MSCs via their CD44 receptor. Animal models showed that local HGF, KGF, GCSF induce proliferation and motility of BM derived progenitor cells in the lung. It was also shown that systemically infused lung epithelial cells derived from human umbilical cord blood MSCs were able to express Clara cell secretory protein, human beta 2 microglobulin and cystic fibrosis transmembrane conductance regulator (CFTR) protein, and also differentiated into alveolar epithelial cells in mice lung [56, 57].

Mechanism of action

BMMSCs have been shown to down regulate proinflammatory cytokines like tumor necrosis factor (TNF)-alpha, interleukine (IL)-1, IL-1 beta, monocyte chemoattractant protein (MCP)-1, IL-6, matrix metalloproteinase (MMP) 9, matrix metalloproteinase (MMP)-12 and upregulation of vascular endothelial growth factor (VEGF), VEGF2 receptor, transforming growth factor beta 1 (TGF B1), hepatocyte growth factor (HGF), epithelial growth factor (EGF), secretory leukocyte protease inhibitor (SLPI) and reduction of pulmonary cell apoptosis [58, 59].

MSCs also have immune effects. MSCs promote generation of Treg cells and inhibit Th2 cells (**Figure 3**). MSCs also upregulate antiinflammatory factors IL-1 receptor antagonist (IL1RN) and TNF-alpha induced protein 6 (TSG-6) and IL-10. Human mesenchymal stem cells (hMSCs) suppress T-cell and dendritic-cell function and represent a promising strategy for cell therapy of autoimmune diseases [60, 61].

MSCs have some antibacterial effects and regulation on wound healing. It reduced total bacterial counts in blood. MSCs inhibited bacterial growth by secretion of antimicrobial peptide LL-37. LL-37 activates airway epithelial cells and IL-8 secretion and regulates epithelial cell proliferation and wound healing. In an LPS-induced model, Toll-like receptor 4 on the surface of macrophages binds with LPS. That activates binding of macrophage-derived TNF alpha with TNF receptor 1 on the surface of BMMSCs. These actions initiate an activation of myeloid differentiation factor-88, nuclear factor kappa B and upregulation of cyclooxygenase 2 (Cox-2) which causes increased synthesis of Prostaglandin E2 from BMMSCs. Then BMMSCs bind to E2 and E4 receptors on the surface of macrophages through a cell to cell contact dependent pathway and reprogram the macrophages to release lower level of TNF-alpha and IL-6 while producing increased levels of IL-10. IL 10 inhibits the neutrophil-mediated tissue damage by downregulating the production of MPO [62].

There is also evidence that MSCs have the ability to reverse pulmonary hypertension. Intratracheal instillation of rat BMMSCs reduced the pulmonary vascular resistance and right ventricular hypertrophy via releasing factors that improve endothelial function or stimulate vascular growth in the monocrotaline-injured lung [63].

MSCs have properties protection against fibrosis and autoimmune conditions. MSCs prevent

fibrosis by reducing neutrophil influx, inflammation and collagen deposition. MSCs downregulate profibrotic cytokines IL-1 beta, TGF beta, VEGF, IL-6, TNF alpha, MIP, IFN gamma and, NOS [64].

MSCs have shown several other remarkable effects. MSCs have shown their ability to control oxidative damage via restoration of heme oxygenase-1 (Hmox-1), glutathione-S-transferase (GST), and nuclear factor-erythroid 2 p45 subunit related factor 20 (Nfr2) in a carbon tetrachloride induced liver injury. In addition to the pathological conditions discussed above it is important to elucidate the potential of MSC therapy against aging and smoking [65].

In summary, MSCs perform action via several ways besides limited engraftment, direct cell to cell connections, release soluble factors and other cellular components such as microvesicles or exosomes. Those effects are probably orchestrated by paracrine MSC-derived mediators [66].

Mesenchymal cells in COPD

Animal models

Several reports have also demonstrated that MSCs have potential therapeutic effects in animal emphysema models. Shigemura et al have showed in a panacinar emphysema rat model that adipose tissue derived MSCs given iv route reduced alveolar cell apoptosis, enhanced epithelial cell proliferation and promote angiogenesis leading to restoration of pulmonary function affected by emphysema. In several other animal emphysema models, intratracheal administration of bone marrow derived MSCs reduced proinflammatory cytokines, increased level of epithelial growth factors, and reduced emphysema severity [67, 68]. In a rat model of smoke-induced lung injury, MSCs improved lung function and protected lungs against cigarette smoke-induced lung damage reducing lung cell apoptosis. In that model MSCs reduced proinflammatory cytokines TNF alpha, IL-1, IL-6, MMP-9, MMP-12, and MCP-1 and upregulated VEGF receptors and TGF 1 [59]. Cho et al [65] mcompared with spheroid human adipose-derived MSC and dissociated MSC and point out that mice injected with spheroid MSC showed improved regeneration of lung tissues, increased expression of growth factors and a reduction in proteases with an induction of protease inhibitors when compared with mice injected with dissociated MSCs. In a recent work, our team has tested the therapeutic potential of stem cells in BMCs, BM derived MSCs and MSC-cell free conditioning medium (MSC-CM)] to repair cigarette smoke-induced emphysema. Inbred female Lewis rats were exposed to cigarette smoke to induced emphysema for six months then received BMCs, MSCs, or MSC-CM. After two months of injection, the BMCs transplantation significantly increased cell proliferation and the small pulmonary vessels and, diminished the cigarette smoke-induced emphysema. Surprisingly, at the 30th day of treatment, the transplanted cells were not detected in the hosts, however; cell free MSC-CM media also showed to induce the differentiation. MSC-CM could be a solution against the threat of MSC transforming tumour associated fibroblasts [27].

Human studies

There are few human studies in the literature. After obtaining encouraging effects on safety and efficacy of MSC in myocardial infarction, pulmonologists focused on MSC treatment in human lung disorders. In a phase 1 clinical trial taken in GOLD 3-4 COPD patients, allogeneic bone marrow-derived MSC transplantation was found to be safe for 90 days follow-up [69]. In 2013, a phase II randomized controlled trial of MSC effect on moderate to severe human COPD has published. Autologous BM derived MSC or placebo has given to 62 patients monthly for 4 months. The patients have been followed with 2 years. There were no toxic effects of infusion. Deaths did occur but were not attributed to the study drugs. With an extensive evaluation of inflammatory markers, exercise capacity, functional assessments with spirometry, quality of life measures, COPD exacerbation rates and CRP, the study failed to show any significant improvement from baseline values. The ineffective dose of MSCs, inappropriate time window of injections, inappropriate route of administration and, relatively severe patient population would be the reasons of that failure. It is very important to note that most of the chronic models coming from animal studies were based on acute expose or injury models and should be taken into account when interpreting COPD studies. Hence there is a need for

Table 2. Selected animal COPD models and human studies

Lung disease	MSCs source	Type of model	Outcome/mechanism	References
Animal models of COPD	BMC-Lewis rat	Cigarette smoke	↑Cell proliferation, pulmonary vascularity, repair of emphysema ↓Apoptosis	[25]
	ADSCs (Mouse and human) (Intravenous)	Cigarette smoke	Reduction in collagen accumulation, fibrosis score, metal-loproteinase levels, weighloss, BM suppression/Nonspecific Potentially soluble mediators	[29]
	Mouse Rat BM-MSCs Plastic adherent (Intratracheal)	Mouse elastase	Reduction in collagen deposition/↓TNF-α, IL-1β, MCP-1, and IL-6, ↓MMP9 and MMP12, ↑VEGF and TGF-β	[58]
	Rat ADSCs Plastic adherent (Intravenous)	Rat elastase	Improved gas exchange and exercise tolerance/Decreased apoptosis, improved histologic repair, hepatocyte growth factor secretion	[72]
	Rat BM-MSCs (Intravenous)	Rat papain	Decreased histologic injury and AEC apoptosis/↑Bcl-2 and BAx Differentiation into type II alveolar epithelial cells	[72]
	Rat BM-MSCs (Intratracheal)	Rat papain	Decreased histologic injury, Restoration of VEGF lung expression/↑VEGF-A and ↓ apoptosis	[72]
COPD	BM-MSCs (Intratracheal)		Marginal improvement in FEV_1	[72]
	BM-MSCs (Intratracheal)		No improvement in FEV_1	[72]

COPD; chronic obstructive pulmonary disease, MSCs; mesenchymal stem cells, ADSCs; adipose-derived stem cells, BM-MSCs; bone marrow-mesenchymal stem cells, AEC; alveolar epithelial cell, VEGF; vascular endothelial growth factor, BM; bone marrow, FEV_1; forced expiratory volüme in 1 s.

to set realistic outcomes and markers (such as molecular markers) to follow potential benefits in human models [70, 71]. **Table 2** summarizes some of the human and animal studies on stem cells in the COPD.

Adverse events and cautious

As mentioned before, there could be several adverse effects of iv MSC administration such as development of osteosarcoma and worsening of fibrosis due to myofibroblast differentiation. The neoplasia effect has been demonstrated with murine MSC but not human MSC. A recent systematic review indicated that MSC administration is relatively safe in the treatment of human with left sided heart failure, myocardial infarction (MI), stroke, spinal cord injury, hematologic malignancy, graft-vs-host disease, or Crohn's disease. The phase II clinical trial in COPD patients showed that iv MSCs (100 million cells in total) is safe for the 2-year follow up periods [73].

The application of MSC in clinical settings could be complicated with several factors [64]. Bone marrow MSCs would display a limited proliferative effect and a low quality when derived from aged or diseased donor. During ex vivo expansion, those cells could lose their differentiation potential. More importantly in a bleomycin-induced fibrosis model, resident mesencyhmal stem cells are replaced with administration of isolated lung MSCs and that treatment attenuated the bleomycin-associated pathology and mitigated the development of PAH [72].

However, when those cells engrafted in the distorted, dysplastic microenvironment which developed in several years in COPD, we should bear in mind that those cells can develop fibrotic phenotype or tumorigenic phenotype. Furthermore, activating endogenous regenerative capacity in detrimental environment could also lead cells from senescense to cell inmortality [71].

Conclusions

Until now there has not been described a curative therapy for COPD with current medications. Stem cell based therapies that have been successfully applied to other diseases are a new approach for COPD and degenerative lung diseases treatment. Stem cells may deliver some signals to host cells, inducing a regenerative mechanism against alveolar destruction in the COPD lung. MSCs are now known to have potent beneficial effects in animal models of many types of lung injury including cigarette smoke-induced or elastase-induced COPD/emphysema, bleomycine-induced fibrosis, bronchopulmonary dysplasia, ventilator-induced lung injury, and bacterial pneumonia. Mesenchymal stem cells (MSCs) effectively reduce airway inflammation and regenerate the alveolus in cigarette- and elastase-induced chronic obstructive pulmonary disease (COPD). Stem cells also effects the regenerative genes [74, 75].

COPD still is a threat worldwide and there is a need to develop more effective therapeutic

strategies. Stem cell based therapies may be the key for the future.

Disclosure of conflict of interest

None.

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