Cellular senescence as a mechanism and target in chronic lung diseases

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Abstract

Cellular senescence is now considered an important driving mechanism for chronic lung diseases, particularly COPD and idiopathic pulmonary fibrosis. Cellular senescence is due to replicative and stress-related senescence with activation of p53 and $p16^{INK4a}$ respectively, leading to activation of $p21^{CIP1}$ and cell cycle arrest. Senescent cells secrete multiple inflammatory proteins known as the senescence-associated secretory phenotype (SASP), leading to low grade chronic inflammation, which further drives senescence. Loss of key anti-aging molecules sirtuin-1 and sirtuin-6 may be important in acceleration of aging and arises from oxidative stress reducing phosphatase PTEN, thereby activating PI3K (phosphoinositide-3-kinase) and mTOR (mammalian target of rapamycin). MicroRNA-34a, which is regulated by PI3K-mTOR signaling, plays a pivotal role in reducing sirtuin-1/6 and its inhibition with an antagomir results in their restoration, reducing markers of senescence, reducing SASP and reversing cell cycle arrest in epithelial cells from peripheral airways of COPD patients. MiR-570 is also involved in reduction of sirtuin-1 and cellular senescence and is activated by p38 MAP kinase. These miRNAs may be released from cells in extracellular vesicles that are taken up by other cells, thereby spreading senescence locally within the lung but outside the lung through the circulation; this may account for comorbidities of COPD and other lung diseases. Understanding the mechanisms of cellular senescence may result in new treatments for chronic lung disease, either by inhibiting PI3K-mTOR signaling, by inhibiting specific miRNAs or by deletion of senescent cells with senolytic therapies, already shown to be effective in experimental lung fibrosis.

Key words: telomere, senescence-associated secretory phenotype, sirtuin, microRNA, senolytic

Chronic lung diseases impose an enormous global health burden, particularly in the elderly (1). Despite important advances in therapy for many of these diseases, there remains a major unmet need for the development of safe and effective disease-modifying treatments. There are no drug treatments that reduce disease progression or mortality in chronic obstructive pulmonary disease (COPD), which now affects about 10% of people over 40 years, or in idiopathic pulmonary fibrosis (IPF) where current treatments have little clinical impact. Accelerated or premature aging appears to be important in several pulmonary diseases, including COPD and IPF, but may also be involved in asthma, bronchiectasis, pulmonary hypertension and lung infections. Although cystic fibrosis (CF) is a disease of childhood, advances in management, mean patients may survive into middle age, therefore the aging process may also be contributory. Increased understanding of accelerated aging pathways in chronic lung diseases is now identifying new therapeutic targets and the prospect of novel disease-modifying therapies.

There is growing knowledge of the complex molecular pathways involved in aging and how this process is accelerated in chronic disease. Hallmarks of aging include genomic instability, telomere shortening, reduced proteostasis (autophagy), mitochondrial dysfunction, defective nutrient sensing, epigenetic changes, exhaustion of stem cells and cellular senescence (2). Cellular senescence describes a state of cell cycle arrest in which the cells undergo phenotypic changes, which drive pathology in many age-related diseases.

Normal lung aging

In normal individuals, FEV_1 and FVC peak at around 25 years in men and then decline slowly with age(3). Changes in the flow-volume loop suggests that loss of lung volumes is largely due to narrowing of peripheral airways. Reduced lung function over time may be associated with decreased lung elasticity and enlargement of alveolar spaces (termed “senile emphysema”), resulting in reduced gas transfer, but insufficient to cause symptoms or impaired oxygenation. The aged lung may also be more susceptible to damage by environmental stresses, such as cigarette smoking and this may be due to increased oxidative stress in aging lungs as a result of reduced antioxidants (4). There may also be a low grade inflammation in the peripheral airways and lung parenchyma (5).

There may be increased susceptibility to lung infections as a result of impaired mucociliary clearance and reduced innate and adaptive immunity associated with aging (6).

Cellular senescence

Cellular senescence is a hallmark of aging and may develop after repeated cellular division due to progressive attrition of telomeres, with activation of DNA damage-response pathways which results in the activation of the tumor suppressor p53 (replicative senescence). Alternatively, it may result from cellular stress, for example oxidative stress, ionizing radiation or cytotoxic therapies, which activate the cyclin-dependent kinase inhibitor $p16^{INK4}$ (stress-related senescence). These pathways may interact and both activate the cyclin-dependent kinase inhibitor $p21^{CIP1}$ leading to cell cycle arrest through inhibition of cyclin-dependent kinase-2/4 (7, 8) (**Fig. 1**). Acute cellular senescence may be beneficial during embryonic development, wound healing and tissue repair processes (8). Cellular senescence was considered to protect against malignancy, however, accumulation of senescent cells over time in aging animals results in tissue dysfunction, age-related diseases and shortening of lifespan (9). Indeed, elimination of naturally occurring senescent cells which express $p16^{INK4a}$ by a caspase-dependent mechanism, results in a marked increase in lifespan (over 30%) of normally aging mice due to reduced organ failure and cancer development (10). Furthermore, transplanting even small numbers of senescent cells into elderly mice leads to the spread of senescence and increased organ failure and mortality, again indicating that senescent cells can drive diseases of aging (11).

Senescent cells, including alveolar epithelial and endothelial cells, accumulate in the lungs of COPD patients (12-14). Oxidative stress, as a result of cigarette smoke exposure, is likely to be important inducer of senescence in COPD, as exposure to cigarette smoke increases senescence markers in airway epithelial cells (15). Similarly, in IPF there is senescence in alveolar epithelial cells and fibroblasts (16), which in some cases is due to shorter telomeres (17). It is likely that the accumulation of senescent cells leads to progression through small airway fibrosis and alveolar cell loss (emphysema) in COPD and progressive lung fibrosis in IPF. The reason why some elderly smokers develop COPD and others IPF (and some both) is not well understood, as there are

common pathways of senescence (18). Cellular senescence may also be linked to an independent increase in risk of lung cancer in COPD, IPF and combined pulmonary fibrosis-emphysema patients (19). Increased lung senescent cells are also seen patients with other chronic lung conditions, including adult cystic fibrosis (20), bronchiectasis (21) and pulmonary arterial hypertension (22), although so far little research into the role of senescence in these diseases.

Cellular senescence is usually linked to other characteristics of aging, such as telomere attrition, mitochondrial dysfunction, and defective autophagy, all of which are found in chronic lung diseases (23). Stem cells, including alveolar type 2 cells and mesenchymal stem cells, are particularly susceptible to senescence, leading to stem cell exhaustion and failure of repair mechanisms. Senescence is also found in cells outside the lung, such as circulating endothelial progenitor cells in COPD (24) and in bone marrow-derived stem cell in IPF (25). Senescence of both adaptive and innate immune cells (immunosenescence) may result in impaired immune responses and increased susceptibility to infections, cancer and autoimmunity (26). In COPD patients, increased senescent $CD28^{null}$ CD8+ T-lymphocytes and macrophages expressing the senescence marker p21 are found in the lungs (27, 28).

Senescence-associated secretory phenotype

In contrast to apoptotic cells, senescent cells are metabolically active and may affect other cells via secretion of multiple inflammatory proteins, described as the senescence-associated secretory phenotype (SASP) (29, 30) (**Fig. 1**) The SASP response is activated by $p21^{CIP1}$, which results in activation of p38 mitogen-activated protein (MAP) kinase and Janus-activated kinases (JAK). This results in the activation of nuclear factor-κB (NF-κB), and secretion of proinflammatory cytokines (IL-1β, IL-6, TNF-α), growth factors (vascular-endothelial growth factor – VEGF, TGF-β) chemokines (CXCL1, CXCL8, CCL2) and matrix metalloproteinases (MMP-2, MMP-9), which are all increased in age-related diseases, including COPD. Plasminogen activator inhibitor-1 (PAI-1), another characteristic SASP protein, is increased in the sputum, sputum macrophages and alveoli of patients with COPD (31) and in IPF (32), suggesting that cellular senescence may be contributory to the chronic inflammation seen in these diseases. CXCL8 binds to the chemokine receptor CXCR2, and

may subsequently induce DNA damage and cellular senescence. Blocking CXCR2 with specific antagonists reduces replicative and stress-induced senescence (33). Activation of $p16^{INK4a}$ also activates NADPH oxidases, leading to a further increase in oxidative stress and NF-κB activation (34). JAK inhibitors inhibit the SASP response and reduce frailty in aging mice (35). The SASP also includes activation of the NRLP3 inflammasome resulting in the secretion of IL-1β further perpetuating the inflammatory response (36). The components of SASP differ between cell types and inducing factors, but nevertheless makes an important contribution to the low grade inflammation seen in many chronic lung diseases (37, 38). It may also account explain the increased baseline secretion of inflammatory mediators characteristic of chronic lung diseases such as COPD.

PI3K-mTOR signaling

Phosphoinositide-3-kinase (PI3K)-mammalian target of rapamycin (mTOR) signaling is pivotal in mediating cellular senescence and aging. Inhibition of this pathway at different points extends lifespan from yeast to mammals (39). Rapamycin Inhibits mTOR and increases longevity in mice (40), possibly via reduced SASP, which attenuates the paracrine spread of senescence (41, 42). mTOR comprises two complexes: mTORC1 is inhibited by rapamycin and activated by cellular stress (including oxidative stress), caloric nutrients and growth factors; mTORC2 is also activated by growth factors but not inhibited by rapamycin. Inhibition of mTORC1 by rapamycin may up-regulate mTORC2, reducing its efficacy. Dual inhibitors of mTORC1 and 2 might therefore be more effective in chronic therapy.

PI3K activation, measured by phosphorylation of the downstream kinase AKT, is increased in lungs, epithelial cells and peripheral blood mononuclear cells (PBMC) of COPD patients (43) (**Fig. 2**). mTOR activation by PI3K has many effects, including inhibition of FOXO transcription factors, which regulate antioxidants and longevity. mTORC1 is activated in COPD peripheral lung and peripheral blood mononuclear cells (PBMC), demonstrated by increased phosphorylated S6 kinase, and is effectively inhibited by rapamycin (44, 45). Gene expression profiling also reveals increased mTOR pathway activation in aged COPD lungs compared to age-matched controls (46). mTOR is also activated in IPF(47, 48).

There are endogenous inhibitory mechanisms to limit the activation of the mTORC1 pathway. PI3K is inhibited by the membrane tyrosine phosphatases, PTEN (phosphatase tensin homologue) and SHIP-1 (SH2-containing inositol-5'-phosphatase-1). The catalytic sites of both phosphatases include cysteines that are susceptible to oxidation and are therefore inhibited by oxidative stress (49), increasing PI3K activity. The activity and expression of PTEN are considerably reduced in the lungs of COPD patients, thus linking oxidative stress to activation of PI3K in COPD (50).

mTOR is endogenously inhibited by 5'-adenosine monophosphate activated kinase (AMPK) which is activated when intracellular ATP concentrations are low. AMPK is activated by caloric restriction, a well-recognized mechanism for extending lifespan (51). Metformin also activates AMPK, thereby inhibiting mTOR and extending lifespan (52). As such, the mTOR pathway maybe important in driving senescence in COPD so that inhibiting this pathway may be a valuable therapeutic approach in the future (53). An AMP activator reduces senescence in small airway epithelial cells in response to cigarette smoke extract *in vitro* and metformin reduces senescence and the development of elastase-induced emphysema and inflammation in mice (54). Metformin also inhibits and reverses fibrosis in the bleomycin mouse model and reduces fibrosis in IPF myofibroblasts (48).

Reduced anti-aging molecules

There are several endogenous molecules that counteract cellular senescence and defective production of these anti-aging molecules may accelerate aging and therefore may play a role in the accelerated lung aging in chronic lung disease (55).

Silent information regulators or sirtuins are NAD^+-dependent protein deacetylases that are highly conserved across all species and prolong lifespan through resisting cellular stress, maintaining genomic stability and regulating metabolism (56). Although 7 sirtuins have been identified in mammals, sirtuin-1 and sirtuin-6, are associated with lifespan prolongation across many species. Sirtuin-1 deacetylates several transcription factors and regulatory proteins that are involved in inflammation, antioxidant expression, DNA repair, mitochondrial function, proteostasis, including autophagy. Sirtuin-1 inhibits cellular senescence and PI3K-mTOR signaling and restores defective

autophagy (**Fig. 2**). More specifically, sirtuin-1 activates FOXO3a, which regulates antioxidants (superoxide dismutases and catalase), activates PGC-1α, a transcription factor that maintains mitochondrial function, inhibits p53-induced senescence and inhibits activated NF-κB thereby suppressing SASP. Sirtuin-1 suppresses K-RAS driven lung adenocarcinomas, indicating that reduced sirtuin-1 in COPD may also increase the risk of lung cancer (57).

There is a great reduction in sirtuin-1 mRNA and protein in peripheral lung, airway epithelial cells and circulating PBMC of COPD patients (58-60). Normally, sirtuin-1 directly inhibits mTOR and through this mechanism also increases autophagy (50), however following oxidative stress, sirtuin-1 is reduced via reduced expression of PTEN, leading to activation of the PI3K-mTOR pathway. The dietary polyphenol resveratrol, found in the skin of red fruits and in red wine, activates sirtuin-1, but is a weak activator and is poorly absorbed from the gut. This has led to the development of more potent, orally bioavailable, sirtuin-1 activating compounds (STACs) and NAD+ supplements that are in development for diseases of premature aging (61). Sirtuin-6, an ADP-ribosylase and protein deacetylase, regulates, telomere length, DNA repair, NF-κB, metabolic homeostasis and extends lifespan (62). Sirtuin-6 also activates the transcription factor Nrf2, which is an important regulator of multiple antioxidant genes (63). Sirtuin-6 mRNA and protein expression are reduced in COPD lungs and small airway epithelial cells (58, 60), and in airway epithelial cells is reduced by cigarette smoke exposure, leading to senescence and impaired autophagy (64).

A role for sirtuins in other lung diseases is less well defined. Sirtuin-1 is reduced in PBMC from elderly patients with severe asthma and associated with increased IL-4 secretion as a result of increased acetylation of the transcription factor GATA-3 (65). Sirtuin-1 knockout in mice enhances lung fibrosis, and sirtuin-1 inhibits TGF-β fibroblast activation. Paradoxically sirtuin-1 is increased in the lungs of IPF patients, perhaps as a compensatory mechanism (66).

Key role of microRNA

MicroRNAs (miRNA) are small non-coding single-stranded RNAs (18-22 nucleotides) that regulate post-transcriptional gene expression through inducing degradation of mRNA or inhibiting translation by binding to complimentary sequences on the 3'-untranslated regulatory region of mRNAs.

Increasing evidence indicates that miRNA may play an key role in in the aging process through the regulation of several regulatory proteins that are involved in senescence, including p16 (miR-24), SASP response (miR-146), p53 (miR-885-5p) and sirtuin-1 expression (miR-34a) (67).

MiR-34a inhibits sirtuin-1 and shows increased expression in peripheral lungs and epithelial cells of COPD patients, and is correlated with increased expression of senescence markers in lung cells (60). MiR-34a also regulates sirtuin-6, but not other sirtuins. MiR-34a is increased by oxidative stress through activation of PI3K-mTOR signaling and leading to a parallel reduction in sirtuin-1 and sirtuin-6 (**Fig. 2**), whereas other sirtuins are unchanged, as in COPD lungs. Importantly, an antagomir of miR-34a restores sirtuin-1 and sirtuin-6 in senescent small airway epithelial cells from COPD patients, reduces markers of cellular senescence (p16, p21, p53), reduces the SASP response (TNF-α, IL-1β, IL-6, CCL2, CXCL8, MMP9), and increases proliferation of senescent epithelial cells by reversing cell cycle arrest (60). MiR-34a is also increased in COPD macrophages and may be associated with impaired phagocytosis and uptake of apoptotic cells (efferocytosis) observed in this disease (68). Another miRNA, miR-570, also inhibits sirtuin-1 (but not sirtuin-6) and is activated via p38 MAP kinase and AP-1. MiR-570 is increased in COPD peripheral lung and small airway epithelial cells. An antagomir restores sirtuin-1, reduces senescence markers and reverses cell cycle arrest (69). This suggests that blocking specific miRNAs may result in rejuvenation of senescent COPD cells. MiR-126, plays an important role in endothelial cell function and maintaining vascular integrity. It is reduced in endothelial progenitor cells and airway epithelial cells from COPD patients and may regulate the DNA damage response pathway that is linked to cellular senescence (70).

Extracellular vesicles as propagators of senescence

Extracellular vesicles (EV) include microvesicles that bud from the cell membrane and exosomes derived from endoplasmic reticulum and contain proteins and RNA, including miRNAs. EV are exported from cells and are taken up by other cells, thus serving as a means of intercellular communication (71). EVs may also reach the circulation from the lung and this spread senescence

to other organs(72). As such, miRNAs, such as miR-34a, miR-570 and miR-126, could be transported to other cells within the lung or through the circulation to other organs, which could provide a mechanism explaining multimorbidity and the comorbidities of COPD and other chronic lung diseases (73) (**Fig. 2**). EVs may also contain could also proteins and other types if RNA. For example, exosomes isolated from bronchial lavage fluid of IPF patients contain the protein WNT-5a, which induces fibroblast proliferation (74).

Implications for therapy

Great progress has been made recently in understanding the molecular mechanisms involved in cellular senescence in chronic lung diseases. It is unrealistic to achieve reversal of normal aging, but may be more likely to fine tune the pathways that result in premature senescence. However, the reversal of senescence (rejuvenation) in small airway epithelial cells of COPD patients by specific antagomirs suggests that some reversal of senescence might be possible in the future (60, 69). Several new therapeutic targets have been identified, leading to the development of senotherapies (**Table 1**) (75). Existing drugs (such as metformin and rapamycin) may be repurposed and novel drugs developed by screening and rational drug design. The potential for these drugs is that they may treat the common pathways of multimorbidities (73). Future therapies may also involve lifestyle interventions, including diet and greater physical activity (76).

Senolytic therapy

Removal of senolytic cells expressing $p16^{INK4a}$, using a caspase-activated system, significantly prolongs lifespan in mice by delaying the development of organ failure and cancers (10). Using this approach, pulmonary fibrosis induced by bleomycin and its associated inflammatory response is prevented in mice (16). Senolytics are drugs that mimic this effect by inducing apoptosis in senescent cells while having little or no effect on proliferating cells (77). Several senolytic drugs have been identified by screening and include the naturally occurring polyphenol quercetin and the cytotoxic agent navitoclax (ABT263), which inhibits the Bcl2 family of anti-apoptotic proteins. The senolytic

cocktail of dasitinib and quercetin reduced senolytic cells and the SASP response in aging mice and intermittent therapy prolonged lifespan by 36% and reduced mortality risk by 65% (11) A combination of quercetin and navitoclax is also effective in protecting against bleomycin-induced pulmonary fibrosis in mice (16) and navitoclax also reduces senescence associated with lung irradiation by selectively eliminating senescent type 2 pneumocytes (78). The combination of quercetin and dasitinib also reduced senescent type 2 pneumocytes in bleomycin-induced fibrosis in mice, and p16 and SASP (79). A recent open-label pilot study in IPF patients showed that the combination of quercitin and dasitinib improved exercise performance and was relatively well tolerated (80). Another approach is to target FOXO4, which maintains senescent cell viability through binding to p53. A cell penetrant D-retro-inverso peptide FOXO4-DRI interferes with this interaction so that cells become apoptotic and *in vivo,* it counteracts chemotherapy-induced senescence in both wild-type and a strain of fast aging mice (81). However, due to different mechanisms of senescence in different cell types, it may be important to select senolytic therapies that are most effective for the predominant mechanism of senescence involved in each disease. Several biotechnology and pharmaceutical companies are now searching for safe and effective senolytic therapies (75). It is envisaged that these treatments may be given intermittently, perhaps yearly by inhalation, to remove accumulated senescent cells, but to prevent healthy cells from becoming senescent it may be important to inhibit the development of senescence by targeting the signaling pathways that produce it.

Targeting senescence pathways

Greater understanding of the mechanisms involved in premature senescence has highlighted several potential therapies for inhibiting accelerated aging in lung disease. Inhibitors of the PI3K-mTOR pathway may extend lifespan. Rapamycin induces autophagy, increases sirtuin-1 and extends the lifespan of all species tested, including mammals (53). Rapamycin effectively inhibits activated mTOR in COPD cells *in vitro* and *in vivo* in mice (44, 45) and inhibits bleomycin-induced pulmonary fibrosis in mice (82). However, rapamycin and its analogues (rapalogs) commonly induce adverse effects, including mouth ulceration, anemia, pneumonitis and may delay wound healing, making long-term administration problematic. Low dose mTORC1 inhibition reduces

immunosenescence, increases responses to influenza vaccination and reduces infections in elderly individuals, and is well tolerated (83). The biguanide, metformin, is commonly used to treat type 2 diabetes, activates AMPK, which inhibits mTOR and prolongs lifespan in mice (52). Metformin reduces elastase-induced emphysema in mice (54) and is well tolerated in COPD patients, although measurements of senescence have not been made (84). Metformin is effective in reversing bleomycin-induced fibrosis (48), but in patients being treated with metformin for diabetes there was no obvious effect on disease progression in IPF (85).

Naturally occurring molecules may also be effective and may be obtained via dietary supplementation (neutraceuticals). Quercetin, a flavonoid found in apples, also activates AMPK as well as being a senolytic (16) and prevents the development of LPS-induced emphysema in mice (86). Quercetin also promotes apoptosis of IPF fibroblasts and reduces bleomycin-induced lung fibrosis in aged mice (87). Resveratrol increases lifespan in several species, including mice through the activation of sirtuin-1. However, resveratrol and related dietary polyphenols are poorly absorbed from the gut, have low potency and are rapidly metabolized. Novel potent synthetic analogs known as STACs, which activate sirtuin-1 have been developed, although there are questions about the specificity of these drugs (61). In cigarette smoke-exposed mice, STAC SRT-2171 suppresses neutrophilic inflammation, restores reduced MMP-9 and improves lung function (58). As oxidative stress appears to drive accelerated aging in lung disease, antioxidants should be effective therapies. However, currently available antioxidants, such as *N*-acetyl cysteine (NAC), are lack efficacy as they are inactivated by oxidants. Novel antioxidants include NADPH oxidase (NOX) inhibitors, superoxide dismutase mimetics and Nrf2 activators. The Nox4 inhibitor GK137831 inhibits the development of lung fibrosis in aged mice, bleomycin-induced fibrosis and induces apoptosis in IPF fibroblasts (88). There is growing evidence that intracellular oxidative stress from mitochondria is important in age-related lung diseases, so a mitochondria-targeted antioxidant may be more useful. SkQ1 is such an antioxidant and reduces senescence biomarkers in rats, whereas an extracellular antioxidant N-acetyl cysteine is not effective (89).

Targeting miRNAs, such as miR-34a and miR-570, may be a future therapeutic strategy for treating and preventing cellular senescence as this has rejuvenates senescent airway epithelial cells

in COPD patients (60, 69). Delivery of miRNA mimics or antagomirs via extracellular vesicles and nanoparticles might be a novel way to deliver these therapies (90).

MiRNA contained in EVs may also serve as biomarkers for cellular senescence and may be measured in plasma, sputum and bronchoalveolar lavage of patients with chronic lung disease (91).

Conclusions

Cellular senescence appears to be critical in the pathogenesis of several chronic lung diseases, and particularly COPD and IPF. Accumulation of senescent cells leads to cellular dysfunction, failure to repair, impaired immunity and chronic, low grade, lung inflammation through the SASP response. Regulation of the molecular pathways controlling senescence in lung cells are now being elucidated, including the critical role of miRNAs. This may lead to new therapeutic strategies, such as senolytic therapies that may be given intermittently, inhibitors of PI3K-mTOR signaling, restoration of anti-aging molecules and targeting specific miRNAs. EVs may propagate senescence within the lung, but also result in extrapulmonary spread to account for comorbidities of chronic lung diseases and multimorbidity. Novel therapies are likely to have a major effect on the progression of chronic lung diseases and on “healthspan” in the future and further translational research is strongly encouraged, since delivery of senotherapies by inhalation may be a good way of testing these new approaches.

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Table 1: *Effects of cellular senescence drugs (senotherapies) in idiopathic pulmonary fibrosis (IPF) and COPD.*

Drug	Proposed mechanism(s)	Effects in lung disease	Ref
Rapamycin Rapalogs	mTORC1 inhibitor	IPF: ↓ bleo fibrosis in mice COPD: ↓ senescence and SASP in COPD lung cells ↓ mTORC1 in lung and PBMC	82 45 44
Metformin	AMPK activation	IPF: ↓bleo fibrosis in mice, ↓ myofibroblasts in IPF. No clinical benefit in IPF COPD: ↓ senescence markers, SASP and emphysema in Mice; Well tolerated in COPD patients	48 85 54 84
Quercetin	AMPK activator Senolytic	IPF: ↓ bleo fibrosis in mice, ↑ apoptosis of IPF fibroblasts COPD: ↓ emphysema and SASP in mice	87 86
Quercetin + Dasatinib	Senolytic	IPF: ↓ bleo fibrosis in mice, ↓ senescence markers and SASP Some clinical improvemant in patients COPD: no studies reported	16 79 80
Navitoclax (ABT-263)	Senolytic (Bcl2 inhibitor)	IPF: ↓ irradiation-induced fibrosis in mice COPD: No studies reported	78
FOXO4-DRI	Senolytic	No studies in lung disease reported	81
GKT37831	Antioxidant (Nox4 inhibitor)	IPF: ↓ fibrosis in aged and bleo fibrosis mice, ↓ senescence markers ↑ apoptosis in IPF fibroblasts COPD: no studies reported	88

Abbreviations: AMPK: AMP-activated kinase; Bcl2: B cell lymphoma-2; bleo: bleomycin; mTORC1: mammalian target of rapamycin complex-1; Nox: NADPH oxidase; NADPH: reduced form of nicotinamide adenine dinucleotide phosphate; PBMC: peripheral blood mononuclear cells; SASP: senescence-associated secretory phenotype.

Figure legends

Fig. 1. *Mechanisms of cellular senescence.* Cell division leads to progressive shortening of the telomeres which eventually leads to the activation of the DNA damage response (DDR) which activates p53 (replicative senescence). Cellular stresses, such as oxidative stress, may also cause DNA damage and activate $p16^{INK4a}$ (stress-related senescence) and together with p53 activates the cyclin kinase inhibitor $p21^{CIP1}$, which induces cell cycle arrest and these cells stain positively for senescence-associated β-galactosidase (SA-β-gal). Senescent cells show activation of nuclear factor-κB (NF-κB), p38 mitogen-activated protein kinase (MAPK) and Janus-activated kinases (JAK), resulting in the secretion of multiple inflammatory proteins known as the senescence-associated secretory phenotype (SASP), which includes inflammatory cytokines, chemokines, proteins, growth factors and plasminogen activator inhibitor-1 (PAI-1). The SASP induces further senescence and senescent cells release reactive oxygen species (ROS), which further drive the senescence process. The SASP also leads to structural changes, including fibrosis and tissue destruction.

Fig. 2. *Signaling pathways that regulate cellular senescence.* Oxidative stress inhibits phosphatase and tensin homolog (PTEN), which activates phosphoinositide-3-kinase (PI3K), which in turn activates mammalian target of rapamycin (mTOR). Decreased activation of AMP kinase (AMPK) also increases mTOR and this leads to activation of microRNA-34a (miR-34a), which inhibits sirtuin-1 and sirtuin-6 in parallel. Decreased sirtuin-1 plays a key role in senescence through activation of nuclear factor-KB (NF-κB) which results in the activation of the senescence-associated secretory phenotype (SASP), as well as peroxisome-proliferator receptor gamma coactivator-1α (PGC-1α) which regulates mitochondrial function, impaired autophagy and DNA repair and activation of p53. Decreased sirtuin-6 also activates NF-κB, as well as decreasing telomeres and reducing β-catenin (β-cat), which regulates cell integrity and vascular-endothelial growth factor (VEGF). Reduced sirtuin-1 inhibits the transcription factor FOXO3a which regulates antioxidants (such as superoxide dismutase), whereas reduced sirtuin-6 reduces Nrf2 (which regulates multiple anti-oxidant genes), thereby result in increased oxidative stress. Activation of p38 mitogen-activated protein kinase (MAPK) leads to increased c-Jun and activation of activator protein-1 (AP-1) which increases miR-570 that also decreases sirtuin-1 (but not sirtuin-6). The miRNAs may be released from senescent cells as extracellular vesicles, which may be taken up by other cells in the lung or travel to other organs, such as the cardiovascular system via the circulation. In this way senescence may spread locally and systemically and may account for comorbidities of chronic lung disease.