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Abstract

Chronic obstructive pulmonary disease (COPD) is characterized by irreversible loss of lung function that stem from two mechanisms, inflammation and senescence. Crosstalk between these two mechanisms accelerate the development of COPD, thus targeting these two pathways may offer benefits in the treatment of COPD. Growing amount of evidence have shown that mesenchymal stem cells as a promising candidate for the treatment of COPD. Over the years, many studies conducted to decipher the therapeutic effect of MSC in COPD and the mechanisms involve, in the hope of utilizing these cells as new therapeutic strategy for COPD. However, the cell-based therapy by using the MSC presented with many obstacles including low engraftment at the site of injury, the risk of microvascular occlusion, unwanted differentiation, and also the risk of malignant transformation. Recently, recently researchers begin to look at the possibility of using MSC derived extracellular vesicles as an alternative to MSC. Here we review the effect of MSC and MSC derived EV in modulating inflammation, and senescence in COPD. We also review current treatment and the side effect in COPD, and senolytic drugs, a new therapeutic strategy targeting the senescent cells.

Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) has a tremendous economic impact on healthcare and is associated with high morbidity, and high mortality rate. Global Initiative for Chronic Obstructive Lung Disease (GOLD) in 2017 reported that more than 3 million people died in 2012 accounting for 6% of total death globally. It is estimated that the prevalence and the burden of COPD to increase in the coming decade and by 2020, COPD will become the third leading cause of death worldwide. Symptoms of COPD include chronic cough, dyspnea and excessive production of sputum, while anorexia, fatigue and weight loss may present in patient with severe COPD. The diagnosis of post-bronchodilator forced expiratory volume in one second (FEV_1)/ forced vital capacity (FVC) ≤0.7 confirms the presence of airflow limitation. The body mass index (BMI) are also useful in predicting outcomes such as survival with the values <21 $kg \cdot m^{-2}$ are associated with increased mortality (Celli et al., 2004). Cigarette smoke is the main risk factor of COPD which accounts for 80-90% of all cases. However, environmental pollution, noxious gases, genetic predisposition, pulmonary infections, and aging can also increase the risk of COPD (Churg et el.,2008).

The hallmark of COPD is chronic inflammation in the lung that leads to chronic bronchitis and emphysema. Chronic bronchitis is linked to the chronic inflammatory cells infiltration in small bronchi, leading to abnormal remodelling and mucus overproduction. Meanwhile emphysema is associated with irreversible alveolar structures that contributes to airspace enlargement without significant pulmonary fibrosis. These pathological changes lead to progressive airflow limitation however, the severity varies between patients (Grove et al., 2009). Apart from inflammation, senescence also plays an important role in the pathogenesis of COPD. Aging individuals are shown to be at higher risk of developing COPD as demonstrated by Hardie et al., (2002) in the study with 35% of healthy, never smoker, asymptomatic aged 70 years and older suffer with at least stage 1 COPD. Increase burden of COPD in elderly might be attributed to 1) Age-related changes in lung structure and function may increase the pathogenetic susceptibility to COPD and 2) Exposure to external insults render the elderly population to become vulnerable to lung injury (Fukuchi et al., 2009). Furthermore, cigarette smoke also contributed to premature lung aging by increasing the oxidative stress and DNA damage thereby inducing senescence (Hara et al., 2013). Senescence cells produced various pro inflammatory cytokines that accelerate inflammation and thus contribute to the chronic condition of COPD (Tsuji et al., 2010).

Inflammation in COPD

Inhalation of cigarette smoke or noxious gases incite the innate and adaptive immune system which in long term will cause destruction to the respiratory system. Cigarette smoke accelerates the production of reactive oxygen species (ROS) and increased the oxidative stress. This will induce cellular dysfunction or cell death and disrupts the proteinase-antiproteinase imbalance by activating proteases and inactivating antiproteinases (D'Agostino et al., 2010). Neutrophils, macrophages, and $CD8^+$ T cells are prominent player in COPD exacerbation, however, $CD4^+$ T cells, regulatory T (T_{reg}) cells, and dendritic cells (DC) may also contribute to the COPD pathogenesis. Acute inflammation takes place at the initial smoke exposure and peaked at the end of first week and chronic inflammation begin after 2 weeks of exposure. Neutrophils influx, thickening of epithelial wall and goblet hyperplasia were also observed during acute inflammation (Stevenson et al. 2007). Exposure to cigarette smoke induced epithelial cells to produce inflammatory mediators including interleukin (IL)-8, GM-CSF, IL-6, IL-1β (Mortaz et al., 2011). Interleukin 8 is a potent chemoattractant to neutrophils which can induce secretion of myeloperoxidase by neutrophils that can attract more inflammatory cells to sustain the inflammation (Quint et al., 2007). Subsequently, tissue macrophages begin to increase, triggering chronic inflammation. Collagen deposition, lymphocytes infiltration and macrophage aggregation were reported to be elevated (Stevenson et al. 2007). Macrophages release IL-8, IL-6, TNF-α, monocyte chemotactic peptide-1 (MCP-1), matrix metalloprotease (MMP)-9, MMP-12, and ROS that leads to the destruction of lung parenchyma (Angelis et al., 2014). Meanwhile, type 1 cytotoxic T (Tc^1) cells that predominate lung parenchyma, release granzyme B and perforins that may induce apoptosis to alveolar epithelial cells and thus contribute to emphysema (Majo et al., 2001). In addition, cigarette smoke exposure increased the accumulation, activation and maturation of DC in lung and in turn, DC communicate with T helper (T_h) 1 cells, T_h2 cells, and T_{reg} cells to release numerous cytokines including IL-4, TNF-α, IFN-γ, increase mucus secretion, and development of tolerance to infection (Tsoumakidou et al., 2008).

There is evidence of persistent inflammation after smoking cessation, although the mechanism has not yet been established (Lapperre et al., 2006). The number of plasma cells, and $CD3^+$ and $CD4^+$ lymphocytes are higher in the ex-smokers with COPD as compared to current smokers with COPD. Additionally, the number of macrophages, neutrophils, mast cells, eosinophils and $CD8^+$ lymphocytes are also similar to the current smokers. Furthermore, elevation of GM-CSF, CSF-1, IL-17A, serum amyloid A after smoking cessation, have been implicated in sustained macrophages proliferation and neutrophil recruitment (Hansen et al., 2014). Persistent apoptosis in airway epithelial cells and T cells were also seen in smoking cessation group as compare to control group. Apoptosis of T cells

may result in reduced immune response to external insults leading to increased risk of infection in COPD (Zuo et al., 2014). Evidence have shown that, reduced number of T_{reg} cells has been implicated in autoimmunity. Regulatory T cells is the key player in regulation of inflammation in autoimmune disorders. Suppression of immune function occur via modulation of maturation and function of antigen presenting cells (APC), metabolic pathway disruption, killing of target cells, and anti-inflammatory cytokine secretion (Grant et al., 2015). In emphysematous lung of COPD patients, reduced level of FOXP3 mRNA, a transcription factor for T_{reg} cells development, reduced number of T_{reg} cells, as well as reduced IL-10 were observed, indicating autoimmune reaction in lung (Lee et al., 2007).

Senescence in COPD

The concept of cellular senescence arose from the research by Leonard Hayflick and Paul Moorhead that demonstrated normal human fibroblast cells in *in vitro* culture have a limited lifespan (Hayflick, 1965). When cells ceased to proliferate, it enters the state of irreversible growth arrest. Morphologically, senescent cells size is bigger than normal cells and adherent cells often exhibit a flattened shape. Senescence cells resist apoptosis and remain metabolically active, as well as excreting senescence associated secretory phenotype (SASP) (Kirkland et al., 2017). Growth arrest of senescent cells happen at G_0/G_1 phase of cell cycle. Senescence cells can be identified by a histochemical staining; Senescence-associated β-galactosidase (SA-βgal). Mammalian cells regardless age express lysosomal β-galactosidase when stained with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) at pH 4. However senescence cells stained positive by X-gal at pH 6 caused by an increased lysosomal β-gal activity but is not demonstrated in actively proliferating cells and quiescent cells (Lee et al., 2006).

The number of senescent cells increase with age, however, senescence can also be induced *in vivo* and *in vitro* by using numerous stimulants including beryllium, bromodeoxyuridine, hydrogen peroxide, and cigarette smoke (Ross et el., 2008, Sun et al., 2015, Coates et al., 2007, Ahmad et al., 2015). These stimulants may induce reactive oxygen species (ROS), telomere attrition, DNA damage response (DDR), oncogenic-induced senescence, CDKN2A derepression, and senescence-associated secretory phenotypes (SASP) which will trigger the activation of p53/p21, and $p16^{INK4A}$/pRb signalling pathways that lead to senescence. Activation of these pathways inhibit the expression of cyclin-dependent kinases (CDKs) including CDK1, CDK2, CDK4, CDK6, Cyclin A, Cyclin E, and Cyclin D, which in turn

suppress the phosphorylation and preventing the activation of retinoblastoma protein (Figure 1) (Campisi et al., 2007, Lujambio et al., 2016).

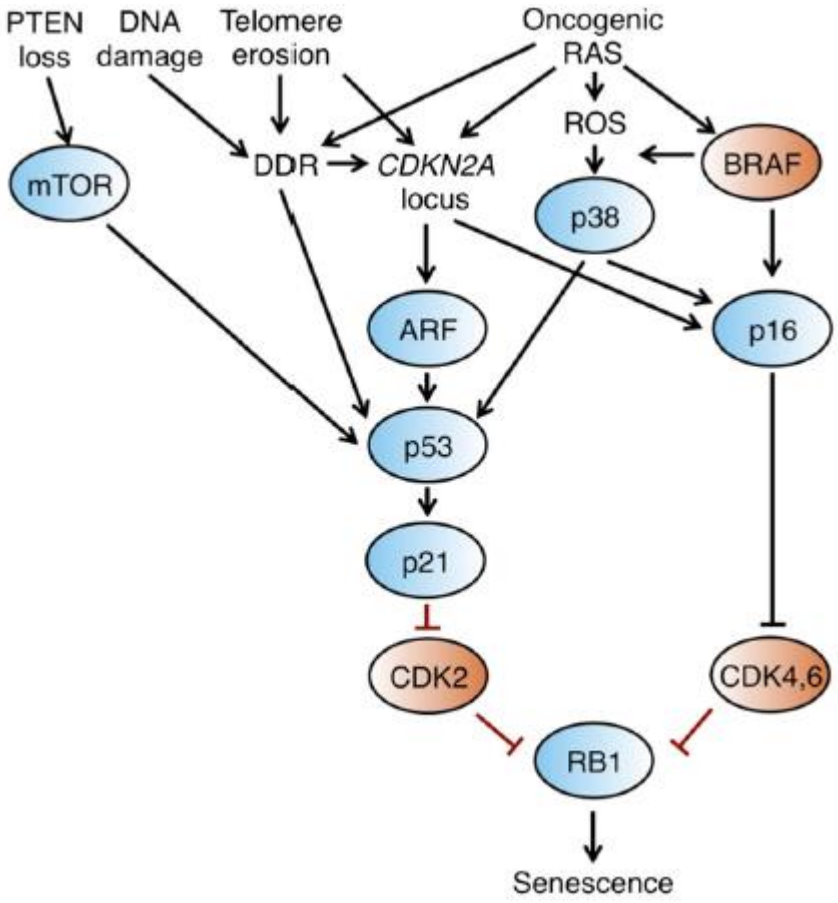

Figure 1: **p53/p21 and p16/pRb signalling pathways**. Multiple stress signal can trigger the activation of p53 and p16, in which both pathway may act downstream to inhibit CDKs and retinoblastoma protein (Lujambio et al., 2016).

This sophisticated mechanism is natural cell response to damaging stimuli that may cause mutation resulting in tumour formation. Senescence plays a role in tumour suppression in the early stage of tumour formation by preventing the proliferation of damaged cells. Activation of p53/p21 through DDR, arrests the human colon adenocarcinoma cells at G_1 phase of cell cycle (Waldman et al., 1995). Meanwhile, the induction of oncogenic H-Ras (V12) in primary human and rodent cells leads to G_1 cell cycle arrest, increased expression of p53 and p16 which result in premature senescence (Serrano et al., 1997). Inactivation of p53 however, bypasses the onset of cellular senescence and leads to oncogenic transformation and restoring p53 function regress the tumour without affecting normal tissues (Ventura et al., 2007). Conversely, the failure of eliminating senescent cells can be damaging. The accumulation of senescent cells over time will cause damage to the tissue and result in age-

related diseases and tumour formation. Senescent fibroblasts have been shown to stimulate the proliferation and progression of preneoplastic and neoplastic cells (Krtolica et al., 2001). This in part appear to be mediated by SASP released by senescence cells which contains numerous soluble factors including proinflammatory cytokines, chemokines, growth factors and matrix degrading proteins (Coppe et al., 2008). SASP has been shown promotes epithelial-mesenchymal transition (EMT) and stimulate the invasion of tumour cells by the action of two secreted cytokine; interleukin (IL) -6 and IL-8 (Coppe et al., 2008).

Senescence play a significant role in the pathogenesis of COPD by altering the cellular functions and contribute to the chronic inflammation. In aging lung, alveolar structures are altered, reduced respiratory muscle strength, modification of extracellular matrix composition, vascular remodelling, reduced lung function, and impaired capacity of gas exchange. This in part, might explain why aging population has higher risk in developing COPD (Karrasch et al., 2008). In addition, long term exposure to cigarette smoke will accelerate telomere shortening in type II alveolar epithelial cells (AEC type II) that will cause senescence in type II alveolar epithelial cells. As a result, senescence AEC type II may contribute to insufficient regeneration of alveolar cells which in turn leads to emphysema (Tsuji et al., 2006). Cigarette smoke disrupt many proteins and enzymes level which may accelerate cellular senescence in lung. Brain type-creatine kinase (CKB) that is involve in energy homeostasis of cells, has been shown to be associated with acceleration of bronchial epithelial cells senescence. Depletion of CKB induced p21 expression, G2/M cell cycle arrest, accumulation of protein involves in mitosis, Cyclin B1, that cause cells to become senescent as well as increased level of IL-8 (Hara et al., 2012). In addition, exposure of lung fibroblast and small airway epithelial cells (SAEC) to cigarette smoke extract also lead to interaction of p53 and E3 ubiquitin ligase (Parkin). This interaction will reduce Parkin translocation to damaged mitochondria, hence impairing damaged mitochondria clearance, thus accumulation of senescent cells (Ahmad et al., 2015). In mice model exposed to cigarette smoke for 6 months, significant decreased of sirtuin 1 (SIRT1) was observed. Sirtuin 1 is NAD^+-dependent protein/histone deacetylase that involves in wide range of processes including aging and inflammation (Rajendrasozhan et al., 2008). Sirtuin 1 has been shown to interact with FOXO3 and p21 in mitigating senescence in emphysematous lung. However, the absence of FOXO3 and p21 deficiency, SIRT1 failed to exert its effect, thus accelerating senescence and emphysema (Yao et al., 2012).

Interaction between inflammation and senescence

Chronic inflammation has been implicated in senescence and accelerate ageing. Many age-related diseases such as Alzheimer's disease, diabetes type-2, atherosclerosis, and Parkinson's disease presented with chronic, low-grade inflammation as an important mechanism responsible for the disease progression (Ikeno et al., 2011). Various pro-inflammatory mediators such as IL-6, TNF-α, CXCR-2, IFN-γ, and TGF-β collectively aggravate the inflammation and senescence. NF-κβ as the master regulator of many gene, cytokines, adhesion molecules, enzymes, many of which related to inflammation as well as apoptosis (Serasanambati et al., 2016), may also play a role in inducing senescence. In addition, chronic inflammation also exhibited increased expression of p21 and p16, and downregulation of anti-inflammatory cytokines, IL-4 and IL-10 (Jurk et al., 2014). Prolonged treatment of IFN-γ in HUVEC enhanced oxidative stress, and arrested cells in G0/G1 phase of cell cycle thus inducing cellular senescence through p53/p21 pathway, and not p16 (Kim et al., 2009). Meanwhile, tumor necrosis factor-α also increase number of senescent endothelial cells, induced p21 expression, and endothelial dysfunction (Yamagata et al.,2016). However, TNF and IFN-γ alone unable to induced senescence in murine breast cancer. Combination of TNF and IFN-γ are needed in order to successfully halt the cancer progression by arresting cell cycle at G_0/G_1 phase, activating $p16^{INK4a}$/Rb pathway, thus inducing senescence (Braumuller et a.,2013).

Senescence has also been shown to contribute in inflammation through the production of SASP. mRNA level of SASP components such as IL-6, IL-8, MCP-1, IL-1β, MMP-3, MMP-12, and TNF-α increased significantly in senescent cells (Xu et al., 2015). In the lung of aging mice, cDNA array analysis demonstrated that 8 genes including CD20, CXCR-3, CD72, IL-8RB, and C-Fgr that are related to inflammation were upregulated. Increased number of CD8 cells, CD4 cells, macrophages, and B cells were also been observed (Aoshiba et al., 2007). Cigarette smoke induced cellular senescence in type II alveolar epithelial cells by increasing the expression of $P16^{INK4a}$ and phosphorylated NF-κB, along with pro-inflammatory cytokines IL-6, IL-8, and TNF-α (Tsuji et al., 2010). Senescence pulmonary vascular endothelial cells exhibited increase p16 and p21 mRNA expression, shorter telomeres, reduced telomerase activity, and also excreted increase level of IL-6, IL-8, MCP-1, Hu-GRO, and soluble ICAM-1. Enhanced secretion of soluble ICAM-1 and MCP-1 led to increase monocyte adherence and migration which in turn will aggravate inflammation (Amsellem et al., 2011).

Current treatment for COPD

The main objectives of COPD management are focusing on reducing symptoms and exacerbation, preserving lung function decline, reducing mortality while increasing exercise capacity and improving health status. Smoking cessation remains to be an effective intervention for COPD as it results in improved FEV_1, reduction in hospital admission, lower prevalence of cough, sputum production, wheezing and shortness of breath (Gotfredsen et al., 2002, Kanner et al., 1999). A wide range of pharmacotherapeutic drugs are also available for the treatment of COPD, such as bronchodilators, muscarinic antagonist, and corticosteroids, however, the administration of these drugs is often associated with adverse effect.

Bronchodilators is a type of drugs that promotes airway smooth muscle relaxation and improved lung emptying during tidal breathing. Common bronchodilators include anticholinergic drugs, $beta_2$ agonists and methylxanthine which can be administered orally, inhalation or by injection. Short-acting $beta_2$ agonists such as salbutamol and fenoterol provides a quick relief from symptoms. On the other hand, long-acting $beta_2$ agonists such as salmeterol and formoterol help to control persistent symptoms but do not provide quick relief (Ejiofor et al., 2013). Theophylline, a type of methylxanthine is a long-acting bronchodilator used to treat airway disease. However, to achieve significant bronchodilatation as $beta_2$ agonist, relatively high plasma concentrations are needed (10–20 mg/l) (Barnes, 2006).

Muscarinic antagonist exerts its action by blocking the bronchoconstrictor effects of acetylcholine on M3 muscarinic receptors. Ipratropium bromide, the most common short-acting muscarinic agonist (SAMA) exerts its action within minutes and last for approximately 4 hours. (Ejiofor et al., 2013). According to the Lung Health Study, regular ipratropium use has no effect on the rate of decline of lung function over time in mild to moderate COPD. Meanwhile, long-acting muscarinic antagonists like tiotropium and aclidinium bromide have a longer duration of action of over 24 hours due to prolong binding to the M3 muscarinic receptor and faster dissociation from M2 muscarinic receptor. On the other hand, inhaled corticosteroids (ICS) are prescribed in high doses for COPD, however beneficial effect in patient is controversial as it does not effective in slowing the reduction in lung function. Combination of ICS and $beta_2$ agonist is effective in improving lung function and reducing exacerbation in patient with moderate to severe COPD as compared to single component (Nannini et al., 2007). In a double-blind cross over study, Culpitt and colleagues (1998) examined the effect of 500µg fluticasone propionate which given twice a day for 4 weeks. There are no changes in sputum supernatant elastase activity, anti-proteases secretory

leukoprotease inhibitor (SLPI), matrix metalloproteinase (MMP)-1, MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-1, suggesting that ICS has no benefit in reducing inflammation.

Side effect of COPD drugs

Generally, bronchodilators are well tolerated. However, several studies reported multiple adverse effect on patient prescribed with bronchodilators. Chest pain, headache, and syncope were experienced by patient prescribed with formoterol (Thomson et al., 1998) and cardiac rhythmic disturbance was reported in patient prescribed with terbutaline (Lipworth et al., 1990). Long acting beta$_2$-agonist may cause higher heart rate, and supraventricular or ventricular premature in COPD patient with pre-existing arrhythmia and hypoxemia (Cazzola et al., 1998). Administration of theophylline is associated with headache, abdominal discomfort, nausea and vomiting, and restlessness, increased acid secretion, gastroesophageal reflux, and diuresis. Convulsions, cardiac arrhythmias, and death may occur at high concentration. These side effects are due to the increase of plasma concentration (Barnes, 2005).

Dry mouth is the most common side effect of muscarinic agonist. A 4-year trial of tiotropium in COPD patient reported adverse effect including pneumonia, dyspnea as well as dry mouth and constipation (Tashkin et al., 2008). A randomized controlled trial on ipratropium and tiotropium suggested an increased risk of myocardial infarction, cardiovascular death, and stroke (Singh et al., 2008), while another study reported no significant effect of triotropium on cardiovascular mortality, myocardial infarction, and respiratory mortality, although dyspnea, dry mouth and upper respiratory tract infection were observed (Kesten et al., 2006). A comparison study between indacaterol and triotropium showed that both drugs produced similar side effects such as cough, nasopharyngitis, COPD exacerbation, headache, influenza, and bronchitis (Dunn et al., 2010). A 24-weeks, randomized, placebo-controlled study demonstrated that aclidinium bromide is associated with multiple adverse effect including cough, headache, nasopharyngitis, urinary tract infection, diarrhea, muscle spasm, dyspnea, and nausea (D D'urzo et al., 2014). Evidence suggested that there is increase of fractures in COPD patient when treated with ICS (Loke et al., 2011). The use of ICS is also associated with increased risk of pneumonia; however, the risk declines gradually after stopping the ICS use (Suissa et al., 2013). A study using fluticasone furoate depicted increase risk of pneumonia, fractures, and nasopharyngitis in fluticasone furoate only group,

and fluticasone furoate/vilanterol group (Dransfield et al., 2013). Skin bruising, oropharyngeal candidiasis and throat irritation were also prominent in COPD patient prescribed with high dose of ICS (Pauwels et al., 1999).

Senolytic drugs as a new therapy in eliminating senescent cells

Recently, research begins to look at the possibility of developing new pharmacological strategies that are able to selectively eliminate senescent cells without harming healthy quiescent and proliferating cells. This strategy is thought to be beneficial as senescent cells have been implicated in the development of chronic diseases and tumour formation. Since senescent cells resist apoptosis, a study was conducted to analyse pro-survival markers in senescent cells including ephrin ligands (EFNB1 and EFNB3), PI3KCD, p21, Bcl-2, PAI-1, and PAI-2. Administration of drugs or silencing these markers, killed senescent cells without harming proliferating cells and quiescent cells (Zhu et al., 2015). Since then, more studies have been done to identify drugs that can potentially be used in killing senescent cells including dasatinib, quercetin, navitoclax, and 17-DMAG.

Zhu and colleagues (2015) found out that dasatinib and quercetin were able to reduce senescent cells number. Dasatinib and quercetin were tested in experimental lung fibrosis model, in which combination of dasatinib and quercetin reduced senescent cells number, decreased level of p16, reduced expression of SASP components such as IL-6, MMP12, SPP1, serpine 1, collagen 1a1, collagen 5a3, fibronectin, increased level of caspase 3, and increased epithelial cell marker E-cadherin, AECII markers SFTPC and SFTPA (Lehmann et al., 2017). Fuhrmann-Stroissnigg and colleagues (2017) recently identified HSP90 inhibitor, 17-DMAG as a potential new senolytic drug in which the treatment of senescent MEF with 17-DMAG selectively induced apoptosis in these cells, as well as murine MSCs, IMR90, and human lung fibroblast cells (WI38). 17-DMAG also reduced the expression of $p16^{INK4a}$, SASP component IL-6, DNA damage marker γH2AX, and reduced p-AKT level. Administration of 17-DMAG in progeroid mouse model, decreased age-related symptoms including, tremor, ataxia, gait disorder, dystonia, and kyphosis (Fuhrmann-Stroissnigg et al., 2017). Navitoclax and TW-37, member of Bcl-2 family inhibitor have been tested in *in vitro* setting by using human umbilical vein epithelial cells (HUVEC), human lung fibroblast (IMR90), murine embryonic fibroblast (MEF) and preadipocytes. Navitoclax induced apoptosis in HUVEC, IMR90, MEF, but not preadipocytes. TW-37 however, has very little senolytic effect over these cells, which may be due to different in level of targets between navitoclax and TW-37 (Zhu et al., 2016). In addition, dasatinib is more effective in eliminating senescent cells in preadipocyte and less effective in senescent HUVEC. Meanwhile quercetin kills senescent

HUVEC but not effective against senescent preadipocyte (Zhu et al., 2015). A more recent study demonstrated that, BCL-XL inhibitors, A1331852 and A1155463 induced apoptosis in senescent HUVEC and IMR90, but not senescent preadipocyte (Zhu et al., 2017). In addition, another HSP90 inhibitor, ganetespib reduced the viability of senescent HUVEC, but not preadipocyte (Fuhrmann-Stroissnigg et al., 2017). This indicates that senolytic drugs is cell specific, targeting different proteins and pathways. However, many of these drugs have been used in treating other diseases such as leukemia, lung cancer, and ovarian cancer, which can cause side effects (Aguilera et al., 2009, Niu et al., 2009, Rudin et al., 2012). It is not clear however, if these drugs can be used in the treatment of COPD.

Mesenchymal Stem Cells

Mesenchymal stem cells are multipotent adult stem cells with fibroblast-like morphology that can be found mainly in the bone marrow. International Society of Cellular Therapy has proposed three minimal criteria to define mesenchymal stem cells (MSCs); I) MSC is plastic adherent under standard culture condition. II) MSC should expressed CD73, CD90 and CD29 and III) MSC are capable of differentiating into osteocytes, chondrocytes, and adipocytes (Wuchter et al., 2013). Mesenchymal stem cells possess the ability to modulate innate and adaptive immune systems mediated by cell-to-cell contact and the secretion of paracrine factors. Interaction of MSC with immune cells produced myriad of effects such as enhanced differentiation of B lymphocytes into plasma cells, impaired function of dendritic cells, inhibition of T lymphocytes proliferation, as well as enhanced respiratory burst activity of macrophages (Vasandan et al., 2016, Bonnaure et al., 2016, Tabera et al., 2008, Jarvinen et al., 2008). In addition, MSC are also capable of homing to the site of injury, and the lack of Major Histocompatibility Complex class II (MHCII) expression and low expression of MHCI makes MSC hypoimmunogenic and thus escape immune recognition (Pittenger et al., 1999, Zhao et al., 2016).

In the past decade, studies have been conducted in preclinical and clinical settings for the treatment of various disease including GVHD, diabetes, neurological disorders, heart diseases, cancers, and lung diseases. Whilst the results have been encouraging, there are still major challenges to overcome before stem cell-based therapy can be translated into clinical settings. One of the major concern of with cell-based therapy using MSC is the risk of malignant transformation. Prolonged culture of MSC have shown to spontaneously transformed itself *in vitro* and when transplanted in mice model, the cells formed fibrosarcoma with p53 mutation (Li et al., 2007). Another study has also reported unwanted differentiation in transplanted MSC in mice model of myocardial infarction which resulted in

calcification and bone formation at the injury site (Breitbach et al., 2007). These unregulated growths are thought to occur due to genetic and epigenetic changes during handling and cultivation of stem cells (Mousavinejad et al., 2016). In addition, the engraftment of MSC in the injury area appeared to be low is insufficient to account for therapeutic response, suggesting paracrine-mediated mechanism of MSC (van Haaften et al., 2009). Furthermore, study demonstrated that administration of MSC resulted in immediate death of the animal due to pulmonary embolism associated with large size of MSC or cellular clumping (Antunes et al., 2014).

Mesenchymal stem cells in the treatment of COPD

Research on MSC as a new therapeutic regiment for COPD is currently focusing more on ameliorating the inflammation. The effect of MSC in mitigating the inflammation have been extensively studied in preclinical settings and tested in clinical trial all over the world. Attenuation of inflammation and increase secretion of cytokines involve in tissue repair by MSC significantly improved lung function, and stimulate the lung tissue regeneration (Liu et al., 2016). Besides ameliorating inflammation, MSC have a remarkable regenerative capability to differentiate into functional type II alveolar epithelial cells with the ability to express surfactant-related genes and proteins (Cerrada et al., 2014). Destruction of alveolar wall in elastase induce emphysema leads to reduction in pressure of arterial oxygen (PaO_2) and alveolar-arterial oxygen gradient ($A\text{-}aDO_2$) which will impair the pulmonary function. Regeneration of alveolar wall by MSC restore the pulmonary function by increase PaO_2 and $A\text{-}aDO_2$ level, and decrease the mean linear intercept (Furuya et al., 2012).

Although MSC have shown a tremendous benefit in preclinical studies, a recent phase II, randomized, double-blind, placebo-controlled study was conducted on 62 moderate-to-severe COPD patients receiving intravenous allogeneic MSC appeared to be safe with no significant adverse effect, and no increase in exacerbation. However, there is no significant difference in pulmonary function test and quality of life, although there is decreased in circulating C-reactive protein which indicate there is anti-inflammatory effect of MSC on systemic inflammation (Weiss et al.,2013). However, a more recent phase I study to evaluate the safety of MSC administration after lung volume reduction surgery for severe emphysema demonstrated an increased in FEV1 after 12 months follow up, and a 3-fold increase of CD31 in alveolar septa which indicates responsiveness of microvascular endothelial cells to MSC treatment (Stolk et al., 2016).

Although MSC are capable of attenuating the inflammation in COPD, it is unknown whether MSC can regulate the senescence in COPD. Recently, an *in vitro* study was conducted to

determine the effect of MSC in hypoxia/reoxygenation-induced premature senescence in cardiomyocyte. Co-culture of MSC have been shown to reduce the number of senescent cardiomyocytes and decreased expression of p53 and p21. However, MSC do not have any effect of p16, suggesting that MSC exert its effect via p53/$p21^{Cip1/Waf1}$ pathway but not $p16^{INK4a}$/Rb pathway (Cai et al., 2012). This encouraging evidence led to *in vivo* study by the same group using natural aging rat model. Four months old and 20 months old Sprague Dawley (SD) rat was used as young and natural aging model respectively. Consistent with previous result, transplantation of MSC inhibit oxidative stress, as well as reduced the expression of senescence markers p53 and p21, but not p16. Mesenchymal stem cells also significantly decreased senescent cells number and normalized endogenous anti-oxidant activity. Attenuation of senescence effect on aging heart improved cardiac function, cardiac hypertrophy and fibrosis as evident in MSCs treated group (Zhang et al., 2015). In addition, another study has been conducted in premature aging model of Bmi-1 deficiency. Mesenchymal stem cells transplantation in Bmi-1 deficiency mice, inhibits apoptosis while migrating into multiple organs, proliferate, and differentiate into various cells, thus promoting growth and delay senescence. Mesenchymal stem cells also downregulate senescence markers p16, p19, and p27 expression as well as Wnt16, prolonged the survival rate, increased body weight, increased proliferation of thymocyte and renal cells, ameliorate skeletal growth and development retardation, improved dysmaturity of T lymphocytes, decreased intracellular ROS level and hydrogen peroxide level, and increased level of anti-oxidant, superoxide dismutase and catalase (Xie et al., 2015).

Therapeutic application of mesenchymal stem cells derived extracellular vesicles in lung diseases

Due to the limitation faced in cell-based therapy, much focus has been diverted into the possibility of using extracellular vesicles (EV) released by MSC as a potential new therapeutic regiment for various diseases. Extracellular vesicles are small membrane vesicles released by various types of cells and also can be found in body fluids such as milk, saliva, urine, amniotic fluid and cerebral spinal fluid that is important for cell-to-cell communication. The size of EV range from 40-1000nm and can be sedimented by centrifugation at 100,000 g. The most prominent extracellular vesicles are exosome and microvesicles that contain various types of proteins, lipids, messenger ribonucleic acid (mRNA) and micro RNA (miRNA). These EV participate in many physiological and pathological processes including inflammation, cancer progression, immune responses, and angiogenesis (Ludwig et. al., 2011, Yu et. al., 2014). Extracellular vesicles are naturally

stable due to its lipid membrane similar to the cells itself can easily be taken up by the cells. MSC derived EV also possess no risk of pulmonary embolism due to its small size, and do not contain nuclei, therefore, EV do not replicate, thus avoiding the risk of forming teratoma and unwanted differentiation as compared to MSC (Chen et al., 2011).

To date, there is no report on the effect of MSC derived EV on inflammation and senescence in COPD, however, understanding how MSC derived EV exerts its function in other diseases may give an insight on the mechanism involved. Administration of MSC derived EV inhibited the vascular remodelling and hypoxic pulmonary hypertension model through the inhibition of transcription factor STAT-3, reduction of pro-inflammatory mediators MCP-1, IL-6, FIZZ-1/HIMF, inhibition of pulmonary artery smooth muscle cells, and suppression of miR-17 (Lee et al., 2012). In influenza-induced lung injury, MSC derived EV inhibited the influenza replication in lung epithelial cells, reduced the level of TNF-α, CXCL-10, and increased the level of pro inflammatory cytokine IL-10, as well as inhibited the apoptosis of lung epithelial cells (Khatri et al., 2018). Similar findings demonstrated the ability of MSC derived EV to ameliorate pulmonary oedema and lung protein permeability in acute lung injury through reduction of total white blood cells count, neutrophils, and MIP-2, as well as increasing the IL-10. This therapeutic effect by MSC derived EV is thought to occur due to the transfer of EV's content into the cells. MSC derived EV contain mRNA for keratinocyte growth factor (KGF) that involve in alveolar fluid clearance and blocking the KGF mRNA expression abrogated the effect of MSC derived EV. In addition, MSC derived EV are also able to provide therapeutic effect, and home to the site of injury following intravenous injection similar to MSC. (Zhu et al., 2014). Notably, MSC derived EV have been reported to induce lung adenocarcinoma cells growth by transferring miRNA-410 to the cells, resulted in increased proliferation rate, and lower apoptosis rate of the cells (Dong et al., 2018). However, conflicting result was reported in which MSC are inhibiting the lung tumour formation induced by chemical carcinogen (Liu et al., 2017). This contradicting effect in part may be due to the different source of MSC and different cargo content of EV. Del Fattore and colleagues (2015) demonstrated that bone marrow and umbilical cord MSC derived EV reduced the proliferation and increase apoptosis rate of glioblastoma cells, while adipose tissue MSC derived EV does not produce any effect, suggesting the role of tissue origin of MSC in mediating MSC derived EV effect (Del Fattore et al., 2015). In addition, a study conducted by Tofino-Vian et al., in 2017 to study the effect of MSC derived EV on osteoarthritic osteoblast induced by IL-1β showing a promising result in which MSC derived EV are shown to reduced the SA-β galactosidase activity and the accumulation of γH2AX foci, as well as downregulated the mitochondrial membrane changes and oxidative stress.

Conclusion

Considering the limitations of MSC, MSC derived EV could be a new therapeutic tool for the treatment of COPD. Although MSC derived EV have shown a tremendous effect in attenuating the inflammation and senescence in other model diseases, the effect of MSC derived EV on senescence in COPD is currently unknown. Thus, it is important to understand how MSC derived EV may exert its effect on inflammation and senescence in COPD. Long-term study should also be conducted to determine the safety and side effect that may occur with the administration of MSC derived EV. More studies should be done to standardize the culture condition of MSC, and methods of isolation of MSC derived EV as to maximize production of EV while maintaining normal phenotype. Optimal dosage, route of administration, and biodistribution of MSC derived EV should also be addressed before it can be translated into clinical settings.

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