Cellular Senescence: The Trojan Horse in Chronic Lung Diseases

Shruthi Hamsanathan¹, Jonathan K. Alder^{2,3}, Jacobo Sellares^{4,5}, Mauricio Rojas^{2,3,6}, Aditi U. Gurkar^{1,7,8}, and Ana L. Mora^{1,2,9}

¹Aging Institute, ²Division of Pulmonary Allergy and Critical Care Medicine, and ⁷Division of Geriatric Medicine, Department of Medicine, ³Dorothy P. and Richard P. Simmons Center for Interstitial Lung Diseases, ⁶McGowan Institute of Regenerative Medicine, and ⁹Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, Pennsylvania; ⁴Interstitial Lung Disease Program, Servei de Pneumologia, Hospital Clínic Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain; ⁵Centro de Investigaciones Biomédicas en Red—Enfermedades Respiratorias (CibeRes CB06/06/0028), Instituto de Salud Carlos III, Barcelona, Spain; and ⁸Geriatric Research Education and Clinical Center, VA Pittsburgh Healthcare System, Pittsburgh, Pennsylvania

ORCID IDs: 0000-0003-3741-6512 (J.K.A.); 0000-0001-7340-4784 (M.R.); 0000-0003-4338-0624 (A.U.G.); 0000-0003-1653-8318 (A.L.M.).

Abstract

Senescence is a cell fate decision characterized by irreversible arrest of proliferation accompanied by a senescence-associated secretory phenotype. Traditionally, cellular senescence has been recognized as a beneficial physiological mechanism during development and wound healing and in tumor suppression. However, in recent years, evidence of negative consequences of cellular senescence has emerged, illuminating its role in several chronic pathologies. In this context, senescent cells persist or accumulate and have detrimental consequences. In this review, we discuss the possibility that in chronic obstructive pulmonary disease, persistent senescence

impairs wound healing in the lung caused by secretion of proinflammatory senescence-associated secretory phenotype factors and exhaustion of progenitor cells. In contrast, in idiopathic pulmonary fibrosis, chronic senescence in alveolar epithelial cells exacerbates the accumulation of senescent fibroblasts together with production of extracellular matrix. We review how cellular senescence may contribute to lung disease pathology.

Keywords: senescence; idiopathic pulmonary fibrosis; chronic obstructive pulmonary disease; senescence-associated secretory phenotype; lung

During the fabled Trojan War, the people of Troy allowed a seemingly harmless wooden horse into their city. Despite its benign appearance, the horse led to the capture and destruction of their city. Analogously, accumulating senescent cells, despite their harmless appearance, can result in organ dysfunction and disease. When normal proliferating cells lose their ability to divide, they either can opt for self-destruction or can undergo an irreversible growth arrest, referred to as “cellular senescence.”

Contrastingly, in cellular quiescence, the cells enter a brief “sleep” phase because of a lack of growth factors or nutrients. However, these cells can “wake up” and

reenter the cell cycle upon stimulation (1, 2). The irreversibility distinguishes cellular senescence from quiescence and transient cell cycle arrest (1, 3, 4). This irreversible growth arrest puts senescence under the spotlight as an anticancer or tumor-suppressive mechanism (5). However, with age, senescent cells accumulate, impacting tissue-regenerative capacity and leading to frailty (6). Thus, it is a prime example of antagonistic pleiotropy, whereby it is beneficial to organismal survival in one context and detrimental to fitness in another (7–9). Cellular senescence can be induced by a number of stressors, including DNA damage, epigenomic stress, oncogene

activation, mitochondrial dysfunction, and redox imbalance. Owing to the direct exposure to environmental factors, the lung is more vulnerable to senescence-inducing conditions leading to loss of elasticity and structural integrity as well as decreased pulmonary function. Accordingly, the effect of senescence has been implicated in the pathobiology of two major age-related lung diseases: chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). With this backdrop, we review cellular senescence, emerging concepts in senescence, and the role of senescence in chronic lung diseases.

(Received in original form December 17, 2018; accepted in final form April 5, 2019)

Supported by National Institutes of Health grants 1R01 HL131789-01 (A.L.M.), 1R01 HL123766-01 (M.R.), R00 AG049126 (A.U.G.), R00 HL113105 (J.K.A.), and R01 HL135062 (J.K.A.).

Correspondence and requests for reprints should be addressed to Ana L. Mora, M.D., Aging Institute, Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, 560 Bridgeside Point 1 Building, 100 Technology Drive, Pittsburgh, PA 15219. E-mail: anamora@pitt.edu.

Am J Respir Cell Mol Biol Vol 61, Iss 1, pp 21–30, Jul 2019

Copyright © 2019 by the American Thoracic Society

Originally Published in Press as DOI: 10.1165/rcmb.2018-0410TR on April 9, 2019

Internet address: www.atsjournals.org

Cellular senescence was initially observed as a response to the gradual loss of telomeres due to an end-replication problem with successive cell division, known as “replicative senescence” (4, 10, 11). When telomeres reach a critically short length, they trigger a persistent DNA damage response (DDR), leading to senescence (12). Cellular senescence typically gives rise to three major phenotypic changes: irreversible proliferative arrest, resistance to apoptosis, and the senescence-associated secretory phenotype (SASP) (12–15). Although senescent cells have permanently exited the cell cycle, they are metabolically active with an altered chromatin organization and gene expression profile (3). Transient accumulation of senescent cells plays an integral role in wound healing and tissue remodeling. However, senescent cells need to be cleared to prevent fibrosis and scar tissue accumulation (16). Clearance of senescent cells is mediated by immune cells that are recruited in response to SASP factors (17).

Senescence Markers

Several morphologic and biochemical changes distinguish senescent cells from normal cells that can serve as markers for senescence (18). Generally, senescent cells appear flattened, large, and multinucleated *in vitro* (19). The most popular marker for senescence is the expression of a lysosomal hydrolase, β -galactosidase, referred to as the “senescence-associated β -galactosidase.” In nonsenescent cells, the activity of β -galactosidase is measured at pH 4.0, but low activity is detected at pH 6.0 (20). However, in cultured cells undergoing replicative or induced senescence, β -galactosidase activity is detectable at pH 6.0. This is because of the increased lysosomal contents in senescent cells with upregulation of autophagy and general enlargement of lysosomes (21). High levels of expression of cell cycle regulators such as p16, ARF (alternative reading frame), p53, p21, p15, and p27 result in irreversible cell cycle arrest, and hypo-pRb (hypophosphorylated retinoblastoma protein) can be detected in senescent cells (22). Senescent cells are also characterized by their condensed chromatin regions. These heterochromatin regions repress expression of proliferation-promoting genes such as E2F (23). Foci of

heterochromatin comprised of a di- or trimethylated lysine 9 of histone H3 (H3K9Me2/3), a histone H2A variant (macroH2A), and HP1 (heterochromatin protein 1) proteins are known as senescence-associated heterochromatin foci (SAHF) (24). Although not a universal senescence marker, the accumulation γ -H2AX (phosphorylated at the C-terminal serine 139 of the H2AX histone), a DNA damage marker is a commonly used SAHF marker to identify senescent cells (22). Although several markers are available for identifying senescent cells, they do not conclusively do so. To date, clear guidelines or definitions on how to identify senescent cells *in vivo* have not been identified or widely applied. Indeed, the most commonly used marker for cellular senescence, p16, is dispensable for cellular senescence *in vivo* for some tissues (25). Ideally, *in vivo* evidence of cellular senescence would include functional readout (proliferation studies); however, these are not always possible in clinical samples. Likely the use of multiple markers, with a focus on functional assays, is the most prudent course for studying senescence *in vivo*.

Another difficulty in studying cell senescence *in vivo* is the relative sparsity of senescent cells compared with nonsenescent cells and their phenotypic heterogeneity. Senescent cells may comprise only a small percentage of cells in a tissue, so studies on bulk gene or protein expression may not detect their contribution. Many of the characteristics of senescent cells—gene expression and secreted proteins—were described in fibroblasts, but changes as mentioned above are cell-type specific. For example, senescent epithelial cells may undergo different morphologic and gene expression changes from those in senescent fibroblasts. Further complicating this is the heterogeneity observed between senescent cells from the same cell type and the distinction between early and late senescence (26). In addition, although cellular senescence was first identified in proliferating cells, recent evidence suggests that postmitotic cells can also undergo senescence (27). This complexity has likely slowed research on the contribution of cellular senescence to disease pathology, especially in the lung, where more than 40 distinct cell types coexist and access to the organ is difficult. Moving past these limitations will likely require the

application of novel techniques that have the capacity to sample rare cell types and characterize diverse responses (i.e., single-cell RNA sequencing).

SASP

SASP composition is complex and varies with cell type and senescence-triggering stimuli. The most common SASP factors are cytokines, especially interleukin family members such as IL-1 α , IL-1 β , IL-6, IL-8, IL-7, IL-13, and IL-15 (13, 28). In addition to cytokines, SASP factors include several growth factors, chemokines, and proteases. Chemokines secreted by senescent cells include MCP-1, -2, -3, and -4 (monocyte chemoattractant proteins 1–4, respectively); HCC-4; eotaxin 3; and MIP-1 α and -3 α (macrophage inflammatory proteins 1 α and 3 α , respectively) (13). SASP-driven extracellular matrix (ECM) components, serine proteases, and matrix metalloproteases aid in tissue remodeling through production and degradation of ECM. SASPs also consist of extracellular vesicles or microvesicles, which include a wide variety of small membranous vesicles ranging from approximately 50 nm to a few micrometers in size. Microvesicles are important mediators of intercellular communication that serve as vehicles transferring proteins, mRNAs, microRNAs, and organelles such as mitochondria (29). Moreover, microvesicles secreted from senescent cells contribute to the changes in the phenotype of the target cells through activating intracellular pathways. Most important, there is increasing evidence that suggests that the quantity and quality of the total microvesicles secreted from senescent cells are quite different from those of microvesicles generated by younger replicative cells (30).

Emerging data suggest that NF- κ B and CEBPB (CCAAT/enhancer-binding protein- β) (31, 32) are required for the induction of SASP factors, especially the proinflammatory cytokines IL-6 and IL-8 (33). These cytokines maintain a self-amplifying secretory program by inducing an autocrine positive feedback loop. SASP only develops in established senescent cells and persists for long intervals. Thus, several pathways regulate SASP by influencing NF- κ B activity. These include the stress-inducible kinase p38MAPK (p38 mitogen-activated protein kinase) (32) and mTOR.

Other important SASP factors are the insulin growth factor (IGF)-binding proteins that inhibit IGF signaling (34), PAI-1 (35), and transforming growth factor- β by generating reactive oxygen species (ROS) and DNA damage by autocrine and paracrine mechanisms (36).

As with the growth arrest response, SASP has both beneficial and detrimental effects, depending on the biological context. For example, SASP plays an important role in normal embryonic growth and patterning, as well as the promotion of vascular remodeling and angiogenesis of the placenta (37). On one hand, recent evidence suggests that cellular senescence and release of SASP, such as IL-6, are crucial for cellular reprogramming of nonsenescent cells and tissue regeneration through Oct4 (Pou domain class 5 transcription factor 1), Sox2, Klf4 (Krüppel-like factor 4), and c-Myc in young animals (38). On the other hand, recent studies indicate that exacerbated SASP induces dedifferentiation, increases cell division of neighboring cells, restructures the stroma, and promotes tumor cell metastasis (13, 39–41). The burden of cellular senescence increases with age in several model organisms, as well as in humans. Partially, this increased burden is due to inefficient clearance of senescent cells, probably owing to an aging immune system (42). Senescent immune cells release SASP and cause secondary “paracrine” senescence in resident tissues. Persistent senescent cells may also have developed strategies to evade immune surveillance, as suggested by studies in which senescent cells were transplanted into young animals. This led to an increase in inflammation, and the “young” immune system was unable to clear all senescent cells (43). Activating the caspase-8 moiety in the ATTAC suicide gene product that is expressed only in p16-positive senescent cells (INK-ATTAC [INK-linked apoptosis through targeted activation of caspase]) improves healthspan and lifespan significantly in accelerated and naturally aged mice by delaying the occurrence of cataracts, age-related mobility problems, and cardiac hypertrophy (44). Similarly, pharmacological elimination of senescent cells also improved healthspan, suggesting that cellular senescence contributes to a number of age-related pathologies (45). These lines of evidence strongly suggest that although intermittent cellular senescence may play a physiological role, persistent senescence contributes to pathology.

Signaling Pathways Regulating Cellular Senescence

Permanent growth arrest is regulated primarily by the p53/p21 pathway and the p16 pathway. Upon stimulation by genotoxic stress (46), p53 induces the expression of cyclin-dependent kinase inhibitor p21. Then, p21 inhibits cyclins D and E and subsequently activates the retinoblastoma protein, pRb. pRb halts cells from entering S phase by inhibiting transcription factor E2F (47, 48). Like p21, p16 is a cyclin-dependent kinase (CDK) inhibitor, and it inhibits CDK4 and CDK6. p16 also activates pRb and inhibits E2F, leading to growth arrest. Thus, pRb can work either through p53→p21, or via the p16 arm, or through both arms in parallel (49). Although senescence can occur through either p53 or p16, in the absence of the latter, early senescence arrest can be reversed by inactivating p53, suggesting that p16 acts as a backup regulator to p53 (47). The p16/pRb pathway promotes additional effectors, such as the SAHFs, that repress proliferation-promoting genes, such as E2F target genes, ensuring permanent cell cycle exit (50). Although not all senescent cells express p16, with age, the expression levels were found to increase in several tissues (51). The p38MAPK pathway is a known mediator of cellular responses to inflammation and stress, and it also regulates senescence and SASP independent of the DDR. Sequential activation of the Raf–MEK (mitogen-activated protein kinase kinase)–ERK (extracellular signal-regulated kinase) and MKK3/6 (mitogen-activated protein kinase kinase 3/6)–p38 pathways in response to *ras*-oncogene-induced senescence (OIS) activates PRAK (p38-regulated/activated kinase) through transcription of p16. PRAK in turn activates p53 that, together with p21, triggers senescence (52).

Factors Driving Cellular Senescence

Telomere Shortening/DDR-induced Cellular Senescence

Telomeres are DNA–protein structures that function to suppress the DDR at the ends of chromosomes (12, 53). Telomeres function as a cellular clock and shorten as cells divide. Shortening of telomeres is

responsible for replicative senescence, and the introduction of exogenous telomerase, the enzyme that synthesizes telomeres, is sufficient to immortalize many cell types (10, 54). Generally, short telomeres can be repaired only by telomerase or fusion with other chromosomes. Because telomerase is not expressed in the majority of somatic cells, short telomeres often trigger a persistent DDR or trigger chromosome fusions that can prevent or halt mitosis, respectively (55). Uncapped telomeres trigger a DDR through the DNA damage sensor, ATM (ataxia telangiectasia mutated) protein kinase. ATM, a serine/threonine kinase, phosphorylates the heterotrimeric complex of NBS1 (nibrin, Nijmegen breakage syndrome 1 protein), Mre11, and Rad50 (MRN complex). ATM also phosphorylates H2AX histone at Ser139 adjacent to the DNA break. The phosphorylated form of H2AX (γ H2AX) attracts several DDR mediators, including MDC1 (mediator of DNA damage checkpoint protein 1) and NBS1, at the damage site. Ultimately, downstream kinases Chk1 and Chk2 (checkpoint kinases 1 and 2, respectively) are activated and trigger p53 and p16-pRb (56).

OIS

A sustained oncogenic signal can elicit replicative stress activation of the DDR in OIS (57). Oncogene activation is a stressor that usually leads to the de-repression of the *CDKN2A* (cyclin-dependent kinase inhibitor 2A) locus (consisting of p16 and ARF tumor suppressor), most often owing to the loss of Polycomb-repressive complexes (58–60). An increase in ROS is another common feature in cancer cells. Apart from replicative stress-driven DNA damage, accumulated ROS can also damage DNA and induce OIS. Serrano and colleagues (5) showed that oncogenic *Ras* induced a permanent cell cycle arrest in primary fibroblasts. However, this cell growth arrest was not immediate and occurred after a brief initial period of proliferation with concomitant accumulation of p53 and p16. Disrupting p53 or p16/pRb pathways prevented *ras*-induced senescence. Several genes, including *Ras*, *Raf*, *Mos*, and *Cdc6* (cell division cycle 6), trigger oncogene-induced senescence (61) and promote cancer progression. OIS displays shared characteristics such as senescence-associated DDR foci; accumulated CHK1,

CHK2, p21, p16, and p53; and hypo-Rb (62, 63).

Mitochondrial Dysfunction–induced Cellular Senescence

There is evidence that factors which induce cellular senescence, such as DNA damage and telomere shortening, require mitochondria, suggesting that there is a role of impaired mitochondrial function in driving cellular senescence (64, 65). Dysfunctional mitochondria are unable to oxidize NADH to NAD⁺, leading to cytoplasmic accumulation of NADH. This leads to chronic activation of AMPK (AMP kinase) and p53, driving cells into senescence (66). There is also increasing indication that senescence is associated with metabolic changes. p53 can repress the mitochondrial malic enzymes ME1 and ME2, which convert the tricarboxylic acid metabolite malate to pyruvate (67). ME2 has key effects in NAD(P)H production, lipogenesis, and glutamine metabolism. Importantly, suppression of ME2 induces senescence, suggesting that p53 may mediate senescence partially through regulation of the trichloroacetic acid cycle. There is also a strong link between mitochondrial metabolism, ROS production, and senescence. Mitochondrial ROS detoxification enzymes, such as GLO1 (glyoxalase I), glutathione S-transferase (GST), and MsrA (methionine sulfoxide reductase), were expressed at lower amounts in senescent cells than in nonsenescent cells (68). Mechanistically, it has been established that mitochondrial ROS production drives a feedback loop to p21 and maintains late senescence (69).

Stress-induced Cellular Senescence

The lung is susceptible to several agents that act as stressors to drive cellular senescence, such as pathogens or hyperoxia. Oxidative stress increases DNA damage and induces mitochondrial dysfunction, leading to a vicious cycle that culminates in cellular senescence (70). Hyperoxic conditions also induce senescence in fibroblasts (71), primary retinal microvascular endothelial cells (72), and fetal airway smooth muscle cells (73). Stress-induced senescence has been also associated with alterations in proteostasis. Inhibition of the proteasome can induce a senescence phenotype (74). Similarly, endoplasmic reticulum stress has been implicated in the stress-induced

senescence via activation of p21-dependent signaling (75).

Cellular Senescence in the Lung

The interest in the role of cellular senescence in lung diseases derives from the observation of markers of senescence in COPD and IPF. There is a logical connection between cellular senescence and the most significant risk factors for them: advanced age, short telomeres, mitochondrial dysfunction, and environmental exposures that can promote senescence. Age is a risk factor for several lung diseases, and the relative abundance of senescent cells is believed to increase with age (76). Oxidative stress due to environmental exposures such as cigarette smoke also promotes cellular senescence (77), together with additional forms of cellular stress such as mitochondrial dysfunction and endoplasmic reticulum stress.

There is a good reason to suspect that the lung may be uniquely susceptible to cellular senescence and that lung disease may be more closely connected than other diseases to senescence. Typically, cells within the body proliferate at approximately 3% oxygen concentrations, and higher levels of oxygen slow their proliferation. Even the proliferative basal cells of the epidermis are maintained at lower oxygen concentrations by a stratified epithelium (77, 78). Stem/progenitor cells of the lung epithelium and mesenchyme are likely the only cells of the body that are required to proliferate at atmospheric levels of oxygen. How this unique environment promotes cellular senescence is not completely understood, but the redox stress likely places these cells in a uniquely challenging environment. Thus, repair and maintenance of the lung must occur not only in an increased oxidative environment but also in one that is highly susceptible to multiple exogenous challenges, such as cigarette smoke, air pollutants, and infections, making it highly prone to cellular senescence. In concordance with this, expression of markers of senescence increase in the lung with physiological aging and with environmental exposures such as cigarette smoke (79, 80). Interestingly, *in vivo* elimination of cells expressing p19^{ARF}, a cell cycle regulator of

the *CDKN2A* locus, prevented increased expression of markers of senescence in aging lungs and improved lung function (81). It is also important to note that although the reprogramming factors Oct4, Sox2, Klf4, and c-Myc successfully rejuvenated several tissues in the presence of senescent cells, the lung did not undergo reprogramming. However, upon an exogenous insult such as increased genotoxic stress, *in vivo* reprogramming of the lung was achievable (82). This suggests that there may be some inherent differences in lung senescence compared with senescence in other tissues.

Senescence and Chronic Lung Diseases

Senescence is recognized as a physiological response in the repair process after injury, promoting the SASP-induced recruitment of fibroblasts and immune cells, angiogenesis, and even proliferative responses (83, 84). However, persistent senescence promotes disrepair. Senescence has been hypothesized to play a pathogenic role in two chronic, age-associated lung diseases: COPD and IPF (Figure 1). IPF and COPD are both complex disorders, and their full etiology is unknown. However, some risk factors, such as smoking and aging, are well established, and investigators have long suspected that they may be linked to cellular senescence (85). In support of this concept, numerous studies in human lung tissues show evidence of the accumulation of senescent cells in lungs from patients with COPD and patients with IPF (86–88), as well as in lungs from animal models of lung emphysema and fibrosis (89).

Growing evidence suggests that telomere shortening is a promoting factor of senescence and disease in COPD and IPF. Mutations in at least seven genes related to telomere maintenance have been found in families with inherited IPF (90–96), and short telomeres are found in the majority of patients (97, 98). Interestingly, in some families with mutations in telomere maintenance genes, emphysema, a component of COPD, can develop independently of or concurrently with IPF (99–101). The precise mechanism responsible for the disparate phenotypes is not known, but the development of emphysema requires a history of cigarette smoke exposure, suggesting that the

Figure 1. Senescence in lung injury and repair and in chronic lung diseases. After injury, senescence is activated during the wound-healing process to induce recruitment of fibroblasts and immune cells via the senescence-associated secretory phenotype (SASP) factors secreted by senescent epithelial cells. In normal lung tissue repair, macrophages and other immune cells clear the senescent cells, and progenitor AEC2 cells promote regeneration of the alveoli. In emphysema, the tissue-healing process is defective because of persistent senescence of progenitor alveolar and airway cells, blood vessel cells, and fibroblasts. Senescent cells release inflammatory SASP factors that promote transmission of senescence to other cells. Tissue repair in idiopathic pulmonary fibrosis lungs is characterized by loss of AEC2 and accumulation of basal epithelial cells and senescent fibroblasts that are resistant to apoptosis. Senescent fibroblasts produce excessive extracellular matrix components in the context of SASP. Illustration by Jacqueline Schaffer. AEC1/2 = alveolar epithelial type I/II; COPD = chronic obstructive pulmonary disease; IPF = idiopathic pulmonary fibrosis.

combination of genetic predisposition and environmental exposure can drive specific pathologies. The influence of environmental exposure in COPD development is further supported by studies which show that prenatal or childhood environmental exposure to cigarette smoke or pesticides might lead to the development of COPD (102).

Early evidence connecting COPD and cellular senescence stems from the observation that fibroblasts from explanted lungs had proliferative defects and expressed several markers of senescence (103, 104). Fibroblasts show a senescent phenotype characterized by high expression of proinflammatory mediators and impaired ability to respond to prorepair growth factors such as transforming growth factor- β (105, 106). In addition, alveolar type II epithelial cells (AEC2), airway basal cells, endothelial cells, and pulmonary artery smooth muscle cells can have a senescent

phenotype in COPD. *In vitro* and mouse model studies demonstrate that cigarette smoke promotes senescence in lung epithelial cells. Interestingly, and consistent with the complexity of the disease, mice with global or airway deficiency of p16 are not protected from cigarette smoke-induced emphysema, inflammation, and deterioration of lung function (25). However, mice with deficiency of p53 have reduced expression of markers of senescence, inflammation, and proapoptotic genes (107). The senescence phenotype in COPD epithelial cells has been found to be associated with increased activation of NF- κ B signaling as well as a robust inflammatory SASP repertoire, including IL-6, IL-8, IL-1 β , and TNF- α . Persistent senescence with the secretion of proinflammatory SASP factors is hypothesized to cause tissue destruction and exhaustion of progenitor epithelial

cells and to halt the wound-healing process in the lung.

In this context of IPF, markers of senescence have been observed in AEC2 cells, bronchial epithelial cells, and fibroblasts (86, 87, 108, 109). Endoplasmic reticulum stress markers and accumulation of dysfunctional mitochondria were observed in AEC2 cells isolated from human IPF lungs (79, 110–113). These varied stressors might enhance the fraction of senescent cells in the lung. Senescent epithelial cells might secrete abundant SASP factors that promote abnormal and persistent fibroblast accumulation. Senescent IPF fibroblasts secrete exaggerated amounts of ECM components and are resistant to apoptosis, promoting the development of fibrosis (Figure 1) (114–116). Recent studies demonstrate that telomere dysfunction can limit the function of the immune system in patients with short telomeres and IPF (117, 118). It is

intriguing to speculate that short telomeres may increase the fraction of senescent cells at the same time that they limit the clearance of these potentially harmful cells by impaired immune cells.

The detrimental role of senescence in lung fibrosis is supported by the bleomycin-induced lung fibrosis model, in which inhibition of NOX4 (NADPH-dependent oxidase 4) reduces markers of senescence and reverts lung fibrosis (119). Deficiency of p53 or elimination of p16-expressing cells does not change bleomycin-induced lung fibrosis, but lung function during exercise is improved by selective ablation of p16-expressing cells or by a senolytic agent (small molecules that cause apoptosis of senescent cells), such as dasatinib or quercetin, administered during the fibrotic phase of the bleomycin-induced injury (Days 14–28) (109). Importantly, earlier administration (Days 7–21) of the senolytic agent quercetin is sufficient to mitigate bleomycin-induced fibrosis in aged mice (120). Similarly, the use of senolytics in an *ex vivo* model of bleomycin-treated mouse lung tissue slice cultures effectively diminished senescence markers and expression of SASP factors (121). The translational potential of these studies is indicated by the well-established accumulation of senescent fibroblasts in IPF (87, 109, 121) and the finding that quercetin can reverse their resistance to death ligand-induced apoptosis (120). Further investigations are needed to understand the differences in the repair process and senescence phenotype in IPF and COPD lung cells.

How Does Cellular Senescence Contribute to Lung Disease Pathogenesis?

It is likely that numerous interrelated aspects contribute to lung disease pathogenesis, but for simplicity, these are organized into the following classes: 1) loss of stem/progenitor function, 2) cell-intrinsic consequences, and 3) cell-extrinsic consequences.

Loss of stem/progenitor function.

Perhaps the most obvious consequence of cellular senescence is loss of proliferative capacity in response to growth-promoting stimuli. After injury, the lung demonstrates a remarkable capacity to repair and for lung cells to proliferate. Under normal conditions, functional stem/progenitor cells are required for the restoration of lung

function after injury. Despite their dormant appearance, experimental models have demonstrated that subtypes of cells within the lung have the capacity to proliferate in response to injury. Indeed, AEC2-specific telomere dysfunction is sufficient to sensitize mice to bleomycin challenge, highlighting the importance of a proliferative response in these cells after challenge (97). More recently, a Wnt-dependent alveolar epithelial progenitor cell lineage—a subpopulation of AEC2—has been identified. These cells have the capacity to restore the alveolar epithelium (122, 123) and appear to be dependent on neighboring mesenchymal cells to maintain their stem cell capacity. How cellular senescence may impact these cells is not known, but these findings highlight the importance of the niche in maintaining epithelial stem/progenitor cells. In addition, during lung injury, bone marrow-derived mesenchymal stem cells have the potential to contribute to the repair of lung tissue. There is accumulating evidence that with aging, mesenchymal stem cell senescence diminishes the capacity to prevent fibrotic changes after bleomycin-induced injury. In these animal models, there is increased illness severity with a proinflammatory and profibrotic response (124). Thus, cellular senescence of stem cells can potentially disrupt the ability to respond to challenge and repair when required.

Cell-intrinsic consequences. Not unexpectedly, senescence can disrupt the normal metabolic function of a cell. Oxidative stress and telomere dysfunction trigger a p53-dependent DDR that has numerous downstream effects. In addition to halting the cell cycle, p53 directly inhibits the expression of PGC1- α (peroxisome proliferative activated receptor- γ coactivator 1 α) and PGC1- β , key regulators of mitochondrial biogenesis (69, 125). Thus, senescent cells likely have compromised ATP production that can disrupt normal metabolic function. This phenomenon could potentially have detrimental consequences for tissue homeostasis. In this context, elimination of senescent cells may have a favorable consequence by eliminating a “deadbeat” cell and a potential source of proinflammatory signals.

Cell-extrinsic consequences. It has recently been demonstrated that the composition of the SASP can differ

significantly in terms of the quantity and quality of secreted proteins, depending on the mechanism by which cell senescence is induced in an individual cell type (3). Consequently, the composition of the SASP associated with aging is highly variable, mostly because senescence occurs simultaneously in different cell types and organs. Differences in the SASP components are also reflected in the capacity to induce paracrine senescence, or the induction of senescence in neighboring cells. Senescence can thus result in sterile inflammation, stem cell dysfunction, and organ disrepair.

Future Considerations

We are left with a question: Is it better to die or senesce? The answer to this cell fate decision is not simple. The capacity of senescent cells to cause damage has recently been illustrated in experimental studies in young mice that received injections of senescent cells (43). These mice showed propagation of senescence and impairment of multiple functions, including physical endurance, food intake, and daily activity. Because a feature of senescent cells is the upregulation of prosurvival factors to inhibit apoptosis, this mechanism (senescent cell antiapoptotic pathways) has been targeted to selectively eliminate such cells (126). Senescent cell antiapoptotic pathways involve Bcl-2/Bcl-X_L, PI3K/AKT, p53/p21/serpins, dependence receptors/tyrosine kinases, and hypoxia-inducible factor-1 α pathways. Currently, senotherapy includes senolytic agents that inhibit antiapoptotic proteins such as navitoclax and ABT-737 (activator of basal transcription 37) that inhibit the antiapoptotic function of BCL-2 family members, as well as HSP90 (heat shock protein 90) inhibitors that allow senescent cells to initiate apoptosis (127–130). Notably, administration of senolytic agents both in young animals and in very old mice attenuated the injury caused by senescent cells, extended lifespan, and delayed age-related conditions. Additional research will be needed to delineate the conditions under which senolytics may be beneficial and when they may be harmful, owing to limited stem cell pools required to promote tissue repair.

Senotherapeutics are currently considered a viable option for therapy; the

idea is that intermittent treatment would be required to eliminate senescent cell accumulation. Recently, a peptide that inhibits MDM2–p53 interactions for triggering elimination of senescent cells is in a phase 1 clinical trial for the treatment of osteoarthritis. In addition to senolytics, SASP suppression or neutralization molecules such as kaempferol and apigenin (131), which act by modulating the NF- κ B pathway (132), are also being explored in senotherapy. In the context of lung fibrosis, animal studies suggest that senolytics might have therapeutic benefits (109, 120). Recently, a first-in-human pilot study was conducted for IPF in which the patients

were orally administered two senolytic drugs: dasatinib and quercetin (133). The patients showed improved mobility gain of up to 5%. These results look promising; however, the study sample was small. We suggest that caution is warranted as therapies are pursued to promote or prevent cellular senescence until it is clear what the full set of consequences will be.

A number of outstanding questions need to be further addressed to delineate the role of cellular senescence in chronic lung diseases and in lung aging: 1) Do mechanisms and markers of senescence and SASP differ according to cell type in the

lung? Which ones contribute to aging versus disease? 2) What factors (environmental and genetic) contribute to the differences observed in senescence, inflammation, injury, and repair in COPD versus IPF? 3) Will certain senolytics (cell type specific) be required to address the differences between these chronic lung conditions? In summary, cellular senescence in the lung needs to be further explored as a causal mechanism and for development of novel therapeutics to address chronic lung diseases. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

- Blagosklonny MV. Cell cycle arrest is not senescence. *Aging (Albany NY)* 2011;3:94–101.
- Terzi MY, Izmirli M, Gogebakan B. The cell fate: senescence or quiescence. *Mol Biol Rep* 2016;43:1213–1220.
- Campisi J, d'Adda di Fagnana F. Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol* 2007;8:729–740.
- Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961;25:585–621.
- Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16^{INK4a}. *Cell* 1997;88:593–602.
- López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013;153:1194–1217.
- Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 2005;120:513–522.
- Giaimo S, d'Adda di Fagnana F. Is cellular senescence an example of antagonistic pleiotropy? *Aging Cell* 2012;11:378–383.
- Wright WE, Shay JW. Time, telomeres and tumours: is cellular senescence more than an anticancer mechanism? *Trends Cell Biol* 1995;5:293–297.
- Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, et al. Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998;279:349–352.
- Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature* 1990;345:458–460.
- d'Adda di Fagnana F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, et al. A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 2003;426:194–198.
- Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol* 2010;5:99–118.
- Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21^{CIP1}, but not p16^{INK4a}. *Mol Cell* 2004;14:501–513.
- Rodier F, Coppé JP, Patil CK, Hoeijmakers WA, Muñoz DP, Raza SR, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol* 2009;11:973–979. [Published erratum appears in *Nat Cell Biol* 11:1272.]
- Rajagopalan S, Long EO. Cellular senescence induced by CD158d reprograms natural killer cells to promote vascular remodeling. *Proc Natl Acad Sci USA* 2012;109:20596–20601.
- Ovadya Y, Landsberger T, Leins H, Vadai E, Gal H, Biran A, et al. Impaired immune surveillance accelerates accumulation of senescent cells and aging. *Nat Commun* 2018;9:5435.
- Campisi J. Aging, cellular senescence, and cancer. *Annu Rev Physiol* 2013;75:685–705.
- Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. *Genes Dev* 2010;24:2463–2479.
- Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA- β gal) activity, a biomarker of senescent cells in culture and in vivo. *Nat Protoc* 2009;4:1798–1806.
- Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin EC, et al. Senescence-associated β -galactosidase is lysosomal β -galactosidase. *Aging Cell* 2006;5:187–195.
- Lawless C, Wang C, Jurk D, Merz A, Zglinicki Tv, Passos JF. Quantitative assessment of markers for cell senescence. *Exp Gerontol* 2010;45:772–778.
- Ciscione SW, Teo YV, Neretti N. The chromatin landscape of cellular senescence. *Trends Genet* 2016;32:751–761.
- Zhang R, Chen W, Adams PD. Molecular dissection of formation of senescence-associated heterochromatin foci. *Mol Cell Biol* 2007;27:2343–2358.
- Sundar IK, Rashid K, Gerloff J, Li D, Rahman I. Genetic ablation of p16^{INK4a} does not protect against cellular senescence in mouse models of chronic obstructive pulmonary disease/emphysema. *Am J Respir Cell Mol Biol* 2018;59:189–199.
- Wiley CD, Flynn JM, Morrissey C, Lebofsky R, Shuga J, Dong X, et al. Analysis of individual cells identifies cell-to-cell variability following induction of cellular senescence. *Aging Cell* 2017;16:1043–1050.
- Sapieha P, Mallette FA. Cellular senescence in postmitotic cells: beyond growth arrest. *Trends Cell Biol* 2018;28:595–607.
- Kuilman T, Michaloglou C, Vredevelde LC, Douma S, van Doorn R, Desmet CJ, et al. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* 2008;133:1019–1031.
- Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, et al. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat Commun* 2015;6:8472.
- Kadota T, Fujita Y, Yoshioka Y, Araya J, Kuwano K, Ochiya T. Emerging role of extracellular vesicles as a senescence-associated secretory phenotype: insights into the pathophysiology of lung diseases. *Mol Aspects Med* 2018;60:92–103.
- Chien Y, Scuoppo C, Wang X, Fang X, Balgley B, Bolden JE, et al. Control of the senescence-associated secretory phenotype by NF- κ B promotes senescence and enhances chemosensitivity. *Genes Dev* 2011;25:2125–2136.
- Freund A, Patil CK, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J* 2011;30:1536–1548.
- Barakat DJ, Zhang J, Barberi T, Denmeade SR, Friedman AD, Paz-Priel I. CCAAT/enhancer binding protein β controls androgen-deprivation-induced senescence in prostate cancer cells. *Oncogene* 2015;34:5912–5922.

34. Elzi DJ, Lai Y, Song M, Hakala K, Weintraub ST, Shiao Y. Plasminogen activator inhibitor 1: insulin-like growth factor binding protein 3 cascade regulates stress-induced senescence. *Proc Natl Acad Sci USA* 2012;109:12052–12057.
35. Kortlever RM, Higgins PJ, Bernards R. Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. *Nat Cell Biol* 2006;8:877–884.
36. Hubackova S, Krejčíková K, Bartek J, Hodny Z. IL1- and TGFβ-Nox4 signaling, oxidative stress and DNA damage response are shared features of replicative, oncogene-induced, and drug-induced paracrine 'bystander senescence'. *Aging (Albany NY)* 2012;4:932–951.
37. Yougbaré I, Tai WS, Zdravac D, Oswald BE, Lang S, Zhu G, *et al.* Activated NK cells cause placental dysfunction and miscarriages in fetal alloimmune thrombocytopenia. *Nat Commun* 2017;8:224.
38. Mosteiro L, Pantoja C, de Martino A, Serrano M. Senescence promotes in vivo reprogramming through p16^{INK4a} and IL-6. *Aging Cell* 2018;17:e12711.
39. Loaiza N, Demaria M. Cellular senescence and tumor promotion: is aging the key? *Biochim Biophys Acta* 2016;1865:155–167.
40. Velarde MC, Demaria M, Campisi J. Senescent cells and their secretory phenotype as targets for cancer therapy. *Interdiscip Top Gerontol* 2013;38:17–27.
41. Davalos AR, Coppe JP, Campisi J, Desprez PY. Senescent cells as a source of inflammatory factors for tumor progression. *Cancer Metastasis Rev* 2010;29:273–283.
42. Kyllis N, Tuza ZA, Stan GB, Polizzi KM. Tools for engineering coordinated system behaviour in synthetic microbial consortia. *Nat Commun* 2018;9:2677.
43. Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, *et al.* Senolytics improve physical function and increase lifespan in old age. *Nat Med* 2018;24:1246–1256.
44. Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, *et al.* Naturally occurring p16^{INK4a}-positive cells shorten healthy lifespan. *Nature* 2016;530:184–189.
45. Zhu Y, Tchikonja T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, *et al.* The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell* 2015;14:644–658.
46. Bargonetti J, Manfredi JJ. Multiple roles of the tumor suppressor p53. *Curr Opin Oncol* 2002;14:86–91.
47. Beauséjour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, *et al.* Reversal of human cellular senescence: roles of the p53 and p16 pathways. *EMBO J* 2003;22:4212–4222.
48. Sherr CJ, Bertwistle D, den Besten W, Kuo ML, Sugimoto M, Tago K, *et al.* p53-Dependent and -independent functions of the Arf tumor suppressor. *Cold Spring Harb Symp Quant Biol* 2005;70:129–137.
49. Deng Y, Chan SS, Chang S. Telomere dysfunction and tumour suppression: the senescence connection. *Nat Rev Cancer* 2008;8:450–458.
50. Salama R, Sadaie M, Hoare M, Narita M. Cellular senescence and its effector programs. *Genes Dev* 2014;28:99–114.
51. Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, Bonner-Weir S, *et al.* p16^{INK4a} induces an age-dependent decline in islet regenerative potential. *Nature* 2006;443:453–457.
52. Xu Y, Li N, Xiang R, Sun P. Emerging roles of the p38 MAPK and PI3K/AKT/mTOR pathways in oncogene-induced senescence. *Trends Biochem Sci* 2014;39:268–276.
53. Greider CW. Telomeres. *Curr Opin Cell Biol* 1991;3:444–451.
54. Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, *et al.* Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci USA* 1992;89:10114–10118.
55. Rossiello F, Herbig U, Longhese MP, Fumagalli M, d'Adda di Fagnaga F. Irreparable telomeric DNA damage and persistent DDR signalling as a shared causative mechanism of cellular senescence and ageing. *Curr Opin Genet Dev* 2014;26:89–95.
56. Valdiglesias V, Giunta S, Fenech M, Neri M, Bonassi S. γH2AX as a marker of DNA double strand breaks and genomic instability in human population studies. *Mutat Res* 2013;753:24–40.
57. Kilbey A, Terry A, Cameron ER, Neil JC. Oncogene-induced senescence: an essential role for Runx. *Cell Cycle* 2008;7:2333–2340.
58. Bracken AP, Kleine-Kohlbrecher D, Dietrich N, Pasini D, Gargiulo G, Beekman C, *et al.* The Polycomb group proteins bind throughout the INK4A-ARF locus and are disassociated in senescent cells. *Genes Dev* 2007;21:525–530.
59. Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* 1999;397:164–168.
60. Kotake Y, Cao R, Viator P, Sage J, Zhang Y, Xiong Y. pRB family proteins are required for H3K27 trimethylation and Polycomb repression complexes binding to and silencing p16^{INK4a} tumor suppressor gene. *Genes Dev* 2007;21:49–54.
61. Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N, *et al.* Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature* 2006;444:633–637.
62. Courtis-Cox S, Jones SL, Cichowski K. Many roads lead to oncogene-induced senescence. *Oncogene* 2008;27:2801–2809.
63. Narita M, Núñez S, Heard E, Narita M, Lin AW, Hearn SA, *et al.* Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* 2003;113:703–716.
64. Barreiro E, Hussain SNA. Protein carbonylation in skeletal muscles: impact on function. *Antioxid Redox Signal* 2010;12:417–429.
65. Marin-Corral J, Minguella J, Ramirez-Sarmiento AL, Hussain SN, Gea J, Barreiro E. Oxidised proteins and superoxide anion production in the diaphragm of severe COPD patients. *Eur Respir J* 2009;33:1309–1319.
66. Wiley CD, Velarde MC, Lecot P, Liu S, Sarnoski EA, Freund A, *et al.* Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metab* 2016;23:303–314.
67. Jiang P, Du W, Mancuso A, Wellen KE, Yang X. Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. *Nature* 2013;493:689–693.
68. Ahmed EK, Rogowska-Wrzesinska A, Roepstorff P, Bulteau AL, Friguet B. Protein modification and replicative senescence of WI-38 human embryonic fibroblasts. *Aging Cell* 2010;9:252–272.
69. Passos JF, Nelson G, Wang C, Richter T, Simillion C, Proctor CJ, *et al.* Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Mol Syst Biol* 2010;6:347.
70. von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. *Ann N Y Acad Sci* 2000;908:99–110.
71. von Zglinicki T, Saretzki G, Döcke W, Lotze C. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? *Exp Cell Res* 1995;220:186–193.
72. Galvin O, Gardiner T, McDonald D. Mechanisms of hyperoxia-induced retinal endothelial cell death and senescence. *Invest Ophthalmol Vis Sci* 2013;54:5573.
73. Parikh P, Britt RD Jr, Manlove LJ, Wicher SA, Roesler A, Ravix J, *et al.* Hyperoxia-induced cellular senescence in fetal airway smooth muscle cells. *Am J Respir Cell Mol Biol* [online ahead of print] 3 Dec 2018; DOI: 10.1165/rcmb.2018-0176OC.
74. Chondrogianni N, Voutetakis K, Kapetanou M, Delitsikou V, Papaevgeniou N, Sakellari M, *et al.* Proteasome activation: an innovative promising approach for delaying aging and retarding age-related diseases. *Ageing Res Rev* 2015;23:37–55.
75. Liu J, Huang K, Cai GY, Chen XM, Yang JR, Lin LR, *et al.* Receptor for advanced glycation end-products promotes premature senescence of proximal tubular epithelial cells via activation of endoplasmic reticulum stress-dependent p21 signaling. *Cell Signal* 2014;26:110–121.
76. Burd CE, Sorrentino JA, Clark KS, Darr DB, Krishnamurthy J, Deal AM, *et al.* Monitoring tumorigenesis and senescence in vivo with a p16^{INK4a}-luciferase model. *Cell* 2013;152:340–351.
77. Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S, Campisi J. Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. *Nat Cell Biol* 2003;5:741–747.
78. Packer L, Fuehr K. Low oxygen concentration extends the lifespan of cultured human diploid cells. *Nature* 1977;267:423–425.
79. Bueno M, Brands J, Voltz L, Fiedler K, Mays B, St Croix C, *et al.* ATF3 represses PINK1 gene transcription in lung epithelial cells to control mitochondrial homeostasis. *Aging Cell* 2018;17:e12720.

80. Rashid K, Sundar IK, Gerloff J, Li D, Rahman I. Lung cellular senescence is independent of aging in a mouse model of COPD/emphysema. *Sci Rep* 2018;8:9023.
81. Hashimoto M, Asai A, Kawagishi H, Mikawa R, Iwashita Y, Kanayama K, et al. Elimination of p19^{ARF}-expressing cells enhances pulmonary function in mice. *JCI Insight* 2016;1:e87732.
82. Mosteiro L, Pantoja C, Alcazar N, Marión RM, Chondronasiou D, Rovira M, et al. Tissue damage and senescence provide critical signals for cellular reprogramming in vivo. *Science* 2016;354:aaf4445.
83. Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev Cell* 2014;31:722–733.
84. Telgenhoff D, Shroot B. Cellular senescence mechanisms in chronic wound healing. *Cell Death Differ* 2005;12:695–698.
85. Tudor RM. Aging and cigarette smoke: fueling the fire. *Am J Respir Crit Care Med* 2006;174:490–491.
86. Minagawa S, Araya J, Numata T, Nojiri S, Hara H, Yumino Y, et al. Accelerated epithelial cell senescence in IPF and the inhibitory role of SIRT6 in TGF- β -induced senescence of human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L391–L401.
87. Álvarez D, Cárdenas N, Sellarés J, Bueno M, Corey C, Hanumanthu VS, et al. IPF lung fibroblasts have a senescent phenotype. *Am J Physiol Lung Cell Mol Physiol* 2017;313:L1164–L1173.
88. Houssaini A, Breaux M, Kebe K, Abid S, Marcos E, Lipskaia L, et al. mTOR pathway activation drives lung cell senescence and emphysema. *JCI Insight* 2018;3:93203.
89. Hecker L, Logsdon NJ, Kurundkar D, Kurundkar A, Bernard K, Hock T, et al. Reversal of persistent fibrosis in aging by targeting Nox4-Nrf2 redox imbalance. *Sci Transl Med* 2014;6:231ra47.
90. Alder JK, Parry EM, Yegnasubramanian S, Wagner CL, Lieblich LM, Auerbach R, et al. Telomere phenotypes in females with heterozygous mutations in the dyskeratosis congenita 1 (DKC1) gene. *Hum Mutat* 2013;34:1481–1485.
91. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 2007;356:1317–1326.
92. Kropski JA, Mitchell DB, Markin C, Polosukhin VV, Choi L, Johnson JE, et al. A novel dyskerin (DKC1) mutation is associated with familial interstitial pneumonia. *Chest* 2014;146:e1–e7.
93. Alder JK, Stanley SE, Wagner CL, Hamilton M, Hanumanthu VS, Armanios M. Exome sequencing identifies mutant TINF2 in a family with pulmonary fibrosis. *Chest* 2015;147:1361–1368.
94. Cogan JD, Kropski JA, Zhao M, Mitchell DB, Rives L, Markin C, et al. Rare variants in RTEL1 are associated with familial interstitial pneumonia. *Am J Respir Crit Care Med* 2015;191:646–655.
95. Stanley SE, Gable DL, Wagner CL, Carlisle TM, Hanumanthu VS, Podlevsky JD, et al. Loss-of-function mutations in the RNA biogenesis factor NAF1 predispose to pulmonary fibrosis-emphysema. *Sci Transl Med* 2016;8:351ra107.
96. Stuart BD, Choi J, Zaidi S, Xing C, Holohan B, Chen R, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. *Nat Genet* 2015;47:512–517.
97. Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proc Natl Acad Sci USA* 2008;105:13051–13056.
98. Cronkhite JT, Xing C, Raghu G, Chin KM, Torres F, Rosenblatt RL, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008;178:729–737.
99. Alder JK, Guo N, Kembou F, Parry EM, Anderson CJ, Gorgy AI, et al. Telomere length is a determinant of emphysema susceptibility. *Am J Respir Crit Care Med* 2011;184:904–912.
100. Diaz de Leon A, Cronkhite JT, Katzenstein AL, Godwin JD, Raghu G, Glazer CS, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. *PLoS One* 2010;5:e10680.
101. Stanley SE, Chen JJ, Podlevsky JD, Alder JK, Hansel NN, Mathias RA, et al. Telomerase mutations in smokers with severe emphysema. *J Clin Invest* 2015;125:563–570.
102. Beyer D, Mitfessell H, Gillissen A. Maternal smoking promotes chronic obstructive lung disease in the offspring as adults. *Eur J Med Res* 2009;14:27–31.
103. Holz O, Zühlke I, Jaksztat E, Müller KC, Welker L, Nakashima M, et al. Lung fibroblasts from patients with emphysema show a reduced proliferation rate in culture. *Eur Respir J* 2004;24:575–579.
104. Müller KC, Welker L, Paasch K, Feindt B, Erpenbeck VJ, Hohlfeld JM, et al. Lung fibroblasts from patients with emphysema show markers of senescence in vitro. *Respir Res* 2006;7:32.
105. Togo S, Holz O, Liu X, Sugiura H, Kamio K, Wang X, et al. Lung fibroblast repair functions in patients with chronic obstructive pulmonary disease are altered by multiple mechanisms. *Am J Respir Crit Care Med* 2008;178:248–260.
106. Zhang J, Wu L, Qu JM, Bai CX, Merrilees MJ, Black PN. Pro-inflammatory phenotype of COPD fibroblasts not compatible with repair in COPD lung. *J Cell Mol Med* 2012;16:1522–1532.
107. Izzotti A, Cartiglia C, Longobardi M, Bagnasco M, Merello A, You M, et al. Gene expression in the lung of p53 mutant mice exposed to cigarette smoke. *Cancer Res* 2004;64:8566–8572.
108. Schuliga M, Pechkovsky DV, Read J, Waters DW, Blokland KEC, Reid AT, et al. Mitochondrial dysfunction contributes to the senescent phenotype of IPF lung fibroblasts. *J Cell Mol Med* 2018;22:5847–5861.
109. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, et al. Cellular senescence mediates fibrotic pulmonary disease. *Nat Commun* 2017;8:14532.
110. Bueno M, Lai YC, Romero Y, Brands J, St Croix CM, Kamga C, et al. PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. *J Clin Invest* 2015;125:521–538.
111. Kirkham PA, Barnes PJ. Oxidative stress in COPD. *Chest* 2013;144:266–273.
112. Meyer A, Zoll J, Charles AL, Charloux A, de Blay F, Diemunsch P, et al. Skeletal muscle mitochondrial dysfunction during chronic obstructive pulmonary disease: central actor and therapeutic target. *Exp Physiol* 2013;98:1063–1078.
113. Zank DC, Bueno M, Mora AL, Rojas M. Idiopathic pulmonary fibrosis: aging, mitochondrial dysfunction, and cellular bioenergetics. *Front Med (Lausanne)* 2018;5:10.
114. Cui H, Ge J, Xie N, Banerjee S, Zhou Y, Antony VB, et al. miR-34a inhibits lung fibrosis by inducing lung fibroblast senescence. *Am J Respir Cell Mol Biol* 2017;56:168–178.
115. Mora AL, Rojas M, Pardo A, Selman M. Emerging therapies for idiopathic pulmonary fibrosis, a progressive age-related disease. *Nat Rev Drug Discov* 2017;16:755–772.
116. Im J, Kim K, Hergert P, Nho RS. Idiopathic pulmonary fibrosis fibroblasts become resistant to Fas ligand-dependent apoptosis via the alteration of decoy receptor 3. *J Pathol* 2016;240:25–37.
117. Popescu I, Mannem H, Winters SA, Hoji A, Silveira F, McNally E, et al. Impaired cytomegalovirus immunity in idiopathic pulmonary fibrosis lung transplant recipients with short telomeres. *Am J Respir Crit Care Med* 2019;199:362–376.
118. Wagner CL, Hanumanthu VS, Talbot CC Jr, Abraham RS, Hamm D, Gable DL, et al. Short telomere syndromes cause a primary T cell immunodeficiency. *J Clin Invest* 2018;128:5222–5234.
119. Hecker L, Logsdon NJ, Kurundkar D, Kurundkar A, Bernard K, Hock T, et al. Reversal of persistent fibrosis in aging by targeting Nox4-Nrf2 redox imbalance. *Sci Transl Med* 2014;6:231ra47.
120. Hohmann MS, Habel DM, Coelho AL, Verri WA Jr, Hogaboam CM. Quercetin enhances ligand-induced apoptosis in senescent idiopathic pulmonary fibrosis fibroblasts and reduces lung fibrosis in vivo. *Am J Respir Cell Mol Biol* 2019;60:28–40.
121. Lehmann M, Korfei M, Mutze K, Klee S, Skronska-Wasek W, Alsafadi HN, et al. Senolytic drugs target alveolar epithelial cell function and attenuate experimental lung fibrosis ex vivo. *Eur Respir J* 2017;50:1602367.
122. Nabhan AN, Brownfield DG, Harbury PB, Krasnow MA, Desai TJ. Single-cell Wnt signaling niches maintain stemness of alveolar type 2 cells. *Science* 2018;359:1118–1123.
123. Zacharias WJ, Frank DB, Zepp JA, Morley MP, Alkhaleel FA, Kong J, et al. Regeneration of the lung alveolus by an evolutionarily conserved epithelial progenitor. *Nature* 2018;555:251–255.

124. Cárdenes N, Álvarez D, Sellarés J, Peng Y, Corey C, Wecht S, *et al.* Senescence of bone marrow-derived mesenchymal stem cells from patients with idiopathic pulmonary fibrosis. *Stem Cell Res Ther* 2018;9:257.
125. Sahin E, Colla S, Liesa M, Moslehi J, Müller FL, Guo M, *et al.* Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature* 2011;470:359–365.
126. Kirkland JL, Tchkonja T. Cellular senescence: a translational perspective. *EBioMedicine* 2017;21:21–28.
127. Zhu Y, Tchkonja T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, *et al.* Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell* 2016; 15:428–435.
128. Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, *et al.* Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med* 2016;22: 78–83.
129. Yosef R, Pilpel N, Tokarsky-Amiel R, Biran A, Ovadya Y, Cohen S, *et al.* Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. *Nat Commun* 2016;7:11190.
130. Fuhrmann-Stroissnigg H, Ling YY, Zhao J, McGowan SJ, Zhu Y, Brooks RW, *et al.* Identification of HSP90 inhibitors as a novel class of senolytics. *Nat Commun* 2017;8:422.
131. Lim H, Park H, Kim HP. Effects of flavonoids on senescence-associated secretory phenotype formation from bleomycin-induced senescence in BJ fibroblasts. *Biochem Pharmacol* 2015;96:337–348.
132. Kim JM, Lee EK, Kim DH, Yu BP, Chung HY. Kaempferol modulates pro-inflammatory NF- κ B activation by suppressing advanced glycation endproducts-induced NADPH oxidase. *Age (Dordr)* 2010; 32:197–208.
133. Justice JN, Nambiar AM, Tchkonja T, LeBrasseur NK, Pascual R, Hashmi SK, *et al.* Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. *EBioMedicine* 2019; 40:554–563.