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Breathe it in – spotlight on senescence and regeneration in the lung

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Abstract

Cellular senescence, a highly coordinated and programmed cellular state, has a functional role in both lung physiology and pathology. While the contribution of senescent cells is recognized in the context of ageing and age-related pulmonary diseases, relatively less is known how cellular senescence of functionally distinct cell types leads to the progression of these pathologies. Recent advances in tools to track and isolate senescent cells from tissues, shed a light on the identity, behavior and function of senescent cells *in vivo*. The transient presence of senescent cells has an indispensable role in limiting lung damage and contributes to organ regenerative capacity upon acute stress insults. In contrast persistent accumulation of senescent cells is a driver of age-related decline in organ function. Here we discuss lung physiology and pathology as an example of seemingly contradictory role of senescence in structural and functional integrity of the tissue upon damage, and in age-related pulmonary diseases.

Introduction

Cellular senescence is a state of prolonged and essentially irreversible cell-cycle withdrawal. It is an alarm response to numerous triggers, including DNA replication stress, telomere dysfunction, oncogene activation, oxidative stress, DNA damage, genomic instability and cell-cell fusion [1]. The stress signal and its duration, type of cell, microenvironment and time after senescence induction, can influence the senescence program and biological function of senescent cells [2]. Senescent cells are heterogeneous and exhibit a complex phenotype. Thus, they are identified by a number of molecular markers representing different characteristics of senescent cells. These molecular markers include: senescence-associated β-galactosidase (SA-β-gal), based on increased lysosomal β-galactosidase activity [3-5], markers of cell cycle arrest machinery (e.g. p16, p53, p21), apoptosis resistance markers (e.g. DCR2, Bcl-xL), secretory factors (e.g. IL-6, IL-8), markers of activation of DNA damage response (DDR) (e.g. p53BP1, γH2AX) and upregulation of immune surveillance genes [1, 6, 7]. Since there is no single biomarker exclusive to senescent cells, combination of senescence-related markers is used for their identification. Still, however, it is challenging to reliably detect and tract senescent cells *in viv*o, what is indispensable to fully understand their role at the organismal level.

High heterogeneity of senescent cells is reflected by their engagement in a number of physiological processes and pathological conditions, with beneficial and detrimental effect respectively [1, 6, 8, 9]. On one hand, senescence response limits propagation of damaged cells and thus tumorigenesis, but it also plays indispensable role upon tissue injury. In the lung, cellular senescence prevents massive cell death by induction of an anti-apoptotic mechanisms and helps in restoration of lung structure following lung damage [10-13]. Conversely, long-term presence of senescent cells in the lungs limits the potential for tissue renewal and promotes lung ageing and age-related diseases [8, 9, 11, 14-16]. To assist these tasks, senescent cells regulate their own immunosurveillance by expression of immune ligands [7, 17]. However, with ageing immune system deteriorates, what might impede the removal of senescent cells and consequently promote paracrine senescence via their secretory milieu [18]. The senescence secretome includes pro-inflammatory cytokines, chemokines, growth factors, matrix remodeling enzymes and angiogenic factors collectively known as Senescent Associated Secretory Phenotype (SASP) [19-21]. SASP is a hallmark of senescent cells and mediates their pathological and physiological effects. SASP can reinforce cell growth arrest of neighboring cells, modulate microenvironment, promote local inflammation, facilitate immunosurveillance of senescent cell, but it can also contribute to tissue remodeling and regeneration [19-25]. Therefore, the functional role of

senescence in the lung might depend on the temporary presence of senescent cells or their accumulation over time.

Cellular senescence contributes to wide spectrum of age-related lung diseases, including inflammatory diseases, tissue fibrosis, degeneration and cancer. Recently, it became appreciated that senescent cells are highly heterogeneous with complex cellular phenotype and can play functional role in both, lung physiology and pathology. Here, we will discuss how senescence of lung specific cell populations drive chronic tissue damage and the mechanisms that regulate this process. We will also discuss recent advances in understanding the role of senescence in limiting lung damage in acute lung injury and its emerging role in lung regeneration.

1. Cellular senescence in lung pathologies

1.1. Idiopathic pulmonary fibrosis (IPF)

Idiopathic pulmonary fibrosis (IPF) is progressive scar tissue formation, which results in thickening and stiffening of lung tissue that eventually leads to irreversible decline of lung function including respiratory failure. Although exact etiology of IPF is unknown, ageing is one of the main risk factors [26, 27]. Moreover, the disease mainly affects the elderly population, with a mean age of over 60 years at the time of diagnosis. Senescent cells accumulate in fibroblastic foci of IPF patients [28-30], suggesting role of senescence in development and progression of the disease.

Functional contribution of fibroblast senescence to fibrogenesis following injury can be beneficial or deleterious depending on senescent cell half-life in the tissue. Transient presence of senescent cells can promote anti-fibrotic effects in different organ systems, including lung [31, 32], kidney [33], liver [34], heart [35] and skin [36]. However, persistent senescent cells in the context of ageing might support pro-fibrotic mechanism leading to non-resolving fibrosis following acute lung injury or in the patients with IPF [37]. Moreover, number of studies demonstrate accumulation of senescent cells at the site of tissue pathology, predominantly in elderly patients with IPF [28-30], liver fibrosis [38], cardiac [39] and kidney fibrosis [40].

What is then the possible explanation of divergent role of senescence in fibrosis?

In response to damage, fibroblasts migrate to the site of injury and differentiate into myofibroblasts, a specialized contractile fibroblast with smooth-muscle (SM)-like features, which synthetize ECM components, but also generate force and establish tissue tension during

wound healing [41, 42]. Under physiological conditions, senescence of ECM-producing myofibroblasts blocks their proliferation, and also converts them into ECM-degrading cells, exerting anti-fibrotic effect [34, 36]. With the course of wound closure, myofibroblast are cleared, probably either by immunosurveillance mechanism or apoptosis, which is critical for termination of the repair process and restoration of tissue structure and function [22]. Moreover, myofibroblasts can de-differentiate and contribute to re-epithelization to reconstitute tissue integrity, process regulated by endogenous levels of myogenic differentiation factor (MyoD) [37, 43]. However, when healing process is dysregulated and activity of myofibroblasts continues unchecked with excessive ECM deposition, it can lead to fibrosis and impairment of organ function. Senescent myofibroblast in IPF patients or in the context of ageing, express elevated levels of MyoD/αSMA, which inhibit their de-differentiation and promotes an apoptosis-resistant phenotype contributing to their accumulation in non-resolving lung fibrosis [37] (Fig. 1). Relationship between senescence and ECM production in fibrosis is complex and might have different impact on the pathology in tissue specific manner [44]. Therefore, cellular senescence is an innate stress response exerting either 'fibrosis-preventing' or 'fibrosis-supporting' mechanisms depending on its duration and microenvironment context of the tissues.

Apart of critical role of senescent myofibroblasts in development of IPF, emerging data from single cell RNA sequencing studies provide insights into contribution of different lung cellular subsets to the onset and progression of disease. A single-cell atlas of the IPF lung discovered population of aberrant basaloid cells, absent in control lungs, but detectable in COPD lungs (1.1% of all epithelial cells) and enriched in IPF lung (3.5% of all epithelial cells), suggesting that they might be a feature of the disease [45]. While these cells express some of the most typical airway basal cell markers, they also show expression of mesenchymal markers suggesting active epithelial-mesenchymal transition (EMT). Intriguingly, these basaloid cells show also expression of senescence markers (CDKN1A (p21), CDKN2A (p16)) together with SOX9, a transcription factor critical for airway development and repair [46, 47], and localize at the edge of the myofibroblast foci in distal lung. Expansion of aberrant basaloid cells lining myofibroblast foci in the distal lung, possibly represents fibroblastic foci with honeycomb cysts also known as basal cell hyperplasia or bronchiolization, two distinct histopathological features of IPF lung [48, 49].

It is possible that these basaloid cells combining senescence and progenitor features drive and sustain IPF pathology. IPF patients show significant shift in epithelial cell composition in distal

lung, with alveolar epithelial cells depletion and basaloid cells enrichment [45]. It might suggest that basaloid cells may serve as progenitors to replenish depleted AT1 and AT2 cells. However, it is unknown if premature senescence of basaloid cells can lead to stem cell exhaustion and impaired regeneration. Such scenario can be supported by work done by Chilosi et al. showing abnormal nuclear accumulation of β-catenin, and aberrant activation of WNT pathway in epithelial – fibroblast junction (most probably referring to aberrant basoloid cells), specifically in IPF, but not other fibrotic pulmonary diseases, strongly suggesting its pathogenic relevance [50]. WNT signaling regulates self-renewal potential, proliferation and differentiation in adult stem cell niches [51-54]. For example, WNT signaling within alveolar – fibroblast niche maintains stemness of alveolar type 2 cells, which can replenish AT1 population and self-renew throughout adult life [55]. However, chronic WNT/β-catenin signaling can also drive senescence of lung epithelial cells [56]. Possibly, if there is extensive alveolar damage, including AT2 cell death, basaloid cells might re-populate depleted populations. This could be facilitated by EMT of basal cells to allow them to dissociate from bronchiolar structures where they originate and migrate to alveolar compartment [57]. However, chronic activation of WNT/β-catenin signaling can trigger DNA damage response and p53/p21 dependent cellular senescence in basaloid cells due to their extensive proliferation leading to dysfunctional tissue repair [58]. In addition, aberrant activation of WNT pathway can induce mesenchymal phenotype in epithelial cells causing fibrogenesis and abnormal, irreversible lung remodeling [59, 60] (Fig. 1).

Progression of pulmonary fibrosis engage p53-dependent senescence of alveolar type 2 cells [61]. Conditional loss of Sin3a in AT2 cells triggered activation of p53-dependent senescence and progression to pulmonary fibrosis. However, therapeutic approaches targeting p53 activation to attenuate senescence-related pathways as well as elimination of senescent cells decrease lung fibrosis and restore pulmonary function [29, 61, 62]. Most widely used senolytics (drugs which selectively kill senescent cells) in pulmonary fibrosis rely on inhibiting antiapoptotic pathways to reduce viability of senescent cells [29, 63, 64]. Interestingly, recent pilot study conducted in humans provided initial evidence that therapies targeting senescent cells for elimination (senotherapy) improved physical functions in IPF patients, without significant change in lung function [64]. This suggests that cellular senescence contributes to lung fibrosis development and targeting senescent cells may be attractive strategy for the IPF treatment, following better characterization of senescent cells in the lung.

1.2. Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease characterized by airflow limitation due to emphysema (enlargement of alveoli, destruction of alveolar walls), chronic obstructive bronchiolitis (narrowing of the small airways) and chronic bronchitis (mucus hypersecretion). Patients diagnosed with COPD experience breathing difficulty, shortness of breath upon exertion, chronic cough and lack of energy. COPD is the third leading cause of death worldwide with limited therapeutic options [65]. Although the most common risk factors for the development of COPD include age and cigarette smoke, factors such as genetics, epigenetics and environmental cues might be involved in determining individual susceptibility to the disease [66, 67]. The mechanisms underlying COPD are not completely understood, however there is a growing body of evidence that senescent cells may play a key pathophysiological role [8, 9, 14, 15].

Cellular senescence contributes to structural and functional changes found in COPD lung. For example, toll-like receptor 4 (TLR4)-mediated epigenetic suppression of $p16^{INK4a}$ in endothelial cells maintains integrity of the lung in mouse model of emphysema [68]. Another study demonstrated that p53-mediated senescence of airway lung epithelium promotes chronic bronchitis, formation of inducible bronchus associated lymphoid tissue (iBALT) composed of T and B lymphocytes, increase of bronchial wall width and enlargement of airspace [69]. Interestingly, these processes were diminished in club cell-specific p53 knockout mice. It suggests that in continuously damaged lungs p53 exerts pro-inflammatory role by inducing senescence program, which intensifies inflammation and lung destruction. These results not only implicate p53 in induction of club cell senescence but also give mechanistic explanation for epithelial cells senescence and development of chronic airway inflammation and lung destruction observed in COPD (Fig. 1). This might be surprising since there is growing body of evidence indicating reciprocal negative regulation of p53 and NF-κB, the central component of inflammation-associated responses including inflammatory phenotype of senescent cells [70, 71]. On one hand, in cancer loss of p53-mediated NF-κB suppression results in unleashed inflammatory responses indicating inhibitory role of p53 in inflammation. On another hand, in response to intrinsic stress (genotoxic, oncogenic activation), p53 positively regulates cyclin kinase inhibitor p21 and recruits cells into senescence with pronounced inflammatory secretome. It demonstrates versatility of p53 function, with tumour suppressive, anti-inflammatory role during tumorigenesis, but pro-inflammatory role in p53-mediated

senescence program. Therefore, nature of p53/NF-κB interaction is context dependent, inhibitory in cancer cells, but co-stimulatory in regulation of proinflammatory response in senescence [14].

The mechanistic target of rapamycin (mTOR) regulates lifespan, ageing and age-related processes such as cellular senescence [72, 73]. Interestingly, there is a causative relationship between mTOR signalling and senescence in COPD development. mTOR activation within lung vascular and epithelial cells by TSC1 deletion (negative mTORC1 regulator) induces cellular senescence load and leads to COPD-like alterations with lung emphysema, inflammation and pulmonary hypertension [74]. This observation is in agreement with widely accepted notion that mTOR inhibition extends lifespan and delays the onset of age-associated diseases in several models [72, 75]. However, in contrary to these results, another study indicated beneficial protective role of mTOR signaling in COPD, as mice with epithelial knockdown of mTOR were sensitive to cigarette smoke-induced inflammation and emphysema development [76]. These discrepancies might be due to different mouse models used or concentration and timing of toxic insult, and require further investigation. In addition, since the mTOR signaling is involved in cell metabolism, survival, and growth [77], it is hard to conclude if observed senescence is direct causative agent of COPD symptoms, or it is by-product of the mTOR activation contributing to lung pathology by promoting non-resolving inflammation.

Two major risk factors for COPD development – ageing and smoking – are known to promote accumulation of senescent cells in the lung. Interestingly, mice exposed to chronic cigarette smoke presented age-dependent increase in inflammatory response, but not in load of cellular senescence [78]. This suggests that ageing and toxins in cigarette smoke might independently contribute to accumulation of senescent cells in the lung. DNA damage caused by the toxic materials present in cigarette smoke might be a stronger trigger for cellular senescence then ageing, however functional decline of immune system with age leads to reduction in senescent cell clearance and thus senescent cell buildup followed by deterioration of tissue architecture [79-81]. Also, cigarette smoke has been shown to affect a wide range of immune system responses skewing towards reduction of immunosurveillance to promote senescent cells accumulation, chronic inflammation, infections, and smoking-related diseases including cancer [82]. This is due to altered expression of genes involved in inflammatory responses, immune signalling and cytolytic activity of immune cells. Exposure to cigarette smoke attenuates activity of innate immune system, the first line of defence against pathogenic microbes and critical player in tumour immunosurveillance. For example, smoke-related reduction of

cytotoxic activity and cytokine production of natural killer (NK) cells, links it to increased rate of infection and cancer [83]. Also, phagocytic activity of alveolar macrophages, key innate immune sentinel of the lung, is significantly reduced, with decreased ability to eliminate pathogens, necrotic or apoptotic cells [84, 85]. Smoke also induces state of T cell anergy through depletion of intracellular calcium ions leading to immunosuppressive mechanisms [86]. Therefore, reduction of immunosurveillance driven by ageing and smoking might explain pathological senescent cells accumulation and COPD prevalence predominantly in elderly people.

1.3. Acute Respiratory Distress Syndrome (ARDS)

Acute respiratory distress syndrome (ARDS) is the most severe form of acute lung injury with destruction and increased permeability of alveolar-capillary barrier, a form of diffuse alveolar injury. In patients with ARDS mechanical ventilation is often necessary to support gas exchange, which can lead to high airway pressures and alveolar overdistension, so-called ventilator-induced lung injury (VILI) [87, 88].

In clinically relevant model of ARDS with VILI, mice exposed to acid aspiration and mechanical ventilation, activate p53/p21 pathway to induce senescence to avoid massive cell apoptosis and limit lung destruction [10]. Mechanical stress during ventilation causes functional alteration to the nuclear envelope and chromatin organization inducing DNA damage response and p53/p21 pathway. Activation of p21 maintains the viability of DNA damage-induced senescent cells [89]. Accordingly, p21-knockout mice show decrease in senescence response, but massive cell apoptosis and overall, more severe lung injury compared to their wildtype counterpart in response to the treatment. Conversely, p21 overexpression due to lopinavir/ritonavir treatment ameliorates cell apoptosis and lung injury. These results suggest that acute lung injury activates p53/p21 to induce senescence as an anti-apoptotic mechanism to ameliorate damage (Fig. 1). In contrary, persistent senescent cells in old mice become hyper-inflammatory in response to acute, often lethal lung damage caused by viral SARS-CoV-2 Spike protein, increasing expression of viral entry proteins and decreasing anti-viral gene expression in their microenvironment in paracrine manner [16] (Fig. 1). Reducing senescent cell burden with senolytics decreases coronavirus-induced mortality in old mice [16]. Therefore, role of senescence in response to acute injury depends on the context, beneficial if induced directly upon damage, but deleterious if persistent in the tissue.

2. Senescence in lung regeneration

Lung presents a remarkable reparative capacity, with resident progenitor and stem cell populations able to replenish diverse cell types in response to injury [90]. Cellular senescence can play role in coordinating lung regeneration process [11-13, 25] (Fig. 1). Mesenchymal cells expressing senescent marker $p16^{INK4a}$ can act as a reparative niche in response to lung injury [13]. Adjacent to epithelial progenitors in the lung, these cells appear short after birth, and upon airway epithelial injury secrete epiregulin (EREG), a member of epidermal growth factor (EGF) enhancing epithelial progenitor cell proliferation and differentiation to secure integrity of tissue following injury. Specific deletion of $p16^{INK4a}$ gene within mesenchymal cells, abrogates their senescence features *in vivo*, but also attenuates epithelial repair. This supports beneficial role of $p16^{INK4a}$ + mesenchymal cells behaving as sentinels for airway epithelial stem cell niche. This phenomenon is similar to positive role of SASP in the wound repair process, where senescent cells accelerate cutaneous wound closure by stimulating myofibroblast differentiation through secretion of platelet-derived growth factor AA (PDGF-AA) [22].

Emerging evidences suggest cellular senescence as a plastic state induced in epithelial cells before their transition to another cellular identity. In the lung a subset of AT2 cells serves as a progenitor pool to generate AT1 cells in response to injury, but also in homeostatic conditions throughout adult life [55]. However, trans-differentiation of cuboidal, mucus secreting AT2 into thin and elongated AT1 cells, specialized in gas exchange, involves transitional state in route to their terminal differentiation [11, 12]. Extensive stretching which assists a dramatic change in shape, structure and mechanical properties, induces a p53-dependent DNA damage response and senescence within transitional progenitor cells. Importantly, senescent progenitor cells do not accumulate in normal lung repair, but are persistently present in patients with IPF [11]. This might be explained by immune surveillance of senescent cells, which slows down with age together with senescent cells turnover resulting in their accumulation in the tissue and subsequent detrimental effect on lung scaring [79]. This suggests that senescent progenitor cells can accompany the regeneration program, but also lead to pathological conditions when persist in tissues.

Concluding remarks

Highly heterogeneous senescent cells play functional role in both, lung physiology and pathology, from limiting lung damage and restoring tissue structure upon injury, to mediating lung ageing and age-related diseases (Fig. 1). This engagement of cellular senescence in diverse biological processes helps to comprehend it as a highly coordinated and programmed cellular state. In this regard, senescence might be an innate mechanism supporting normal lung-maintenance programs and enhancing robust regeneration and repair responses in damaged tissue. Once homeostasis is disturbed, uncontrolled and persistent presence of senescent cells potentially leads to senescence-mediated pathological conditions. Further investigations of molecular mechanisms underlying cellular senescence induction, heterogeneity and function in acute versus chronic conditions may have implications for new therapeutic interventions to abrogate deleterious impact of senescent cells while preserving their beneficial effect.

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Fig. 1. Contradictory roles of senescence in lung physiology and pathology.

Transient presence of senescent cells and components of SASP stimulate lung repair and regeneration. Also, induction of senescence-assisted growth arrest in response to acute respiratory distress prevents massive death of lung resident cells, preserving functional and structural integrity of the organ. However, chronic presence of senescent cells in the context of ageing, disease or persistent damage has deleterious effect by promoting non-resolving inflammation, fibrotic tissue remodeling, retarded regeneration/repair and tissue degeneration. Persistent senescent cells compromise viral defense mechanisms and promote susceptibility to viral infection. Reducing persistent senescence and its paracrine effect on microenvironment by senescent cell elimination, either by senolytic approaches or by immune-mediated interventions, as well as SASP inhibition might be attractive strategies for maintaining cellular homeostasis in the lung.