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Mitochondria, telomeres and cell senescence: Implications for lung ageing and disease

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ABSTRACT

Cellular senescence, the irreversible loss of replicative capacity in somatic cells, plays a causal role in the development of age-related pathology and in a number of age-related chronic inflammatory diseases. The ageing lung is marked by an increasing number of senescent cells, and evidence is mounting that senescence may directly contribute to a number of age-related respiratory diseases, including chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). Telomere dysfunction and alterations in mitochondrial homeostasis frequently occur in cellular senescence and are important to the development of the often detrimental senescence-associated secretory phenotype (SASP). The roles of telomeres, the mitochondria and cellular senescence in lung ageing and disease are discussed. Therapeutic interventions targeting cellular senescence are considered for delaying or potentially reversing age-related respiratory disease.

1. Introduction

Cellular senescence is generally defined as irreversible cell-cycle arrest. However, it is now accepted that senescence is a multifaceted phenotype that includes time-dependent changes in global gene expression, epigenetic profile and metabolism. Importantly, senescence is characterised by the development of a pro-inflammatory secretory phenotype, termed the senescence-associated secretory phenotype (SASP)(Campisi & d'Adda di Fagagna, 2007). The SASP is thought to be important for the immune-mediated clearance of senescent cells, however, may also be a contributor to tissue dysfunction. Senescence can be beneficial: it acts as a tumour suppressor mechanism, inhibiting the proliferation of potentially transformed cells, and has been implicated in essential biological processes, such as wound healing, tissue repair and embryonic development (van Deursen, 2014). However, evidence suggests that accumulation of senescent cells with time, leads to age-related loss of tissue function (Baker et al., 2016; Baker et al., 2011). Accordingly, senescent cells are found at sites of chronic age-related disease (Munoz-Espin & Serrano, 2014) and have been causally implicated in the development of osteoarthritis (OA) (Jeon et al., 2017), atherosclerosis (Childs et al., 2016), liver steatosis (Ogrodnik et al., 2017) and pulmonary fibrosis (Schafer et al., 2017). The lung is particularly affected by the ageing process, showing clear decline in structure and function with age (Janssens, Pache, & Nicod, 1999). Moreover, the ageing lung is characterised by the presence of senescent cells and several respiratory diseases have been identified as

Abbreviations: SASP, senescence-associated secretory phenotype; OA, osteoarthritis; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; ROS, reactive oxygen species; DDR, DNA damage response; ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3-related protein; p38 MAPK, p38 mitogen-activated protein kinase; VEGF, vascular endothelial growth factor; MCP-1, monocyte chemoattractant protein 1; OIS, oncogene-induced senescence; Sen-β-Gal, senescence-associated β-galactosidase; TGF- β, transforming growth factor-β; PDGF-AA, platelet-derived growth factor AA; MMPs, matrix metalloproteinases; ECM, extracellular matrix; IL, interleukin; PAI-1, plasminogen activator inhibitor 1; BubR1, mitotic checkpoint kinase budding uninhibited by benzimidazole-related 1; EMT, epithelial-to-mesenchymal transition; TNF- α, tumour necrosis factor- α; Nox4, NADPH oxidase-4; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; mH2A, macro H2A; CSE, cigarette smoke extract; CPFE, combined pulmonary fibrosis emphysema; CF, cystic fibrosis; NF-κB, nuclear factor-κB; TRF1, telomeric repeat binding factor 1; TRF2, telomeric repeat binding factor 2; RAP1, TRF2 interacting protein; TIN2, TRF1-interacting nuclear factor 2; TPP1, adrenocortical dysplasia protein homolog; POT1, protection of telomeres 1; TAF, telomere-associated DNA damage foci; HR, homologous recombination; mTOR, mechanistic target of rapamycin; TERT, telomerase reverse transcriptase; mtDNA, mitochondrial DNA; SIRT3, sirtuin 3; ETC, electron transport chain; MiDAS, mitochondrial dysfunction-associated senescence; AMPK, AMP-activated protein kinase; PARP, poly-ADP ribose polymerase; TCA, tricarboxylic acid; PDH, pyruvate dehydrogenase; ME2, mitochondrial malic enzyme 2; DAMPs, damage-associated molecular patterns; PRRs, pattern recognition receptors; CCF, cytoplasmic chromatin fragment; PINK1, PTEN-induced putative kinase 1; 4-HNE, 4-hydroxy-2-nonenal; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; IRP2, iron-responsive element-binding protein 2; SIRT, Sirtuin; SDH, succinate dehydrogenase; ACOS, Asthma-COPD overlap syndrome; NAC, *N*-Acetyl Cysteine; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinases; PH, pulmonary hypertension; BOS, bronchiolitis obliterans syndrome; CPP, cell penetrating peptide

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diseases of accelerated lung ageing (Adnot et al., 2015; Mercado, Ito, & Barnes, 2015). Chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) are classic examples of respiratory diseases that increase in prevalence with age and have been associated with senescence (Amsellem et al., 2011; Birch et al., 2015; Minagawa et al., 2011; Schafer et al., 2017; Tsuji, Aoshiba, & Nagai, 2006). Telomeres, the protective structures at the ends of linear chromosomes, are particularly important to the senescence state and can interact with other significant regulators of the senescence phenotype, including the mitochondria (Passos, Saretzki, & von Zglinicki, 2007). Both telomere dysfunction and alterations in mitochondrial homeostasis occur in the lung with age and in several age-related respiratory diseases. Understanding the mechanisms involved in these processes and how they impact on senescence and its associated phenotypes could be pivotal to the generation of therapies to dampen, or prevent, age-associated lung disease.

2. Cellular senescence: the good, the bad and the ugly

Cellular senescence was first described more than 50 years ago following the observation that normal human fibroblasts undergo a finite number of divisions before ceasing to proliferate in culture, a concept termed the 'Hayflick Limit' (Hayflick & Moorhead, 1961). It was subsequently hypothesised that senescence is a barrier to malignant transformation, since transformed cells proliferate indefinitely (Hayflick, 1965). There is now considerable evidence to support the notion that senescence is a potent anti-cancer mechanism, both in preventing malignant transformation and in limiting tumour progression (Kang et al., 2011; Serrano, Lin, McCurrach, Beach, & Lowe, 1997; Sharpless, Ramsey, Balasubramanian, Castrillon, & DePinho, 2004; Suram et al., 2012; Xue et al., 2007). In this context, the induction of cellular senescence is believed to be a beneficial process, something which is supported by recent discoveries suggesting that short-term or 'acute' senescence is advantageous during embryonic development (Munoz-Espin et al., 2013; Storer et al., 2013), wound healing and tissue repair (Demaria et al., 2014; Krizhanovsky et al., 2008). However, with advanced age, senescent cells accumulate and evidence suggests that senescent cells which remain for prolonged periods are major drivers of age-related tissue deterioration and chronic age-related disease (van Deursen, 2014). In this respect, senescent cells may be beneficial or deleterious to the organism, depending on context.

Cellular senescence is a complex process that can be induced by a vast array of stressors. Replicative senescence occurs following prolonged periods of division leading to an eventual plateau in cell proliferation. However, an increasing repertoire of senescence inducers is continually being identified, which cause senescence prior to the onset of replicative exhaustion, termed "stress-induced premature senescence"; these include oncogenes, reactive oxygen species (ROS) and cytotoxic compounds (Kuilman, Michaloglou, Mooi, & Peeper, 2010). Cell cycle arrest is usually accompanied by the activation of a DNA damage response (DDR), initiated by ataxia-telangiectasia mutated (ATM) and AT and Rad3-related protein (ATR) kinases, then mediated through activation of the tumour suppressor protein p53 and upregulation of the cyclin-dependent kinase inhibitors (CDKis) $p21^{CIP1}$, $p16^{INK4a}$ or $p14^{ARF}$ (known as $p19^{ARF}$ in mice). The CDKis supress the phosphorylation and inactivation of pRb leading to blockade of S-phase entry and cell cycle arrest (Campisi & d'Adda di Fagagna, 2007) (Fig. 1). While proliferation arrest is a major hallmark of senescent cells, these cells are not inert; they are highly metabolically active and generally continue to enlarge in size. In addition, senescent cells develop a secretory phenotype, characterised by release of cytokines, chemokines, growth factors and matrix remodelling proteases; the so-called SASP (Coppe et al., 2008). The SASP can have potent effects on neighbouring cells and surrounding tissue, and on the very cells that produce it, thus the SASP can act in both paracrine and autocrine fashions. Indeed, it has been shown that the SASP can induce senescence in nearby healthy cells, mediated by vascular endothelial growth factor (VEGF), monocyte chemoattractant protein 1 (MCP-1) and CCL20 (Acosta et al., 2013). Moreover, the SASP can reinforce both stress-induced, replicative and oncogene-induced senescence (OIS) growth arrest via IL-6 and IL-8 signaling, in a self-amplifying secretory network (Acosta et al., 2008; Kojima, Kunimoto, Inoue, & Nakajima, 2012). It is the potent effects of the SASP that likely implicate senescence in a range of biological processes. For instance, data suggests that cells with senescence-like characteristics, including activity of the lysosomal enzyme senescence-associated β-galactosidase (Sen-β-Gal) and p21 expression, without detectable DDR activation, signal the immune system leading to macrophage infiltration and clearance of senescent cells during embryonic development, which contributes to tissue remodeling processes (Munoz-Espin et al., 2013). The specific SASP components are undefined but are believed to involve transforming growth factor-β (TGF-β). Similarly, evidence suggests that senescent tumour cells signal and are cleared by the immune system, limiting liver carcinogenesis in a process mediated by release of MCP-1 (Iannello, Thompson, Ardolino, Lowe, & Raulet, 2013; Kang et al., 2011; Xue et al., 2007). Indeed, the constituents of the SASP could differ depending on the biological process and the origin of the senescent cell. The SASP associated with senescent fibroblasts involved in cutaneous wound healing is characterised by release of platelet-derived growth factor AA (PDGF-AA) and proteases, such as matrix metalloproteinases (MMP) 2, 3 and 9, which act to induce myofibroblast differentiation to accelerate wound closure and to limit fibrosis occurring through rapid synthesis of extracellular matrix (ECM) (Demaria et al., 2014; Jun & Lau, 2010). Similar results demonstrate that hepatic senescent cells act to limit liver fibrosis following acute liver injury through production of proteases and activation of immune surveillance mechanisms (Krizhanovsky et al., 2008). The composition of the SASP associated with ageing is highly variable, likely since senescence occurs in a number of different cell types, in various organs, and in several organisms with age (Birch et al., 2015; Dimri et al., 1995; Fumagalli et al., 2012; Herbig, Ferreira, Condel, Carey, & Sedivy, 2006; Hewitt et al., 2012; Jeyapalan, Ferreira, Sedivy, & Herbig, 2007; Krishnamurthy et al., 2004; C. Wang et al., 2009). Furthermore, while senescence was initially believed to predominantly affect cells with proliferative capacity, such as epithelial cells and fibroblasts, more recently senescent-like phenotypes have been described in post-mitotic cells, including neurons, slowly-dividing hepatocytes and in adipose tissue, suggesting that the contribution of cellular senescence to the ageing process is more widespread than initially conceived (Fumagalli et al., 2012; Jurk et al., 2012; Schafer et al., 2016). Nevertheless, studies suggest that the consistently upregulated SASP factors associated with senescence in ageing include IL-6, IL-1α, plasminogen activator inhibitor 1 (PAI-1) and MCP-1 (Baker et al., 2011; Baker et al., 2016; Childs et al., 2016). The mechanisms underlying senescent cell accumulation with ageing are incompletely understood. However, an age-associated reduction in immune system function leading to impaired senescent cell clearance is one probable factor (Ovadya & Krizhanovsky, 2014). While age-related increases in senescent cell frequencies have been observed for some time, only recently has senescence been causally implicated in the ageing process. Using a transgenic mouse model (INK-ATTAC), Baker and colleagues found that elimination of $p16^{INK4a}$-positive senescent cells delayed the onset of age-related pathologies including sarcopenia, loss of adipose tissue and cataracts in both progeroid (BubR1 (mitotic checkpoint kinase budding uninhibited by benzimidazole-related 1) insufficient mice) and wild-type mice and increased lifespan of wild-type mice (Baker et al., 2011; Baker et al., 2016). Senescent cells may contribute to a loss of tissue homeostasis and impaired organ function through a reduction in regenerative capacity of tissues due to proliferative arrest (Choudhury et al., 2007) or through altering functions of surrounding cells and tissues by the deleterious properties of the SASP, such as the pro-inflammatory aspects (Ovadya & Krizhanovsky, 2014). Indeed, and paradoxically, cellular senescence can contribute to malignant

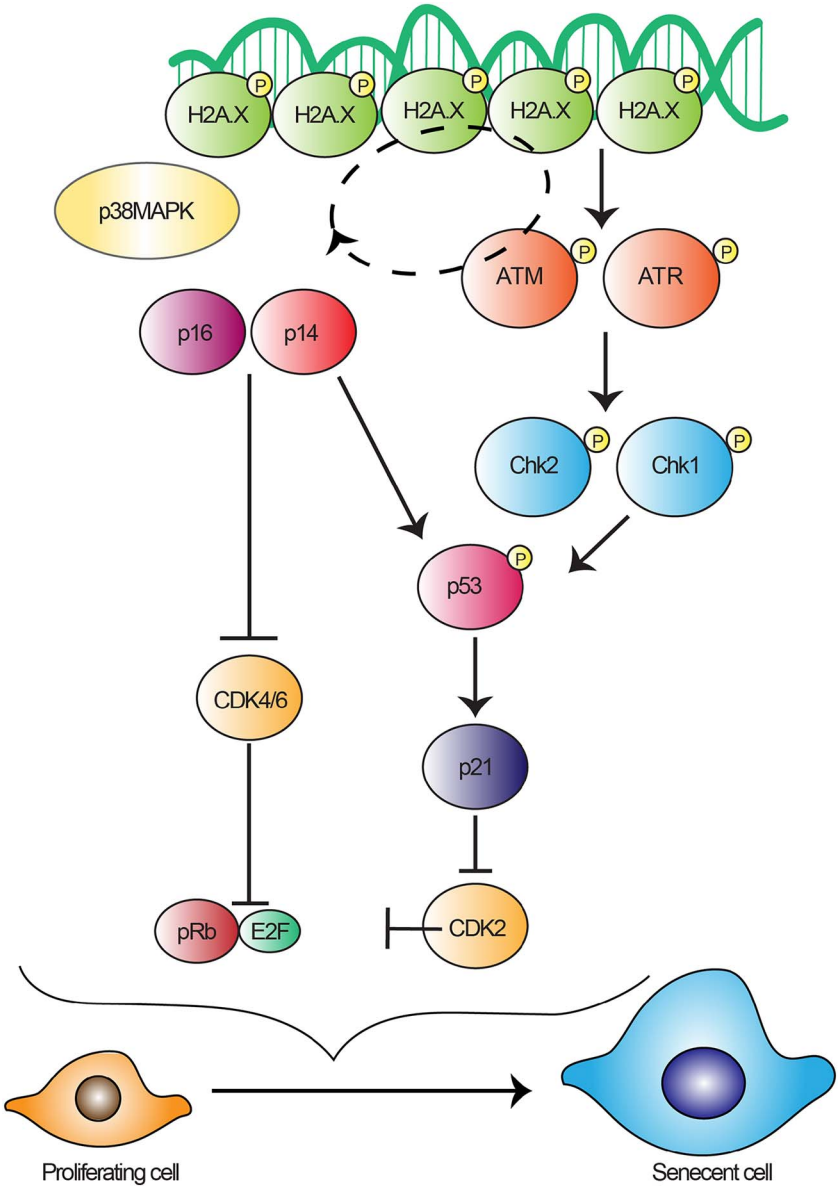

Fig. 1. Pathways involved in senescence induction. Senescent cell cycle arrest is usually triggered by the activation of a DNA damage response (DDR), initiated by ataxia-telangiectasia mutated (ATM) and AT and Rad3-related protein (ATR) kinases leading to the formation of DNA damage foci, characterised by modification of histone proteins, such as the phosphorylation of histone 2A.X. This then impacts on the p53-p21 and p16/p14-pRB tumour suppressor pathways. ATM and ATR kinases phosphorylate and stabilise p53, which establishes senescence by inducing expression of the cyclin-dependent kinase inhibitor p21. p14 (p19 in mice) also stabilises p53 by inhibiting the ubiquitin ligase mouse double minute 2 (MDM2). p21 supresses the phosphorylation and inactivation of pRb, leading to cell cycle arrest. pRb halts cell proliferation by inhibiting the activation of E2F, a transcription factor required for the expression of cell-cycle progression genes. Cell cycle arrest is maintained by p16, which is transcriptionally upregulated under the control of p38 mitogen-activated protein kinase (p38MAPK), and also supresses pRB inactivation by inhibiting CDK4 and CDK6. Chk1/2, checkpoint kinase 1/2; CDK, cyclin-dependent kinase inhibitor.

transformation and cancer progression by promoting transformation and invasiveness of pre-malignant cells through the factors they release (Coppe et al., 2008). Additionally, the secretome of senescent stromal cells can stimulate growth, angiogenesis and epithelial-to-mesenchymal transition (EMT); all of which can promote malignancy (Capparelli et al., 2012; Coppe et al., 2008; Coppe, Kauser, Campisi, & Beausejour, 2006). The SASP can also generate an inflammatory environment, which is believed to be a factor by which senescent cells may contribute to 'inflammaging' and to the development of chronic age-related diseases. Indeed, senescent cells have been found in the articular cartilage from patients with OA, in the brains of patients with Alzheimer's disease and in atherosclerotic lesions (McShea, Harris, Webster, Wahl, & Smith, 1997; Price et al., 2002; Vasile, Tomita, Brown, Kocher, & Dvorak, 2001; J. Wang et al., 2015). Interestingly, a recent study demonstrated that transplantation of senescent cells into the knee joints of mice induces an OA-like-phenotype (M. Xu et al., 2016). Furthermore, OA occurring following cruciate ligament injury is preceded by the accumulation of senescent cells in the knee joint of mice and selective elimination of senescent cells by either genetic or pharmacological means prevents the development of post-traumatic OA in mice (Jeon et al., 2017). Similarly, clearance of senescent cells in mice prone to atherosclerosis reduces formation and progression of atherosclerotic plaques and inhibits processes that promote plaque rupture (Childs et al., 2016). While evidence suggests that senescent cells are causal to the development of some age-related diseases, whether the SASP *per se* plays a causal role in the process is more challenging to test experimentally, particularly given its heterogeneity. Despite this, genetic and pharmacological removal of senescent cells leads to a concomitant decrease in commonly described SASP factors, such as IL-6, tumour necrosis factor (TNF)-α, IL-1α, and MCP-1 in tissues that show functional improvements in age-associated pathologies (Baker et al., 2011; Baker et al., 2016; Childs et al., 2016). This suggests that amelioration of senescence-associated inflammation could be leading to beneficial effects and that targeting the SASP could offer therapeutic potential for age-associated pathologies and age-related diseases.

3. Cellular senescence in lung ageing and disease

Lung maturity typically peaks between the ages of 20 and 25 years, and then undergoes an age-progressive decline in function with reductions in forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), with progressively more inefficient gas exchange across

the alveolar wall. In addition, a range of physiological changes occur in the ageing lung, including alveolar dilatation and distal airspace enlargement (emphysema) and loss of supporting tissue for peripheral airways (Janssens et al., 1999). This is associated with an increase in compliance as well as pathogenic susceptibly to pulmonary diseases (L. Wang, Green, Smiley-Jewell, & Pinkerton, 2010). Despite this, some of the mechanisms underlying lung ageing remain elusive. A number of studies have shown that cells positive for senescence-associated markers, such as the DDR protein γH2A.X, the heterochromatin protein macro H2A (mH2A) and the CDKis p21 and p16 accumulate in the murine lung during physiological ageing, including in the alveoli (Kreiling et al., 2011; C. Wang et al., 2009), the airway epithelium (Birch et al., 2015), in vascular smooth muscle cells (Calhoun et al., 2016) and in lysates of whole murine lung (Kreiling et al., 2011; Krishnamurthy et al., 2004). In addition, several factors associated with the SASP, in particular TGF-β and IL-6 increase in the murine lung with age (Calhoun et al., 2016). In contrast, the role of senescence during lung ageing in humans is less well defined. Using whole tissue lysates, one group described increased p16 and mH2A in the lungs of older humans (Shivshankar, Boyd, Le Saux, Yeh, & Orihuela, 2011). Another study has demonstrated that mH2A and p16 increase in the lungs of humans between 50 and 60 years of age, as compared to 20-30-year-old persons, with expression predominantly increasing in the vasculature (Calhoun et al., 2016). Interestingly, in the murine lung, senescent cell frequencies correlate with changes in lung structure, such as emphysematous-like changes (Birch et al., 2015) and deposition of collagen (Calhoun et al., 2016). However, it is unclear if senescent cells causally contribute to lung dysfunction or are merely associated with it. Recent work from Hashimoto et al. provides evidence that senescent cells are indeed responsible, at least in part, for the age-associated decline in lung function. Using a transgenic mouse model whereby naturally occurring $p19^{ARF}$-positive cells could be eliminated by a toxin-mediated cell knockout system, it was demonstrated that removal of $p19^{ARF}$-positive cells from the lungs of 12-month-old mice abolished expression of senescence-associated markers and restored lung compliance, structure, and elasticity (Hashimoto et al., 2016). These findings suggest that cellular senescence is causal to physiological lung ageing and could potentially be targeted to combat age-associated phenotypes in the lung or indeed development of diseases associated with lung ageing. A number of respiratory diseases are classified as diseases of accelerated lung ageing, with cellular senescence thought to contribute to their development and pathophysiology.

COPD is one such age-related disease and one of the most common respiratory conditions globally, causing chronic morbidity and high mortality (Mannino & Buist, 2007). COPD is typically characterised by imbalances in inflammatory processes and repair events, affecting both the peripheral airways and lung parenchyma and leading to structural changes, including peri-airway fibrosis and destruction of alveolar airspaces (Barnes, 2016a, 2016b; Chung & Adcock, 2008). Thus patients often have a combination of chronic bronchitis and emphysema. Some of the structural changes that occur in the COPD lung reflect those which occur during normal lung ageing. Indeed, the decline in lung function that occurs as COPD progresses resembles that which occurs during ageing, however at a more advanced rate (Fletcher & Peto, 1977; K. Ito & Barnes, 2009). In accordance with accelerated ageing processes taking place in COPD, a number of studies have described increased markers of senescence in the COPD lung. Higher percentages of alveolar type II cells and pulmonary vascular endothelial cells positive for p16 and p21 expression have been observed in lung tissue from patients with COPD (Amsellem et al., 2011; Aoshiba & Nagai, 2009; Birch et al., 2015; Tsuji et al., 2006; Tsuji, Aoshiba, & Nagai, 2010). Similarly, there are significantly more DNA damage foci in type I and type II alveolar epithelial cells, airway epithelial cells and endothelial cells in the lungs of patients with COPD (Aoshiba, Zhou, Tsuji, & Nagai, 2012; Birch et al., 2015). Cells isolated from and cultured outside of the COPD lung, such as pulmonary vascular endothelial cells and airway epithelial cells, retain markers of senescence including, Sen-β-Gal activity and high p21 and p16 mRNA expression (Amsellem et al., 2011; Birch et al., 2015). Similarly, lung fibroblasts from patients with advanced COPD appear senescent in culture, exhibiting reduced proliferation capacity and Sen-β-Gal activity (Holz et al., 2004; Muller et al., 2006). Moreover, cigarette smoke exposure, the main risk factor for COPD, induces senescence-associated phenotypic changes *in vitro*. In cultured alveolar epithelial cells, cigarette smoke extract (CSE) causes growth arrest, an enlarged cellular morphology, Sen-β-Gal activity and increased p21 expression (Tsuji, Aoshiba, & Nagai, 2004). Similar findings have been reported in lung fibroblasts, with CSE exposure leading to an enlarged and flattened morphology, increased Sen-β-Gal activity, increased p16 expression and premature cell-cycle arrest (Aoshiba & Nagai, 2009; Birch et al., 2015; Nyunoya et al., 2006). Long-term exposure to CSE (35 days) also triggers a surge in release of key SASP factors IL-6 and IL-8 (Aoshiba & Nagai, 2009; Birch et al., 2015; Nyunoya et al., 2006). Cigarette smoke exposure triggers H2A.X phosphorylation in normal bronchial epithelial cells *in vitro* (Albino et al., 2004; Birch et al., 2015) and *in vivo* (Birch et al., 2015) while also increasing levels of p16 in the lungs and nasal cavity, as determined using a p16-luciferase reporter mouse model (Sorrentino et al., 2014). The effects of senescent cells in chronic lung diseases are two-fold; 1) the accumulation of cells exhibiting limited replicative potential would impair lung tissue maintenance and repair, promoting progressive destruction of the lung and likely explaining lung function decline in COPD and 2) by contributing to a pro-inflammatory and pro-ageing environment via SASP factor release and bystander effects. To this end, the chronic inflammatory processes that occur in the lungs of patients with COPD, and those that are detectable in the circulation, involve many inflammatory cytokines akin to the SASP, including IL-1, IL-6, CXCL8, TNF-α, TGF-β and proteases such as MMP2 and MMP9 (Aldonyte, Jansson, Piitulainen, & Janciauskiene, 2003; Bhowmik, Seemungal, Sapsford, & Wedzicha, 2000; Keatings, Collins, Scott, & Barnes, 1996; Russell et al., 2002; Song, Zhao, & Li, 2001). Therefore, the SASP may contribute to lung structural alterations and may promote senescence in surrounding cells. The potent effects of the SASP could also extend to extra-pulmonary environments, contributing to systemic complications and co-morbidities of which there are many associated with COPD, including sarcopenia, insulin resistance and kidney dysfunction (Boyer et al., 2015). While current data does not definitively implicate cellular senescence and the SASP as causal to COPD, understanding the underlying mechanisms involved in COPD-associated senescence could be important for development of therapies to dampen COPD progression.

Similarly to COPD, IPF is a progressive and fatal lung disease classically dubbed a 'disease of ageing' but contrastingly is characterised by fibrosis of the interstitium, leading to scarring of the lung parenchyma, with limited airway-centered pathology (Alder et al., 2008; Alder et al., 2011; Minagawa et al., 2011; Raghu, Weycker, Edelsberg, Bradford, & Oster, 2006). While IPF occurs more commonly with age, the underlying mechanisms are still poorly understood. However, a number of studies implicate cellular senescence processes in IPF pathogenesis. It has been described that in the bronchial epithelium of patients with IPF, there is an increased number of cells positive for p21 and Sen-β-Gal activity (Minagawa et al., 2011). Similar findings have been described in alveolar epithelial cells of the IPF lung (Kuwano et al., 1996) and in fibroblasts isolated from the lungs of IPF patients (Yanai et al., 2015). A recent study describes increased p16 and p21 levels in alveolar epithelial cells present in *ex vivo* lung tissue from patients with IPF, with p16 levels negatively correlating with diffusing capacity of the lung for carbon monoxide, suggesting that p16 levels correlate with disease severity (Lehmann et al., 2017). Indeed, another recent study reports a severity-dependent increase in p16 in the lungs of patients with IPF (Schafer et al., 2017). Moreover, *ex vivo* models of pulmonary fibrosis are characterized by increased senescent alveolar type II cells and elevated levels of key SASP factors (Lehmann et al., 2017). Intratracheal delivery of bleomycin, a chemotherapeutic drug,

induces lung fibrosis in mice with similar features to human IPF, along with increased frequencies of senescent alveolar epithelial cells and fibroblasts (Aoshiba, Koinuma, Yokohori, & Nagai, 2003; Schafer et al., 2017) and increased levels of key SASP factor PAI-1 (W. T. Huang et al., 2015). Interestingly, accumulation of senescent myofibroblasts in the lungs of aged mice following bleomycin injury is associated with an impaired capacity for resolution of fibrosis (Hecker et al., 2014), suggesting that the presence of senescent cells is causal to the fibrotic phenotype in IPF. Indeed, a more recent proof-of-concept study causally implicates cellular senescence in IPF pathogenesis and demonstrates the therapeutic potential of senescence-targeted therapies or 'senotherapies' for pulmonary fibrosis. Schafer and colleagues showed that clearance of p16-positive senescent cells in the INK-ATTAC genetic mouse model improves lung function, body composition and physical health of mice treated with bleomycin to induce pulmonary fibrosis (Schafer et al., 2017). Notably, removal of senescent cells reduced expression of SASP factors with established roles in regulating fibrotic and pulmonary aspects of IPF, including IL-6, TGF-β and MMP12, suggesting the SASP is a major mediator of IPF pathology. Moreover, treatment of mice with drugs that induce apoptosis specifically in senescent cells, termed senolytics, mirrored the results observed with transgenic cell clearance (Schafer et al., 2017). While Schafer and colleagues report no observable reduction in lung fibrosis *per se* following senescent cell elimination, another recent study reported that pulmonary fibrosis, induced in mice following irradiation, was reversed following treatment with ABT-263, a senolytic agent that selectively killed senescent type II alveolar epithelial cells (Pan et al., 2017). This suggests that targeting senescent cells is a plausible therapy for IPF, both for improving physical health, and in limiting fibrotic lesions.

COPD and IPF increase with age and occur later in life (Faner, Rojas, Macnee, & Agusti, 2012). Therefore it is unsurprising that senescence processes are activated in the COPD and IPF lung. However, why COPD and IPF are so phenotypically different, despite both being characterized by the presence of senescent cells, is poorly understood. So far, studies in mouse models where an IPF-like phenotype can be induced by bleomycin, support the concept that senescence is a contributor to the process. However, in the context of COPD, such experiments have not been conducted. Moreover, some patients with IPF also have COPD-associated changes such as emphysema and this overlap syndrome has been termed combined pulmonary fibrosis emphysema (CPFE), which is often accompanied by pulmonary hypertension and lung cancer, and has a poor prognosis (Lin & Jiang, 2015). Although the mechanisms are poorly understood, it may reflect a common pathogenetic pathway, such as cellular senescence, which should be investigated further using models to determine causality, as discussed above. More in-depth investigation into the roles of senescence in other respiratory diseases, not typically associated with ageing, is also required. For instance, cells with senescence-like properties, including p16 and p21 expression, increase in the airway epithelium of patients with cystic fibrosis (CF) (Fischer et al., 2013) and bronchiectasis (Birch et al., 2016). CF patients suffer recurrent inflammation in the lung from an early age, resulting in decline in lung function (Elborn, 2016), whereas bronchiectasis typically affects the adult population and can occur in conjunction with CF or can develop independently of CF, commonly following childhood respiratory infection (O'Donnell, 2008). Both CF and bronchiectasis are characterised by cycles of infection and chronic inflammation. Our group has previously demonstrated that the ageing process and cellular senescence are accelerated under conditions of chronic low-grade inflammation (Jurk et al., 2014). Using a mouse model whereby nuclear factor-κB (NF-κB) activity is enhanced due to knockdown of the inhibitory nfkb1 subunit (*nfkb1*$^{-/-}$), it was found that markers of cellular senescence were increased in the gut and liver (Jurk et al., 2014). Moreover, *nfkb1*$^{-/-}$ mice showed signs of premature ageing including fur loss and greying, epidermal thinning and cachexia (Jurk et al., 2014). This is not the first report of inflammatory processes impacting on rates of cellular senescence. A previous study using the same *nfkb1*$^{-/-}$ mice reported increased frequencies of hepatocytes harboring telomere damage, a major hallmark of senescent cells, in hepatocellular carcinoma, which could be reduced upon neutrophil-specific depletion (Wilson et al., 2015). Moreover, other pro-inflammatory stimuli, such as neutrophil elastase, induce senescence *in vitro* (Fischer et al., 2013). Given the results of these studies, it is highly plausible that chronic inflammatory processes occurring in the lungs of patients with CF and bronchiectasis may lead to senescence induction, which could act in a vicious cycle to perpetuate inflammation, via the SASP and paracrine effects, contributing to disease progression and chronicity. The underlying mechanisms involved in senescence occurring in bronchiectasis and CF should be elucidated further. The concept of inflammation-induced senescence may also be relevant to asthma; a disease of airway hyper-responsiveness characterised by chronic inflammation and structural alterations to the airways (Burrows, Martinez, Halonen, Barbee, & Cline, 1989; Halwani, Al-Muhsen, & Hamid, 2010). It has been shown that p16 and p21 are increased in the airway epithelium of adult patients with asthma with loss of proliferation marker Ki67 (Wu et al., 2013). Additionally, cellular senescence was induced in the airway epithelium of a mouse model of asthma following chronic allergen exposure (Wu et al., 2013). Senescence-related changes are not only present in the lungs of adults with asthma, with senescence markers including p21 and loss of Ki67 already detectable in the airways of asthmatic children (Fedorov, Wilson, Davies, & Holgate, 2005). Therefore understanding whether and what senescence-associated pathways are involved in the pathogenesis of asthma may assist in the development of therapeutics.

4. Telomeres and the mitochondria: major players in senescence

4.1. The role of telomeres in senescence

Telomeres are specialised structures consisting of repetitive DNA (5′-TTAGGG-3′) repeats, which function to protect the ends of linear chromosomes from erosion or fusion by DNA repair processes (Blackburn, 1991). Telomeres progressively shorten with each cell division due to the "end-replication problem", whereby standard DNA polymerases are unable to synthesise DNA in a 3′-5' direction, leading to incomplete replication of the lagging strand. Telomere shortening was shown to be causal to replicative senescence when ectopic expression of the catalytic subunit of telomerase, which maintains telomere length, prevented senescence of normal human fibroblasts *in vitro* (Bodnar et al., 1998). The progressive loss of telomere repeats during replicative senescence destabilises the telomere structure, which is usually arranged in a telomere loop conformation by a complex of six proteins: telomeric repeat binding factor 1 (TRF1), telomeric repeat binding factor 2 (TRF2), TRF2 interacting protein (RAP1), TRF1-interacting nuclear factor 2 (TIN2), adrenocortical dysplasia protein homolog (TPP1) and protection of telomeres 1 (POT1); collectively known as shelterin (de Lange, 2005; Griffith et al., 1999). Loss of shelterin due to telomere shortening "telomere uncapping" has been shown to trigger a classical DDR due to exposure of the telomere end and recognition by the DNA repair machinery as a double-strand break, leading to cell-cycle arrest (d'Adda di Fagagna et al., 2003). Indeed, it was shown that replicatively senescent human fibroblasts accumulate proteins involved in the DDR at telomere regions, including γH2A.X, 53BP1, MDC1 and NBS1 (d'Adda di Fagagna et al., 2003). Telomere shortening is not solely dictated by the end-replication problem however, with studies showing that the rate of telomere shortening, and therefore induction of senescence, can be accelerated by oxidative stress (Richter & von Zglinicki, 2007; von Zglinicki, 2002). Accordingly, telomeres are known to be particularly sensitive to oxidative stress, acquiring oxidative single-strand damage at a much faster rate than the bulk of the genome, possibly due to the high content of guanine triplets (Henle et al., 1999; Petersen, Saretzki, & von Zglinicki, 1998). This, coupled with the fact that repair of telomere damage is inhibited by

components of the shelterin complex, in order to prevent telomere fusions, places telomeres as key inducers of cellular senescence (Bae & Baumann, 2007; Smogorzewska & de Lange, 2002). Indeed, our group and others have demonstrated that while the great majority of DDR foci are resolved soon after their formation, telomere-associated damage is relatively long-lived (Fumagalli et al., 2012; Hewitt et al., 2012). Furthermore, our group have shown that genotoxic and oxidative damage induces a DDR at telomere regions, referred to as telomere-associated DNA damage foci (TAF), in a length-independent and telomerase-independent manner, suggesting that loss of shelterin may not be a requirement for telomere-induced senescence (Hewitt et al., 2012). Telomere dysfunction fuels persistent DDR signalling and is therefore considered particularly important to senescence and its associated phenotypes. For instance, it has been shown that persistent DDR signalling is required for release of key SASP factors, such as IL-6, with transient DDR activation having little effect on IL-6 release (Rodier et al., 2009). Mechanistically, ATM activation, a key event in DDR signalling, leads to activation of NEMO which then activates the IKK complex resulting in nuclear translocation of NF-κB, the major factor regulating transcription of many SASP-associated components (McCool & Miyamoto, 2012). However, other evidence suggests that the SASP can be influenced by DDR-independent mechanisms, such as through activation of p38MAPK, which then increases transcriptional activity of NF-κB (Freund, Patil, & Campisi, 2011), or through cytosolic DNA sensing leading to upregulation of interferon-stimulated genes that drive the production of inflammatory SASP components (Gluck et al., 2017).

4.2. Telomere-mitochondria crosstalk in senescence

Telomere damage may also impact on senescence and associated phenotypes via the mitochondria (Fig. 2). The mitochondria are the main generators of reactive oxygen species (ROS) within the cell. Our group have previously shown that activation of a DDR can induce mitochondrial ROS generation via signalling pathways involving ATM, p21 and the mechanistic target of rapamycin (mTOR) (Correia-Melo et al., 2016; Passos et al., 2010). We reported that changes in mitochondrial size and ROS production were dependent on PGC-1β-dependent mitochondrial biogenesis. In contrast, it has been documented that short telomeres signal and activate p53, which represses PGC-1α and PGC-1β promoters, leading to mitochondrial dysfunction (Sahin et al., 2011). Conversely, telomerase, the enzyme responsible for maintaining telomere length, may impact directly on mitochondrial function since the reverse transcriptase component (TERT) can translocate to the mitochondria under conditions of mild oxidative stress and protect mitochondrial function by reducing ROS, protecting mitochondrial DNA (mtDNA) and increasing mitochondrial membrane potential (Ahmed et al., 2008).

On the other hand, mitochondria can influence DDR signalling and potentiate telomeric damage; mitochondrial-derived ROS are involved in induction and maintenance of senescence through feedback loops replenishing DNA damage and maintaining the DDR and senescence (Passos et al., 2007; Passos et al., 2010). Moreover, mitochondrial ROS can lead to the generation of single-strand breaks in telomere regions, with the mitochondrial-targeted antioxidant Mito-Q being able to reduce telomere shortening and increase replicative lifespan of fibroblasts under mild oxidative stress (Saretzki, Murphy, & von Zglinicki, 2003). Additionally, mild chronic uncoupling of mitochondria, which reduces accumulation of mitochondrial superoxide, improves telomere maintenance and extends telomere-dependent lifespan (Passos et al., 2007). On the other hand, FCCP treatment, which causes severe mitochondrial depolarisation and thus mitochondrial dysfunction, increases ROS generation and leads to telomere attrition and chromosome fusions in murine embryos, further supporting a role for mitochondrial dysfunction impacting on telomere health (Liu, Trimarchi, Smith, & Keefe, 2002).

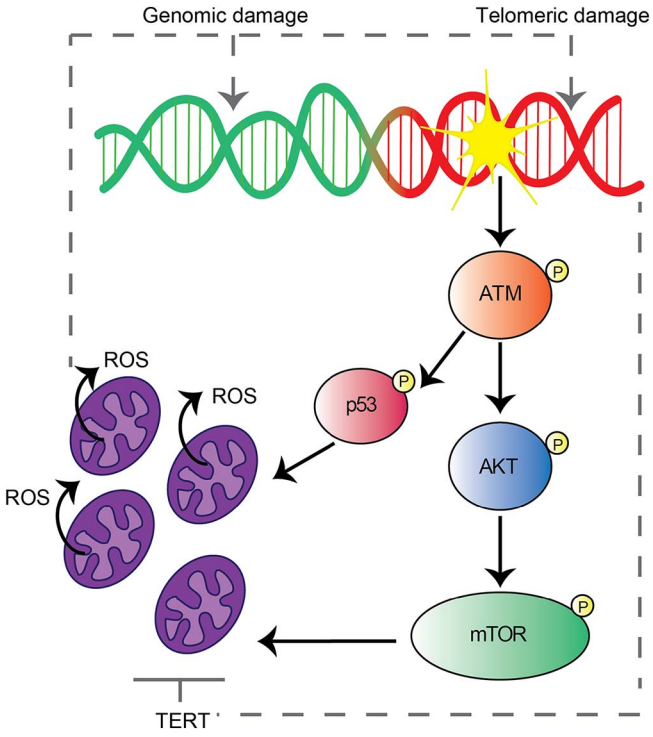

Fig. 2. Crosstalk between telomeres and the mitochondria. Damage at telomere regions, either due to telomere shortening or length-independent damage, elicits a DNA damage response (DDR) in a similar manner to double-strand breaks (DSBs). Activation of ATM kinase can impact on mTOR activity via AKT kinase and p21 leading to PGC-1β-dependent mitochondrial biogenesis and associated ROS generation. The activation of p53 can also impact on PGC-1α and PGC-1β, leading to mitochondrial dysfunction. Mitochondrial-derived ROS can feedback and directly affect DDR signalling by replenishing damage at genomic and telomere regions. Telomerase, the enzyme responsible for maintaining telomere length, may impact directly on mitochondrial function since the reverse transcriptase component (TERT) can translocate to the mitochondria under conditions of mild oxidative stress and reduce ROS, protecting mitochondrial DNA (mtDNA) and increasing mitochondrial membrane potential. ATM, ataxia-telangiectasia mutated; mTOR, mechanistic target of rapamycin; ROS, reactive oxygen species.

4.3. The role of the mitochondria in senescence

The mitochondria can impact on aspects of the senescence phenotype in a number of possible ways and it has been suggested that dysfunctional mitochondria are an additional feature of senescent cells that enable them to mediate paracrine effects (Birch & Passos, 2017; Korolchuk, Miwa, Carroll, & von Zglinicki, 2017). Therefore, crosstalk between telomeres and the mitochondria may have knock-on effects on a number of pathways that regulate senescence.

4.3.1. Are the mitochondria causal to senescence?

Mitophagy, the selective degradation of defective mitochondria by autophagy, is reduced in senescent cells (Garcia-Prat et al., 2016). This could, in part, be responsible for the increase in mitochondrial mass that has been described in senescence both *in vitro* and *in vivo* (Correia-Melo et al., 2016; Passos et al., 2010). mTOR-dependent mitochondrial biogenesis may also play a role, since treatment of cells and mice with rapamycin, an inhibitor of mTOR, reduces mitochondrial mass (Correia-Melo et al., 2016). Moreover, a reduction in mitochondrial fission events in senescent cells contributes to increased connectivity of the mitochondrial network (Dalle Pezze et al., 2014). The accumulation of the mitochondrial compartment and of dysfunctional mitochondria in particular, may be an important contributor to the pro-inflammatory aspects of cellular senescence. It has been shown that mitochondrial dysfunction induced by mtDNA depletion, knockdown of mitochondrial sirtuin 3 (SIRT3) or through inhibition of the electron transport chain

(ETC) induces senescence with a distinct phenotype, termed MiDAS (mitochondrial dysfunction-associated senescence) (Wiley et al., 2016). Our group recently designed a proof-of-principle experiment, which interrogated whether mitochondria are truly necessary for senescence. Utilising the parkin-mediated mitophagy system to completely remove mitochondria upon their depolarisation, we found that following a variety of senescence triggers (e.g. oxidative stress and oncogene activation) features of cellular senescence, including Sen-β-Gal activity and the SASP, were suppressed (Correia-Melo et al., 2016). Paradoxically, despite decreases in p21 and p16, mitochondrial clearance did not rescue cell-cycle arrest. Additionally, treating senescent cells with rapamycin supresses the senescence phenotype, including major SASP components, in a similar fashion to mitochondrial clearance by impacting on mTOR-driven PGC1-β-dependent mitochondrial biogenesis (Correia-Melo et al., 2016). This suggests that mTOR can regulate the SASP through mitochondria-dependent mechanisms. Indeed, data suggest that mTOR has an important role in regulating mitophagy; it has been described that reduced mTOR activity limits ROS generation within the cell by increasing autophagic flux and mitochondrial membrane potential, thereby extending replicative lifespan of fibroblasts (Lerner et al., 2013). The mitochondria may therefore be key to the regulation of some aspects of cellular senescence, such as the pro-inflammatory phenotype, and may be promising targets for SASP modulation (Birch & Passos, 2017). Additionally, data suggests that mTOR can regulate the SASP by mechanisms independent of the mitochondria. For instance, mTOR has been shown to regulate the translation of MAPKAPK2 kinase, which phosphorylates and inhibits the RNA binding protein ZFP36L1 during senescence, limiting its ability to degrade mRNA transcripts of numerous SASP factors (Herranz et al., 2015). mTOR also promotes translation of certain early SASP factors, including IL-1α, which is a membrane bound cytokine that can promote activity of NF-κB (Laberge et al., 2015).

4.3.2. Do alterations in mitochondrial metabolism impact on senescence?

How the mitochondria may regulate the SASP needs to be fully elucidated. A number of studies provide evidence to suggest that the metabolic state of the cell is drastically shifted in senescence and may influence senescence-associated inflammation (Wiley & Campisi, 2016). For example, senescent cells have enhanced glycolytic activity, with relatively less ATP being generated by oxidative phosphorylation (Zwerschke et al., 2003). Increases in AMP:ATP and ADP:ATP ratios have been linked to senescence induction; AMP-activated protein kinase (AMPK) is a regulator of cellular responses to energy stress and is activated by increased AMP and ADP, which then regulates a series of responses including mitochondrial biogenesis (Hardie, Ross, & Hawley, 2012). AMPK activation can also lead to phosphorylation and activation of p53, promoting cell-cycle arrest via transcriptional upregulation of p21 (Jiang, Du, Mancuso, Wellen, & Yang, 2013) and can inhibit HuR-dependent degradation of p21 and p16 mRNA (W. Wang, Yang, Lopez de Silanes, Carling, & Gorospe, 2003). Additionally, a low NAD^+/NADH ratio leading to AMPK activation was proposed as a mechanism mediating MiDAS, in the aforementioned study (Wiley et al., 2016). Low NAD^+/NADH ratios have also been shown to promote senescence by impacting on NAD^+-dependent poly-ADP ribose polymerase (PARP) activity, the main repairers of genotoxic stress-induced damage. Inhibition of PARP-1 maintains DNA damage and sensitises cells to senescence *in vitro* (Efimova et al., 2010). NAD^+ is also a co-factor for Sirtuins, a family of histone and protein deacetylases. Sirtuins impact on senescence in various ways, including regulating NF-κB activity and in maintaining transcriptionally silent chromatin of NF-κB-dependent genes, such as SASP factor transcripts (Haigis & Sinclair, 2010). Incidentally, Sirtuin levels are reduced in senescent cells (Sasaki, Maier, Bartke, & Scrable, 2006). Alterations in tricarboxylic acid (TCA) cycle activity have also been described in cellular senescence, however the extent to which this occurs is still not fully understood (Fig. 3). In OIS, pyruvate dehydrogenase (PDH) kinase 1 is suppressed, leading to increased activity of PDH and enhanced usage of pyruvate in the TCA cycle (Kaplon et al., 2013). In contrast, pyruvate assists in scavenging ROS and contributes to cellular anti-oxidant defences (Desagher, Glowinski, & Premont, 1997). However, the role of pyruvate in oxidant defence in senescence is unknown. Interestingly, pyruvate supplementation at superphysiological levels limits the replicative lifespan of fibroblasts *in vitro* (D. Xu & Finkel, 2002). Malate metabolism has also been implicated in senescence regulation, with loss of mitochondrial malic enzyme 2 (ME2) inducing senescence (Jiang et al., 2013). Additionally, overexpression of ME2 extends replicative lifespan of fibroblasts. Mechanistically, ME2 depletion increases ROS, leading to AMPK-dependent p53 activation and senescence induction (Jiang et al., 2013). Thus, malate metabolism may be involved in senescence by regulating anti-oxidant defences. Whether malate metabolism is involved in SASP regulation is undetermined. Interestingly, macrophages activated by pro-inflammatory stimuli undergo metabolic reprogramming, whereby mitochondria shift to a more glycolytic state and produce ROS, promoting a pro-inflammatory response (Mills et al., 2016), directly linking mitochondrial metabolism to pro-inflammatory cellular responses. Therefore, it is possible that shifts in mitochondrial metabolism could be important in SASP regulation; understanding metabolic control of senescence and the SASP may be crucial for development of therapies for age-related disease.

4.3.3. Mitochondrial damage-associated molecular patterns

Dysfunctional mitochondria release multiple forms of damage-associated molecular patterns (DAMPS), such as ATP and mtDNA. Similarly to pathogenic stimuli, mtDNA can activate innate immune receptors, known as pattern recognition receptors (PRRs). Therefore when present outside of the mitochondrial matrix, mtDNA is an immunostimulatory factor. Indeed, several signalling pathways sense mtDNA in the cytoplasm, including the NLRP3 inflammasome, which converges on NF-κB leading to production of pro-inflammatory cytokines IL-1β and IL-18 (Horng, 2014). The cytosolic sensor of DNA: the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway also senses mtDNA that has leaked into the cyotsol, which ultimately leads to type I interferon responses and an anti-viral innate immune response (West et al., 2015). Recently, the cGAS-STING pathway has been identified as a crucial regulator of senescence and the SASP, which is engaged in response to cytosolic chromatin fragments (CCFs) occurring due to Lamin B1 degradation in senescence, leading to production of inflammatory SASP factors (Gluck et al., 2017). Whether mtDNA plays a role in cGAS-STING-mediated SASP regulation is yet to be assessed. However, it has been shown that in cells lacking mtDNA, key SASP factors are attenuated (IL-1-depdendent arm) while other features of the senescence phenotype are present, such as Sen-β-Gal activity, suggesting that mtDNA might have some involvement in SASP regulation (Wiley et al., 2016). However, whether mtDNA leakage occurs in senescence or has a role in SASP regulation is unknown.

5. Telomere dysfunction in lung ageing and disease

Telomere dysfunction is strongly implicated in induction and maintenance of cellular senescence. While studies describe increased markers of senescence in the lungs with age, the role of telomere dysfunction is less well documented. Our lab has found that TAF increase in the airway epithelium of the murine lung with age (Birch et al., 2015). However, significant changes to telomere length were not detected. Mice ubiquitously express telomerase and have long telomeres, therefore telomere shortening may not play a major role in murine lung ageing. In fact, we have previously reported that while TAF significantly correlated with emphysematous-like changes in the murine lung with age, telomere length did not (Birch et al., 2015). In contrast, telomere shortening and telomere dysfunction may heavily influence the ageing human lung, however little research into telomere dysfunction and physiological human lung ageing exists.

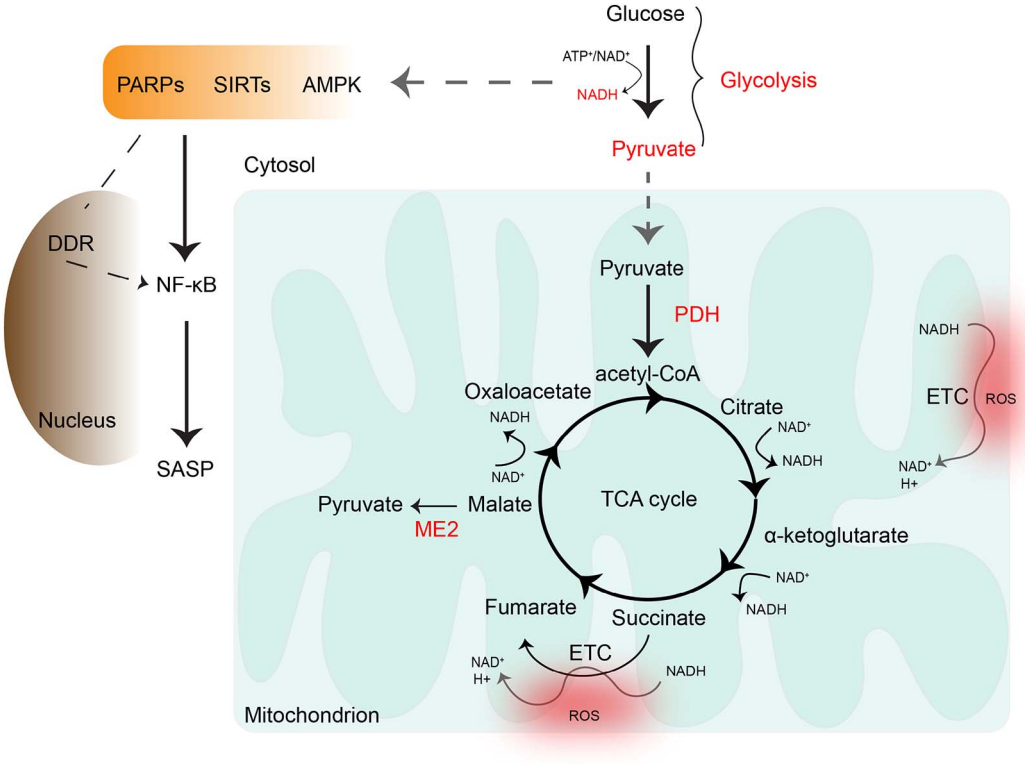

Fig. 3. Simplified depiction of key metabolic pathways and their involvement in cellular senescence. A number of metabolic processes have been found to be altered in cellular senescence (highlighted in red text). Glycolysis, which takes place in the cytosol and leads to the generation of pyruvate from glucose, is increased in senescent cells in culture. Pyruvate metabolism has also been implicated in cellular senescence. Pyruvate serves as the primary carbon source for acetyl-CoA, which feeds into the tricarboxylic acid (TCA) cycle; a series of reactions that take place in the matrix of the mitochondria to generate energy and NADH for use in the electron transport chain (ETC). While the ETC leads to generation of ATP, it is also the major source of mitochondrial reactive oxygen species (ROS). A number of intermediates are generated during the TCA cycle (not all are shown for simplicity) which have roles in various biological processes, such as amino acid and fatty acid synthesis. Malate, produced from fumarate by fumarase, is decarboxylated to pyruvate via mitochondrial malic enzyme 2 (ME2). ME2 expression is also altered in senescent cells. Changes in metabolic processes leading to alterations in NAD^+: NADH and AMP/ADP:ATP ratios impact on enzymes such as poly-ADP-ribose polymerases (PARPs) and sirtuins, which are dependent on NAD^+, and AMP-activated protein kinase (AMPK), which responds to high levels of AMP and ADP. PARPs are the main repairers of genotoxic stress-induced damage while sirtuins impact on senescence in various ways, including regulating NF-κB activity and in maintaining transcriptionally silent chromatin of NF-κB-dependent genes, such as SASP factor transcripts. AMPK promotes cell cycle arrest by impacting on activity and stability of cyclin-dependent kinase inhibitors p53, p21 and p16. DDR, DNA damage response; SASP, senescence-associated secretory phenotype; NF-κB, nuclear factor-κB.

5.1. *Telomere dysfunction in COPD*

In contrast to physiological lung ageing, telomere dysfunction is well-documented in a number of age-related lung diseases, such to a degree that they have been dubbed “telomere disorders” (Adnot et al., 2015). A number of studies have described accelerated telomere shortening in circulating leukocytes from patients with COPD (Houben et al., 2009; Mui et al., 2009). Savale and colleagues have shown that telomere length in circulating leukocytes of patients with COPD negatively correlates with plasma IL-6 levels (Savale et al., 2009). Interestingly, a dose-dependent relationship between smoking history and telomere length of circulating lymphocytes has also been described (Morla et al., 2006). In one study of over 40,000 individuals, shorter telomere length was associated with decreased lung function and increased risk of COPD (Rode, Bojesen, Weischer, Vestbo, & Nordestgaard, 2013). However, other studies have failed to find associations between telomere length and FEV1 or smoking history (Houben et al., 2009; Savale et al., 2009). Additionally, accelerated telomere shortening has been described in resident lung cells in patients with COPD, including type II alveolar cells and endothelial cells (Tsuji et al., 2006). In contrast, our group has previously reported a significant increase in TAF in small airway epithelial cells of patients with COPD, without significant changes in telomere length detected (Birch et al., 2015). It is highly plausible that both telomere shortening and length-independent telomere damage occur in the lungs of patients with COPD, since imbalances in oxidative stress and anti-oxidant defenses are a major feature of the disease (Houben et al., 2009), with telomeres being particularly susceptible to oxidative modifications and less efficiently repaired compared to the rest of the genome (Hewitt et al., 2012; Petersen et al., 1998; Richter & von Zglinicki, 2007). Indeed, there are an estimated $10^{14–17}$ oxidative particles per puff of cigarette smoke and patients with COPD show increased markers of oxidative stress both systemically and in the lung (Kirkham & Barnes, 2013; MacNee, 2001). Levels of 4-hydroxy-2-nonenal (4-HNE) (a lipid peroxidation end-product that reacts with extracellular proteins forming adducts) modified proteins were increased in airway and alveolar epithelial cells, endothelial cells and neutrophils present in lung tissue from patients with COPD (Rahman et al., 2002). Both RNA and DNA oxidation have been described in the alveolar walls of patients with COPD (Deslee et al., 2009). A common byproduct of guanine oxidation by ROS and reactive nitrogen species is 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Kasai, 1997). 8-OHdG is increased in the peripheral lungs of patients with COPD and smokers without COPD (Caramori et al., 2011; Tzortzaki et al., 2012). The high proportion of guanine triplets within telomere repeats in addition to their remarkable sensitivity to oxidative modifications suggests that telomeres could be the likely site of 8-OHdG occurrence. Accordingly, exposure to CSE leads to telomere shortening and TAF in embryonic stem cells, fibroblasts and airway epithelial cells *in vitro* (Birch et al., 2015; J. Huang et al., 2013) and *in vivo* exposure to cigarette smoke leads to increased TAF in the murine lung (Birch et al., 2015). In addition, exposure to cigarette smoke increases 8-OHdG and 4-HNE in the lungs of mice (Aoshiba et al., 2003; Yao et al., 2014). These findings suggest that telomere-associated damage is highly important to the pathogenesis of COPD – the properties of telomeres that

render them particularly susceptible to oxidative damage, plus protection from repair activities at telomere regions, places telomeres as key signalers of damage occurring as a consequence of cigarette smoke exposure and during the aging process.

5.2. Telomere dysfunction in IPF

One of the major indicators of telomeres being particularly important to development of chronic lung disease comes from studies using mouse models of telomere dysfunction. In two murine models of telomere dysfunction, whereby telomeres were critically short (telomerase-deficient mice) or a DNA damage response was activated at telomere regions without shortening (TRF1 deletion in type II alveolar cells), pulmonary fibrosis developed (Povedano, Martinez, Flores, Mulero, & Blasco, 2015). In patients with pulmonary fibrosis, telomere shortening is detectable in circulating leukocytes (Alder et al., 2008) and in alveolar type II cells in the lungs of patients with sporadic and familial pulmonary fibrosis (Alder et al., 2008). In addition, levels of TAF increase significantly in alveolar cells in lung tissue from patients with IPF as compared to controls (Schafer et al., 2017). However, some of the most compelling evidence for telomere shortening and dysfunction contributing to IPF, and COPD alike, comes from genetic studies showing that mutations in telomere-related genes are common in patients with familial pulmonary fibrosis and severe emphysema. Armanios et al. found that mutations in hTERT and hTR, the RNA component of telomerase, were common in patients with familial pulmonary fibrosis and lead to shorter leukocyte telomere length in these patients (Armanios et al., 2007). Similarly, deleterious mutations in hTERT have been found in cohorts of patients with severe COPD and have been identified as a risk factor for emphysema susceptibility in smokers, showing an autosomal dominant inheritance pattern in families (Stanley et al., 2015). Interestingly, an observational study of 149 patients with IPF found that shorter telomere length in circulating leukocytes was associated with a reduced transplant-free survival time (Stuart et al., 2014).

5.3. Telomere dysfunction in bronchiectasis and asthma

While limited studies are available investigating the role of telomeres in bronchiectasis and asthma, there are some data to suggest that telomere dysfunction may be involved in the pathogenesis of these diseases. Our lab has recently discovered that large airway epithelial cells in the bronchiectatic lung have shortened telomeres compared to aged-matched controls and a significantly higher number of TAF (Birch et al., 2016). Given this is the only documented investigation into telomere health in bronchiectasis, further research is required to understand the importance of this observation. Telomere shortening has been documented in circulating leukocytes from adults with asthma (Hadj Salem, Dube, Boulet, & Chakir, 2015). Accordingly, life-long persistent asthma is characterised by shorter leukocyte telomere length in comparison to childhood- and adult-onset, non-persistent asthma, suggesting telomere shortening could be important to disease chronicity (Belsky et al., 2014). However, the relevance of these observations to the cells directly involved in the disease process in the airways of asthmatics is unknown, since no published studies have examined telomere function in resident lung cells in the asthmatic lung. Our group has found that large airway epithelial cells have significantly more TAF in a small cohort of patients with adult-onset asthma as compared to controls (unpublished). Further research into the role of telomere dysfunction in the development and progression of asthma is required. While some markers of cellular senescence are increased in the lungs of patients with CF, telomere shortening was not detected in all patients (Fischer et al., 2013). Further study into telomere length and dysfunction in the CF lung and in circulating leukocytes of patients with CF is warranted.

6. Mitochondria in lung ageing and dysfunction

The mitochondria are particularly important for several features of the senescence state (Correia-Melo et al., 2016). In addition, altered mitochondrial homeostasis is documented in the healthy lung with age, including increases in mitochondrial mass and frequencies of fused mitochondria (Bueno et al., 2015), impaired respiration and increased mitochondrial-derived ROS (Braidy et al., 2011). Moreover, a number of mitochondrial changes associated with senescence occur in age-related lung diseases, including in COPD and IPF. Therefore, understanding the mechanisms behind mitochondrial dysfunction could offer potential therapeutic avenues for treatment of age-related lung dysfunction and chronic lung disease.

Since it is difficult to discern whether mitochondrial-derived ROS *per se* are upregulated in the lungs of patients with chronic lung disease, the majority of data implicating mitochondrial ROS in COPD is in isolated cells. For instance, exposure to CSE has been shown to increase mitochondrial-derived ROS in vascular endothelial cells and lung fibroblasts (Birch et al., 2015; Csiszar et al., 2008), which can be supressed by cultivation of cells under low oxygen concentrations, also limiting TAF and Sen-β-Gal activity (Birch et al., 2015). Additionally, airway smooth muscle cells from patients with COPD produce greater amounts of mitochondrial-derived ROS compared to controls at baseline and following inflammatory stress, such as treatment with IL-1 or TNF-α (Wiegman et al., 2015). Nevertheless, since markers of oxidative stress are upregulated in the lungs of patients with COPD and mitochondria are the main producers of ROS within the cell, it is highly likely that mitochondrial ROS are a major source of COPD-associated oxidative stress. Indeed, in the ozone-induced mouse model of COPD, airway hyper-responsiveness and inflammation was attenuated by the mitochondrial-targeted antioxidant Mito-Q (Wiegman et al., 2015). Increased oxidative stress has also been described in IPF. Hecker and colleagues showed that ROS-generating NADPH oxidase-4 (NOX-4) is up-regulated in IPF lung tissue and in IPF-derived lung fibroblasts, along with other markers of senescence (Hecker et al., 2014). Interestingly, in aged mice, pharmacological inactivation of NOX-4 attenuates myofibroblast senescence and reverses persistence of fibrosis (Hecker et al., 2014). These results suggest that restoring redox homeostasis, or interfering with other key effectors of senescence, could ameliorate age-related fibrotic phenotypes.

Changes in mitochondrial morphology have been described in the ageing lung and in age-related lung disease. Bronchial epithelial cells from patients with COPD harbour swollen, elongated mitochondria in addition to fragmentation and disruption of cristae (Hoffmann et al., 2013). Moreover, treatment of human bronchial epithelial cells with CSE leads to long-term changes in mitochondrial structure (Hoffmann et al., 2013) and causes mitochondrial fragmentation (Hara et al., 2013). Interestingly, PGC1-α-mediated mitochondrial biogenesis is increased in mild COPD but levels of PGC1-α steadily decrease with increasing COPD severity (Li, Dai, Hu, Zhu, & Tan, 2010). Increased mitochondrial mass observed following CSE exposure could be due to decreases in mitophagy, since key mitophagy-mediating factors like Parkin are reduced in the lungs of patients with COPD and non-COPD smokers (Ahmad et al., 2015; S. Ito et al., 2015). In addition, knockdown of Parkin and its activator PTEN-induced putative kinase 1 (PINK1), which are responsible for promoting mitophagy of damaged mitochondria, leads to increased mitochondrial ROS produced as a consequence of CSE exposure (S. Ito et al., 2015) In contrast, some reports describe an increase in Parkin and PINK1 in airway epithelial cells from the COPD lung, however this could be a compensatory effect (Hoffmann et al., 2013; Mizumura et al., 2014). However, PINK1$^{-/-}$ mice are protected against mitochondrial dysfunction and airspace enlargement following exposure to cigarette smoke (Mizumura et al., 2014), suggesting that mitophagy-dependent events are disadvantageous in the development of emphysema following cigarette smoke exposure (Mizumura et al., 2014). In the IPF lung, Bueno and

colleagues showed that type II alveolar epithelial cells harbour dysmorphic and dysfunctional mitochondria with reduced expression of PINK1 (Bueno et al., 2015). Hawkins and colleagues also demonstrated that mutations causing defective clearance of mitochondria are linked to familial pulmonary fibrosis (Hawkins et al., 2015). In addition, research by Patel and collaborators demonstrated that PINK1-deficient mice have enhanced susceptibility to pulmonary fibrosis (Patel et al., 2015). This could suggest that mitochondrial dysfunction could be mediated, in part, by impaired mitophagy as a consequence of cigarette smoke exposure or during disease progression. However, further work is required to fully elucidate the role of mitophagy in age-related lung disease, since reports are conflicting; something that has previously been described with autophagy being beneficial in some instances, while detrimental in others (Korolchuk et al., 2017).

Intricate changes in mitochondrial metabolism, such as shifts in glycolysis or TCA cycle activity are relatively understudied in the ageing lung and in age-related respiratory diseases. In a model of progressive murine emphysema, L-carnitine was found to be significantly reduced (Conlon et al., 2016). Carnitine is involved in fatty acid transfer into the mitochondria, which may suggest that alterations in fatty acid metabolism are linked to mitochondrial dysfunction in certain pathologies, such as emphysema. In another study, it was found that knockdown of iron-responsive element-binding protein 2 (IRP2) protected mice from development of CS-induced COPD (Cloonan et al., 2016). IRP2 increases mitochondrial iron loading and mitochondrial dysfunction through elevating levels of mitochondrial cytochrome C oxidase, which then causes COPD (Cloonan et al., 2016). Mice on a low-iron diet or treated with an iron chelator were protected against CS-induced inflammation and lung injury, suggesting that the mitochondrial-iron axis is important to development of cigarette smoke-induced COPD (Cloonan et al., 2016). Changes in NAD^+/NADH ratios occur in cellular senescence. Reductions in NAD^+-dependent SIRT1, SIRT3 and SIRT6 have been implicated in COPD and IPF, respectively (Birch et al., 2015; Nakamaru et al., 2009; Rajendrasozhan, Yang, Kinnula, & Rahman, 2008; Sosulski, Gongora, Feghali-Bostwick, Lasky, & Sanchez, 2017). SIRT1 is decreased in the airway epithelium and peripheral lung of patients with COPD (Birch et al., 2015; Nakamaru et al., 2009; Rajendrasozhan et al., 2008). Moreover, CSE reduces SIRT1 expression *in vitro* (Caito et al., 2010; Rajendrasozhan et al., 2008). SIRT6 is also reduced in the COPD lung, in isolated small airway epithelial cells from COPD patients, and following exposure to oxidative stress *in vitro* (Baker et al., 2016; Nakamaru et al., 2009). Levels of SIRT3 are decreased in fibrotic regions of the IPF lung and SIRT3 deficiency has been shown to augment pulmonary fibrosis in mice (Sosulski et al., 2017). SIRT1 is also reduced in the epithelium of patients with bronchiectasis (Birch et al., 2016). Sirtuins are involved in histone and protein post-translational modifications and SIRT1 supresses the expression of SASP factors via histone deacetylation at their promoter regions and via deacetylation of NF-κB (Hayakawa et al., 2015). Interestingly, it was reported that pharmacological inhibition of histone deacetylases ameliorates bleomycin-induced pulmonary fibrosis in mice (Sanders et al., 2014), suggesting that activation or inhibition of sirtuins is a potential therapeutic option in chronic lung disease, which may be disease- or severity-dependent. In line with this, a sirtuin-activating compound is able to prevent the decrease in SIRT1 that occurs in mice following cigarette smoke exposure, while also inhibiting neutrophilic inflammation in the lung (Nakamaru et al., 2009).

How the mitochondrial metabolome is altered in the ageing lung or in conditions of accelerated lung ageing is largely unknown. Some data suggests that cigarette smoke exposure leads to reduced glycolytic activity *in vitro* (Agarwal et al., 2012). Whereas, cigarette smoke exposure leads to increases in succinate dehydrogenase (SDH) gene expression in the murine lung (Agarwal et al., 2012). Moreover, citrate synthase activity is reduced in muscle from patients with COPD (Konokhova et al., 2016). Research into the role of glycolysis and TCA cycle activity is required in lung ageing and disease, since the mitochondria are particularly important in regulating a number of cellular functions and alterations in mitochondrial metabolism may be linked to SASP modulation.

As previously mentioned, mtDNA is an immunustimulatory factor that could potentially be important to senescence and senescence-associated inflammation. Interestingly, CSE exposure causes DAMP release from neutrophils, including HMGB1 and mtDNA, inducing innate immune responses (Heijink et al., 2015; Pouwels et al., 2016). Moreover, mtDNA is increased in saliva of smokers, even decades after smoking cessation, suggesting long-term changes induced by cigarette smoke exposure (Masayesva et al., 2006). Asthma-COPD overlap syndrome (ACOS) is a respiratory condition including features of both COPD and asthma (Barnes, 2016a, 2016b). Levels of mtDNA are increased relative to nuclear DNA in the blood of patients with ACOS. While the role of DAMPs, including mtDNA, in IPF have not particularly been studied, it has been shown that activation of the NLRP3 inflammasome, a sensor of cytosolic mtDNA, is relevant to the development of lung fibrosis, since deficiency of NLRP3 diminishes bleomycin-induced lung injury in aged animals (Stout-Delgado et al., 2016). Understanding whether mtDNA acts as an immunostimulatory factor, or contributes to senescence, in the context of chronic lung disease may therefore be of therapeutic interest.

7. Therapeutic opportunities

There is currently no cure for chronic inflammatory lung diseases like COPD and IPF, and the use of anti-inflammatories has in general lead to disappointing results. Numerous studies strongly support the concept that cellular senescence is involved in the pathogenesis of COPD and IPF, and is potentially relevant to other respiratory conditions. Therefore, therapies that harness pathways involved in cellular senescence may be on the horizon for respiratory diseases (Barnes, 2017). While oxidative stress is a major feature of COPD, the use of anti-oxidants such as *N*-Acetyl Cysteine (NAC) has not produced clear benefits in trials and only has moderate effects on patients with COPD (Shen, Cai, Lei, & Zhang, 2014). This is possibly due to the fact that NAC is inactivated by high levels of oxidative stress, hence more effective antioxidants are required. Indeed, novel, more stable, anti-oxidants are now being considered including SOD mimetics and Nrf2 activators (Barnes, 2017) Indeed, restoration of the NOX4-Nrf2 redox imbalance was sufficient to reverse persistent fibrosis, at least in mice (Hecker et al., 2014).

Studies demonstrating genetic elimination of already senescent cells have provided a proof-of-concept that removal of senescent cells could be a viable therapy for treating aspects of age-related functional decline, age-related diseases or potential side-effects of therapies that lead to the generation of senescent cells. While Baker and colleagues have demonstrated that genetic elimination of $p16^{INK4a}$-positive senescent cells delays onset of a number of age-related pathologies and extends lifespan during accelerated and physiological ageing (Baker et al., 2011; Baker et al., 2016), the same transgenic mouse model has also been utilised to demonstrate that removal of senescent cells can improve lung function and fitness of mice with pulmonary fibrosis following bleomycin injury (Schafer et al., 2017). In both of these studies no overt side effects of senescent cell clearance were reported. Genetic elimination of senescent cells is an unlikely viable strategy in humans. However, the development of pharmacological drivers of senescent-cell apoptosis, known as senolytics, offers a potential alternative. Senolytics act by inhibiting pro-survival and anti-apoptotic proteins that are specifically upregulated in senescent cells. It has been shown *in vitro* that a combination of dasatinib, a tyrosine kinase inhibitor, and quercetin, a flavanoid and inhibitor of phosphatidylinositol-4,5-bisphosphate 3-kinases (PI3Ks), (D + Q) reduces expression of BCL-xL in both senescent pre-adipocytes and HUVEC cells (Zhu et al., 2016). D + Q has also been shown to reduce senescent cell burden in aged, radiation-exposed and progeroid mice and improves healthspan, including cardiac function

(Zhu et al., 2015). D + Q administration reduces senescent cell frequencies and improves established age-associated vascular phenotypes, such as aortic calcification, in aged and hypercholesterolaemic mice (Roos et al., 2016). Interestingly, D + Q administration improves the health of mice with pulmonary fibrosis to the same degree as genetic elimination of senescent cells (Schafer et al., 2017) and has been shown to reduce fibrotic burden and key SASP factors, while upregulating markers of type II alveolar epithelial cells, in an *ex vivo* model of pulmonary fibrosis derived from mice treated with bleomycin (Lehmann et al., 2017). ABT-263, otherwise known as navitoclax, is an inhibitor of the BCL-2 family, which has been shown to induce apoptosis in senescent HUVECs, human and murine fibroblasts *in vitro* (Zhu et al., 2016). Navitoclax has also been shown to effectively deplete senescent cells, including senescent bone marrow hematopoietic stem cells and senescent muscle stem cells, in sub-lethally irradiated and physiologically aged mice (Chang et al., 2016). Moreover, navitoclax has recently been shown to reverse pulmonary fibrosis induced by thoracic irradiation in mice (Pan et al., 2017). These results suggest that senolytics could be a viable option for treatment of pathologies involving cellular senescence, including age-related lung disease. Strengthening this notion are data from Schafer and colleagues that showed both early administration of senolytics, at onset of disease pathogenesis, and administration at a later time point when the pathology had fully developed, lead to beneficial effects (Schafer et al., 2017). This suggests that senescent cell clearance may be beneficial even at later stages of disease progression. Whether senolytics are beneficial in mouse models of COPD still needs to be determined. It must be noted that the potential pitfalls of senolytic drug use are still not fully understood, with possible “side effects” suspected in non-senescent healthy cells (Birch & Passos, 2017). In addition, senolytics have shown cell specificity in their apoptosis-inducing efficacy (Zhu et al., 2016) and so determining the senolytic of choice, depending on the senescent cell type involved, adds another layer of complexity. This point particularly resonates for diseases where multiple cell types are involved in the senescence programme, as has been described in COPD and IPF. Another potential option could be to harness the immune system to induce senescent cell clearance by immunotherapy methods. Studies have provided strong evidence to suggest that components of the immune system are responsible for senescent cell clearance. While no current data exit showing immune-mediated clearance of senescent cells induced by specific activation of the immune response, such as using a senescence-associated peptide or DAMP, it is a possibility that the immune system could be driven in this direction in a similar manner to cancer immunotherapy. Senescence has some beneficial effects, as previously alluded to, therefore complete removal of senescent cells may not be desirable. This is also the case in the context of lung ageing and disease, since some studies show that senescence is beneficial in proliferative diseases such as cancer and pulmonary hypertension (PH). Inducing cellular senescence by blocking p53 inactivation prevented and reversed PH in animal models (Mouraret et al., 2013). Furthermore, telomerase inactivation in mice reduces the severity of experimental PH, by inhibiting cell proliferation and by promoting senescence in pulmonary vascular smooth muscle cells (Mouraret et al., 2015). PH is a common feature of both COPD and IPF and the presence of PH is associated with a poorer prognosis, therefore the use of senolytic therapies would need to be very carefully considered. Moreover, around one-third of patients with COPD die from lung cancer and IPF patients also have increased cancer risk, which is an important consideration when advocating anti-senescence therapies for use in these diseases.

Interfering with pathways important to senescence regulation, such as those that orchestrate SASP modulation, could be a better strategy, or indeed, inhibition of SASP factors themselves. The mTOR pathway has previously been considered a potential druggable target for age-related lung disease. Inhibition of mTOR with rapamycin has been shown to increase lifespan in various model organisms (Harrison et al., 2009; Miller et al., 2011) and attenuates a number of age-related changes in mice (Wilkinson et al., 2012). It has previously been suggested that the beneficial effects of rapamycin *in vivo* could be due to its ability to acts as a SASP suppressor, since rapamycin inhibits IL-1 translation and reduces stability of SASP factor mRNA transcripts *in vitro* (Herranz et al., 2015; Laberge et al., 2015). Indeed, mTOR activity is upregulated in the murine lung with age (Calhoun et al., 2016) and in fibroblasts from patients with IPF (Romero et al., 2016). Moreover, Calhoun and colleagues demonstrated that rapamcyin reduces collagen deposition that occurs in the murine lungs with age, along with reducing senescent cell frequencies (Calhoun et al., 2016). The role of mTOR in COPD is less well defined, however our lab have found that inhibition of mTOR with rapamycin reduces the SASP induced following CSE exposure (unpublished). Similar results have been documented in mice (Yoshida et al., 2010). Additionally, metformin, which activates the AMP kinase and subsequently inhibits mTOR has beneficial effects in patients with COPD. The safety of metformin in patients with COPD and type 2 diabetes mellitus was assessed recently, and improved patient survival was documented (Hitchings, Archer, Srivastava, & Baker, 2014; Sexton, Metcalf, & Kolbe, 2014). Moreover, mTOR activation has been described in peripheral blood mononuclear cells from patients with COPD, with rapamycin treatment restoring corticosteroid sensitivity (Mitani, Ito, Vuppusetty, Barnes, & Mercado, 2016). Furthermore, a prospective study showed improvements in some symptoms and health status of patients with COPD given metformin for 6 months (Sexton et al., 2014). Therefore, rapamycin and metformin use could potentially be beneficial in COPD. Additionally, the use of a combined mTOR and PI3K inhibitor, known as GSK2126458, reduces pro-fibrotic collagen formation in IPF lung tissue slices (Mercer et al., 2016), suggesting that mTOR inhibition could be considered as a method to interfere with fibrogenic signaling in IPF. This is in line with another study showing beneficial effects of targeting the PI3K/AKT/mTOR pathway with rapamycin and PP42 in IPF-derived fibroblasts, which activated autophagy and induced apoptosis in usually apoptosis-resistant IPF fibroblasts under conditions of serum starvation (Romero et al., 2016). Whether these drugs could act as novel senolytics under certain conditions should be further investigated. Indeed, another study demonstrated that inhibition of the SASP factor PAI-1, which is overexpressed in murine lung fibroblasts with age and following bleoymycin challenge, eliminated resistance to apoptosis in fibroblasts derived from fibrotic murine lungs (W. T. Huang et al., 2015). It could be possible therefore that targeting of key SASP factors, some of which are known to maintain senescence in an autocrine fashion (Acosta et al., 2008), is a form of senotherapy that should be explored. Mitochondria-targeted therapies, including regulation of mitophagy, altering mitochondrial homeostasis or impacting on mitochondrial biogenesis are other possible alternatives. To this end, inhibitors of mitochondrial fission such as the DRP1 inhibitor Mdivi-1 have been shown to rescue abnormal mitochondrial processes in models of COPD (Mizumura et al., 2014). Interestingly, in murine cancer models, alterations in amino acid availability, such as serine and glycine starvation reduces tumour growth and leads to improved survival (Maddocks et al., 2017). While only limited data exist suggesting alterations in the mitochondrial metabolome in lung disease, mechanisms underlying these changes may potentially be exploited for the development of future therapies for respiratory disease, particularly since evidence suggests that alterations in mitochondrial function may impact on specific SASP factors (Correia-Melo et al., 2016). However, the exact roles of these processes in lung ageing and disease must first be elucidated.

The future is bright for the generation of innovative therapies to treat age-related respiratory diseases, such as COPD and IPF, with therapeutic advances likely to extend to other lung conditions (Fig. 4). The comorbidities associated with COPD and IPF are also largely due to accelerated ageing and appear to share common molecular pathways. Therefore, it is possible that therapies could be developed that would also treat associated comorbidities, such as cardiovascular disease and type 2 diabetes (Barnes, 2015). Other respiratory disorders that could

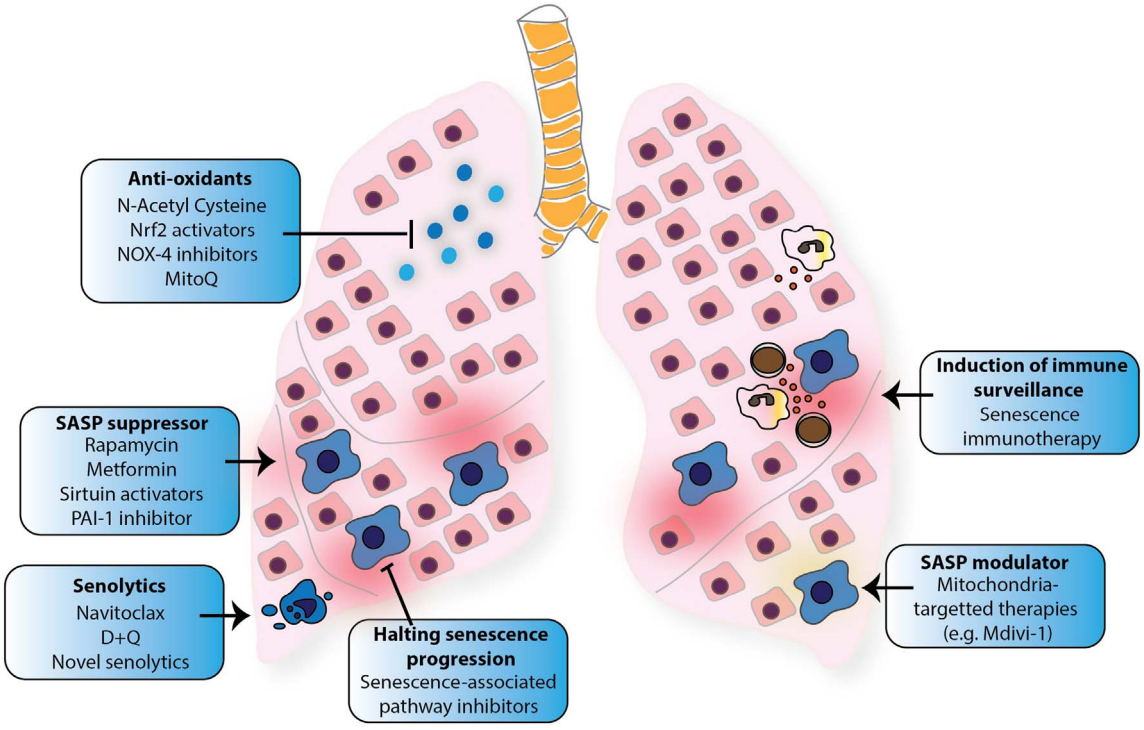

Fig. 4. Potential methods to counteract senescence-associated processes for therapeutic gain in respiratory disease. Cellular senescence and associated processes have been implicated in a range of respiratory diseases, including chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). Therefore, anti-senescence therapies or 'senotherapies' could be utilised to counteract disease progression. The high levels of oxidative stress present in the lungs of patients with age-related lung disease, such as COPD, could mean that effective antioxidants could be beneficial in dampening inflammation and further progression of senescence-associated pathways. Specific induction of apoptosis in senescent cells using senolytics, which act by inhibiting anti-apoptotic proteins in the mitochondrial membrane, is another viable option. Suppressors of the senescence-associated secretory phenotype (SASP), such as rapamycin, metformin or sirtuin activators could also be considered. Inducing senescent cell clearance by manipulating the immune system to recognise and clear senescent cells is another potential senotherapy that could be explored in age-related respiratory disease. Moreover, halting the senescence programme or modifying the components of the SASP could be exploited as a "senostatic" therapy, however, understanding of the processes involved, including the role of mitochondrial metabolism and associated pathways is crucial for these regulators to be manipulated for therapeutic gain. D + Q, dasatinib + quercetin; Nrf2, nuclear factor erythroid 2–related factor 2; NOX-4, NADPH oxidase-4; PAI-1, plasminogen activator inhibitor 1.

potentially benefit from senotherapies include pathologies arising following lung transplantation, such as bronchiolitis obliterans syndrome (BOS) (Parker et al., 2008) and senescence-related pathologies arising following lung cancer treatment. Indeed, a recent study has demonstrated that a cell penetrating peptide (CPP) designed around a specific amino acid sequence excludes p53 from the nucleus of senescent cells and induces apoptosis. This CPP is also able to reduce doxorubicin-induced chemo-toxicity *in vivo* (Baar et al., 2017), suggesting that anti-senescence therapies may be useful to counteract senescence induced by cancer therapy. While the use of senolytics for treatment of age-related disease is an attractive concept, and no overt side effects have been recorded in control mice or *ex vivo* lung tissues when administered in the context of pulmonary fibrosis (Lehmann et al., 2017; Schafer et al., 2017), reports of undesirable side-effects have been recorded in human cancer patients, including thrombocytopenia (Rudin et al., 2012). Hence, the full extent of the off-target effects of senolytics needs to be uncovered. Another area for consideration relates to the beneficial functions of senescent cells that have been identified. Therefore the context in which senescent cells are targeted needs to be carefully fine-tuned. Molecules that interfere with regulatory processes active in senescent cells, such as those that govern the SASP, may be a better alternative. The use of aged and senescence-prone mice, which have so far shown to recapitulate some of the key features of human respiratory disease, to conduct preclinical studies of such interventions is an exciting possibility. However, how humans and mice differ in the key processes that regulate senescence, such as mTOR signalling and mitochondrial metabolism, needs to be determined if any of the described senotherapies are to be truly translational to humans. The roles of such processes in governing cellular senescence in relation to age-related lung disease also requires further study, and whether senescence is an important process contributing to the pathogenesis of respiratory diseases not typically associated with ageing, such as CF and asthma, needs also to be clearly understood.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Acosta, J. C., Banito, A., Wuestefeld, T., Georgilis, A., Janich, P., Morton, J. P., ... Gil, J. (2013). A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nature Cell Biology, 15*, 978–990.

Acosta, J. C., O'Loghlen, A., Banito, A., Guijarro, M. V., Augert, A., Raguz, S., ... Gil, J. (2008). Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell, 133*, 1006–1018.

Adnot, S., Amsellem, V., Boyer, L., Marcos, E., Saker, M., Houssaini, A., ... Boczkowski, J. (2015). Telomere dysfunction and cell senescence in chronic lung diseases: Therapeutic potential. *Pharmacology & Therapeutics, 153*, 125–134.

Agarwal, A. R., Zhao, L., Sancheti, H., Sundar, I. K., Rahman, I., & Cadenas, E. (2012). Short-term cigarette smoke exposure induces reversible changes in energy metabolism and cellular redox status independent of inflammatory responses in mouse lungs. *American Journal of Physiology - Lung Cellular and Molecular Physiology, 303*, L889–L898.

Ahmad, T., Sundar, I. K., Lerner, C. A., Gerloff, J., Tormos, A. M., Yao, H., & Rahman, I. (2015). Impaired mitophagy leads to cigarette smoke stress-induced cellular senescence: Implications for chronic obstructive pulmonary disease. *The FASEB Journal,*

29, 2912–2929.
Ahmed, S., Passos, J. F., Birket, M. J., Beckmann, T., Brings, S., Peters, H., ... Saretzki, G. (2008). Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. *Journal of Cell Science, 121*, 1046–1053.
Albino, A. P., Huang, X., Jorgensen, E., Yang, J., Gietl, D., Traganos, F., & Darzynkiewicz, Z. (2004). Induction of H2AX phosphorylation in pulmonary cells by tobacco smoke: A new assay for carcinogens. *Cell Cycle, 3*, 1062–1068.
Alder, J. K., Chen, J. J., Lancaster, L., Danoff, S., Su, S. C., Cogan, J. D., ... Armanios, M. Y. (2008). Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 13051–13056.
Alder, J. K., Cogan, J. D., Brown, A. F., Anderson, C. J., Lawson, W. E., Lansdorp, P. M., ... Armanios, M. (2011). Ancestral mutation in telomerase causes defects in repeat addition processivity and manifests as familial pulmonary fibrosis. *PLoS Genetics, 7*, e1001352.
Aldonyte, R., Jansson, L., Piitulainen, E., & Janciauskiene, S. (2003). Circulating monocytes from healthy individuals and COPD patients. *Respiratory Research, 4*, 11.
Amsellem, V., Gary-Bobo, G., Marcos, E., Maitre, B., Chaar, V., Validire, P., ... Adnot, S. (2011). Telomere dysfunction causes sustained inflammation in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine, 184*, 1358–1366.
Aoshiba, K., Koinuma, M., Yokohori, N., & Nagai, A. (2003). Immunohistochemical evaluation of oxidative stress in murine lungs after cigarette smoke exposure. *Inhalation Toxicology, 15*, 1029–1038.
Aoshiba, K., & Nagai, A. (2009). Senescence hypothesis for the pathogenetic mechanism of chronic obstructive pulmonary disease. *Proceedings of the American Thoracic Society, 6*, 596–601.
Aoshiba, K., Zhou, F., Tsuji, T., & Nagai, A. (2012). DNA damage as a molecular link in the pathogenesis of COPD in smokers. *The European Respiratory Journal, 39*, 1368–1376.
Armanios, M. Y., Chen, J. J., Cogan, J. D., Alder, J. K., Ingersoll, R. G., Markin, C., ... Loyd, J. E. (2007). Telomerase mutations in families with idiopathic pulmonary fibrosis. *The New England Journal of Medicine, 356*, 1317–1326.
Baar, M. P., Brandt, R. M., Putavet, D. A., Klein, J. D., Derks, K. W., Bourgeois, B. R., ... de Keizer, P. L. (2017). Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. *Cell, 169*(132-147), e116.
Bae, N. S., & Baumann, P. (2007). A RAP1/TRF2 complex inhibits nonhomologous end-joining at human telomeric DNA ends. *Molecular Cell, 26*, 323–334.
Baker, D. J., Childs, B. G., Durik, M., Wijers, M. E., Sieben, C. J., Zhong, J., ... van Deursen, J. M. (2016). Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature, 530*, 184–189.
Baker, D. J., Wijshake, T., Tchkonia, T., LeBrasseur, N. K., Childs, B. G., van de Sluis, B., ... van Deursen, J. M. (2011). Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature, 479*, 232–236.
Baker, J. R., Vuppusetty, C., Colley, T., Papaioannou, A. I., Fenwick, P., Donnelly, L., ... Barnes, P. J. (2016). Oxidative stress dependent microRNA-34a activation via PI3Kalpha reduces the expression of sirtuin-1 and sirtuin-6 in epithelial cells. *Scientific Reports, 6*, 35871.
Barnes, P. J. (2015). Mechanisms of development of multimorbidity in the elderly. *The European Respiratory Journal, 45*, 790–806.
Barnes, P. J. (2016a). Asthma-COPD overlap. *Chest, 149*, 7–8.
Barnes, P. J. (2016b). Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. *Journal of Allergy and Clinical Immunology, 138*, 16–27.
Barnes, P. J. (2017). Senescence in COPD and its comorbidities. *Annual Review of Physiology, 79*, 517–539.
Belsky, D. W., Shalev, I., Sears, M. R., Hancox, R. J., Lee Harrington, H., Houts, R., ... Caspi, A. (2014). Is chronic asthma associated with shorter leukocyte telomere length at midlife? *American Journal of Respiratory and Critical Care Medicine, 190*, 384–391.
Bhowmik, A., Seemungal, T. A., Sapsford, R. J., & Wedzicha, J. A. (2000). Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. *Thorax, 55*, 114–120.
Birch, J., Anderson, R. K., Correia-Melo, C., Jurk, D., Hewitt, G., Marques, F. M., ... Passos, J. F. (2015). DNA damage response at telomeres contributes to lung ageing and chronic obstructive pulmonary disease. *American Journal of Physiology. Lung Cellular and Molecular Physiology, AJPLung, 00293*, 02015.
Birch, J., & Passos, J. F. (2017). Targeting the SASP to combat ageing: Mitochondria as possible intracellular allies? *Bioessays*.
Birch, J., Victorelli, S., Rahmatika, D., Anderson, R. K., Jiwa, K., Moisey, E., ... Passos, J. F. (2016). Telomere dysfunction and senescence-associated pathways in bronchiectasis. *American Journal of Respiratory and Critical Care Medicine, 193*, 929–932.
Blackburn, E. H. (1991). Structure and function of telomeres. *Nature, 350*, 569–573.
Bodnar, A. G., Ouellette, M., Frolkis, M., Holt, S. E., Chiu, C. P., Morin, G. B., ... Wright, W. E. (1998). Extension of life-span by introduction of telomerase into normal human cells. *Science, 279*, 349–352.
Boyer, L., Chouaid, C., Bastuji-Garin, S., Marcos, E., Margarit, L., Le Corvoisier, P., ... Adnot, S. (2015). Aging-related systemic manifestations in COPD patients and cigarette smokers. *PLoS One, 10*, e0121539.
Braidy, N., Guillemin, G. J., Mansour, H., Chan-Ling, T., Poljak, A., & Grant, R. (2011). Age related changes in NAD + metabolism oxidative stress and Sirt1 activity in wistar rats. *PLoS One, 6*, e19194.
Bueno, M., Lai, Y. C., Romero, Y., Brands, J., St Croix, C. M., Kamga, C., ... Mora, A. L. (2015). PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. *The Journal of Clinical Investigation, 125*, 521–538.
Burrows, B., Martinez, F. D., Halonen, M., Barbee, R. A., & Cline, M. G. (1989). Association of asthma with serum IgE levels and skin-test reactivity to allergens. *The New England Journal of Medicine, 320*, 271–277.
Caito, S., Rajendrasozhan, S., Cook, S., Chung, S., Yao, H., Friedman, A. E., ... Rahman, I. (2010). SIRT1 is a redox-sensitive deacetylase that is post-translationally modified by oxidants and carbonyl stress. *The FASEB Journal, 24*, 3145–3159.
Calhoun, C., Shivshankar, P., Saker, M., Sloane, L. B., Livi, C. B., Sharp, Z. D., ... Le Saux, C. J. (2016). Senescent cells contribute to the physiological remodeling of aged lungs. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 71*, 153–160.
Campisi, J., & d'Adda di Fagagna, F. (2007). Cellular senescence: When bad things happen to good cells. *Nature Reviews. Molecular Cell Biology, 8*, 729–740.
Capparelli, C., Chiavarina, B., Whitaker-Menezes, D., Pestell, T. G., Pestell, R. G., Hulit, J., ... Lisanti, M. P. (2012). CDK inhibitors (p16/p19/p21) induce senescence and autophagy in cancer-associated fibroblasts, "fueling" tumor growth via paracrine interactions, without an increase in neo-angiogenesis. *Cell Cycle, 11*, 3599–3610.
Caramori, G., Adcock, I. M., Casolari, P., Ito, K., Jazrawi, E., Tsaprouni, L., ... Papi, A. (2011). Unbalanced oxidant-induced DNA damage and repair in COPD: A link towards lung cancer. *Thorax, 66*, 521–527.
Chang, J., Wang, Y., Shao, L., Laberge, R. M., Demaria, M., Campisi, J., ... Zhou, D. (2016). Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nature Medicine, 22*, 78–83.
Childs, B. G., Baker, D. J., Wijshake, T., Conover, C. A., Campisi, J., & van Deursen, J. M. (2016). Senescent intimal foam cells are deleterious at all stages of atherosclerosis. *Science, 354*, 472–477.
Choudhury, A. R., Ju, Z., Djojosubroto, M. W., Schienke, A., Lechel, A., Schaetzlein, S., ... Rudolph, K. L. (2007). Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. *Nature Genetics, 39*, 99–105.
Chung, K. F., & Adcock, I. M. (2008). Multifaceted mechanisms in COPD: Inflammation, immunity, and tissue repair and destruction. *The European Respiratory Journal, 31*, 1334–1356.
Cloonan, S. M., Glass, K., Laucho-Contreras, M. E., Bhashyam, A. R., Cervo, M., Pabon, M. A., ... Choi, A. M. (2016). Mitochondrial iron chelation ameliorates cigarette smoke-induced bronchitis and emphysema in mice. *Nature Medicine, 22*, 163–174.
Conlon, T. M., Bartel, J., Ballweg, K., Gunter, S., Prehn, C., Krumsiek, J., ... Yildirim, A. O. (2016). Metabolomics screening identifies reduced L-carnitine to be associated with progressive emphysema. *Clinical Science (London, England), 130*, 273–287.
Coppe, J. P., Kauser, K., Campisi, J., & Beausejour, C. M. (2006). Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. *The Journal of Biological Chemistry, 281*, 29568–29574.
Coppe, J. P., Patil, C. K., Rodier, F., Sun, Y., Munoz, D. P., Goldstein, J., ... Campisi, J. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biology, 6*, 2853–2868.
Correia-Melo, C., Marques, F. D., Anderson, R., Hewitt, G., Hewitt, R., Cole, J., ... Passos, J. F. (2016). Mitochondria are required for pro-ageing features of the senescent phenotype. *The EMBO Journal, 35*, 724–742.
Csiszar, A., Labinskyy, N., Podlutsky, A., Kaminski, P. M., Wolin, M. S., Zhang, C., ... Ungvari, Z. (2008). Vasoprotective effects of resveratrol and SIRT1: Attenuation of cigarette smoke-induced oxidative stress and proinflammatory phenotypic alterations. *American Journal of Physiology. Heart and Circulatory Physiology, 294*, H2721–2735.
d'Adda di Fagagna, F., Reaper, P. M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., ... Jackson, S. P. (2003). A DNA damage checkpoint response in telomere-initiated senescence. *Nature, 426*, 194–198.
Dalle Pezze, P., Nelson, G., Otten, E. G., Korolchuk, V. I., Kirkwood, T. B., von Zglinicki, T., & Shanley, D. P. (2014). Dynamic modelling of pathways to cellular senescence reveals strategies for targeted interventions. *PLoS Computational Biology, 10*, e1003728.
de Lange, T. (2005). Shelterin: The protein complex that shapes and safeguards human telomeres. *Genes & Development, 19*, 2100–2110.
Demaria, M., Ohtani, N., Youssef, S. A., Rodier, F., Toussaint, W., Mitchell, J. R., ... Campisi, J. (2014). An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Developmental Cell, 31*, 722–733.
Desagher, S., Glowinski, J., & Premont, J. (1997). Pyruvate protects neurons against hydrogen peroxide-induced toxicity. *The Journal of Neuroscience, 17*, 9060–9067.
Deslee, G., Woods, J. C., Moore, C., Conradi, S. H., Gierada, D. S., Atkinson, J. J., ... Pierce, R. A. (2009). Oxidative damage to nucleic acids in severe emphysema. *Chest, 135*, 965–974.
Dimri, G. P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E. E., Linskens, M., Rubelj, I., Pereira-Smith, O., et al. (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 9363–9367.
Efimova, E. V., Mauceri, H. J., Golden, D. W., Labay, E., Bindokas, V. P., Darga, T. E., ... Weichselbaum, R. R. (2010). Poly(ADP-ribose) polymerase inhibitor induces accelerated senescence in irradiated breast cancer cells and tumors. *Cancer Research, 70*, 6277–6282.
Elborn, J. S. (2016). Cystic fibrosis. *The Lancet, 388*, 2519–2531.
Faner, R., Rojas, M., Macnee, W., & Agusti, A. (2012). Abnormal lung aging in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine, 186*, 306–313.
Fedorov, I. A., Wilson, S. J., Davies, D. E., & Holgate, S. T. (2005). Epithelial stress and structural remodelling in childhood asthma. *Thorax, 60*, 389–394.
Fischer, B. M., Wong, J. K., Degan, S., Kummarapurugu, A. B., Zheng, S., Haridass, P., & Voynow, J. A. (2013). Increased expression of senescence markers in cystic fibrosis airways. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 304*, L394–400.
Fletcher, C., & Peto, R. (1977). The natural history of chronic airflow obstruction. *British Medical Journal, 1*, 1645–1648.

Freund, A., Patil, C. K., & Campisi, J. (2011). p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *The EMBO Journal, 30*, 1536–1548.

Fumagalli, M., Rossiello, F., Clerici, M., Barozzi, S., Cittaro, D., Kaplunov, J. M., ... d'Adda di Fagagna, F. (2012). Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. *Nature Cell Biology, 14*, 355–365.

Garcia-Prat, L., Martinez-Vicente, M., Perdiguero, E., Ortet, L., Rodriguez-Ubreva, J., Rebollo, E., ... Munoz-Canoves, P. (2016). Autophagy maintains stemness by preventing senescence. *Nature, 529*, 37–42.

Gluck, S., Guey, B., Gulen, M. F., Wolter, K., Kang, T.-W., Schmacke, N. A., ... Ablasser, A. (2017). Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. *Nature Cell Biology* advance online publication.

Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H., & de Lange, T. (1999). Mammalian telomeres end in a large duplex loop. *Cell, 97*, 503–514.

Hadj Salem, I., Dube, J., Boulet, L. P., & Chakir, J. (2015). Telomere shortening correlates with accelerated replicative senescence of bronchial fibroblasts in asthma. *Clinical & Experimental Allergy, 45*, 1713–1715.

Haigis, M. C., & Sinclair, D. A. (2010). Mammalian sirtuins: Biological insights and disease relevance. *Annual Review of Pathology, 5*, 253–295.

Halwani, R., Al-Muhsen, S., & Hamid, Q. (2010). Airway remodeling in asthma. *Current Opinion in Pharmacology, 10*, 236–245.

Hara, H., Araya, J., Ito, S., Kobayashi, K., Takasaka, N., Yoshii, Y., ... Kuwano, K. (2013). Mitochondrial fragmentation in cigarette smoke-induced bronchial epithelial cell senescence. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 305*, L737–746.

Hardie, D. G., Ross, F. A., & Hawley, S. A. (2012). AMPK: A nutrient and energy sensor that maintains energy homeostasis. *Nature Reviews. Molecular Cell Biology, 13*, 251–262.

Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., Flurkey, K., ... Miller, R. A. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature, 460*, 392–395.

Hashimoto, M., Asai, A., Kawagishi, H., Mikawa, R., Iwashita, Y., Kanayama, K., ... Sugimoto, M. (2016). Elimination of p19ARF-expressing cells enhances pulmonary function in mice. *JCI Insight, 1*, e87732.

Hawkins, A., Guttentag, S. H., Deterding, R., Funkhouser, W. K., Goralski, J. L., Chatterjee, S., ... Beers, M. F. (2015). A non-BRICHOS SFTPC mutant (SP-CI73T) linked to interstitial lung disease promotes a late block in macroautophagy disrupting cellular proteostasis and mitophagy. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 308*, L33–47.

Hayakawa, T., Iwai, M., Aoki, S., Takimoto, K., Maruyama, M., Maruyama, W., & Motoyama, N. (2015). SIRT1 suppresses the senescence-associated secretory phenotype through epigenetic gene regulation. *PLoS One, 10*, e0116480.

Hayflick, L. (1965). The limited in vitro lifetime of human diploid cell strains. *Experimental Cell Research, 37*, 614–636.

Hayflick, L., & Moorhead, P. S. (1961). The serial cultivation of human diploid cell strains. *Experimental Cell Research, 25*, 585–621.

Hecker, L., Logsdon, N. J., Kurundkar, D., Kurundkar, A., Bernard, K., Hock, T., ... Thannickal, V. J. (2014). Reversal of persistent fibrosis in aging by targeting Nox4-Nrf2 redox imbalance. *Science Translational Medicine, 6*, 231ra247.

Heijink, I. H., Pouwels, S. D., Leijendekker, C., de Bruin, H. G., Zijlstra, G. J., van der Vaart, H., ... van der Toorn, M. (2015). Cigarette smoke-induced damage-associated molecular pattern release from necrotic neutrophils triggers proinflammatory mediator release. *American Journal of Respiratory Cell and Molecular Biology, 52*, 554–562.

Henle, E. S., Han, Z., Tang, N., Rai, P., Luo, Y., & Linn, S. (1999). Sequence-specific DNA cleavage by Fe2 + -mediated Fenton reactions has possible biological implications. *The Journal of Biological Chemistry, 274*, 962–971.

Herbig, U., Ferreira, M., Condel, L., Carey, D., & Sedivy, J. M. (2006). Cellular senescence in aging primates. *Science, 311*, 1257.

Herranz, N., Gallage, S., Mellone, M., Wuestefeld, T., Klotz, S., Hanley, C. J., ... Gil, J. (2015). mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nature Cell Biology, 17*, 1205–1217.

Hewitt, G., Jurk, D., Marques, F. D., Correia-Melo, C., Hardy, T., Gackowska, A., ... Passos, J. F. (2012). Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence. *Nature Communications, 3*, 708.

Hitchings, A. W., Archer, J. R., Srivastava, S. A., & Baker, E. H. (2014). *Safety of metformin in patients with chronic obstructive pulmonary disease and type 2 diabetes mellitus. COPD*.

Hoffmann, R. F., Zarrintan, S., Brandenburg, S. M., Kol, A., de Bruin, H. G., Jafari, S., ... Heijink, I. H. (2013). Prolonged cigarette smoke exposure alters mitochondrial structure and function in airway epithelial cells. *Respiratory Research, 14*, 97.

Holz, O., Zuhlke, I., Jaksztat, E., Muller, K. C., Welker, L., Nakashima, M., ... Jorres, R. A. (2004). Lung fibroblasts from patients with emphysema show a reduced proliferation rate in culture. *The European Respiratory Journal, 24*, 575–579.

Horng, T. (2014). Calcium signaling and mitochondrial destabilization in the triggering of the NLRP3 inflammasome. *Trends in Immunology, 35*, 253–261.

Houben, J. M., Mercken, E. M., Ketelslegers, H. B., Bast, A., Wouters, E. F., Hageman, G. J., & Schols, A. M. (2009). Telomere shortening in chronic obstructive pulmonary disease. *Respiratory Medicine, 103*, 230–236.

Huang, J., Okuka, M., Lu, W., Tsibris, J. C., McLean, M. P., Keefe, D. L., & Liu, L. (2013). Telomere shortening and DNA damage of embryonic stem cells induced by cigarette smoke. *Reproductive Toxicology, 35*, 89–95.

Huang, W. T., Akhter, H., Jiang, C., MacEwen, M., Ding, Q., Antony, V., ... Liu, R. M. (2015). Plasminogen activator inhibitor 1, fibroblast apoptosis resistance, and aging-related susceptibility to lung fibrosis. *Experimental Gerontology, 61*, 62–75.

Iannello, A., Thompson, T. W., Ardolino, M., Lowe, S. W., & Raulet, D. H. (2013). p53-Dependent chemokine production by senescent tumor cells supports NKG2D-dependent tumor elimination by natural killer cells. *The Journal of Experimental Medicine, 210*, 2057–2069.

Ito, K., & Barnes, P. J. (2009). COPD as a disease of accelerated lung aging. *Chest, 135*, 173–180.

Ito, S., Araya, J., Kurita, Y., Kobayashi, K., Takasaka, N., Yoshida, M., ... Kuwano, K. (2015). PARK2-mediated mitophagy is involved in regulation of HBEC senescence in COPD pathogenesis. *Autophagy, 11*, 547–559.

Janssens, J. P., Pache, J. C., & Nicod, L. P. (1999). Physiological changes in respiratory function associated with ageing. *The European Respiratory Journal, 13*, 197–205.

Jeon, O. H., Kim, C., Laberge, R. M., Demaria, M., Rathod, S., Vasserot, A. P., ... Elisseeff, J. H. (2017). Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nature Medicine*.

Jeyapalan, J. C., Ferreira, M., Sedivy, J. M., & Herbig, U. (2007). Accumulation of senescent cells in mitotic tissue of aging primates. *Mechanisms of Ageing and Development, 128*, 36–44.

Jiang, P., Du, W., Mancuso, A., Wellen, K. E., & Yang, X. (2013). Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. *Nature, 493*, 689–693.

Jun, J. I., & Lau, L. F. (2010). The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nature Cell Biology, 12*, 676–685.

Jurk, D., Wang, C., Miwa, S., Maddick, M., Korolchuk, V., Tsolou, A., ... von Zglinicki, T. (2012). Postmitotic neurons develop a p21-dependent senescence-like phenotype driven by a DNA damage response. *Aging Cell, 11*, 996–1004.

Jurk, D., Wilson, C., Passos, J. F., Oakley, F., Correia-Melo, C., Greaves, L., ... von Zglinicki, T. (2014). Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. *Nature Communications, 2*, 4172.

Kang, T. W., Yevsa, T., Woller, N., Hoenicke, L., Wuestefeld, T., Dauch, D., ... Zender, L. (2011). Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature, 479*, 547–551.

Kaplon, J., Zheng, L., Meissl, K., Chaneton, B., Selivanov, V. A., Mackay, G., ... Peeper, D. S. (2013). A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. *Nature, 498*, 109–112.

Kasai, H. (1997). Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutation Research, 387*, 147–163.

Keatings, V. M., Collins, P. D., Scott, D. M., & Barnes, P. J. (1996). Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *American Journal of Respiratory and Critical Care Medicine, 153*, 530–534.

Kirkham, P. A., & Barnes, P. J. (2013). Oxidative Stress in COPD. *Chest, 144*, 266–273.

Kojima, H., Kunimoto, H., Inoue, T., & Nakajima, K. (2012). The STAT3-IGFBP5 axis is critical for IL-6/gp130-induced premature senescence in human fibroblasts. *Cell Cycle, 11*, 730–739.

Konokhova, Y., Spendiff, S., Jagoe, R. T., Aare, S., Kapchinsky, S., MacMillan, N. J., ... Taivassalo, T. (2016). Failed upregulation of TFAM protein and mitochondrial DNA in oxidatively deficient fibers of chronic obstructive pulmonary disease locomotor muscle. *Skeletal Muscle, 6*, 10.

Korolchuk, V. I., Miwa, S., Carroll, B., & von Zglinicki, T. (2017). Mitochondria in cell senescence: Is mitophagy the weakest link? *eBioMedicine*.

Kreiling, J. A., Tamamori-Adachi, M., Sexton, A. N., Jeyapalan, J. C., Munoz-Najar, U., Peterson, A. L., ... Sedivy, J. M. (2011). Age-associated increase in heterochromatic marks in murine and primate tissues. *Aging Cell, 10*, 292–304.

Krishnamurthy, J., Torrice, C., Ramsey, M. R., Kovalev, G. I., Al-Regaiey, K., Su, L., & Sharpless, N. E. (2004). Ink4a/Arf expression is a biomarker of aging. *The Journal of Clinical Investigation, 114*, 1299–1307.

Krizhanovsky, V., Yon, M., Dickins, R. A., Hearn, S., Simon, J., Miething, C., ... Lowe, S. W. (2008). Senescence of activated stellate cells limits liver fibrosis. *Cell, 134*, 657–667.

Kuilman, T., Michaloglou, C., Mooi, W. J., & Peeper, D. S. (2010). The essence of senescence. *Genes & Development, 24*, 2463–2479.

Kuwano, K., Kunitake, R., Kawasaki, M., Nomoto, Y., Hagimoto, N., Nakanishi, Y., & Hara, N. (1996). P21Waf1/Cip1/Sdi1 and p53 expression in association with DNA strand breaks in idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine, 154*, 477–483.

Laberge, R. M., Sun, Y., Orjalo, A. V., Patil, C. K., Freund, A., Zhou, L., ... Campisi, J. (2015). MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. *Nature Cell Biology, 17*, 1049–1061.

Lehmann, M., Korfei, M., Mutze, K., Klee, S., Skronska-Wasek, W., Alsafadi, H. N., ... Konigshoff, M. (2017). Senolytic drugs target alveolar epithelial cell function and attenuate experimental lung fibrosis ex vivo. *The European Respiratory Journal, 50*.

Lerner, C., Bitto, A., Pulliam, D., Nacarelli, T., Konigsberg, M., Van Remmen, H., ... Sell, C. (2013). Reduced mammalian target of rapamycin activity facilitates mitochondrial retrograde signaling and increases life span in normal human fibroblasts. *Aging Cell, 12*, 966–977.

Li, J., Dai, A., Hu, R., Zhu, L., & Tan, S. (2010). Positive correlation between PPARgamma/PGC-1alpha and gamma-GCS in lungs of rats and patients with chronic obstructive pulmonary disease. *Acta Biochimica et Biophysica Sinica Shanghai, 42*, 603–614.

Lin, H., & Jiang, S. (2015). Combined pulmonary fibrosis and emphysema (CPFE): An entity different from emphysema or pulmonary fibrosis alone. *Journal of Thoracic Disease, 7*, 767–779.

Liu, L., Trimarchi, J. R., Smith, P. J., & Keefe, D. L. (2002). Mitochondrial dysfunction leads to telomere attrition and genomic instability. *Aging Cell, 1*, 40–46.

MacNee, W. (2001). Oxidants/antioxidants and chronic obstructive pulmonary disease: Pathogenesis to therapy. *Novartis Foundation Symposium, 234*, 169–185 discussion 185-168.

Maddocks, O. D. K., Athineos, D., Cheung, E. C., Lee, P., Zhang, T., van den Broek, N. J. F., ... Vousden, K. H. (2017). Modulating the therapeutic response of tumours to dietary serine and glycine starvation. *Nature, 544*, 372–376.

Mannino, D. M., & Buist, A. S. (2007). Global burden of COPD: Risk factors, prevalence, and future trends. *Lancet, 370*, 765–773.

Masayesva, B. G., Mambo, E., Taylor, R. J., Goloubeva, O. G., Zhou, S., Cohen, Y., ... Califano, J. (2006). Mitochondrial DNA content increase in response to cigarette smoking. *Cancer Epidemiology, Biomarkers & Prevention, 15*, 19–24.

McCool, K. W., & Miyamoto, S. (2012). DNA damage-dependent NF-kappaB activation: NEMO turns nuclear signaling inside out. *Immunological Reviews, 246*, 311–326.

McShea, A., Harris, P. L., Webster, K. R., Wahl, A. F., & Smith, M. A. (1997). Abnormal expression of the cell cycle regulators P16 and CDK4 in Alzheimer's disease. *The American Journal of Pathology, 150*, 1933–1939.

Mercado, N., Ito, K., & Barnes, P. J. (2015). Accelerated ageing of the lung in COPD: New concepts. *Thorax, 70*, 482–489.

Mercer, P. F., Woodcock, H. V., Eley, J. D., Plate, M., Sulikowski, M. G., Durrenberger, P. F., ... Chambers, R. C. (2016). Exploration of a potent PI3 kinase/mTOR inhibitor as a novel anti-fibrotic agent in IPF. *Thorax, 71*, 701–711.

Miller, R. A., Harrison, D. E., Astle, C. M., Baur, J. A., Boyd, A. R., de Cabo, R., ... Strong, R. (2011). Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 66*, 191–201.

Mills, E. L., Kelly, B., Logan, A., Costa, A. S., Varma, M., Bryant, C. E., ... O'Neill, L. A. (2016). Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell, 167*(457-470), e413.

Minagawa, S., Araya, J., Numata, T., Nojiri, S., Hara, H., Yumino, Y., ... Kuwano, K. (2011). Accelerated epithelial cell senescence in IPF and the inhibitory role of SIRT6 in TGF-beta-induced senescence of human bronchial epithelial cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 300*, L391–401.

Mitani, A., Ito, K., Vuppusetty, C., Barnes, P. J., & Mercado, N. (2016). Restoration of corticosteroid sensitivity in chronic obstructive pulmonary disease by inhibition of mammalian target of rapamycin. *American Journal of Respiratory and Critical Care Medicine, 193*, 143–153.

Mizumura, K., Cloonan, S. M., Nakahira, K., Bhashyam, A. R., Cervo, M., Kitada, T., ... Choi, A. M. (2014). Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD. *The Journal of Clinical Investigation, 124*, 3987–4003.

Morla, M., Busquets, X., Pons, J., Sauleda, J., MacNee, W., & Agusti, A. G. (2006). Telomere shortening in smokers with and without COPD. *The European Respiratory Journal, 27*, 525–528.

Mouraret, N., Houssaini, A., Abid, S., Quarck, R., Marcos, E., Parpaleix, A., ... Adnot, S. (2015). Role for telomerase in pulmonary hypertension. *Circulation, 131*, 742–755.

Mouraret, N., Marcos, E., Abid, S., Gary-Bobo, G., Saker, M., Houssaini, A., ... Adnot, S. (2013). Activation of lung p53 by Nutlin-3a prevents and reverses experimental pulmonary hypertension. *Circulation, 127*, 1664–1676.

Mui, T. S., Man, J. M., McElhaney, J. E., Sandford, A. J., Coxson, H. O., Birmingham, C. L., ... Sin, D. D. (2009). Telomere length and chronic obstructive pulmonary disease: evidence of accelerated aging. *Journal of the American Geriatrics Society, 57*, 2372–2374.

Muller, K. C., Welker, L., Paasch, K., Feindt, B., Erpenbeck, V. J., Hohlfeld, J. M., ... Holz, O. (2006). Lung fibroblasts from patients with emphysema show markers of senescence in vitro. *Respiratory Research, 7*, 32.

Munoz-Espin, D., Canamero, M., Maraver, A., Gomez-Lopez, G., Contreras, J., Murillo-Cuesta, S., ... Serrano, M. (2013). Programmed cell senescence during mammalian embryonic development. *Cell, 155*, 1104–1118.

Munoz-Espin, D., & Serrano, M. (2014). Cellular senescence: from physiology to pathology. *Nature Reviews. Molecular Cell Biology, 15*, 482–496.

Nakamaru, Y., Vuppusetty, C., Wada, H., Milne, J. C., Ito, M., Rossios, C., ... Ito, K. (2009). A protein deacetylase SIRT1 is a negative regulator of metalloproteinase-9. *The FASEB Journal, 23*, 2810–2819.

Nyunoya, T., Monick, M. M., Klingelhutz, A., Yarovinsky, T. O., Cagley, J. R., & Hunninghake, G. W. (2006). Cigarette smoke induces cellular senescence. *American Journal of Respiratory Cell and Molecular Biology, 35*, 681–688.

O'Donnell, A. E. (2008). Bronchiectasis. *Chest, 134*, 815–823.

Ogrodnik, M., Miwa, S., Tchkonia, T., Tiniakos, D., Wilson, C. L., Lahat, A., ... Jurk, D. (2017). Cellular senescence drives age-dependent hepatic steatosis. *Nature Communications, 8*, 15691.

Ovadya, Y., & Krizhanovsky, V. (2014). Senescent cells: SASPected drivers of age-related pathologies. *Biogerontology*.

Pan, J., Li, D., Xu, Y., Zhang, J., Wang, Y., Chen, M., ... Meng, A. (2017). Inhibition of Bcl-2/xl with ABT-263 selectively kills senescent Type II pneumocytes and reverses persistent pulmonary fibrosis induced by ionizing radiation in mice. *International Journal of Radiation Oncology, Biology, Physics*.

Parker, S. M., Goriwiec, M. R., Borthwick, L. A., Johnson, G., Ward, C., Lordan, J. L., ... Fisher, A. J. (2008). Airway epithelial cell senescence in the lung allograft. *American Journal of Transplantation, 8*, 1544–1549.

Passos, J. F., Nelson, G., Wang, C., Richter, T., Simillion, C., Proctor, C. J., ... von Zglinicki, T. (2010). Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Molecular Systems Biology, 6*, 347.

Passos, J. F., Saretzki, G., Ahmed, S., Nelson, G., Richter, T., Peters, H., ... von Zglinicki, T. (2007). Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. *PLoS Biology, 5*, e110.

Passos, J. F., Saretzki, G., & von Zglinicki, T. (2007). DNA damage in telomeres and mitochondria during cellular senescence: Is there a connection? *Nucleic Acids Research, 35*, 7505–7513.

Patel, A. S., Song, J. W., Chu, S. G., Mizumura, K., Osorio, J. C., Shi, Y., ... Morse, D. (2015). Epithelial cell mitochondrial dysfunction and PINK1 are induced by transforming growth factor-beta1 in pulmonary fibrosis. *PLoS One, 10*, e0121246.

Petersen, S., Saretzki, G., & von Zglinicki, T. (1998). Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. *Experimental Cell Research, 239*, 152–160.

Pouwels, S. D., Zijlstra, G. J., van der Toorn, M., Hesse, L., Gras, R., Ten Hacken, N. H., ... Nawijn, M. C. (2016). Cigarette smoke-induced necroptosis and DAMP release trigger neutrophilic airway inflammation in mice. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 310*, L377–386.

Povedano, J. M., Martinez, P., Flores, J. M., Mulero, F., & Blasco, M. A. (2015). Mice with pulmonary fibrosis driven by telomere dysfunction. *Cell Reports, 12*, 286–299.

Price, J. S., Waters, J. G., Darrah, C., Pennington, C., Edwards, D. R., Donell, S. T., & Clark, I. M. (2002). The role of chondrocyte senescence in osteoarthritis. *Aging Cell, 1*, 57–65.

Raghu, G., Weycker, D., Edelsberg, J., Bradford, W. Z., & Oster, G. (2006). Incidence and prevalence of idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine, 174*, 810–816.

Rahman, I., van Schadewijk, A. A., Crowther, A. J., Hiemstra, P. S., Stolk, J., MacNee, W., & De Boer, W. I. (2002). 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine, 166*, 490–495.

Rajendrasozhan, S., Yang, S. R., Kinnula, V. L., & Rahman, I. (2008). SIRT1, an antiinflammatory and antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine, 177*, 861–870.

Richter, T., & von Zglinicki, T. (2007). A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Experimental Gerontology, 42*, 1039–1042.

Rode, L., Bojesen, S. E., Weischer, M., Vestbo, J., & Nordestgaard, B. G. (2013). Short telomere length, lung function and chronic obstructive pulmonary disease in 46,396 individuals. *Thorax, 68*, 429–435.

Rodier, F., Coppe, J. P., Patil, C. K., Hoeijmakers, W. A., Munoz, D. P., Raza, S. R., ... Campisi, J. (2009). Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nature Cell Biology, 11*, 973–979.

Romero, Y., Bueno, M., Ramirez, R., Alvarez, D., Sembrat, J. C., Goncharova, E. A., ... Pardo, A. (2016). mTORC1 activation decreases autophagy in aging and idiopathic pulmonary fibrosis and contributes to apoptosis resistance in IPF fibroblasts. *Aging Cell*.

Roos, C. M., Zhang, B., Palmer, A. K., Ogrodnik, M. B., Pirtskhalava, T., Thalji, N. M., ... Miller, J. D. (2016). Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. *Aging Cell, 15*, 973–977.

Rudin, C. M., Hann, C. L., Garon, E. B., Ribeiro de Oliveira, M., Bonomi, P. D., Camidge, D. R., ... Gandhi, L. (2012). Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. *Clinical Cancer Research, 18*, 3163–3169.

Russell, R. E., Culpitt, S. V., DeMatos, C., Donnelly, L., Smith, M., Wiggins, J., & Barnes, P. J. (2002). Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. *American Journal of Respiratory Cell and Molecular Biology, 26*, 602–609.

Sahin, E., Colla, S., Liesa, M., Moslehi, J., Muller, F. L., Guo, M., ... DePinho, R. A. (2011). Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature, 470*, 359–365.

Sanders, Y. Y., Hagood, J. S., Liu, H., Zhang, W., Ambalavanan, N., & Thannickal, V. J. (2014). Histone deacetylase inhibition promotes fibroblast apoptosis and ameliorates pulmonary fibrosis in mice. *The European Respiratory Journal, 43*, 1448–1458.

Saretzki, G., Murphy, M. P., & von Zglinicki, T. (2003). MitoQ counteracts telomere shortening and elongates lifespan of fibroblasts under mild oxidative stress. *Aging Cell, 2*, 141–143.

Sasaki, T., Maier, B., Bartke, A., & Scrable, H. (2006). Progressive loss of SIRT1 with cell cycle withdrawal. *Aging Cell, 5*, 413–422.

Savale, L., Chaouat, A., Bastuji-Garin, S., Marcos, E., Boyer, L., Maitre, B., ... Adnot, S. (2009). Shortened telomeres in circulating leukocytes of patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine, 179*, 566–571.

Schafer, M. J., White, T. A., Evans, G., Tonne, J. M., Verzosa, G. C., Stout, M. B., ... LeBrasseur, N. K. (2016). Exercise prevents diet-induced cellular senescence in adipose tissue. *Diabetes, 65*, 1606–1615.

Schafer, M. J., White, T. A., Iijima, K., Haak, A. J., Ligresti, G., Atkinson, E. J., ... LeBrasseur, N. K. (2017). Cellular senescence mediates fibrotic pulmonary disease. *Nature Communications, 8*, 14532.

Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D., & Lowe, S. W. (1997). Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell, 88*, 593–602.

Sexton, P., Metcalf, P., & Kolbe, J. (2014). Respiratory effects of insulin sensitisation with metformin: a prospective observational study. *COPD, 11*, 133–142.

Sharpless, N. E., Ramsey, M. R., Balasubramanian, P., Castrillon, D. H., & DePinho, R. A. (2004). The differential impact of p16(INK4a) or p19(ARF) deficiency on cell growth and tumorigenesis. *Oncogene, 23*, 379–385.

Shen, Y., Cai, W., Lei, S., & Zhang, Z. (2014). Effect of high/low dose *N*-acetylcysteine on chronic obstructive pulmonary disease: A systematic review and meta-analysis. *COPD, 11*, 351–358.

Shivshankar, P., Boyd, A. R., Le Saux, C. J., Yeh, I. T., & Orihuela, C. J. (2011). Cellular senescence increases expression of bacterial ligands in the lungs and is positively correlated with increased susceptibility to pneumococcal pneumonia. *Aging Cell, 10*, 798–806.

Smogorzewska, A., & de Lange, T. (2002). Different telomere damage signaling pathways in human and mouse cells. *The EMBO Journal, 21*, 4338–4348.

Song, W., Zhao, J., & Li, Z. (2001). Interleukin-6 in bronchoalveolar lavage fluid from patients with COPD. *Chinese Medical Journal, 114*, 1140–1142.

Sorrentino, J. A., Krishnamurthy, J., Tilley, S., Alb, J. G., Jr., Burd, C. E., & Sharpless, N. E. (2014). p16INK4a reporter mice reveal age-promoting effects of environmental toxicants. *The Journal of Clinical Investigation, 124*, 169–173.

Sosulski, M. L., Gongora, R., Feghali-Bostwick, C., Lasky, J. A., & Sanchez, C. G. (2017). Sirtuin 3 deregulation promotes pulmonary fibrosis. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 72*, 595–602.

Stanley, S. E., Chen, J. J., Podlevsky, J. D., Alder, J. K., Hansel, N. N., Mathias, R. A., ... Armanios, M. (2015). Telomerase mutations in smokers with severe emphysema. *The Journal of Clinical Investigation, 125*, 563–570.

Storer, M., Mas, A., Robert-Moreno, A., Pecoraro, M., Ortells, M. C., Di Giacomo, V., ... Keyes, W. M. (2013). Senescence is a developmental mechanism that contributes to embryonic growth and patterning. *Cell, 155*, 1119–1130.

Stout-Delgado, H. W., Cho, S. J., Chu, S. G., Mitzel, D. N., Villalba, J., El-Chemaly, S., ... Rosas, I. O. (2016). Age-dependent susceptibility to pulmonary fibrosis is associated with NLRP3 inflammasome activation. *American Journal of Respiratory Cell and Molecular Biology, 55*, 252–263.

Stuart, B. D., Lee, J. S., Kozlitina, J., Noth, I., Devine, M. S., Glazer, C. S., ... Garcia, C. K. (2014). Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: An observational cohort study with independent validation. *The Lancet Respiratory Medicine, 2*, 557–565.

Suram, A., Kaplunov, J., Patel, P. L., Ruan, H., Cerutti, A., Boccardi, V., ... Herbig, U. (2012). Oncogene-induced telomere dysfunction enforces cellular senescence in human cancer precursor lesions. *The EMBO Journal, 31*, 2839–2851.

Tsuji, T., Aoshiba, K., & Nagai, A. (2004). Cigarette smoke induces senescence in alveolar epithelial cells. *American Journal of Respiratory Cell and Molecular Biology, 31*, 643–649.

Tsuji, T., Aoshiba, K., & Nagai, A. (2006). Alveolar cell senescence in patients with pulmonary emphysema. *American Journal of Respiratory and Critical Care Medicine, 174*, 886–893.

Tsuji, T., Aoshiba, K., & Nagai, A. (2010). Alveolar cell senescence exacerbates pulmonary inflammation in patients with chronic obstructive pulmonary disease. *Respiration, 80*, 59–70.

Tzortzaki, E. G., Dimakou, K., Neofytou, E., Tsikritsaki, K., Samara, K., Avgousti, M., ... Siafakas, N. M. (2012). Oxidative DNA damage and somatic mutations: A link to the molecular pathogenesis of chronic inflammatory airway diseases. *Chest, 141*, 1243–1250.

van Deursen, J. M. (2014). The role of senescent cells in ageing. *Nature, 509*, 439–446.

Vasile, E., Tomita, Y., Brown, L. F., Kocher, O., & Dvorak, H. F. (2001). Differential expression of thymosin beta-10 by early passage and senescent vascular endothelium is modulated by VPF/VEGF: Evidence for senescent endothelial cells in vivo at sites of atherosclerosis. *The FASEB Journal, 15*, 458–466.

von Zglinicki, T. (2002). Oxidative stress shortens telomeres. *Trends in Biochemical Sciences, 27*, 339–344.

Wang, C., Jurk, D., Maddick, M., Nelson, G., Martin-Ruiz, C., & von Zglinicki, T. (2009). DNA damage response and cellular senescence in tissues of aging mice. *Aging Cell, 8*, 311–323.

Wang, J., Uryga, A. K., Reinhold, J., Figg, N., Baker, L., Finigan, A., ... Bennett, M. (2015). Vascular smooth muscle cell senescence promotes atherosclerosis and features of plaque vulnerability. *Circulation, 132*, 1909–1919.

Wang, L., Green, F. H., Smiley-Jewell, S. M., & Pinkerton, K. E. (2010). Susceptibility of the aging lung to environmental injury. *Seminars in Respiratory and Critical Care Medicine, 31*, 539–553.

Wang, W., Yang, X., Lopez de Silanes, I., Carling, D., & Gorospe, M. (2003). Increased AMP:ATP ratio and AMP-activated protein kinase activity during cellular senescence linked to reduced HuR function. *The Journal of Biological Chemistry, 278*, 27016–27023.

West, A. P., Khoury-Hanold, W., Staron, M., Tal, M. C., Pineda, C. M., Lang, S. M., ... Shadel, G. S. (2015). Mitochondrial DNA stress primes the antiviral innate immune response. *Nature, 520*, 553–557.

Wiegman, C. H., Michaeloudes, C., Haji, G., Narang, P., Clarke, C. J., Russell, K. E., ... Adcock, I. M. (2015). Oxidative stress-induced mitochondrial dysfunction drives inflammation and airway smooth muscle remodeling in patients with chronic obstructive pulmonary disease. *The Journal of Allergy and Clinical Immunology, 136*, 769–780.

Wiley, C. D., & Campisi, J. (2016). From ancient pathways to aging cells-connecting metabolism and cellular senescence. *Cell Metabolism, 23*, 1013–1021.

Wiley, C. D., Velarde, M. C., Lecot, P., Liu, S., Sarnoski, E. A., Freund, A., ... Campisi, J. (2016). Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metabolism, 23*, 303–314.

Wilkinson, J. E., Burmeister, L., Brooks, S. V., Chan, C. C., Friedline, S., Harrison, D. E., ... Miller, R. A. (2012). Rapamycin slows aging in mice. *Aging Cell, 11*, 675–682.

Wilson, C. L., Jurk, D., Fullard, N., Banks, P., Page, A., Luli, S., ... Mann, D. A. (2015). NFkappaB1 is a suppressor of neutrophil-driven hepatocellular carcinoma. *Nature Communications, 6*, 6818.

Wu, J., Dong, F., Wang, R. A., Wang, J., Zhao, J., Yang, M., ... Dong, L. (2013). Central role of cellular senescence in TSLP-induced airway remodeling in asthma. *PLoS One, 8*, e77795.

Xu, D., & Finkel, T. (2002). A role for mitochondria as potential regulators of cellular life span. *Biochemical and Biophysical Research Communications, 294*, 245–248.

Xu, M., Bradley, E. W., Weivoda, M. M., Hwang, S. M., Pirtskhalava, T., Decklever, T., ... Kirkland, J. L. (2016). *Transplanted senescent cells induce an osteoarthritis-like condition in mice.* J Gerontol A Biol Sci Med Sci.

Xue, W., Zender, L., Miething, C., Dickins, R. A., Hernando, E., Krizhanovsky, V., ... Lowe, S. W. (2007). Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature, 445*, 656–660.

Yanai, H., Shteinberg, A., Porat, Z., Budovsky, A., Braiman, A., Ziesche, R., & Fraifeld, V. E. (2015). Cellular senescence-like features of lung fibroblasts derived from idiopathic pulmonary fibrosis patients. *Aging (Albany NY), 7*, 664–672.

Yao, H., Sundar, I. K., Ahmad, T., Lerner, C., Gerloff, J., Friedman, A. E., ... Rahman, I. (2014). SIRT1 protects against cigarette smoke-induced lung oxidative stress via a FOXO3-dependent mechanism. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 306*, L816–828.

Yoshida, T., Mett, I., Bhunia, A. K., Bowman, J., Perez, M., Zhang, L., ... Tuder, R. M. (2010). Rtp801, a suppressor of mTOR signaling, is an essential mediator of cigarette smoke-induced pulmonary injury and emphysema. *Nature Medicine, 16*, 767–773.

Zhu, Y., Tchkonia, T., Fuhrmann-Stroissnigg, H., Dai, H. M., Ling, Y. Y., Stout, M. B., ... Kirkland, J. L. (2016). Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell, 15*, 428–435.

Zhu, Y., Tchkonia, T., Pirtskhalava, T., Gower, A. C., Ding, H., Giorgadze, N., ... Kirkland, J. L. (2015). The Achilles' heel of senescent cells: From transcriptome to senolytic drugs. *Aging Cell, 14*, 644–658.

Zwerschke, W., Mazurek, S., Stockl, P., Hutter, E., Eigenbrodt, E., & Jansen-Durr, P. (2003). Metabolic analysis of senescent human fibroblasts reveals a role for AMP in cellular senescence. *Biochemical Journal, 376*, 403–411.